Degradable Clostridial Toxins

[01] This application claims the benefit of U.S. Provisional Patent Application Serial Number 61/346,578, filed on May 20, 2010, the entire disclosure of which is incorporated herein by this specific reference.

[02] The ability of Clostridial toxins, such as, *e.g.*, Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see *e.g.*, William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). Clostridial toxins commercially available as pharmaceutical compositions include, BoNT/A preparations, such as, *e.g.*, BOTOX® (Allergan, Inc., Irvine, CA), DYSPORT®/RELOXIN®, (Beaufour Ipsen, Porton Down, England), NEURONOX® (Medy-Tox, Inc., Ochang-myeon, South Korea) BTX-A (Lanzhou Institute Biological Products, China) and XEOMIN® (Merz Pharmaceuticals, GmbH., Frankfurt, Germany); and BoNT/B preparations, such as, *e.g.*, MYOBLOC™/NEUROBLOC™ (Elan Pharmaceuticals, San Francisco, CA). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder.

[03] A Clostridial toxin treatment inhibits neurotransmitter release by disrupting the exocytotic process used to secrete the neurotransmitter into the synaptic cleft. There is a great desire by the pharmaceutical industry to expand the use of Clostridial toxin therapies beyond its current myo-relaxant applications to treat sensory nerve-based ailments, such as, *e.g.*, various kinds of chronic pain, neurogenic inflammation and urogentital disorders, as well as other disorders, such as, *e.g.*, pancreatitis. One approach that is currently being exploited to expand Clostridial toxin-based therapies involves modifying a Clostridial toxin so that the modified toxin has an altered cell targeting capability for a non-Clostridial toxin target cell. This re-targeted capability is achieved by replacing a naturally-occurring targeting domain of a Clostridial toxin with a targeting domain showing a preferential binding activity for a non-Clostridial toxin receptor present in a non-Clostridial toxin target cell. Such modifications to a targeting domain result in a Clostridial toxin chimeric called a Targeted Vesicular Exocytosis Modulating Protein (TVEMP) that is able to selectively bind to a non-Clostridial toxin receptor (target receptor) present on a non-Clostridial toxin target cell (re-targeted). A Clostridial toxin chimeric with a targeting activity for a non-Clostridial toxin target cell can bind to a receptor present on the non-Clostridial toxin target cell, translocate into the cytoplasm, and exert its proteolytic effect on the SNARE complex of the non-Clostridial toxin target cell.

[04] Clostridial toxin and Clostridial toxin chimeric therapies are successfully used for many indications. Generally, administration of a Clostridial toxin or Clostridial toxin chimeric is well tolerated. However, administration in some applications can be challenging because of the larger doses required to achieve a beneficial effect. Larger doses can increase the likelihood that the toxin or Clostridial toxin chimeric may move through the interstitial fluids and the circulatory systems, such as, *e.g.*, the cardiovascular system and the lymphatic system, of the body, resulting in the undesirable dispersal of the toxin or Clostridial toxin chimeric to areas not targeted for treatment. Such dispersal can lead to undesirable side effects,

such as, *e.g.*, inhibition of neurotransmitter release in neurons not targeted for toxin treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. Thus, there remains a need for improved Clostridial toxins and/or Clostridial toxin chimeras that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for toxin treatment.

[05] The growing clinical, therapeutic, and cosmetic use of Clostridial toxins and Clostridial toxin chimeras in therapies requiring larger doses necessitates the pharmaceutical industry to develop modified Clostridial toxins and Clostridial toxin chimeras that are effective at the target site of application, but reduce or prevent the possible side-effects associated with the dispersal of the toxins to an unwanted location. The present specification provides novel modified Clostridial toxins and Clostridial toxin chimeras that reduce or prevent unwanted side-effects associated with toxin dispersal into non-targeted areas. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuromuscular disorders, neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders and hyperkinetic facial lines, as well as, other disorders where a Clostridial toxin or a Clostridial toxin chimeric administration to a mammal can produce a beneficial effect.

SUMMARY

[05a] In one aspect, the present invention refers to A BoNT/A Clostridial toxin comprising a least one inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the 871 - 895 domain of SEQ ID NO: 1, wherein the at least one inactivation cleavage site comprises a dual Thrombin-Thrombin site.

[05b] In another aspect, the present invention refers to a polynucleotide molecule encoding a Clostridial toxin of the present invention.

BRIEF DESCRIPTION OF THE DRAWING

[06] FIG. 1 shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain

translocation, where multiple events are thought to occur, including, e.g., changes in the internal pH of the vesicle, formation of a channel pore comprising the HN domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, e.g., SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[07] FIG. 2 shows the domain organization of naturally-occurring Clostridial toxins. The single-chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, and a binding domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as

e.g., an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment, converts the single-chain form of the toxin into the di-chain form. Above the single-chain form, the H_{CC} region of the Clostridial toxin binding domain is depicted. This region comprises the β-trefoil domain which comprises in an amino to carboxyl linear organization an α-fold, a β4/β5 hairpin turn, a β-fold, a β8/β9 hairpin turn, and a γ-fold.

[08] FIG. 3 shows Clostridial toxins or Clostridial toxn chimeras with a binding domain located at the amino terminus of the toxin. FIG. 3A depicts the single-chain polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a binding element, a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form. FIG. 3B depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a binding element, a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form.

[09] FIG. 4 shows Clostridial toxins or Clostridial toxn chimeras with a binding domain located at the amino terminus of the toxin. FIG. 4A depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), a binding element, and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form. FIG. 4B depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), a binding element, and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form. FIG. 4C depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a therapeutic element, a binding element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form. FIG. 4D depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a translocation element, a binding element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form.

[010] FIG. 5 shows Clostridial toxins or Clostridial toxin chimeras with a binding domain located at the amino terminus of the toxin. FIG. 5A depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), a translocation element, and a binding element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 5B depicts the single polypeptide form of a toxin or chimera with an amino to carboxyl linear organization comprising a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), a therapeutic element, and a binding element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin or chimera is converted to the di-chain form.

DETAILED DESCRIPTION

[011] The present specification discloses modified Clostridial toxins and modified Clostridial toxin chimeras that can be rapidly inactivated from an unwanted location or locations by exploiting the presence of proteases present in interstitial fluids and circulatory systems, such as, e.g., the cardiovascular system and the lymphatic system. This is because the modified Clostridial toxins and modified Clostridial toxin chimeras disclosed in the present specification comprise a protease cleavage site for a protease present in an interstitial fluid and/or a circulatory system. The presence of such a protease cleavage site makes the modified Clostridial toxin or modified Clostridial toxin chimeric susceptible to proteolytic cleavage by its cognate protease, which renders such modified toxins inactive. For example, in situations where a Clostridial toxin or Clostridial toxin chimeric modified to comprise a cleavage site for an extracellular matrix protease has diffused into the interstitial fluid, this modified toxin or modified Clostridial toxin chimeric can be effectively cleaved by the cognate extracellular matrix protease. As another example, in situations where a Clostridial toxin or Clostridial toxin chimeric modified to comprise a cleavage site for a blood protease has diffused into the cardiovascular system, this modified toxin or modified Clostridial toxin chimeric can be effectively cleaved by the cognate blood protease. As yet another example, in situations where a Clostridial toxin or Clostridial toxin chimeric modified to comprise a cleavage site for a lymphatic protease has diffused into the lymphatic system, this modified toxin or modified Clostridial toxin chimeric can be effectively cleaved by the cognate lymphatic protease. Thus utilizing a Clostridial toxin or Clostridial toxin chimeric comprising a cleavage site(s) for proteases present the interstitial fluid and/or circulatory system will lessen or remove such Clostridial toxin or Clostridial toxin chimeric from an unwanted location, thereby reducing or preventing the undesirable side-effects associated with the diffusion of a Clostridial toxin or Clostridial toxin chimeric to an unwanted location.

[012] Thus, aspects of the present specification provide a Clostridial toxin comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain or the H_{CN} binding subdomain. Such disclosed toxins can comprise a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, a di-chain loop region comprising an exogenous protease cleavage site, and an inactivation cleavage site located within an inactivation cleavage site region. Non-limiting examples of inactivation cleavage sites include Thrombin cleavage sites, Plasmin cleavage sites, Coagulation Factor VIIa cleavage sites, Coagulation Factor IXa cleavage sites, Coagulation Factor Xa cleavage sites, Coagulation Factor XIa cleavage sites, Coagulation Factor XIIa cleavage sites, plasma kallikrein cleavage sites, protease-activated G protein-coupled receptor-1 (PAR1) cleavage sites, PAR 2 cleavage sites, PAR3 cleavage sites, PAR4 cleavage sites, Matrix Metalloproteinase-2 (MMP-2) cleavage sites, Matrix Metalloproteinase-9 (MMP-9) cleavage sites, Furin cleavage sites, urokinase-type Plasminogen activator (uPA) cleavage sites, tissue-type Plasminogen activator (tPA) cleavage sites, Tryptase-ε cleavage sites, Mouse mast cell protease-7 (mMCP-7) cleavage sites, endothelin-converting enzyme-1 (ECE-1) cleavage sites, Kell blood group cleavage sites, DPPIV cleavage sites, ADAM metallopeptidase with thrombospondin type 1 motif-13 (ADAMTS13) cleavage sites, and Cathepsin L cleavage sites. The addition of the inactivation cleavage site increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the

additional inactivation cleavage site.

[013] Other aspects of the present specification provide a Clostridial toxin chimeric comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain or the H_{CN} binding subdomain. Such disclosed toxins can comprise a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, a di-chain loop region comprising an exogenous protease cleavage site, and an inactivation cleavage site located within an inactivation cleavage site region. Non-limiting examples of inactivation cleavage sites include Thrombin cleavage sites, Plasmin cleavage sites, Coagulation Factor VIIa cleavage sites, Coagulation Factor IXa cleavage sites, Coagulation Factor Xa cleavage sites, Coagulation Factor XIa cleavage sites, Coagulation Factor XIIa cleavage sites, plasma kallikrein cleavage sites, protease-activated G protein-coupled receptor-1 (PAR1) cleavage sites, PAR 2 cleavage sites, PAR3 cleavage sites, PAR4 cleavage sites, Matrix Metalloproteinase-2 (MMP-2) cleavage sites, Matrix Metalloproteinase-9 (MMP-9) cleavage sites, Furin cleavage sites, urokinase-type Plasminogen activator (uPA) cleavage sites, tissue-type Plasminogen activator (tPA) cleavage sites, Tryptase-ε cleavage sites, Mouse mast cell protease-7 (mMCP-7) cleavage sites, endothelin-converting enzyme-1 (ECE-1) cleavage sites, Kell blood group cleavage sites, DPPIV cleavage sites, ADAM metallopeptidase with thrombospondin type 1 motif-13 (ADAMTS13) cleavage sites, and Cathepsin L cleavage sites. The addition of the inactivation cleavage site increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site.

[014] Other aspects of the present specification provide polynucleotide molecules encoding a Clostridial toxin or a Clostridial toxin chimeric disclosed in the present specification. A polynucleotide molecule encoding such a Clostridial toxin or a Clostridial toxin chimeric can further comprise an expression vector.

[015] Other aspects of the present specification provide a composition comprising a Clostridial toxin or a Clostridial toxin chimeric disclosed in the present specification. A composition comprising such a Clostridial toxin or a Clostridial toxin chimeric can be a pharmaceutical composition. Such a pharmaceutical composition can comprise, in addition to a modified Clostridial toxin disclosed in the present specification a pharmaceutical carrier, a pharmaceutical component, or both.

[016] Other aspects of the present specification provide a method of producing a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, the method comprising the step of expressing in a cell a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, wherein expression from the polynucleotide molecule produces the encoded Clostridial toxin or Clostridial toxin chimeric. In other aspects, the method comprises the steps of introducing into a cell a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, and expressing the polynucleotide molecule, wherein expression from the polynucleotide molecule produces the encoded Clostridial toxin or Clostridial toxin chimeric.

[017] Clostridia toxins produced by *Clostridium botulinum*, *Clostridium tetani*, *Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and

other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). BoNTs possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. It is recognized by those of skill in the art that within each type of Clostridial toxin there can be subtypes which differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific subtypes showing about 84% to 93% amino acid identity when compared to the BoNT/A subtype of SEQ ID NO: 1. As another example, there are presently five BoNT/B subtypes, BoNT/B1, BoNT/B2, BoNT/B3, BoNT/Bnp, and BoNT/Bbv, with specific subtypes showing about 93% to 96% amino acid identity when compared to the BoNT/B subtype of SEQ ID NO: 6. As yet another example, there are presently three BoNT/E subtypes, BoNT/E1, BoNT/E2, and BoNT/E3, with specific subtypes showing about 95% to 99% amino acid identity when compared to the BoNT/E subtype of SEQ ID NO: 15. While all seven BoNT serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other Clostridia species, *C. baratii* and *C. butyricum*, produce toxins, BaNT and BuNT, which are similar to BoNT/F and BoNT/E, respectively.

[018] Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease (FIG. 1). This cleavage occurs within the discrete di-chain loop region created between two cysteine residues that form a disulfide bridge. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by the single disulfide bond and non-covalent interactions between the two chains. The naturally-occurring protease used to convert the single chain molecule into the di-chain is currently not known. In some serotypes, such as, *e.g.*, BoNT/A, the naturally-occurring protease is produced endogenously by the bacteria serotype and cleavage occurs within the cell before the toxin is released into the environment. However, in other serotypes, such as, *e.g.*, BoNT/E, the bacterial strain appears not to produce an endogenous protease capable of converting the single chain form of the toxin into the di-chain form. In these situations, the toxin is released from the cell as a single-chain toxin which is subsequently converted into the di-chain form by a naturally-occurring protease found in the environment.

[019] Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus; 2) a translocation domain contained within the amino-terminal half of the HC (H_N) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell; and 3) a binding domain found within the carboxyl-terminal half of the HC (H_C) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell. D. B. Lacy and R. C. Stevens, Sequence Homology and Structural Analysis of the Clostridial Neurotoxins, J. Mol. Biol. 291: 1091-1104 (1999). The H_C domain comprises two distinct structural features of roughly equal size, separated by an α-helix, designated the H_{CN} and H_{CC} subdomains. Table 1 gives approximate boundary regions for each domain and subdomain found in exemplary Clostridial toxins.

Table 1. Clostridial Toxin Reference Sequences and Regions							
Toxin	SEQ ID NO:	LC	Di-Chain Loop	H_N	H_C		
					H_{CN}	α-Linker	H_{CC}
BoNT/A	1	M1/P2-L429	C430-C454	I455-I873	I874-N1080	E1081-Q1091	S1092-L1296
BoNT/B	6	M1/P2-M436	C437-C446	I447-I860	L861-S1067	Q1068-Q1078	S1079-E1291
BoNT/C1	11	M1/P2-F436	C437-C453	R454-I868	N869-D1081	G1082-L1092	Q1093-E1291
BoNT/D	13	M1/T2-V436	C437-C450	I451-I864	N865-S1069	N1069-Q1079	I1080-E1276
BoNT/E	15	M1/P2-F411	C412-C426	I427-I847	K848-D1055	E1056-E1066	P1067-K1252
BoNT/F	18	M1/P2-F428	C429-C445	I446-I865	K866-D1075	K1076-E1086	P1087-E1274
BoNT/G	21	M1/P2-M435	C436-C450	I451-I865	S866-N1075	A1076-Q1086	S1087-E1297
TeNT	22	M1/P2-L438	C439-C467	I468-L881	K882-N1097	P1098-Y1108	L1109-D1315
BaNT	23	M1/P2-L420	C421-C435	I436-I857	I858-D1064	K1065-E1075	P1076-E1268
BuNT	24	M1/P2-F411	C412-C426	I427-I847	K848-D1055	E1056-E1066	P1067-K1251

[020] The binding, translocation, and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of serotype or subtype. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (FIG. 3). The process is initiated when the H_C domain of a Clostridial toxin binds to a toxin-specific receptor system located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it appears to specifically target one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble *N*-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the

cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo*. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, *e.g.*, Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) Biochimie. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) Trends Biochem. Sci. 552-558. (2002); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) Trends Microbiol. 431-437, (2003).

[021] The three-dimensional crystal structures of BoNT/A, BoNT/B and the H_C domain of TeNT indicate that the three functional domains of Clostridial neurotoxins are structurally distinct domains that are shared by all Clostridial toxins. The HEXXH consensus motif of the light chain forms the tetrahedral zinc binding pocket of the catalytic site located in a deep cleft on the protein surface that is accessible by a channel. The structure of the H_N and H_C domains consists primarily of β-sheet topologies that are linked by a single α-helix. The cylindrical-shaped H_N domain comprises two long amphipathic α-helices that resemble the coiled-coil motif found in some viral proteins. The H_N domain also forms a long unstructured loop called the 'translocation belt,' which wraps around a large negatively charged cleft of the light chain that blocks access of the zinc atom to the catalytic-binding pocket of active site. The H_C domain comprises two distinct structural features of roughly equal size that indicate function. The first, designated the H_{CN} domain, is located in the amino half of the H_C domain. The H_{CN} domain forms a β-barrel, jelly-roll fold. The H_{CC} domain is the second domain that comprises the H_C domain. This carboxyl-terminal domain comprises a modified β-trefoil domain which forms three distinct carbohydrate binding regions that resembles the carbohydrate binding moiety found in many sugar-binding proteins, such as, *e.g.*, serum amyloid P, sialidase, cryia, insecticidal ∂-endotoxin and lectins. Biochemical studies indicate that the β-trefoil domain structure of the H_{CC} domain appears to mediate the binding to specific carbohydrate containing components of the Clostridial toxin receptor on the cell surface, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000). The H_C domain tilts away from the H_N domain exposing the surface loops and making them accessible for binding. No contacts occur between the light chain and the H_C domain.

[022] Aspects of the present specification provide, in part, a Clostridial toxin. As used herein, the term "Clostridial toxin" refers to any neurotoxin produced by a Clostridial toxin strain that can execute the overall cellular mechanism whereby a Clostridial toxin intoxicates a cell and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. A Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, and a Clostridial toxin binding domain. Exemplary Clostridial toxins include those produced by a *Clostridium botulinum*, a *Clostridium tetani*, a *Clostridium baratii* and a *Clostridium butyricum*.

[023] A Clostridial toxin includes, without limitation, naturally occurring Clostridial toxin variants, such as, *e.g.*, Clostridial toxin isoforms and Clostridial toxin subtypes; non-naturally occurring Clostridial toxin

variants, such as, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, and active Clostridial toxin fragments thereof, or any combination thereof. As used herein, the term "Clostridial toxin variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. As non-limiting examples, a BoNT/A variant of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 1; a BoNT/B variant of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 6; a BoNT/C1 variant of SEQ ID NO: 11 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 11; a BoNT/D variant of SEQ ID NO: 13 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 13; a BoNT/E variant of SEQ ID NO: 15 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 15; a BoNT/F variant of SEQ ID NO: 18 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 18; a BoNT/G variant of SEQ ID NO: 21 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 21; a TeNT variant c of SEQ ID NO: 22 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 22; a BaNT variant of SEQ ID NO: 23 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 23; and a BuNT variant of SEQ ID NO: 24 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the corresponding position(s) of SEQ ID NO: 24.

[024] As used herein, the term "naturally occurring Clostridial toxin variant" refers to any Clostridial toxin produced without the aid of any human manipulation, including, without limitation, Clostridial toxin isoforms produced from alternatively-spliced transcripts, Clostridial toxin isoforms produced by spontaneous mutation and Clostridial toxin subtypes. Non-limiting examples of a Clostridial toxin isoform include, *e.g.*, BoNT/A isoforms, BoNT/B isoforms, BoNT/C1 isoforms, BoNT/D isoforms, BoNT/E isoforms, BoNT/F isoforms, BoNT/G isoforms, TeNT isoforms, BaNT isoforms and BuNT isoforms. Non-limiting examples of a Clostridial toxin subtype include, *e.g.*, BoNT/A subtypes BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5; BoNT/B subtypes BoNT/B1, BoNT/B2, BoNT/B3, BoNT/B bivalent and BoNT/B nonproteolytic; BoNT/C1 subtypes BoNT/C1-1 and BoNT/C1-2; BoNT/E subtypes BoNT/E1, BoNT/E2, and BoNT/E3; BoNT/F subtypes BoNT/F1, BoNT/F2, and BoNT/F3; and BuNT subtypes BuNT-1, and BuNT-2. Other non-limiting examples of a Clostridial toxin subtype include, *e.g.*, BoNT/A subtypes SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5; BoNT/B subtypes SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; BoNT/C1 subtypes SEQ ID NO: 11 and SEQ ID NO: 12; BoNT/E subtypes SEQ ID NO: 15, SEQ ID NO: 16, and

SEQ ID NO: 17; BoNT/F subtypes SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; and BuNT subtypes SEQ ID NO: 24 and SEQ ID NO: 25.

[025] As used herein, the term "non-naturally occurring Clostridial toxin variant" refers to any Clostridial toxin produced with the aid of human manipulation, including, without limitation, Clostridial toxins produced by genetic engineering using random mutagenesis or rational design and Clostridial toxins produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin variants include, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, and active Clostridial toxin fragments.

[026] As used herein, the term "conservative Clostridial toxin variant" refers to a Clostridial toxin that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present specification. A conservative Clostridial toxin variant may substitute 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, or 500 or more amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. A conservative Clostridial toxin variant can also substitute at least 5, 10, 15, 20, or 25 contiguous amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. Non-limiting examples of a conservative Clostridial toxin variant include, *e.g.*, conservative BoNT/A variants, conservative BoNT/B variants, conservative BoNT/C1 variants, conservative BoNT/D variants, conservative BoNT/E variants, conservative BoNT/F variants, conservative BoNT/G variants, conservative TeNT variants, conservative BaNT variants and conservative BuNT variants.

[027] As used herein, the term "non-conservative Clostridial toxin variant" refers to a Clostridial toxin in which 1) at least one amino acid is deleted from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based; 2) at least one amino acid added to the reference Clostridial toxin on which the non-conservative Clostridial toxin is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). A non-conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present specification. A non-conservative Clostridial toxin variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant may substitute 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75, 100, 200, 300,

400, or 500 or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can also substitute at least 5, 10, 15, 20, or 25 contiguous amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. Non-limiting examples of a non-conservative Clostridial toxin variant include, *e.g.*, non-conservative BoNT/A variants, non-conservative BoNT/B variants, non-conservative BoNT/C1 variants, non-conservative BoNT/D variants, non-conservative BoNT/E variants, non-conservative BoNT/F variants, non-conservative BoNT/G variants, non-conservative TeNT variants, non-conservative BaNT variants and non-conservative BuNT variants.

[028] It is also envisioned that any of a variety of Clostridial toxin fragments can be useful in aspects of the present specification with the proviso that these active fragments can execute the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Thus, aspects of this embodiment can include Clostridial toxin fragments having a length of, *e.g.*, at least 600, 700, 800, 900, 1000, 1100, or at least 1200 amino acids. Other aspects of this embodiment, can include Clostridial toxin fragments having a length of, *e.g.*, at most 600, 700, 800, 900, 1000, 1100, or at most 1200 amino acids.

[029] It is also envisioned that any of a variety of Clostridial toxin fragments comprising the light chain can be useful in aspects of the present specification with the proviso that these light chain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The light chains of Clostridial toxins are approximately 420-460 amino acids in length and comprise a Clostridial toxin enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin light chain is not necessary for the enzymatic activity of the Clostridial toxin enzymatic domain. As a non-limiting example, the first eight amino acids of a BoNT/A light chain are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT light chain are not required for enzymatic activity. Likewise, the carboxyl-terminus of the light chain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A light chain are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT light chain are not required for enzymatic activity. Thus, aspects of this embodiment include a Clostridial toxin light chain comprising a Clostridial toxin enzymatic domain having a length of, *e.g.*, at least 350, 375, 400, 425, or 450 amino acids. Other aspects of this embodiment include a Clostridial toxin light chain comprising a Clostridial toxin enzymatic domain having a length of, *e.g.*, at most 350, 375, 400, 425, or 450 amino acids.

[030] It is also envisioned that any of a variety of Clostridial toxin H_N regions comprising a Clostridial toxin translocation domain can be useful in aspects of the present specification with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_N regions from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a Clostridial toxin translocation domain (Table 1). Research has shown that the entire length of a H_N region from a Clostridial toxin heavy chain is not necessary for the translocating activity of the Clostridial toxin translocation domain. Thus, aspects of this embodiment can include Clostridial toxin H_N regions comprising a Clostridial toxin translocation domain having a length of, *e.g.*, at least 350, 375, 400, or 425 amino acids. Other aspects of this embodiment

can include Clostridial toxin H_N regions comprising Clostridial toxin translocation domain having a length of, *e.g.*, at most 350, 375, 400, or 425 amino acids.

[031] It is also envisioned that any of a variety of Clostridial toxin H_C regions comprising a Clostridial toxin binding domain can be useful in aspects of the present specification with the proviso that these active fragments can determine the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell and facilitate the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_C regions from the heavy chains of Clostridial toxins are approximately 400-440 amino acids in length and comprise a binding domain (Table 1). Research has shown that the entire length of a H_C region from a Clostridial toxin heavy chain is not necessary for the binding activity of the Clostridial toxin binding domain. Thus, aspects of this embodiment can include Clostridial toxin H_C regions comprising a binding domain having a length of, *e.g.*, at least 350, 375, 400, or 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H_C regions comprising a binding domain having a length of, *e.g.*, at most 350, 375, 400, or 425 amino acids.

[032] Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[033] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.*, CLUSTAL W, see, *e.g.*, Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice*, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, *e.g.*, Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments*, 264(4) J. Mol. Biol. 823-838 (1996).

[034] Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.*, Match-box, see, *e.g.*, Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences*, 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.*, C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment*, 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., *Align-M – A New Algorithm for Multiple Alignment of Highly Divergent Sequences*, 20(9) Bioinformatics,:1428-1435 (2004).

[035] Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.*, segment-to-segment comparison, see, *e.g.*, Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison*, 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, *e.g.*, Cédric Notredame et al., *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment*, 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, *e.g.*, Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput*, 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, *e.g.*, Amarendran R

Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment*, 6(1) BMC Bioinformatics 66 (2005).

[036] The present specification describes various polypeptide variants where one amino acid is substituted for another, such as, *e.g.*, Clostridial toxin variants, Clostridial toxin enzymatic domain variants, Clostridial toxin translocation domain variants, Clostridial toxin binding domain variants, non-Clostridial toxin binding domain variants, and protease cleavage site variants. A substitution can be assessed by a variety of factors, such as, *e.g.*, the physical properties of the amino acid being substituted (Table 2) or how the original amino acid would tolerate a substitution (Table 3). The selections of which amino acid can be substituted for another amino acid in a polypeptide are known to a person of ordinary skill in the art.

TABLE 2. Amino Acid Properties

Property	Amino Acids
Aliphatic	G, A, I, L, M, P, V
Aromatic	F, H, W, Y
C-beta branched	I, V, T
Hydrophobic	C, F, I, L, M, V, W
Small polar	D, N, P
Small non-polar	A, C, G, S, T
Large polar	E, H, K, Q, R, W, Y
Large non-polar	F, I, L, M, V
Charged	D, E, H, K, R
Uncharged	C, S, T
Negative	D, E
Positive	H, K, R
Acidic	D, E
Basic	K, R
Amide	N, Q

TABLE 3. Amino Acid Substitutions

Amino Acid	Favored Substitution	Neutral Substitutions	Disfavored substitution
A	G, S, T	C, E, I, K, M, L, P, Q, R, V	D, F, H, N, Y, W
C	F, S, Y, W	A, H, I, M, L, T, V	D, E, G, K, N, P, Q, R
D	E, N	G, H, K, P, Q, R, S, T	A, C, I, L,
E	D, K, Q	A, H, N, P, R, S, T	C, F, G, I, L, M, V, W, Y
F	M, L, W, Y	C, I, V	A, D, E, G, H, K, N, P, Q, R, S, T
G	A, S	D, K, N, P, Q, R	C, E, F, H, I, L, M, T, V, W, Y
H	N, Y	C, D, E, K, Q, R, S, T, W	A, F, G, I, L, M, P, V
I	V, L, M	A, C, T, F, Y	D, E, G, H, K, N, P, Q, R, S, W
K	Q, E, R	A, D, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
L	F, I, M, V	A, C, W, Y	D, E, G, H, K, N, P, Q, R, S, T
M	F, I, L, V	A, C, R, Q, K, T, W, Y	D, E, G, H, N, P, S

N	D, H, S	E, G, K, Q, R, T	A, C, F, I, L, M, P, V, W, Y
P	—	A, D, E, G, K, Q, R, S, T	C, F, H, I, L, M, N, V, W, Y
Q	E, K, R	A, D, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
R	K, Q	A, D, E, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
S	A, N, T	C, D, E, G, H, K, P, Q, R, T	F, I, L, M, V, W, Y
T	S	A, C, D, E, H, I, K, M, N, P, Q, R, V	F, G, L, W, Y
V	I, L, M	A, C, F, T, Y	D, E, G, H, K, N, P, Q, R, S, W
W	F, Y	H, L, M	A, C, D, E, G, I, K, N, P, Q, R, S, T, V
Y	F, H, W	C, I, L, M, V	A, D, E, G, K, N, P, Q, R, S, T
Matthew J. Betts and Robert, B. Russell, Amino Acid Properties and Consequences of Substitutions, pp. 289-316, In Bioinformatics for Geneticists, (eds Michael R. Barnes, Ian C. Gray, Wiley, 2003).			

[037] Thus, in an embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, and a Clostridial toxin binding domain. In an aspect of this embodiment, a Clostridial toxin comprises a naturally occurring Clostridial toxin variant, such as, *e.g.*, a Clostridial toxin isoform or a Clostridial toxin subtype. In another aspect of this embodiment, a Clostridial toxin comprises a non-naturally occurring Clostridial toxin variant, such as, *e.g.*, a conservative Clostridial toxin variant, a non-conservative Clostridial toxin variant or an active Clostridial toxin fragment, or any combination thereof. In another aspect of this embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain or an active fragment thereof, a Clostridial toxin translocation domain or an active fragment thereof, a Clostridial toxin binding domain or an active fragment thereof, or any combination thereof. In other aspects of this embodiment, a Clostridial toxin can comprise a BoNT/A, a BoNT/B, a BoNT/C1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G, a TeNT, a BaNT, or a BuNT.

[038] In another embodiment, a hydrophobic amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.*, A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted

with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another small amino acid. Examples of small amino acids include, *e.g.*, A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branched amino acid at one particular position in the polypeptide chain of the Clostridial toxin can be substituted with another C-beta branched amino acid. Examples of C-beta branched amino acids include, *e.g.*, I, T and V.

[039] In another embodiment, a Clostridial toxin comprises a BoNT/A. In an aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain, a BoNT/A translocation domain, and a BoNT/A binding domain. In another aspect of this embodiment, a BoNT/A comprises SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant or an active BoNT/A fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant, an active BoNT/A fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain or an active fragment thereof, a BoNT/A translocation domain or an active fragment thereof, a BoNT/A binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprising a BoNT/A enzymatic domain SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, or an active fragment thereof, a BoNT/A translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, or an active fragment thereof, a BoNT/A binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, or an active fragment thereof, or any combination thereof.

[040] In other aspects of this embodiment, a BoNT/A comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20,

30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5.

[041] In another embodiment, a Clostridial toxin comprises a BoNT/B. In an aspect of this embodiment, a BoNT/B comprises a BoNT/B enzymatic domain, a BoNT/B translocation domain, and a BoNT/B binding domain. In another aspect of this embodiment, a BoNT/B comprises SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant, an active BoNT/B fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant, an active BoNT/B fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprises a BoNT/B enzymatic domain or an active fragment thereof, a BoNT/B translocation domain or active fragment thereof, a BoNT/B binding domain or active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprises a BoNT/B enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, or active fragment thereof, a BoNT/B translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, or active fragment thereof, a BoNT/B binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, or active fragment thereof, or any combination thereof.

[042] In other aspects of this embodiment, a BoNT/B comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 102; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In still other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino

acid deletions, additions, and/or substitutions relative to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.

[043] In another embodiment, a Clostridial toxin comprises a BoNT/C1. In an aspect of this embodiment, a BoNT/C1comprises a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain, and a BoNT/C1 binding domain. In another aspect of this embodiment, a BoNT/C1 comprises SEQ ID NO: 11 or SEQ ID NO: 12. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant, such as, e.g., a BoNT/C1 isoform or a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a BoNT/C1 isoform or a BoNT/C1 subtype. In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant, an active BoNT/C1 fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant, an active BoNT/C1 fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain or active fragment thereof, a BoNT/C1 translocation domain or active fragment thereof, a BoNT/C1 binding domain, active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12, or active fragment thereof, a BoNT/C1 translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12, or active fragment thereof, a BoNT/C1 binding domain of SEQ ID NO: 11 or SEQ ID NO: 12, or active fragment thereof, or any combination thereof.

[044] In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 11 or SEQ ID NO: 12; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 11 or SEQ ID NO: 12. In still other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 11 or SEQ ID NO: 12.

[045] In another embodiment, a Clostridial toxin comprises a BoNT/D. In an aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain, a BoNT/D translocation domain, and a BoNT/D binding domain. In another aspect of this embodiment, a BoNT/D comprises SEQ ID NO: 13 or SEQ ID NO: 14. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In still another aspect of this

embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant, an active BoNT/D fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant, an active BoNT/D fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain or an active fragment thereof, a BoNT/D translocation domain or an active fragment thereof, a BoNT/D binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14, or an active fragment thereof, a BoNT/D translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14, or an active fragment thereof, a BoNT/D binding domain of SEQ ID NO: 13 or SEQ ID NO: 14, or an active fragment thereof, or any combination thereof.

[046] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14. In still other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 13 or SEQ ID NO: 14.

[047] In another embodiment, a Clostridial toxin comprises a BoNT/E. In an aspect of this embodiment, a BoNT/E comprises a BoNT/E enzymatic domain, a BoNT/E translocation domain, and a BoNT/E binding domain. In another aspect of this embodiment, a BoNT/E comprises SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant, such as, *e.g.*, a BoNT/E isoform or a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a BoNT/E isoform or a BoNT/E subtype. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant, an active BoNT/E fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant, an active BoNT/E fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprises a BoNT/E enzymatic domain or an active fragment thereof, a BoNT/E translocation domain or active fragment thereof, a BoNT/E binding domain or active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprises a BoNT/E enzymatic domain of SEQ ID NO: 15, SEQ ID

NO: 16, or SEQ ID NO: 17, or active fragment thereof, a BoNT/E translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, or active fragment thereof, a BoNT/E binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, or active fragment thereof, or any combination thereof.

[048] In other aspects of this embodiment, a BoNT/E comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In still other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

[049] In another embodiment, a Clostridial toxin comprises a BoNT/F. In an aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain, a BoNT/F translocation domain, and a BoNT/F binding domain. In another aspect of this embodiment, a BoNT/F comprises SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant, an active BoNT/F fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant, an active BoNT/F fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain or active fragment thereof, a BoNT/F translocation domain or active fragment thereof, a BoNT/F binding domain or active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or active fragment thereof, a BoNT/F translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or active fragment thereof, a BoNT/F binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or active fragment thereof, or any combination thereof.

[050] In other aspects of this embodiment, a BoNT/F comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8,

9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In still other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

[051] In another embodiment, a Clostridial toxin comprises a BoNT/G. In an aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain, a BoNT/G translocation domain, and a BoNT/G binding domain. In another aspect of this embodiment, a BoNT/G comprises SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype of SEQ ID NO: 21. In still another aspect of this embodiment, a BoNT/G comprises a non-naturally occurring BoNT/G variant, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant or an active BoNT/G fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/G variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant, an active BoNT/G fragment, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain or an active fragment thereof, a BoNT/G translocation domain or an active fragment thereof, a BoNT/G binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain of SEQ ID NO: 21 or an active fragment thereof, a BoNT/G translocation domain of SEQ ID NO: 21 or an active fragment thereof, a BoNT/G binding domain of SEQ ID NO: 21 or an active fragment thereof, or any combination thereof.

[052] In other aspects of this embodiment, a BoNT/G comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 21; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 21; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 21.

[053] In another embodiment, a Clostridial toxin comprises a TeNT. In an aspect of this embodiment, a TeNT comprises a TeNT enzymatic domain, a TeNT translocation domain, and a TeNT binding domain.

In an aspect of this embodiment, a TeNT comprises SEQ ID NO: 22. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant, an active TeNT fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant, an active TeNT fragment, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprises a TeNT enzymatic domain or an active fragment thereof, a TeNT translocation domain or active fragment thereof, a TeNT binding domain or active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprises a TeNT enzymatic domain of SEQ ID NO: 22 or active fragment thereof, a TeNT translocation domain of SEQ ID NO: 22 or active fragment thereof, a TeNT binding domain of SEQ ID NO: 22 or active fragment thereof, or any combination thereof.

[054] In other aspects of this embodiment, a TeNT comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 22; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 22; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 22.

[055] In another embodiment, a Clostridial toxin comprises a BaNT. In an aspect of this embodiment, a BaNT comprises a BaNT enzymatic domain, a BaNT translocation domain, and a BaNT binding domain. In another aspect of this embodiment, a BaNT comprises SEQ ID NO: 23. In another aspect of this embodiment, a BaNT comprises a naturally occurring BaNT variant, such as, *e.g.*, a BaNT isoform or a BaNT subtype. In another aspect of this embodiment, a BaNT comprises a naturally occurring BaNT variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform or a BaNT subtype. In still another aspect of this embodiment, a BaNT comprises a non-naturally occurring BaNT variant, such as, *e.g.*, a conservative BaNT variant, a non-conservative BaNT variant or an active BaNT fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT comprises a non-naturally occurring BaNT variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT variant, a non-conservative BaNT variant, an active BaNT fragment, or any combination thereof. In yet another aspect of this embodiment, a BaNT comprises a BaNT enzymatic domain or an active fragment thereof, a BaNT translocation domain or an active fragment thereof, a BaNT binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BaNT comprises a BaNT enzymatic domain of SEQ ID NO: 23 or an active fragment thereof, a BaNT translocation domain of SEQ

ID NO: 23 or an active fragment thereof, a BaNT binding domain of SEQ ID NO: 23 or an active fragment thereof, or any combination thereof.

[056] In other aspects of this embodiment, a BaNT comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 23; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 23; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 23.

[057] In another embodiment, a Clostridial toxin comprises a BuNT. In an aspect of this embodiment, a BuNT comprises a BuNT enzymatic domain, a BuNT translocation domain, and a BuNT binding domain. In another aspect of this embodiment, a BuNT comprises SEQ ID NO: 24 or SEQ ID NO: 25. In another aspect of this embodiment, a BuNT comprises a naturally occurring BuNT variant, such as, *e.g.*, a BuNT isoform or a BuNT subtype. In another aspect of this embodiment, a BuNT comprises a naturally occurring BuNT variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a BuNT isoform or a BuNT subtype. In still another aspect of this embodiment, a BuNT comprises a non-naturally occurring BuNT variant, such as, *e.g.*, a conservative BuNT variant, a non-conservative BuNT variant, an active BuNT fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT comprises a non-naturally occurring BuNT variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, e.g., a conservative BuNT variant, a non-conservative BuNT variant, an active BuNT fragment, or any combination thereof. In yet another aspect of this embodiment, a BuNT comprises a BuNT enzymatic domain or an active fragment thereof, a BuNT translocation domain or an active fragment thereof, a BuNT binding domain, an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BuNT comprises a BuNT enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25, or an active fragment thereof, a BuNT translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25, or an active fragment thereof, a BuNT binding domain of SEQ ID NO: 24 or SEQ ID NO: 25, or an active fragment thereof, or any combination thereof.

[058] In other aspects of this embodiment, a BuNT comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 24 or SEQ ID NO: 25; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT comprises a polypeptide having, *e.g.*, at least

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 24 or SEQ ID NO: 25; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, or 500 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 24 or SEQ ID NO: 25.

[059] As used herein, the term "Clostridial toxin chimeric" or "Clostridial toxin chimeras" refers to a molecule comprising at least a portion from one Clostridial toxin and a portion from at least one other protein to form a toxin with at least one property different from the reference Clostridial toxins of Table 1. Non-limiting examples of Clostridial toxin chimeras include a Clostridial toxin comprising a non-Clostridial toxin enzymatic domain, a Clostridial toxin comprising a non-Clostridial toxin translocation domain, a Clostridial toxin comprising a non-Clostridial toxin binding domain, or any combination thereof. Other non-limiting example of a Clostridial toxin chimeras include a Clostridial toxin comprising a enzymatic domain from a different Clostridial toxin, a Clostridial toxin comprising a translocation domain from a different Clostridial toxin, a Clostridial toxin comprising a binding domain from a different Clostridial toxin, or any combination thereof.

[060] One class of Clostridial toxin chimeric comprises a modified Clostridial toxin were the enzymatic domain or portion thereof, translocation domain or portion thereof, and/or binding domain or portion thereof of a naturally-occurring Clostridial toxin is either modified or replaced with an enzymatic domain or portion thereof, translocation domain or portion thereof, and/or binding domain or portion thereof of a different Clostridial toxin. As non-limiting example, the binding domain of BoNT/A can be replaced with the binding domain of BoNT/B producing a Clostridial toxin chimeric comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain, and a BoNT/B binding domain. Such Clostridial toxin chimeras are described in, *e.g.*, J. Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, U.S. Patent 7,132,259, which is incorporated by reference in its entirety. As another non-limiting example, the leucine motif from BoNT/A can be inserted into the light chain of a BoNT/E in order to increase biological persistence. Such Clostridial toxin chimeras are described in, *e.g.*, Lance E. Steward et al., *Leucine-based Motif and Clostridial Toxins*, U.S. Patent Publication 2003/0027752 (Feb. 6, 2003); Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2003/0219462 (Nov. 27, 2003); and Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2004/0220386 (Nov. 4, 2004), each of which is incorporated by reference in its entirety.

[061] Another class of Clostridial toxin chimeric comprises a Clostridial toxin where the binding domain of a naturally-occurring Clostridial toxin is either modified or replaced with a binding domain of a non-Clostridial toxin. Such Clostridial toxin chimeras possesses an altered cell binding activity because the modified toxin can either, *e.g.*, 1) use the same receptor present on the surface of a naturally occurring Clostridial toxin target cell as that used by the naturally-occurring Clostridial toxin, referred to as an enhanced cell binding activity for a naturally-occurring Clostridial toxin target cell; 2) use a different receptor present on the surface of a naturally occurring Clostridial toxin target cell, referred to as an altered cell binding activity for a naturally-occurring Clostridial toxin target cell; or 3) use a different receptor present on the surface of the non-Clostridial toxin target cell, referred to as an altered cell binding activity for a non-naturally-occurring Clostridial toxin target cell, a re-targeted toxin or a TVEMP.

[062] A Clostridial toxin chimeric can be a Clostridial toxin with an enhanced cell binding activity capable of intoxicating a naturally occurring Clostridial toxin target cell, *e.g.*, a motor neuron. One way this enhanced binding activity is achieved by modifying the endogenous binding domain of a naturally-occurring Clostridial toxin in order to enhance a cell binding activity of the toxin for its naturally-occurring receptor. Such modifications to a targeting domain result in, *e.g.*, a enhanced cell binding activity that increases binding affinity for an endogenous Clostridial toxin receptor present on a naturally-occurring Clostridial toxin target cell; an enhanced cell binding activity that increases binding specificity for a subgroup of endogenous Clostridial toxin receptors present on a naturally-occurring Clostridial toxin target cell; or an enhanced cell binding activity that increases both binding affinity and binding specificity. Non-limiting examples of modified Clostridial toxins an enhanced cell binding activity for a naturally-occurring Clostridial toxin receptor are described in, *e.g.*, Lance E. Steward et al., *Modified Clostridial Toxins with Enhanced Targeting Capabilities For Endogenous Clostridial Toxin Receptor Systems*, U.S. Patent Publication 2008/0096248; Lance E. Steward, *Modified Clostridial Toxins with Enhanced Translocation Capabilities and Enhanced Targeting Activity for Clostridial Toxin Target Cells*, International Patent Publication 2008/105901; each of which is hereby incorporated by reference in its entirety.

[063] A Clostridial toxin chimeric can be a Clostridial toxin with an altered cell binding activity capable of intoxicating a naturally occurring Clostridial toxin target cell, *e.g.*, a motor neuron. One way this altered capability is achieved by replacing the endogenous binding domain of a naturally-occurring Clostridial toxin with a binding domain of another molecule that preferentially binds to a different receptor present on the surface of a Clostridial toxin target cell. Such a modification to a binding domain results in a modified toxin that is able to preferentially bind to a non-Clostridial toxin receptor present on a Clostridial toxin target cell. This enhanced binding activity for a naturally occurring Clostridial toxin target cell allows for lower effective doses of a modified Clostridial toxin to be administered to an individual because more toxin will be delivered to the target cell. Thus, modified Clostridial toxins with an enhanced binding activity will reduce the undesirable dispersal of the toxin to areas not targeted for treatment, thereby reducing or preventing the undesirable side-effects associated with diffusion of a Clostridial toxin to an unwanted location. Non-limiting examples of modified Clostridial toxins with an altered cell binding capability for a Clostridial toxin target cell are described in, *e.g.*, Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent 7,514,088; Lance E. Steward et al., *Modified Clostridial Toxins with Altered Targeting Capabilities For Clostridial Toxin Target Cells*, U.S. Patent Publication 2008/0161543; Lance E. Steward, *Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity for Clostridial Toxin Target Cells*, U.S. Patent Publication 2008/0241881; Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent Publication 2009/0048431; Lance E. Steward et al., *Modified Clostridial Toxins with Altered Targeting Capabilities For Clostridial Toxin Target Cells*, International Patent Publication WO 2007/106115; each of which is hereby incorporated by reference in its entirety.

[064] A Clostridial toxin chimeric can be a Clostridial toxin with an altered cell binding activity capable of intoxicating a cell other than a Clostridial toxin target cell, *e.g.*, a cell other than a motor neuron. Called TVEMPs, these molecules achieve this intoxication by using a target receptor present on non-Clostridial toxin target cell. This re-targeted capability is achieved by replacing a naturally-occurring binding domain

of a Clostridial toxin with a binding domain showing a preferential binding activity for a non-Clostridial toxin receptor present in a non-Clostridial toxin target cell. Such modifications to a binding domain result in a modified toxin that is able to preferentially bind to a non-Clostridial toxin receptor present on a non-Clostridial toxin target cell. A Clostridial toxin chimeric with an altered targeting activity for a non-Clostridial toxin target cell can bind to a target receptor, translocate into the cytoplasm, and exert its proteolytic effect on the SNARE complex of the non-Clostridial toxin target cell. Non-limiting examples of Clostridial toxin chimeras with an altered targeting activity for a non-Clostridial toxin target cell are described in, *e.g.*, Keith A. Foster et al., *Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions*, U.S. Patent 5,989,545; Clifford C. Shone et al., *Recombinant Toxin Fragments*, U.S. Patent 6,461,617; Conrad P. Quinn et al., *Methods and Compounds for the Treatment of Mucus Hypersecretion*, U.S. Patent 6,632,440; Lance E. Steward et al., *Methods and Compositions for the Treatment of Pancreatitis*, U.S. Patent 6,843,998; J. Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, U.S. Patent 7,132,259; Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent 7,244,437; Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent 7,413,742; Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent 7,415,338; Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent 7,514,088; Keith A. Foster et al., *Inhibition of Secretion from Non-neural Cells*, U.S. Patent Publication 2006/0216283; Keith A. Foster, *Fusion Proteins*, U.S. Patent Publication 2008/0064092; Keith A. Foster, *Fusion Proteins*, U.S. Patent Publication 2009/0035822; Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent Publication 2009/0048431; Keith A. Foster, *Non-Cytotoxic Protein Conjugates*, U.S. Patent Publication 2009/0162341; Keith A. Foster et al., *Re-targeted Toxin Conjugates*, International Patent Publication WO 2005/023309; and Lance E. Steward, *Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Capabilities for Non-Clostridial Toxin Target Cells*, International Patent Application WO 2008/008805; each of which is hereby incorporated by reference in its entirety.

[065] Aspects of the present specification provide, in part, a Clostridial toxin enzymatic domain. As used herein, the term "Clostridial toxin enzymatic domain" refers to any Clostridial toxin polypeptide that can execute the enzymatic target modification step of the intoxication process. Thus, a Clostridial toxin enzymatic domain specifically targets a Clostridial toxin substrate and encompasses the proteolytic cleavage of a Clostridial toxin substrate, such as, *e.g.*, SNARE proteins like a SNAP-25 substrate, a VAMP substrate, and a Syntaxin substrate. Non-limiting examples of a Clostridial toxin enzymatic domain include, *e.g.*, a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, and a BuNT enzymatic domain.

[066] A Clostridial toxin enzymatic domain includes, without limitation, naturally occurring Clostridial toxin enzymatic domain variants, such as, *e.g.*, Clostridial toxin enzymatic domain isoforms and Clostridial toxin enzymatic domain subtypes; and non-naturally occurring Clostridial toxin enzymatic domain variants, such as, *e.g.*, conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, active Clostridial toxin enzymatic domain fragments thereof, or any combination thereof.

[067] As used herein, the term "Clostridial toxin enzymatic domain variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin enzymatic domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin enzymatic domain variants useful to practice disclosed embodiments are variants that execute the enzymatic target modification step of the intoxication process. As non-limiting examples, a BoNT/A enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-429 of SEQ ID NO: 1; a BoNT/B enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 6; a BoNT/C1 enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 11; a BoNT/D enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 13; a BoNT/E enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-411 of SEQ ID NO: 15; a BoNT/F enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-428 of SEQ ID NO: 18; a BoNT/G enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-438 of SEQ ID NO: 21; a TeNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-438 of SEQ ID NO: 22; a BaNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-420 of SEQ ID NO: 23; and a BuNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 1/2-411 of SEQ ID NO: 24.

[068] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin enzymatic domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific enzymatic domain subtypes showing about 80% to 95% amino acid identity when compared to the BoNT/A enzymatic domain of SEQ ID NO: 1. As used herein, the term "naturally occurring Clostridial toxin enzymatic domain variant" refers to any Clostridial toxin enzymatic domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin enzymatic domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin enzymatic domain isoforms produced by spontaneous mutation and Clostridial toxin enzymatic domain subtypes. A naturally occurring Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification.

[069] A non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain isoform such as, *e.g.*, a BoNT/A enzymatic domain isoform, a BoNT/B enzymatic domain isoform, a BoNT/C1 enzymatic domain isoform, a BoNT/D enzymatic domain isoform, a BoNT/E enzymatic domain isoform, a BoNT/F enzymatic domain isoform, a BoNT/G enzymatic domain isoform, a TeNT enzymatic domain isoform, a BaNT enzymatic domain isoform, and a BuNT enzymatic domain isoform. Another non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain subtype such as, *e.g.*, an enzymatic domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, or BoNT/A5; an enzymatic domain from subtype BoNT/B1, BoNT/B2, BoNT/Bbv, or BoNT/Bnp; an enzymatic domain from subtype BoNT/C1-1 or BoNT/C1-2; an enzymatic domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; an enzymatic domain from subtype BoNT/F1, BoNT/F2, or BoNT/F3; and an enzymatic domain from subtype BuNT-1 or BuNT-2.

[070] As used herein, the term "non-naturally occurring Clostridial toxin enzymatic domain variant" refers to any Clostridial toxin enzymatic domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin enzymatic domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin enzymatic domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin enzymatic domain variants include, *e.g.*, conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, Clostridial toxin enzymatic domain chimeric variants, and active Clostridial toxin enzymatic domain fragments.

[071] As used herein, the term "conservative Clostridial toxin enzymatic domain variant" refers to a Clostridial toxin enzymatic domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification. Non-limiting examples of a conservative Clostridial toxin enzymatic domain variant include, *e.g.*, conservative BoNT/A enzymatic domain variants, conservative BoNT/B enzymatic domain variants, conservative BoNT/C1 enzymatic domain variants, conservative BoNT/D enzymatic domain variants, conservative BoNT/E enzymatic domain variants, conservative BoNT/F enzymatic domain variants, conservative BoNT/G enzymatic domain variants, conservative TeNT enzymatic domain variants, conservative BaNT enzymatic domain variants, and conservative BuNT enzymatic domain variants.

[072] As used herein, the term "non-conservative Clostridial toxin enzymatic domain variant" refers to a Clostridial toxin enzymatic domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain is based; or 3) at least one amino acid is

substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). A non-conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification. Non-limiting examples of a non-conservative Clostridial toxin enzymatic domain variant include, *e.g.*, non-conservative BoNT/A enzymatic domain variants, non-conservative BoNT/B enzymatic domain variants, non-conservative BoNT/C1 enzymatic domain variants, non-conservative BoNT/D enzymatic domain variants, non-conservative BoNT/E enzymatic domain variants, non-conservative BoNT/F enzymatic domain variants, non-conservative BoNT/G enzymatic domain variants, and non-conservative TeNT enzymatic domain variants, non-conservative BaNT enzymatic domain variants, and non-conservative BuNT enzymatic domain variants.

[073] As used herein, the term "active Clostridial toxin enzymatic domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the enzymatic domain can be useful in aspects of the present specification with the proviso that these enzymatic domain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The enzymatic domains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin enzymatic domain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A enzymatic domain are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT enzymatic domain are not required for enzymatic activity. Likewise, the carboxyl-terminus of the enzymatic domain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A enzymatic domain are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT enzymatic domain are not required for enzymatic activity. Thus, aspects of this embodiment include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, *e.g.*, at least 350, 375, 400, 425, or 450 amino acids. Other aspects of this embodiment include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, *e.g.*, at most 350, 375, 400, 425, or 450 amino acids.

[074] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin enzymatic domain variants and non-naturally-occurring Clostridial toxin enzymatic domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[075] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a Clostridial toxin enzymatic domain isoform or a Clostridial toxin enzymatic domain subtype. In

another aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a conservative Clostridial toxin enzymatic domain variant, a non-conservative Clostridial toxin enzymatic domain variant, an active Clostridial toxin enzymatic domain fragment, or any combination thereof.

[076] In another embodiment, a hydrophobic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, e.g., A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another small amino acid. Examples of small amino acids include, e.g., A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, *e.g.*, I, T and V.

[077] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain. In an aspect of this embodiment, a BoNT/A enzymatic domain comprises the enzymatic domains of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises a naturally occurring BoNT/A enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/A isoform or an enzymatic domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises a naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a BoNT/A isoform enzymatic domain or

a BoNT/A subtype enzymatic domain. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of a naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform enzymatic domain or a BoNT/A subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises a non-naturally occurring BoNT/A enzymatic domain variant, such as, *e.g.*, a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of a non-naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof.

[078] In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-429 of SEQ ID NO: 1; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-429 of SEQ ID NO: 1.

[079] In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In further other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having,

e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1.

[080] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/B enzymatic domain. In an aspect of this embodiment, a BoNT/B enzymatic domain comprises the enzymatic domains of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In other aspects of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises a naturally occurring BoNT/B enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/B isoform or an enzymatic domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises a naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a BoNT/B isoform enzymatic domain or a BoNT/B subtype enzymatic domain. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/B isoform enzymatic domain or a BoNT/B subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises a non-naturally occurring BoNT/B enzymatic domain variant, such as, *e.g.*, a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof.

[081] In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 6; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 6.

[082] In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50,

or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In further other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6.

[083] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/C1 enzymatic domain. In an aspect of this embodiment, a BoNT/C1 enzymatic domain comprises the enzymatic domains of SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 11. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a naturally occurring BoNT/C1 enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/C1 isoform or an enzymatic domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a BoNT/C1 isoform enzymatic domain or a BoNT/C1 subtype enzymatic domain. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11, such as, *e.g.*, a BoNT/C1 isoform enzymatic domain or a BoNT/C1 subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a non-naturally occurring BoNT/C1 enzymatic domain variant, such as, *e.g.*, a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11, such as, *e.g.*, a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof.

[084] In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 11; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 11.

[085] In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11. In still other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In further other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11.

[086] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/D enzymatic domain. In an aspect of this embodiment, a BoNT/D enzymatic domain comprises the enzymatic domains of SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 13. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises a naturally occurring BoNT/D enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/D isoform or an enzymatic domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises a naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a BoNT/D isoform enzymatic domain or a BoNT/D subtype enzymatic domain. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13, such as, *e.g.*, a BoNT/D isoform enzymatic domain or a BoNT/D subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises a non-naturally occurring BoNT/D enzymatic domain variant, such as, *e.g.*, a

conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof.

[087] In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 13; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 13.

[088] In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13. In still other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In further other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13.

[089] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/E enzymatic domain. In an aspect of this embodiment, a BoNT/E enzymatic domain comprises the enzymatic domains of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In other aspects of this embodiment, a

BoNT/E enzymatic domain comprises amino acids 1/2-411 of SEQ ID NO: 15. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises a naturally occurring BoNT/E enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/E isoform or an enzymatic domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises a naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, e.g., a BoNT/E isoform enzymatic domain or a BoNT/E subtype enzymatic domain. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1/2-411 of a naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, such as, *e.g.*, a BoNT/E isoform enzymatic domain or a BoNT/E subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises a non-naturally occurring BoNT/E enzymatic domain variant, such as, *e.g.*, a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1/2-411 of a non-naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, such as, *e.g.*, a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof.

[090] In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-411 of SEQ ID NO: 15; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-411 of SEQ ID NO: 15.

[091] In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15. In still other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or

substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In further other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15.

[092] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/F enzymatic domain. In an aspect of this embodiment, a BoNT/F enzymatic domain comprises the enzymatic domains of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In other aspects of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of SEQ ID NO: 18. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises a naturally occurring BoNT/F enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/F isoform or an enzymatic domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises a naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a BoNT/F isoform enzymatic domain or a BoNT/F subtype enzymatic domain. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of a naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, such as, *e.g.*, a BoNT/F isoform enzymatic domain or a BoNT/F subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises a non-naturally occurring BoNT/F enzymatic domain variant, such as, *e.g.*, a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of a non-naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, such as, *e.g.*, a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof.

[093] In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-428 of SEQ ID NO: 18; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-428 of SEQ ID NO: 18.

[094] In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18. In still other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In further other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18.

[095] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/G enzymatic domain. In an aspect of this embodiment, a BoNT/G enzymatic domain comprises the enzymatic domains of SEQ ID NO: 21. In other aspects of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises a naturally occurring BoNT/G enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BoNT/G isoform or an enzymatic domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises a naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform enzymatic domain or a BoNT/G subtype enzymatic domain. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of a naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform enzymatic domain or a BoNT/G subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises a non-naturally occurring BoNT/G enzymatic domain variant, such as, *e.g.*, a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of a non-naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.*, a

conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof.

[096] In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-4435 of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-4435 of SEQ ID NO: 21.

[097] In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21. In further other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21.

[098] In another embodiment, a Clostridial toxin enzymatic domain comprises a TeNT enzymatic domain. In an aspect of this embodiment, a TeNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 22. In other aspects of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of SEQ ID NO: 22. In another aspect of this embodiment, a TeNT enzymatic domain comprises a naturally occurring TeNT enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a TeNT isoform or an enzymatic domain from a TeNT subtype. In another aspect of this embodiment, a TeNT enzymatic domain comprises a naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform enzymatic domain or a TeNT subtype enzymatic domain. In another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of a naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform enzymatic domain or a TeNT subtype enzymatic domain. In still another aspect of this embodiment, a TeNT enzymatic domain comprises a non-naturally occurring TeNT enzymatic domain

variant, such as, *e.g.*, a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of a non-naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof.

[099] In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-438 of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-438 of SEQ ID NO: 22.

[0100] In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22. In further other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22.

[0101] In another embodiment, a Clostridial toxin enzymatic domain comprises a BaNT enzymatic domain. In an aspect of this embodiment, a BaNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 23. In other aspects of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of SEQ ID NO: 23. In another aspect of this embodiment, a BaNT enzymatic domain

comprises a naturally occurring BaNT enzymatic domain variant, such as, e.g., an enzymatic domain from a BaNT isoform or an enzymatic domain from a BaNT subtype. In another aspect of this embodiment, a BaNT enzymatic domain comprises a naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform enzymatic domain or a BaNT subtype enzymatic domain. In another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of a naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform enzymatic domain or a BaNT subtype enzymatic domain. In still another aspect of this embodiment, a BaNT enzymatic domain comprises a non-naturally occurring BaNT enzymatic domain variant, such as, *e.g.*, a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of a non-naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof.

[0102] In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-420 of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-420 of SEQ ID NO: 23.

[0103] In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23. In further other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7,

8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23.

[0104] In another embodiment, a Clostridial toxin enzymatic domain comprises a BuNT enzymatic domain. In an aspect of this embodiment, a BuNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 24 or SEQ ID NO: 25. In other aspects of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of SEQ ID NO: 24. In another aspect of this embodiment, a BuNT enzymatic domain comprises a naturally occurring BuNT enzymatic domain variant, such as, *e.g.*, an enzymatic domain from a BuNT isoform or an enzymatic domain from a BuNT subtype. In another aspect of this embodiment, a BuNT enzymatic domain comprises a naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a BuNT isoform enzymatic domain or a BuNT subtype enzymatic domain. In another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of a naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24, such as, *e.g.*, a BuNT isoform enzymatic domain or a BuNT subtype enzymatic domain. In still another aspect of this embodiment, a BuNT enzymatic domain comprises a non-naturally occurring BuNT enzymatic domain variant, such as, *e.g.*, a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of a non-naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof.

[0105] In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0106] In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 OR SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at most 1,

2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25. In further other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0107] The "translocation domain" comprises a portion of a Clostridial neurotoxin heavy chain having a translocation activity. By "translocation" is meant the ability to facilitate the transport of a polypeptide through a vesicular membrane, thereby exposing some or all of the polypeptide to the cytoplasm. In the various botulinum neurotoxins translocation is thought to involve an allosteric conformational change of the heavy chain caused by a decrease in pH within the endosome. This conformational change appears to involve and be mediated by the N terminal half of the heavy chain and to result in the formation of pores in the vesicular membrane; this change permits the movement of the proteolytic light chain from within the endosomal vesicle into the cytoplasm. See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902 (October 1998).

[0108] The amino acid sequence of the translocation-mediating portion of the botulinum neurotoxin heavy chain is known to those of skill in the art; additionally, those amino acid residues within this portion that are known to be essential for conferring the translocation activity are also known. It would therefore be well within the ability of one of ordinary skill in the art, for example, to employ the naturally occurring N-terminal peptide half of the heavy chain of any of the various *Clostridium tetanus* or *Clostridium botulinum* neurotoxin subtypes as a translocation domain, or to design an analogous translocation domain by aligning the primary sequences of the N-terminal halves of the various heavy chains and selecting a consensus primary translocation sequence based on conserved amino acid, polarity, steric and hydrophobicity characteristics between the sequences.

[0109] Aspects of the present specification provide, in part, a Clostridial toxin translocation domain. As used herein, the term "Clostridial toxin translocation domain" refers to any Clostridial toxin polypeptide that can execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. Thus, a Clostridial toxin translocation domain facilitates the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridial toxin light chain through the membrane an intracellular vesicle into the cytoplasm of a cell. Non-limiting examples of a Clostridial toxin translocation domain include, *e.g.*, a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a

BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, and a BuNT translocation domain.

[0110] A Clostridial toxin translocation domain includes, without limitation, naturally occurring Clostridial toxin translocation domain variants, such as, *e.g.*, Clostridial toxin translocation domain isoforms and Clostridial toxin translocation domain subtypes; non-naturally occurring Clostridial toxin translocation domain variants, such as, *e.g.*, conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, active Clostridial toxin translocation domain fragments thereof, or any combination thereof.

[0111] As used herein, the term "Clostridial toxin translocation domain variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin translocation domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin translocation domain variants useful to practice disclosed embodiments are variants that execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 455-873 of SEQ ID NO: 1; a BoNT/B translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 447-860 of SEQ ID NO: 6; a BoNT/C1 translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 454-868 of SEQ ID NO: 11; a BoNT/D translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 451-864 of SEQ ID NO: 13; a BoNT/E translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 427-847 of SEQ ID NO: 15; a BoNT/F translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 446-865 of SEQ ID NO: 18; a BoNT/G translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 451-865 of SEQ ID NO: 21; a TeNT translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 468-881 of SEQ ID NO: 22; a BaNT translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 436-857 of SEQ ID NO: 23; and a BuNT translocation domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 427-847 of SEQ ID NO: 24.

[0112] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin translocation domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific translocation domain subtypes showing about 85-87% amino acid identity when compared to the BoNT/A translocation domain subtype of SEQ ID NO: 1. As used herein, the term "naturally occurring Clostridial toxin

translocation domain variant" refers to any Clostridial toxin translocation domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin translocation domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin translocation domain isoforms produced by spontaneous mutation and Clostridial toxin translocation domain subtypes. A naturally occurring Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification.

[0113] A non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain isoform such as, *e.g.*, a BoNT/A translocation domain isoform, a BoNT/B translocation domain isoform, a BoNT/C1 translocation domain isoform, a BoNT/D translocation domain isoform, a BoNT/E translocation domain isoform, a BoNT/F translocation domain isoform, a BoNT/G translocation domain isoform, a TeNT translocation domain isoform, a BaNT translocation domain isoform, and a BuNT translocation domain isoform. Another non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain subtype such as, *e.g.*, a translocation domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5; a translocation domain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a translocation domain from subtype BoNT/C1-1 and BoNT/C1-2; a translocation domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; a translocation domain from subtype BoNT/F1, BoNT/F2, BoNT/F3; and a translocation domain from subtype BuNT-1 and BuNT-2.

[0114] As used herein, the term "non-naturally occurring Clostridial toxin translocation domain variant" refers to any Clostridial toxin translocation domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin translocation domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin translocation domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin translocation domain variants include, *e.g.*, conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, and active Clostridial toxin translocation domain fragments.

[0115] As used herein, the term "conservative Clostridial toxin translocation domain variant" refers to a Clostridial toxin translocation domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification. Non-limiting examples of a conservative Clostridial toxin translocation domain variant include, *e.g.*, conservative BoNT/A translocation domain variants, conservative BoNT/B translocation domain variants, conservative BoNT/C1 translocation domain variants, conservative BoNT/D translocation domain variants, conservative BoNT/E translocation

domain variants, conservative BoNT/F translocation domain variants, conservative BoNT/G translocation domain variants, conservative TeNT translocation domain variants, conservative BaNT translocation domain variants, and conservative BuNT translocation domain variants.

[0116] As used herein, the term "non-conservative Clostridial toxin translocation domain variant" refers to a Clostridial toxin translocation domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). A non-conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification. Non-limiting examples of a non-conservative Clostridial toxin translocation domain variant include, e.g., non-conservative BoNT/A translocation domain variants, non-conservative BoNT/B translocation domain variants, non-conservative BoNT/C1 translocation domain variants, non-conservative BoNT/D translocation domain variants, non-conservative BoNT/E translocation domain variants, non-conservative BoNT/F translocation domain variants, non-conservative BoNT/G translocation domain variants, and non-conservative TeNT translocation domain variants, non-conservative BaNT translocation domain variants, and non-conservative BuNT translocation domain variants.

[0117] As used herein, the term "active Clostridial toxin translocation domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the translocation domain can be useful in aspects of the present specification with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The translocation domains from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a translocation domain from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment include a Clostridial toxin translocation domain having a length of, *e.g.*, at least 350, 375, 400, or 425 amino acids. Other aspects of this embodiment include a Clostridial toxin translocation domain having a length of, *e.g.*, at most 350, 375, 400, or 425 amino acids.

[0118] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin translocation domain variants and non-naturally-occurring Clostridial toxin translocation domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0119] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation domain. In an aspect of this embodiment, a Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin translocation domain variant, such

as, *e.g.*, a Clostridial toxin translocation domain isoform or a Clostridial toxin translocation domain subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.*, a conservative Clostridial toxin translocation domain variant, a non-conservative Clostridial toxin translocation domain variant, an active Clostridial toxin translocation domain fragment, or any combination thereof.

[0120] In another embodiment, a hydrophobic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.*, A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another small amino acid. Examples of small amino acids include, *e.g.*, A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, *e.g.*, I, T and V.

[0121] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/A translocation domain. In an aspect of this embodiment, a BoNT/A translocation domain comprises the translocation domains of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A translocation domain comprises a naturally occurring BoNT/A translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/A isoform or an translocation domain from a BoNT/A subtype. In another aspect of this

embodiment, a BoNT/A translocation domain comprises a naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a BoNT/A isoform translocation domain or a BoNT/A subtype translocation domain. In another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of a naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform translocation domain or a BoNT/A subtype translocation domain. In still another aspect of this embodiment, a BoNT/A translocation domain comprises a non-naturally occurring BoNT/A translocation domain variant, such as, *e.g.*, a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A translocation domain comprises the translocation domain of a non-naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of a non-naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof.

[0122] In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 455-873 of SEQ ID NO: 1; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 455-873 of SEQ ID NO: 1.

[0123] In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1,

2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In further other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1.

[0124] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/B translocation domain. In an aspect of this embodiment, a BoNT/B translocation domain comprises the translocation domains of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In other aspects of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/B translocation domain comprises a naturally occurring BoNT/B translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/B isoform or an translocation domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B translocation domain comprises a naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a BoNT/B isoform translocation domain or a BoNT/B subtype translocation domain. In another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of a naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/B isoform translocation domain or a BoNT/B subtype translocation domain. In still another aspect of this embodiment, a BoNT/B translocation domain comprises a non-naturally occurring BoNT/B translocation domain variant, such as, *e.g.*, a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B translocation domain comprises the translocation domain of a non-naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of a non-naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof.

[0125] In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 447-860 of SEQ ID NO: 6; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 447-860 of SEQ ID NO: 6.

[0126] In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In further other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6.

[0127] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/C1 translocation domain. In an aspect of this embodiment, a BoNT/C1 translocation domain comprises the translocation domains of SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of SEQ ID NO: 11. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises a naturally occurring BoNT/C1 translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/C1 isoform or an translocation domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises a naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a BoNT/C1 isoform translocation domain or a BoNT/C1 subtype translocation domain. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of a naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11, such as, *e.g.*, a BoNT/C1 isoform translocation domain or a BoNT/C1 subtype translocation domain. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises a non-naturally occurring BoNT/C1 translocation domain variant, such as, *e.g.*, a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises the translocation domain of a non-naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof. In still another aspect of this

embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of a non-naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11, such as, *e.g.*, a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof.

[0128] In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 454-868 of SEQ ID NO: 11; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 454-868 of SEQ ID NO: 11.

[0129] In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11. In still other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In further other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11.

[0130] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/D translocation domain. In an aspect of this embodiment, a BoNT/D translocation domain comprises the translocation domains of SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of SEQ ID NO: 13. In another aspect of this embodiment, a BoNT/D translocation domain comprises a naturally occurring BoNT/D translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/D isoform or an translocation domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D translocation domain comprises a naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13 or SEQ ID NO:

14, such as, e.g., a BoNT/D isoform translocation domain or a BoNT/D subtype translocation domain. In another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of a naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13, such as, e.g., a BoNT/D isoform translocation domain or a BoNT/D subtype translocation domain. In still another aspect of this embodiment, a BoNT/D translocation domain comprises a non-naturally occurring BoNT/D translocation domain variant, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D translocation domain comprises the translocation domain of a non-naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of a non-naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof.

[0131] In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 451-864 of SEQ ID NO: 13; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 451-864 of SEQ ID NO: 13.

[0132] In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13. In still other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In further other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5,

6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13.

[0133] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/E translocation domain. In an aspect of this embodiment, a BoNT/E translocation domain comprises the translocation domains of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In other aspects of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of SEQ ID NO: 15. In another aspect of this embodiment, a BoNT/E translocation domain comprises a naturally occurring BoNT/E translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/E isoform or an translocation domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E translocation domain comprises a naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a BoNT/E isoform translocation domain or a BoNT/E subtype translocation domain. In another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of a naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, such as, *e.g.*, a BoNT/E isoform translocation domain or a BoNT/E subtype translocation domain. In still another aspect of this embodiment, a BoNT/E translocation domain comprises a non-naturally occurring BoNT/E translocation domain variant, such as, *e.g.*, a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E translocation domain comprises the translocation domain of a non-naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of a non-naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, such as, *e.g.*, a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof.

[0134] In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 427-847 of SEQ ID NO: 15; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 427-847 of SEQ ID NO: 15.

[0135] In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous

amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15. In still other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In further other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15.

[0136] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/F translocation domain. In an aspect of this embodiment, a BoNT/F translocation domain comprises the translocation domains of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In other aspects of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of SEQ ID NO: 18. In another aspect of this embodiment, a BoNT/F translocation domain comprises a naturally occurring BoNT/F translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/F isoform or an translocation domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F translocation domain comprises a naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a BoNT/F isoform translocation domain or a BoNT/F subtype translocation domain. In another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of a naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, such as, *e.g.*, a BoNT/F isoform translocation domain or a BoNT/F subtype translocation domain. In still another aspect of this embodiment, a BoNT/F translocation domain comprises a non-naturally occurring BoNT/F translocation domain variant, such as, *e.g.*, a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F translocation domain comprises the translocation domain of a non-naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of a non-naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, such as, *e.g.*, a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof.

[0137] In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 446-865 of SEQ ID NO: 18; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 446-865 of SEQ ID NO: 18.

[0138] In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18. In still other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In further other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18.

[0139] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/G translocation domain. In an aspect of this embodiment, a BoNT/G translocation domain comprises the translocation domains of SEQ ID NO: 21. In other aspects of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G translocation domain comprises a naturally occurring BoNT/G translocation domain variant, such as, *e.g.*, an translocation domain from a BoNT/G isoform or an translocation domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G translocation domain comprises a naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, e.g., a BoNT/G isoform translocation domain or a BoNT/G subtype translocation domain. In another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of a naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform translocation domain or a BoNT/G subtype translocation domain. In still another aspect of this embodiment, a BoNT/G

translocation domain comprises a non-naturally occurring BoNT/G translocation domain variant, such as, *e.g.*, a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G translocation domain comprises the translocation domain of a non-naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of a non-naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof.

[0140] In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 451-865 of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 451-865 of SEQ ID NO: 21.

[0141] In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21. In further other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21.

[0142] In another embodiment, a Clostridial toxin translocation domain comprises a TeNT translocation domain. In an aspect of this embodiment, a TeNT translocation domain comprises the translocation domains of SEQ ID NO: 22. In other aspects of this embodiment, a TeNT translocation domain

comprises amino acids 468-881 of SEQ ID NO: 22. In another aspect of this embodiment, a TeNT translocation domain comprises a naturally occurring TeNT translocation domain variant, such as, *e.g.*, an translocation domain from a TeNT isoform or an translocation domain from a TeNT subtype. In another aspect of this embodiment, a TeNT translocation domain comprises a naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, e.g., a TeNT isoform translocation domain or a TeNT subtype translocation domain. In another aspect of this embodiment, a TeNT translocation domain comprises amino acids 468-881 of a naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform translocation domain or a TeNT subtype translocation domain. In still another aspect of this embodiment, a TeNT translocation domain comprises a non-naturally occurring TeNT translocation domain variant, such as, *e.g.*, a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT translocation domain comprises the translocation domain of a non-naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT translocation domain comprises amino acids 468-881 of a non-naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof.

[0143] In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 468-881 of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 468-881 of SEQ ID NO: 22.

[0144] In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22. In further other aspects of this

embodiment, a TeNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22.

[0145] In another embodiment, a Clostridial toxin translocation domain comprises a BaNT translocation domain. In an aspect of this embodiment, a BaNT translocation domain comprises the translocation domains of SEQ ID NO: 23. In other aspects of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of SEQ ID NO: 23. In another aspect of this embodiment, a BaNT translocation domain comprises a naturally occurring BaNT translocation domain variant, such as, *e.g.*, an translocation domain from a BaNT isoform or an translocation domain from a BaNT subtype. In another aspect of this embodiment, a BaNT translocation domain comprises a naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform translocation domain or a BaNT subtype translocation domain. In another aspect of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of a naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform translocation domain or a BaNT subtype translocation domain. In still another aspect of this embodiment, a BaNT translocation domain comprises a non-naturally occurring BaNT translocation domain variant, such as, *e.g.*, a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT translocation domain comprises the translocation domain of a non-naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of a non-naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof.

[0146] In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 436-857 of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 436-857 of SEQ ID NO: 23.

[0147] In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6,

7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23. In further other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23.

[0148] In another embodiment, a Clostridial toxin translocation domain comprises a BuNT translocation domain. In an aspect of this embodiment, a BuNT translocation domain comprises the translocation domains of SEQ ID NO: 24 or SEQ ID NO: 25. In other aspects of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of SEQ ID NO: 24. In another aspect of this embodiment, a BuNT translocation domain comprises a naturally occurring BuNT translocation domain variant, such as, *e.g.*, an translocation domain from a BuNT isoform or an translocation domain from a BuNT subtype. In another aspect of this embodiment, a BuNT translocation domain comprises a naturally occurring BuNT translocation domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a BuNT isoform translocation domain or a BuNT subtype translocation domain. In another aspect of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of a naturally occurring BuNT translocation domain variant of SEQ ID NO: 24, such as, *e.g.*, a BuNT isoform translocation domain or a BuNT subtype translocation domain. In still another aspect of this embodiment, a BuNT translocation domain comprises a non-naturally occurring BuNT translocation domain variant, such as, *e.g.*, a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT translocation domain comprises the translocation domain of a non-naturally occurring BuNT translocation domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of a non-naturally occurring BuNT translocation domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof.

[0149] In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT translocation

domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0150] In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 OR SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25. In further other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0151] Aspects of the present specification provide, in part, a binding domain. As used herein, the term "binding domain" is synonymous with "ligand" or "targeting moiety" and refers to any molecule that can preferentially interact with another molecule present on the surface of a cell under physiological conditions. The cell surface molecule may comprise a polypeptide, a polysaccharide, a lipid, or may have structural characteristics of more than one of these. As used herein, the term "preferentially interacts" refers to molecule is able to bind its target receptor under physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree relative to other, non-target receptor. With reference to a Clostridial toxin binding domain disclosed in the present specification, there is a discriminatory binding of the Clostridial toxin binding domain to its cognate receptor relative to other receptors. With reference to a non-Clostridial toxin binding domain disclosed in the present specification, there is a discriminatory binding of the non-Clostridial toxin binding domain to it cognate receptor relative to other receptors.

[0152] Thus, in an embodiment, a binding domain that selectively binds a target receptor has a dissociation equilibrium constant (K_D) that is greater for the target receptor relative to a non-target receptor by, *e.g.*, at least one-fold, at least two-fold, at least three-fold, at least four fold, at least five-fold, at least 10 fold, at least 50 fold, at least 100 fold, at least 1000, at least 10,000,or at least 100,000 fold.

[0153] Aspects of the present specification provide, in part, a Clostridial toxin binding domain. As used herein, the term "Clostridial toxin binding domain" refers to any Clostridial toxin polypeptide that can execute the binding step of the intoxication process that initiates the overall internalization mechanism whereby the modified Clostridial toxin disclosed in the present specification intoxicates a target cell. Non-limiting examples of a Clostridial toxin binding domain include, *e.g.*, a BoNT/A binding domain, a BoNT/B binding domain, a BoNT/C1 binding domain, a BoNT/D binding domain, a BoNT/E binding domain, a BoNT/F binding domain, a BoNT/G binding domain, a TeNT binding domain, a BaNT binding domain, and a BuNT binding domain. Other non-limiting examples of a Clostridial toxin binding domain include, *e.g.*, amino acids 874-1296 of SEQ ID NO: 1, amino acids 861-1291 of SEQ ID NO: 2, amino acids 869-1291 of SEQ ID NO: 3, amino acids 865-1291 of SEQ ID NO: 4, amino acids 848-1252 of SEQ ID NO: 5, amino acids 866-1274 of SEQ ID NO: 6, amino acids 866-1297 of SEQ ID NO: 7, amino acids 882-1315 of SEQ ID NO: 8, amino acids 858-1268 of SEQ ID NO: 9, and amino acids 848-1251 of SEQ ID NO: 10.

[0154] A Clostridial toxin binding domain includes, without limitation, naturally occurring Clostridial toxin binding domain variants, such as, *e.g.*, Clostridial toxin binding domain isoforms and Clostridial toxin binding domain subtypes; non-naturally occurring Clostridial toxin binding domain variants, such as, *e.g.*, conservative Clostridial toxin binding domain variants, non-conservative Clostridial toxin binding domain variants, active Clostridial toxin binding domain fragments thereof, or any combination thereof.

[0155] As used herein, the term "Clostridial toxin binding domain variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin binding domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin binding domain variants useful to practice disclosed embodiments are variants that execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 874-1296 of SEQ ID NO: 1; a BoNT/B binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 861-1291 of SEQ ID NO: 6; a BoNT/C1 binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 869-1291 of SEQ ID NO: 11; a BoNT/D binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 865-1291 of SEQ ID NO: 13; a BoNT/E binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 848-1252 of SEQ ID NO: 15; a BoNT/F binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 866-1274 of SEQ ID NO: 18; a BoNT/G binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 866-1297 of SEQ ID NO: 21; a TeNT binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 882-1315 of SEQ ID NO: 22; a BaNT binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 858-1268 of SEQ ID

NO: 23; and a BuNT binding domain variant will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to amino acids 848-1251 of SEQ ID NO: 24.

[0156] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin binding domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific binding domain subtypes showing about 83-97% amino acid identity when compared to the BoNT/A binding domain subtype of SEQ ID NO: 1. As another example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific binding domain subtypes showing about 83-97% amino acid identity when compared to the BoNT/A binding domain subtype of SEQ ID NO: 1. As used herein, the term "naturally occurring Clostridial toxin binding domain variant" refers to any Clostridial toxin binding domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin binding domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin binding domain isoforms produced by spontaneous mutation and Clostridial toxin binding domain subtypes. A naturally occurring Clostridial toxin binding domain variant can function in substantially the same manner as the reference Clostridial toxin binding domain on which the naturally occurring Clostridial toxin binding domain variant is based, and can be substituted for the reference Clostridial toxin binding domain in any aspect of the present specification.

[0157] A non-limiting examples of a naturally occurring Clostridial toxin binding domain variant is a Clostridial toxin binding domain isoform such as, *e.g.*, a BoNT/A binding domain isoform, a BoNT/B binding domain isoform, a BoNT/C1 binding domain isoform, a BoNT/D binding domain isoform, a BoNT/E binding domain isoform, a BoNT/F binding domain isoform, a BoNT/G binding domain isoform, a TeNT binding domain isoform, a BaNT binding domain isoform, and a BuNT binding domain isoform. Another non-limiting examples of a naturally occurring Clostridial toxin binding domain variant is a Clostridial toxin binding domain subtype such as, *e.g.*, a binding domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5; a binding domain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a binding domain from subtype BoNT/C1-1 and BoNT/C1-2; a binding domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a binding domain from subtype BoNT/F1, BoNT/F2, and BoNT/F3; and a binding domain from subtype BuNT-1 and BuNT-2.

[0158] As used herein, the term "non-naturally occurring Clostridial toxin binding domain variant" refers to any Clostridial toxin binding domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin binding domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin binding domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin binding domain variants include, *e.g.*, conservative Clostridial toxin binding domain variants, non-conservative Clostridial toxin binding domain variants, Clostridial toxin binding domain chimeric variants and active Clostridial toxin binding domain fragments.

[0159] As used herein, the term "conservative Clostridial toxin binding domain variant" refers to a Clostridial toxin binding domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin binding domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding

capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin binding domain variant can function in substantially the same manner as the reference Clostridial toxin binding domain on which the conservative Clostridial toxin binding domain variant is based, and can be substituted for the reference Clostridial toxin binding domain in any aspect of the present specification. Non-limiting examples of a conservative Clostridial toxin binding domain variant include, *e.g.*, conservative BoNT/A binding domain variants, conservative BoNT/B binding domain variants, conservative BoNT/C1 binding domain variants, conservative BoNT/D binding domain variants, conservative BoNT/E binding domain variants, conservative BoNT/F binding domain variants, conservative BoNT/G binding domain variants, conservative TeNT binding domain variants, conservative BaNT binding domain variants, and conservative BuNT binding domain variants.

[0160] As used herein, the term "non-conservative Clostridial toxin binding domain variant" refers to a Clostridial toxin binding domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin binding domain on which the non-conservative Clostridial toxin binding domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin binding domain on which the non-conservative Clostridial toxin binding domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin binding domain sequence (Table 1). A non-conservative Clostridial toxin binding domain variant can function in substantially the same manner as the reference Clostridial toxin binding domain on which the non-conservative Clostridial toxin binding domain variant is based, and can be substituted for the reference Clostridial toxin binding domain in any aspect of the present specification. Non-limiting examples of a non-conservative Clostridial toxin binding domain variant include, e.g., non-conservative BoNT/A binding domain variants, non-conservative BoNT/B binding domain variants, non-conservative BoNT/C1 binding domain variants, non-conservative BoNT/D binding domain variants, non-conservative BoNT/E binding domain variants, non-conservative BoNT/F binding domain variants, non-conservative BoNT/G binding domain variants, and non-conservative TeNT binding domain variants, non-conservative BaNT binding domain variants, and non-conservative BuNT binding domain variants.

[0161] As used herein, the term "active Clostridial toxin binding domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the binding domain can be useful in aspects of the present specification with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The binding domains from the heavy chains of Clostridial toxins are approximately 400-440 amino acids in length and comprise a binding domain (Table 1). Research has shown that the entire length of a binding domain from a Clostridial toxin heavy chain is not necessary for the translocating activity of the binding domain. Thus, aspects of this embodiment include a Clostridial toxin binding domain having a length of, *e.g.*, at least 350, 375, 400, or 425 amino acids. Other aspects of this embodiment include a Clostridial toxin binding domain having a length of, e.g., at most 350, 375, 400, or 425 amino acids.

[0162] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin binding domain variants and non-naturally-occurring Clostridial toxin binding domain variants, including, without limitation, global methods, local methods, and hybrid

methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0163] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin binding domain. In an aspect of this embodiment, a Clostridial toxin binding domain comprises a naturally occurring Clostridial toxin binding domain variant, such as, *e.g.*, a Clostridial toxin binding domain isoform or a Clostridial toxin binding domain subtype. In another aspect of this embodiment, a Clostridial toxin binding domain comprises a non-naturally occurring Clostridial toxin binding domain variant, such as, *e.g.*, a conservative Clostridial toxin binding domain variant, a non-conservative Clostridial toxin binding domain variant, an active Clostridial toxin binding domain fragment, or any combination thereof.

[0164] In another embodiment, a hydrophobic amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.*, A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another small amino acid. Examples of small amino acids include, *e.g.*, A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the Clostridial toxin binding domain can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, *e.g.*, I, T and V.

[0165] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/A binding domain. In an aspect of this embodiment, a BoNT/A binding domain comprises the binding domains of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In other aspects of this

embodiment, a BoNT/A binding domain comprises amino acids 874-1296 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A binding domain comprises a naturally occurring BoNT/A binding domain variant, such as, *e.g.*, an binding domain from a BoNT/A isoform or an binding domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A binding domain comprises a naturally occurring BoNT/A binding domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a BoNT/A isoform binding domain or a BoNT/A subtype binding domain. In another aspect of this embodiment, a BoNT/A binding domain comprises amino acids 874-1296 of a naturally occurring BoNT/A binding domain variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform binding domain or a BoNT/A subtype binding domain. In still another aspect of this embodiment, a BoNT/A binding domain comprises a non-naturally occurring BoNT/A binding domain variant, such as, *e.g.*, a conservative BoNT/A binding domain variant, a non-conservative BoNT/A binding domain variant, an active BoNT/A binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A binding domain comprises the binding domain of a non-naturally occurring BoNT/A binding domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/A binding domain variant, a non-conservative BoNT/A binding domain variant, an active BoNT/A binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A binding domain comprises amino acids 874-1296 of a non-naturally occurring BoNT/A binding domain variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A binding domain variant, a non-conservative BoNT/A binding domain variant, an active BoNT/A binding domain fragment, or any combination thereof.

[0166] In other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 874-1296 of SEQ ID NO: 1; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 874-1296 of SEQ ID NO: 1.

[0167] In other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 874-1296 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 874-1296 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid

deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In further other aspects of this embodiment, a BoNT/A binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 874-1296 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 874-1296 of SEQ ID NO: 1.

[0168] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/B binding domain. In an aspect of this embodiment, a BoNT/B binding domain comprises the binding domains of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In other aspects of this embodiment, a BoNT/B binding domain comprises amino acids 861-1291 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/B binding domain comprises a naturally occurring BoNT/B binding domain variant, such as, *e.g.*, an binding domain from a BoNT/B isoform or an binding domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B binding domain comprises a naturally occurring BoNT/B binding domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a BoNT/B isoform binding domain or a BoNT/B subtype binding domain. In another aspect of this embodiment, a BoNT/B binding domain comprises amino acids 861-1291 of a naturally occurring BoNT/B binding domain variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/B isoform binding domain or a BoNT/B subtype binding domain. In still another aspect of this embodiment, a BoNT/B binding domain comprises a non-naturally occurring BoNT/B binding domain variant, such as, *e.g.*, a conservative BoNT/B binding domain variant, a non-conservative BoNT/B binding domain variant, an active BoNT/B binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B binding domain comprises the binding domain of a non-naturally occurring BoNT/B binding domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.*, a conservative BoNT/B binding domain variant, a non-conservative BoNT/B binding domain variant, an active BoNT/B binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B binding domain comprises amino acids 861-1291 of a non-naturally occurring BoNT/B binding domain variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/B binding domain variant, a non-conservative BoNT/B binding domain variant, an active BoNT/B binding domain fragment, or any combination thereof.

[0169] In other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 861-1291 of SEQ ID NO: 6; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 861-1291 of SEQ ID NO: 6.

[0170] In other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 861-1291 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 861-1291 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In further other aspects of this embodiment, a BoNT/B binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 861-1291 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 861-1291 of SEQ ID NO: 6.

[0171] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/C1 binding domain. In an aspect of this embodiment, a BoNT/C1 binding domain comprises the binding domains of SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, a BoNT/C1 binding domain comprises amino acids 869-1291 of SEQ ID NO: 11. In another aspect of this embodiment, a BoNT/C1 binding domain comprises a naturally occurring BoNT/C1 binding domain variant, such as, *e.g.*, an binding domain from a BoNT/C1 isoform or an binding domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 binding domain comprises a naturally occurring BoNT/C1 binding domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a BoNT/C1 isoform binding domain or a BoNT/C1 subtype binding domain. In another aspect of this embodiment, a BoNT/C1 binding domain comprises amino acids 869-1291 of a naturally occurring BoNT/C1 binding domain variant of SEQ ID NO: 11, such as, *e.g.*, a BoNT/C1 isoform binding domain or a BoNT/C1 subtype binding domain. In still another aspect of this embodiment, a BoNT/C1 binding domain comprises a non-naturally occurring BoNT/C1 binding domain variant, such as, *e.g.*, a conservative BoNT/C1 binding domain variant, a non-conservative BoNT/C1 binding domain variant, an active BoNT/C1 binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 binding domain comprises the binding domain of a non-naturally occurring BoNT/C1 binding domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.*, a conservative BoNT/C1 binding domain variant, a non-conservative BoNT/C1 binding domain variant, an active BoNT/C1 binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 binding domain comprises amino acids 869-1291 of a non-naturally occurring BoNT/C1 binding domain variant of SEQ ID NO: 11, such as, *e.g.*,

a conservative BoNT/C1 binding domain variant, a non-conservative BoNT/C1 binding domain variant, an active BoNT/C1 binding domain fragment, or any combination thereof.

[0172] In other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 869-1291 of SEQ ID NO: 11; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 869-1291 of SEQ ID NO: 11.

[0173] In other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 869-1291 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 869-1291 of SEQ ID NO: 11. In still other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 11 or SEQ ID NO: 12. In further other aspects of this embodiment, a BoNT/C1 binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 869-1291 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 869-1291 of SEQ ID NO: 11.

[0174] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/D binding domain. In an aspect of this embodiment, a BoNT/D binding domain comprises the binding domains of SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, a BoNT/D binding domain comprises amino acids 865-1291 of SEQ ID NO: 13. In another aspect of this embodiment, a BoNT/D binding domain comprises a naturally occurring BoNT/D binding domain variant, such as, *e.g.*, an binding domain from a BoNT/D isoform or an binding domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D binding domain comprises a naturally occurring BoNT/D binding domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a BoNT/D isoform binding domain or a BoNT/D subtype binding domain. In another aspect of this embodiment, a BoNT/D binding domain comprises amino acids 865-1291 of a naturally occurring BoNT/D binding domain variant of SEQ ID NO: 13, such as, *e.g.*, a BoNT/D isoform binding domain or a BoNT/D subtype binding domain. In still another aspect of this embodiment, a BoNT/D binding domain comprises a non-naturally occurring BoNT/D binding

domain variant, such as, *e.g.*, a conservative BoNT/D binding domain variant, a non-conservative BoNT/D binding domain variant, an active BoNT/D binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D binding domain comprises the binding domain of a non-naturally occurring BoNT/D binding domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, *e.g.*, a conservative BoNT/D binding domain variant, a non-conservative BoNT/D binding domain variant, an active BoNT/D binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D binding domain comprises amino acids 865-1291 of a non-naturally occurring BoNT/D binding domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative BoNT/D binding domain variant, a non-conservative BoNT/D binding domain variant, an active BoNT/D binding domain fragment, or any combination thereof.

[0175] In other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 865-1291 of SEQ ID NO: 13; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 865-1291 of SEQ ID NO: 13.

[0176] In other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 865-1291 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 865-1291 of SEQ ID NO: 13. In still other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 13 or SEQ ID NO: 14. In further other aspects of this embodiment, a BoNT/D binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 865-1291 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 865-1291 of SEQ ID NO: 13.

[0177] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/E binding domain. In an aspect of this embodiment, a BoNT/E binding domain comprises the binding domains of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In other aspects of this embodiment, a BoNT/E binding domain comprises amino acids 848-1252 of SEQ ID NO: 15. In another aspect of this embodiment, a

BoNT/E binding domain comprises a naturally occurring BoNT/E binding domain variant, such as, *e.g.*, an binding domain from a BoNT/E isoform or an binding domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E binding domain comprises a naturally occurring BoNT/E binding domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a BoNT/E isoform binding domain or a BoNT/E subtype binding domain. In another aspect of this embodiment, a BoNT/E binding domain comprises amino acids 848-1252 of a naturally occurring BoNT/E binding domain variant of SEQ ID NO: 15, such as, *e.g.*, a BoNT/E isoform binding domain or a BoNT/E subtype binding domain. In still another aspect of this embodiment, a BoNT/E binding domain comprises a non-naturally occurring BoNT/E binding domain variant, such as, *e.g.*, a conservative BoNT/E binding domain variant, a non-conservative BoNT/E binding domain variant, an active BoNT/E binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E binding domain comprises the binding domain of a non-naturally occurring BoNT/E binding domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, *e.g.*, a conservative BoNT/E binding domain variant, a non-conservative BoNT/E binding domain variant, an active BoNT/E binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E binding domain comprises amino acids 848-1252 of a non-naturally occurring BoNT/E binding domain variant of SEQ ID NO: 15, such as, *e.g.*, a conservative BoNT/E binding domain variant, a non-conservative BoNT/E binding domain variant, an active BoNT/E binding domain fragment, or any combination thereof.

[0178] In other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 848-1252 of SEQ ID NO: 15; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 848-1252 of SEQ ID NO: 15.

[0179] In other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1252 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1252 of SEQ ID NO: 15. In still other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to

the binding domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In further other aspects of this embodiment, a BoNT/E binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1252 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1252 of SEQ ID NO: 15.

[0180] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/F binding domain. In an aspect of this embodiment, a BoNT/F binding domain comprises the binding domains of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In other aspects of this embodiment, a BoNT/F binding domain comprises amino acids 866-1274 of SEQ ID NO: 18. In another aspect of this embodiment, a BoNT/F binding domain comprises a naturally occurring BoNT/F binding domain variant, such as, *e.g.*, an binding domain from a BoNT/F isoform or an binding domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F binding domain comprises a naturally occurring BoNT/F binding domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a BoNT/F isoform binding domain or a BoNT/F subtype binding domain. In another aspect of this embodiment, a BoNT/F binding domain comprises amino acids 866-1274 of a naturally occurring BoNT/F binding domain variant of SEQ ID NO: 18, such as, *e.g.*, a BoNT/F isoform binding domain or a BoNT/F subtype binding domain. In still another aspect of this embodiment, a BoNT/F binding domain comprises a non-naturally occurring BoNT/F binding domain variant, such as, *e.g.*, a conservative BoNT/F binding domain variant, a non-conservative BoNT/F binding domain variant, an active BoNT/F binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F binding domain comprises the binding domain of a non-naturally occurring BoNT/F binding domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, *e.g.*, a conservative BoNT/F binding domain variant, a non-conservative BoNT/F binding domain variant, an active BoNT/F binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F binding domain comprises amino acids 866-1274 of a non-naturally occurring BoNT/F binding domain variant of SEQ ID NO: 18, such as, *e.g.*, a conservative BoNT/F binding domain variant, a non-conservative BoNT/F binding domain variant, an active BoNT/F binding domain fragment, or any combination thereof.

[0181] In other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 866-1274 of SEQ ID NO: 18; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 866-1274 of SEQ ID NO: 18.

[0182] In other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid

deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1274 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1274 of SEQ ID NO: 18. In still other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In further other aspects of this embodiment, a BoNT/F binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1274 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1274 of SEQ ID NO: 18.

[0183] In another embodiment, a Clostridial toxin binding domain comprises a BoNT/G binding domain. In an aspect of this embodiment, a BoNT/G binding domain comprises the binding domains of SEQ ID NO: 21. In other aspects of this embodiment, a BoNT/G binding domain comprises amino acids 866-1297 of SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G binding domain comprises a naturally occurring BoNT/G binding domain variant, such as, *e.g.*, an binding domain from a BoNT/G isoform or an binding domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G binding domain comprises a naturally occurring BoNT/G binding domain variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform binding domain or a BoNT/G subtype binding domain. In another aspect of this embodiment, a BoNT/G binding domain comprises amino acids 866-1297 of a naturally occurring BoNT/G binding domain variant of SEQ ID NO: 21, such as, *e.g.*, a BoNT/G isoform binding domain or a BoNT/G subtype binding domain. In still another aspect of this embodiment, a BoNT/G binding domain comprises a non-naturally occurring BoNT/G binding domain variant, such as, *e.g.*, a conservative BoNT/G binding domain variant, a non-conservative BoNT/G binding domain variant, an active BoNT/G binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G binding domain comprises the binding domain of a non-naturally occurring BoNT/G binding domain variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G binding domain variant, a non-conservative BoNT/G binding domain variant, an active BoNT/G binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G binding domain comprises amino acids 866-1297 of a non-naturally occurring BoNT/G binding domain variant of SEQ ID NO: 21, such as, *e.g.*, a conservative BoNT/G binding domain variant, a non-conservative BoNT/G binding domain variant, an active BoNT/G binding domain fragment, or any combination thereof.

[0184] In other aspects of this embodiment, a BoNT/G binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 21. In yet other aspects of this

embodiment, a BoNT/G binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 866-1297 of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 866-1297 of SEQ ID NO: 21.

[0185] In other aspects of this embodiment, a BoNT/G binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1297 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1297 of SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 21. In further other aspects of this embodiment, a BoNT/G binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1297 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 866-1297 of SEQ ID NO: 21.

[0186] In another embodiment, a Clostridial toxin binding domain comprises a TeNT binding domain. In an aspect of this embodiment, a TeNT binding domain comprises the binding domains of SEQ ID NO: 22. In other aspects of this embodiment, a TeNT binding domain comprises amino acids 882-1315 of SEQ ID NO: 22. In another aspect of this embodiment, a TeNT binding domain comprises a naturally occurring TeNT binding domain variant, such as, *e.g.*, an binding domain from a TeNT isoform or an binding domain from a TeNT subtype. In another aspect of this embodiment, a TeNT binding domain comprises a naturally occurring TeNT binding domain variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform binding domain or a TeNT subtype binding domain. In another aspect of this embodiment, a TeNT binding domain comprises amino acids 882-1315 of a naturally occurring TeNT binding domain variant of SEQ ID NO: 22, such as, *e.g.*, a TeNT isoform binding domain or a TeNT subtype binding domain. In still another aspect of this embodiment, a TeNT binding domain comprises a non-naturally occurring TeNT binding domain variant, such as, *e.g.*, a conservative TeNT binding domain variant, a non-conservative TeNT binding domain variant, an active TeNT binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT binding domain comprises the binding domain of a non-naturally occurring TeNT binding domain variant of SEQ ID NO: 22, such as, *e.g.*, a conservative TeNT binding domain variant, a non-conservative TeNT binding domain variant, an active TeNT binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT binding domain comprises amino acids 882-1315 of a non-naturally occurring TeNT binding domain variant of SEQ ID

NO: 22, such as, *e.g.*, a conservative TeNT binding domain variant, a non-conservative TeNT binding domain variant, an active TeNT binding domain fragment, or any combination thereof.

[0187] In other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 882-1315 of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 882-1315 of SEQ ID NO: 22.

[0188] In other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 882-1315 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 882-1315 of SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 22. In further other aspects of this embodiment, a TeNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 882-1315 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 882-1315 of SEQ ID NO: 22.

[0189] In another embodiment, a Clostridial toxin binding domain comprises a BaNT binding domain. In an aspect of this embodiment, a BaNT binding domain comprises the binding domains of SEQ ID NO: 23. In other aspects of this embodiment, a BaNT binding domain comprises amino acids 858-1268 of SEQ ID NO: 23. In another aspect of this embodiment, a BaNT binding domain comprises a naturally occurring BaNT binding domain variant, such as, *e.g.*, an binding domain from a BaNT isoform or an binding domain from a BaNT subtype. In another aspect of this embodiment, a BaNT binding domain comprises a naturally occurring BaNT binding domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform binding domain or a BaNT subtype binding domain. In another aspect of this embodiment, a BaNT binding domain comprises amino acids 858-1268 of a naturally occurring BaNT binding domain variant of SEQ ID NO: 23, such as, *e.g.*, a BaNT isoform binding domain or a BaNT subtype binding domain. In still another aspect of this embodiment, a BaNT binding domain comprises a non-naturally occurring BaNT binding domain variant, such as, *e.g.*, a conservative BaNT binding domain variant, a non-conservative BaNT binding domain variant, an active BaNT binding domain fragment, or any combination

thereof. In still another aspect of this embodiment, a BaNT binding domain comprises the binding domain of a non-naturally occurring BaNT binding domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT binding domain variant, a non-conservative BaNT binding domain variant, an active BaNT binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT binding domain comprises amino acids 858-1268 of a non-naturally occurring BaNT binding domain variant of SEQ ID NO: 23, such as, *e.g.*, a conservative BaNT binding domain variant, a non-conservative BaNT binding domain variant, an active BaNT binding domain fragment, or any combination thereof.

[0190] In other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 858-1268 of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 858-1268 of SEQ ID NO: 23.

[0191] In other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 858-1268 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 858-1268 of SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 23. In further other aspects of this embodiment, a BaNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 858-1268 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 858-1268 of SEQ ID NO: 23.

[0192] In another embodiment, a Clostridial toxin binding domain comprises a BuNT binding domain. In an aspect of this embodiment, a BuNT binding domain comprises the binding domains of SEQ ID NO: 24 or SEQ ID NO: 25. In other aspects of this embodiment, a BuNT binding domain comprises amino acids 848-1251 of SEQ ID NO: 24. In another aspect of this embodiment, a BuNT binding domain comprises a naturally occurring BuNT binding domain variant, such as, *e.g.*, an binding domain from a BuNT isoform or an binding domain from a BuNT subtype. In another aspect of this embodiment, a BuNT binding domain comprises a naturally occurring BuNT binding domain variant of SEQ ID NO: 24 or SEQ ID NO:

25, such as, *e.g.*, a BuNT isoform binding domain or a BuNT subtype binding domain. In another aspect of this embodiment, a BuNT binding domain comprises amino acids 848-1251 of a naturally occurring BuNT binding domain variant of SEQ ID NO: 24, such as, e.g., a BuNT isoform binding domain or a BuNT subtype binding domain. In still another aspect of this embodiment, a BuNT binding domain comprises a non-naturally occurring BuNT binding domain variant, such as, *e.g.*, a conservative BuNT binding domain variant, a non-conservative BuNT binding domain variant, an active BuNT binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT binding domain comprises the binding domain of a non-naturally occurring BuNT binding domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.*, a conservative BuNT binding domain variant, a non-conservative BuNT binding domain variant, an active BuNT binding domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT binding domain comprises amino acids 848-1251 of a non-naturally occurring BuNT binding domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative BuNT binding domain variant, a non-conservative BuNT binding domain variant, an active BuNT binding domain fragment, or any combination thereof.

[0193] In other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the binding domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the binding domain of SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0194] In other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 24 OR SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the binding domain of SEQ ID NO: 24 or SEQ ID NO: 25. In further other aspects of this embodiment, a BuNT binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100

contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 848-1251 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0195] Aspects of the present specification provide, in part, a non-Clostridial toxin binding domain. As used herein, the term "non-Clostridial toxin binding domain" refers to any polypeptide that can execute the binding step of the intoxication process that initiates the overall internalization mechanism whereby the modified Clostridial toxin disclosed in the present specification intoxicates a target cell. Examples of binding domains are described in, *e.g.*, Keith A. Foster et al., *Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions*, U.S. Patent 5,989,545; Clifford C. Shone et al., *Recombinant Toxin Fragments*, U.S. Patent 6,461,617; Conrad P. Quinn et al., *Methods and Compounds for the Treatment of Mucus Hypersecretion*, U.S. Patent 6,632,440; Lance E. Steward et al., *Methods And Compositions For The Treatment Of Pancreatitis*, U.S. Patent 6,843,998; J. Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, U.S. Patent 7,132,259; Stephan Donovan, *Clostridial Toxin Derivatives and Methods For Treating Pain*, U.S. Patent Publication 2002/0037833; Keith A. Foster et al., *Inhibition of Secretion from Non-neural Cells*, U.S. Patent Publication 2003/0180289; Lance E. Steward et al., *Multivalent Clostridial Toxin Derivatives and Methods of Their Use*, U.S. Patent Publication 2006/0211619; Keith A. Foster et al., *Non-Cytotoxic Protein Conjugates*, U.S. Patent Publication 2008/0187960; Steward, L.E. et al., *Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells*, U.S. Patent Application No. 11/776,075; Keith A. Foster et al., *Re-targeted Toxin Conjugates*, U.S. Patent Application No. 11/792,210; each of which is incorporated by reference in its entirety.

[0196] A non-Clostridial toxin binding domain includes, without limitation, naturally occurring non-Clostridial toxin binding domain variants, such as, *e.g.*, non-Clostridial toxin binding domain isoforms and non-Clostridial toxin binding domain subtypes; and non-naturally occurring non-Clostridial toxin binding domain variants, such as, *e.g.*, conservative non-Clostridial toxin binding domain variants, non-conservative non-Clostridial toxin binding domain variants, non-Clostridial toxin binding domain chimeras, active non-Clostridial toxin binding domain fragments thereof, or any combination thereof.

[0197] As used herein, the term "non-Clostridial toxin binding domain variant," whether naturally-occurring or non-naturally-occurring, refers to a non-Clostridial toxin binding domain that has at least one amino acid change from the corresponding region of a reference sequence and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, non-Clostridial toxin binding domain variants useful to practice the disclosed embodiments are variants that execute the binding step of the intoxication process.

[0198] It is recognized by those of skill in the art that within each non-Clostridial toxin binding domain there can be naturally occurring variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. As used herein, the term "naturally occurring non-Clostridial toxin binding domain variant" refers to any non-Clostridial toxin binding domain produced by a naturally-occurring process, including, without limitation, non-Clostridial toxin binding domain isoforms produced from alternatively-spliced transcripts and non-Clostridial toxin binding domain isoforms produced by spontaneous mutation. A naturally occurring non-Clostridial toxin binding domain variant can function in substantially the same manner as the reference non-Clostridial toxin binding domain on which the naturally occurring non-Clostridial toxin binding domain variant is based, and can be substituted for the

reference non-Clostridial toxin binding domain in any aspect of the present specification. A non-limiting examples of a naturally occurring non-Clostridial toxin binding domain variant is a non-Clostridial toxin binding domain isoform. Non-limiting examples of a non-Clostridial toxin binding domain isoform include, *e.g.*, opiod binding domain isoforms, tachykinin binding domain isoforms, melanocortin binding domain isoforms, galanin binding domain isoforms, granin binding domain isoforms, Neuropeptide Y related peptide binding domain isoforms, neurohormone binding domain isoforms, neuroregulatory cytokine binding domain isoforms, kinin peptide binding domain isoforms, growth factor binding domain isoforms, and glucagon like hormone binding domain isoforms.

[0199] As used herein, the term "non-naturally occurring non-Clostridial toxin binding domain variant" refers to any non-Clostridial toxin binding domain produced with the aid of human manipulation, including, without limitation, non-Clostridial toxin binding domains produced by genetic engineering using random mutagenesis or rational design and non-Clostridial toxin binding domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring non-Clostridial toxin binding domain variants include, *e.g.*, conservative non-Clostridial toxin binding domain variants, non-conservative non-Clostridial toxin binding domain variants, non-Clostridial toxin binding domain chimeric variants and active non-Clostridial toxin binding domain fragments.

[0200] As used herein, the term "conservative non-Clostridial toxin binding domain variant" refers to a non-Clostridial toxin binding domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from a reference non-Clostridial toxin binding domain sequence. Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative non-Clostridial toxin binding domain variant can function in substantially the same manner as the reference non-Clostridial toxin binding domain on which the conservative non-Clostridial toxin binding domain variant is based, and can be substituted for the reference non-Clostridial toxin binding domain in any aspect of the present specification. Non-limiting examples of a conservative non-Clostridial toxin binding domain variant include, *e.g.*, conservative opiod binding domain variants, conservative tachykinin binding domain variants, conservative melanocortin binding domain variants, conservative galanin binding domain variants, conservative granin binding domain variants, conservative Neuropeptide Y related peptide binding domain variants, conservative neurohormone binding domain variants, conservative neuroregulatory cytokine binding domain variants, conservative kinin peptide binding domain variants, conservative growth factor binding domain variants, and conservative glucagon like hormone binding domain variants.

[0201] As used herein, the term "non-conservative non-Clostridial toxin binding domain variant" refers to a non-Clostridial toxin binding domain in which 1) at least one amino acid is deleted from the reference non-Clostridial toxin binding domain on which the non-conservative non-Clostridial toxin binding domain variant is based; 2) at least one amino acid added to the reference non-Clostridial toxin binding domain on which the non-conservative non-Clostridial toxin binding domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from a reference non-Clostridial toxin binding domain sequence. A non-conservative non-Clostridial toxin binding domain variant can function in substantially the same

manner as the reference non-Clostridial toxin binding domain on which the non-conservative non-Clostridial toxin binding domain variant is based, and can be substituted for the reference non-Clostridial toxin binding domain in any aspect of the present specification. Non-limiting examples of a non-conservative non-Clostridial toxin binding domain variant include, *e.g.*, non-conservative opiod binding domain variants, non-conservative tachykinin binding domain variants, non-conservative melanocortin binding domain variants, non-conservative galanin binding domain variants, non-conservative granin binding domain variants, non-conservative Neuropeptide Y related peptide binding domain variants, non-conservative neurohormone binding domain variants, non-conservative neuroregulatory cytokine binding domain variants, non-conservative kinin peptide binding domain variants, non-conservative growth factor binding domain variants, and non-conservative glucagon like hormone binding domain variants.

[0202] As used herein, the term "active non-Clostridial toxin binding domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the binding domain can be useful in aspects of the present specification with the proviso that these biding domain fragments can preferentially interact with the cognate receptor, and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

[0203] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin binding domain variants and non-naturally-occurring Clostridial toxin binding domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0204] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a non-Clostridial toxin binding domain. In an aspect of this embodiment, a non-Clostridial toxin binding domain comprises a naturally occurring non-Clostridial toxin binding domain variant, such as, *e.g.*, a non-Clostridial toxin binding domain isoform. In another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a non-naturally occurring non-Clostridial toxin binding domain variant, such as, *e.g.*, a conservative non-Clostridial toxin binding domain variant, a non-conservative non-Clostridial toxin binding domain variant, an active non-Clostridial toxin binding domain fragment, or any combination thereof.

[0205] In another embodiment, a hydrophobic amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.*, A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another polar amino acid. Examples of polar

amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another small amino acid. Examples of small amino acids include, *e.g.*, A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the non-Clostridial toxin binding domain can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, *e.g.*, I, T and V.

[0206] In another embodiment, a non-Clostridial toxin binding domain comprises an opiod binding domain, such as, *e.g.*, an enkephalin, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin. In yet another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a tachykinin binding domain, such as, *e.g.*, a Substance P, a neuropeptide K (NPK), a neuropeptide gamma (NP gamma), a neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), a neurokinin B (NKB), a hemokinin or a endokinin. In still another aspect of this embodiment, a non-Clostridial toxin comprises a melanocortin binding domain, such as, *e.g.*, a melanocyte stimulating hormone, adrenocorticotropin, or a lipotropin. In still another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a galanin binding domain, such as, *e.g.*, a galanin or a galanin message-associated peptide. In a further aspect of this embodiment, a non-Clostridial toxin binding domain comprises a granin binding domain, such as, *e.g.*, a Chromogranin A, a Chromogranin B, or a Chromogranin C. In another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a Neuropeptide Y related peptide binding domain, such as, *e.g.*, a Neuropeptide Y, a Peptide YY, Pancreatic peptide or a Pancreatic icosapeptide. In yet another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a neurohormone binding domain, such as, *e.g.*, a corticotropin-releasing hormone, a parathyroid hormone, a thyrotropin-releasing hormone, or a somatostatin. In still another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a neuroregulatory cytokine binding domain, such as, *e.g.*, a ciliary neurotrophic factor, a glycophorin-A, a leukemia inhibitory factor, a cholinergic differentiation factor, an interleukin, an onostatin M, a cardiotrophin-1, a cardiotrophin-like cytokine, or a neuroleukin. In a further aspect of this embodiment, a non-Clostridial toxin binding domain comprises a kinin peptide binding domain, such as, *e.g.*, a bradykinin, a kallidin, a desArg9 bradykinin, or a desArg10 bradykinin. In another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a growth factor binding domain, such as, e.g., a fibroblast growth factor binding domain, a nerve growth factor binding domain, an insulin growth factor binding domain, an epidermal growth factor binding domain, a vascular endothelial growth factor

binding domain, a brain derived neurotrophic factor binding domain, a growth derived neurotrophic factor binding domain, a neurotrophin binding domain, such as, *e.g.*, a neurotrophin-3, a neurotrophin-4/5, a head activator peptide binding domain, a neurturin binding domain, a persephrin binding domain, an artemin binding domain, a transformation growth factor β binding domain, such as, *e.g.*, a TGFβ1, a TGFβ2, a TGFβ3 or a TGFβ4, a bone morphogenic protein binding domain, such as, e.g., a BMP2, a BMP3, a BMP4, a BMP5, a BMP6, a BMP7, a BMP8 or a BMP10, a growth differentiation factor binding domain, such as, *e.g.*, a GDF1, a GDF2, a GDF3, a GDF5, a GDF6, a GDF7, a GDF8, a GDF10, a GDF11 or a GDF15, or an activin binding domain, such as, *e.g.*, an activin A, an activin B, an activin C, an activin E or an inhibin A. In another aspect of this embodiment, a non-Clostridial toxin binding domain comprises a glucagon like hormone binding domain, such as, *e.g.*, a secretin, a glucagon-like peptide, like a GLP-1 and a GLP-2, a pituitary adenylate cyclase activating peptide binding domain, a growth hormone-releasing hormone binding domain, vasoactive intestinal peptide binding domain like a VIP1 or a VIP2, a gastric inhibitory polypeptide binding domain, a calcitonin-related peptidesvisceral gut peptide binding domain like a gastrin, a gastrin-releasing peptide or a cholecystokinin, or a PAR peptide binding domain like a PAR1 peptide, a PAR2 peptide, a PAR3 peptide or a PAR4 peptide.

[0207] In another embodiment, an opioid peptide comprises an enkephalin peptide. In aspects of this embodiment, an enkephalin peptide comprises a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF. In other aspects of this embodiment, an enkephalin peptide comprises SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29.

[0208] In other aspects of this embodiment, an enkephalin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29. In yet other aspects of this embodiment, an enkephalin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29; or at most 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29. In still other aspects of this embodiment, an enkephalin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29; or at most 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 or SEQ ID NO: 29.

[0209] In another embodiment, an opioid peptide comprises a bovine adrenomedullary-22 (BAM22) peptide. In aspects of this embodiment, a BAM22 peptide comprises a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22). In other aspects of this embodiment, a BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35.

[0210] In other aspects of this embodiment, a BAM22 peptide comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35.

[0211] In yet other aspects of this embodiment, a BAM22 peptide comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35; or at most 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35.

[0212] In still other aspects of this embodiment, a BAM22 peptide comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 31; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35; or at most 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 30; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 71; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 32; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 33;

amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 34 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 35.

[0213] In another embodiment, an opioid peptide comprises an endomorphin peptide. In aspects of this embodiment, an endomorphin peptide comprises an endomorphin-1 or an endomorphin-2. In other aspects of this embodiment, an endomorphin peptide comprises SEQ ID NO: 36 or SEQ ID NO: 37.

[0214] In other aspects of this embodiment, an endomorphin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 36 or SEQ ID NO: 37; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 36 or SEQ ID NO: 37. In yet other aspects of this embodiment, an endomorphin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 36 or SEQ ID NO: 37; or at most 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 36 or SEQ ID NO: 37. In still other aspects of this embodiment, an endomorphin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 36 or SEQ ID NO: 37; or at most 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 36 or SEQ ID NO: 37.

[0215] In another embodiment, an opioid peptide comprises an endorphin peptide. In aspects of this embodiment, an endorphin peptide comprises an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ. In other aspects of this embodiment, an endorphin peptide comprises SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43.

[0216] In other aspects of this embodiment, an endorphin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43. In yet other aspects of this embodiment, an endorphin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43; or at most 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43. In still other aspects of this embodiment, an endorphin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43; or at most 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, or SEQ ID NO: 43.

[0217] In another embodiment, an opioid peptide comprises a dynorphin peptide. In aspects of this embodiment, a dynorphin peptide comprises a dynorphin A, a dynorphin B (leumorphin) or a rimorphin. In other aspects of this embodiment, a dynorphin peptide comprises SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO:

64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, or SEQ ID NO: 74.

[0218] In other aspects of this embodiment, a dynorphin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69. In yet other aspects of this embodiment, a dynorphin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69. In still other aspects of this embodiment, a dynorphin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 53, or SEQ ID NO: 69.

[0219] In another embodiment, an opioid peptide comprises a nociceptin peptide. In aspects of this embodiment, a nociceptin peptide comprises a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2, or a neuropeptide 3. In other aspects of this embodiment, a nociceptin peptide comprises SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84.

[0220] In other aspects of this embodiment, a nociceptin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84. In yet other aspects of this embodiment, a nociceptin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84. In still other aspects of this embodiment, a nociceptin comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or SEQ ID NO: 84.

[0221] In another embodiment, an opioid peptide comprises a hemorphin peptide. In aspects of this embodiment, a hemorphin peptide comprises a LVVH7, a VVH7, a VH7, a H7, a LVVH6, a LVVH5, a VVH5, a LVVH4, and a LVVH3. In other aspects of this embodiment, a hemorphin peptide comprises SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93.

[0222] In other aspects of this embodiment, a hemorphin comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93. In yet other aspects of this embodiment, a nociceptin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 1, 2, or 3 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93. In still other aspects of this embodiment, a nociceptin comprises a polypeptide having, *e.g.*, at least 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 1, 2, or 3 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93.

[0223] In yet another embodiment, a non-Clostridial toxin binding domain comprises a galanin peptide binding domain. In aspects of this embodiment, a galanin peptide binding domain comprises a galanin or a galanin message-associated peptide (GMAP). In other aspects of this embodiment, a galanin peptide binding domain comprises SEQ ID NO: 94 or SEQ ID NO: 95.

[0224] In other aspects of this embodiment, a galanin binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 94 or SEQ ID NO: 95. In yet other aspects of this embodiment, a galanin binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95. In still other aspects of this embodiment, a galanin binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95.

[0225] In still another embodiment, a non-Clostridial toxin binding domain comprises a tachykinin peptide binding domain. In aspects of this embodiment, a tachykinin peptide binding domain comprises a

Substance P, a neuropeptide K (NPK), a neuropeptide gamma (NP gamma), a neurokinin A (NKA; Substance K, neurokinin alpha, neuromedin L), a neurokinin B (NKB), a hemokinin or a endokinin. In other aspects of this embodiment, a tachykinin peptide binding domain comprises SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107.

[0226] In other aspects of this embodiment, a tachykinin peptide binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107. In yet other aspects of this embodiment, a tachykinin peptide binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107; or at most 1, 2, 3, 4, or 5 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107. In still other aspects of this embodiment, a tachykinin peptide binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107; or at most 1, 2, 3, 4, or 5 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107.

[0227] In still another embodiment, a non-Clostridial toxin binding domain comprises a Neuropeptide Y related peptide binding domain. In aspects of this embodiment, a Neuropeptide Y related peptide binding domain comprises a Neuropeptide Y (NPY), a Peptide YY (PYY), Pancreatic peptide (PP) or a Pancreatic icosapeptide (PIP). In other aspects of this embodiment, a Neuropeptide Y related peptide binding domain comprises SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112.

[0228] In other aspects of this embodiment, a Neuropeptide Y related peptide binding domain comprises a polypeptide having an amino acid identity of, *e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112. In yet other aspects of this embodiment, a Neuropeptide Y related peptide binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID

NO: 111, or SEQ ID NO: 112; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112. In still other aspects of this embodiment, a Neuropeptide Y related peptide binding domain comprises a polypeptide having, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112.

[0229] It is envisioned that a Clostridial toxin chimeric disclosed in the present specification can comprise a non-Clostridial binding domain in any and all locations with the proviso that Clostridial toxin chimeric can perform the intoxication process. Non-limiting examples include, locating a non-Clostridial binding domain at the amino terminus of a modified Clostridial toxin; locating a non-Clostridial binding domain between a Clostridial toxin enzymatic domain and a translocation domain of a modified Clostridial toxin; and locating a non-Clostridial binding domain at the carboxyl terminus of a modified Clostridial toxin. Other non-limiting examples include, locating a non-Clostridial binding domain between a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain of a modified Clostridial toxin. The enzymatic domain of naturally-occurring Clostridial toxins contains the native start methionine. Thus, in domain organizations where the enzymatic domain is not in the amino-terminal location an amino acid sequence comprising the start methionine should be placed in front of the amino-terminal domain. Likewise, where a non-Clostridial binding domain is in the amino-terminal position, an amino acid sequence comprising a start methionine and a protease cleavage site may be operably-linked in situations in which a non-Clostridial binding domain requires a free amino terminus, see, *e.g.*, Shengwen Li et al., *Degradable Clostridial Toxins*, U.S. Patent Application 11/572,512 (Jan. 23, 2007), which is hereby incorporated by reference in its entirety. In addition, it is known in the art that when adding a polypeptide that is operably-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted.

[0230] Thus, in an embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a non-Clostridial binding domain, a translocation domain, an exogenous protease cleavage site and an enzymatic domain (FIG. 3A). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a non-Clostridial binding domain, a Clostridial toxin translocation domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0231] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a non-Clostridial binding domain, an enzymatic domain, an exogenous protease cleavage site, and a translocation domain (FIG. 3B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a non-Clostridial binding domain, a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

[0232] In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, a non-Clostridial binding domain, and a translocation domain (FIG. 4A). In an aspect of this embodiment, a

modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a non-Clostridial binding domain, and a Clostridial toxin translocation domain.

[0233] In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, a non-Clostridial binding domain, and an enzymatic domain (FIG. 4B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a non-Clostridial binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0234] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, a non-Clostridial binding domain, an exogenous protease cleavage site, and a translocation domain (FIG. 4C). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a non-Clostridial binding domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

[0235] In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, a non-Clostridial binding domain, an exogenous protease cleavage site and an enzymatic domain (FIG. 4D). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a non-Clostridial binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0236] In still another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, a translocation domain, and a non-Clostridial binding domain (FIG. 5A). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain, and a non-Clostridial binding domain.

[0237] In still another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, an enzymatic domain and a non-Clostridial binding domain, (FIG. 5B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a non-Clostridial binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0238] Aspects of the present specification provide, in part, an inactivation cleavage site. As used herein, the term "inactivation cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for selective proteolysis at the scissile bond by a protease present in interstitial fluids or circulatory systems, such as, e.g., the cardiovascular system or the lymphatic system. Such an inactivation cleavage site is operably-linked as a fusion protein to a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. By definition, an inactivation cleavage site is susceptible to selective cleavage by at least one protease present in interstitial fluids or circulatory systems. Non-limiting examples of inactivation cleavage sites include

Thrombin cleavage sites, Plasmin cleavage sites, Coagulation Factor VIIa cleavage sites, Coagulation Factor IXa cleavage sites, Coagulation Factor Xa cleavage sites, Coagulation Factor XIa cleavage sites, Coagulation Factor XIIa cleavage sites, plasma kallikrein cleavage sites, protease-activated G protein-coupled receptor-1 (PAR1) cleavage sites, PAR 2 cleavage sites, PAR3 cleavage sites, PAR4 cleavage sites, Matrix Metalloproteinase-2 (MMP-2) cleavage sites, Matrix Metalloproteinase-9 (MMP-9) cleavage sites, Furin cleavage sites, urokinase-type Plasminogen activator (uPA) cleavage sites, tissue-type Plasminogen activator (tPA) cleavage sites, Tryptase-ε cleavage sites, Mouse mast cell protease-7 (mMCP-7) cleavage sites, endothelin-converting enzyme-1 (ECE-1) cleavage sites, Kell blood group cleavage sites, DPPIV cleavage sites, ADAM metallopeptidase with thrombospondin type 1 motif-13 (ADAMTS13) cleavage sites, and Cathepsin L cleavage sites (Table 4).

TABLE 4. Inactivation Cleavage Sites

Protease Cleavage Site	Reference Sequences	SEQ ID NO:
Thrombin	LVPR*GS	114
	LVPK*GS	115
	FIPR*TF	116
	VLPR*SF	117
	IVPR*SF	118
	IVPR*GY	119
	VVPR*GV	120
	VLPR*LI	121
	VMPR*SL	122
	MFPR*SL	123
Coagulation Factor VIIa (FVIIA)	KLTR*AETV	125
	DFTR*VVGG	126
	LSPR*TFHP	127
	LIQR*NLSP	128
	MATR*KMHD	129
	LGIR*SFRN	130
	PQGR*IVGG	131
	NLTR*IVGG	132
	QVVR*IVGG	133
Coagulation Factor IXa (FIXa)	PQGR*IVGG	135
	PQLR*MKNN	136
	NLTR*IVGG	137
	QVVR*IVGG	138
Coagulation Factor Xa (FXa)	IDGR*	140
	IEGR*	141
	IDGR*SVGG	142
	IDGR*TVGG	143
	IDGR*IVGG	144
	IEGR*SVGG	145
	IEGR*TVGG	146
	IEGR*IVGG	147
	PQGR*IVGG	148
	IEGR*TSED	149
	IEGR*IVEG	150
	IDGR*IVEG	151
	FNPR*TFGS	152
	FDER*TFGL	153
	IDER*IVGG	154
	FNEK*TFGL	155

Coagulation Factor XIa (FXIa)	AFWK*TDAS	157
	KLTR*AETV	158
	KLTR*AETI	159
	DFTR*VVGG	160
	EFSR*VVGG	161
	KLTR*AETV	162
	DFTR*VVGG	163
	IKPR*IVGG	164
	DLHR*HIFW	165
	KQLR*VVNG	166
Coagulation Factor XIIa (FXIIa)	PQGR*IVGG	168
	IKPR*IVGG	169
	SMTR*VVGG	170
	TSTR*IVGG	171
	PMKR*LTLG	172
Kallikrein 1	SMTR*VVGG	174
	SPFR*SSDI	175
	SLMK*RPPG	176
	YDWR*TPYL	177
	SPFR*SVQV	178
	SPFR*TPYL	179
	TFHK*AEYR	180
	PRFK*IIGG	181
	ISLM*KRPP	182
	LEAR*SAYH	183
	EAKR*SYHS	184
	PNRW*STGA	185
	EAFY*SQFG	186
	NAAR*STGA	187
	SSEW*SMPY	188
	GTLF*RSGN	189
	ARLY*SRGA	190
	EASR*SATL	191
	EASY*RRKQ	192
	TTFY*RRGA	193
	AAWY*RTSR	194
	SFHY*RMVG	195
	ASSY*RTSR	196
	TRFY*SRGR	197
	IKFF*SAQT	198
Protein C	KKTR*NLKK	200
	LDRR*GLQR	201
	MATR*KMHD	202
	RLKK*SQFL	203
	PQLR*MKNN	204
	VDQR*GNQI	205
	IEPR*SPSQ	206
	KKTR*SPKT	207
	LDQR*GVQR	208
	PDPR*SKNN	209
Plasminogen	GEAR*GSVI	211
	GHAR*LVHV	212
	AEFR*HDSG	213
	HHQK*LVFF	214
	GSNK*GALL	215
	RAQR*SAGA	216
	AFWK*TDAS	217
	MSMR*VRRH	218
	RGVR*RTAS	219

	RAAR*SQCT	220
	PQSR*SVPP	221
	PYLK*VFNP	222
	LSFR*ARAY	223
	PQLR*RGWR	224
	EDNR*DSSM	225
	LSFR*ARAY	226
	FRAR*AYGF	227
	YGFR*GPGP	228
	ITFR*MNVA	229
	THEK*GRQS	230
	PRLK*ARAG	231
	PKAK*SHAP	232
	PSHK*EGPQ	233
	LFEK*KVYL	234
	ADGK*KPSS	235
	PRFK*IIGG	236
	PQFR*IKGG	237
	PRCR*HRPH	238
	KGYR*SQRG	239
	DVAQ*FVLT	240
Matrix Metalloproteinase-2 (MMP-2)	QPVS*VKVG	242
	RGVG*IKST	243
	FVDC*LIEQ	244
	VPAG*NWVL	245
	YHAD*IYDK	246
	RACR*LAKA	247
	QGAY*QEAF	248
	DVLS*LLEK	249
	TLDD*LIMA	250
	HISS*LIKL	251
	DPNN*LLND	252
	PVQP*QQSP	253
	KPKT*ITGP	254
	VVHP*LVLL	255
	HPLV*LLSV	256
	AVAL*LIGP	257
	QPLQ*LLDA	258
	YIQG*INLV	259
	LPQE*IKAN	260
	NISD*LTAA	261
	KPRA*LTAL	262
	APSW*LLTA	263
	AVRW*LLTA	264
	AVSW*LLTA	265
	SLRR*LTAA	266
	SLSR*LTAL	267
	RYSS*LTAA	268
	SLAY*YTAL	269
	SLRY*YTAA	270
	SPAY*YTAL	271
	MHKA*LTAA	272
	LRLA*ITAL	273
Matrix Metalloproteinase-9 (MMP-9)	IPEN*FFGV	275
	MDIA*IHHP	276
	SPSR*LFDQ	277
	SEMR*LEKD	278
	FSVN*LDVK	279
	RLFD*QFFG	280

	FFGE*HLLE	281
	GLSE*MRLE	282
	SPEE*LKVK	283
	DVIE*VHGK	284
	EVHG*KHEE	285
	DEHG*FISR	286
	GEHL*LESD	287
	FHRK*YRIP	288
	GPRK*QVSG	289
	LSPF*YLRP	290
	PPSF*LRAP	291
	NPLE*NSGF	292
	VPYG*LGSP	293
	PPLK*LMHS	294
	GPEG*LRVG	295
	FMKG*LSKA	296
	VVTG*VTAV	297
	AIIG*LMVG	298
	SDLG*LTGI	299
	VPYG*LGSP	300
	GAAG*VKGD	301
	GPTG*KQGD	302
	GPSG*DQGA	303
	GPSG*FPFP	304
	GAPG*FPGP	305
	GAPG*NRGF	306
	GLRG*ERGE	307
	GPPG*SQGN	308
	GPAG*QQGA	309
	GPPG*KDGT	310
	GQPG*SPGS	311
	GSPG*YQGP	312
	GPVS*AVLT	313
	GPLG*MLSQ	314
	GPLG*MWAQ	315
	GPQG*IFGQ	316
	LPRS*AKEL	317
	NSFG*LRFG	318
	RAIH*INAE	319
Furin	RPRR*AKRF	321
	RKKR*GLYA	322
	RERR*RKKR	323
	RKKR*GLYA	324
	RKKR*TTSA	325
	RHKR*ETLK	326
	RLKR*DVVT	327
	RMKR*EDLN	328
	RAKR*FASL	329
	RKKR*FVSS	330
	RTKR*FLSY	331
	RRAR*SVDG	332
	VFRR*DAHK	333
	VFRR*EAHK	334
	RVAR*DITM	335
	RISR*SLPQ	336
	RSRR*AATS	337
	RAKR*SPKH	338
	FWHR*GVTK	339
	AKRR*TKRD	340
	AKRR*AKRD	341

	AKQR*AKRD	342
	RDVR*GFAS	343
	RKRR*SVNP	344
	RQKR*FVLS	345
	RSKR*SLSC	346
u-Plasminogen Activator (u-PA)	GSGK*SATL	348
	QRGR*SATL	349
	RGSV*ILTV	350
	PSSR*RRVN	351
	CPGR*VVGG	352
	PGAR*GRAF	353
	SSSR*GPTH	354
	VSNK*YFSN	355
	NSGR*AVTY	356
	TYSR*SRYL	357
	NSGR*AVTY	358
	PSGR*GRTL	359
	AGSR*AVYY	360
	TYGR*SRTN	361
	NSSR*GVYL	362
	PSSR*SVYN	363
	ASGR*GRTY	364
	TSSR*AVYL	365
	NSGR*SRTL	366
	VSGR*IRTG	367
	SSGR*IRTV	368
t-Plasminogen Activator (t-PA)	NALR*YAPD	370
	CPGR*VVGG	371
	PQFR*IKGG	372
	ALSR*MAVL	373
Tryptase-ε (Prosemin)	*RVVGGE	375
	*RIVGGE	376
	*RIIGGE	377
	*RVVGGD	378
	*RIVGGD	379
	*RIIGGD	380
	*KVVGGE	381
	*KIVGGE	382
	*KIIGGE	383
	*KVVGGD	384
	*KIVGGD	385
	*KIIGGD	386
Mouse mast cell protease-7 (mMCP-7)	LSSR*QSPG	388
	LQAR*GASL	389
	LGPK*AITM	390
	LGPR*SAVY	391
Endothelin-converting enzyme-1 (ECE-1)	HQKL*VFFA	393
	HHQK*LVFF	394
	KLVF*FAED	395
	DRVY*IHPF	396
	YIHP*FHLV	397
	YGLG*SPRS	398
	TPEH*VVPY	399
	DIIW*VNTP	400
	DIIW*INTP	401
	CHLD*IIWV	402
	HLDI*IWVN	403
	CVYF*CHLD	404

	SCSS*LMDK	405
	ECVY*FCHL	406
	RSKR*CSCS	407
	RSKR*ALEN	408
	GFSP*FRSS	409
	PRRP*YILP	410
	KPQQ*FFGL	411
	PQQF*FGLM	412
Kell blood-group protein (KBGP)	DIIW*VNTP	414
	DIIW*INTP	415
Cathepsin L	MFLE*AIPM	417
	KVFQ*EPLF	418
	ATLT*FDHS	419
	PLFY*EAPR	420
	TGLR*DPFN	421
	KILH*LPTS	422
	AHLK*NSQE	423
	APLT*AEIQ	424
	EALF*AERK	425
	EPLA*AERK	426
	GTFT*SDYS	427
	KYLD*SRRA	428
	QDFV*QWLM	429
	KQLA*TKAA	430
	STFE*ERSY	431
	LRLE*WPYQ	432
	RGFF*YTPK	433
	GFFY*TPKA	434
	HFFK*NIVT	435
	RGLS*LSRF	436
	QWLG*APVP	437
	NMLK*RGLP	438
	LSLA*HTHQ	439
	TPFA*ATSS	440
	KLLA*VSGP	441
	QLFR*RAVL	442
	PRFK*IIGG	443
PAR1	*SFLLRN	445
	*SFFLRN	446
	*SFFLKN	447
	*TFLLRN	448
	*GFPGKF	449
	*GYPAKF	450
	*GYPLKF	451
	*GYPIKF	452
PAR2	*SLIGKV	454
	*SLIGRL	455
PAR3	*TFRGAP	457
	*SFNGGP	458
	*SFNGNE	459
PAR4	*GYPGQV	461
	*AYPGKF	462
	*TYPGKF	463
	*GYPGKY	464
	*GYPGKW	465
	*GYPGKK	466
	*GYPGKF	467
	*GYPGRF	468

	*GYPGFK	469
	*GYPAKF	470
	*GFPGKF	471
	*GFPGKP	472
	*SYPGKF	473
	*SYPAKF	474
	*SYPGRF	475
	*SYAGKF	476
	*SFPGQP	477
	*SFPGQA	478
ADAMTS13	NLVY*MVTG	479
An asterisks (*) indicates the peptide bond of the P_1-$P_{1'}$ cleavage site that is cleaved by the indicated protease.		

[0239] It is envisioned that an inactivation cleavage site of any and all lengths can be useful in aspects of the present specification with the proviso that the inactivation cleavage site is capable of being cleaved by a interstitial fluid or circulatory system protease. Thus, in aspects of this embodiment, an inactivation cleavage site can be, *e.g.*, at least 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 amino acids in length. In other aspects of this embodiment, an inactivation cleavage site can be, *e.g.*, at most 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 amino acids in length.

[0240] An inactivation cleavage site useful in aspects of the specification includes, without limitation, naturally occurring inactivation cleavage site; naturally occurring inactivation cleavage site variants; and non-naturally-occurring inactivation cleavage site variants, such as, *e.g.*, conservative inactivation cleavage site variants, non-conservative inactivation cleavage site variants and inactivation cleavage site peptidomimetics. As used herein, the term "inactivation cleavage site variant," whether naturally-occurring or non-naturally-occurring, refers to an inactivation cleavage site that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence. Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0241] As used herein, the term "naturally occurring inactivation cleavage site variant" refers to any inactivation cleavage site produced without the aid of any human manipulation. Non-limiting examples of inactivation cleavage sites include Thrombin cleavage site variants, Plasmin cleavage site variants, Coagulation Factor V cleavage site variants, Coagulation Factor VII cleavage site variants, Coagulation Factor VIII cleavage site variants, Coagulation Factor IXa cleavage site variants, Coagulation Factor Xa cleavage site variants, Coagulation Factor XIa cleavage site variants, Coagulation Factor XIIa cleavage site variants, plasma kallikrein cleavage site variants, MMP-2 cleavage site variants, MMP-9 cleavage site variants, Furin cleavage site variants, u-Plasminogen activator cleavage site variants, t-Plasminogen activator cleavage site variants, Tryptase-ε cleavage site variants, mMCP-7 cleavage site variants, ECE-1 cleavage site variants, KBGP cleavage site variants, Cathepsin L cleavage site variants, PAR1 cleavage site variants, PAR2 cleavage site variants, PAR3 cleavage site variants, PAR4 cleavage site variants, and ADAM-TS13 cleavage site variants.

[0242] As used herein, the term "non-naturally occurring inactivation cleavage site variant" refers to any inactivation cleavage site produced with the aid of human manipulation, including, without limitation, inactivation cleavage site variants produced by genetic engineering using random mutagenesis or rational design and inactivation cleavage site variants produced by chemical synthesis. Non-limiting examples of non-naturally occurring inactivation cleavage site variants include, *e.g.*, conservative inactivation cleavage site variants, non-conservative inactivation cleavage site variants, and inactivation cleavage site peptidomimetics.

[0243] As used herein, the term "conservative inactivation cleavage site variant" refers to an inactivation cleavage site that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference inactivation cleavage site sequence. Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative inactivation cleavage site variant can function in substantially the same manner as the reference inactivation cleavage site on which the conservative inactivation cleavage site variant is based, and can be substituted for the reference inactivation cleavage site in any aspect of the present specification. Non-limiting examples of a conservative inactivation cleavage site variant include, *e.g.*, conservative Thrombin cleavage site variants, conservative Plasmin cleavage site variants, conservative Coagulation Factor V cleavage site variants, conservative Coagulation Factor VII cleavage site variants, conservative Coagulation Factor VIII cleavage site variants, conservative Coagulation Factor IXa cleavage site variants, conservative Coagulation Factor Xa cleavage site variants, conservative Coagulation Factor XIa cleavage site variants, conservative Coagulation Factor XIIa cleavage site variants, conservative plasma kallikrein cleavage site variants, conservative MMP-2 cleavage site variants, conservative MMP-9 cleavage site variants, conservative Furin cleavage site variants, conservative u-Plasminogen activator cleavage site variants, conservative t-Plasminogen activator cleavage site variants, conservative Tryptase-ε cleavage site variants, conservative mMCP-7 cleavage site variants, conservative ECE-1 cleavage site variants, conservative KBGP cleavage site variants, conservative Cathepsin L cleavage site variants, conservative PAR1 cleavage site variants, conservative PAR2 cleavage site variants, conservative PAR3 cleavage site variants, conservative PAR4 cleavage site variants, and conservative ADAM-TS13 cleavage site variants.

[0244] As used herein, the term "non-conservative inactivation cleavage site variant" refers to an inactivation cleavage site in which 1) at least one amino acid is deleted from the reference inactivation cleavage site on which the non-conservative inactivation cleavage site variant is based; 2) at least one amino acid added to the reference inactivation cleavage site on which the non-conservative inactivation cleavage site is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference inactivation cleavage site sequence (Table 4). A non-conservative inactivation cleavage site variant can function in substantially the same manner as the reference inactivation cleavage site on which the non-conservative inactivation cleavage site is based, and can be substituted for the reference inactivation cleavage site in any aspect of the present specification. Non-limiting examples of a non-conservative inactivation cleavage site variant include, *e.g.*, non-conservative Thrombin cleavage site variants, non-

conservative Plasmin cleavage site variants, non-conservative Coagulation Factor V cleavage site variants, non-conservative Coagulation Factor VII cleavage site variants, non-conservative Coagulation Factor VIII cleavage site variants, non-conservative Coagulation Factor IXa cleavage site variants, non-conservative Coagulation Factor Xa cleavage site variants, non-conservative Coagulation Factor XIa cleavage site variants, non-conservative Coagulation Factor XIIa cleavage site variants, non-conservative plasma kallikrein cleavage site variants, non-conservative MMP-2 cleavage site variants, non-conservative MMP-9 cleavage site variants, non-conservative Furin cleavage site variants, non-conservative u-Plasminogen activator cleavage site variants, non-conservative t-Plasminogen activator cleavage site variants, non-conservative Tryptase-ε cleavage site variants, non-conservative mMCP-7 cleavage site variants, non-conservative ECE-1 cleavage site variants, non-conservative KBGP cleavage site variants, non-conservative Cathepsin L cleavage site variants, non-conservative PAR1 cleavage site variants, non-conservative PAR2 cleavage site variants, non-conservative PAR3 cleavage site variants, non-conservative PAR4 cleavage site variants, and non-conservative ADAM-TS13 cleavage site variants.

[0245] As used herein, the term "inactivation cleavage site peptidomimetic" refers to an inactivation cleavage site that has at least one amino acid substituted by a non-natural oligomer that has at least one property similar to that of the first amino acid. Examples of properties include, without limitation, topography of a peptide primary structural element, functionality of a peptide primary structural element, topology of a peptide secondary structural element, functionality of a peptide secondary structural element, of the like, or any combination thereof. An inactivation cleavage site peptidomimetic can function in substantially the same manner as the reference inactivation cleavage site on which the inactivation cleavage site peptidomimetic is based, and can be substituted for the reference inactivation cleavage site in any aspect of the present specification. For examples of peptidomimetic methods see, *e.g.*, Amy S. Ripka & Daniel H. Rich, Peptidomimetic design, 2(4) CURR. OPIN. CHEM. BIOL. 441-452 (1998); and M. Angels Estiarte & Daniel H. Rich, *Peptidomimetics for Drug Design*, 803-861 (BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY Vol. 1 PRINCIPLE AND PRACTICE, Donald J. Abraham ed., Wiley-Interscience, 6th ed 2003). Non-limiting examples of an inactivation cleavage site peptidomimetic include, *e.g.*, Thrombin cleavage site peptidomimetics, Plasmin cleavage site peptidomimetics, Coagulation Factor V cleavage site peptidomimetics, Coagulation Factor VII cleavage site peptidomimetics, Coagulation Factor VIII cleavage site peptidomimetics, Coagulation Factor IXa cleavage site peptidomimetics, Coagulation Factor Xa cleavage site peptidomimetics, Coagulation Factor XIa cleavage site peptidomimetics, Coagulation Factor XIIa cleavage site peptidomimetics, plasma kallikrein cleavage site peptidomimetics, MMP-2 cleavage site peptidomimetics, MMP-9 cleavage site peptidomimetics, Furin cleavage site peptidomimetics, u-Plasminogen activator cleavage site peptidomimetics, t-Plasminogen activator cleavage site peptidomimetics, Tryptase-ε cleavage site peptidomimetics, mMCP-7 cleavage site variants, ECE-1 cleavage site peptidomimetics, KBGP cleavage site peptidomimetics, Cathepsin L cleavage site peptidomimetics, PAR1 cleavage site peptidomimetics, PAR2 cleavage site peptidomimetics, PAR3 cleavage site peptidomimetics, PAR4 cleavage site peptidomimetics, and ADAM-TS13 cleavage site peptidomimetics.

[0246] Thus, in an embodiment, a Clostridial toxin comprises an inactivation cleavage site. In an aspect of this embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, and an inactivation cleavage site. In another

aspect of this embodiment, a Clostridial toxin comprises a naturally occurring inactivation cleavage site variant, such as, *e.g.*, an inactivation cleavage site isoform. In another aspect of this embodiment, a Clostridial toxin comprises a non-naturally occurring inactivation cleavage site variant, such as, *e.g.*, a conservative inactivation cleavage site variant, a non-conservative inactivation cleavage site variant or an active inactivation cleavage site fragment, or any combination thereof.

[0247] In another embodiment, a Clostridial toxin chimeric comprises an inactivation cleavage site. In an aspect of this embodiment, a Clostridial toxin chimeric comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site. In another aspect of this embodiment, a Clostridial toxin chimeric comprises a naturally occurring inactivation cleavage site variant, such as, *e.g.*, an inactivation cleavage site isoform. In another aspect of this embodiment, a Clostridial toxin chimeric comprises a non-naturally occurring inactivation cleavage site variant, such as, *e.g.*, a conservative inactivation cleavage site variant, a non-conservative inactivation cleavage site variant or an active inactivation cleavage site fragment, or any combination thereof.

[0248] In another embodiment, a hydrophobic amino acid at one particular position in the inactivation cleavage site can be substituted with another hydrophobic amino acid. Examples of hydrophobic amino acids include, *e.g.*, C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the inactivation cleavage site can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.*, A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the inactivation cleavage site can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.*, F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the inactivation cleavage site can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.*, F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the inactivation cleavage site can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.*, D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the inactivation cleavage site can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.*, A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the inactivation cleavage site can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.*, K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the inactivation cleavage site can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.*, D and E. In another aspect of this embodiment, a small amino acid at one particular position in the inactivation cleavage site can be substituted with another small amino acid. Examples of small amino acids include, *e.g.*, A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the inactivation cleavage site can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, *e.g.*, I, T and V.

[0249] Aspects of the present specification disclose, in part, a Thrombin cleavage site as an inactivation cleavage site. As used herein, the term "Thrombin cleavage site" refers to a scissile bond together with

adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Thrombin under conditions suitable for Thrombin protease activity. It is envisioned that any amino acid sequence cleaved by Thrombin can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Thrombin cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 113), where X_1 is preferentially S, T, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is preferentially F, S, T, an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially S, T, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5, X_6, and X_7, are independently any amino acid. Table 4 lists exemplary reference cleavage sites for Thrombin (SEQ ID NO: 114-123). Additional Thrombin cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0250] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Thrombin cleavage site. In an aspect of this embodiment, a Thrombin cleavage site comprises the consensus sequence SEQ ID NO: 113, where X_1 is S, T, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is F, S, T, an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is S, T, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5, X_6, and X_7, are independently any amino acid. In another aspect of this embodiment, a Thrombin cleavage site comprises the consensus sequence SEQ ID NO: 113, where X_1 is S, Q, K, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, an amidic amino acid like N and Q, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is N, Q, G, P, A, V, L, or I; X_4 is S, T, H, G, A, L, or I; X_5 is S, T, Q, K, R, F, Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is S, T, Q, K, R, G, P, A, V, L, or I; and X_7 is S, T, Q, K, R, G, P, A, V, L, or I. In another aspect of this embodiment, a Thrombin cleavage site comprises the consensus sequence SEQ ID NO: 113, where X_1 is Q, G, P, A, V, L, I, or M; X_2 is S, T, D, E, G, A, V, or I; X_3 is G, P, A, V, or L; X_4 is S, G, A, or L; X_5 is Q, K, F, A, V, or L; X_6 is S, Q, K, R, G, P, V, or L; and X_7 is S, T, K, G, V, L, or I. In other aspects of this embodiment, a Thrombin cleavage site comprises, *e.g.*, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, or SEQ ID NO: 123.

[0251] Aspects of the present specification disclose, in part, a Plasmin cleavage site as an inactivation cleavage site. As used herein, the term "Plasmin cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile

bond by Plasmin under conditions suitable for Plasmin protease activity. It is envisioned that any amino acid sequence cleaved by Plasmin can be useful in aspects of the present specification. Plasmin catalyzes cleavage of Lys| and Arg| bonds, with a specificity similar to that of Trypsin. However, Plasmin is a much less efficient enzyme than Trypsin, and cleaves only some of these bonds in proteins. Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline.

[0252] Aspects of the present specification disclose, in part, a Coagulation Factor VIIa cleavage site as an inactivation cleavage site. As used herein, the term "Coagulation Factor VIIa cleavage site" or "FVIIa cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by FVIIa under conditions suitable for FVIIa protease activity. It is envisioned that any amino acid sequence cleaved by FVIIa can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a FVIIa cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 124), where X_1 is preferentially an acidic amino acid like D and E, an amidic amino acid like N and Q, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is Q, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially Q, S, T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4, X_5, X_6, and X_7, are independently any amino acid. Table 4 lists exemplary reference cleavage sites for FVIIa (SEQ ID NO: 125-133). Additional FVIIa cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, J. H. Morrissey, *Coagulation Factor VIIa*. In HANDBOOK OF PROTEOLYTIC ENZYMES, pp.1659-1662 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0253] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Coagulation Factor VIIa cleavage site. In an aspect of this embodiment, a Coagulation Factor VIIa cleavage site comprises the consensus sequence SEQ ID NO: 124, where X_1 is an acidic amino acid like D and E, an amidic amino acid like N and Q, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is Q, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is Q, S, T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_4, X_5, X_6, and X_7, are independently any amino acid. In other aspects of this embodiment, a Coagulation Factor VIIa cleavage site comprises, *e.g.*, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133

[0254] Aspects of the present specification disclose, in part, a Coagulation Factor IXa cleavage site as an inactivation cleavage site. As used herein, the term "Coagulation Factor IXa cleavage site" or "FIXa cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by FIXa under conditions suitable for FIXa

protease activity. It is envisioned that any amino acid sequence cleaved by FIXa can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a FIXa cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 134), where X_1 is preferentially an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_4, X_5, X_6, and X_7, are independently any amino acid. Table 4 lists exemplary reference cleavage sites for FIXa (SEQ ID NO: 135-138). Additional FIXa cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, A. T. Thompson, *Molecular Biology of Factor IX*. In HEMOSTASIS AND THROMBOSIS, BASIC PRINCIPLES AND CLINICAL PRACTICE, pp. 128-129 (R. W. Colman, J. Hirsh, V. J. Marder, A. W Clowes, J. N. George, eds; Lippincott Williams & Wilkins, Philadelphia, PA, 2d, 2001); S. Kawabata and S. Iwanaga, *Russellysin*. In HANDBOOK OF PROTEOLYTIC ENZYMES, pp.683-684 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); A. E. Schmidt and S. P. Bajaj, *Coagulation factor IXa*. In HANDBOOK OF PROTEOLYTIC ENZYMES, pp. 1655-1659 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0255] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Coagulation Factor IXa cleavage site. In an aspect of this embodiment, a Coagulation Factor IXa cleavage site comprises the consensus sequence SEQ ID NO: 134, where X_1 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_4, X_5, X_6, and X_7, are independently any amino acid. In another aspect of this embodiment, a Coagulation Factor IXa cleavage site comprises the consensus sequence SEQ ID NO: 134, where X_1 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4, X_5, X_6, and X_7, are independently an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In other aspects of this embodiment, a Coagulation Factor IXa cleavage site comprises, *e.g.*, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, or SEQ ID NO: 138.

[0256] Aspects of the present specification disclose, in part, a Coagulation Factor Xa cleavage site as an inactivation cleavage site. As used herein, the term "Coagulation Factor Xa cleavage site" or "FXa cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or

both, sufficient for detectable proteolysis at the scissile bond by FXa under conditions suitable for FXa protease activity. It is envisioned that any amino acid sequence cleaved by FXa can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a FXa cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 139), where X_1 is any amino acid, X_2 is preferentially G, A, S, an acidic amino acid like D and E, an amidic amino acid like Q and N, or an aromatic hydrophobic amino acid like F, W and Y, X_3 is preferentially an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5, X_6, and X_7, are independently any amino acid. Table 4 lists exemplary reference cleavage sites for FXa (SEQ ID NO: 140-155). Additional FXa cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, D. L. Greenberg and E. W. Davie, Blood Coagulation Factors: Their Complementary DNAs, Genes, and Expression. In HEMOSTASIS AND THROMBOSIS, BASIC PRINCIPLES AND CLINICAL PRACTICE, pp. 34-35 (R. W. Colman, J. Hirsh, V. J. Marder, A. W Clowes, J. N. George, eds; Lippincott Williams & Wilkins, Philadelphia, PA, 2d, 2001); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0257] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Coagulation Factor Xa cleavage site. In an aspect of this embodiment, a Coagulation Factor Xa cleavage site comprises the consensus sequence SEQ ID NO: 139, where X_1 is any amino acid, X_2 is G, A, S, an acidic amino acid like D and E, an amidic amino acid like Q and N, or an aromatic hydrophobic amino acid like F, W and Y, X_3 is an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5, X_6, and X_7, are independently any amino acid. In another aspect of this embodiment, a Coagulation Factor Xa cleavage site comprises the consensus sequence SEQ ID NO: 139, where X_1 is E, F, P, A, L, or I; X_2 is S, Q, D, E, F, G, or A; X_3 is F, G, or P; X_4 is S, T, L, or I; X_5 is S, F, A, or V; X_6 is S, T, E, N, H, G, A, or M; and X_7 is S, N, D, Q, K, R, or G. In another aspect of this embodiment, a Coagulation Factor Xa cleavage site comprises the consensus sequence SEQ ID NO: 139, where X_1 is I or A; X_2 is E or F; X_3 is F, G, or P; X_4 is S, T, or I; X_5 is S, F, or V; X_6 is E or G; and X_7 is S or G. In other aspects of this embodiment, a Coagulation Factor Xa cleavage site comprises, *e.g.*, the amino acid sequence SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, or SEQ ID NO: 155.

[0258] Aspects of the present specification disclose, in part, a Coagulation Factor XIa cleavage site as an inactivation cleavage site. As used herein, the term "Coagulation Factor XIa cleavage site" or "FXIa cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or

both, sufficient for detectable proteolysis at the scissile bond by FXIa under conditions suitable for FXIa protease activity. It is envisioned that any amino acid sequence cleaved by FXIa can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a FXIa cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 156), where X_1 is preferentially an acidic amino acid like D or E, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially an acidic amino acid like D or E, an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially H, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially H, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is preferentially an acidic amino acid like D and E, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is preferentially an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is preferentially an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for FXIa (SEQ ID NO: 157-166). Additional FXIa cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, P. N. Walsh, *Coagulation Factor XIa*. In Handbook of Proteolytic Enzymes, pp.1651-1655 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0259] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Coagulation Factor XIa cleavage site. In an aspect of this embodiment, a Coagulation Factor XIa cleavage site comprises the consensus sequence SEQ ID NO: 156, where X_1 is an acidic amino acid like D or E, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D or E, an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is H, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is H, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is an acidic amino acid like D and E, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Coagulation Factor XIa cleavage site comprises the consensus sequence SEQ ID NO: 156, where X_1 is an acidic amino acid like D or E, or a basic amino acid like K and R; X_2 is an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic

amino acid like, G, P, A, V, L, I, and M; X_3 is an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is an acidic amino acid like D and E, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Coagulation Factor XIa cleavage site comprises the consensus sequence SEQ ID NO: 156, where X_1 is D or K; X_2 is F or L; X_3 is T or P; X_4 is A or V; X_5 is E or V; X_6 is T or G; and X_7 is G or V. In other aspects of this embodiment, a Coagulation Factor XIa cleavage site comprises, *e.g.*, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, or SEQ ID NO: 166.

[0260] Aspects of the present specification disclose, in part, a Coagulation Factor XIIa cleavage site as an inactivation cleavage site. As used herein, the term "Coagulation Factor XIIa cleavage site" or "FXIIa cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by FXIIa under conditions suitable for FXIIa protease activity. It is envisioned that any amino acid sequence cleaved by FXIIa can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a FXIIa cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 167), where X_1 is preferentially an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially an acidic amino acid like D and E, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is preferentially an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for FXIIa (SEQ ID NO: 168-172). Additional FXIIa cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. D. Ratnoff, *Coagulation Factor XIIa*. In Handbook of Proteolytic Enzymes, pp.1642-1644 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0261] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Coagulation Factor XIIa cleavage site. In an aspect of this embodiment, a Coagulation Factor XIIa cleavage site comprises the consensus sequence SEQ ID NO: 167, where X_1 is an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic

hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In other aspect of this embodiment, a Coagulation Factor XIIa cleavage site comprises the consensus sequence SEQ ID NO: 167, where X_1 is S, T, P, or I; X_2 is Q, K, S, or M; X_3 is K, T, G, or P; X_4 is L, I, or V; X_5 is T or V; X_6 is G or L; and X_7 is G. In other aspects of this embodiment, a Coagulation Factor XIIa cleavage site comprises, *e.g.*, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, or SEQ ID NO: 172.

[0262] Aspects of the present specification disclose, in part, a Kallikrein 1 cleavage site as an inactivation cleavage site. As used herein, the term "Kallikrein 1 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Kallikrein 1 under conditions suitable for Kallikrein 1 protease activity. It is envisioned that any amino acid sequence cleaved by Kallikrein 1 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Kallikrein 1 cleavage site is $X_1X_2X_3X_4$*(R/K/S)$X_5X_6X_7$ (SEQ ID NO: 173), where X_1 is preferentially an acidic amino acid like D and E, an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is any amino acid; X_4 is preferentially a positive amino acid like H, K, and R, a large non-polar amino acid like F, I, L, M and V, or an aromatic hydrophobic amino acid like F, W and Y; X_5 is any amino acid; X_6 is any amino acid; and X_7 is any amino acid. Table 4 lists exemplary reference cleavage sites for Kallikrein 1 (SEQ ID NO: 174-198). Additional Kallikrein 1 cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, R. W. Colman, *Contact Activation Pathway: Inflammation, Fibrinolytic, Anticoagulant, Antiadhesive, and Antiangiogenic Activities*. In HEMOSTASIS AND THROMBOSIS, BASIC PRINCIPLES AND CLINICAL PRACTICE, pp. 103-104 (R. W. Colman, J. Hirsh, V. J. Marder, A. W Clowes, J. N. George, eds; Lippincott Williams & Wilkins, Philadelphia, PA, 2d, 2001); J. Chao, *Human Kallikrein 1, Tissue Kallikrein*. In Handbook of Proteolytic Enzymes, pp.1577-1580 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); H. X. Li, et al., *Substrate Specificity of Human Kallikreins 1 and 6 Determined by Phage Display*, Protein Sci. 17: 664-672 (2008); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0263] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Kallikrein 1 cleavage site. In an aspect of this embodiment, a Kallikrein 1 cleavage site comprises the consensus sequence SEQ ID NO: 173, where X_1 is an acidic amino acid like D and E, an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is any amino acid; X_4 is a positive amino acid like H, K, and R, a

large non-polar amino acid like F, I, L, M and V, or an aromatic hydrophobic amino acid like F, W and Y; X_5 is any amino acid; X_6 is any amino acid; and X_7 is any amino acid. In another aspect of this embodiment, a Kallikrein 1 cleavage site comprises the consensus sequence SEQ ID NO: 173, where X_1 is D, S, T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is S, T, A, P, or V; X_3 is S, F, or L; X_4 is R or an aromatic hydrophobic amino acid like F, W and Y; X_5 is R, S, T, or A; X_6 is R, S, or G; and X_7 is R, G, or A. In other aspects of this embodiment, a Kallikrein 1 cleavage site comprises, *e.g.*, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, or SEQ ID NO: 198.

[0264] Aspects of the present specification disclose, in part, a Protein C cleavage site as an inactivation cleavage site. As used herein, the term "Protein C cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Protein C under conditions suitable for Protein C protease activity. It is envisioned that any amino acid sequence cleaved by Protein C can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Protein C cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 199), where X_1 is preferentially a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is preferentially an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is preferentially an amidic amino acid like Q and N, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aromatic hydrophobic amino acid like F, W and Y; X_7 is preferentially an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for Protein C (SEQ ID NO: 200-209). Additional Protein C cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, L. Shen and B. Dahiback, *Protein C*. In Handbook of Proteolytic Enzymes, pp.1673-1677 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0265] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Protein C

cleavage site. In an aspect of this embodiment, a Protein C cleavage site comprises the consensus sequence SEQ ID NO: 199, where X_1 is a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 and X_4 are independently an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an amidic amino acid like Q and N, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aromatic hydrophobic amino acid like F, W and Y; X_7 is an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Protein C cleavage site comprises the sequence SEQ ID NO: 199, where X_1 is K, R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is D, E, Q, N, or K; X_3 is P, L, T, Q, K, or R; X_4 is G, I, S, N, or K; X_5 is Q, N, K, F, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is F, S, N, Q, K, or H; X_7 is L, I, T, K, D, E, Q, or N. In other aspects of this embodiment, a Protein C cleavage site comprises, *e.g.*, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, or SEQ ID NO: 209.

[0266] Aspects of the present specification disclose, in part, a Plasminogen cleavage site as an inactivation cleavage site. As used herein, the term "Plasminogen cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Plasminogen under conditions suitable for Plasminogen protease activity. It is envisioned that any amino acid sequence cleaved by Plasminogen can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Plasminogen cleavage site is $X_1X_2X_3$(R/K)*$X_4X_5X_6X_7$ (SEQ ID NO: 210), where X_1 is preferentially a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially an amidic amino acid like N and Q, a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is preferentially a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is any amino acid; X_7 is preferentially H, F, Y, R, an uncharged amino acid like C, S, and T, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for Plasminogen (SEQ ID NO: 211-240). Additional Plasminogen cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-

D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0267] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Plasminogen cleavage site. In an aspect of this embodiment, a Plasminogen cleavage site comprises the consensus sequence SEQ ID NO: 211, where X_1 is a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an amidic amino acid like N and Q, a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is any amino acid; X_7 is H, F, Y, R, an uncharged amino acid like C, S, and T, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Plasminogen cleavage site comprises the sequence SEQ ID NO: 211, where X_1 is K, R, S, T, A, G, L, or P; X_2 is D, E, Q, N, K, R, S, T, A, G, I or L; X_3 is N, Q, S, F, Y, A or L; X_4 is K, R, S, A, G, L, or V; X_5 is K, R, N, S, F, Y, A, I, L, P, or V; X_6 is K, R, N, S, F, Y, A, G, L, P or V; X_7 is R, S, T, F, Y, A, G, I, L, or P. In other aspects of this embodiment, a Plasminogen cleavage site comprises, e.g., SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, or SEQ ID NO: 240.

[0268] Aspects of the present specification disclose, in part, a Matrix Metalloproteinase-2 cleavage site as an inactivation cleavage site. As used herein, the term "Matrix Metalloproteinase-2 cleavage site" or "MMP-2 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by MMP-2 under conditions suitable for MMP-2 protease activity. It is envisioned that any amino acid sequence cleaved by MMP-2 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a MMP-2 cleavage site is X_1(P/A/V/L/I)X_2X_3*(V/L/I/F/Q)$X_4X_5X_6$ (SEQ ID NO: 241), where X_1, X_2, X_3, X_4, X_5, and X_6 are any amino acid. Table 4 lists exemplary reference cleavage sites for MMP-2 (SEQ ID NO: 242-273). Additional MMP-2 cleavage sites are well known in the art or can be defined by routine methods. See, e.g., O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each

of which is incorporated by reference in its entirety.

[0269] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Matrix Metalloproteinase-2 cleavage site. In an aspect of this embodiment, a Matrix Metalloproteinase-2 cleavage site comprises the consensus sequence SEQ ID NO: 241, where X_1, X_2, X_3, X_4, X_5, and X_6 are any amino acid. In another aspect of this embodiment, a Matrix Metalloproteinase-2 cleavage site comprises the consensus sequence SEQ ID NO: 241, where X_1 is a an acidic amino acid like D and E, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an acidic amino acid like D and E, a basic amino acid like K and R, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is H, an acidic amino acid like D and E, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is a basic amino acid like K and R, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is an acidic amino acid like D and E, a basic amino acid like K and R, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an acidic amino acid like D and E, a basic amino acid like K and R, an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Matrix Metalloproteinase-2 cleavage site comprises the sequence SEQ ID NO: 241, where X_1 is G, P, A, V, L, I, S, T, E, or Q; X_2 is G, A, L, S, N, Q, W, or K; X_3 is G, P, A, S, Q, D, E, or H; X_4 is G, A, V, L, I, F, S, T, Q, or K; X_5 is G, A, V, S, T, Q, or K; X_6 is G, P, A, V, L, I, S, T, D, E, K, N, or Q. In another aspect of this embodiment, a Matrix Metalloproteinase-2 cleavage site comprises the sequence SEQ ID NO: 241, where X_1 is G, A, or L, or Q; X_2 is G, A, or S; X_3 is G, A, S, or N; X_4 is A, V, L, I, or K; X_5 is G, A, or S; X_6 is G, P, A, V, L, or D. In other aspects of this embodiment, a Matrix Metalloproteinase-2 cleavage site comprises, *e.g.*, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, SEQ ID NO: 254, SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, SEQ ID NO: 266, SEQ ID NO: 267, SEQ ID NO: 268, SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 271, SEQ ID NO: 272, or SEQ ID NO: 273.

[0270] Aspects of the present specification disclose, in part, a Matrix Metalloproteinase-9 cleavage site as an inactivation cleavage site. As used herein, the term "Matrix Metalloproteinase-9 cleavage site" or "MMP-2 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by MMP-9 under conditions suitable for MMP-9 protease activity. It is envisioned that any amino acid sequence cleaved by MMP-9 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a MMP-9 cleavage site is $X_1X_2X_3X_4{*}X_5X_6X_7X_8$ (SEQ ID NO: 274), where X_1 is preferentially F, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is preferentially F, Y, S, T, an acidic amino acid like D and E, an amidic

amino acid like N and Q, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is any amino acid; X_5 is preferentially S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is any amino acid; X_7 is any amino acid; X_8 is preferentially F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for MMP-9 (SEQ ID NO: 275-319). Additional MMP-9 cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, S. L. Kridel, et al., *Substrate Hydrolysis by Matrix Metalloproteinase-9*, J. Biol. Chem. 276: 20572-20578 (2001); E. Y. Zhen, et al., *Characterization of Metalloprotease Cleavage Products of Human Articular Cartilage*, Arthritis Rheum. 58: 2420-2431 (2008); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0271] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Matrix Metalloproteinase-9 cleavage site. In an aspect of this embodiment, a Matrix Metalloproteinase-9 cleavage site comprises the consensus sequence SEQ ID NO: 274, where X_1 is F, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is F, Y, S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is any amino acid; X_5 is S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is any amino acid; X_7 is any amino acid; X_8 is F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Matrix Metalloproteinase-9 cleavage site comprises the consensus sequence SEQ ID NO: 274, where X_1 is G, V, L, I, F, S, Q, K, or R; X_2 is P, A, V, L, I, or S; X_3 is G, P, A, V, L, S, Q, E, K, or R; X_4 is G, P, A, V, L, F, S, N, E, or K; X_5 is A, V, L, I, M, F, S, Q, or K; X_6 is P, A, V, L, I, S, T, Q, E, K, or R; X_7 is G, A, V, L, S, or T; X_8 is G, P, A, V, L, F, T, D, E, K, or R. In another aspect of this embodiment, a Matrix Metalloproteinase-9 cleavage site comprises the consensus sequence SEQ ID NO: 274, where X_1 is G or L; X_2 is P, A, or V; X_3 is P, A, R, K, or S; X_4 is G; X_5 is A, V, L, or I; X_6 is T, Q, K, or R; X_7 is G, A, or S; X_8 is G, P, A, V, or E. In other aspects of this

embodiment, a Matrix Metalloproteinase-9 cleavage site comprises, *e.g.*, SEQ ID NO: 275, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 281, SEQ ID NO: 282, SEQ ID NO: 283, SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 288, SEQ ID NO: 289, SEQ ID NO: 290, SEQ ID NO: 291, SEQ ID NO: 292, SEQ ID NO: 293, SEQ ID NO: 294, SEQ ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299, SEQ ID NO: 300, SEQ ID NO: 301, SEQ ID NO: 302, SEQ ID NO: 303, SEQ ID NO: 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309, SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 314, SEQ ID NO: 315, SEQ ID NO: 316, SEQ ID NO: 317, SEQ ID NO: 318, or SEQ ID NO: 319.

[0272] Aspects of the present specification disclose, in part, a Furin cleavage site as an inactivation cleavage site. As used herein, the term "Furin cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Furin under conditions suitable for Furin protease activity. It is envisioned that any amino acid sequence cleaved by Furin can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Furin cleavage site is (R/I/A)X_1(R/K/A/P)R*X_2*$X_3X_4X_5$ (SEQ ID NO: 320), where X_1, X_2, X_3, X_4, and X_5 are any amino acid. Table 4 lists exemplary reference cleavage sites for Furin (SEQ ID NO: 321-346). Additional Furin cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, A. Basak, et al., *Implication of the Proprotein Convertases Furin, PC5 And PC7 in the Cleavage of Surface Glycoproteins of Hong Kong, Ebola and Respiratory Syncytial Viruses: A Comparative Analysis with Fluorogenic Peptides*, Biochem. J. 353: 537-545 (2001); O. Bader, et al., *Processing of Predicted Substrates of Fungal Kex2 Proteinases from Candida albicans, C. glabrata, Saccharomyces cerevisiae and Pichia pastoris*, BMC Microbiol. 8: 116 (2008); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0273] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Furin cleavage site. In an aspect of this embodiment, a Furin cleavage site comprises the consensus sequence SEQ ID NO: 320, where X_1, X_2, X_3, X_4, and X_5 are any amino acid. In another aspect of this embodiment, a Furin cleavage site comprises the consensus sequence SEQ ID NO: 320, where X_1 is F, S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is G, P, M, F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an uncharged amino acid like C, S, and T; X_3 is G, P, A, V, L, I, F, W, S, T, N, Q, D, H, K, or R; X_4 is F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic

hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Furin cleavage site comprises the consensus sequence SEQ ID NO: 320, where X_1 is K, R, S or T; X_2 is D, E, S, A or G; X_3 is A, V, L, or I; and X_4 is S, G, D, E or R; and X_5 is G, P, A, S, T, Q, D, or E. In other aspects of this embodiment, a Furin cleavage site comprises, *e.g.*, SEQ ID NO: 321, SEQ ID NO: 322, SEQ ID NO: 323, SEQ ID NO: 324, SEQ ID NO: 325, SEQ ID NO: 326, SEQ ID NO: 327, SEQ ID NO: 328, SEQ ID NO: 329, SEQ ID NO: 330, SEQ ID NO: 331, SEQ ID NO: 332, SEQ ID NO: 333, SEQ ID NO: 334, SEQ ID NO: 335, SEQ ID NO: 336, SEQ ID NO: 337, SEQ ID NO: 338, SEQ ID NO: 339, SEQ ID NO: 340, SEQ ID NO: 341, SEQ ID NO: 342, SEQ ID NO: 343, SEQ ID NO: 344, SEQ ID NO: 345, or SEQ ID NO: 346.

[0274] Aspects of the present specification disclose, in part, a u-Plasminogen Activator cleavage site as an inactivation cleavage site. As used herein, the term "u-Plasminogen Activator cleavage site" or "u-PA cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by u-PA under conditions suitable for u-PA protease activity. It is envisioned that any amino acid sequence cleaved by u-Plasminogen Activator can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a u-PA cleavage site is $X_1X_2X_3$(R/K)*X_4*$X_5X_6X_7$ (SEQ ID NO: 347), where X_1 is any amino acid; , X_2 is preferentially an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is any amino acid; X_5 is preferentially a basic amino acid like K and R, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is preferentially an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is any amino acid. Table 4 lists exemplary reference cleavage sites for u-PA (SEQ ID NO: 347-368). Additional u-PA cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, V. Ellis, *u-Plasminogen Activator*. In Handbook of Proteolytic Enzymes, pp. 1677-1683 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0275] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a u-Plasminogen Activator cleavage site. In an aspect of this embodiment, a u-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 347, where X_1 is any amino acid; , X_2 is an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is any amino acid; X_5 is a basic amino acid like K and R, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an uncharged amino acid like C,

S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is any amino acid. In another aspect of this embodiment, a u-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 347, where X_1 is P, A, L, S, T, C, N, or R; X_2 is G, P, L, Y, S, or T; X_3 is G, A, S, or N; and X_4 is G, A, V, I, Y,S, or R; X_5 is P, V, L, F, or R; X_6 is G, A, V, Y, S, or T; and X_7 is G, V, L, F, Y, N, or H. In another aspect of this embodiment, a u-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 347, where X_1 is P, A, L, S, T, C, N, or R; X_2 is G, Y, or S; X_3 is G or S; and X_4 is G, A, V, I, Y,S, or R; X_5 is V or R; X_6 is T or Y; and X_7 is G, V, L, F, Y, N, or H. In other aspects of this embodiment, a u-Plasminogen Activator cleavage site comprises, *e.g.*, SEQ ID NO: 348, SEQ ID NO: 349, NO: 350, SEQ ID NO: 351, SEQ ID NO: 352, SEQ ID NO: 353, SEQ ID NO: 354, SEQ ID NO: 355, or SEQ ID NO: 356, SEQ ID NO: 357, SEQ ID NO: 358, SEQ ID NO: 359, SEQ ID NO: 360, SEQ ID NO: 361, SEQ ID NO: 362, SEQ ID NO: 363, SEQ ID NO: 364, SEQ ID NO: 365, SEQ ID NO: 366, SEQ ID NO: 367, or SEQ ID NO: 368.

[0276] Aspects of the present specification disclose, in part, a t-Plasminogen Activator cleavage site as an inactivation cleavage site. As used herein, the term "t-Plasminogen Activator cleavage site" or "t-PA cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by t-PA under conditions suitable for t-PA protease activity. It is envisioned that any amino acid sequence cleaved by t-PA can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a t-PA cleavage site is $X_1X_2X_3$(R/K)*X_4*$X_5X_6X_7$ (SEQ ID NO: 369), where X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are any amino acid. Table 4 lists exemplary reference cleavage sites for t-PA (SEQ ID NO: 370-373). Additional t-PA cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, H. R. Lijnen and D. Collen, *t-Plasminogen Activator.* In Handbook of Proteolytic Enzymes, pp. 1684-1689 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0277] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a t-Plasminogen Activator cleavage site. In an aspect of this embodiment, a t-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 369, where X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are any amino acid. In another aspect of this embodiment, a t-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 369, where X_1 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is an amidic amino acid like N and Q, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_7 is an acidic amino acid like D and E,

or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a t-Plasminogen Activator cleavage site comprises the consensus sequence SEQ ID NO: 369, where X_1 is A, P, C, or N; X_2 is A, L, P, or Q; X_3 is G, L, S, or F; X_4 is I, V, M, or Y; X_5 is A, V, or K; X_6 is G, V, or P; and X_7 is G, L, or D. In other aspects of this embodiment, a t-Plasminogen Activator cleavage site comprises, *e.g.*, SEQ ID NO: 370, SEQ ID NO: 371, SEQ ID NO: 372, or SEQ ID NO: 373.

[0278] Aspects of the present specification disclose, in part, a Tryptase-ε cleavage site as an inactivation cleavage site. As used herein, the term "Tryptase-ε cleavage site" or "Prosemin cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Tryptase-ε under conditions suitable for Tryptase-ε protease activity. It is envisioned that any amino acid sequence cleaved by Tryptase-ε can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Tryptase-ε cleavage site is *(R/K)$X_1X_2X_3X_4$(D/E) (SEQ ID NO: 374), where X_1, X_2, X_3, and X_4, are independently an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for Tryptase-ε (SEQ ID NO: 375-386). Additional Tryptase-ε cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0279] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Tryptase-ε cleavage site. In an aspect of this embodiment, a Tryptase-ε cleavage site comprises the consensus sequence SEQ ID NO: 374, where X_1, X_2, X_3, and X_4, are independently an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Tryptase-ε cleavage site comprises the consensus sequence SEQ ID NO: 374, where X_1 is I or V; X_2 is I or V; X_3 is G or S; X_4 is G or S. In other aspects of this embodiment, a Tryptase-ε cleavage site comprises, *e.g.*, SEQ ID NO: 375, SEQ ID NO: 376, SEQ ID NO: 377, SEQ ID NO: 378, SEQ ID NO: 379, SEQ ID NO: 380, SEQ ID NO: 381, SEQ ID NO: 382, SEQ ID NO: 383, SEQ ID NO: 384, SEQ ID NO: 385, or SEQ ID NO: 386.

[0280] Aspects of the present specification disclose, in part, a Mouse Mast Cell Protease-7 cleavage site as an inactivation cleavage site. As used herein, the term "Mouse Mast Cell Protease-7 cleavage site" or "mMCP-7 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by mMCP-7 under conditions suitable for mMCP-7 protease activity. It is envisioned that any amino acid sequence cleaved by mMCP-7 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a mMCP-7 cleavage site is $X_1X_2X_3$(K/R)*$X_4X_5X_6X_7$ (SEQ ID NO: 387), where X_1 is any amino acid; X_2 is preferentially an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_4, X_5, X_6, X_7 are any amino acid. Table 4 lists exemplary reference cleavage sites for mMMCP-7 (SEQ ID NO: 388-391). Additional mMMCP-7 cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. Schilling and C. M. Overall,

Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0281] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Mouse Mast Cell Proteas-7 cleavage site. In an aspect of this embodiment, a Mouse Mast Cell Proteas-7 cleavage site comprises the consensus sequence SEQ ID NO: 387, where X_1 is any amino acid; X_2 is an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_4, X_5, X_6, X_7 are independently any amino acid. In another aspect of this embodiment, a Mouse Mast Cell Proteas-7 cleavage site comprises the consensus sequence SEQ ID NO: 387, where X_1 is any amino acid; X_2 is G, S, or Q; X_3 is A, P or S; and X_4, X_5, X_6, X_7 are any amino acid. In other aspects of this embodiment, a Mouse Mast Cell Proteas-7 cleavage site comprises, *e.g.*, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, or SEQ ID NO: 391.

[0282] Aspects of the present specification disclose, in part, an Endothelin-Converting Enzyme-1 cleavage site as an inactivation cleavage site. As used herein, the term "Endothelin-Converting Enzyme-1 cleavage site" or "ECE-1 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by ECE-1 under conditions suitable for ECE-1 protease activity. It is envisioned that any amino acid sequence cleaved by ECE-1 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for an ECE-1 cleavage site is $X_1X_2X_3X_4$*$(F/L/I/V/Y)_5X_6X_7$ (SEQ ID NO: 392), where X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are any amino acid. Table 4 lists exemplary reference cleavage sites for ECE-1 (SEQ ID NO: 393-412). Additional ECE-1 cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, K. Ahn and G. D. Johnson, *Endothelin-Converting Enzyme-1*. In Handbook of Proteolytic Enzymes, pp. 429-434 (A. J. Barrett, N. D. Rawlings, and J. F. Woessner, eds; Elsevier, London, 2d, 2004); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0283] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises an Endothelin-Converting Enzyme-1 cleavage site. In an aspect of this embodiment, an Endothelin-Converting Enzyme-1 cleavage site comprises the consensus sequence SEQ ID NO: 392, where X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are independently any amino acid. In another aspect of this embodiment, an Endothelin-Converting Enzyme-1 cleavage site comprises the consensus sequence SEQ ID NO: 392, where X_1 is G, P, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an uncharged amino acid like C, S, and T; X_2 is F, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C,

S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is S, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is S, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_5 is F, W, S, C, N, E, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is G, P, V, L, F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an uncharged amino acid like C, S, and T; and X_7 is P, A, V, L, M, F, Y, S, N, D, or K. In another aspect of this embodiment, an Endothelin-Converting Enzyme-1 cleavage site comprises the consensus sequence SEQ ID NO: 392, where X_1 is G, P, Y, C, D, K, R, or H; X_2 is P, L, I, F, S, C, Q, D, R, or H; X_3 is V, L, I, S, Q, K, or R; X_4 is G, P, L, F, Y, W, or R; X_5 is V, I, M, F, N, R, or H; X_6 is P, L, F, T, E, or H; and X_7 is P, V, L, F, S, N, D, or K. In another aspect of this embodiment, an Endothelin-Converting Enzyme-1 cleavage site comprises the consensus sequence SEQ ID NO: 392, where X_1 is G, D, or H; X_2 is I or F; X_3 is V, I, S, Q or K; X_4 is P, F, or W; X_5 is I, N, R, or H; X_6 is L, T, or H; and X_7 is P, S, or D. In other aspects of this embodiment, an Endothelin-Converting Enzyme-1 cleavage site comprises, *e.g.*, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, SEQ ID NO: 406, SEQ ID NO: 407, SEQ ID NO: 408, SEQ ID NO: 409, SEQ ID NO: 410, SEQ ID NO: 411, or SEQ ID NO: 412.

[0284] Aspects of the present specification disclose, in part, a Kell blood-group protein cleavage site as an inactivation cleavage site. As used herein, the term "Kell blood-group protein cleavage site" or KBGP cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by KBGP under conditions suitable for KBGP protease activity. It is envisioned that any amino acid sequence cleaved by KBGP can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a KBGP cleavage site is $X_1X_2X_3X_4{*}X_5X_6X_7X_8$ (SEQ ID NO: 413), where X_1 is preferentially an acidic amino acid like D and E; X_2 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially an aromatic amino acid like F, W, and Y; X_5 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is preferentially an amidic amino acid like N and Q; X_7 is an uncharged amino acid like C, S, and T; X_8 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for KBGP (SEQ ID NO: 414-415). Additional KBGP cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0285] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Kell blood-

group protein cleavage site. In an aspect of this embodiment, a Kell blood-group protein cleavage site comprises the consensus sequence SEQ ID NO: 413, where X_1 is an acidic amino acid like D and E; X_2 is T or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an aromatic amino acid like F, W, and Y; X_5 is T or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_6 is an amidic amino acid like N and Q; X_7 is an uncharged amino acid like C, S, and T, or a C-beta branched amino acid like I, V, or T; X_8 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a Kell blood-group protein cleavage site comprises the consensus sequence SEQ ID NO: 413, where X_1 is D; X_2 is I, V, or T; X_3 is I, V, or T; X_4 is W; X_5 is I, V, or T; X_6 is N; X_7 is T; X_8 is P. In other aspects of this embodiment, a Kell blood-group protein cleavage site comprises, *e.g.*, SEQ ID NO: 414 or SEQ ID NO: 415.

[0286] Aspects of the present specification disclose, in part, a Cathepsin L cleavage site as an inactivation cleavage site. As used herein, the term "Cathepsin L cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by Cathepsin L under conditions suitable for Cathepsin L protease activity. It is envisioned that any amino acid sequence cleaved by Cathepsin L can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a Cathepsin L cleavage site is $X_1X_2X_3X_4{*}X_5X_6X_7X_8$ (SEQ ID NO: 416), where X_1 is preferentially W, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is preferentially L, V, F or Y; and X_4, X_5, X_6, X_7, and X_8 are any amino acid. Table 4 lists exemplary reference cleavage sites for Cathepsin L (SEQ ID NO: 417-443). Additional Cathepsin L cleavage sites are well known in the art or can be defined by routine methods. See, *e.g.*, J. C. Kelly, et al., *Profiling of Calpain Activity with a Series of FRET-Based Substrates*, Biochim. Biophys. Acta 1794: 1505-1509 (2009); O. Schilling and C. M. Overall, *Proteome-Derived, Database-Searchable Peptide Libraries for Identifying Protease Cleavage Sites*, Nat. Biotechnol. 26: 685-694 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 36(Database issue): D320-D325 (2008); Neil D. Rawlings, et al., *MEROPS: The Peptidase Database*, Nucleic Acids Res. 38(Database issue): D227-D233 (2010); Neil D. Rawlings, et al., *A Large and Accurate Collection of Peptidase Cleavages in the MEROPS Database*, Database in press (2010), each of which is incorporated by reference in its entirety.

[0287] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a Cathepsin L cleavage site. In an aspect of this embodiment, a Cathepsin L cleavage site comprises the consensus sequence SEQ ID NO: 416, where X_1 is W, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_2 is any amino acid; X_3 is L, V, F or Y; and X_4, X_5, X_6, X_7, and X_8 are any amino acid. In another aspect of this embodiment, a Cathepsin L cleavage site comprises the consensus sequence SEQ ID NO: 416, where X_1 is G, P, A, L, Q, E, or K; X_2 is an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_3 is L, V, F or Y; X_4 is G, A, F, T, Q, E, K, or R; X_5 is G, A, S, an acidic amino acid like D and E, an amidic amino acid like N and Q, or a positive amino acid like H, K, and R; X_6 is P, A, L, I, S, Q, an acidic

amino acid like D and E, or a positive amino acid like H, K, and R; X_7 is a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_8 is P, L, S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, or a basic amino acid like K, and R. In another aspect of this embodiment, a Cathepsin L cleavage site comprises the consensus sequence SEQ ID NO: 416, where X_1 is G, A, Q, E, or K; X_2 is G, P, L, or F; X_3 is L, V, F or Y; X_4 is G, A, F, T, Q, E, K, or R; X_5 is A, S, Q, E, K, or R; X_6 is P, A, L, I, S, or E; X_7 P, L, or R; and X_8 is P, L, S, or K. In other aspects of this embodiment, a Cathepsin L cleavage site comprises, *e.g.*, SEQ ID NO: 417, SEQ ID NO: 418, SEQ ID NO: 419, SEQ ID NO: 420, SEQ ID NO: 421, SEQ ID NO: 422, SEQ ID NO: 423, SEQ ID NO: 424, SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO: 435, SEQ ID NO: 436, SEQ ID NO: 437, SEQ ID NO: 438, SEQ ID NO: 439, SEQ ID NO: 440, SEQ ID NO: 441, SEQ ID NO: 442, or SEQ ID NO: 443.

[0288] Aspects of the present specification disclose, in part, a PAR1 cleavage site as an inactivation cleavage site. As used herein, the term "PAR1 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by PAR1 under conditions suitable for PAR1 protease activity. It is envisioned that any amino acid sequence cleaved by PAR1 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a PAR1 cleavage site is $X_1X_2X_3X_4(K/R)X_5$ (SEQ ID NO: 444), where X_1 is preferentially a small non-polar amino acid like A, C G, S, and T; X_2 is preferentially a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_3 is preferentially a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_4 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is preferentially an amidic amino acid like N and Q, or an aromatic hydrophobic amino acid like F, W, or Y. Table 4 lists exemplary reference cleavage sites for PAR1 (SEQ ID NO: 445-452). Additional PAR1 cleavage sites are well known in the art or can be defined by routine methods.

[0289] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a PAR1 cleavage site. In an aspect of this embodiment, a PAR1 cleavage site comprises the consensus sequence SEQ ID NO: 444, where X_1 is a small non-polar amino acid like A, C G, S, and T; X_2 is a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_3 is a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_4 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is an amidic amino acid like N and Q, or an aromatic hydrophobic amino acid like F, W, or Y. In another aspect of this embodiment, a PAR1 cleavage site comprises the consensus sequence SEQ ID NO: 444, where X_1 is S, T, or G; X_2 is F or Y; X_3 is L, P, or F; X_4 is A, G, I, or L; and X_5 is F or N. In other aspects of this embodiment, a PAR1 cleavage site comprises, *e.g.*, SEQ ID NO: 445, SEQ ID NO: 446, SEQ ID NO: 447, SEQ ID NO: 448, SEQ ID NO: 449, SEQ ID NO: 450, SEQ ID NO: 451, or SEQ ID NO: 452.

[0290] Aspects of the present specification disclose, in part, a PAR2 cleavage site as an inactivation cleavage site. As used herein, the term "PAR2 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by PAR2 under conditions suitable for PAR2 protease activity. It is envisioned that any amino acid sequence cleaved by PAR2 can be useful in aspects of the present specification. Although exceptions

are known, a generalized consensus sequence for a PAR2 cleavage site is $X_1X_2X_3X_4$(K/R)X_5 (SEQ ID NO: 453), where X_1 is preferentially a small non-polar amino acid like A, C G, S, and T; X_2 is preferentially a large non-polar amino acid like F, I, L, M, V; X_3 is preferentially a large non-polar amino acid like F, I, L, M, V; X_4 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is preferentially a large non-polar amino acid like F, I, L, M, V. Table 4 lists exemplary reference cleavage sites for PAR2 (SEQ ID NO: 454-455). Additional PAR2 cleavage sites are well known in the art or can be defined by routine methods.

[0291] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a PAR2 cleavage site. In an aspect of this embodiment, a PAR2 cleavage site comprises the consensus sequence SEQ ID NO: 453, where X_1 is a small non-polar amino acid like A, C G, S, and T; X_2 is a large non-polar amino acid like F, I, L, M, V; X_3 is a large non-polar amino acid like F, I, L, M, V; X_4 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is a large non-polar amino acid like F, I, L, M, V. In another aspect of this embodiment, a PAR2 cleavage site comprises the consensus sequence SEQ ID NO: 453, where X_1 is S; X_2 is I or L; X_3 is I or L; X_4 is A or G; X_5 is L or V. In other aspects of this embodiment, a PAR2 cleavage site comprises, *e.g.*, SEQ ID NO: 454 or SEQ ID NO: 455.

[0292] Aspects of the present specification disclose, in part, a PAR3 cleavage site as an inactivation cleavage site. As used herein, the term "PAR3 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by PAR3 under conditions suitable for PAR3 protease activity. It is envisioned that any amino acid sequence cleaved by PAR3 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a PAR3 cleavage site is $X_1X_2X_3X_4X_5X_6$ (SEQ ID NO: 456), where X_1 is preferentially a small non-polar amino acid like A, C G, S, and T; X_2 is preferentially a large non-polar amino acid like F, I, L, M, V; X_3 is preferentially an amidic amino acid like N and Q, or a basic amino acid like K and R; X_4 is preferentially a small non-polar amino acid like A, C G, S, and T; X_5 is preferentially a small non-polar amino acid like A, C G, S, and T, or a small polar amino acid like D, N, or P; and X_6 is preferentially an acidic amino acid like D and E, or a small polar amino acid like D, N, or P. Table 4 lists exemplary reference cleavage sites for PAR3 (SEQ ID NO: 457-459). Additional PAR3 cleavage sites are well known in the art or can be defined by routine methods.

[0293] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a PAR3 cleavage site. In an aspect of this embodiment, a PAR3 cleavage site comprises the consensus sequence SEQ ID NO: 456, where X_1 is a small non-polar amino acid like A, C G, S, and T; X_2 is a large non-polar amino acid like F, I, L, M, V; X_3 is an amidic amino acid like N and Q, or a basic amino acid like K and R; X_4 is a small non-polar amino acid like A, C G, S, and T; X_5 is a small non-polar amino acid like A, C G, S, and T, or a small polar amino acid like D, N, or P; and X_6 is an acidic amino acid like D and E, or a small polar amino acid like D, N, or P. In another aspect of this embodiment, a PAR3 cleavage site comprises the consensus sequence SEQ ID NO: 456, where X_1 is S or T; X_2 is F; X_3 is N or R; X_4 is A or G; X_5 is A,G, or N and X_6 is P or E. In other aspects of this embodiment, a PAR3 cleavage site comprises, *e.g.*, SEQ ID NO: 457, SEQ ID NO: 458, or SEQ ID NO: 459.

[0294] Aspects of the present specification disclose, in part, a PAR4 cleavage site as an inactivation cleavage site. As used herein, the term "PAR4 cleavage site" refers to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile

bond by PAR4 under conditions suitable for PAR4 protease activity. It is envisioned that any amino acid sequence cleaved by PAR4 can be useful in aspects of the present specification. Although exceptions are known, a generalized consensus sequence for a PAR4 cleavage site is $X_1X_2X_3X_4$(K/R/Q/F)X_5 (SEQ ID NO: 460), where X_1 is preferentially a small non-polar amino acid like A, C G, S, and T; X_2 is preferentially a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_3 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is preferentially an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is preferentially a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W, or Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. Table 4 lists exemplary reference cleavage sites for PAR4 (SEQ ID NO: 461-478). Additional PAR4 cleavage sites are well known in the art or can be defined by routine methods.

[0295] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises a PAR4 cleavage site. In an aspect of this embodiment, a PAR4 cleavage site comprises the consensus sequence SEQ ID NO: 460, where X_1 is a small non-polar amino acid like A, C G, S, and T; X_2 is a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y; X_3 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; X_4 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M; and X_5 is a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W, or Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M. In another aspect of this embodiment, a PAR4 cleavage site comprises the consensus sequence SEQ ID NO: 460, where X_1 is A, G, S, or T; X_2 is F or Y; X_3 is A or P; X_4 is A or G; and X_5 is A, V, P, F, W, Y, or K. In other aspects of this embodiment, a PAR4 cleavage site comprises, *e.g.*, SEQ ID NO: 461, SEQ ID NO: 462, SEQ ID NO: 463, SEQ ID NO: 464, SEQ ID NO: 465, SEQ ID NO: 466, SEQ ID NO: 467, SEQ ID NO: 468, SEQ ID NO: 469, SEQ ID NO: 470, SEQ ID NO: 471, SEQ ID NO: 472, SEQ ID NO: 473, SEQ ID NO: 474, SEQ ID NO: 475, SEQ ID NO: 476, SEQ ID NO: 477, or SEQ ID NO: 478.

[0296] The location of an inactivation cleavage site is a critical aspect that is governed by several criteria. First, the placement of the inactivation cleavage site should not substantially affect the ability of a Clostridial toxin or Clostridial toxin chimeric to intoxicate its target cell. As used herein, the term "not substantially affect," with regards to intoxication, refers to a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification that can still execute the overall intoxication mechanism whereby a Clostridial toxin or Clostridial toxin chimeric enters a target cell and proteolytically cleaves a target substrate and encompasses the binding of a Clostridial toxin or Clostridial toxin chimeric to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the light chain into the cytoplasm and the enzymatic modification of a target substrate.

[0297] In an aspect of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site can intoxicate a target cell to the same extent as the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site modification. In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site can intoxicate a target cell by, *e.g.*, at least 50%, 60%, 70%, 80%, 90% or 95% the extent as the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site modification. In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site can intoxicate a target cell by, *e.g.*, at most 50%, 60%, 70%, 80%, 90% or 95% the extent as the same or similar Clostridial toxin or Clostridial toxin chimeric, but

without the inactivation cleavage site modification.

[0298] Second, the placement of an inactivation cleavage site should be at a surface exposed region of the toxin or Clostridial toxin chimeric and not buried internally within the protein or masked by secondary structure elements. Proper surface exposure of the inactivation cleavage site facilitates proper access of the site to its corresponding protease, thereby enabling proteolytic cleavage. Proteolytic cleavage of the inactivation cleavage site by its corresponding protease substantially inactivates the ability of the Clostridial toxin or Clostridial toxin chimeric to intoxicate the cell. As used herein, the term "substantially inactivates," with regards to intoxication, refers to a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification that, after cleavage at an inactivation cleavage site, has a reduced ability to execute the overall intoxication mechanism whereby a Clostridial toxin or Clostridial toxin chimeric enters a target cell and proteolytically cleaves a target substrate and encompasses the binding of a Clostridial toxin or Clostridial toxin chimeric to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the light chain into the cytoplasm and the enzymatic modification of a target substrate.

[0299] In one aspect of this embodiment, proteolytic cleavage of a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification at an inactivation cleavage site results in complete inability of the toxin to intoxicate a target cell as compared to the same or similar Clostridial toxin or Clostridial toxin chimeric, but in a proteolytic uncleaved state (*i.e.*, the intoxication cleavage site is intact or uncleaved). In other aspects of this embodiment, proteolytic cleavage of a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification at an inactivation cleavage site results in, *e.g.*, at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% decreased ability to intoxicate a target cell as compared to the same or similar Clostridial toxin or Clostridial toxin chimeric, but in a proteolytic uncleaved state. In other aspects of this embodiment, proteolytic cleavage of a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification at an inactivation cleavage site results in, *e.g.*, at most a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% decreased ability to intoxicate a target cell as compared to the same or similar Clostridial toxin or Clostridial toxin chimeric, but in a proteolytic uncleaved state.

[0300] In an aspect of the present specification, an inactivation cleavage site is located within an inactivation cleavage site region. As used herein, the term "inactivation cleavage site region" refers to an amino acid sequence of a Clostridial toxin or Clostridial toxin chimeric that can be modified to contain an inactivation cleavage site because such modification will not substantially disrupt the ability of the protein to intoxicate a target cell; and upon exposure to its cognate protease, the inactivation cleavage site will be cleaved and substantially inactivate the Clostridial toxin or Clostridial toxin chimeric. The location of an inactivation cleavage site can be anywhere within the inactivation cleavage site region, with the proviso that such location will not substantially affect the ability of the Clostridial toxin or Clostridial toxin chimeric to intoxicate a target cell; and upon exposure to its cognate protease, cleavage of the inactivation cleavage site will substantially inactivate the Clostridial toxin or Clostridial toxin chimeric. Table 5 lists exemplary inactivation cleavage site regions suitable for use with a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification.

[0301] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site located within inactivation cleavage site region. In

aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site located within inactivation cleavage site region from the translocation domain or the H_{CN} subdomain.

[0302] In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 462-496 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 458-492 of SEQ ID NO: 3; amino acids 464-487 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 463-496 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 458-491 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 434-467 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 453-486 of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; amino acids 458-491 of SEQ ID NO: 21; amino acids 475-508 of SEQ ID NO: 22; amino acids 443-476 of SEQ ID NO: 23; or amino acids 434-467 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0303] In yet other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 618-634 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 614-630 of SEQ ID NO: 3; amino acids 605-621 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 613-629 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 609-625 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 587-603 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 604-620 of SEQ ID NO: 18; amino acids 605-621 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 610-626 of SEQ ID NO: 21; amino acids 627-643 of SEQ ID NO: 22; amino acids 596-612 of SEQ ID NO: 23; or amino acids 587-603 of SEQ ID NO: 24 or SEQ ID NO: 25.

Table 5. Inactivation Cleavage Site Regions of Clostridial Toxins

Toxin	SEQ ID NO:	Inactivation Cleavage Site Regions							
		1	2	3	4	5	6	7	8
BoNT/A	1	L462-L496	T618-I634	G638-D651	L665-N687	N752-N765	N826-D835	T844-L863	K871-A895
BoNT/B	2	L464-P487	A605-V621	G625-N638	L652-N674	N739-D752	N813-A824	Y831-I850	S858-G882
BoNT/C1	3	L463-S496	I613-I629	G633-N646	L660-E682	K747-Q760	H821-D830	S839-K858	N866-N890
BoNT/D	4	L458-S491	I609-I625	G629-N642	L656-E678	K743-Q756	H817-D826	S835-K854	N862-N886
BoNT/E	5	L434-D467	A587-V603	G607-N620	L634-N659	N724-D739	H800-Q809	T818-I837	K845-D869
BoNT/F	6	L453-N486	A605-V621	G625-N638	L652-N677	N742-N757	H818-N827	T836-I855	K863-G887
BoNT/G	7	L458-S491	S610-I626	G630-N643	M657-N679	N744-D757	N818-N827	H836-I855	S863-G887
TeNT	8	L475-S508	S627-V643	G647-N660	L674-Q696	K761-E774	N835-K844	V854-V871	V879-N903
BaNT	9	L443-N476	A596-V612	G616-N629	L643-S668	N733-N748	N809-P819	T828-I847	K855-G879

BuNT	10	L434-D467	A587-V603	G607-N620	L634-S659	N724-D739	H800-Q809	T818-I837	K845-D869

[0304] In still other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 638-651 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 634-647 of SEQ ID NO: 3; amino acids 625-638 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 633-646 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 629-642 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 607-620 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 624-637 of SEQ ID NO: 18; amino acids 625-638 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 630-643 of SEQ ID NO: 21; amino acids 647-660 of SEQ ID NO: 22; amino acids 616-629 of SEQ ID NO: 23; or amino acids 607-620 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0305] In further aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 665-687 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 661-683 of SEQ ID NO: 3; amino acids 652-674 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 660-682 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 656-678 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 634-659 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 651-676 of SEQ ID NO: 18; amino acids 652-677 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 657-679 of SEQ ID NO: 21; amino acids 674-696 of SEQ ID NO: 22; amino acids 643-668 of SEQ ID NO: 23; or amino acids 634-659 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0306] In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 752-765 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 748-761 of SEQ ID NO: 3; amino acids 739-752 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 747-760 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 743-756 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 724-739 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 741-756 of SEQ ID NO: 18; amino acids 742-757 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 744-757 of SEQ ID NO: 21; amino acids 761-774 of SEQ ID NO: 22; amino acids 733-748 of SEQ ID NO: 23; or amino acids 724-739 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0307] In yet other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 826-835 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 824-831 of SEQ ID NO: 3; amino acids 813-824 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 821-830 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 817-826 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 800-809 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 817-826 of SEQ ID NO: 18; amino acids 818-827 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 818-827 of SEQ ID NO: 21; amino acids 835-844

of SEQ ID NO: 22; amino acids 809-819 of SEQ ID NO: 23; or amino acids 800-809 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0308] In still other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 844-863 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 840-859 of SEQ ID NO: 3;; amino acids 831-850 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 839-858 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 835-854 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 818-837 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 835-854 of SEQ ID NO: 18; amino acids 836-855 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 836-855 of SEQ ID NO: 21; amino acids 854-871 of SEQ ID NO: 22; amino acids 828-847 of SEQ ID NO: 23; or amino acids 818-837 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0309] In further aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 871-895 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; amino acids 867-891 of SEQ ID NO: 3; amino acids 858-882 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; amino acids 866-890 of SEQ ID NO: 11 or SEQ ID NO: 12; amino acids 862-886 of SEQ ID NO: 13 or SEQ ID NO: 14; amino acids 845-869 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; amino acids 862-886 of SEQ ID NO: 18; amino acids 863-887 of SEQ ID NO: 19 or SEQ ID NO: 20; amino acids 863-887 of SEQ ID NO: 21; amino acids 879-903 of SEQ ID NO: 22; amino acids 855-879 of SEQ ID NO: 23; or amino acids 845-869 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0310] In another aspect of this embodiment, a BoNT/A or BoNT/A chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/A or BoNT/A chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 462-496, 618-634, 638-651, 665-687, 752-765, 826-835, 844-863, or 871-895 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 5; or comprising amino acids 458-492, 614-630, 634-647, 665-687, 748-761, 822-831, 840-859, or 867-891 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/A comprising an inactivation cleavage site located within inactivation cleavage site region is encoded by SEQ ID NO: 530, SEQ ID NO: 532, SEQ ID NO: 534, or SEQ ID NO: 536. In still other aspects of this embodiment, a BoNT/A comprising an inactivation cleavage site located within inactivation cleavage site region comprises SEQ ID NO: 531, SEQ ID NO: 533, SEQ ID NO: 535, or SEQ ID NO: 537.

[0311] In yet another aspect of this embodiment, a BoNT/B or BoNT/B chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/B or BoNT/B chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 464-487, 605-621, 625-638, 652-674, 739-752, 813-824, 831-850, or 858-882 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.

[0312] In still another aspect of this embodiment, a BoNT/C1 or BoNT/C1 chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/C1 or BoNT/C1 chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 463-496, 613-629, 633-646, 660-682, 747-760, 821-830, 839-858, or 866-890 of SEQ ID NO: 11 or SEQ ID NO: 12.

[0313] In a further aspect of this embodiment, a BoNT/D or BoNT/D chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/D or BoNT/D chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 458-491, 609-625, 629-642, 656-678, 743-756, 817-826, 835-854, or 862-886 of SEQ ID NO: 13 or SEQ ID NO: 14.

[0314] In another aspect of this embodiment, a BoNT/E or BoNT/E chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/E or BoNT/E chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 434-467, 587-603, 607-620, 634-659, 724-739, 800-809, 818-837, or 845-869 of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

[0315] In still another aspect of this embodiment, a BoNT/F or BoNT/F chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/F or BoNT/F chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 453-486, 604-620, 624-637, 651-676, 741-756, 817-826, 835-854, or 862-886 of SEQ ID NO: 18; or comprising amino acids 453-486, 605-621, 625-638, 652-677, 742-757, 818-827, 836-855, or 863-887 of SEQ ID NO: 19 or SEQ ID NO: 20.

[0316] In a further aspect of this embodiment, a BoNT/G or BoNT/G chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BoNT/G or BoNT/G chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 458-491, 610-626, 630-643, 657-679, 744-757, 818-827, 836-855, or 863-887 of SEQ ID NO: 21.

[0317] In another aspect of this embodiment, a TeNT or TeNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a TeNT or TeNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 475-508, 627-643, 647-660, 674-696, 761-774, 835-844, 854-871, or 879-903 of SEQ ID NO: 22.

[0318] In yet another aspect of this embodiment, a BaNT or BaNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BaNT or BaNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located

within an inactivation cleavage site region comprising amino acids 443-476, 596-612, 616-629, 643-668, 733-748, 809-819, 828-847, or 855-879 of SEQ ID NO: 23.

[0319] In still another aspect of this embodiment, a BuNT or BuNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region from the translocation domain or the H_{CN} subdomain. In other aspects of this embodiment, a BuNT or BuNT chimeric disclosed in the present specification comprises an inactivation cleavage site is located within an inactivation cleavage site region comprising amino acids 434-467, 587-603, 607-620, 634-659, 724-739, 800-809, 818-837, or 845-869 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0320] In an aspect of the present specification, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin greater than the safety margin for the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In other words, the addition of an inactivation cleavage site increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site.

[0321] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than, *e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, or at least 300%, relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than, *e.g.*, at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90%, at most 100%, 110%, at most 120%, at most 130%, at most 140%, at most 150%, at most 160%, at most 170%, at most 180%, at most 190%, at most 200%, 210%, at most 220%, at most 230%, at most 240%, at most 250%, at most 260%, at most 270%, at most 280%, at most 290%, or at most 300%, relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In yet other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater by, *e.g.*, about 10% to about 300%, about 20% to about 300%, about 30% to about 300%, about 40% to about 300%, about 50% to about 300%, about 60% to about 300%, about 70% to about 300%, about 80% to about 300%, about 90% to about 300%, or about 100% to about 300%, relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site.

[0322] In other aspects embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than, *e.g.*, at least 1-fold, at least 1-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold, relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In yet other aspects embodiment, a Clostridial toxin or Clostridial toxin

chimeric comprising an inactivation cleavage site has a safety margin that is greater than, *e.g.*, at least 1-fold, at most 1-fold, at most 3-fold, at most 4-fold, at most 5-fold, at most 6-fold, at most 7-fold, at most 8-fold, at most 9-fold, or at most 10-fold, relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the inactivation cleavage site. In still other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater by, *e.g.*, about 1-fold to about 10-fold, about 1-fold to about 9-fold, about 1-fold to about 8-fold, about 1-fold to about 7-fold, about 1-fold to about 6-fold, about 1-fold to about 5-fold, about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, or about 2-fold to about 5-fold.

[0323] In another embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site. In aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, or at least 300%. In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90%, at most 100%, 110%, at most 120%, at most 130%, at most 140%, at most 150%, at most 160%, at most 170%, at most 180%, at most 190%, at most 200%, 210%, at most 220%, at most 230%, at most 240%, at most 250%, at most 260%, at most 270%, at most 280%, at most 290%, or at most 300%. In yet other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, about 10% to about 300%, about 20% to about 300%, about 30% to about 300%, about 40% to about 300%, about 50% to about 300%, about 60% to about 300%, about 70% to about 300%, about 80% to about 300%, about 90% to about 300%, or about 100% to about 300%.

[0324] In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, at least 1-fold, at least 1-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold. In yet other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or

Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, at most 1-fold, at most 3-fold, at most 4-fold, at most 5-fold, at most 6-fold, at most 7-fold, at most 8-fold, at most 9-fold, or at most 10-fold. In still other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin or Clostridial toxin chimeric relative to the same or similar Clostridial toxin or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, about 1-fold to about 10-fold, about 1-fold to about 9-fold, about 1-fold to about 8-fold, about 1-fold to about 7-fold, about 1-fold to about 6-fold, about 1-fold to about 5-fold, about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, or about 2-fold to about 5-fold.

[0325] In another embodiment, an inactivation cleavage site region can be modified to include a single inactivation cleavage site. In yet another embodiment, an inactivation cleavage site region can be modified to include a plurality inactivation cleavage site cleavage sites. In aspects of this embodiment, an inactivation cleavage site cleavage site region can comprise, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 inactivation cleavage sites. In other aspects of this embodiment, an inactivation cleavage site cleavage site region can comprise, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 inactivation cleavage sites. In yet other aspects of this embodiment, an inactivation cleavage site cleavage site region can comprise, *e.g.*, 2-10 inactivation cleavage sites, 2-8 inactivation cleavage sites, 2-6 inactivation cleavage sites, 2-4 inactivation cleavage sites, 2-3 inactivation cleavage sites, 3-9 inactivation cleavage sites, 3-7 inactivation cleavage sites, 3-5 inactivation cleavage sites, or 3-4 inactivation cleavage sites.

[0326] In another embodiment, an inactivation cleavage site region can be modified to include only one type of inactivation cleavage site, such as, *e.g.*, a thrombin cleavage site. In still another embodiment, an inactivation cleavage site region can be modified to include a plurality of different types of inactivation cleavage sites, such as, *e.g.*, a thrombin cleavage site, a Factor Xa cleavage site, MMP-2 cleavage site, and a MMP-9 cleavage site. In aspects of this embodiment, an inactivation cleavage site region can comprise, *e.g.*, at least 2, 3, 4, or 5 different types of inactivation cleavage sites. In other aspects of this embodiment, an inactivation cleavage site region can comprise, *e.g.*, at most 2, 3, 4, or 5 different types of inactivation cleavage sites. In other aspects of this embodiment, an inactivation cleavage site region can comprise, *e.g.*, 2-5 different types of inactivation cleavage sites, 2-4 different types of inactivation cleavage sites, 2-3 different types of inactivation cleavage sites, 3-5 different types of inactivation cleavage sites, or 3-4 different types of inactivation cleavage sites.

[0327] Modification of an inactivation cleavage site region to include a inactivation cleavage site can be accomplished by altering at least one of the amino acids within the inactivation cleavage site region. Non-limiting examples of an amino acid alteration include a deletion of an amino acid, an addition of an amino acid, or a substitution of an original amino acid with a different amino acid. In aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by altering, *e.g.*, at least 1, 2, 3, 4, or 5 amino acids within the inactivation cleavage site region. In other aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by altering, *e.g.*, at most 1, 2, 3, 4, or 5 amino acids within the inactivation cleavage site region. In yet aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by altering, *e.g.*, 1-5 amino acids within the inactivation cleavage site region, 1-

4 amino acids within the inactivation cleavage site region, 1-3 amino acids within the inactivation cleavage site region, 1-2 amino acids within the inactivation cleavage site region, 2-5 amino acids within the inactivation cleavage site region, 2-4 amino acids within the inactivation cleavage site region, 2-3 amino acids within the inactivation cleavage site region, 3-5 amino acids within the inactivation cleavage site region, or 4-5 amino acids within the inactivation cleavage site region.

[0328] In aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by deleting, adding, substituting, or any combination thereof, *e.g.*, at least 1, 2, 3, 4, or 5 amino acids within the inactivation cleavage site region. In other aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by deleting, adding, substituting, or any combination thereof, *e.g.*, at most 1, 2, 3, 4, or 5 amino acids within the inactivation cleavage site region. In yet aspects of this embodiment, an inactivation cleavage site region is modified to include an inactivation cleavage site by deleting, adding, substituting, or any combination thereof, *e.g.*, 1-5 amino acids within the inactivation cleavage site region, 1-4 amino acids within the inactivation cleavage site region, 1-3 amino acids within the inactivation cleavage site region, 1-2 amino acids within the inactivation cleavage site region, 2-5 amino acids within the inactivation cleavage site region, 2-4 amino acids within the inactivation cleavage site region, 2-3 amino acids within the inactivation cleavage site region, 3-5 amino acids within the inactivation cleavage site region, or 4-5 amino acids within the inactivation cleavage site region.

[0329] Modification of an inactivation cleavage site region to include an inactivation cleavage site can be achieved using standard mutagenesis procedures known to a person skilled in the art. Non-limiting examples of mutagenesis procedures, as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0330] As mentioned above, Clostridial toxins and Clostridial toxin chimeras disclosed in the present specification are translated as single-chain polypeptides that are subsequently cleaved by proteolytic scission within a disulfide loop region. This posttranslational processing yields a di-chain molecule held together by a single disulphide bond and noncovalent interactions. The proteolytic scission within a disulfide loop region can be achieved by using the endogenous protease cleavage sites naturally-occurring within the di-chain loop region, or by engineering the di-chain loop region to comprise an exogenous protease cleavage site.

[0331] Aspects of the present specification disclose, in part, a di-chain loop region. As used herein, the term "di-chain loop region" refers to an amino acid sequence of a Clostridial toxin or Clostridial toxin chimeric flanked by cysteine amino acids and containing a protease cleavage site used to convert the single-chain form of a Clostridial toxin or Clostridial toxin chimeric into its di-chain form (Table 6). Non-limiting examples of a di-chain loop region, include, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F

comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; and a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8 (Table 6).

Table 6. Di-chain Loop Region

Toxin	Di-chain Loop Region Containing the Naturally-occurring Protease Cleavage Site
BoNT/A	CVRGIITSKTKSLDKGYNK*----ALNDLC
BoNT/B	CKSVK*----------APGIC
BoNT/C1	CHKAIDGRSLYNK*----------TLDC
BoNT/D	CLRLTKNSR*----------DDSTC
BoNT/E	CKNIVSVKGIR*----------KSIC
BoNT/F	CKSVIPRKGTK*----------APPRLC
BoNT/G	CKPVMYKNTGK*----------SEQC
TeNT	CKKIIPPTNIRENLYNRTA*SLTDLGGELC
BaNT	CKS-IVSKKGTK*----------NSLC
BuNT	CKN-IVSVKGIR*----------KSIC

The amino acid sequence displayed are as follows: BoNT/A, residues 430-454 of SEQ ID NO: 1; BoNT/B, residues 437-446 of SEQ ID NO: 2; BoNT/C1, residues 437-453 of SEQ ID NO: 3; BoNT/D, residues 437-450 of SEQ ID NO: 4; BoNT/E, residues 412-426 of SEQ ID NO: 5; BoNT/F, residues 429-445 of SEQ ID NO: 6; BoNT/G, residues 436-450 of SEQ ID NO: 7; TeNT, residues 439-467 of SEQ ID NO: 8; BaNT, residues 421-435 of SEQ ID NO: 9; and BuNT, residues 412-426 of SEQ ID NO: 10. An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0332] Thus, in an embodiment, a di-chain loop region comprises a Clostridial toxin di-chain loop region. In aspects of this embodiment, a di-chain loop region comprises, *e.g.*, a BoNT/A di-chain loop region, a BoNT/B di-chain loop region, a BoNT/C1 di-chain loop region, a BoNT/D di-chain loop region, a BoNT/E di-chain loop region, a BoNT/F di-chain loop region, a BoNT/G di-chain loop region, a TeNT di-chain loop region, a BaNT di-chain loop region, or a BuNT di-chain loop region. In other aspects of this embodiment, a di-chain loop region comprises, *e.g.*, a BoNT/A di-chain loop region comprising amino acids 430-454 of SEQ ID NO: 1; a BoNT/B di-chain loop region comprising amino acids 437-446 of SEQ ID NO: 2; a BoNT/C1 di-chain loop region comprising amino acids 437-453 of SEQ ID NO: 3; a BoNT/D di-chain loop region comprising amino acids 437-450 of SEQ ID NO: 4; a BoNT/E di-chain loop region comprising amino acids 412-426 of SEQ ID NO: 5; a BoNT/F di-chain loop region comprising amino acids 429-445 of SEQ ID NO: 6; a BoNT/G di-chain loop region comprising amino acids 436-450 of SEQ ID NO: 7; or a TeNT di-chain loop region comprising amino acids 439-467 of SEQ ID NO: 8. a BaNT di-chain loop region comprising amino acids 421-435 of SEQ ID NO: 9; or a BuNT di-chain loop region comprising amino acids 412-426 of SEQ ID NO: 10.

[0333] Aspects of the present specification disclose, in part, an endogenous di-chain loop protease cleavage site. As used herein, the term "endogenous di-chain loop protease cleavage site" is synonymous with a "naturally occurring di-chain loop protease cleavage site" and refers to a naturally occurring protease cleavage site found within the di-chain loop region of a naturally occurring Clostridial toxin or Clostridial toxin chimeric and includes, without limitation, naturally occurring Clostridial toxin di-chain loop protease cleavage site variants, such as, *e.g.*, Clostridial toxin di-chain loop protease cleavage

site isoforms and Clostridial toxin di-chain loop protease cleavage site subtypes. Non-limiting examples of an endogenous protease cleavage site, include, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

[0334] While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BaNT at K431-N432 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BuNT at R422-K423 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked to a Clostridial toxin or Clostridial toxin chimeric as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleave ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

[0335] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises a di-chain loop region including an endogenous di-chain loop protease cleavage site. In aspects of this embodiment, an endogenous di-chain loop protease cleavage site located within the di-chain loop region comprises, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site, a TeNT di-chain loop protease cleavage site, a BaNT di-chain loop protease cleavage site, or a BuNT di-chain loop protease cleavage site. In other aspects of this embodiment, an endogenous di-chain loop protease cleavage site located within the di-chain loop region comprises, *e.g.*, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; or a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8. a di-chain loop region of BaNT comprising amino acids 421-435 of SEQ ID NO: 9; or a di-chain loop region of BuNT comprising amino acids 412-426 of SEQ ID NO: 10.

[0336] Aspects of the present specification disclose, in part, an exogenous protease cleavage site. As used herein, the term "exogenous protease cleavage site" is synonymous with "engineered protease cleavage site", "non-naturally occurring protease cleavage site", or "non-native protease cleavage site" and refers to a protease cleavage site that is not normally present in a di-chain loop region from a naturally occurring Clostridial toxin. Such engineered or exogenous protease cleavage sites within the di-chain loop region are used to convert the single-chain polypeptide form of a Clostridial toxin of Clostridial toxin chimeric disclosed in the present specification into its di-chain form. It is envisioned that any and all exogenous protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin or Clostridial toxin chimeric into its active di-chain form are useful to practice aspects of the present specification. Non-limiting examples of exogenous protease cleavage sites include, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus (TEV) protease cleavage site, a Tobacco Vein Mottling Virus (TVMV) cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site. Engineered protease cleavage sites located within the di-chain loop are described in, *e.g.*, Dolly, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent 7,419,676, Dolly, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent 7,422,877, Steward, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent Publication 2009/0069238, Steward, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent Publication 2008/0032930, Steward, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent Publication 2009/0018081, Steward, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent Publication 2009/0005313, Steward, et al., *Activatable Recombinant Neurotoxins*, U.S. Patent Publication 2009/0004224; each of which is hereby incorporated by reference in its entirety.

[0337] It is envisioned that an exogenous protease cleavage site of any and all lengths can be useful in aspects of the present specification with the proviso that the exogenous protease cleavage site can be cleaved by its respective protease. Thus, in aspects of this embodiment, an exogenous protease cleavage site can have a length of, e.g., at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or at least 60 amino acids; or at most 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or at least 60 amino acids.

[0338] In an embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification comprises a di-chain loop region including an exogenous protease cleavage site. In aspects of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, a non-human enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Tobacco Vein Mottling Virus protease cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, a SUMO/ULP-1 protease cleavage site, and a non-human Caspase 3 cleavage site.

[0339] In an aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a non-human enterokinase cleavage site. In another aspect of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a bovine enterokinase protease cleavage site. In yet another aspect of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 480.

[0340] In another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a Tobacco Etch Virus protease cleavage. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence $EX_1X_2YX_3Q^*G$ (SEQ ID NO: 481) or $EX_1X_2YX_3Q^*S$ (SEQ ID NO: 482), where X_1, X_2 and X_3 is any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 483, SEQ ID NO: 484, SEQ ID NO: 485, SEQ ID NO: 486, SEQ ID NO: 487, SEQ ID NO: 488, SEQ ID NO: 489, SEQ ID NO: 490, SEQ ID NO: 491, or SEQ ID NO: 492.

[0341] In another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a Tobacco Vein Mottling Virus protease cleavage site. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence $X_1X_2VRFQ^*G$ (SEQ ID NO: 493) or $X_1X_2VRFQ^*S$ (SEQ ID NO: 494), where X_1 and X_2 are independently any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 495, SEQ ID NO: 496, SEQ ID NO: 497, or SEQ ID NO: 498.

[0342] In still another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a human rhinovirus 3C protease cleavage site. In another aspect of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence $X_1X_2LFQ^*GP$ (SEQ ID NO: 499), where X_1 is any amino acid with an acidic amino acid like D or E preferred; and X_2 is preferentially S, T, and an aliphatic hydrophobic amino acid like G, P, A, V, L, I, and M. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 500, SEQ ID NO: 501, SEQ ID NO: 502, SEQ ID NO: 503, SEQ ID NO: 504, or SEQ ID NO: 505. In another aspect of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a human rhinovirus 3C protease cleaved by PRESCISSION®.

[0343] In yet another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a subtilisin cleavage site. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence $X_1X_2X_3X_4H^*Y$ (SEQ ID NO: 506) or $X_1X_2X_3X_4YH^*$ (SEQ ID NO: 507), where X_1, X_2, X_3, and X_4 are independently any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 508, SEQ ID NO: 509, or SEQ ID NO: 510. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a subtilisin cleavage site cleaved by GENENASE®.

[0344] In yet another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a hydroxylamine cleavage site. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the dipeptide N*G. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 511 or SEQ ID NO: 512.

[0345] In yet another aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a SUMO/ULP-1 protease cleavage site. In other aspects of the

embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence GG*$X_1X_2X_3$ (SEQ ID NO: 513), where X_1, X_2, and X_3 are independently any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 514.

[0346] In an aspect of this embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a Caspase 3 cleavage site. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., a non-human Caspase 3 protease cleavage site. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., the consensus sequence DX_1X_2D*X_3 (SEQ ID NO: 515), where X_1 is any amino acid, with an acidic amino acid like D and E preferred, X_2 is any amino acid and X_3 is amino acid, with a small non-polar amino acid like A, C, G, S, and T preferred. In other aspects of the embodiment, an exogenous protease cleavage site located within the di-chain loop region comprises, e.g., SEQ ID NO: 516, SEQ ID NO: 517, SEQ ID NO: 518, SEQ ID NO: 519, SEQ ID NO: 520, or SEQ ID NO: 521.

[0347] A di-chain loop region can be modified so that a naturally-occurring di-chain loop protease cleavage site is replaced by an exogenous protease cleavage site. In this modification, the naturally-occurring di-chain loop protease cleavage site is made inoperable and thus cannot be cleaved by its protease. Only the exogenous protease cleavage site can be cleaved by its corresponding exogenous protease. In this type of modification, the exogenous protease site is operably-linked to a Clostridial toxin or Clostridial toxin chimeric as a fusion protein and the site can be cleaved by its respective exogenous protease. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be a substitution of the sites where the exogenous site is engineered at the position approximating the cleavage site location of the endogenous site. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be an addition of an exogenous site where the exogenous site is engineered at the position different from the cleavage site location of the endogenous site, the endogenous site being engineered to be inoperable. The location and kind of protease cleavage site may be critical because certain binding domains require a free amino-terminal or carboxyl-terminal amino acid. For example, when a peptide binding domain is placed between two other domains, e.g., see FIG. 4, a criterion for selection of a protease cleavage site could be whether the protease that cleaves its site leaves a flush cut, exposing the free amino-terminal or carboxyl-terminal of the binding domain necessary for selective binding of the binding domain to its receptor.

[0348] A naturally-occurring protease cleavage site can be made inoperable by altering at least one of the two amino acids flanking the peptide bond cleaved by the naturally-occurring di-chain loop protease. More extensive alterations can be made, with the proviso that the two cysteine residues of the di-chain loop region remain intact and the region can still form the disulfide bridge. Non-limiting examples of an amino acid alteration include deletion of an amino acid or replacement of the original amino acid with a different amino acid. Thus, in one embodiment, a naturally-occurring protease cleavage site is made inoperable by altering at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 amino acids including at least one of the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease. In another embodiment, a naturally-occurring protease cleavage site is made inoperable by altering at most 1, 2, 3,

4, 5, 6, 7, 8, 9, 10, 15, 20 amino acids including at least one of the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0349] It is understood that a modified Clostridial toxin disclosed in the present specification can optionally further comprise a flexible region comprising a flexible spacer. A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better present a peptide binding domain, thereby facilitating the binding of that binding domain to its receptor.

[0350] A flexible space comprising a peptide is at least one amino acid in length and comprises non-charged amino acids with small side-chain R groups, such as, *e.g.*, small non-polar amino acids like A, C, G, S, and T. Thus, in an embodiment a flexible spacer can have a length of, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In still another embodiment, a flexible spacer can be, *e.g.*, between 1-3 amino acids, between 2-4 amino acids, between 3-5 amino acids, between 4-6 amino acids, or between 5-7 amino acids. Non-limiting examples of a flexible spacer include, *e.g.*, a G-spacers such as GGG, GGGG (SEQ ID NO: 522), and GGGGS (SEQ ID NO: 523) or an A-spacers such as AAA, AAAA (SEQ ID NO: 524) and AAAAT (SEQ ID NO: 525). Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein.

[0351] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1, 2, 3, 4, or 5 G-spacers; or at most 1, 2, 3, 4, or 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1, 2, 3, 4, or 5 A-spacers; or at most 1, 2, 3, 4, or 5 A-spacers. In another aspect of this embodiment, a Clostridial toxin or Clostridial toxin chimeric can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0352] It is envisioned that a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can comprise a flexible spacer in any and all locations with the proviso that the Clostridial toxin or Clostridial toxin chimeric is capable of performing the overall intoxication process. In aspects of this embodiment, a flexible spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and a binding domain, an enzymatic domain and an exogenous protease cleavage site. In other aspects of this embodiment, a flexible spacer is positioned between, e.g., a binding domain and a translocation domain, a binding domain and an enzymatic domain, a binding domain and an exogenous protease cleavage site. In yet other aspects of this embodiment, a flexible spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and a binding domain, a translocation domain and an exogenous protease cleavage site.

[0353] As another non-limiting example of an optional component, a Clostridial toxin or Clostridial toxin

chimeric can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™, human Influenza virus hemagluttinin (HA), human $p62^{c\text{-}Myc}$ protein (c-MYC), Vesicular Stomatitis Virus Glycoprotein (VSV-G), glycoprotein-D precursor of Herpes simplex virus (HSV), V5, AU1, and AU5; affinity-binding , such as. *e.g.*, polyhistidine (HIS), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as. *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., MOLECULAR CLONING A LABORATORY MANUAL, Vol. 3, 3rd ed. 2001); ANTIBODIES: A LABORATORY MANUAL (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and USING ANTIBODIES: A LABORATORY MANUAL: PORTABLE PROTOCOL NO. I (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0354] Thus, in an embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric can comprise, *e.g.*, at least 1, 2, 3, 4, or 5 epitope-binding regions. In other aspects of this embodiment, a Clostridial toxin or Clostridial toxin chimeric can comprise, *e.g.*, at most 1, 2, 3, 4, or 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof.

[0355] The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus, within, or at the carboxyl terminus of a Clostridial toxin or Clostridial toxin chimeric. Thus, in an embodiment, an epitope-binding region is located at the amino-terminus of a Clostridial toxin or Clostridial toxin chimeric. In another embodiment, an epitope-binding region is located at the carboxyl-terminus of a modified Clostridial toxin.

[0356] Aspects of the present specification provide, in part, polynucleotide molecules. As used herein, the term "polynucleotide molecule" is synonymous with "nucleic acid molecule" and refers to a polymeric form of nucleotides, such as, *e.g.*, ribonucleotides and deoxyribonucleotides. It is envisioned that any and all polynucleotide molecules that can encode a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can be useful, including, without limitation naturally-occurring and non-naturally-occurring DNA molecules and naturally-occurring and non-naturally-occurring RNA molecules. Non-limiting examples of naturally-occurring and non-naturally-occurring DNA molecules include single-

stranded DNA molecules, double-stranded DNA molecules, genomic DNA molecules, cDNA molecules, vector constructs, such as, *e.g.*, plasmid constructs, phagmid constructs, bacteriophage constructs, retroviral constructs and artificial chromosome constructs. Non-limiting examples of naturally-occurring and non-naturally-occurring RNA molecules include single-stranded RNA, double stranded RNA and mRNA.

[0357] Well-established molecular biology techniques that may be necessary to make a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification including, but not limited to, procedures involving polymerase chain reaction (PCR) amplification, restriction enzyme reactions, agarose gel electrophoresis, nucleic acid ligation, bacterial transformation, nucleic acid purification, nucleic acid sequencing and recombination-based techniques are routine procedures well within the scope of one skilled in the art and from the teaching herein. Non-limiting examples of specific protocols necessary to make a polynucleotide molecule encoding a modified Clostridial toxin are described in *e.g.*, MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Frederick M. Ausubel et al., eds. John Wiley & Sons, 2004). Additionally, a variety of commercially available products useful for making a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification are widely available. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0358] Thus, in an embodiment, a polynucleotide molecule encodes a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification.

[0359] Another aspect of the present specification provides, in part, a method of producing a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, such method comprising the step of expressing a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric in a cell. Another aspect of the present specification provides a method of producing a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, such method comprising the steps of introducing an expression construct comprising a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric into a cell and expressing the expression construct in the cell.

[0360] The methods disclosed in the present specification include, in part, a Clostridial toxin or Clostridial toxin chimeric. It is envisioned that any and all Clostridial toxins or Clostridial toxin chimeras disclosed in the present specification can be produced using the methods disclosed in the present specification. It is also envisioned that any and all polynucleotide molecules encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can be useful in producing a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification using the methods disclosed in the present specification.

[0361] The methods disclosed in the present specification include, in part, an expression construct. An expression construct comprises a polynucleotide molecule disclosed in the present specification operably-linked to an expression vector useful for expressing the polynucleotide molecule in a cell or cell-free extract. A wide variety of expression vectors can be employed for expressing a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, including, without limitation, a viral expression vector; a prokaryotic expression vector; eukaryotic expression vectors, such as, *e.g.*, a yeast expression vector, an insect expression vector and a

mammalian expression vector; and a cell-free extract expression vector. It is further understood that expression vectors useful to practice aspects of these methods may include those which express a Clostridial toxin or Clostridial toxin chimeric under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both. Non-limiting examples of expression vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

[0362] Thus, in aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification operably-linked to an expression vector. In aspects of this embodiment, the expression vector is, *e.g.*, a viral expression vector, a prokaryotic expression vector, a yeast expression vector, an insect expression vector, or a mammalian expression vector. On other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification operably-linked to a cell-free extract expression vector.

[0363] The methods disclosed in the present specification include, in part, a cell. It is envisioned that any and all cells can be used. Thus, aspects of this embodiment include, without limitation, prokaryotic cells including, without limitation, strains of aerobic, microaerophilic, capnophilic, facultative, anaerobic, gram-negative and gram-positive bacteria cells such as those derived from, *e.g.*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacteroides fragilis*, *Clostridia perfringens*, *Clostridia difficile*, *Caulobacter crescentus*, *Lactococcus lactis*, *Methylobacterium extorquens*, *Neisseria meningirulls*, *Neisseria meningitidis*, *Pseudomonas fluorescens* and *Salmonella typhimurium*; and eukaryotic cells including, without limitation, yeast strains, such as, *e.g.*, those derived from *Pichia pastoris*, *Pichia methanolica*, *Pichia angusta*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; insect cells and cell lines derived from insects, such as, *e.g.*, those derived from *Spodoptera frugiperda*, *Trichoplusia ni*, *Drosophila melanogaster* and *Manduca sexta*; and mammalian cells and cell lines derived from mammalian cells, such as, *e.g.*, those derived from mouse, rat, hamster, porcine, bovine, equine, primate and human. Cell lines may be obtained from the American Type Culture Collection, European Collection of Cell Cultures and the German Collection of Microorganisms and Cell Cultures. Non-limiting examples of specific protocols for selecting, making and using an appropriate cell line are described in *e.g.*, INSECT CELL CULTURE ENGINEERING (Mattheus F. A. Goosen et al. eds., Marcel Dekker, 1993); INSECT CELL CULTURES: FUNDAMENTAL AND APPLIED ASPECTS (J. M. Vlak et al. eds., Kluwer Academic Publishers, 1996); Maureen A. Harrison & Ian F. Rae, GENERAL TECHNIQUES OF CELL CULTURE (Cambridge University Press, 1997); CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Alan Doyle et al eds., John Wiley and Sons, 1998); R. Ian Freshney, CULTURE OF ANIMAL CELLS: A MANUAL OF BASIC TECHNIQUE (Wiley-Liss, 4th ed. 2000); ANIMAL CELL CULTURE: A PRACTICAL APPROACH (John R. W. Masters ed., Oxford University Press, 3rd ed. 2000); MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); BASIC CELL CULTURE: A PRACTICAL APPROACH (John M. Davis, Oxford Press, 2nd ed. 2002); and CURRENT

PROTOCOLS IN MOLECULAR BIOLOGY, *supra*, (2004). These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0364] The methods disclosed in the present specification include, in part, introducing into a cell a polynucleotide molecule. A polynucleotide molecule introduced into a cell can be transiently or stably maintained by that cell. Stably-maintained polynucleotide molecules may be extra-chromosomal and replicate autonomously, or they may be integrated into the chromosomal material of the cell and replicate non-autonomously. It is envisioned that any and all methods for introducing a polynucleotide molecule disclosed in the present specification into a cell can be used. Methods useful for introducing a polynucleotide molecule into a cell include, without limitation, chemical-mediated transfection or transformation such as, *e.g.*, calcium choloride-mediated, calcium phosphate-mediated, diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polylysine-mediated and polybrene-mediated; physical-mediated tranfection or transformation, such as, *e.g.*, biolistic particle delivery, microinjection, protoplast fusion and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection, see, *e.g.*, Introducing Cloned Genes into Cultured Mammalian Cells, pp. 16.1-16.62 (Sambrook & Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001). One skilled in the art understands that selection of a specific method to introduce an expression construct into a cell will depend, in part, on whether the cell will transiently contain an expression construct or whether the cell will stably contain an expression construct. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0365] In an aspect of this embodiment, a chemical-mediated method, termed transfection, is used to introduce into a cell a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. In chemical-mediated methods of transfection the chemical reagent forms a complex with the nucleic acid that facilitates its uptake into the cells. Such chemical reagents include, without limitation, calcium phosphate-mediated, see, *e.g.*, Martin Jordan & Florian Worm, *Transfection of adherent and suspended cells by calcium phosphate*, 33(2) Methods 136-143 (2004); diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, cationic polymer-mediated like polyethyleneimine (PEI)-mediated and polylysine-mediated and polybrene-mediated, see, *e.g.*, Chun Zhang et al., *Polyethylenimine strategies for plasmid delivery to brain-derived cells*, 33(2) Methods 144-150 (2004). Such chemical-mediated delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, CellPhect Transfection Kit (Amersham Biosciences, Piscataway, NJ); Mammalian Transfection Kit, Calcium phosphate and DEAE Dextran, (Stratagene, Inc., La Jolla, CA); LIPOFECTAMINE™ Transfection Reagent (Invitrogen, Inc., Carlsbad, CA); ExGen 500 Transfection kit (Fermentas, Inc., Hanover, MD), and SuperFect and Effectene Transfection Kits (Qiagen, Inc., Valencia, CA).

[0366] In another aspect of this embodiment, a physical-mediated method is used to introduce into a cell a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. Physical techniques include, without limitation, electroporation, biolistic and microinjection. Biolistics and microinjection techniques perforate the cell wall in order to introduce the nucleic acid molecule into the cell, see, *e.g.*, Jeike E. Biewenga et al., *Plasmid-mediated gene transfer in neurons using the biolistics technique*, 71(1) J. Neurosci. Methods 67-75 (1997); and John O'Brien & Sarah C. R. Lummis, Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic

dyes into mammalian cells, 33(2) Methods 121-125 (2004). Electroporation, also termed electropermeabilization, uses brief, high-voltage, electrical pulses to create transient pores in the membrane through which the nucleic acid molecules enter and can be used effectively for stable and transient transfections of all cell types, see, *e.g.*, M. Golzio et al., *In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression*, 33(2) Methods 126-135 (2004); and Oliver Greschet al., *New non-viral method for gene transfer into primary cells*, 33(2) Methods 151-163 (2004).

[0367] In another aspect of this embodiment, a viral-mediated method, termed transduction, is used to introduce into a cell a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. In viral-mediated methods of transient transduction, the process by which viral particles infect and replicate in a host cell has been manipulated in order to use this mechanism to introduce a polynucleotide molecule into the cell. Viral-mediated methods have been developed from a wide variety of viruses including, without limitation, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, picornaviruses, alphaviruses and baculoviruses, see, *e.g.*, Armin Blesch, *Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer*, 33(2) Methods 164-172 (2004); and Maurizio Federico, *From lentiviruses to lentivirus vectors*, 229 Methods Mol. Biol. 3-15 (2003); E. M. Poeschla, *Non-primate lentiviral vectors*, 5(5) Curr. Opin. Mol. Ther. 529-540 (2003); Karim Benihoud et al, *Adenovirus vectors for gene delivery*, 10(5) Curr. Opin. Biotechnol. 440-447 (1999); H. Bueler, *Adeno-associated viral vectors for gene transfer and gene therapy*, 380(6) Biol. Chem. 613-622 (1999); Chooi M. Lai et al., *Adenovirus and adeno-associated virus vectors*, 21(12) DNA Cell Biol. 895-913 (2002); Edward A. Burton et al., *Gene delivery using herpes simplex virus vectors*, 21(12) DNA Cell Biol. 915-936 (2002); Paola Grandi et al., *Targeting HSV amplicon vectors*, 33(2) Methods 179-186 (2004); Ilya Frolov et al., *Alphavirus-based expression vectors: strategies and applications*, 93(21) Proc. Natl. Acad. Sci. U. S. A. 11371-11377 (1996); Markus U. Ehrengruber, *Alphaviral gene transfer in neurobiology*, 59(1) Brain Res. Bull. 13-22 (2002); Thomas A. Kost & J. Patrick Condreay, *Recombinant baculoviruses as mammalian cell gene-delivery vectors*, 20(4) Trends Biotechnol. 173-180 (2002); and A. Huser & C. Hofmann, *Baculovirus vectors: novel mammalian cell gene-delivery vehicles and their applications*, 3(1) Am. J. Pharmacogenomics 53-63 (2003).

[0368] Adenoviruses, which are non-enveloped, double-stranded DNA viruses, are often selected for mammalian cell transduction because adenoviruses handle relatively large polynucleotide molecules of about 36 kb, are produced at high titer, and can efficiently infect a wide variety of both dividing and non-dividing cells, see, *e.g.*, Wim T. J. M. C. Hermens et al., *Transient gene transfer to neurons and glia: analysis of adenoviral vector performance in the CNS and PNS*, 71(1) J. Neurosci. Methods 85-98 (1997); and Hiroyuki Mizuguchi et al., *Approaches for generating recombinant adenovirus vectors*, 52(3) Adv. Drug Deliv. Rev. 165-176 (2001). Transduction using adenoviral-based system do not support prolonged protein expression because the nucleic acid molecule is carried by an episome in the cell nucleus, rather than being integrated into the host cell chromosome. Adenoviral vector systems and specific protocols for how to use such vectors are disclosed in, *e.g.*, VIRAPOWER™ Adenoviral Expression System (Invitrogen, Inc., Carlsbad, CA) and VIRAPOWER™ Adenoviral Expression System Instruction Manual 25-0543 version A, Invitrogen, Inc., (Jul. 15, 2002); and ADEASY™ Adenoviral Vector System (Stratagene, Inc., La Jolla, CA) and ADEASY™ Adenoviral Vector System Instruction Manual 064004f, Stratagene, Inc..

[0369] Polynucleotide molecule delivery can also use single-stranded RNA retroviruses, such as, *e.g.*, oncoretroviruses and lentiviruses. Retroviral-mediated transduction often produce transduction efficiencies close to 100%, can easily control the proviral copy number by varying the multiplicity of infection (MOI), and can be used to either transiently or stably transduce cells, see, *e.g.*, Tiziana Tonini et al., *Transient production of retroviral- and lentiviral-based vectors for the transduction of Mammalian cells*, 285 Methods Mol. Biol. 141-148 (2004); Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) Methods 164-172 (2004); Félix Recillas-Targa, *Gene transfer and expression in mammalian cell lines and transgenic animals*, 267 Methods Mol. Biol. 417-433 (2004); and Roland Wolkowicz et al., *Lentiviral vectors for the delivery of DNA into mammalian cells*, 246 Methods Mol. Biol. 391-411 (2004). Retroviral particles consist of an RNA genome packaged in a protein capsid, surrounded by a lipid envelope. The retrovirus infects a host cell by injecting its RNA into the cytoplasm along with the reverse transcriptase enzyme. The RNA template is then reverse transcribed into a linear, double stranded cDNA that replicates itself by integrating into the host cell genome. Viral particles are spread both vertically (from parent cell to daughter cells via the provirus) as well as horizontally (from cell to cell via virions). This replication strategy enables long-term persistent expression since the nucleic acid molecules of interest are stably integrated into a chromosome of the host cell, thereby enabling long-term expression of the protein. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year, see, *e.g.*, Luigi Naldini et al., *In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector*, 272(5259) Science 263-267 (1996). The Oncoretroviruses-derived vector systems, such as, *e.g.*, Moloney murine leukemia virus (MoMLV), are widely used and infect many different non-dividing cells. Lentiviruses can also infect many different cell types, including dividing and non-dividing cells and possess complex envelope proteins, which allows for highly specific cellular targeting.

[0370] Retroviral vectors and specific protocols for how to use such vectors are disclosed in, *e.g.*, Manfred Gossen & Hermann Bujard, *Tight control of gene expression in eukaryotic cells by tetracycline-responsive promoters*, U.S. Patent No. 5,464,758 (Nov. 7, 1995) and Hermann Bujard & Manfred Gossen, *Methods for regulating gene expression*, U.S. Patent No. 5,814,618 (Sep. 29, 1998) David S. Hogness, *Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same*, U.S. Patent No. 5,514,578 (May 7, 1996) and David S. Hogness, *Polynucleotide encoding insect ecdysone receptor*, U.S. Patent 6,245,531 (Jun. 12, 2001); Elisabetta Vegeto et al., *Progesterone receptor having C. terminal hormone binding domain truncations*, U.S. Patent No. 5,364,791 (Nov. 15, 1994), Elisabetta Vegeto et al., *Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy*, U.S. Patent No. 5,874,534 (Feb. 23, 1999) and Elisabetta Vegeto et al., *Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy*, U.S. Patent No. 5,935,934 (Aug. 10, 1999). Furthermore, such viral delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, BD™ Tet-Off and Tet-On Gene Expression Systems (BD Biosciences-Clonetech, Palo Alto, CA) and BD™ Tet-Off and Tet-On Gene Expression Systems User Manual, PT3001-1, BD Biosciences Clonetech, (Mar. 14, 2003), GENESWITCH™ System (Invitrogen, Inc., Carlsbad, CA) and GENESWITCH™ System A Mifepristone-Regulated Expression System for Mammalian Cells version D, 25-0313, Invitrogen, Inc., (Nov. 4, 2002); VIRAPOWER™

Lentiviral Expression System (Invitrogen, Inc., Carlsbad, CA) and VIRAPOWER™ Lentiviral Expression System Instruction Manual 25-0501 version E, Invitrogen, Inc., (Dec. 8, 2003); and COMPLETE CONTROL® Retroviral Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and COMPLETE CONTROL® Retroviral Inducible Mammalian Expression System Instruction Manual, 064005e.

[0371] The methods disclosed in the present specification include, in part, expressing from a polynucleotide molecule a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. It is envisioned that any of a variety of expression systems may be useful for expressing from a polynucleotide molecule a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification, including, without limitation, cell-based systems and cell-free expression systems. Cell-based systems include, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems, and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts, and *E. coli* extracts and generally are equivalent to the method disclosed herein. Expression of a polynucleotide molecule using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in *e.g.*, PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0372] A variety of cell-based expression procedures are useful for expressing a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. Examples included, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems, and mammalian expression systems. Viral expression systems include, without limitation, the VIRAPOWER™ Lentiviral (Invitrogen, Inc., Carlsbad, CA), the Adenoviral Expression Systems (Invitrogen, Inc., Carlsbad, CA), the ADEASY™ XL Adenoviral Vector System (Stratagene, La Jolla, CA) and the VIRAPORT® Retroviral Gene Expression System (Stratagene, La Jolla, CA). Non-limiting examples of prokaryotic expression systems include the CHAMPION™ pET Expression System (EMD Biosciences-Novagen, Madison, WI), the TRIEX™ Bacterial Expression System (EMD Biosciences-Novagen, Madison, WI), the QIAEXPRESS® Expression System (QIAGEN, Inc.), and the AFFINITY® Protein Expression and Purification System (Stratagene, La Jolla, CA). Yeast expression systems include, without limitation, the EASYSELECT™ *Pichia* Expression Kit (Invitrogen, Inc., Carlsbad, CA), the YES-ECHO™ Expression

Vector Kits (Invitrogen, Inc., Carlsbad, CA) and the SPECTRA™ *S. pombe* Expression System (Invitrogen, Inc., Carlsbad, CA). Non-limiting examples of baculoviral expression systems include the BACULODIRECT™ (Invitrogen, Inc., Carlsbad, CA), the BAC-TO-BAC® (Invitrogen, Inc., Carlsbad, CA), and the BD BACULOGOLD™ (BD Biosciences-Pharmigen, San Diego, CA). Insect expression systems include, without limitation, the *Drosophila* Expression System (DES®) (Invitrogen, Inc., Carlsbad, CA), INSECTSELECT™ System (Invitrogen, Inc., Carlsbad, CA) and INSECTDIRECT™ System (EMD Biosciences-Novagen, Madison, WI). Non-limiting examples of mammalian expression systems include the T-REX™ (Tetracycline-Regulated Expression) System (Invitrogen, Inc., Carlsbad, CA), the FLP-IN™ T-REX™ System (Invitrogen, Inc., Carlsbad, CA), the pcDNA™ system (Invitrogen, Inc., Carlsbad, CA), the pSecTag2 system (Invitrogen, Inc., Carlsbad, CA), the EXCHANGER® System, INTERPLAY™ Mammalian TAP System (Stratagene, La Jolla, CA), COMPLETE CONTROL® Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and LACSWITCH® II Inducible Mammalian Expression System (Stratagene, La Jolla, CA).

[0373] Another procedure of expressing a polynucleotide molecule encoding a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification employs a cell-free expression system such as, without limitation, prokaryotic extracts and eukaryotic extracts. Non-limiting examples of prokaryotic cell extracts include the RTS 100 *E. coli* HY Kit (Roche Applied Science, Indianapolis, IN), the ActivePro In Vitro Translation Kit (Ambion, Inc., Austin, TX), the ECOPRO™ System (EMD Biosciences-Novagen, Madison, WI) and the EXPRESSWAY™ Plus Expression System (Invitrogen, Inc., Carlsbad, CA). Eukaryotic cell extract include, without limitation, the RTS 100 Wheat Germ CECF Kit (Roche Applied Science, Indianapolis, IN), the TNT® Coupled Wheat Germ Extract Systems (Promega Corp., Madison, WI), the Wheat Germ IVT™ Kit (Ambion, Inc., Austin, TX), the Retic Lysate IVT™ Kit (Ambion, Inc., Austin, TX), the PROTEINSCRIPT® II System (Ambion, Inc., Austin, TX) and the TNT® Coupled Reticulocyte Lysate Systems (Promega Corp., Madison, WI).

[0374] The Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification disclosed in the present specification are produced by the cell in a single-chain form. In order to achieve full activity, this single-chain form has to be converted into its di-chain form. As discussed above, this conversion process is achieved by cleaving a protease cleavage site located within the di-chain loop region of the Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. This conversion process can be performed using a standard in vitro proteolytic cleavage assay or in a cell-based proteolytic cleavage system as described in patent application Ghanshani, et al., *Methods of Intracellular Conversion of Single-Chain Proteins into their Di-chain Form*, Attorney Docket No. 18469 PROV (BOT), which is hereby incorporated by reference in its entirety.

[0375] Aspects of the present specification disclose, in part, a composition comprising a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. In a further aspect, the composition is a pharmaceutical acceptable composition. As used herein, the term "pharmaceutically acceptable" refers to any molecular entity or composition that does not produce an adverse, allergic, or other untoward or unwanted reaction when administered to an individual. As used herein, the term "pharmaceutically acceptable composition" is synonymous with "pharmaceutical composition" and refers to a therapeutically effective concentration of an active ingredient, such as, *e.g.*, any of the Clostridial toxins or Clostridial toxin chimeras disclosed in the present specification. A pharmaceutical composition comprising a

Clostridial toxin or Clostridial toxin chimeric is useful for medical and veterinary applications. A pharmaceutical composition may be administered to a patient alone, or in combination with other supplementary active ingredients, agents, drugs or hormones. The pharmaceutical compositions may be manufactured using any of a variety of processes, including, without limitation, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, and lyophilizing. The pharmaceutical composition can take any of a variety of forms including, without limitation, a sterile solution, suspension, emulsion, lyophilizate, tablet, pill, pellet, capsule, powder, syrup, elixir or any other dosage form suitable for administration.

[0376] It is also envisioned that a pharmaceutical composition comprising a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification can optionally include a pharmaceutically acceptable carriers that facilitate processing of an active ingredient into pharmaceutically acceptable compositions. As used herein, the term "pharmacologically acceptable carrier" is synonymous with "pharmacological carrier" and refers to any carrier that has substantially no long term or permanent detrimental effect when administered and encompasses terms such as "pharmacologically acceptable vehicle, stabilizer, diluent, additive, auxiliary, or excipient." Such a carrier generally is mixed with an active compound or is permitted to dilute or enclose the active compound and can be a solid, semi-solid, or liquid agent. It is understood that the active ingredients can be soluble or can be delivered as a suspension in the desired carrier or diluent. Any of a variety of pharmaceutically acceptable carriers can be used including, without limitation, aqueous media such as, *e.g.*, water, saline, glycine, hyaluronic acid and the like; solid carriers such as, *e.g.*, mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like; solvents; dispersion media; coatings; antibacterial and antifungal agents; isotonic and absorption delaying agents; or any other inactive ingredient. Selection of a pharmacologically acceptable carrier can depend on the mode of administration. Except insofar as any pharmacologically acceptable carrier is incompatible with the active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of specific uses of such pharmaceutical carriers can be found in PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed. 1999); REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20th ed. 2000); GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and HANDBOOK OF PHARMACEUTICAL EXCIPIENTS (Raymond C. Rowe et al., APhA Publications, 4th edition 2003). These protocols are routine procedures and any modifications are well within the scope of one skilled in the art and from the teaching herein.

[0377] It is further envisioned that a pharmaceutical composition disclosed in the present specification can optionally include, without limitation, other pharmaceutically acceptable components (or pharmaceutical components), including, without limitation, buffers, preservatives, tonicity adjusters, salts, antioxidants, osmolality adjusting agents, physiological substances, pharmacological substances, bulking agents, emulsifying agents, wetting agents, sweetening or flavoring agents, and the like. Various buffers and refers to for adjusting pH can be used to prepare a pharmaceutical composition disclosed in the present specification, provided that the resulting preparation is pharmaceutically acceptable. Such

buffers include, without limitation, acetate buffers, citrate buffers, phosphate buffers, neutral buffered saline, phosphate buffered saline and borate buffers. It is understood that acids or bases can be used to adjust the pH of a composition as needed. Pharmaceutically acceptable antioxidants include, without limitation, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene. Useful preservatives include, without limitation, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric nitrate, a stabilized oxy chloro composition, such as, *e.g.*, PURITE® and chelants, such as, *e.g.*, DTPA or DTPA-bisamide, calcium DTPA, and CaNaDTPA-bisamide. Tonicity adjustors useful in a pharmaceutical composition include, without limitation, salts such as, *e.g.*, sodium chloride, potassium chloride, mannitol or glycerin and other pharmaceutically acceptable tonicity adjustor. The pharmaceutical composition may be provided as a salt and can be formed with many different acids, including, but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. It is understood that these and other substances known in the art of pharmacology can be included in a pharmaceutical composition useful in the specification.

[0378] In an embodiment, a composition comprises a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. In an aspect of this embodiment, the composition is a pharmaceutical composition comprising a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification. In aspects of this embodiment, a pharmaceutical composition comprising Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification further comprises a pharmacological carrier, a pharmaceutical component, or both a pharmacological carrier and a pharmaceutical component. In other aspects of this embodiment, a pharmaceutical composition comprising a Clostridial toxin or Clostridial toxin chimeric disclosed in the present specification further comprises at least one pharmacological carrier, at least one pharmaceutical component, or at least one pharmacological carrier and at least one pharmaceutical component.

[0379] Aspects of the present specification can also be described as follows:

1. A Clostridial toxin comprising a least one inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain, wherein the at least one inactivation cleavage site comprises a dual Thrombin-Thrombin site, a Factor Xa site, a dual Factor Xa-Thrombin site, and/or a MMP-9 site.
2. A Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, a di-chain loop region, an exogenous protease cleavage site, and at least two inactivation cleavage sites located within an inactivation cleavage site region; wherein the exogenous protease cleavage site located within the di-chain loop region.
3. A Clostridial toxin of aspect 2, wherein the inactivation cleavage sites comprise a dual Thrombin-Thrombin site and/or a dual Factor Xa-Thrombin site.
4. A Clostridial toxin chimeric comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

5. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids amino acids 462-496 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 458-492 of SEQ ID NO: 3; amino acids 464-487 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 463-496 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 458-491 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 434-467 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 453-486 of SEQ ID NO: 18, SEQ ID NO: 19, and/or SEQ ID NO: 20; amino acids 458-491 of SEQ ID NO: 21; amino acids 443-476 of SEQ ID NO: 23; and/or amino acids 434-467 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
6. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 618-634 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 614-630 of SEQ ID NO: 3; amino acids 605-621 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 613-629 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 609-625 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 587-603 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 604-620 of SEQ ID NO: 18; amino acids 605-621 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 610-626 of SEQ ID NO: 21; amino acids 596-612 of SEQ ID NO: 23; and/or amino acids 587-603 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
7. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 638-651 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 634-647 of SEQ ID NO: 3; amino acids 625-638 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 633-646 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 629-642 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 607-620 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 624-637 of SEQ ID NO: 18; amino acids 625-638 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 630-643 of SEQ ID NO: 21; amino acids 616-629 of SEQ ID NO: 23; and/or amino acids 607-620 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
8. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 665-687 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 661-683 of SEQ ID NO: 3; amino acids 652-674 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 660-682 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 656-678 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 634-659 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 651-676 of SEQ ID NO: 18; amino acids 652-677 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 657-679 of SEQ ID NO: 21; amino acids 643-668 of SEQ ID NO: 23; and/or amino acids 634-659 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
9. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 752-765 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 748-761 of SEQ ID NO: 3; amino acids 739-752 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 747-760 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 743-756 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino

acids 724-739 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 741-756 of SEQ ID NO: 18; amino acids 742-757 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 744-757 of SEQ ID NO: 21; amino acids 733-748 of SEQ ID NO: 23; and/or amino acids 724-739 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

10. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 826-835 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 824-831 of SEQ ID NO: 3; amino acids 813-824 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 821-830 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 817-826 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 800-809 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 817-826 of SEQ ID NO: 18; amino acids 818-827 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 818-827 of SEQ ID NO: 21; amino acids 809-819 of SEQ ID NO: 23; and/or amino acids 800-809 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
11. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 844-863 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 840-859 of SEQ ID NO: 3;; amino acids 831-850 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 839-858 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 835-854 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 818-837 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 835-854 of SEQ ID NO: 18; amino acids 836-855 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 836-855 of SEQ ID NO: 21; amino acids 828-847 of SEQ ID NO: 23; and/or amino acids 818-837 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
12. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-4, wherein the inactivation cleavage site region comprises amino acids 871-895 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 867-891 of SEQ ID NO: 3; amino acids 858-882 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 866-890 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 862-886 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 845-869 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 862-886 of SEQ ID NO: 18; amino acids 863-887 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 863-887 of SEQ ID NO: 21; amino acids 855-879 of SEQ ID NO: 23; and/or amino acids 845-869 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
13. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-12, wherein the Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a BaNT enzymatic domain, and/or a BuNT enzymatic domain.
14. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-13, wherein the inactivation cleavage site comprises Thrombin cleavage sites, Plasmin cleavage sites, Coagulation Factor VIIa cleavage sites, Coagulation Factor IXa cleavage sites, Coagulation Factor Xa cleavage sites, Coagulation Factor XIa cleavage sites, Coagulation Factor XIIa cleavage sites, plasma kallikrein cleavage sites, protease-activated G protein-coupled receptor-1 (PAR1) cleavage sites, PAR 2 cleavage sites, PAR3 cleavage sites, PAR4 cleavage sites, Matrix Metalloproteinase-2 (MMP-2)

cleavage sites, Matrix Metalloproteinase-9 (MMP-9) cleavage sites, Furin cleavage sites, urokinase-type Plasminogen activator (uPA) cleavage sites, tissue-type Plasminogen activator (tPA) cleavage sites, Tryptase-ε cleavage sites, Mouse mast cell protease-7 (mMCP-7) cleavage sites, endothelin-converting enzyme-1 (ECE-1) cleavage sites, Kell blood group cleavage sites, DPPIV cleavage sites, ADAM metallopeptidase with thrombospondin type 1 motif-13 (ADAMTS13) cleavage sites, and/or Cathepsin L cleavage sites.

15. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-14, wherein the Clostridial toxin translocation domain comprises a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, and/or a BuNT translocation domain.
16. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-15, wherein the inactivation cleavage site comprises a dual Thrombin-Thrombin site, a Factor Xa site, a dual Factor Xa-Thrombin site, and/or a MMP-9 site.
17. The Clostridial toxin and/or Clostridial toxin chimeric of aspects 1-16, wherein the, a non-Clostridial toxin binding domain, comprises a opiod binding domain, a tachykinin binding domain, a melanocortin binding domain, a galanin binding domain, a granin binding domain, a Neuropeptide Y related peptide binding domain, a neurohormone binding domain, a neuroregulatory cytokine binding domain, a kinin peptide binding domain, a growth factor binding domain, and/or a glucagon like hormone binding domain.
18. A BoNT/A comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
19. A Clostridial toxin comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain, a BoNT/A binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
20. A Clostridial toxin comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain, a BoNT/A binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
21. A Clostridial toxin comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
22. A Clostridial toxin comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

23. The toxin and/or chimeric of aspects 18-22, wherein the inactivation cleavage site region comprises amino acids 462-496, 618-634, 638-651, 665-687, 752-765, 826-835, 844-863, and/or 871-895 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5, and/or amino acids 458-492, 614-630, 634-647, 665-687, 748-761, 822-831, 840-859, and/or 867-891 of SEQ ID NO: 3.
24. A BoNT/B comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
25. A Clostridial toxin comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain, a BoNT/B binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
26. A Clostridial toxin comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain, a BoNT/B binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
27. A Clostridial toxin comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
28. A Clostridial toxin comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
29. The toxin and/or chimeric of aspects 24-28, wherein the inactivation cleavage site region comprises amino acids 464-487, 605-621, 625-638, 652-674, 739-752, 813-824, 831-850, and/or 858-882 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10.
30. A BoNT/C1 comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
31. A Clostridial toxin comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain, a BoNT/C1 binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
32. A Clostridial toxin comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain, a BoNT/C1 binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

33. A Clostridial toxin comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
34. A Clostridial toxin comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
35. The toxin and/or chimeric of aspects 30-34, wherein the inactivation cleavage site region comprises amino acids 463-496, 613-629, 633-646, 660-682, 747-760, 821-830, 839-858, and/or 866-890 of SEQ ID NO: 11 and/or SEQ ID NO: 12.
36. A BoNT/D comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
37. A Clostridial toxin comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain, a BoNT/D binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
38. A Clostridial toxin comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain, a BoNT/D binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
39. A Clostridial toxin comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
40. A Clostridial toxin comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
41. The toxin and/or chimeric of aspects 36-40, wherein the inactivation cleavage site region comprises amino acids 458-491, 609-625, 629-642, 656-678, 743-756, 817-826, 835-854, and/or 862-886 of SEQ ID NO: 13 and/or SEQ ID NO: 14.
42. A BoNT/E comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
43. A Clostridial toxin comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain, a BoNT/E binding domain, and an inactivation cleavage site located within an inactivation cleavage site

region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

44. A Clostridial toxin comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain, a BoNT/E binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
45. A Clostridial toxin comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
46. A Clostridial toxin comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
47. The toxin and/or chimeric of aspects 42-46, wherein the inactivation cleavage site region comprises amino acids 434-467, 587-603, 607-620, 634-659, 724-739, 800-809, 818-837, and/or 845-869 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17.
48. A BoNT/F comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
49. A Clostridial toxin comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain, a BoNT/F binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
50. A Clostridial toxin comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain, a BoNT/F binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
51. A Clostridial toxin comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
52. A Clostridial toxin comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

53. The toxin and/or chimeric of aspects 48-52, wherein the inactivation cleavage site region comprises amino acids 453-486, 604-620, 624-637, 651-676, 741-756, 817-826, 835-854, and/or 862-886 of SEQ ID NO: 18; and/or amino acids 453-486, 605-621, 625-638, 652-677, 742-757, 818-827, 836-855, and/or 863-887 of SEQ ID NO: 19 and/or SEQ ID NO: 20.
54. A BoNT/G comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
55. A Clostridial toxin comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain, a BoNT/G binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
56. A Clostridial toxin comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain, a BoNT/G binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
57. A Clostridial toxin comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
58. A Clostridial toxin comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
59. The toxin and/or chimeric of aspects 54-58, wherein the inactivation cleavage site region comprises amino acids 458-491, 610-626, 630-643, 657-679, 744-757, 818-827, 836-855, and/or 863-887 of SEQ ID NO: 21.
60. A BaNT comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
61. A Clostridial toxin comprising a BaNT enzymatic domain, a BaNT translocation domain, a BaNT binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
62. A Clostridial toxin comprising a BaNT enzymatic domain, a BaNT translocation domain, a BaNT binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

63. A Clostridial toxin comprising a BaNT enzymatic domain, a BaNT translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
64. A Clostridial toxin comprising a BaNT enzymatic domain, a BaNT translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
65. The toxin and/or chimeric of aspects 60-64, wherein the inactivation cleavage site region comprises amino acids 443-476, 596-612, 616-629, 643-668, 733-748, 809-819, 828-847, and/or 855-879 of SEQ ID NO: 23.
66. A BuNT comprising an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
67. A Clostridial toxin comprising a BuNT enzymatic domain, a BuNT translocation domain, a BuNT binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
68. A Clostridial toxin comprising a BuNT enzymatic domain, a BuNT translocation domain, a BuNT binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
69. A Clostridial toxin comprising a BuNT enzymatic domain, a BuNT translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
70. A Clostridial toxin comprising a BuNT enzymatic domain, a BuNT translocation domain, a non-Clostridial toxin binding domain, an exogenous protease cleavage site, a di-chain loop region, and an inactivation cleavage site located within an inactivation cleavage site region, wherein the exogenous protease cleavage site is located within the di-chain loop region; the wherein inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.
71. The toxin and/or chimeric of aspects 66-70, wherein the inactivation cleavage site region comprises amino acids 434-467, 587-603, 607-620, 634-659, 724-739, 800-809, 818-837, and/or 845-869 of SEQ ID NO: 24 and/or SEQ ID NO: 25.
72. The toxin and/or chimeric of aspects 1-71, wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater relative to the same and/or similar to the Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site.

73. The toxin and/or chimeric of aspect 72, wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, and/or at least 300%, relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site, and/or

wherein Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90%, at most 100%, 110%, at most 120%, at most 130%, at most 140%, at most 150%, at most 160%, at most 170%, at most 180%, at most 190%, at most 200%, 210%, at most 220%, at most 230%, at most 240%, at most 250%, at most 260%, at most 270%, at most 280%, at most 290%, and/or at most 300%, relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site, and/or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater by about 10% to about 300%, about 20% to about 300%, about 30% to about 300%, about 40% to about 300%, about 50% to about 300%, about 60% to about 300%, about 70% to about 300%, about 80% to about 300%, about 90% to about 300%, and/or about 100% to about 300%, relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site, or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than at least 1-fold, at least 1-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, and/or at least 10-fold, relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site, and/or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater than at least 1-fold, at most 1-fold, at most 3-fold, at most 4-fold, at most 5-fold, at most 6-fold, at most 7-fold, at most 8-fold, at most 9-fold, and/or at most 10-fold, relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the inactivation cleavage site, and/or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprising an inactivation cleavage site has a safety margin that is greater by about 1-fold to about 10-fold, about 1-fold to about 9-fold, about 1-fold to about 8-fold, about 1-fold to about 7-fold, about 1-fold to about 6-fold, about 1-fold to about 5-fold, about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, and/or about 2-fold to about 5-fold.

74. The toxin and/or chimeric of aspects 1-73, wherein the addition of the inactivation cleavage site increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site.

75. The toxin and/or chimeric of aspect 74, wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, 110%, at least 120%, at least 130%, at least 140%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, at least 200%, 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, and/or at least 300%, or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90%, at most 100%, 110%, at most 120%, at most 130%, at most 140%, at most 150%, at most 160%, at most 170%, at most 180%, at most 190%, at most 200%, 210%, at most 220%, at most 230%, at most 240%, at most 250%, at most 260%, at most 270%, at most 280%, at most 290%, and/or at most 300%, or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by about 10% to about 300%, about 20% to about 300%, about 30% to about 300%, about 40% to about 300%, about 50% to about 300%, about 60% to about 300%, about 70% to about 300%, about 80% to about 300%, about 90% to about 300%, and/or about 100% to about 300%, or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by at least 1-fold, at least 1-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, and/or at least 10-fold, and/or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by, *e.g.*, at most 1-fold, at most 3-fold, at most 4-fold, at most 5-fold, at most 6-fold, at most 7-fold, at most 8-fold, at most 9-fold, and/or at most 10-fold, and/or

wherein the Clostridial toxin and/or Clostridial toxin chimeric comprises the addition of an inactivation cleavage site that increases the safety margin of the Clostridial toxin and/or Clostridial toxin chimeric relative to the same and/or similar Clostridial toxin and/or Clostridial toxin chimeric, but without the additional inactivation cleavage site by about 1-fold to about 10-fold, about 1-fold to about 9-fold, about 1-fold to about 8-fold, about 1-fold to about 7-fold, about 1-fold to about 6-fold, about 1-fold to about 5-fold, about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, and/or about 2-fold to about 5-fold.

76. A polynucleotide molecule encoding a toxin and/or chimeric according to any one of aspects 1-75.
77. The polynucleotide molecule, wherein the molecule comprises SEQ ID NO: 530, SEQ ID NO: 532, SEQ ID NO: 534, and/or SEQ IS NO: 536.
78. A method of producing a Clostridial toxin and/or Clostridial toxin chimeric comprising the step of expressing in a cell a polynucleotide molecule according to aspect 76 and/or 77, wherein expression from the polynucleotide molecule produces the encoded Clostridial toxin and/or Clostridial toxin chimeric.
79. A method of producing a modified Clostridial toxin comprising the steps of:
    a. introducing into a cell a polynucleotide molecule according to aspect 76 and/or 77; and
    b. expressing the polynucleotide molecule, wherein expression from the polynucleotide molecule produces the encoded Clostridial toxin and/or Clostridial toxin chimeric.
80. A Clostridial toxin comprising SEQ ID NO: 531, SEQ ID NO: 533, SEQ ID NO: 535, and/or SEQ ID NO: 537.
81. A Clostridial toxin comprising SEQ ID NO: 531.
82. A Clostridial toxin comprising SEQ ID NO: 533.
83. A Clostridial toxin comprising SEQ ID NO: 535.
84. A Clostridial toxin comprising SEQ ID NO: 537.

EXAMPLES

[0380] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of disclosed embodiments and are in no way intended to limit any of the embodiments disclosed in the present specification.

Example 1

Identification of Inactivation Cleavage Site Regions

[0381] This example illustrates how to identify regions within a Clostridial toxin or Clostridial toxin chimeric suitable for modifying the toxin to comprise an inactivation cleavage site and how to make a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site.

[0382] To identify a location or locations in the protein structure suitable as a potential inactivation cleavage site region, the three-dimensional structure of a BoNT/A was initially analyzed by computer software to identify surface exposed loops or extended regions that would be more accessible to a protease. Of the regions predicted to be accessible, eight were selected for further analysis: amino acids 462-496 of SEQ ID NO: 1, amino acids 618-634 of SEQ ID NO: 1, amino acids 638-651 of SEQ ID NO: 1, amino acids 665-687 of SEQ ID NO: 1, amino acids 752-765 of SEQ ID NO: 1, and amino acids 826-835 of SEQ IN NO: 1, amino acids 844-863 of SEQ ID NO: 1, and amino acids 871-895 of SEQ ID NO: 1.

[0383] To determine whether a region identified by computer analysis could function as an inactivation cleavage site region, thrombin cleavage sites were genetically engineered into these regions using multi-primer mutagenesis and assayed for its ability to be cleaved by thrombin. A 50 µL reaction was assembled comprising a primer pool of unidirectional oligonucleotide primers each containing the desired modification (125 ng of each primer) mixed in different ratios with a DNA template comprising an expression construct encoding a BoNT/A, such as, *e.g.*, an expression construct comprising SEQ ID NO: 526 encoding SEQ ID NO: 527, or an expression construct comprising SEQ ID NO: 528 encoding SEQ

ID NO: 529, that was hypermethylated with *dam* methylase. To this mixture was added 5 µL of 10x PCR Buffer, 1 µL of deoxyribonucleotides (dNTPs), 1µL of 2.5 units/µL PFUULTRA™ High Fidelity DNA polymerase (Stratagene, La Jolla, CA), *Pfu* DNA ligase, ATP, and nuclease-free water to a final volume of 50 µL. The thermocycler conditions were: 30 cycles of 96 °C for 1 minute, 60 °C for 30 seconds, and 68 °C for 20 minutes. Following thermocycling, 1 µL of *Dpn*I restriction enzyme (Stratagene, La Jolla, CA) was added to the reaction and incubated for 1 hour at 37 °C to digest the template DNA and reduce the recovery of wild-type clones. The digested reaction mixture was transformed into electro-competent *E. coli* BL21(DE3) Acella cells (Edge BioSystems, Gaithersburg, MD) by electroporation, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs were identified as kanamycin resistant colonies. Candidate constructs were isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by sequencing to determine the frequency and identity of the mutations incorporated. Table 7 lists each BoNT/A comprising a Thrombin cleavage site (BoNT/A-TCS) made and tested in this thrombin scanning analysis.

TABLE 7. Thrombin Scanning Analysis

Region	Modification	Expression	Thrombin Sensitivity	BoNT/A Potency
462-496	T482insLVPRGS	+	ND	ND
462-496	A489insLVPRGS	++	++	ND
618-634	E620insLVPRGS	+	ND	ND
638-651	M646insLVPRGS	–/+	ND	ND
665-687	I673insLVPRGS	+	ND	ND
752-765	E758insLVPRGS	–	ND	ND
826-835	delR827GT-insLVPRGS	–/+	ND	ND
844-863	T844insLVPRGS	+++	+	ND
844-863	D848insLVPRGS	+++	+	ND
844-863	Q852insLVPRGS	–/+	ND	ND
844-863	L862insLVPRGS	+++	++	ND
871-895	E868insLVPRGS			ND
871-895	delE868YIKNI-insLVPRGS			ND
871-895	K871insLVPRGS	+++	+++	ND
871-895	I873insLVPRGS	+++	++++	ND
871-895	delN872IINTS-insLVPRGS			ND
871-895	T876insLVPRGS			ND
871-895	L879insVPRGS			ND
871-895	delL879NLRYE-insLVPRGS			ND
871-895	N880insLVPRGS	+++	++++	4.05
871-895	L881insVPRGS			ND
871-895	delL881RYESN-insLVPRGS			ND
871-895	Y883insLVPRGS			ND
871-895	E884insLVPRGS	+++	+++	>50

871-895	S885insLVPRGS			ND
871-895	delH887LIDLS-insLVPRGS			ND
871-895	L888insVPRGS			ND
871-895	D890insLVPRGS	++	++++	3.15
871-895	L891insVPRG			ND
871-895	delS892RYA-insVPRG			ND
467-496	T482insLVPRGS A489insLVPRGS	+	ND	ND
618-634 665-687	E620insLVPRGS I673insLVPRGS	+	ND	ND
638-651 665-687	M646insLVPRGS I673insLVPRGS	+	ND	ND
825-832 871-895	delR827GT-insLVPRGS K871insLVPRGS	+	+++	ND
844-863	T844insLVPRGS Q852insLVPRGS	–/+	ND	ND
844-863	Q852insLVPRGS L862insLVPRGS	–	ND	ND
825-832 871-895	delR827GT-insLVPRGS K871insLVPRGS	+	ND	ND
825-832 871-895	delR827GT-insLVPRGS K880insLVPRGS	–	ND	ND
871-895	E868insLVPRGS I873insLVPRGS			1.32
871-895	delE868YIKNI-insLVPRGS delL881RYESN-insLVPRGS			0.86
871-895	I873insLVPRGS E884insLVPRGS			1.32
871-895	L881insVPRGS L891insVPRG	+++	++++	4.20
Control	Backbone	+++	–	Yes

Protease sensitivity: +, less than 25% of toxin proteolyzed within about 1 to about 4 hours; ++, from 25% to 50% of toxin proteolyzed within about 1 to about 4 hours; +++, from 51% to 75% of toxin proteolyzed within about 1 to about 4 hours; ++++, more than 75% of toxin proteolyzed within about 1 to about 4 hours.
BoNT/A potency is calculated by dividing the EC_{50} value of the toxin into the EC_{50} value of the backbone control.
ND is not determined.

[0384] To determine the expression level of soluble protein for each BoNT/A-TCS, an expression construct comprising each BoNT/A-TCS was expressed, purified by immobilized metal affinity chromatography and analyzed by SDS-PAGE analysis. First, using a 96-well plate, 100 µL of PA-0.5G media containing 50 µg/mL Kanamycin was inoculated with a single colony of BL21(DE3) cells harboring the appropriate expression construct and grown at 37 °C with shaking overnight. A 5 µL aliquot from this starter culture was used to inoculate 1 mL of ZYP-5052 containing 50 µg/mL kanamycin and grown at 37 °C with shaking for 3.5 hours and then 22 °C for 16 hours. A 110 µL aliquot of Protein Extraction Reagent comprising 10X FASTBREAK™ Cell Lysis Reagent (Promega Corp., Madison, WI), 250 U/mL Benzonase nuclease (EMD Biosciences-Novagen, Madison, WI), and 10X Protease Inhibitor Cocktail III

(EMD Biosciences-Calbiochem, Gibbstown, NJ) was added to each 1 mL expression culture in a 96-well plate. 75 µL of HISLINK™ resin (Promega Corp., Madison, WI) was next transferred to each well and the mixture was alternately mixed by pipetting and by shaking at 900 rpm for 30 minutes. The lysates were transferred to a filter plate with a 25 µm pore size (Promega Corp., Madison, WI), with membranes pre-wetted with water, and the liquid was removed by vacuum filtration. The resin was washed three times with 200 µL Wash Buffer comprising 100 mM HEPES (pH 7.5), 10 mM imidazole. The protein was eluted by adding 200 µL Elution Buffer comprising 100 mM HEPES (pH 7.5), 500 mM imidazole, incubating for 5 minutes and the elute collected by vacuum filtration into a 96-well plate.

[0385] To perform SDS-PAGE, an equal volume of 2 x Laemmli Sample Buffer was added to the IMAC purified BoNT/A comprising a thrombin cleavage site, and the mixture incubated at 95 °C for 5 minutes. A 15 µL aliquot was loaded and separated by MOPS polyacrylamide gel electrophoresis using NUPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. The gel was washed and fixed in 10% methanol and 7% acetic acid for 30 minutes. The wash solution was removed and the gel incubated in SYPRO Ruby protein gel stain solution for 3 hours to overnight at room temperature. The stained gel was destained in 10% methanol and 7% acetic acid for 30 minutes. The destained gel was visualized with a Fluro-S-Max digital imager (Bio-Rad).

[0386] The results of the expression analysis are given in Table 7. In general, toxins harboring an inserted thrombin cleavage site in the inactivation regions comprising amino acids 462-496 of SEQ ID NO: 1, amino acids 844-863, or amino acids 871-895 of SEQ ID NO: 1 were expressed well. For example, toxins comprising A489insLVPRGS was expressed at about 50% that of a wild-type control construct and toxins comprising D848insLVPRGS or N880insLVPRGS were expressed at, or near, control levels (Table 7). These results reveal that inactivation cleavage site regions located within the translocation domain and/or the H_{CN} binding subdomain tolerated the modification of regions to include a protease cleavage site.

[0387] To further explore the extent to which the inactivation cleavage site regions identified could tolerate modifications that introduce a protease cleavage site, toxins were modified to include thrombin cleavage sites throughout the region. For example, toxins comprising T884insLVPRGS or L862ins LVPRGS were made to examine the inactivation cleavage site region comprising 844-863 of SEQ ID NO: 1. Similarly, toxins comprising E868insLVPRGS, delE868YIKNI-insLVPRGS, delN872IINTS-insLVPRGS, T876insLVPRGS, L879insVPRGS, delL879NLRYE-insLVPRGS, L881insVPRGS, delL881RYESN-insLVPRGS, Y883insLVPRGS, E884insLVPRGS, S885insLVPRGS, delH887LIDLS-insLVPRGS, L888insLVPRGS, L891insVPRG, and delS892RYA-insVPRG were made to examine the inactivation cleavage site region comprising 871-895 of SEQ ID NO: 1. Both insertion and substitution modifications were made to examine whether the type of modification had any affect. In general, all toxins harboring an inserted thrombin cleavage site in these inactivation regions were expressed at, or near, the levels of a wild-type control construct. These results reveal that inactivation cleavage site regions within the translocation domain and/or the H_{CN} binding subdomain can tolerate modifications placed anywhere within an inactivation site region.

[0388] Lastly, the ability of an inactivation site region to tolerate the presence of two or more protease cleavage sites was examined (Table 7). These results indicate that inactivation cleavage site regions

within the translocation domain and/or the H_{CN} binding subdomain can tolerate modifications placing two or more protease cleavage sites within an inactivation site region.

[0389] To determine whether a BoNT/A comprising a thrombin cleavage site could be cleaved by thrombin, an in vitro thrombin cleavage assay was performed. 5 µg of each purified BoNT/A-TCS was incubated with 1 U of Thrombin (Novagen) at 23 °C for 1 hour, 3 hours, and 18.5 hours. A zero-enzyme control was also set up in parallel for each BoNT/A-TCS. Samples were taken at each time point and quenched with SDS-Loading Buffer including DTT and analyzed by SDS-PAGE as described above.

[0390] The results of the expression analysis are given in Table 7. In general, modification of an inactivation cleavage site region comprising amino acids 467-496, 844-863, or 871-895 of SEQ ID NO: 1 to include a protease cleavage site resulted in a toxin that was susceptible to proteolytic cleavage by the appropriate protease.

[0391] To determine whether a BoNT/A comprising a thrombin cleavage site maintained its potency, a BoNT/A activity assay was performed using a cell-based activity assay. To conduct a cell-based activity assay, about 1.2 x 10^6 Neuro-2a or SiMa cells were plated into the wells of 24-well tissue culture plates containing 1 mL of serum-free medium containing Minimum Essential Medium, 2 mM GLUTAMAX™ I with Earle's salts, 1 x B27 supplement, 1 x N2 supplement, 0.1 mM Non-Essential Amino Acids, 10 mM HEPES and 25 µg/mL GT1b. The cells were incubated in a 37 °C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest and neurite extension (approximately 3 days). The media was aspirated from each well and replaced with either 1) fresh media containing no toxin (untreated cell line) or 2) fresh media containing 1 nM of a BoNT/A complex (treated cell line). After an overnight incubation, the cells were washed by aspirating the media and rinsing each well with 200 µL of 1 x PBS. To harvest the cells, the 1 x PBS was aspirated, the cells were lysed by adding 50 µl of 2 x SDS Loading Buffer, the lysate was transferred to a clean test tube and the sample was heated to 95 °C for 5 minutes.

[0392] To detect for the presence of cleaved SNAP-25 products, an aliquot from each harvested sample was analyzed by Western blot. In this analysis, a 12 µl aliquot of the harvested sample was separated by MOPS polyacrylamide gel electrophoresis using NUPAGE® Novex 12% Bis-Tris precast polyacrylamide gels (Invitrogen Inc., Carlsbad, CA) under denaturing, reducing conditions. Separated peptides were transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen Inc., Carlsbad, CA) by Western blotting using a TRANS-BLOT® SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes were blocked by incubating at room temperature for 2 hours in a solution containing Tris-Buffered Saline (TBS) (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20® (polyoxyethylene (20) sorbitan monolaureate), 2% Bovine Serum Albumin (BSA), 5% nonfat dry milk. Blocked membranes were incubated at 4 °C for overnight in TBS, 0.1% TWEEN-20® (polyoxyethylene (20) sorbitan monolaureate), 2% BSA, and 5% nonfat dry milk containing either 1) a 1:5,000 dilution of an α-SNAP-25 mouse monoclonal antibody as the primary antibody (SMI-81; Sternberger Monoclonals Inc., Lutherville, MD); or 2) a 1:5,000 dilution of S9684 α-SNAP-25 rabbit polyclonal antiserum as the primary antibody (Sigma, St. Louis, MO). Both α-SNAP-25 mouse monoclonal and rabbit polyclonal antibodies can detect both the uncleaved SNAP-25 substrate and the SNAP-25 cleavage product, allowing for the assessment of overall SNAP-25 expression in each cell line

and the percent of SNAP-25 cleaved after BoNT/A treatment as a parameter to assess the amount of BoNT/A uptake. Primary antibody probed blots were washed three times for 15 minutes each time in TBS, TWEEN-20® (polyoxyethylene (20) sorbitan monolaureate). Washed membranes were incubated at room temperature for 2 hours in TBS, 0.1% TWEEN-20® (polyoxyethylene (20) sorbitan monolaureate), 2% BSA, and 5% nonfat dry milk containing either 1) a 1:10,000 dilution of goat polyclonal anti-mouse immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Zymed, South San Francisco, CA) as a secondary antibody; or 2) a 1:10,000 dilution of goat polyclonal anti-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Zymed, South San Francisco, CA) as a secondary antibody. Secondary antibody-probed blots were washed three times for 15 minutes each time in TBS, 0.1% TWEEN-20® (polyoxyethylene (20) sorbitan monolaureate). Signal detection of the labeled SNAP-25 products were visualized using the ECL Plus™ Western Blot Detection System (GE Healthcare, Amersham Biosciences, Piscataway, NJ) and the membrane was imaged and the percent of cleaved quantified with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (GE Healthcare, Amersham Biosciences, Piscataway, NJ). The choice of pixel size (100 to 200 pixels) and PMT voltage settings (350 to 600, normally 400) depended on the individual blot.

[0393] The results of the expression analysis are given in Table 7. In general, modification of an inactivation cleavage site region comprising amino acids 467-496, 844-863, or 871-895 of SEQ ID NO: 1 to include a protease cleavage site resulted in a potent toxin that was able to execute the overall intoxication process.

[0394] Taken together, these results indicate that although eight different inactivation cleavage regions were identified, not all were able to support the insertion of a functional thrombin cleavage site. In general, modification of the inactivation cleavage site regions comprising amino acids 467-496, 844-863 and 871-895 of SEQ ID NO: 1 to include a protease cleavage site resulted in a stably produced toxin that that was able to execute the overall intoxication process and was sensitive to proteolytic cleavage by the appropriate protease.

[0395] Because the three-dimensional structure of all Clostridial toxins are simila, the corresponding locations in BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G, TeNT, BaNT, and BuNT are also suitable as inactivation cleavage site regions. Table 5 lists these regions.

Example 2

Protease Cleavage Site Analysis

[0396] This example illustrates how to make a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site.

[0397] To explore whether protease claeavge sites other than thrombin could be useful as an inactivation site, toxins comprising many different protease cleavage sites were examined.

[0398] To make a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site, protease cleavage sites were genetically engineered into inactivation cleavage site regions using multi-primer mutagenesis as described in Example 1. Table 8 lists the expression constructs modified to contain a protease cleavage site.

[0399] To determine whether a BoNT/A comprising a protease cleavage site could be cleaved by its cognate protease, in vitro protease cleavage assays was performed essentially as described above, but

using the appropriate protease instead of thrombin. Samples were taken at each time point and quenched with SDS-Loading Buffer including DTT, and analyzed by SDS-PAGE as described in Example 1.

[0400] The results of the expression analysis are given in Table 7. In general, modification of an inactivation cleavage site region comprising amino acids 467-496, 844-863, or 871-895 of SEQ ID NO: 1 to include a protease cleavage site resulted in a toxin that was susceptible to proteolytic cleavage by the appropriate protease.

TABLE 8. Protease Cleavage Site Analysis

Protease Cleavage Site	Region	Modification	Protease Sensitivity	BoNT/A Potency
Factor Xa	535	E535insG	+	2.70
Factor Xa	844-863	L863insIEGR	+	>50
Factor Xa	871-895	K871insIEGR	++	6.15
Factor Xa	871-895	I873insEGR	+	3.97
Factor Xa	871-895	L881insIEG	ND	ND
Factor Xa	871-895	E884insIEGR	+	2.95
Factor Xa	871-895	L891insIEGR	++	ND
Factor Xa	1272	E1272insG	+	ND
Factor Xa x 2	535 1272	E535insG E1272insG	+	ND
Factor Xa x 2	871-895	K871insIEGR L891insIEGR	++	4.35
Factor Xa x 2	871-895	I873insEGR L891insIEGR	+	7.63
Factor Xa x 2	871-895	L881insIEG L891insIEGR	++	>50
Factor Xa tPA	871-895	I873insEGR delS885NHLIDL-insPQRGRSA	ND	ND
Factor Xa Thrombin	871-895	I873insEGR E884insLVPRG	+ ++++	3.29
MMP-2	871-895	S885insGPLGMLSQ	+	6.55
MMP-2	871-895	delK871NIINTSI-insGPLGMLSQ	++	5.27
MMP-2	871-895	delS885NHLIDLS-insGPLGMLSQ	++	4.76
MMP-9	871-895	K871insGPLGLWAQ	ND	ND
MMP-9	871-895	delK871NIINTSI-insGPLGLWAQ	+	3.36
MMP-9	871-895	I873insGPLGLWAQ		22.8
MMP-9	871-895	delI874NTSILNL-insGPLGLWAQ		37.7
MMP-9	871-895	delL881RYESNHL-insGPLGLWAQ	ND	ND
MMP-9	871-895	E884insGPLGLWAQ	ND	ND
MMP-9	871-895	delS885NHLIDLS-insGPLGLWAQ	+	4.38
MMP-9	871-895	S885insGPLGLWAQ		3.38
MMP-9	871-895	L891insGPLGLWAQ		20.61
MMP-9	871-895	delK871NIINTSI-insGPLGLWAQ	ND	ND

Thrombin		E884insLVPRG		
MMP-9 Factor Xa	871-895	delK871NIINTSI-insGPLGLWAQ E884insIEGR		19.62
u-PA	871-895	delN872IINTSI-insPGSGKSA	+	ND
u-PA	871-895	S885insPGSGKSA	++	3.00
u-PA	871-895	delN886HLIDL-insPGSGKSA	++	4.90
t-PA	871-895	delN872IINTSI-insPQRGRSA	++	3.65
t-PA	871-895	S885insPQRGRSA	+++	3.30
t-PA	871-895	delS885NHLIDL-insPQRGRSA	++	4.80
Thrombin tPA	871-895	I873LVPRGS delS885NHLIDL-insPQRGRSA	ND	ND
Furin	871-895	I870insRKKR	+++	6.70
Furin	871-895	delK871NII-insRKKR	+	3.50
Furin	871-895	L881insRKK	+	7.20
Furin	871-895	delY883ES-insKKR	+	12.1
Furin	871-895	S892RKK	+	15.2
Furin x 2	871-895	delK871NII-insRKKR delY883ES-insKKR	+	12.6
Furin x 2	871-895	delK871NII-insRKKR S892RKK	++	6.00
Furin x 3	871-895	delK871NII-insRKKR delY883ES-insKKR S892RKK	ND	ND
Kell	871-895	L891insAAF	+	10.8
Kell	871-895	delI889DL-insAAF	+	4.80
Tryptase ε	871-895	K871insIVGGE	+	9.45
Tryptase ε	871-895	K871insRIVGGE	+	6.48
Tryptase ε	871-895	delN886HLIDL-insRIVGGE		5.50
Tryptase ε	871-895	delN886HLIDL-insKIVGGE	ND	ND
mMMCP-7	871-895	K871insSLSSRQSP		3.90
mMMCP-7	871-895	delN886HLIDLS-insLSSRQSP		4.80
ECE-1	871-895	I870insRPPGFSAF	+	5.70
ECE-1	871-895	K871insAFA	+	3.85
ECE-1	871-895	K871insDIIWVNTPEHVVPYGLGS	+	>50
ECE-1	871-895	K871insRPKPQQFFGLM	ND	ND
ECE-1	871-895	delYES885NHLIDLS- insPKPQQFFGLM	+	9.20
ECE-1	871-895	E884insKAFA	+	2.95
ECE-1	871-895	delS885NHLIDLS-insRPPGFSAF	+	3.70
Cathespin L	871-895	I870insRGFFYTPK	++++	10.3
Cathespin L	871-895	K871insLR	++++	2.25
Cathespin L	871-895	K871insFR	++++	3.05
Cathespin L Thrombin	871-895	K871insLR L891insLVPRGS		12.6
PolyArg	844-863	R861insRR	ND	ND

PolyArg	871-895	R882insRRR		Yes
PolyArg	871-895	S885insRRR		2.22
PolyArg	871-895	S892insRRR		3.02
PolyArg x 2	844-863 871-895	R861insRR K871insRKR	ND	ND
PolyArg x 2	844-863 871-895	R861insRR I873insRRRR	ND	ND
PolyArg x 2	844-863 871-895	R861insRR R882insRRR	ND	ND
PolyArg x 2	871-895	K871insRKR S885insRRR		1.92
PolyArg x 2	871-895	R882insRRR S892insRRR	ND	ND
Protease sensitivity: +, less than 25% of toxin proteolyzed within about 1 to about 4 hours; ++, from 25% to 50% of toxin proteolyzed within about 1 to about 4 hours; +++, from 51% to 75% of toxin proteolyzed within about 1 to about 4 hours; ++++, more than 75% of toxin proteolyzed within about 1 to about 4 hours. BoNT/A potency is calculated by dividing the EC_{50} value of the toxin into the EC_{50} value of the backbone control. ND is not determined.				

[0401] To determine whether a BoNT/A comprising a protease cleavage site maintained its potency, the cell-based activity assay described above was performed (Table 8). In general, toxins comprising a protease cleavage site that exhibited an EC_{50} of about 20 or less were deemed to retain enough potency to warranted evaluation using an animal-based assay.

Example 3

In vivo Analysis

[0402] This example illustrates how to evaluate a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site using an animal-based assay analysis.

[0403] Although the cell-based activity assay is a good assessment of whether a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site can be cleaved by its cognate protease, certain candidates were selected for evaluation in an animal-based assay.

[0404] To test the activity of a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site using an animal-based assay, an *in vivo* Digit Abduction Score (DAS) assay was initially performed. CD-1 Fe mice were weighed and placed into subsets of 10 animals for each discrete DAS assay. Mice were included into a particular subset based on the following criteria: 1) good health; 2) robust baseline DAS response of 0; 3) inclusion in a median weight range of X ± 2 g established for the selected subset and 4) weight greater than 17.0 g.

[0405] Each mouse was injected using a 30-gauge needle in the gastrocnemius muscle of the right hind limb with either 1) 5 µL of 10.0 nM BoNT/A comprising an inactivation cleavage site (single-dose DAS study); or 2) 5 µL of one of seven different doses of BoNT/A comprising an inactivation cleavage site (0.01 nM, 0.04 nM, 0.12 nM, 0.37 nM, 1.11 nM, 3.33 nM and 10.0 nM; Full-Dosing DAS study). As a control, the gastrocnemius muscle of the left hind limb was injected with 5 µL of a solution not containing any toxin. Mice were observed for the DAS response consecutively for the first 4 days. The DAS was read by lifting each mouse by the tail and precisely observing the injected hind limbs. The abduction or

no abduction of the hind digits reveals the effect of paralysis due to the test toxin injected in the muscle. The digit abduction of the injected hind limb was compared with that of the non-injected hind limb and scored accordingly. DAS data was analyzed by calculating the ED_{50} dose based on peak mean DAS score and AUC (area under the curve) in terms of u/Kg and/or ng/Kg. This was accomplished as follows: 1) the mean peak DAS score for each dose was calculated in each study; 2) any dose that elicited more than five deaths in any study was eliminated from consideration; 3) the highest dose used in a given individual study was the lowest dose which elicited an average peak of 4.0; 4) the lowest dose used in a given individual study was the highest dose which elicited an average peak of 0; 5) curves were constructed for each individual study of average peak DAS vs. log (dose); 6) an AUC value was calculated for each group of 10 mice of the multiple groups in some studies; 7) curves were constructed for each individual study of average AUC vs. log (dose); 8) an x, y replicate response curve was constructed for each set of multiple identical studies; for each test toxin; 9) dose-response data were analyzed by non-linear regression (non-weighted) using a three-parameter logistic equation (Sigma Plot v 8.0; SPSS Science, Chicago, Illinois) using the following equation:

$$y = a/(1 + (x/x0)^{b})$$

where y is the response, a is the asymptotic y_{max}, b is the slope, x is the dose, and 0 is the ED_{50} dose, For peak ED_{50} determinations, Ymax was set to 4 (maximum DAS reading on scale). Mean (peak and/or AUC) ED_{50} values were computed for each eight-dose study performed.

[0406] The results indicate that (Table 9). In general, toxins comprising an inactivation cleavage site that exhibited a relative potency of about 10 or above were deemed to retain enough potency to warranted evaluation of its safety margin.

[0407] To determine the safety margin of a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site, a mouse lethality assay was performed.

[0408] To calculate the safety margin of a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site, the LD_{50} value obtained from the mouse lethality assay was divided by the EC_{50} value obtained from a full-dosing DAS study. A toxin comprising an inactivation cleavage site was deemed to possess enough activity at the inactivation cleavage site if it exhibited a safety margin value of about 15 or more.

TABLE 9. Animal-based Assay Analysis

Protease Cleavage Site	Region	Modification	Single-Dose DAS		Full-Dosing DAS		Lethality Assay	Safety Margin
			EC_{50}	Relative	EC_{50}	Relative	LD_{50}	LD_{50}/DAS ED_{50}
Thrombin	871-895	I873insLVPGRS	1.08	30.5	ND	ND	ND	ND
Thrombin	871-895	L881insVPRGS	0.37	7.38	ND	ND	ND	ND
Thrombin	871-895	E884insLVPRGS	0.16	25.3	0.15	46.7	1.90	12.5
Thrombin	871-895	L891insVPRG	0.12	23.3	0.19	36.8	2.74	14.8
Thrombin x 2	871-895	L881insVPRGS L891insVPRG	0.25	11.0	0.15	34.5	4.20	26.9
Factor Xa	871-895	I873insEGR	0.11	46.3	0.10	70.0	2.39	23.0
Factor Xa Thrombin	871-895	I873insEGR E884insLVPRG	0.09	37.2	0.26	15.3	6.69	26.9

MMP-2	871-895	delK871NIINTSI-insGPLGMLSQ	0.33	10.0	ND	ND	ND	ND
MMP-2	871-895	delS885NHLIDLS-insGPLGMLSQ	0.10	34.5	ND	ND	ND	ND
MMP-9	871-895	delK871NIINTSI-insGPLGLWAQ	0.11	29.1	0.16	27.7	5.04	23.9
MMP-9	871-895	delS885NHLIDLS-insGPLGLWAQ	0.08	40.8	ND	ND	ND	ND
u-PA	871-895	S885insPGSGKSA	0.03	36.6	ND	ND	ND	ND
u-PA	871-895	delN886HLIDL-insPGSGKSA	0.35	3.52	ND	ND	ND	ND
t-PA	871-895	delN872IINTSI-insPQRGRSA	0.04	30.0	ND	ND	ND	ND
t-PA	871-895	S885insPQRGRSA	0.12	10.1	ND	ND	ND	ND
t-PA	871-895	delS885NHLIDL-insPQRGRSA	0.08	16.0	0.27	25.9	4.46	17.2
Furin	871-895	I870insRKKR	0.80	2.68	ND	ND	ND	ND
Furin x 2	871-895	delK871NII-insRKKR delY883ES-insKKR	0.24	8.93	ND	ND	ND	ND
Furin x 2	871-895	delK871NII-insRKKR S892RKK	0.34	6.25	ND	ND	ND	ND
Tryptase ε	871-895	K871insIVGGE	0.14	37.3	ND	ND	ND	ND
Tryptase ε	871-895	K871insRIVGGE	0.21	10.4	ND	ND	ND	ND
Tryptase ε	871-895	delN886HLIDL-insRIVGGE	0.13	17.2	ND	ND	ND	ND
ECE-1	871-895	E884insKAFA	0.05	43.1	ND	ND	ND	ND
Cathespin L	871-895	K871insLR	0.10	34.3	ND	ND	ND	ND
Cathespin L	871-895	K871insFR	0.27	13.0	ND	ND	ND	ND
Control	—	WT	0.05	57.0	0.07	32.4	0.88	14.2
ND is not determined.								

[0409] After the DAS analysis, a Clostridial toxin or Clostridial toxin chimeric comprising an inactivation cleavage site was evaluated using a mouse lethality assay in order to determine the safety margin by comparing the ED_{50} with the LD_{50}.

[0410] Although aspects of the present specification have been described with reference to the disclosed embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of these aspects and in no way limit the present specification. Various modifications can be made without departing from the spirit of the present specification.

CLAIMS

1. A BoNT/A Clostridial toxin comprising a least one inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the 871 - 895 domain of SEQ ID NO: 1, wherein the at least one inactivation cleavage site comprises a dual Thrombin-Thrombin site.

2. A Clostridial toxin of Claim 1 comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, a di-chain loop region, an exogenous protease cleavage site, and at least two inactivation cleavage sites located within said inactivation cleavage site region; wherein the exogenous protease cleavage site is located within the di-chain loop region.

3. The Clostridial toxin of any one of Claim 1 - 2, wherein the inactivation site has the sequence of SEQ ID NO: 114.

4. A polynucleotide molecule encoding a Clostridial toxin of claim 1.

5. A polynucleotide molecule of Claim 4 encoding a BoNT/A toxin, comprising a Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, a di-chain loop region, an exogenous protease cleavage site, and at least two inactivation cleavage sites located within an inactivation cleavage site region; wherein the exogenous protease cleavage site located within the di-chain loop region.

6. The polynucleotide molecule of any one of Claim 4-5, wherein the polynucleotide molecule encodes an inactivation cleavage site which has the sequence of SEQ ID NO:114.

7. A polynucleotide molecule of Claim 6 comprising SEQ ID NO: 530.

SEQUENCE LISTING

<110> Steward, Lance E.
Ghanshani, Sanjiv
Fernandez-Salas, Ester
Gilmore, Marcella A.
Francis, Joseph
Aoki, Kei Roger

<120> Degradable Clostridial Toxins

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<150> US 61/346,578
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Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
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Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
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Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
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Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
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Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
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Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
660 665 670
Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
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Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
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Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
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Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
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Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
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Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser
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Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly
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65                  70                  75                  80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
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Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
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    210                 215                 220
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Val Ile Asp Thr Asn Cys Ile Asn Val Ile Glu Pro Gly Gly Ser Tyr
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Arg Ser Glu Glu Leu Asn Leu Val Ile Thr Gly Pro Ser Ala Asp Ile
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Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Asp Ser
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Asp Ile Thr Ile Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly
625 630 635 640
Asn Met Val Ser Lys Gly Glu Phe Val Glu Ala Ile Leu Phe Thr Gly
645 650 655
Val Val Ala Leu Leu Glu Phe Ile Pro Glu Tyr Ser Leu Pro Val Phe
660 665 670
Gly Thr Phe Ala Ile Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val
675 680 685
Gln Thr Ile Asn Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu
690 695 700
Val Tyr Lys Tyr Thr Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln
705 710 715 720
Ile Asp Leu Ile Arg Glu Lys Met Lys Lys Ala Leu Glu Asn Gln Ala
725 730 735
Glu Ala Thr Arg Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu
740 745 750
Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys
755 760 765
Leu Asn Arg Ser Ile Asn Arg Ala Met Ile Asn Ile Asn Lys Phe Leu
770 775 780
Asp Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Ala
785 790 795 800
Val Lys Arg Leu Lys Asp Phe Asp Ala Ser Val Arg Asp Val Leu Leu
805 810 815
Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Leu Gln Val Asp Arg
820 825 830
Leu Lys Asp Glu Val Asn Asn Thr Leu Ser Ala Asp Ile Pro Phe Gln
835 840 845
Leu Ser Lys Tyr Val Asn Asp Lys Lys Leu Leu Ser Thr Phe Thr Glu
850 855 860
Tyr Ile Lys Asn Ile Val Asn Thr Ser Ile Leu Ser Ile Val Tyr Lys
865 870 875 880
Lys Asp Asp Leu Ile Asp Leu Ser Arg Tyr Gly Ala Lys Ile Asn Ile
885 890 895
Gly Asp Arg Val Tyr Tyr Asp Ser Ile Asp Lys Asn Gln Ile Lys Leu
900 905 910
Ile Asn Leu Glu Ser Ser Thr Ile Glu Val Ile Leu Lys Asn Ala Ile
915 920 925
Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Lys
930 935 940
Ile Pro Lys Tyr Phe Ser Lys Ile Asn Leu Asn Asn Glu Tyr Thr Ile
945 950 955 960
Ile Asn Cys Ile Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr
965 970 975
Gly Glu Ile Ile Trp Thr Leu Gln Asp Asn Lys Gln Asn Ile Gln Arg
980 985 990
Val Val Phe Lys Tyr Ser Gln Met Val Asn Ile Ser Asp Tyr Ile Asn
995 1000 1005
Arg Trp Met Phe Val Thr Ile Thr Asn Asn Arg Leu Thr Lys Ser Lys
1010 1015 1020
Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu
1025 1030 1035 1040
Gly Asn Ile His Ala Ser Asn Lys Ile Met Phe Lys Leu Asp Gly Cys
1045 1050 1055
Arg Asp Pro Arg Arg Tyr Ile Met Ile Lys Tyr Phe Asn Leu Phe Asp
1060 1065 1070
Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Ser Gln Ser
1075 1080 1085

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Asn Pro Gly Ile Leu Lys Asp Phe Trp Gly Asn Tyr Leu Gln Tyr Asp
    1090                1095                1100
Lys Pro Tyr Tyr Met Leu Asn Leu Phe Asp Pro Asn Lys Tyr Val Asp
1105                1110                1115                1120
Val Asn Asn Ile Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg
                1125                1130                1135
Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Thr Leu Tyr Met
            1140                1145                1150
Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Glu Asp Asn
        1155                1160                1165
Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn
    1170                1175                1180
Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys
1185                1190                1195                1200
Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val
                1205                1210                1215
Val Val Met Lys Ser Lys Asp Asp Gln Gly Ile Arg Asn Lys Cys Lys
            1220                1225                1230
Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Val Gly Phe
        1235                1240                1245
His Leu Tyr Asp Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn
    1250                1255                1260
Arg Gln Val Gly Lys Ala Ser Arg Thr Phe Gly Cys Ser Trp Glu Phe
1265                1270                1275                1280
Ile Pro Val Asp Asp Gly Trp Gly Glu Ser Ser Leu
                1285                1290
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<211> 1296
<212> PRT
<213> Clostridium botulinum A4

<400> 4

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 1                5                  10                  15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Lys Met Gln Pro
            20                  25                  30
Val Lys Ala Phe Lys Ile His Asn Lys Val Trp Val Ile Pro Glu Arg
        35                  40                  45
Asp Ile Phe Thr Asn Pro Glu Glu Val Asp Leu Asn Pro Pro Pro Glu
    50                  55                  60
Ala Lys Gln Val Pro Ile Ser Tyr Tyr Asp Ser Ala Tyr Leu Ser Thr
65                  70                  75                  80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Ile Lys Leu Phe Glu
                85                  90                  95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Ile Ser Ile Val
            100                 105                 110
Arg Gly Ile Pro Phe Trp Gly Gly Gly Lys Ile Asp Thr Glu Leu Lys
        115                 120                 125
Val Ile Asp Thr Asn Cys Ile Asn Ile Ile Gln Leu Asp Asp Ser Tyr
    130                 135                 140
Arg Ser Glu Glu Leu Asn Leu Ala Ile Ile Gly Pro Ser Ala Asn Ile
145                 150                 155                 160
Ile Glu Ser Gln Cys Ser Ser Phe Arg Asp Asp Val Leu Asn Leu Thr
                165                 170                 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190
Thr Val Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205
Gly Ala Gly Lys Phe Ala Gln Asp Pro Ala Val Ala Leu Ala His Glu
    210                 215                 220
Leu Ile His Ala Glu His Arg Leu Tyr Gly Ile Ala Ile Asn Thr Asn
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225 230 235 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ala Gly Leu
245 250 255
Glu Val Ser Leu Glu Glu Leu Ile Thr Phe Gly Gly Asn Asp Ala Lys
260 265 270
Phe Ile Asp Ser Leu Gln Lys Lys Glu Phe Ser Leu Tyr Tyr Tyr Asn
275 280 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320
Tyr Leu Leu Ser Glu Asp Ala Thr Gly Lys Phe Leu Val Asp Arg Leu
325 330 335
Lys Phe Asp Glu Leu Tyr Lys Leu Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Asp Val Asn Tyr
370 375 380
Thr Ile His Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400
Phe Asn Gly Gln Asn Ile Glu Ile Asn Asn Lys Asn Phe Asp Lys Leu
405 410 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Glu Gly Tyr Asn Lys
435 440 445
Ala Leu Asn Glu Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
450 455 460
Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asp Lys Val Glu Glu
465 470 475 480
Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
485 490 495
Asp Leu Ile Gln Gln Tyr Tyr Leu Asn Phe Asn Phe Asp Asn Glu Pro
500 505 510
Glu Asn Thr Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
515 520 525
Glu Pro Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
530 535 540
Leu Asn Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Lys
545 550 555 560
His Ser Asn Ser Arg Ile Ile Leu Thr Asn Ser Ala Lys Glu Ala Leu
565 570 575
Leu Lys Pro Asn Ile Val Tyr Thr Phe Phe Ser Ser Lys Tyr Ile Lys
580 585 590
Ala Ile Asn Lys Ala Val Glu Ala Val Thr Phe Val Asn Trp Ile Glu
595 600 605
Asn Leu Val Tyr Asp Phe Thr Asp Glu Thr Asn Glu Val Ser Thr Met
610 615 620
Asp Lys Ile Ala Asp Ile Thr Ile Val Ile Pro Tyr Ile Gly Pro Ala
625 630 635 640
Leu Asn Ile Gly Asn Met Ile Tyr Lys Gly Glu Phe Val Glu Ala Ile
645 650 655
Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Ile Val Pro Glu Ile Ala
660 665 670
Leu Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Val Ser Asn Lys
675 680 685
Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
690 695 700
Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Ile
705 710 715 720
Val Asn Thr Gln Ile Asn Leu Ile Arg Glu Lys Met Lys Lys Ala Leu
725 730 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
740 745 750
Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
755 760 765
Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Ser Ala Met Ile Asn Ile
770 775 780
Asn Lys Phe Leu Asp Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785 790 795 800
Ile Pro Tyr Ala Val Lys Arg Leu Lys Asp Phe Asp Ala Ser Val Arg
805 810 815
Asp Val Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
820 825 830
Gln Val Asn Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Ala Asp
835 840 845
Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Lys Lys Leu Leu Ser
850 855 860
Thr Phe Thr Glu Tyr Ile Lys Asn Ile Thr Asn Ala Ser Ile Leu Ser
865 870 875 880
Ile Val Tyr Lys Asp Asp Asp Leu Ile Asp Leu Ser Arg Tyr Gly Ala
885 890 895
Glu Ile Tyr Asn Gly Asp Lys Val Tyr Tyr Asn Ser Ile Asp Lys Asn
900 905 910
Gln Ile Arg Leu Ile Asn Leu Glu Ser Ser Thr Ile Glu Val Ile Leu
915 920 925
Lys Lys Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940
Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
945 950 955 960
Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
965 970 975
Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Phe Gln Asp Thr Gln Glu
980 985 990
Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
995 1000 1005
Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Ile
1010 1015 1020
Thr Lys Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro
1025 1030 1035 1040
Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Lys Ile Met Phe Lys
1045 1050 1055
Leu Asp Gly Cys Arg Asp Pro His Arg Tyr Ile Val Ile Lys Tyr Phe
1060 1065 1070
Asn Leu Phe Asp Lys Glu Leu Ser Glu Lys Glu Ile Lys Asp Leu Tyr
1075 1080 1085
Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr
1090 1095 1100
Leu Gln Tyr Asp Lys Ser Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn
1105 1110 1115 1120
Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu
1125 1130 1135
Lys Gly Pro Arg Asp Asn Val Met Thr Thr Asn Ile Tyr Leu Asn Ser
1140 1145 1150
Ser Leu Tyr Met Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly
1155 1160 1165
Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val
1170 1175 1180
Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala
1185 1190 1195 1200
Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn
1205 1210 1215
Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr
1220 1225 1230
Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly

1235 1240 1245
Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser
1250 1255 1260
Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys
1265 1270 1275 1280
Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Arg Glu Arg Pro Leu
1285 1290 1295

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<212> PRT
<213> Clostridium botulinum A5

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Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
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Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95
Arg Ile Tyr Ser Thr Glu Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Asp Val Leu Asn Leu Thr
165 170 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
180 185 190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Glu His Asp Ala Lys
260 265 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Glu Val Asn Tyr
370 375 380

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Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Glu Gly Tyr Asn Lys
        435                 440                 445
Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460
Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480
Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495
Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510
Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525
Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540
Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560
His Gly Lys Ser Arg Ile Val Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575
Leu Asn Pro Ser Ser Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Arg
            580                 585                 590
Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605
Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620
Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640
Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655
Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670
Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685
Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700
Lys Trp Gly Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720
Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735
Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750
Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Gly Asp
        755                 760                 765
Leu Ser Ser Lys Leu Asn Asp Ser Ile Asn Lys Ala Met Ile Asn Ile
    770                 775                 780
Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785                 790                 795                 800
Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
                805                 810                 815
Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
            820                 825                 830
Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
        835                 840                 845
Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
    850                 855                 860
Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865                 870                 875                 880
Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
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885 890 895
Glu Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
900 905 910
Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Ile Ile Leu
915 920 925
Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940
Phe Trp Ile Lys Ile Pro Lys Tyr Phe Ser Lys Ile Asn Leu Asn Asn
945 950 955 960
Glu Tyr Thr Ile Ile Asn Cys Ile Glu Asn Asn Ser Gly Trp Lys Val
965 970 975
Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Asn Lys Gln
980 985 990
Asn Ile Gln Arg Val Val Phe Lys Tyr Ser Gln Met Val Ala Ile Ser
995 1000 1005
Asp Tyr Ile Asn Arg Trp Ile Phe Ile Thr Ile Thr Asn Asn Arg Leu
1010 1015 1020
Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro
1025 1030 1035 1040
Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys
1045 1050 1055
Leu Asp Gly Cys Arg Asp Pro Gln Arg Tyr Ile Trp Ile Lys Tyr Phe
1060 1065 1070
Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr
1075 1080 1085
Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asn Tyr
1090 1095 1100
Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn
1105 1110 1115 1120
Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu
1125 1130 1135
Lys Gly Pro Arg Gly Ser Ile Val Thr Thr Asn Ile Tyr Leu Asn Ser
1140 1145 1150
Ser Leu Tyr Met Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly
1155 1160 1165
Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val
1170 1175 1180
Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala
1185 1190 1195 1200
Gly Val Glu Lys Ile Leu Ser Val Leu Glu Ile Pro Asp Val Gly Asn
1205 1210 1215
Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Arg
1220 1225 1230
Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245
Phe Ile Gly Phe His Gln Phe Asn Asn Ile Asp Lys Leu Val Ala Ser
1250 1255 1260
Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Phe Gly Cys
1265 1270 1275 1280
Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Ser Pro Leu
1285 1290 1295

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<212> PRT
<213> Clostridium botulinum B1

<400> 6
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Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
20 25 30

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Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
        35                  40                  45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
    50                  55                  60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65                  70                  75                  80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
                85                  90                  95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
            100                 105                 110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
        115                 120                 125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
    130                 135                 140
Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145                 150                 155                 160
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
                165                 170                 175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
            180                 185                 190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
        195                 200                 205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
    210                 215                 220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240
Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
                245                 250                 255
Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270
Gly Gly Gln Asp Pro Ser Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile
        275                 280                 285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
    290                 295                 300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305                 310                 315                 320
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
                325                 330                 335
Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
            340                 345                 350
Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
        355                 360                 365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
    370                 375                 380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385                 390                 395                 400
Ser Asp Lys Asp Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
                405                 410                 415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
            420                 425                 430
Lys Ile Gln Met Cys Lys Ser Val Lys Ala Pro Gly Ile Cys Ile Asp
        435                 440                 445
Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
    450                 455                 460
Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asn Thr Gln Ser Asn
465                 470                 475                 480
Tyr Ile Glu Asn Asp Phe Pro Ile Asn Glu Leu Ile Leu Asp Thr Asp
                485                 490                 495
Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
            500                 505                 510
Asp Phe Asn Val Asp Val Pro Ala Tyr Glu Lys Gln Pro Ala Ile Lys
        515                 520                 525
Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
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530 535 540
Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545 550 555 560
Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe Ser Met Asp
565 570 575
Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
580 585 590
Trp Val Lys Gln Ile Val Asn Asp Phe Val Ile Glu Ala Asn Lys Ser
595 600 605
Asn Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
610 615 620
Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625 630 635 640
Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
645 650 655
Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
660 665 670
Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
675 680 685
Arg Asn Glu Lys Trp Ser Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
690 695 700
Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705 710 715 720
Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
725 730 735
Arg Tyr Asn Ile Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp
740 745 750
Phe Asn Asp Ile Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
755 760 765
Asp Asn Ile Asn Asn Phe Ile Asn Gly Cys Ser Val Ser Tyr Leu Met
770 775 780
Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785 790 795 800
Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
805 810 815
Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asn Lys Tyr Leu
820 825 830
Lys Thr Ile Met Pro Phe Asp Leu Ser Ile Tyr Thr Asn Asp Thr Ile
835 840 845
Leu Ile Glu Met Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
850 855 860
Ile Leu Asn Leu Arg Tyr Lys Asp Asn Asn Leu Ile Asp Leu Ser Gly
865 870 875 880
Tyr Gly Ala Lys Val Glu Val Tyr Asp Gly Val Glu Leu Asn Asp Lys
885 890 895
Asn Gln Phe Lys Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Thr
900 905 910
Gln Asn Gln Asn Ile Ile Phe Asn Ser Val Phe Leu Asp Phe Ser Val
915 920 925
Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
930 935 940
Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser
945 950 955 960
Gly Trp Lys Ile Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Ile
965 970 975
Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Asn Ile Arg
980 985 990
Glu Asp Ile Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
995 1000 1005
Asn Asn Leu Asn Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser
1010 1015 1020
Asn Thr Asp Ile Lys Asp Ile Arg Glu Val Ile Ala Asn Gly Glu Ile
1025 1030 1035 1040

Ile Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met
1045 1050 1055
Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Glu
1060 1065 1070
Glu Arg Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
1075 1080 1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
1090 1095 1100
Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Pro Val Gly Glu
1105 1110 1115 1120
Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr
1125 1130 1135
Arg Asp Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
1140 1145 1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
1155 1160 1165
Leu Asp Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr Ile Tyr Lys
1170 1175 1180
Tyr Phe Lys Lys Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp
1185 1190 1195 1200
Ser Asp Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
1205 1210 1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
1220 1225 1230
Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile
1235 1240 1245
Val Phe Lys Glu Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
1250 1255 1260
Lys Glu Val Lys Arg Lys Pro Tyr Asn Ser Lys Leu Gly Cys Asn Trp
1265 1270 1275 1280
Gln Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
1285 1290

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<212> PRT
<213> Clostridium botulinum B2

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Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
20 25 30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
35 40 45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
50 55 60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65 70 75 80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
85 90 95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
100 105 110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
115 120 125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
130 135 140
Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145 150 155 160
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
165 170 175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln

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                180                 185                 190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
        195                 200                 205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
    210                 215                 220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240
Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
                245                 250                 255
Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270
Gly Gly Gln Asp Pro Ser Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile
        275                 280                 285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
    290                 295                 300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305                 310                 315                 320
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
                325                 330                 335
Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
            340                 345                 350
Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
        355                 360                 365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
    370                 375                 380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385                 390                 395                 400
Ser Asp Lys Asn Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
                405                 410                 415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
            420                 425                 430
Lys Ile Gln Met Cys Lys Ser Val Arg Ala Pro Gly Ile Cys Ile Asp
        435                 440                 445
Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
    450                 455                 460
Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asp Thr Gln Ser Asn
465                 470                 475                 480
Tyr Ile Glu Asn Arg Ser Ser Ile Asp Glu Leu Ile Leu Asp Thr Asn
                485                 490                 495
Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
            500                 505                 510
Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro Ala Ile Lys
        515                 520                 525
Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
    530                 535                 540
Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545                 550                 555                 560
Asp Ala Leu Leu Phe Ser Lys Lys Val Tyr Ser Phe Phe Ser Met Asp
                565                 570                 575
Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
            580                 585                 590
Trp Val Lys Gln Ile Val Asp Asp Phe Val Ile Glu Ala Asn Lys Ser
        595                 600                 605
Ser Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
    610                 615                 620
Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625                 630                 635                 640
Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
                645                 650                 655
Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
            660                 665                 670
Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
        675                 680                 685
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Arg Asp Glu Lys Trp Ile Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
    690                 695                 700
Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705                 710                 715                 720
Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
                725                 730                 735
Lys Tyr Asn Ile Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp
            740                 745                 750
Phe Asn Asp Ile Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
        755                 760                 765
Asp Asn Ile Asn Asn Phe Ile Asn Glu Cys Ser Val Ser Tyr Leu Met
    770                 775                 780
Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785                 790                 795                 800
Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
                805                 810                 815
Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asp Lys His Leu
            820                 825                 830
Lys Thr Ile Ile Pro Phe Asp Leu Ser Lys Tyr Thr Asn Asn Thr Ile
        835                 840                 845
Leu Ile Glu Ile Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
    850                 855                 860
Ile Leu Asn Leu Arg Tyr Arg Asp Asn Asn Leu Ile Asp Leu Ser Gly
865                 870                 875                 880
Tyr Gly Ala Asn Val Glu Val Tyr Asp Gly Val Glu Leu Asn Asp Lys
                885                 890                 895
Asn Gln Phe Lys Leu Thr Ser Ser Thr Asn Ser Glu Ile Arg Val Thr
            900                 905                 910
Gln Asn Gln Asn Ile Ile Phe Asn Ser Met Phe Leu Asp Phe Ser Val
        915                 920                 925
Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
    930                 935                 940
Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Ile Lys Asn Asn Ser
945                 950                 955                 960
Gly Trp Lys Ile Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Thr
                965                 970                 975
Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Ser Ile Arg
            980                 985                 990
Lys Asp Val Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
        995                 1000                1005
Asn Asn Ser Asp Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser
    1010                1015                1020
Asn Ile Asp Ile Lys Asp Ile Gly Glu Val Ile Ala Asn Gly Glu Ile
1025                1030                1035                1040
Ile Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met
                1045                1050                1055
Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Lys
            1060                1065                1070
Glu Ile Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
        1075                1080                1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
    1090                1095                1100
Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Ser Val Gly Glu
1105                1110                1115                1120
Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Asn Tyr Ile Asn Tyr
                1125                1130                1135
Arg Asn Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
            1140                1145                1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
        1155                1160                1165
Leu Asp Phe Phe Asn Ser Asn Arg Glu Trp Arg Val Tyr Ala Tyr Lys
    1170                1175                1180
Asp Phe Lys Glu Glu Glu Lys Lys Leu Phe Leu Ala Asn Ile Tyr Asp
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1185 1190 1195 1200
Ser Asn Glu Phe Tyr Lys Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
1205 1210 1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
1220 1225 1230
Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Thr
1235 1240 1245
Val Phe Lys Asn Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
1250 1255 1260
Lys Glu Val Lys Arg Lys Pro Tyr Asn Ser Asp Leu Gly Cys Asn Trp
1265 1270 1275 1280
Lys Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
1285 1290

<210> 8
<211> 1291
<212> PRT
<213> Clostridium botulinum B3

<400> 8
Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
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Asp Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
20 25 30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
35 40 45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
50 55 60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65 70 75 80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
85 90 95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
100 105 110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
115 120 125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
130 135 140
Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145 150 155 160
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
165 170 175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
180 185 190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
195 200 205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
210 215 220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225 230 235 240
Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
245 250 255
Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
260 265 270
Gly Gly Gln Asp Pro Arg Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile
275 280 285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
290 295 300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305 310 315 320
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
325 330 335

Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
340 345 350
Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
355 360 365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
370 375 380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385 390 395 400
Ser Asp Lys Asn Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
405 410 415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
420 425 430
Lys Ile Gln Met Cys Lys Ser Val Arg Ala Pro Gly Ile Cys Ile Asp
435 440 445
Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
450 455 460
Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asp Thr Gln Ser Asn
465 470 475 480
Tyr Ile Glu Asn Arg Ser Ser Ile Asp Glu Leu Ile Leu Asp Thr Asn
485 490 495
Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
500 505 510
Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro Ala Ile Lys
515 520 525
Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
530 535 540
Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545 550 555 560
Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe Ser Met Asp
565 570 575
Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
580 585 590
Trp Val Lys Gln Ile Val Asp Asp Phe Val Ile Glu Ala Asn Lys Ser
595 600 605
Ser Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
610 615 620
Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625 630 635 640
Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
645 650 655
Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
660 665 670
Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
675 680 685
Arg Asp Glu Lys Trp Ile Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
690 695 700
Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705 710 715 720
Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
725 730 735
Lys Tyr Asn Ile Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp
740 745 750
Phe Asn Asp Ile Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
755 760 765
Asp Asn Ile Asn Asn Phe Ile Asn Glu Cys Ser Val Ser Tyr Leu Met
770 775 780
Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785 790 795 800
Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
805 810 815
Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asp Lys His Leu
820 825 830
Lys Thr Ile Ile Pro Phe Asp Leu Ser Met Tyr Thr Asn Asn Thr Ile

835 840 845
Leu Ile Glu Ile Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
850 855 860
Ile Leu Asn Leu Arg Tyr Arg Asp Asn Asn Leu Ile Asp Leu Ser Gly
865 870 875 880
Tyr Gly Ala Lys Val Glu Val Tyr Asn Gly Val Glu Leu Asn Asp Lys
885 890 895
Asn Gln Phe Lys Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Thr
900 905 910
Gln Asn Gln Asp Ile Ile Phe Asn Ser Met Phe Leu Asp Phe Ser Val
915 920 925
Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
930 935 940
Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Ile Lys Asn Asn Ser
945 950 955 960
Gly Trp Lys Ile Ser Ile Arg Gly Asn Lys Ile Ile Trp Thr Leu Thr
965 970 975
Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Ser Ile Arg
980 985 990
Lys Asp Val Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
995 1000 1005
Asn Asn Ser Asp Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser
1010 1015 1020
Asn Ile Asp Ile Lys Asp Ile Gly Glu Val Ile Ala Asn Gly Glu Ile
1025 1030 1035 1040
Ile Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met
1045 1050 1055
Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Lys
1060 1065 1070
Glu Ile Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
1075 1080 1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
1090 1095 1100
Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Ser Val Gly Glu
1105 1110 1115 1120
Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Asn Tyr Ile Asn Tyr
1125 1130 1135
Arg Asn Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
1140 1145 1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
1155 1160 1165
Leu Asp Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr Ala Tyr Lys
1170 1175 1180
Asp Phe Lys Lys Lys Glu Glu Lys Leu Phe Leu Ala Asn Ile Tyr Asp
1185 1190 1195 1200
Ser Asn Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
1205 1210 1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
1220 1225 1230
Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile
1235 1240 1245
Val Phe Lys Asp Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
1250 1255 1260
Lys Glu Val Lys Arg Lys Pro Tyr Asn Pro Asn Leu Gly Cys Asn Trp
1265 1270 1275 1280
Gln Phe Ile Pro Lys Asp Glu Gly Trp Ile Glu
1285 1290

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<213> Clostridium botulinum Bnp

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            20                  25                  30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
        35                  40                  45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
    50                  55                  60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65                  70                  75                  80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
                85                  90                  95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
            100                 105                 110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
        115                 120                 125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
    130                 135                 140
Pro Gly Glu Val Glu Gln Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145                 150                 155                 160
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
                165                 170                 175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
            180                 185                 190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
        195                 200                 205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
    210                 215                 220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240
Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
                245                 250                 255
Phe Met Gln Ser Thr Asp Thr Ile Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270
Gly Gly Gln Asp Pro Ser Ile Ile Ser Pro Ser Thr Asp Lys Ser Ile
        275                 280                 285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
    290                 295                 300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305                 310                 315                 320
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
                325                 330                 335
Lys Tyr Ser Ile Asp Val Glu Ser Phe Asn Lys Leu Tyr Lys Ser Leu
            340                 345                 350
Met Phe Gly Phe Thr Glu Ile Asn Ile Ala Glu Asn Tyr Lys Ile Lys
        355                 360                 365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
    370                 375                 380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385                 390                 395                 400
Ser Asp Lys Asn Met Gly Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
                405                 410                 415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
            420                 425                 430
Lys Ile Gln Met Cys Lys Ser Val Lys Val Pro Gly Ile Cys Ile Asp
        435                 440                 445
Val Asp Asn Glu Asn Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
    450                 455                 460
Asp Asp Leu Ser Lys Asn Glu Arg Val Glu Tyr Asn Thr Gln Asn Asn
465                 470                 475                 480
Tyr Ile Gly Asn Asp Phe Pro Ile Asn Glu Leu Ile Leu Asp Thr Asp
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485 490 495
Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
500 505 510
Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro Ala Ile Lys
515 520 525
Lys Val Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
530 535 540
Thr Phe Pro Leu Asn Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545 550 555 560
Asp Ala Leu Leu Val Ser Ser Lys Val Tyr Ser Phe Phe Ser Met Asp
565 570 575
Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
580 585 590
Trp Val Lys Gln Ile Val Asp Asp Phe Val Ile Glu Ala Asn Lys Ser
595 600 605
Ser Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
610 615 620
Gly Leu Ala Leu Asn Val Gly Asp Glu Thr Ala Lys Gly Asn Phe Glu
625 630 635 640
Ser Ala Phe Glu Ile Ala Gly Ser Ser Ile Leu Leu Glu Phe Ile Pro
645 650 655
Glu Leu Leu Ile Pro Val Val Gly Val Phe Leu Leu Glu Ser Tyr Ile
660 665 670
Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
675 680 685
Arg Val Glu Lys Trp Ile Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
690 695 700
Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705 710 715 720
Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
725 730 735
Lys Tyr Asn Ile Tyr Ser Glu Glu Glu Lys Ser Asn Ile Asn Ile Asn
740 745 750
Phe Asn Asp Ile Asn Ser Lys Leu Asn Asp Gly Ile Asn Gln Ala Met
755 760 765
Asp Asn Ile Asn Asp Phe Ile Asn Glu Cys Ser Val Ser Tyr Leu Met
770 775 780
Lys Lys Met Ile Pro Leu Ala Val Lys Lys Leu Leu Asp Phe Asp Asn
785 790 795 800
Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
805 810 815
Leu Ile Gly Ser Val Glu Asp Glu Lys Ser Lys Val Asp Lys Tyr Leu
820 825 830
Lys Thr Ile Ile Pro Phe Asp Leu Ser Thr Tyr Thr Asn Asn Glu Ile
835 840 845
Leu Ile Lys Ile Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
850 855 860
Ile Leu Asn Leu Arg Tyr Arg Asp Asn Asn Leu Ile Asp Leu Ser Gly
865 870 875 880
Tyr Gly Ala Lys Val Glu Val Tyr Asp Gly Val Lys Leu Asn Asp Lys
885 890 895
Asn Gln Phe Lys Leu Thr Ser Ser Ala Asp Ser Lys Ile Arg Val Thr
900 905 910
Gln Asn Gln Asn Ile Ile Phe Asn Ser Met Phe Leu Asp Phe Ser Val
915 920 925
Ser Phe Trp Ile Arg Ile Pro Lys Tyr Arg Asn Asp Asp Ile Gln Asn
930 935 940
Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser
945 950 955 960
Gly Trp Lys Ile Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Ile
965 970 975
Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Asn Ile Arg
980 985 990

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Glu Asp Ile Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
        995                 1000                1005
Asn Asn Leu Asp Asn Ala Lys Ile Tyr Ile Asn Gly Thr Leu Glu Ser
    1010                1015                1020
Asn Met Asp Ile Lys Asp Ile Gly Glu Val Ile Val Asn Gly Glu Ile
1025                1030                1035                1040
Thr Phe Lys Leu Asp Gly Asp Val Asp Arg Thr Gln Phe Ile Trp Met
                1045                1050                1055
Lys Tyr Phe Ser Ile Phe Asn Thr Gln Leu Asn Gln Ser Asn Ile Lys
            1060                1065                1070
Glu Ile Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
        1075                1080                1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
    1090                1095                1100
Asn Lys Asn Ser Tyr Ile Lys Leu Val Lys Asp Ser Ser Val Gly Glu
1105                1110                1115                1120
Ile Leu Ile Arg Ser Lys Tyr Asn Gln Asn Ser Asn Tyr Ile Asn Tyr
                1125                1130                1135
Arg Asn Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
            1140                1145                1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile His
        1155                1160                1165
Leu Asp Phe Val Asn Ser Asn Glu Glu Trp Arg Val Tyr Ala Tyr Lys
    1170                1175                1180
Asn Phe Lys Glu Gln Glu Gln Lys Leu Phe Leu Ser Ile Ile Tyr Asp
1185                1190                1195                1200
Ser Asn Glu Phe Tyr Lys Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
                1205                1210                1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
            1220                1225                1230
Asp Asp Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Val
        1235                1240                1245
Leu Arg Lys Lys Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
    1250                1255                1260
Lys Glu Val Lys Arg Lys Pro Tyr Lys Ser Asn Leu Gly Cys Asn Trp
1265                1270                1275                1280
Gln Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
                1285                1290


<210> 10
<211> 1291
<212> PRT
<213> Clostridium botulinum Bbv

<400> 10
Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
 1               5                  10                  15
Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Met Gly Arg
            20                  25                  30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
        35                  40                  45
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
    50                  55                  60
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65                  70                  75                  80
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
                85                  90                  95
Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
            100                 105                 110
Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
        115                 120                 125
Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
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130 135 140
Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145 150 155 160
Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
165 170 175
Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
180 185 190
Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
195 200 205
Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
210 215 220
Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225 230 235 240
Gly Ile Lys Val Asn Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
245 250 255
Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
260 265 270
Gly Gly Gln Asp Pro Ser Ile Ile Ser Pro Ser Thr Asp Lys Ser Ile
275 280 285
Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
290 295 300
Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305 310 315 320
Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
325 330 335
Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
340 345 350
Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
355 360 365
Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
370 375 380
Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385 390 395 400
Ser Asp Lys Asn Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
405 410 415
Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
420 425 430
Lys Ile Gln Met Cys Lys Ser Val Lys Ala Pro Gly Ile Cys Ile Asp
435 440 445
Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
450 455 460
Asp Asp Leu Ser Lys Asn Glu Arg Ile Ala Tyr Asn Thr Gln Asn Asn
465 470 475 480
Tyr Ile Glu Asn Asp Phe Ser Ile Asn Glu Leu Ile Leu Asp Thr Asp
485 490 495
Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
500 505 510
Asp Phe Asn Val Tyr Val Pro Val Tyr Lys Lys Gln Pro Ala Ile Lys
515 520 525
Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
530 535 540
Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545 550 555 560
Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe Ser Met Asp
565 570 575
Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
580 585 590
Trp Val Lys Gln Ile Val Asp Asp Phe Val Ile Glu Ala Asn Lys Ser
595 600 605
Ser Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
610 615 620
Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625 630 635 640

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Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
                645                 650                 655
Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
            660                 665                 670
Asp Asn Lys Asn Lys Ile Ile Glu Thr Ile Asn Ser Ala Leu Thr Lys
        675                 680                 685
Arg Asp Glu Lys Trp Ile Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
    690                 695                 700
Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705                 710                 715                 720
Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
                725                 730                 735
Lys Tyr Asn Ile Tyr Ser Glu Lys Glu Arg Ser Asn Ile Asn Ile Asp
            740                 745                 750
Phe Asn Asp Val Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
        755                 760                 765
Asp Asn Ile Asn Asn Phe Ile Asn Glu Cys Ser Val Ser Tyr Leu Met
    770                 775                 780
Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785                 790                 795                 800
Thr Leu Arg Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
                805                 810                 815
Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asp Lys Tyr Leu
            820                 825                 830
Lys Thr Ser Ile Pro Phe Asp Leu Ser Thr Tyr Thr Asn Asn Thr Ile
        835                 840                 845
Leu Ile Glu Ile Phe Asn Lys Tyr Asn Ser Asp Ile Leu Asn Asn Ile
    850                 855                 860
Ile Leu Asn Leu Arg Tyr Arg Asp Asn Lys Leu Ile Asp Leu Ser Gly
865                 870                 875                 880
Tyr Gly Ala Lys Val Glu Val Tyr Asp Gly Val Lys Leu Asn Asp Lys
                885                 890                 895
Asn Gln Phe Lys Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Ile
            900                 905                 910
Gln Asn Gln Asn Ile Ile Phe Asn Ser Met Phe Leu Asp Phe Ser Val
        915                 920                 925
Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
    930                 935                 940
Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser
945                 950                 955                 960
Gly Trp Lys Ile Ser Ile Arg Gly Asn Met Ile Ile Trp Thr Leu Ile
                965                 970                 975
Asp Ile Asn Gly Lys Ile Lys Ser Val Phe Phe Glu Tyr Ser Ile Lys
            980                 985                 990
Glu Asp Ile Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
        995                 1000                1005
Asn Asn Ser Asp Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser
    1010                1015                1020
His Ile Asp Ile Arg Asp Ile Arg Glu Val Ile Ala Asn Asp Glu Ile
1025                1030                1035                1040
Ile Phe Lys Leu Asp Gly Asn Ile Asp Arg Thr Gln Phe Ile Trp Met
                1045                1050                1055
Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Glu
            1060                1065                1070
Glu Ile Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
        1075                1080                1085
Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
    1090                1095                1100
Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Ser Val Gly Glu
1105                1110                1115                1120
Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr
                1125                1130                1135
Arg Asp Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
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            1140                1145                1150
Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
        1155                1160                1165
Leu Asp Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr Met Tyr Lys
    1170                1175                1180
Tyr Phe Lys Lys Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp
1185                1190                1195                1200
Ser Asp Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
                1205                1210                1215
Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
            1220                1225                1230
Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile
        1235                1240                1245
Val Phe Lys Glu Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
    1250                1255                1260
Lys Glu Val Lys Arg Lys Pro Tyr Asn Ser Lys Leu Gly Cys Asn Trp
1265                1270                1275                1280
Gln Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
                1285                1290


<210> 11
<211> 1291
<212> PRT
<213> Clostridium botulinum C1-1

<400> 11
Met Pro Ile Thr Ile Asn Asn Phe Asn Tyr Ser Asp Pro Val Asp Asn
 1               5                  10                  15
Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala Asn Glu
            20                  25                  30
Pro Glu Lys Ala Phe Arg Ile Thr Gly Asn Ile Trp Val Ile Pro Asp
        35                  40                  45
Arg Phe Ser Arg Asn Ser Asn Pro Asn Leu Asn Lys Pro Pro Arg Val
    50                  55                  60
Thr Ser Pro Lys Ser Gly Tyr Tyr Asp Pro Asn Tyr Leu Ser Thr Asp
65                  70                  75                  80
Ser Asp Lys Asp Thr Phe Leu Lys Glu Ile Ile Lys Leu Phe Lys Arg
                85                  90                  95
Ile Asn Ser Arg Glu Ile Gly Glu Glu Leu Ile Tyr Arg Leu Ser Thr
            100                 105                 110
Asp Ile Pro Phe Pro Gly Asn Asn Asn Thr Pro Ile Asn Thr Phe Asp
        115                 120                 125
Phe Asp Val Asp Phe Asn Ser Val Asp Val Lys Thr Arg Gln Gly Asn
    130                 135                 140
Asn Trp Val Lys Thr Gly Ser Ile Asn Pro Ser Val Ile Ile Thr Gly
145                 150                 155                 160
Pro Arg Glu Asn Ile Ile Asp Pro Glu Thr Ser Thr Phe Lys Leu Thr
                165                 170                 175
Asn Asn Thr Phe Ala Ala Gln Glu Gly Phe Gly Ala Leu Ser Ile Ile
            180                 185                 190
Ser Ile Ser Pro Arg Phe Met Leu Thr Tyr Ser Asn Ala Thr Asn Asp
        195                 200                 205
Val Gly Glu Gly Arg Phe Ser Lys Ser Glu Phe Cys Met Asp Pro Ile
    210                 215                 220
Leu Ile Leu Met His Glu Leu Asn His Ala Met His Asn Leu Tyr Gly
225                 230                 235                 240
Ile Ala Ile Pro Asn Asp Gln Thr Ile Ser Ser Val Thr Ser Asn Ile
                245                 250                 255
Phe Tyr Ser Gln Tyr Asn Val Lys Leu Glu Tyr Ala Glu Ile Tyr Ala
            260                 265                 270
Phe Gly Gly Pro Thr Ile Asp Leu Ile Pro Lys Ser Ala Arg Lys Tyr
        275                 280                 285
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Phe Glu Glu Lys Ala Leu Asp Tyr Tyr Arg Ser Ile Ala Lys Arg Leu
    290                 295                 300
Asn Ser Ile Thr Thr Ala Asn Pro Ser Ser Phe Asn Lys Tyr Ile Gly
305                 310                 315                 320
Glu Tyr Lys Gln Lys Leu Ile Arg Lys Tyr Arg Phe Val Val Glu Ser
                325                 330                 335
Ser Gly Glu Val Thr Val Asn Arg Asn Lys Phe Val Glu Leu Tyr Asn
            340                 345                 350
Glu Leu Thr Gln Ile Phe Thr Glu Phe Asn Tyr Ala Lys Ile Tyr Asn
        355                 360                 365
Val Gln Asn Arg Lys Ile Tyr Leu Ser Asn Val Tyr Thr Pro Val Thr
    370                 375                 380
Ala Asn Ile Leu Asp Asp Asn Val Tyr Asp Ile Gln Asn Gly Phe Asn
385                 390                 395                 400
Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu Ser
                405                 410                 415
Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr Leu
            420                 425                 430
Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn
        435                 440                 445
Lys Thr Leu Asp Cys Arg Glu Leu Leu Val Lys Asn Thr Asp Leu Pro
    450                 455                 460
Phe Ile Gly Asp Ile Ser Asp Val Lys Thr Asp Ile Phe Leu Arg Lys
465                 470                 475                 480
Asp Ile Asn Glu Glu Thr Glu Val Ile Tyr Tyr Pro Asp Asn Val Ser
                485                 490                 495
Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu
            500                 505                 510
Asp Leu Leu Tyr Pro Ser Ile Asp Ser Glu Ser Glu Ile Leu Pro Gly
        515                 520                 525
Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu
    530                 535                 540
Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu
545                 550                 555                 560
Asp Phe Thr Phe Thr Arg Ser Ile Glu Glu Ala Leu Asp Asn Ser Ala
                565                 570                 575
Lys Val Tyr Thr Tyr Phe Pro Thr Leu Ala Asn Lys Val Asn Ala Gly
            580                 585                 590
Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp
        595                 600                 605
Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp
    610                 615                 620
Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn
625                 630                 635                 640
Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val
                645                 650                 655
Thr Ile Leu Leu Glu Ala Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly
            660                 665                 670
Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
        675                 680                 685
Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
    690                 695                 700
Tyr Glu Trp Met Met Gly Thr Trp Leu Ser Arg Ile Ile Thr Gln Phe
705                 710                 715                 720
Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Gly
                725                 730                 735
Ala Ile Lys Ala Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
            740                 745                 750
Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
        755                 760                 765
Asp Val Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
    770                 775                 780
Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
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785 790 795 800
Asp Glu Leu Asn Glu Phe Asp Arg Asn Thr Lys Ala Lys Leu Ile Asn
805 810 815
Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Lys Leu
820 825 830
Lys Ala Lys Val Asn Asn Ser Phe Gln Asn Thr Ile Pro Phe Asn Ile
835 840 845
Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr
850 855 860
Phe Asn Asn Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Arg Lys
865 870 875 880
Asn Thr Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Ser Glu Glu
885 890 895
Gly Asp Val Gln Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly
900 905 910
Ser Ser Gly Glu Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn
915 920 925
Ile Val Tyr Asn Ser Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile
930 935 940
Arg Ile Asn Lys Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp
945 950 955 960
Ser Val Lys Asn Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe
965 970 975
Leu Val Phe Thr Leu Lys Gln Asn Glu Asp Ser Glu Gln Ser Ile Asn
980 985 990
Phe Ser Tyr Asp Ile Ser Asn Asn Ala Pro Gly Tyr Asn Lys Trp Phe
995 1000 1005
Phe Val Thr Val Thr Asn Asn Met Met Gly Asn Met Lys Ile Tyr Ile
1010 1015 1020
Asn Gly Lys Leu Ile Asp Thr Ile Lys Val Lys Glu Leu Thr Gly Ile
1025 1030 1035 1040
Asn Phe Ser Lys Thr Ile Thr Phe Glu Ile Asn Lys Ile Pro Asp Thr
1045 1050 1055
Gly Leu Ile Thr Ser Asp Ser Asp Asn Ile Asn Met Trp Ile Arg Asp
1060 1065 1070
Phe Tyr Ile Phe Ala Lys Glu Leu Asp Gly Lys Asp Ile Asn Ile Leu
1075 1080 1085
Phe Asn Ser Leu Gln Tyr Thr Asn Val Val Lys Asp Tyr Trp Gly Asn
1090 1095 1100
Asp Leu Arg Tyr Asn Lys Glu Tyr Tyr Met Val Asn Ile Asp Tyr Leu
1105 1110 1115 1120
Asn Arg Tyr Met Tyr Ala Asn Ser Arg Gln Ile Val Phe Asn Thr Arg
1125 1130 1135
Arg Asn Asn Asn Asp Phe Asn Glu Gly Tyr Lys Ile Ile Ile Lys Arg
1140 1145 1150
Ile Arg Gly Asn Thr Asn Asp Thr Arg Val Arg Gly Gly Asp Ile Leu
1155 1160 1165
Tyr Phe Asp Met Thr Ile Asn Asn Lys Ala Tyr Asn Leu Phe Met Lys
1170 1175 1180
Asn Glu Thr Met Tyr Ala Asp Asn His Ser Thr Glu Asp Ile Tyr Ala
1185 1190 1195 1200
Ile Gly Leu Arg Glu Gln Thr Lys Asp Ile Asn Asp Asn Ile Ile Phe
1205 1210 1215
Gln Ile Gln Pro Met Asn Asn Thr Tyr Tyr Tyr Ala Ser Gln Ile Phe
1220 1225 1230
Lys Ser Asn Phe Asn Gly Glu Asn Ile Ser Gly Ile Cys Ser Ile Gly
1235 1240 1245
Thr Tyr Arg Phe Arg Leu Gly Gly Asp Trp Tyr Arg His Asn Tyr Leu
1250 1255 1260
Val Pro Thr Val Lys Gln Gly Asn Tyr Ala Ser Leu Leu Glu Ser Thr
1265 1270 1275 1280
Ser Thr His Trp Gly Phe Val Pro Val Ser Glu
1285 1290

<210> 12
<211> 1280
<212> PRT
<213> Clostridium botulinum C1-2

<400> 12
Met Pro Ile Thr Ile Asn Asn Phe Asn Tyr Ser Asp Pro Val Asp Asn
 1   5   10   15
Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala Asn Glu
            20   25   30
Pro Glu Lys Ala Phe Arg Ile Ile Gly Asn Ile Trp Val Ile Pro Asp
        35   40   45
Arg Phe Ser Arg Asp Ser Asn Pro Asn Leu Asn Lys Pro Pro Arg Val
    50   55   60
Thr Ser Pro Lys Ser Gly Tyr Tyr Asp Pro Asn Tyr Leu Ser Thr Asp
65   70   75   80
Ser Glu Lys Asp Thr Phe Leu Lys Glu Ile Ile Lys Leu Phe Lys Arg
                85   90   95
Ile Asn Ser Arg Glu Ile Gly Glu Glu Leu Ile Tyr Arg Leu Ala Thr
            100   105   110
Asp Ile Pro Phe Pro Gly Asn Asn Asn Thr Pro Ile Asn Thr Phe Asp
        115   120   125
Phe Asp Val Asp Phe Asn Ser Val Asp Val Lys Thr Arg Gln Gly Asn
    130   135   140
Asn Trp Val Lys Thr Gly Ser Ile Asn Pro Ser Val Ile Ile Thr Gly
145   150   155   160
Pro Arg Glu Asn Ile Ile Asp Pro Glu Thr Ser Thr Phe Lys Leu Thr
                165   170   175
Asn Asn Thr Phe Ala Ala Gln Glu Gly Phe Gly Ala Leu Ser Ile Ile
            180   185   190
Ser Ile Ser Pro Arg Phe Met Leu Thr Tyr Ser Asn Ala Thr Asn Asn
        195   200   205
Val Gly Glu Gly Arg Phe Ser Lys Ser Glu Phe Cys Met Asp Pro Ile
    210   215   220
Leu Ile Leu Met His Glu Leu Asn His Thr Met His Asn Leu Tyr Gly
225   230   235   240
Ile Ala Ile Pro Asn Asp Gln Arg Ile Ser Ser Val Thr Ser Asn Ile
                245   250   255
Phe Tyr Ser Gln Tyr Lys Val Lys Leu Glu Tyr Ala Glu Ile Tyr Ala
            260   265   270
Phe Gly Gly Pro Thr Ile Asp Leu Ile Pro Lys Ser Gly Arg Lys Tyr
        275   280   285
Phe Glu Glu Lys Ala Leu Asp Tyr Tyr Arg Ser Ile Ala Lys Arg Leu
    290   295   300
Asn Ser Ile Thr Thr Ala Asn Pro Ser Ser Phe Asn Lys Tyr Ile Gly
305   310   315   320
Glu Tyr Lys Gln Lys Leu Ile Arg Lys Tyr Arg Phe Val Val Glu Ser
                325   330   335
Ser Gly Glu Val Ala Val Asp Arg Asn Lys Phe Ala Glu Leu Tyr Lys
            340   345   350
Glu Leu Thr Gln Ile Phe Thr Glu Phe Asn Tyr Ala Lys Ile Tyr Asn
        355   360   365
Val Gln Asn Arg Lys Ile Tyr Leu Ser Asn Val Tyr Thr Pro Val Thr
    370   375   380
Ala Asn Ile Leu Asp Asp Asn Val Tyr Asp Ile Gln Asn Gly Phe Asn
385   390   395   400
Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu Ser
                405   410   415
Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr Leu
            420   425   430
Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn

435 440 445
Lys Thr Leu Asp Cys Arg Glu Leu Leu Val Lys Asn Thr Asp Leu Pro
450 455 460
Phe Ile Gly Asp Ile Ser Asp Ile Lys Thr Asp Ile Phe Leu Ser Lys
465 470 475 480
Asp Ile Asn Val Glu Thr Glu Val Ile Asp Tyr Pro Asp Asn Val Ser
485 490 495
Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu
500 505 510
Asp Leu Leu Tyr Pro Ile Ile Glu Gly Glu Ser Gln Val Leu Pro Gly
515 520 525
Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu
530 535 540
Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu
545 550 555 560
Asp Phe Thr Phe Thr Thr Ser Ile Glu Glu Ala Leu Asp Asn Ser Gly
565 570 575
Lys Val Tyr Thr Tyr Phe Pro Lys Leu Ala Asp Lys Val Asn Thr Gly
580 585 590
Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp
595 600 605
Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp
610 615 620
Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn
625 630 635 640
Ser Val Arg Arg Glu Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val
645 650 655
Thr Ile Leu Leu Glu Ala Phe Gln Glu Phe Thr Ile Pro Ala Leu Gly
660 665 670
Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
675 680 685
Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
690 695 700
Tyr Glu Trp Met Ile Gly Thr Trp Leu Ser Arg Ile Thr Thr Gln Phe
705 710 715 720
Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Asp
725 730 735
Ala Ile Lys Asp Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
740 745 750
Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
755 760 765
Asp Ile Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
770 775 780
Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
785 790 795 800
Asp Glu Leu Asn Lys Phe Asp Leu Lys Thr Lys Thr Glu Leu Ile Asn
805 810 815
Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu
820 825 830
Lys Ala Lys Val Asn Glu Ser Phe Glu Asn Thr Ile Pro Phe Asn Ile
835 840 845
Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr
850 855 860
Phe Asn Ser Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys
865 870 875 880
Asn Ala Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Leu Glu
885 890 895
Gly Asp Val Gln Val Asn Thr Ile Tyr Thr Asn Asp Phe Lys Leu Ser
900 905 910
Ser Ser Gly Asp Lys Ile Ile Val Asn Leu Asn Asn Asn Ile Leu Tyr
915 920 925
Ser Ala Ile Tyr Glu Asn Ser Ser Val Ser Phe Trp Ile Lys Ile Ser
930 935 940

Lys Asp Leu Thr Asn Ser His Asn Glu Tyr Thr Ile Ile Asn Ser Ile
945 950 955 960
Lys Gln Asn Ser Gly Trp Lys Leu Cys Ile Arg Asn Gly Asn Ile Glu
965 970 975
Trp Ile Leu Gln Asp Ile Asn Arg Lys Tyr Lys Ser Leu Ile Phe Asp
980 985 990
Tyr Ser Glu Ser Leu Ser His Thr Gly Tyr Thr Asn Lys Trp Phe Phe
995 1000 1005
Val Thr Ile Thr Asn Asn Ile Met Gly Tyr Met Lys Leu Tyr Ile Asn
1010 1015 1020
Gly Glu Leu Lys Gln Ser Glu Arg Ile Glu Asp Leu Asn Glu Val Lys
1025 1030 1035 1040
Leu Asp Lys Thr Ile Val Phe Gly Ile Asp Glu Asn Ile Asp Glu Asn
1045 1050 1055
Gln Met Leu Trp Ile Arg Asp Phe Asn Ile Phe Ser Lys Glu Leu Ser
1060 1065 1070
Asn Glu Asp Ile Asn Ile Val Tyr Glu Gly Gln Ile Leu Arg Asn Val
1075 1080 1085
Ile Lys Asp Tyr Trp Gly Asn Pro Leu Lys Phe Asp Thr Glu Tyr Tyr
1090 1095 1100
Ile Ile Asn Asp Asn Tyr Ile Asp Arg Tyr Ile Ala Pro Lys Ser Asn
1105 1110 1115 1120
Ile Leu Val Leu Val Gln Tyr Pro Asp Arg Ser Lys Leu Tyr Thr Gly
1125 1130 1135
Asn Pro Ile Thr Ile Lys Ser Val Ser Asp Lys Asn Pro Tyr Ser Arg
1140 1145 1150
Ile Leu Asn Gly Asp Asn Ile Met Phe His Met Leu Tyr Asn Ser Gly
1155 1160 1165
Lys Tyr Met Ile Ile Arg Asp Thr Asp Thr Ile Tyr Ala Ile Glu Gly
1170 1175 1180
Arg Glu Cys Ser Lys Asn Cys Val Tyr Ala Leu Lys Leu Gln Ser Asn
1185 1190 1195 1200
Leu Gly Asn Tyr Gly Ile Gly Ile Phe Ser Ile Lys Asn Ile Val Ser
1205 1210 1215
Gln Asn Lys Tyr Cys Ser Gln Ile Phe Ser Ser Phe Met Lys Asn Thr
1220 1225 1230
Met Leu Leu Ala Asp Ile Tyr Lys Pro Trp Arg Phe Ser Phe Glu Asn
1235 1240 1245
Ala Tyr Thr Pro Val Ala Val Thr Asn Tyr Glu Thr Lys Leu Leu Ser
1250 1255 1260
Thr Ser Ser Phe Trp Lys Phe Ile Ser Arg Asp Pro Gly Trp Val Glu
1265 1270 1275 1280

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<211> 1276
<212> PRT
<213> Clostridium botulinum D1

<400> 13
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1 5 10 15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr
20 25 30
Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
35 40 45
Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
50 55 60
Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65 70 75 80
Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
85 90 95
Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val

100 105 110
Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
115 120 125
Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
130 135 140
Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145 150 155 160
Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
165 170 175
Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
180 185 190
Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
195 200 205
Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
210 215 220
Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225 230 235 240
Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
245 250 255
Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
260 265 270
Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Ser Gln
275 280 285
Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
290 295 300
Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser Asn Ile Asp
305 310 315 320
Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
325 330 335
Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
340 345 350
Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
355 360 365
Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu Pro Val Phe
370 375 380
Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp Gly Phe Asn
385 390 395 400
Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
405 410 415
Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu
420 425 430
Phe Thr Lys Val Cys Leu Arg Leu Thr Lys Asn Ser Arg Asp Asp Ser
435 440 445
Thr Cys Ile Lys Val Lys Asn Asn Arg Leu Pro Tyr Val Ala Asp Lys
450 455 460
Asp Ser Ile Ser Gln Glu Ile Phe Glu Asn Lys Ile Ile Thr Asp Glu
465 470 475 480
Thr Asn Val Gln Asn Tyr Ser Asp Lys Phe Ser Leu Asp Glu Ser Ile
485 490 495
Leu Asp Gly Gln Val Pro Ile Asn Pro Glu Ile Val Asp Pro Leu Leu
500 505 510
Pro Asn Val Asn Met Glu Pro Leu Asn Leu Pro Gly Glu Glu Ile Val
515 520 525
Phe Tyr Asp Asp Ile Thr Lys Tyr Val Asp Tyr Leu Asn Ser Tyr Tyr
530 535 540
Tyr Leu Glu Ser Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu
545 550 555 560
Thr Thr Ser Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr
565 570 575
Phe Leu Pro Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly
580 585 590
Leu Phe Leu Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn
595 600 605

Ile Met Lys Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Val Ile
610 615 620
Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg
625 630 635 640
Gly Asn Phe Asn Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu
645 650 655
Glu Gly Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe
660 665 670
Tyr Ser Ser Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn
675 680 685
Cys Leu Glu Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met
690 695 700
Val Ser Asn Trp Leu Ser Arg Ile Thr Thr Gln Phe Asn His Ile Asn
705 710 715 720
Tyr Gln Met Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala
725 730 735
Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn
740 745 750
Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile
755 760 765
Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val
770 775 780
Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn
785 790 795 800
Lys Phe Asp Leu Arg Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser
805 810 815
His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val
820 825 830
Asn Glu Ser Phe Glu Asn Thr Met Pro Phe Asn Ile Phe Ser Tyr Thr
835 840 845
Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr Phe Asn Ser Ile
850 855 860
Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys Asn Ala Leu Val
865 870 875 880
Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Val Gly Asp Asn Val Gln
885 890 895
Leu Asn Thr Ile Tyr Thr Asn Asp Phe Lys Leu Ser Ser Ser Gly Asp
900 905 910
Lys Ile Ile Val Asn Leu Asn Asn Asn Ile Leu Tyr Ser Ala Ile Tyr
915 920 925
Glu Asn Ser Ser Val Ser Phe Trp Ile Lys Ile Ser Lys Asp Leu Thr
930 935 940
Asn Ser His Asn Glu Tyr Thr Ile Ile Asn Ser Ile Glu Gln Asn Ser
945 950 955 960
Gly Trp Lys Leu Cys Ile Arg Asn Gly Asn Ile Glu Trp Ile Leu Gln
965 970 975
Asp Val Asn Arg Lys Tyr Lys Ser Leu Ile Phe Asp Tyr Ser Glu Ser
980 985 990
Leu Ser His Thr Gly Tyr Thr Asn Lys Trp Phe Phe Val Thr Ile Thr
995 1000 1005
Asn Asn Ile Met Gly Tyr Met Lys Leu Tyr Ile Asn Gly Glu Leu Lys
1010 1015 1020
Gln Ser Gln Lys Ile Glu Asp Leu Asp Glu Val Lys Leu Asp Lys Thr
1025 1030 1035 1040
Ile Val Phe Gly Ile Asp Glu Asn Ile Asp Glu Asn Gln Met Leu Trp
1045 1050 1055
Ile Arg Asp Phe Asn Ile Phe Ser Lys Glu Leu Ser Asn Glu Asp Ile
1060 1065 1070
Asn Ile Val Tyr Glu Gly Gln Ile Leu Arg Asn Val Ile Lys Asp Tyr
1075 1080 1085
Trp Gly Asn Pro Leu Lys Phe Asp Thr Glu Tyr Tyr Ile Ile Asn Asp
1090 1095 1100
Asn Tyr Ile Asp Arg Tyr Ile Ala Pro Glu Ser Asn Val Leu Val Leu

1105 1110 1115 1120
Val Gln Tyr Pro Asp Arg Ser Lys Leu Tyr Thr Gly Asn Pro Ile Thr
1125 1130 1135
Ile Lys Ser Val Ser Asp Lys Asn Pro Tyr Ser Arg Ile Leu Asn Gly
1140 1145 1150
Asp Asn Ile Ile Leu His Met Leu Tyr Asn Ser Arg Lys Tyr Met Ile
1155 1160 1165
Ile Arg Asp Thr Asp Thr Ile Tyr Ala Thr Gln Gly Gly Glu Cys Ser
1170 1175 1180
Gln Asn Cys Val Tyr Ala Leu Lys Leu Gln Ser Asn Leu Gly Asn Tyr
1185 1190 1195 1200
Gly Ile Gly Ile Phe Ser Ile Lys Asn Ile Val Ser Lys Asn Lys Tyr
1205 1210 1215
Cys Ser Gln Ile Phe Ser Ser Phe Arg Glu Asn Thr Met Leu Leu Ala
1220 1225 1230
Asp Ile Tyr Lys Pro Trp Arg Phe Ser Phe Lys Asn Ala Tyr Thr Pro
1235 1240 1245
Val Ala Val Thr Asn Tyr Glu Thr Lys Leu Leu Ser Thr Ser Ser Phe
1250 1255 1260
Trp Lys Phe Ile Ser Arg Asp Pro Gly Trp Val Glu
1265 1270 1275

<210> 14
<211> 1285
<212> PRT
<213> Clostridium botulinum D2

<400> 14
Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
1 5 10 15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr
20 25 30
Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
35 40 45
Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
50 55 60
Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65 70 75 80
Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
85 90 95
Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
100 105 110
Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
115 120 125
Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
130 135 140
Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145 150 155 160
Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
165 170 175
Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
180 185 190
Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
195 200 205
Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
210 215 220
Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225 230 235 240
Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
245 250 255
Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
260 265 270

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Phe Gly Gly Ser Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Leu Gln
        275                 280                 285
Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
    290                 295                 300
Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ser Ser Asn Ile Asp
305                 310                 315                 320
Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
                325                 330                 335
Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
            340                 345                 350
Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
        355                 360                 365
Val Lys Asn Arg Thr His Tyr Phe Ser Lys His Tyr Leu Pro Val Phe
    370                 375                 380
Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Ile Asn Gly Phe Asn
385                 390                 395                 400
Leu Thr Thr Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
                405                 410                 415
Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu
            420                 425                 430
Phe Thr Lys Val Cys Leu Arg Leu Thr Arg Asn Ser Arg Asp Asp Ser
        435                 440                 445
Thr Cys Ile Gln Val Lys Asn Asn Thr Leu Pro Tyr Val Ala Asp Lys
    450                 455                 460
Asp Ser Ile Ser Gln Glu Ile Phe Glu Ser Gln Ile Ile Thr Asp Glu
465                 470                 475                 480
Thr Asn Val Glu Asn Tyr Ser Asp Asn Phe Ser Leu Asp Glu Ser Ile
                485                 490                 495
Leu Asp Ala Lys Val Pro Thr Asn Pro Glu Ala Val Asp Pro Leu Leu
            500                 505                 510
Pro Asn Val Asn Met Glu Pro Leu Asn Val Pro Gly Glu Glu Glu Val
        515                 520                 525
Phe Tyr Asp Asp Ile Thr Lys Asp Val Asp Tyr Leu Asn Ser Tyr Tyr
    530                 535                 540
Tyr Leu Glu Ala Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu
545                 550                 555                 560
Thr Thr Ser Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr
                565                 570                 575
Phe Leu Pro Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly
            580                 585                 590
Leu Phe Leu Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn
        595                 600                 605
Ile Met Lys Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Ala Ile
    610                 615                 620
Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg
625                 630                 635                 640
Gly Asn Phe Lys Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu
                645                 650                 655
Glu Gly Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe
            660                 665                 670
Tyr Ser Ser Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn
        675                 680                 685
Cys Leu Glu Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met
    690                 695                 700
Val Ser Asn Trp Leu Ser Arg Ile Thr Thr Arg Phe Asn His Ile Ser
705                 710                 715                 720
Tyr Gln Met Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala
                725                 730                 735
Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn
            740                 745                 750
Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile
        755                 760                 765
Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val
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770 775 780
Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn
785 790 795 800
Lys Phe Asp Leu Lys Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser
805 810 815
His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val
820 825 830
Asn Glu Ser Phe Glu Asn Thr Ile Pro Phe Asn Ile Phe Ser Tyr Thr
835 840 845
Asn Asn Ser Leu Leu Lys Asp Met Ile Asn Glu Tyr Phe Asn Ser Ile
850 855 860
Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys Asn Thr Leu Met
865 870 875 880
Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Val Glu Gly Asn Val Gln
885 890 895
Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly Ser Ser Gly Asp
900 905 910
Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn Ile Val Tyr Asn
915 920 925
Ala Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile Arg Ile Asn Lys
930 935 940
Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp Ser Val Lys Asn
945 950 955 960
Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe Leu Val Phe Thr
965 970 975
Leu Lys Gln Asn Glu Asn Ser Glu Gln Asp Ile Asn Phe Ser Tyr Asp
980 985 990
Ile Ser Lys Asn Ala Ala Gly Tyr Asn Lys Trp Phe Phe Val Thr Ile
995 1000 1005
Thr Thr Asn Met Met Gly Asn Met Met Ile Tyr Ile Asn Gly Lys Leu
1010 1015 1020
Ile Asp Thr Ile Lys Val Lys Glu Leu Thr Gly Ile Asn Phe Ser Lys
1025 1030 1035 1040
Thr Ile Thr Phe Gln Met Asn Lys Ile Pro Asn Thr Gly Leu Ile Thr
1045 1050 1055
Ser Asp Ser Asp Asn Ile Asn Met Trp Ile Arg Asp Phe Tyr Ile Phe
1060 1065 1070
Ala Lys Glu Leu Asp Asp Lys Asp Ile Asn Ile Leu Phe Asn Ser Leu
1075 1080 1085
Gln Tyr Thr Asn Val Val Lys Asp Tyr Trp Gly Asn Asp Leu Arg Tyr
1090 1095 1100
Asp Lys Glu Tyr Tyr Met Ile Asn Val Asn Tyr Met Asn Arg Tyr Met
1105 1110 1115 1120
Ser Lys Lys Gly Asn Gly Ile Val Phe Asn Thr Arg Lys Asn Asn Asn
1125 1130 1135
Asp Phe Asn Glu Gly Tyr Lys Ile Ile Ile Lys Arg Ile Arg Gly Asn
1140 1145 1150
Thr Asn Asp Thr Arg Val Arg Gly Glu Asn Val Leu Tyr Phe Asn Thr
1155 1160 1165
Thr Ile Asp Asn Lys Gln Tyr Ser Leu Gly Met Tyr Lys Pro Ser Arg
1170 1175 1180
Asn Leu Gly Thr Asp Leu Val Pro Leu Gly Ala Leu Asp Gln Pro Met
1185 1190 1195 1200
Asp Glu Ile Arg Lys Tyr Gly Ser Phe Ile Ile Gln Pro Cys Asn Thr
1205 1210 1215
Phe Asp Tyr Tyr Ala Ser Gln Leu Phe Leu Ser Ser Asn Ala Thr Thr
1220 1225 1230
Asn Arg Leu Gly Ile Leu Ser Ile Gly Ser Tyr Ser Phe Lys Leu Gly
1235 1240 1245
Asp Asp Tyr Trp Phe Asn His Glu Tyr Leu Ile Pro Val Ile Lys Ile
1250 1255 1260
Glu His Tyr Ala Ser Leu Leu Glu Ser Thr Ser Thr His Trp Val Phe
1265 1270 1275 1280

Val Pro Ala Ser Glu
1285

<210> 15
<211> 1252
<212> PRT
<213> Clostridium botulinum E1

<400> 15

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Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
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Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr Lys Ser
            20                  25                  30
Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
        35                  40                  45
Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
    50                  55                  60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65                  70                  75                  80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
                85                  90                  95
Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
            100                 105                 110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
        115                 120                 125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
    130                 135                 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145                 150                 155                 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
                165                 170                 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
            180                 185                 190
Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
        195                 200                 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
    210                 215                 220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225                 230                 235                 240
Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
                245                 250                 255
Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
            260                 265                 270
Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
        275                 280                 285
Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
    290                 295                 300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350
Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
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420 425 430

Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
435 440 445

Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
450 455 460

Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465 470 475 480

Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
485 490 495

Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
500 505 510

Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
515 520 525

Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
530 535 540

Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545 550 555 560

Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
565 570 575

Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
580 585 590

Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
595 600 605

Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
610 615 620

Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625 630 635 640

Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
645 650 655

Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
660 665 670

Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
675 680 685

Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
690 695 700

Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705 710 715 720

Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
725 730 735

Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
740 745 750

Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
755 760 765

Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
770 775 780

Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
785 790 795 800

Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
805 810 815

Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
820 825 830

Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
835 840 845

Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
850 855 860

Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865 870 875 880

Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
885 890 895

Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
900 905 910

Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
915 920 925

Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
930 935 940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945 950 955 960
Trp Thr Leu Gln Asp Asn Ala Gly Ile Asn Gln Lys Leu Ala Phe Asn
965 970 975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
980 985 990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
995 1000 1005
Gly Asn Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His
1010 1015 1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025 1030 1035 1040
Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
1045 1050 1055
Thr Glu Ile Gln Thr Leu Tyr Ser Asn Glu Pro Asn Thr Asn Ile Leu
1060 1065 1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
1075 1080 1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Lys Asp Ser
1090 1095 1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105 1110 1115 1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
1125 1130 1135
Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
1140 1145 1150
Val Ala Ser Lys Thr His Leu Phe Pro Leu Tyr Ala Asp Thr Ala Thr
1155 1160 1165
Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe
1170 1175 1180
Asn Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn
1185 1190 1195 1200
Phe Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala
1205 1210 1215
Asp Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp His
1220 1225 1230
Thr Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly
1235 1240 1245
Trp Gln Glu Lys
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20 25 30
Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45
Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65 70 75 80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
85 90 95
Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro

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                100                 105                 110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
            115                 120                 125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ile Gln Asp Ile Leu
        130                 135                 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145                 150                 155                 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
                165                 170                 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
            180                 185                 190
Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
        195                 200                 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
    210                 215                 220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225                 230                 235                 240
Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
                245                 250                 255
Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
            260                 265                 270
Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
        275                 280                 285
Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
    290                 295                 300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350
Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430
Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445
Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460
Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480
Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495
Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510
Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525
Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540
Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560
Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
                565                 570                 575
Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590
Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605
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Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
610 615 620
Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625 630 635 640
Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
645 650 655
Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
660 665 670
Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
675 680 685
Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
690 695 700
Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705 710 715 720
Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
725 730 735
Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
740 745 750
Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
755 760 765
Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
770 775 780
Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
785 790 795 800
Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
805 810 815
Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
820 825 830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
835 840 845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
850 855 860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865 870 875 880
Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
885 890 895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
900 905 910
Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
915 920 925
Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
930 935 940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945 950 955 960
Trp Thr Leu Gln Asp Asn Ala Gly Ile Asn Gln Lys Leu Ala Phe Asn
965 970 975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
980 985 990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
995 1000 1005
Gly Asn Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His
1010 1015 1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025 1030 1035 1040
Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
1045 1050 1055
Thr Glu Ile Gln Thr Leu Tyr Asn Asn Glu Pro Asn Ala Asn Ile Leu
1060 1065 1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
1075 1080 1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Thr Asp Ser
1090 1095 1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg

1105 1110 1115 1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
1125 1130 1135
Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
1140 1145 1150
Val Ala Ser Lys Thr His Leu Phe Pro Leu Tyr Ala Asp Thr Asn Thr
1155 1160 1165
Thr Asn Lys Glu Lys Thr Ile Lys Ser Ser Ser Ser Gly Asn Arg Phe
1170 1175 1180
Asn Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn
1185 1190 1195 1200
Phe Lys Asn Asn Asn Gly Asn Asn Ile Gly Met Leu Gly Phe Lys Asp
1205 1210 1215
Asn Thr Leu Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp Asn
1220 1225 1230
Thr Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly
1235 1240 1245
Trp Gln Glu Lys
1250

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<213> Clostridium botulinum E3

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Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45
Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65 70 75 80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
85 90 95
Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
100 105 110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
115 120 125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln His Ile Leu
130 135 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190
Arg Phe Asn Asp Asn Ser Ile Asn Glu Phe Ile Gln Asp Pro Ala Leu
195 200 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
210 215 220
Lys Gly Ile Thr Thr Thr Cys Ile Ile Thr Gln Gln Gln Asn Pro Leu
225 230 235 240
Ile Thr Asn Arg Lys Gly Ile Asn Ile Glu Glu Phe Leu Thr Phe Gly
245 250 255
Gly Asn Asp Leu Asn Ile Ile Thr Val Ala Gln Tyr Asn Asp Ile Tyr
260 265 270
Thr Asn Leu Leu Asn Asp Tyr Arg Lys Ile Ala Ser Lys Leu Ser Lys
275 280 285

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Val Gln Val Ser Asn Pro Gln Leu Asn Pro Tyr Lys Asp Ile Phe Gln
    290                 295                 300
Glu Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320
Ile Asn Lys Phe Asp Asp Ile Leu Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Glu Thr Tyr Ile
            340                 345                 350
Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Lys Pro Ile Thr
385                 390                 395                 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430
Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445
Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460
Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480
Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495
Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510
Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525
Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540
Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560
Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
                565                 570                 575
Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590
Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605
Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620
Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625                 630                 635                 640
Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655
Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670
Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
        675                 680                 685
Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700
Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705                 710                 715                 720
Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
                725                 730                 735
Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
            740                 745                 750
Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765
Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780
Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
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785 790 795 800
Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
805 810 815
Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
820 825 830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
835 840 845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
850 855 860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865 870 875 880
Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
885 890 895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
900 905 910
Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
915 920 925
Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
930 935 940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945 950 955 960
Trp Thr Leu Gln Asp Asn Ala Gly Ile Asn Gln Lys Leu Ala Phe Asn
965 970 975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
980 985 990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
995 1000 1005
Gly Asn Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His
1010 1015 1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025 1030 1035 1040
Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
1045 1050 1055
Thr Glu Ile Gln Thr Leu Tyr Ser Asn Glu Pro Asn Thr Asn Ile Leu
1060 1065 1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
1075 1080 1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Lys Asp Ser
1090 1095 1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105 1110 1115 1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
1125 1130 1135
Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
1140 1145 1150
Val Ala Ser Lys Thr His Leu Phe Pro Leu Tyr Ala Asp Thr Ala Thr
1155 1160 1165
Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe
1170 1175 1180
Asn Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn
1185 1190 1195 1200
Phe Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala
1205 1210 1215
Asp Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp His
1220 1225 1230
Thr Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly
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Trp Gln Glu Lys
1250

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<212> PRT
<213> Clostridium botulinum F1

<400> 18

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Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser Lys Lys
            20                  25                  30
Tyr Tyr Lys Ala Phe Glu Ile Met Arg Asn Val Trp Ile Ile Pro Glu
        35                  40                  45
Arg Asn Thr Ile Gly Thr Asn Pro Ser Asp Phe Asp Pro Pro Ala Ser
    50                  55                  60
Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65                  70                  75                  80
Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys
                85                  90                  95
Arg Ile Asn Ser Asn Pro Ala Gly Lys Val Leu Leu Gln Glu Ile Ser
            100                 105                 110
Tyr Ala Lys Pro Tyr Leu Gly Asn Asp His Thr Pro Ile Asp Glu Phe
        115                 120                 125
Ser Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Leu Ser Thr Asn
    130                 135                 140
Val Glu Ser Ser Met Leu Leu Asn Leu Leu Val Leu Gly Ala Gly Pro
145                 150                 155                 160
Asp Ile Phe Glu Ser Cys Cys Tyr Pro Val Arg Lys Leu Ile Asp Pro
                165                 170                 175
Asp Val Val Tyr Asp Pro Ser Asn Tyr Gly Phe Gly Ser Ile Asn Ile
            180                 185                 190
Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
        195                 200                 205
Gly His Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
    210                 215                 220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
225                 230                 235                 240
Gly Val Thr Tyr Glu Glu Thr Ile Glu Val Lys Gln Ala Pro Leu Met
                245                 250                 255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
            260                 265                 270
Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
        275                 280                 285
Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Glu Val
    290                 295                 300
Asn Ser Ala Pro Pro Glu Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
305                 310                 315                 320
Gln Trp Lys Tyr Gly Leu Asp Lys Asn Ala Asp Gly Ser Tyr Thr Val
                325                 330                 335
Asn Glu Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr
            340                 345                 350
Glu Ser Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
        355                 360                 365
Phe Ile Lys Tyr Glu Phe Leu Lys Val Pro Asn Leu Leu Asp Asp Asp
    370                 375                 380
Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385                 390                 395                 400
Asn Arg Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile
                405                 410                 415
Pro Asp Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val
            420                 425                 430
Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Arg Val
        435                 440                 445
Asn Asn Ser Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
    450                 455                 460
Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
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465                 470                 475                 480
Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
                485                 490                 495
Gln Thr Ile Pro Gln Ile Ser Asn Arg Thr Leu Asn Thr Leu Val Gln
            500                 505                 510
Asp Asn Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile
        515                 520                 525
Glu Glu Tyr Asp Val Val Asp Phe Asn Val Phe Phe Tyr Leu His Ala
    530                 535                 540
Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545                 550                 555                 560
Asp Thr Ala Leu Leu Glu Glu Ser Lys Asp Ile Phe Phe Ser Ser Glu
                565                 570                 575
Phe Ile Asp Thr Ile Asn Lys Pro Val Asn Ala Ala Leu Phe Ile Asp
            580                 585                 590
Trp Ile Ser Lys Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln Lys
        595                 600                 605
Ser Thr Val Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Val
    610                 615                 620
Gly Leu Ala Leu Asn Ile Ile Ile Glu Ala Glu Lys Gly Asn Phe Glu
625                 630                 635                 640
Glu Ala Phe Glu Leu Leu Gly Val Gly Ile Leu Leu Glu Phe Val Pro
                645                 650                 655
Glu Leu Thr Ile Pro Val Ile Leu Val Phe Thr Ile Lys Ser Tyr Ile
            660                 665                 670
Asp Ser Tyr Glu Asn Lys Asn Lys Ala Ile Lys Ala Ile Asn Asn Ser
        675                 680                 685
Leu Ile Glu Arg Glu Ala Lys Trp Lys Glu Ile Tyr Ser Trp Ile Val
    690                 695                 700
Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu
705                 710                 715                 720
Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr Ala
                725                 730                 735
Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Lys Asn Arg Leu
            740                 745                 750
Glu Ser Glu Tyr Asn Ile Asn Asn Ile Glu Glu Glu Leu Asn Lys Lys
        755                 760                 765
Val Ser Leu Ala Met Lys Asn Ile Glu Arg Phe Met Thr Glu Ser Ser
    770                 775                 780
Ile Ser Tyr Leu Met Lys Leu Ile Asn Glu Ala Lys Val Gly Lys Leu
785                 790                 795                 800
Lys Lys Tyr Asp Asn His Val Lys Ser Asp Leu Leu Asn Tyr Ile Leu
                805                 810                 815
Asp His Arg Ser Ile Leu Gly Glu Gln Thr Asn Glu Leu Ser Asp Leu
            820                 825                 830
Val Thr Ser Thr Leu Asn Ser Ser Ile Pro Phe Glu Leu Ser Ser Tyr
        835                 840                 845
Thr Asn Asp Lys Ile Leu Ile Ile Tyr Phe Asn Arg Leu Tyr Lys Lys
    850                 855                 860
Ile Lys Asp Ser Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys Phe
865                 870                 875                 880
Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asn Val
                885                 890                 895
Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Asn Ser Arg
            900                 905                 910
Leu Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn Ser
        915                 920                 925
Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Lys His
    930                 935                 940
Tyr Lys Pro Met Asn His Asn Arg Glu Tyr Thr Ile Ile Asn Cys Met
945                 950                 955                 960
Gly Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Arg Thr Val Arg Asp
                965                 970                 975
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Cys Glu Ile Ile Trp Thr Leu Gln Asp Thr Ser Gly Asn Lys Glu Asn
980 985 990
Leu Ile Phe Arg Tyr Glu Glu Leu Asn Arg Ile Ser Asn Tyr Ile Asn
995 1000 1005
Lys Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg
1010 1015 1020
Ile Tyr Ile Asn Gly Asn Leu Ile Val Glu Lys Ser Ile Ser Asn Leu
1025 1030 1035 1040
Gly Asp Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly Cys
1045 1050 1055
Asp Asp Glu Thr Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asn Thr
1060 1065 1070
Glu Leu Asp Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asn Glu Pro Asp
1075 1080 1085
Pro Ser Ile Leu Lys Asn Tyr Trp Gly Asn Tyr Leu Leu Tyr Asn Lys
1090 1095 1100
Lys Tyr Tyr Leu Phe Asn Leu Leu Arg Lys Asp Lys Tyr Ile Thr Leu
1105 1110 1115 1120
Asn Ser Gly Ile Leu Asn Ile Asn Gln Gln Arg Gly Val Thr Glu Gly
1125 1130 1135
Ser Val Phe Leu Asn Tyr Lys Leu Tyr Glu Gly Val Glu Val Ile Ile
1140 1145 1150
Arg Lys Asn Gly Pro Ile Asp Ile Ser Asn Thr Asp Asn Phe Val Arg
1155 1160 1165
Lys Asn Asp Leu Ala Tyr Ile Asn Val Val Asp Arg Gly Val Glu Tyr
1170 1175 1180
Arg Leu Tyr Ala Asp Thr Lys Ser Glu Lys Glu Lys Ile Ile Arg Thr
1185 1190 1195 1200
Ser Asn Leu Asn Asp Ser Leu Gly Gln Ile Ile Val Met Asp Ser Ile
1205 1210 1215
Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly Ser Asn Ile
1220 1225 1230
Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser Ser Trp Tyr
1235 1240 1245
Tyr Asn Asn Ile Arg Arg Asn Thr Ser Ser Asn Gly Cys Phe Trp Ser
1250 1255 1260
Ser Ile Ser Lys Glu Asn Gly Trp Lys Glu
1265 1270

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20 25 30
Tyr Tyr Lys Ala Phe Glu Ile Met Pro Asn Val Trp Ile Met Pro Glu
35 40 45
Arg Asp Thr Ile Gly Thr Lys Pro Asp Glu Phe Gln Val Pro Asp Ser
50 55 60
Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65 70 75 80
Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Met Ile Lys Leu Phe Asn
85 90 95
Arg Ile Asn Ser Asn Pro Thr Gly Lys Val Leu Leu Glu Glu Val Ser
100 105 110
Asn Ala Arg Pro Tyr Leu Gly Asp Asp Asp Thr Leu Ile Asn Glu Phe
115 120 125
Leu Pro Val Asn Val Thr Thr Ser Val Asn Ile Lys Phe Ser Thr Asp

130 135 140
Val Glu Ser Ser Ile Ile Ser Asn Leu Leu Val Leu Gly Ala Gly Pro
145 150 155 160
Asp Ile Phe Lys Ala Tyr Cys Thr Pro Leu Val Arg Phe Asn Lys Ser
165 170 175
Asp Lys Leu Ile Glu Pro Ser Asn His Gly Phe Gly Ser Ile Asn Ile
180 185 190
Leu Thr Phe Ser Pro Glu Tyr Glu His Ile Phe Asn Asp Ile Ser Gly
195 200 205
Gly Asn His Asn Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
210 215 220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Lys
225 230 235 240
Ala Val Thr His Lys Glu Ser Leu Val Ala Glu Arg Gly Pro Leu Met
245 250 255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
260 265 270
Glu Asp Leu Asn Ile Ile Pro Ser Ala Met Lys Glu Lys Ile Tyr Asn
275 280 285
Asp Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Arg Glu Val
290 295 300
Asn Thr Ala Pro Pro Gly Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
305 310 315 320
Gln Trp Lys Tyr Gly Leu Asp Arg Asn Ala Asp Gly Ser Tyr Thr Val
325 330 335
Asn Arg Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr
340 345 350
Glu Ile Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
355 360 365
Phe Ile Lys Tyr Gly Phe Val Lys Val Pro Asn Leu Leu Asp Asp Asp
370 375 380
Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385 390 395 400
Asn Arg Gly Gln Asn Ile Asn Leu Asn Pro Lys Ile Ile Asp Ser Ile
405 410 415
Pro Asp Lys Gly Leu Val Glu Lys Ile Ile Lys Phe Cys Lys Ser Ile
420 425 430
Ile Pro Arg Lys Gly Thr Lys Gln Ser Pro Ser Leu Cys Ile Arg Val
435 440 445
Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
450 455 460
Ser Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
465 470 475 480
Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
485 490 495
Glu Thr Ile Pro Gln Ile Ser Asn Arg Thr Leu Asn Thr Leu Val Gln
500 505 510
Asp Asn Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile
515 520 525
Glu Glu Tyr Asp Val Val Asp Phe Asn Val Phe Phe Tyr Leu His Ala
530 535 540
Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545 550 555 560
Asp Thr Ala Leu Leu Glu Glu Ser Lys Val Tyr Thr Phe Phe Ser Ser
565 570 575
Glu Phe Ile Asp Thr Ile Asn Lys Pro Val Asn Ala Ala Leu Phe Ile
580 585 590
Asp Trp Ile Ser Lys Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln
595 600 605
Lys Ser Thr Val Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr
610 615 620
Val Gly Leu Ala Leu Asn Ile Val Ile Glu Ala Glu Lys Gly Asn Phe
625 630 635 640

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Glu Glu Ala Phe Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Val
                645                 650                 655
Pro Glu Leu Thr Ile Pro Val Ile Leu Val Phe Thr Ile Lys Ser Tyr
            660                 665                 670
Ile Asp Ser Tyr Glu Asn Lys Asn Lys Ala Ile Lys Ala Ile Asn Asn
        675                 680                 685
Ser Leu Ile Glu Arg Glu Ala Lys Trp Lys Glu Ile Tyr Ser Trp Ile
    690                 695                 700
Val Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys
705                 710                 715                 720
Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr
                725                 730                 735
Ala Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Lys Asn Arg
            740                 745                 750
Leu Glu Ser Lys Tyr Asn Ile Asn Asn Ile Glu Glu Glu Leu Asn Lys
        755                 760                 765
Lys Val Ser Leu Ala Met Lys Asn Ile Glu Arg Phe Met Thr Glu Ser
    770                 775                 780
Ser Ile Ser Tyr Leu Met Lys Leu Ile Asn Glu Ala Glu Val Gly Lys
785                 790                 795                 800
Leu Lys Glu Tyr Asp Lys His Val Lys Ser Asp Leu Leu Asp Tyr Ile
                805                 810                 815
Leu Tyr His Lys Leu Ile Leu Gly Glu Gln Thr Lys Glu Leu Ile Asp
            820                 825                 830
Leu Val Thr Ser Thr Leu Asn Ser Ser Ile Pro Phe Glu Leu Ser Ser
        835                 840                 845
Tyr Thr Asn Asp Lys Ile Leu Ile Ile Tyr Phe Asn Arg Leu Tyr Lys
    850                 855                 860
Lys Ile Lys Asp Ser Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys
865                 870                 875                 880
Phe Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asn
                885                 890                 895
Val Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Ser Gly
            900                 905                 910
Arg Leu Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn
        915                 920                 925
Ser Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Thr Ile Pro Lys
    930                 935                 940
His Tyr Arg Pro Met Asn Arg Asn Arg Glu Tyr Thr Ile Ile Asn Cys
945                 950                 955                 960
Met Gly Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Arg Thr Ile Arg
                965                 970                 975
Asp Cys Glu Ile Ile Trp Thr Leu Gln Asp Thr Ser Gly Asn Lys Glu
            980                 985                 990
Lys Leu Ile Phe Arg Tyr Glu Glu Leu Ala Ser Ile Ser Asp Tyr Ile
        995                 1000                1005
Asn Lys Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser
    1010                1015                1020
Arg Ile Tyr Ile Asn Gly Asn Leu Ile Val Glu Lys Ser Ile Ser Asn
1025                1030                1035                1040
Leu Gly Asp Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly
                1045                1050                1055
Cys Asp Asp Glu Thr Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asn
            1060                1065                1070
Thr Glu Leu Asp Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asn Glu Pro
        1075                1080                1085
Asp Pro Ser Ile Leu Lys Asp Tyr Trp Gly Asn Tyr Leu Leu Tyr Asn
    1090                1095                1100
Lys Lys Tyr Tyr Leu Phe Asn Leu Leu Arg Lys Asp Lys Tyr Ile Thr
1105                1110                1115                1120
Arg Asn Ser Gly Ile Leu Asn Ile Asn Gln Gln Arg Gly Val Thr Gly
                1125                1130                1135
Gly Ile Ser Val Phe Leu Asn Tyr Lys Leu Tyr Glu Gly Val Glu Val
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            1140                1145                1150
Ile Ile Arg Lys Asn Ala Pro Ile Asp Ile Ser Asn Thr Asp Asn Phe
        1155                1160                1165
Val Arg Lys Asn Asp Leu Ala Tyr Ile Asn Val Val Asp His Gly Val
    1170                1175                1180
Glu Tyr Arg Leu Tyr Ala Asp Ile Ser Ile Thr Lys Ser Glu Lys Ile
1185                1190                1195                1200
Ile Lys Leu Ile Arg Thr Ser Asn Pro Asn Asp Ser Leu Gly Gln Ile
                1205                1210                1215
Ile Val Met Asp Ser Ile Gly Asn Asn Cys Thr Met Asn Phe Gln Asn
            1220                1225                1230
Asn Asp Gly Ser Asn Ile Gly Leu Leu Gly Phe His Ser Asp Asp Leu
        1235                1240                1245
Val Ala Ser Ser Trp Tyr Tyr Asn His Ile Arg Arg Asn Thr Ser Ser
    1250                1255                1260
Asn Gly Cys Phe Trp Ser Phe Ile Ser Lys Glu His Gly Trp Lys Glu
1265                1270                1275                1280


<210> 20
<211> 1278
<212> PRT
<213> Clostridium botulinum F3

<400> 20
Met Pro Val Val Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
 1               5                  10                  15
Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser Lys Lys
            20                  25                  30
Tyr Tyr Lys Ala Phe Glu Ile Met Arg Asn Val Trp Ile Ile Pro Glu
        35                  40                  45
Arg Asn Thr Ile Gly Thr Asp Pro Ser Asp Phe Asp Pro Pro Ala Ser
    50                  55                  60
Leu Glu Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65                  70                  75                  80
Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys
                85                  90                  95
Arg Ile Asn Ser Asn Pro Ala Gly Glu Val Leu Leu Gln Glu Ile Ser
            100                 105                 110
Tyr Ala Lys Pro Tyr Leu Gly Asn Glu His Thr Pro Ile Asn Glu Phe
        115                 120                 125
His Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Ser Ser Thr Asn
    130                 135                 140
Val Lys Ser Ser Ile Ile Leu Asn Leu Leu Val Leu Gly Ala Gly Pro
145                 150                 155                 160
Asp Ile Phe Glu Asn Ser Ser Tyr Pro Val Arg Lys Leu Met Asp Ser
                165                 170                 175
Gly Gly Val Tyr Asp Pro Ser Asn Asp Gly Phe Gly Ser Ile Asn Ile
            180                 185                 190
Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
        195                 200                 205
Gly Tyr Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
    210                 215                 220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
225                 230                 235                 240
Gly Val Thr Tyr Lys Glu Thr Ile Lys Val Lys Gln Ala Pro Leu Met
                245                 250                 255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
            260                 265                 270
Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
        275                 280                 285
Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Arg Val
    290                 295                 300
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Asn Ser Ala Pro Pro Glu Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
305                 310                 315                 320
Gln Trp Lys Tyr Gly Leu Asp Lys Asn Ala Asp Gly Ser Tyr Thr Val
                325                 330                 335
Asn Glu Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr
            340                 345                 350
Glu Ile Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
        355                 360                 365
Phe Ile Lys Tyr Gly Phe Leu Lys Val Pro Asn Leu Leu Asp Asp Asp
    370                 375                 380
Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385                 390                 395                 400
Asn Arg Gly Gln Asn Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile
                405                 410                 415
Pro Asp Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val
            420                 425                 430
Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Arg Val
        435                 440                 445
Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
    450                 455                 460
Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
465                 470                 475                 480
Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
                485                 490                 495
Glu Thr Ile Pro Gln Ile Ser Asn Gln Thr Leu Asn Thr Leu Val Gln
            500                 505                 510
Asp Asp Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile
        515                 520                 525
Glu Glu His Asn Val Val Asp Leu Asn Val Phe Phe Tyr Leu His Ala
    530                 535                 540
Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545                 550                 555                 560
Asp Thr Ala Leu Ser Glu Glu Ser Gln Val Tyr Thr Phe Phe Ser Ser
                565                 570                 575
Glu Phe Ile Asn Thr Ile Asn Lys Pro Val His Ala Ala Leu Phe Ile
            580                 585                 590
Ser Trp Ile Asn Gln Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln
        595                 600                 605
Lys Ser Thr Phe Asp Lys Ile Ala Asp Ile Ser Leu Val Val Pro Tyr
    610                 615                 620
Val Gly Leu Ala Leu Asn Ile Gly Asn Glu Val Gln Lys Glu Asn Phe
625                 630                 635                 640
Lys Glu Ala Phe Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Val
                645                 650                 655
Pro Glu Leu Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe
            660                 665                 670
Ile Gly Ser Ser Glu Asn Lys Asn Lys Ile Ile Lys Ala Ile Asn Asn
        675                 680                 685
Ser Leu Met Glu Arg Glu Thr Lys Trp Lys Glu Ile Tyr Ser Trp Ile
    690                 695                 700
Val Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys
705                 710                 715                 720
Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr
                725                 730                 735
Val Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Arg Asn Arg
            740                 745                 750
Leu Glu Ser Glu Tyr Asn Ile Asn Asn Ile Arg Glu Glu Leu Asn Lys
        755                 760                 765
Lys Val Ser Leu Ala Met Glu Asn Ile Glu Arg Phe Ile Thr Glu Ser
    770                 775                 780
Ser Ile Phe Tyr Leu Met Lys Leu Ile Asn Glu Ala Lys Val Ser Lys
785                 790                 795                 800
Leu Arg Glu Tyr Asp Glu Gly Val Lys Glu Tyr Leu Leu Asp Tyr Ile
```

805 810 815
Ser Glu His Arg Ser Ile Leu Gly Asn Ser Val Gln Glu Leu Asn Asp
820 825 830
Leu Val Thr Ser Thr Leu Asn Asn Ser Ile Pro Phe Glu Leu Ser Ser
835 840 845
Tyr Thr Asn Asp Lys Ile Leu Ile Leu Tyr Phe Asn Lys Leu Tyr Lys
850 855 860
Lys Ile Lys Asp Asn Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys
865 870 875 880
Phe Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asp
885 890 895
Val Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Ser Ser
900 905 910
Lys Pro Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn
915 920 925
Gly Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Lys
930 935 940
Tyr Phe Asn Lys Val Asn Leu Asn Asn Glu Tyr Thr Ile Ile Asp Cys
945 950 955 960
Ile Arg Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Asn Tyr Asn Lys
965 970 975
Ile Ile Trp Thr Leu Gln Asp Thr Ala Gly Asn Asn Gln Lys Leu Val
980 985 990
Phe Asn Tyr Thr Gln Met Ile Ser Ile Ser Asp Tyr Ile Asn Lys Trp
995 1000 1005
Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg Ile Tyr
1010 1015 1020
Ile Asn Gly Asn Leu Ile Asp Glu Lys Ser Ile Ser Asn Leu Gly Asp
1025 1030 1035 1040
Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly Cys Asn Asp
1045 1050 1055
Thr Arg Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asp Thr Glu Leu
1060 1065 1070
Gly Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asp Glu Pro Asp Pro Ser
1075 1080 1085
Ile Leu Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asn Lys Arg Tyr
1090 1095 1100
Tyr Leu Leu Asn Leu Leu Arg Thr Asp Lys Ser Ile Thr Gln Asn Ser
1105 1110 1115 1120
Asn Phe Leu Asn Ile Asn Gln Gln Arg Gly Val Tyr Gln Lys Pro Asn
1125 1130 1135
Ile Phe Ser Asn Thr Arg Leu Tyr Thr Gly Val Glu Val Ile Ile Arg
1140 1145 1150
Lys Asn Gly Ser Thr Asp Ile Ser Asn Thr Asp Asn Phe Val Arg Lys
1155 1160 1165
Asn Asp Leu Ala Tyr Ile Asn Val Val Asp Arg Asp Val Glu Tyr Arg
1170 1175 1180
Leu Tyr Ala Asp Ile Ser Ile Ala Lys Pro Glu Lys Ile Ile Lys Leu
1185 1190 1195 1200
Ile Arg Thr Ser Asn Ser Asn Asn Ser Leu Gly Gln Ile Ile Val Met
1205 1210 1215
Asp Ser Ile Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly
1220 1225 1230
Gly Asn Ile Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser
1235 1240 1245
Ser Trp Tyr Tyr Asn Asn Ile Arg Lys Asn Thr Ser Ser Asn Gly Cys
1250 1255 1260
Phe Trp Ser Phe Ile Ser Lys Glu His Gly Trp Gln Glu Asn
1265 1270 1275

<210> 21
<211> 1297

<212> PRT
<213> Clostridium botulinum G

<220>
<221> VARIANT
<222> 7
<223> Identifty of amino acid is unknown

<400> 21

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Met Pro Val Asn Ile Lys Xaa Phe Asn Tyr Asn Asp Pro Ile Asn Asn
 1               5                  10                  15
Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro Gly Thr
            20                  25                  30
Tyr Tyr Lys Ala Phe Arg Ile Ile Asp Arg Ile Trp Ile Val Pro Glu
        35                  40                  45
Arg Phe Thr Tyr Gly Phe Gln Pro Asp Gln Phe Asn Ala Ser Thr Gly
    50                  55                  60
Val Phe Ser Lys Asp Val Tyr Glu Tyr Tyr Asp Pro Thr Tyr Leu Lys
65                  70                  75                  80
Thr Asp Ala Glu Lys Asp Lys Phe Leu Lys Thr Met Ile Lys Leu Phe
                85                  90                  95
Asn Arg Ile Asn Ser Lys Pro Ser Gly Gln Arg Leu Leu Asp Met Ile
            100                 105                 110
Val Asp Ala Ile Pro Tyr Leu Gly Asn Ala Ser Thr Pro Pro Asp Lys
        115                 120                 125
Phe Ala Ala Asn Val Ala Asn Val Ser Ile Asn Lys Lys Ile Ile Gln
    130                 135                 140
Pro Gly Ala Glu Asp Gln Ile Lys Gly Leu Met Thr Asn Leu Ile Ile
145                 150                 155                 160
Phe Gly Pro Gly Pro Val Leu Ser Asp Asn Phe Thr Asp Ser Met Ile
                165                 170                 175
Met Asn Gly His Ser Pro Ile Ser Glu Gly Phe Gly Ala Arg Met Met
            180                 185                 190
Ile Arg Phe Cys Pro Ser Cys Leu Asn Val Phe Asn Asn Val Gln Glu
        195                 200                 205
Asn Lys Asp Thr Ser Ile Phe Ser Arg Arg Ala Tyr Phe Ala Asp Pro
    210                 215                 220
Ala Leu Thr Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240
Gly Ile Lys Ile Ser Asn Leu Pro Ile Thr Pro Asn Thr Lys Glu Phe
                245                 250                 255
Phe Met Gln His Ser Asp Pro Val Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270
Gly Gly His Asp Pro Ser Val Ile Ser Pro Ser Thr Asp Met Asn Ile
        275                 280                 285
Tyr Asn Lys Ala Leu Gln Asn Phe Gln Asp Ile Ala Asn Arg Leu Asn
    290                 295                 300
Ile Val Ser Ser Ala Gln Gly Ser Gly Ile Asp Ile Ser Leu Tyr Lys
305                 310                 315                 320
Gln Ile Tyr Lys Asn Lys Tyr Asp Phe Val Glu Asp Pro Asn Gly Lys
                325                 330                 335
Tyr Ser Val Asp Lys Asp Lys Phe Asp Lys Leu Tyr Lys Ala Leu Met
            340                 345                 350
Phe Gly Phe Thr Glu Thr Asn Leu Ala Gly Glu Tyr Gly Ile Lys Thr
        355                 360                 365
Arg Tyr Ser Tyr Phe Ser Glu Tyr Leu Pro Pro Ile Lys Thr Glu Lys
    370                 375                 380
Leu Leu Asp Asn Thr Ile Tyr Thr Gln Asn Glu Gly Phe Asn Ile Ala
385                 390                 395                 400
Ser Lys Asn Leu Lys Thr Glu Phe Asn Gly Gln Asn Lys Ala Val Asn
                405                 410                 415
Lys Glu Ala Tyr Glu Glu Ile Ser Leu Glu His Leu Val Ile Tyr Arg
            420                 425                 430
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```
Ile Ala Met Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu
        435                 440                 445
Gln Cys Ile Ile Val Asn Asn Glu Asp Leu Phe Phe Ile Ala Asn Lys
    450                 455                 460
Asp Ser Phe Ser Lys Asp Leu Ala Lys Ala Glu Thr Ile Ala Tyr Asn
465                 470                 475                 480
Thr Gln Asn Asn Thr Ile Glu Asn Asn Phe Ser Ile Asp Gln Leu Ile
                485                 490                 495
Leu Asp Asn Asp Leu Ser Ser Gly Ile Asp Leu Pro Asn Glu Asn Thr
            500                 505                 510
Glu Pro Phe Thr Asn Phe Asp Asp Ile Asp Ile Pro Val Tyr Ile Lys
        515                 520                 525
Gln Ser Ala Leu Lys Lys Ile Phe Val Asp Gly Asp Ser Leu Phe Glu
    530                 535                 540
Tyr Leu His Ala Gln Thr Phe Pro Ser Asn Ile Glu Asn Leu Gln Leu
545                 550                 555                 560
Thr Asn Ser Leu Asn Asp Ala Leu Arg Asn Asn Asn Lys Val Tyr Thr
                565                 570                 575
Phe Phe Ser Thr Asn Leu Val Glu Lys Ala Asn Thr Val Val Gly Ala
            580                 585                 590
Ser Leu Phe Val Asn Trp Val Lys Gly Val Ile Asp Asp Phe Thr Ser
        595                 600                 605
Glu Ser Thr Gln Lys Ser Thr Ile Asp Lys Val Ser Asp Val Ser Ile
    610                 615                 620
Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Val Gly Asn Glu Thr Ala
625                 630                 635                 640
Lys Glu Asn Phe Lys Asn Ala Phe Glu Ile Gly Gly Ala Ala Ile Leu
                645                 650                 655
Met Glu Phe Ile Pro Glu Leu Ile Val Pro Ile Val Gly Phe Phe Thr
            660                 665                 670
Leu Glu Ser Tyr Val Gly Asn Lys Gly His Ile Ile Met Thr Ile Ser
        675                 680                 685
Asn Ala Leu Lys Lys Arg Asp Gln Lys Trp Thr Asp Met Tyr Gly Leu
    690                 695                 700
Ile Val Ser Gln Trp Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile
705                 710                 715                 720
Lys Glu Arg Met Tyr Asn Ala Leu Asn Asn Gln Ser Gln Ala Ile Glu
                725                 730                 735
Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met
            740                 745                 750
Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser
        755                 760                 765
Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser
    770                 775                 780
Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu
785                 790                 795                 800
Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp
                805                 810                 815
Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys
            820                 825                 830
Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr
        835                 840                 845
Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn
    850                 855                 860
Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu
865                 870                 875                 880
Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val
                885                 890                 895
Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu
            900                 905                 910
Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser
        915                 920                 925
Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr
```

```
    930                 935                 940
Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile
945                 950                 955                 960
Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn
                965                 970                 975
Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile
            980                 985                 990
Phe Phe Glu Tyr Ser Ile Lys Asp Asn Ile Ser Asp Tyr Ile Asn Lys
        995                 1000                1005
Trp Phe Ser Ile Thr Ile Thr Asn Asp Arg Leu Gly Asn Ala Asn Ile
    1010                1015                1020
Tyr Ile Asn Gly Ser Leu Lys Lys Ser Glu Lys Ile Leu Asn Leu Asp
1025                1030                1035                1040
Arg Ile Asn Ser Ser Asn Asp Ile Asp Phe Lys Leu Ile Asn Cys Thr
                1045                1050                1055
Asp Thr Thr Lys Phe Val Trp Ile Lys Asp Phe Asn Ile Phe Gly Arg
            1060                1065                1070
Glu Leu Asn Ala Thr Glu Val Ser Ser Leu Tyr Trp Ile Gln Ser Ser
        1075                1080                1085
Thr Asn Thr Leu Lys Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr
    1090                1095                1100
Gln Tyr Tyr Leu Phe Asn Gln Gly Met Gln Asn Ile Tyr Ile Lys Tyr
1105                1110                1115                1120
Phe Ser Lys Ala Ser Met Gly Glu Thr Ala Pro Arg Thr Asn Phe Asn
                1125                1130                1135
Asn Ala Ala Ile Asn Tyr Gln Asn Leu Tyr Leu Gly Leu Arg Phe Ile
            1140                1145                1150
Ile Lys Lys Ala Ser Asn Ser Arg Asn Ile Asn Asn Asp Asn Ile Val
        1155                1160                1165
Arg Glu Gly Asp Tyr Ile Tyr Leu Asn Ile Asp Asn Ile Ser Asp Glu
    1170                1175                1180
Ser Tyr Arg Val Tyr Val Leu Val Asn Ser Lys Glu Ile Gln Thr Gln
1185                1190                1195                1200
Leu Phe Leu Ala Pro Ile Asn Asp Asp Pro Thr Phe Tyr Asp Val Leu
                1205                1210                1215
Gln Ile Lys Lys Tyr Tyr Glu Lys Thr Thr Tyr Asn Cys Gln Ile Leu
            1220                1225                1230
Cys Glu Lys Asp Thr Lys Thr Phe Gly Leu Phe Gly Ile Gly Lys Phe
        1235                1240                1245
Val Lys Asp Tyr Gly Tyr Val Trp Asp Thr Tyr Asp Asn Tyr Phe Cys
    1250                1255                1260
Ile Ser Gln Trp Tyr Leu Arg Arg Ile Ser Glu Asn Ile Asn Lys Leu
1265                1270                1275                1280
Arg Leu Gly Cys Asn Trp Gln Phe Ile Pro Val Asp Glu Gly Trp Thr
                1285                1290                1295
Glu
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<210> 22
<211> 1315
<212> PRT
<213> Clostridium tetani

<400> 22

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Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn
 1               5                  10                  15
Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile
            20                  25                  30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu
        35                  40                  45
Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
    50                  55                  60
```

```
Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
65                  70                  75                  80
Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
                85                  90                  95
Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile
            100                 105                 110
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe
        115                 120                 125
Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
    130                 135                 140
Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe
145                 150                 155                 160
Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
                165                 170                 175
Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser
            180                 185                 190
Ile Met Gln Met Ala Phe Cys Pro Glu Tyr Val Pro Thr Phe Asp Asn
        195                 200                 205
Val Ile Glu Asn Ile Thr Ser Leu Thr Ile Gly Lys Ser Lys Tyr Phe
    210                 215                 220
Gln Asp Pro Ala Leu Leu Leu Met His Glu Leu Ile His Val Leu His
225                 230                 235                 240
Gly Leu Tyr Gly Met Gln Val Ser Ser His Glu Ile Ile Pro Ser Lys
                245                 250                 255
Gln Glu Ile Tyr Met Gln His Thr Tyr Pro Ile Ser Ala Glu Glu Leu
            260                 265                 270
Phe Thr Phe Gly Gly Gln Asp Ala Asn Leu Ile Ser Ile Asp Ile Lys
        275                 280                 285
Asn Asp Leu Tyr Glu Lys Thr Leu Asn Asp Tyr Lys Ala Ile Ala Asn
    290                 295                 300
Lys Leu Ser Gln Val Thr Ser Cys Asn Asp Pro Asn Ile Asp Ile Asp
305                 310                 315                 320
Ser Tyr Lys Gln Ile Tyr Gln Gln Lys Tyr Gln Phe Asp Lys Asp Ser
                325                 330                 335
Asn Gly Gln Tyr Ile Val Asn Glu Asp Lys Phe Gln Ile Leu Tyr Asn
            340                 345                 350
Ser Ile Met Tyr Gly Phe Thr Glu Ile Glu Leu Gly Lys Lys Phe Asn
        355                 360                 365
Ile Lys Thr Arg Leu Ser Tyr Phe Ser Met Asn His Asp Pro Val Lys
    370                 375                 380
Ile Pro Asn Leu Leu Asp Asp Thr Ile Tyr Asn Asp Thr Glu Gly Phe
385                 390                 395                 400
Asn Ile Glu Ser Lys Asp Leu Lys Ser Glu Tyr Lys Gly Gln Asn Met
                405                 410                 415
Arg Val Asn Thr Asn Ala Phe Arg Asn Val Asp Gly Ser Gly Leu Val
            420                 425                 430
Ser Lys Leu Ile Gly Leu Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile
        435                 440                 445
Arg Glu Asn Leu Tyr Asn Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly
    450                 455                 460
Glu Leu Cys Ile Lys Ile Lys Asn Glu Asp Leu Thr Phe Ile Ala Glu
465                 470                 475                 480
Lys Asn Ser Phe Ser Glu Glu Pro Phe Gln Asp Glu Ile Val Ser Tyr
                485                 490                 495
Asn Thr Lys Asn Lys Pro Leu Asn Phe Asn Tyr Ser Leu Asp Lys Ile
            500                 505                 510
Ile Val Asp Tyr Asn Leu Gln Ser Lys Ile Thr Leu Pro Asn Asp Arg
        515                 520                 525
Thr Thr Pro Val Thr Lys Gly Ile Pro Tyr Ala Pro Glu Tyr Lys Ser
    530                 535                 540
Asn Ala Ala Ser Thr Ile Glu Ile His Asn Ile Asp Asp Asn Thr Ile
545                 550                 555                 560
Tyr Gln Tyr Leu Tyr Ala Gln Lys Ser Pro Thr Thr Leu Gln Arg Ile
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565 570 575
Thr Met Thr Asn Ser Val Asp Asp Ala Leu Ile Asn Ser Thr Lys Ile
580 585 590
Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val Asn Gln Gly Ala Gln
595 600 605
Gly Ile Leu Phe Leu Gln Trp Val Arg Asp Ile Ile Asp Asp Phe Thr
610 615 620
Asn Glu Ser Ser Gln Lys Thr Thr Ile Asp Lys Ile Ser Asp Val Ser
625 630 635 640
Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Val Lys Gln Gly
645 650 655
Tyr Glu Gly Asn Phe Ile Gly Ala Leu Glu Thr Thr Gly Val Val Leu
660 665 670
Leu Leu Glu Tyr Ile Pro Glu Ile Thr Leu Pro Val Ile Ala Ala Leu
675 680 685
Ser Ile Ala Glu Ser Ser Thr Gln Lys Glu Lys Ile Ile Lys Thr Ile
690 695 700
Asp Asn Phe Leu Glu Lys Arg Tyr Glu Lys Trp Ile Glu Val Tyr Lys
705 710 715 720
Leu Val Lys Ala Lys Trp Leu Gly Thr Val Asn Thr Gln Phe Gln Lys
725 730 735
Arg Ser Tyr Gln Met Tyr Arg Ser Leu Glu Tyr Gln Val Asp Ala Ile
740 745 750
Lys Lys Ile Ile Asp Tyr Glu Tyr Lys Ile Tyr Ser Gly Pro Asp Lys
755 760 765
Glu Gln Ile Ala Asp Glu Ile Asn Asn Leu Lys Asn Lys Leu Glu Glu
770 775 780
Lys Ala Asn Lys Ala Met Ile Asn Ile Asn Ile Phe Met Arg Glu Ser
785 790 795 800
Ser Arg Ser Phe Leu Val Asn Gln Met Ile Asn Glu Ala Lys Lys Gln
805 810 815
Leu Leu Glu Phe Asp Thr Gln Ser Lys Asn Ile Leu Met Gln Tyr Ile
820 825 830
Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Glu
835 840 845
Ser Lys Ile Asn Lys Val Phe Ser Thr Pro Ile Pro Phe Ser Tyr Ser
850 855 860
Lys Asn Leu Asp Cys Trp Val Asp Asn Glu Glu Asp Ile Asp Val Ile
865 870 875 880
Leu Lys Lys Ser Thr Ile Leu Asn Leu Asp Ile Asn Asn Asp Ile Ile
885 890 895
Ser Asp Ile Ser Gly Phe Asn Ser Ser Val Ile Thr Tyr Pro Asp Ala
900 905 910
Gln Leu Val Pro Gly Ile Asn Gly Lys Ala Ile His Leu Val Asn Asn
915 920 925
Glu Ser Ser Glu Val Ile Val His Lys Ala Met Asp Ile Glu Tyr Asn
930 935 940
Asp Met Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys
945 950 955 960
Val Ser Ala Ser His Leu Glu Gln Tyr Gly Thr Asn Glu Tyr Ser Ile
965 970 975
Ile Ser Ser Met Lys Lys His Ser Leu Ser Ile Gly Ser Gly Trp Ser
980 985 990
Val Ser Leu Lys Gly Asn Asn Leu Ile Trp Thr Leu Lys Asp Ser Ala
995 1000 1005
Gly Glu Val Arg Gln Ile Thr Phe Arg Asp Leu Pro Asp Lys Phe Asn
1010 1015 1020
Ala Tyr Leu Ala Asn Lys Trp Val Phe Ile Thr Ile Thr Asn Asp Arg
1025 1030 1035 1040
Leu Ser Ser Ala Asn Leu Tyr Ile Asn Gly Val Leu Met Gly Ser Ala
1045 1050 1055
Glu Ile Thr Gly Leu Gly Ala Ile Arg Glu Asp Asn Asn Ile Thr Leu
1060 1065 1070

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Lys Leu Asp Arg Cys Asn Asn Asn Asn Gln Tyr Val Ser Ile Asp Lys
        1075                1080                1085
Phe Arg Ile Phe Cys Lys Ala Leu Asn Pro Lys Glu Ile Glu Lys Leu
    1090                1095                1100
Tyr Thr Ser Tyr Leu Ser Ile Thr Phe Leu Arg Asp Phe Trp Gly Asn
1105                1110                1115                1120
Pro Leu Arg Tyr Asp Thr Glu Tyr Tyr Leu Ile Pro Val Ala Ser Ser
                1125                1130                1135
Ser Lys Asp Val Gln Leu Lys Asn Ile Thr Asp Tyr Met Tyr Leu Thr
            1140                1145                1150
Asn Ala Pro Ser Tyr Thr Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg
        1155                1160                1165
Leu Tyr Asn Gly Leu Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn
    1170                1175                1180
Glu Ile Asp Ser Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val
1185                1190                1195                1200
Ser Tyr Asn Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn
                1205                1210                1215
Ala Phe Asn Asn Leu Asp Arg Ile Leu Arg Val Gly Tyr Asn Ala Pro
            1220                1225                1230
Gly Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu
        1235                1240                1245
Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala Ser
    1250                1255                1260
Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp Pro Asn
1265                1270                1275                1280
Arg Asp Ile Leu Ile Ala Ser Asn Trp Tyr Phe Asn His Leu Lys Asp
                1285                1290                1295
Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr Asp Glu Gly Trp
            1300                1305                1310
Thr Asn Asp
        1315


<210> 23
<211> 1268
<212> PRT
<213> Clostridium baratii

<400> 23
Met Pro Val Asn Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asn Asn
 1               5                  10                  15
Thr Thr Ile Leu Tyr Met Lys Met Pro Tyr Tyr Glu Asp Ser Asn Lys
            20                  25                  30
Tyr Tyr Lys Ala Phe Glu Ile Met Asp Asn Val Trp Ile Ile Pro Glu
        35                  40                  45
Arg Asn Ile Ile Gly Lys Lys Pro Ser Asp Phe Tyr Pro Pro Ile Ser
    50                  55                  60
Leu Asp Ser Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65                  70                  75                  80
Asp Ala Glu Lys Asp Arg Phe Leu Lys Thr Val Ile Lys Leu Phe Asn
                85                  90                  95
Arg Ile Asn Ser Asn Pro Ala Gly Gln Val Leu Leu Glu Glu Ile Lys
            100                 105                 110
Asn Gly Lys Pro Tyr Leu Gly Asn Asp His Thr Ala Val Asn Glu Phe
        115                 120                 125
Cys Ala Asn Asn Arg Ser Thr Ser Val Glu Ile Lys Glu Ser Asn Gly
    130                 135                 140
Thr Thr Asp Ser Met Leu Leu Asn Leu Val Ile Leu Gly Pro Gly Pro
145                 150                 155                 160
Asn Ile Leu Glu Cys Ser Thr Phe Pro Val Arg Ile Phe Pro Asn Asn
                165                 170                 175
Ile Ala Tyr Asp Pro Ser Glu Lys Gly Phe Gly Ser Ile Gln Leu Met
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                180                 185                 190
Ser Phe Ser Thr Glu Tyr Glu Tyr Ala Phe Asn Asp Asn Thr Asp Leu
        195                 200                 205
Phe Ile Ala Asp Pro Ala Ile Ser Leu Ala His Glu Leu Ile His Val
    210                 215                 220
Leu His Gly Leu Tyr Gly Ala Lys Gly Val Thr Asn Lys Lys Val Ile
225                 230                 235                 240
Glu Val Asp Gln Gly Ala Leu Met Ala Ala Glu Lys Asp Ile Lys Ile
                245                 250                 255
Glu Glu Phe Ile Thr Phe Gly Gly Gln Asp Leu Asn Ile Ile Thr Asn
            260                 265                 270
Ser Thr Asn Gln Lys Ile Tyr Val Ile Leu Leu Ser Asn Tyr Thr Ala
        275                 280                 285
Ile Ala Ser Arg Leu Ser Gln Val Asn Arg Asn Asn Ser Ala Leu Asn
    290                 295                 300
Thr Thr Tyr Tyr Lys Asn Phe Phe Gln Trp Lys Tyr Gly Leu Asp Gln
305                 310                 315                 320
Asp Ser Asn Gly Asn Tyr Thr Val Asn Ile Ser Lys Phe Asn Ala Ile
                325                 330                 335
Tyr Lys Lys Leu Phe Ser Phe Thr Glu Cys Asp Leu Ala Gln Lys Phe
            340                 345                 350
Gln Val Lys Asn Arg Ser Asn Tyr Leu Phe His Phe Lys Pro Phe Arg
        355                 360                 365
Leu Leu Asp Leu Leu Asp Asp Asn Ile Tyr Ser Ile Ser Glu Gly Phe
    370                 375                 380
Asn Ile Gly Ser Leu Arg Val Asn Asn Asn Gly Gln Asn Ile Asn Leu
385                 390                 395                 400
Asn Ser Arg Ile Val Gly Pro Ile Pro Asp Asn Gly Leu Val Glu Arg
                405                 410                 415
Phe Val Gly Leu Cys Lys Ser Ile Val Ser Lys Lys Gly Thr Lys Asn
            420                 425                 430
Ser Leu Cys Ile Lys Val Asn Asn Arg Asp Leu Phe Phe Val Ala Ser
        435                 440                 445
Glu Ser Ser Tyr Asn Glu Asn Gly Ile Asn Ser Pro Lys Glu Ile Asp
    450                 455                 460
Asp Thr Thr Ile Thr Asn Asn Asn Tyr Lys Lys Asn Leu Asp Glu Val
465                 470                 475                 480
Ile Leu Asp Tyr Asn Ser Asp Ala Ile Pro Asn Leu Ser Ser Arg Leu
                485                 490                 495
Leu Asn Thr Thr Ala Gln Asn Asp Ser Tyr Val Pro Lys Tyr Asp Ser
            500                 505                 510
Asn Gly Thr Ser Glu Ile Lys Glu Tyr Thr Val Asp Lys Leu Asn Val
        515                 520                 525
Phe Phe Tyr Leu Tyr Ala Gln Lys Ala Pro Glu Gly Glu Ser Ala Ile
    530                 535                 540
Ser Leu Thr Ser Ser Val Asn Thr Ala Leu Leu Asp Ala Ser Lys Val
545                 550                 555                 560
Tyr Thr Phe Phe Ser Ser Asp Phe Ile Asn Thr Val Asn Lys Pro Val
                565                 570                 575
Gln Ala Ala Leu Phe Ile Ser Trp Ile Gln Gln Val Ile Asn Asp Phe
            580                 585                 590
Thr Thr Glu Ala Thr Gln Lys Ser Thr Ile Asp Lys Ile Ala Asp Ile
        595                 600                 605
Ser Leu Ile Val Pro Tyr Val Gly Leu Ala Leu Asn Ile Gly Asn Glu
    610                 615                 620
Val Gln Lys Gly Asn Phe Lys Glu Ala Ile Glu Leu Leu Gly Ala Gly
625                 630                 635                 640
Ile Leu Leu Glu Phe Val Pro Glu Leu Leu Ile Pro Thr Ile Leu Val
                645                 650                 655
Phe Thr Ile Lys Ser Phe Ile Asn Ser Asp Asp Ser Lys Asn Lys Ile
            660                 665                 670
Ile Lys Ala Ile Asn Asn Ala Leu Arg Glu Arg Glu Leu Lys Trp Lys
        675                 680                 685
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Glu Val Tyr Ser Trp Ile Val Ser Asn Trp Leu Thr Arg Ile Asn Thr
    690                 695                 700
Gln Phe Asn Lys Arg Lys Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln
705                 710                 715                 720
Val Asp Gly Ile Lys Lys Ile Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr
                725                 730                 735
Leu Asp Glu Lys Asn Arg Leu Arg Ala Glu Tyr Asn Ile Tyr Ser Ile
            740                 745                 750
Lys Glu Glu Leu Asn Lys Lys Val Ser Leu Ala Met Gln Asn Ile Asp
        755                 760                 765
Arg Phe Leu Thr Glu Ser Ser Ile Ser Tyr Leu Met Lys Leu Ile Asn
    770                 775                 780
Glu Ala Lys Ile Asn Lys Leu Ser Glu Tyr Asp Lys Arg Val Asn Gln
785                 790                 795                 800
Tyr Leu Leu Asn Tyr Ile Leu Glu Asn Ser Ser Thr Leu Gly Thr Ser
                805                 810                 815
Ser Val Pro Glu Leu Asn Asn Leu Val Ser Asn Thr Leu Asn Asn Ser
            820                 825                 830
Ile Pro Phe Glu Leu Ser Glu Tyr Thr Asn Asp Lys Ile Leu Ile His
        835                 840                 845
Ile Leu Ile Arg Phe Tyr Lys Arg Ile Ile Asp Ser Ser Ile Leu Asn
    850                 855                 860
Met Lys Tyr Glu Asn Asn Arg Phe Ile Asp Ser Ser Gly Tyr Gly Ser
865                 870                 875                 880
Asn Ile Ser Ile Asn Gly Asp Ile Tyr Ile Tyr Ser Thr Asn Arg Asn
                885                 890                 895
Gln Phe Gly Ile Tyr Ser Ser Arg Leu Ser Glu Val Asn Ile Thr Gln
            900                 905                 910
Asn Asn Thr Ile Ile Tyr Asn Ser Arg Tyr Gln Asn Phe Ser Val Ser
        915                 920                 925
Phe Trp Val Arg Ile Pro Lys Tyr Asn Asn Leu Lys Asn Leu Asn Asn
    930                 935                 940
Glu Tyr Thr Ile Ile Asn Cys Met Arg Asn Asn Asn Ser Gly Trp Lys
945                 950                 955                 960
Ile Ser Leu Asn Tyr Asn Asn Ile Ile Trp Thr Leu Gln Asp Thr Thr
                965                 970                 975
Gly Asn Asn Gln Lys Leu Val Phe Asn Tyr Thr Gln Met Ile Asp Ile
            980                 985                 990
Ser Asp Tyr Ile Asn Lys Trp Thr Phe Val Thr Ile Thr Asn Asn Arg
        995                 1000                1005
Leu Gly His Ser Lys Leu Tyr Ile Asn Gly Asn Leu Thr Asp Gln Lys
    1010                1015                1020
Ser Ile Leu Asn Leu Gly Asn Ile His Val Asp Asp Asn Ile Leu Phe
1025                1030                1035                1040
Lys Ile Val Gly Cys Asn Asp Thr Arg Tyr Val Gly Ile Arg Tyr Phe
                1045                1050                1055
Lys Ile Phe Asn Met Glu Leu Asp Lys Thr Glu Ile Glu Thr Leu Tyr
            1060                1065                1070
His Ser Glu Pro Asp Ser Thr Ile Leu Lys Asp Phe Trp Gly Asn Tyr
        1075                1080                1085
Leu Leu Tyr Asn Lys Lys Tyr Tyr Leu Leu Asn Leu Leu Lys Pro Asn
    1090                1095                1100
Met Ser Val Thr Lys Asn Ser Asp Ile Leu Asn Ile Asn Arg Gln Arg
1105                1110                1115                1120
Gly Ile Tyr Ser Lys Thr Asn Ile Phe Ser Asn Ala Arg Leu Tyr Thr
                1125                1130                1135
Gly Val Glu Val Ile Ile Arg Lys Val Gly Ser Thr Asp Thr Ser Asn
            1140                1145                1150
Thr Asp Asn Phe Val Arg Lys Asn Asp Thr Val Tyr Ile Asn Val Val
        1155                1160                1165
Asp Gly Asn Ser Glu Tyr Gln Leu Tyr Ala Asp Val Ser Thr Ser Ala
    1170                1175                1180
Val Glu Lys Thr Ile Lys Leu Arg Arg Ile Ser Asn Ser Asn Tyr Asn
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1185 1190 1195 1200
Ser Asn Gln Met Ile Ile Met Asp Ser Ile Gly Asp Asn Cys Thr Met
1205 1210 1215
Asn Phe Lys Thr Asn Asn Gly Asn Asp Ile Gly Leu Leu Gly Phe His
1220 1225 1230
Leu Asn Asn Leu Val Ala Ser Ser Trp Tyr Tyr Lys Asn Ile Arg Asn
1235 1240 1245
Asn Thr Arg Asn Asn Gly Cys Phe Trp Ser Phe Ile Ser Lys Glu His
1250 1255 1260
Gly Trp Gln Glu
1265

<210> 24
<211> 1251
<212> PRT
<213> Clostridium butyricum 1

<400> 24
Met Pro Thr Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asn Arg
1 5 10 15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Gln Phe Tyr Lys Ser
20 25 30
Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45
Gly Thr Ile Pro Gln Asp Phe Leu Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Gln Glu Lys
65 70 75 80
Asp Lys Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asp
85 90 95
Asn Leu Ser Gly Arg Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
100 105 110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Gly Asp Phe Ile Ile Asn Asp
115 120 125
Ala Ser Ala Val Pro Ile Gln Phe Ser Asn Gly Ser Gln Ser Ile Leu
130 135 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190
Arg Phe Lys Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
195 200 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
210 215 220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225 230 235 240
Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
245 250 255
Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
260 265 270
Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
275 280 285
Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
290 295 300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305 310 315 320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
325 330 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
340 345 350

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Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430
Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445
Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460
Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480
Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495
Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510
Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525
Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540
Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560
Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Gly Trp Ile
                565                 570                 575
Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590
Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605
Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620
Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625                 630                 635                 640
Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655
Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670
Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
        675                 680                 685
Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700
Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Leu Lys Ala Ile Ile Glu
705                 710                 715                 720
Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
                725                 730                 735
Lys Tyr Asp Ile Glu Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
            740                 745                 750
Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765
Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780
Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asp Tyr Ile Ile Lys His
785                 790                 795                 800
Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Ile
                805                 810                 815
Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
            820                 825                 830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
        835                 840                 845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
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    850                 855                 860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865                 870                 875                 880
Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
                885                 890                 895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
            900                 905                 910
Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
        915                 920                 925
Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
    930                 935                 940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945                 950                 955                 960
Trp Thr Leu Gln Asp Asn Ser Gly Ile Asn Gln Lys Leu Ala Phe Asn
                965                 970                 975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
            980                 985                 990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
        995                 1000                1005
Gly Asn Leu Ile Asp Lys Lys Ser Ile Leu Asn Leu Gly Asn Ile His
    1010                1015                1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025                1030                1035                1040
Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
                1045                1050                1055
Thr Glu Ile Gln Thr Leu Tyr Asn Asn Glu Pro Asn Ala Asn Ile Leu
            1060                1065                1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
        1075                1080                1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asn Arg Arg Thr Asp Ser
    1090                1095                1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105                1110                1115                1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
                1125                1130                1135
Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
            1140                1145                1150
Val Ala Ser Lys Thr His Leu Leu Pro Leu Tyr Ala Asp Thr Ala Thr
        1155                1160                1165
Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe
    1170                1175                1180
Asn Gln Val Val Val Met Asn Ser Val Gly Asn Cys Thr Met Asn Phe
1185                1190                1195                1200
Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala Asp
                1205                1210                1215
Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp Asn Thr
            1220                1225                1230
Asn Ser Asn Gly Phe Phe Trp Asn Phe Ile Ser Glu Glu His Gly Trp
        1235                1240                1245
Gln Glu Lys
    1250


<210> 25
<211> 1251
<212> PRT
<213> Clostridium butyricum 2

<400> 25
Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
 1               5                  10                  15
Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr Lys Ser
            20                  25                  30
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Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
        35                  40                  45
Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
    50                  55                  60
Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65                  70                  75                  80
Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
                85                  90                  95
Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
            100                 105                 110
Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
        115                 120                 125
Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
    130                 135                 140
Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145                 150                 155                 160
Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
                165                 170                 175
Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
            180                 185                 190
Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
        195                 200                 205
Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
    210                 215                 220
Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225                 230                 235                 240
Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
                245                 250                 255
Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
            260                 265                 270
Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
        275                 280                 285
Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
    290                 295                 300
Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320
Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335
Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350
Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365
Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380
Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400
Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415
Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430
Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445
Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460
Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480
Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495
Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510
Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525
Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
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    530                 535                 540
Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560
Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
                565                 570                 575
Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590
Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605
Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620
Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Val Pro Glu Leu
625                 630                 635                 640
Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655
Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670
Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
        675                 680                 685
Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700
Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Leu Lys Thr Ile Ile Glu
705                 710                 715                 720
Phe Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Lys Glu Leu Lys Asn
                725                 730                 735
Asn Tyr Asp Ile Glu Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
            740                 745                 750
Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765
Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780
Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asp Tyr Ile Ile Gln His
785                 790                 795                 800
Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Ile
                805                 810                 815
Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
            820                 825                 830
Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
        835                 840                 845
Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
    850                 855                 860
Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Glu Ile Phe Ile
865                 870                 875                 880
Tyr Pro Thr Asn Lys Asn Gln Phe Thr Ile Phe Asn Ser Lys Pro Ser
                885                 890                 895
Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
            900                 905                 910
Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
        915                 920                 925
Lys Ile Val Asn Ile Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
    930                 935                 940
Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945                 950                 955                 960
Trp Thr Leu Gln Asp Asn Ala Arg Ile Asn Gln Lys Leu Val Phe Lys
                965                 970                 975
Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
            980                 985                 990
Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
        995                 1000                1005
Gly His Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His
    1010                1015                1020
Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025                1030                1035                1040
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Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
1045 1050 1055
Thr Glu Ile Gln Thr Leu Tyr Ser Asn Glu Pro Asn Thr Asn Ile Leu
1060 1065 1070
Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Gly Tyr Tyr Leu
1075 1080 1085
Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Lys Asp Ser
1090 1095 1100
Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105 1110 1115 1120
Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asp Ser Ser
1125 1130 1135
Thr Asn Asp Arg Phe Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Tyr
1140 1145 1150
Ile Ser Asn Ser Ser Ser Tyr Ser Leu Tyr Ala Asp Thr Asn Thr Thr
1155 1160 1165
Asp Lys Glu Lys Thr Ile Lys Ser Ser Ser Ser Gly Asn Arg Phe Asn
1170 1175 1180
Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn Phe
1185 1190 1195 1200
Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala Asp
1205 1210 1215
Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp His Thr
1220 1225 1230
Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly Trp
1235 1240 1245
Gln Glu Lys
1250

<210> 26
<211> 5
<212> PRT
<213> Homo sapiens

<400> 26
Tyr Gly Gly Phe Leu
1 5

<210> 27
<211> 5
<212> PRT
<213> Homo sapiens

<400> 27
Tyr Gly Gly Phe Met
1 5

<210> 28
<211> 8
<212> PRT
<213> Homo sapiens

<400> 28
Tyr Gly Gly Phe Met Arg Gly Leu
1 5

<210> 29
<211> 7
<212> PRT

<213> Homo sapiens

<400> 29
Tyr Gly Gly Phe Met Arg Phe
 1   5

<210> 30
<211> 22
<212> PRT
<213> Homo sapiens

<400> 30
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Met Asp
 1   5   10   15
Tyr Gln Lys Arg Tyr Gly
            20

<210> 31
<211> 22
<212> PRT
<213> Necturus maculosus

<400> 31
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Leu Asp
 1   5   10   15
Tyr Gln Lys Arg Tyr Gly
            20

<210> 32
<211> 22
<212> PRT
<213> Bombina orientalis

<400> 32
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Gln Asp
 1   5   10   15
Tyr Gln Lys Arg Tyr Gly
            20

<210> 33
<211> 22
<212> PRT
<213> Xenopus laevis

<400> 33
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Glu Asp
 1   5   10   15
Tyr Gln Lys Arg Tyr Gly
            20

<210> 34
<211> 22
<212> PRT
<213> Neoceratodus forsteri

<400> 34
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Lys Leu Asp
 1   5   10   15

Asn Gln Lys Arg Tyr Gly
20

<210> 35
<211> 21
<212> PRT
<213> Danio rerio

<400> 35
Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Asp Trp Trp Gln Glu
1 5 10 15
Ser Lys Arg Tyr Gly
20

<210> 36
<211> 4
<212> PRT
<213> Homo sapiens

<400> 36
Tyr Pro Trp Phe
1

<210> 37
<211> 4
<212> PRT
<213> Homo sapiens

<400> 37
Tyr Pro Phe Phe
1

<210> 38
<211> 16
<212> PRT
<213> Homo sapiens

<400> 38
Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
1 5 10 15

<210> 39
<211> 10
<212> PRT
<213> Homo sapiens

<400> 39
Tyr Gly Gly Phe Leu Arg Lys Tyr Pro Lys
1 5 10

<210> 40
<211> 31
<212> PRT
<213> Homo sapiens

<400> 40
Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr

1 5 10 15
Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala Tyr Lys Lys Gly Glu
20 25 30

<210> 41
<211> 31
<212> PRT
<213> Homo sapiens

<400> 41
Tyr Gly Gly Phe Met Ser Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
1 5 10 15
Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala His Lys Lys Gly Gln
20 25 30

<210> 42
<211> 9
<212> PRT
<213> Homo sapiens

<400> 42
Tyr Gly Gly Phe Leu Arg Lys Tyr Pro
1 5

<210> 43
<211> 16
<212> PRT
<213> Homo sapiens

<400> 43
Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
1 5 10 15

<210> 44
<211> 16
<212> PRT
<213> Homo sapiens

<400> 44
Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn
1 5 10 15

<210> 45
<211> 13
<212> PRT
<213> Homo sapiens

<400> 45
Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys
1 5 10

<210> 46
<211> 16
<212> PRT
<213> Homo sapiens

<400> 46

Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn Gln
1 5 10 15

<210> 47
<211> 12
<212> PRT
<213> Homo sapiens

<400> 47
Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys
1 5 10

<210> 48
<211> 17
<212> PRT
<213> Xenopus laevis

<400> 48
Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Arg Trp Asp Asn
1 5 10 15
Gln

<210> 49
<211> 17
<212> PRT
<213> Xenopus laevis

<400> 49
Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Arg Leu Arg Trp Asp Asn
1 5 10 15
Gln

<210> 50
<211> 17
<212> PRT
<213> Protopterus annectens

<400> 50
Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Ile Arg Trp Asp Asn
1 5 10 15
Gln

<210> 51
<211> 17
<212> PRT
<213> Danio rerio

<400> 51
Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Leu Arg Trp Asp Asn
1 5 10 15
Gln

<210> 52

<211> 17
<212> PRT
<213> Anguilla rostrata

<400> 52
Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Ser
 1   5   10   15
Gln

<210> 53
<211> 29
<212> PRT
<213> Homo sapiens

<400> 53
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asp Pro Asn Ala Tyr Ser Gly Glu Leu Phe Asp Ala
            20   25

<210> 54
<211> 28
<212> PRT
<213> Rattus norvegicus

<400> 54
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asn Pro Asn Thr Tyr Ser Glu Asp Leu Asp Val
            20   25

<210> 55
<211> 28
<212> PRT
<213> Mus musculus

<400> 55
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Ser Pro Asn Thr Tyr Ser Glu Asp Leu Asp Val
            20   25

<210> 56
<211> 29
<212> PRT
<213> Cavia porcellus

<400> 56
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asp Pro Asn Ala Tyr Ser Glu Glu Phe Phe Asp Val
            20   25

<210> 57
<211> 29
<212> PRT
<213> Sus scrofa

<400> 57
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asp Pro Asn Ala Tyr Tyr Glu Glu Leu Phe Asp Val
            20   25

<210> 58
<211> 29
<212> PRT
<213> Canis familiaris

<400> 58
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asp Pro Asn Ala Tyr Ser Gly Glu Leu Leu Asp Gly
            20   25

<210> 59
<211> 29
<212> PRT
<213> Bos taurus

<400> 59
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15
Glu Asp Pro Ser Ala Tyr Tyr Glu Glu Leu Phe Asp Val
            20   25

<210> 60
<211> 29
<212> PRT
<213> Bufo marinus

<400> 60
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr Arg Ser Glu
 1   5   10   15
Glu Asp Pro Ser Thr Phe Ser Gly Glu Leu Ser Asn Leu
            20   25

<210> 61
<211> 29
<212> PRT
<213> Bombina orientalis

<400> 61
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr Arg Ser Glu
 1   5   10   15
Glu Glu Pro Gly Ser Phe Ser Gly Glu Ile Ser Asn Leu
            20   25

<210> 62
<211> 29
<212> PRT
<213> Xenopus laevis

<400> 62
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala Arg Ser Glu

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 1               5                  10                  15
Glu Asp Pro Thr Met Phe Ser Asp Glu Leu Ser Tyr Leu
            20                  25
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<210> 63
<211> 29
<212> PRT
<213> Xenopus laevis

<400> 63

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Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala Arg Ser Glu
 1               5                  10                  15
Glu Asp Pro Thr Met Phe Ser Gly Glu Leu Ser Tyr Leu
            20                  25
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<210> 64
<211> 29
<212> PRT
<213> Polypterus senegalus

<400> 64

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Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
 1               5                  10                  15
Glu Glu Pro Ser Ser Tyr Ser Asp Glu Val Leu Glu Leu
            20                  25
```

<210> 65
<211> 27
<212> PRT
<213> Danio rerio

<400> 65

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Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
 1               5                  10                  15
Glu Glu Pro Ser Ser Tyr Glu Asp Tyr Ala Leu
            20                  25
```

<210> 66
<211> 27
<212> PRT
<213> Anguilla rostrata

<400> 66

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Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
 1               5                  10                  15
Glu Glu Pro Gly Ser Tyr Asp Val Ile Gly Leu
            20                  25
```

<210> 67
<211> 29
<212> PRT
<213> Neoceratodus forsteri

<400> 67

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Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Thr Val Arg Ser Asp
 1               5                  10                  15
Glu Asp Pro Ser Pro Tyr Leu Asp Glu Phe Ser Asp Leu
            20                  25
```

<210> 68
<211> 27
<212> PRT
<213> Oncorhynchus masou

<400> 68
Tyr Gly Gly Phe Leu Arg Arg His Tyr Lys Leu Ser Val Arg Ser Asp
 1   5   10   15
Glu Glu Pro Ser Ser Tyr Asp Asp Phe Gly Leu
            20   25

<210> 69
<211> 13
<212> PRT
<213> Homo sapiens

<400> 69
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr
 1   5   10

<210> 70
<211> 13
<212> PRT
<213> Bufo marinus

<400> 70
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr
 1   5   10

<210> 71
<211> 13
<212> PRT
<213> Xenopus laevis

<400> 71
Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala
 1   5   10

<210> 72
<211> 13
<212> PRT
<213> Polypterus senegalus

<400> 72
Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val
 1   5   10

<210> 73
<211> 13
<212> PRT
<213> Neoceratodus forsteri

<400> 73
Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Thr Val
 1   5   10

<210> 74
<211> 13
<212> PRT
<213> Oncorhynchus masou

<400> 74
Tyr Gly Gly Phe Leu Arg Arg His Tyr Lys Leu Ser Val
 1   5   10

<210> 75
<211> 17
<212> PRT
<213> Homo sapiens

<400> 75
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Arg Lys Asn
 1   5   10   15
Gln

<210> 76
<211> 17
<212> PRT
<213> Homo sapiens

<400> 76
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Leu Ala Asn
 1   5   10   15
Gln

<210> 77
<211> 17
<212> PRT
<213> Homo sapiens

<400> 77
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Tyr Ala Asn
 1   5   10   15
Gln

<210> 78
<211> 11
<212> PRT
<213> Homo sapiens

<400> 78
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala
 1   5   10

<210> 79
<211> 11
<212> PRT
<213> Homo sapiens

<400> 79

Phe Gly Gly Phe Thr Gly Ala Arg Lys Tyr Ala
1 5 10

<210> 80
<211> 11
<212> PRT
<213> Homo sapiens

<400> 80
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Tyr
1 5 10

<210> 81
<211> 13
<212> PRT
<213> Homo sapiens

<400> 81
Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys
1 5 10

<210> 82
<211> 30
<212> PRT
<213> Homo sapiens

<400> 82
Met Pro Arg Val Arg Ser Leu Phe Gln Glu Gln Glu Glu Pro Glu Pro
1 5 10 15
Gly Met Glu Glu Ala Gly Glu Met Glu Gln Lys Gln Leu Gln
20 25 30

<210> 83
<211> 17
<212> PRT
<213> Homo sapiens

<400> 83
Phe Ser Glu Phe Met Arg Gln Tyr Leu Val Leu Ser Met Gln Ser Ser
1 5 10 15
Gln

<210> 84
<211> 8
<212> PRT
<213> Homo sapiens

<400> 84
Thr Leu His Gln Asn Gly Asn Val
1 5

<210> 85
<211> 10
<212> PRT
<213> Homo sapiens

<400> 85
Leu Val Val Tyr Pro Trp Thr Gln Arg Phe
 1   5   10

<210> 86
<211> 9
<212> PRT
<213> Homo sapiens

<400> 86
Val Val Tyr Pro Trp Thr Gln Arg Phe
 1   5

<210> 87
<211> 8
<212> PRT
<213> Homo sapiens

<400> 87
Val Tyr Pro Trp Thr Gln Arg Phe
 1   5

<210> 88
<211> 7
<212> PRT
<213> Homo sapiens

<400> 88
Tyr Pro Trp Thr Gln Arg Phe
 1   5

<210> 89
<211> 9
<212> PRT
<213> Homo sapiens

<400> 89
Leu Val Val Tyr Pro Trp Thr Gln Arg
 1   5

<210> 90
<211> 8
<212> PRT
<213> Homo sapiens

<400> 90
Leu Val Val Tyr Pro Trp Thr Gln
 1   5

<210> 91
<211> 7
<212> PRT
<213> Homo sapiens

<400> 91
Val Val Tyr Pro Trp Thr Gln
 1   5

<210> 92
<211> 7
<212> PRT
<213> Homo sapiens

<400> 92
Leu Val Val Tyr Pro Trp Thr
 1   5

<210> 93
<211> 6
<212> PRT
<213> Homo sapiens

<400> 93
Leu Val Val Tyr Pro Trp
 1   5

<210> 94
<211> 30
<212> PRT
<213> Homo sapiens

<400> 94
Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Pro His Ala Val
 1   5   10   15
Gly Asn His Arg Ser Phe Ser Asp Lys Asn Gly Leu Thr Ser
            20   25   30

<210> 95
<211> 59
<212> PRT
<213> Homo sapiens

<400> 95
Glu Leu Arg Pro Glu Asp Asp Met Lys Pro Gly Ser Phe Asp Arg Ser
 1   5   10   15
Ile Pro Glu Asn Asn Ile Met Arg Thr Ile Ile Glu Phe Leu Ser Phe
            20   25   30
Leu His Leu Lys Glu Ala Gly Ala Leu Asp Arg Leu Leu Asp Leu Pro
        35   40   45
Ala Ala Ala Ser Ser Glu Asp Ile Glu Arg Ser
    50   55

<210> 96
<211> 11
<212> PRT
<213> Homo sapiens

<400> 96
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met
 1   5   10

<210> 97
<211> 12
<212> PRT

<213> Homo sapiens

<400> 97
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met Gly
 1   5   10

<210> 98
<211> 13
<212> PRT
<213> Homo sapiens

<400> 98
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met Gly Lys
 1   5   10

<210> 99
<211> 14
<212> PRT
<213> Homo sapiens

<400> 99
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met Gly Lys Arg
 1   5   10

<210> 100
<211> 36
<212> PRT
<213> Homo sapiens

<400> 100
Asp Ala Asp Ser Ser Ile Glu Lys Gln Val Ala Leu Leu Lys Ala Leu
 1   5   10   15
Tyr Gly His Gly Gln Ile Ser His Lys Arg His Lys Thr Asp Ser Phe
            20   25   30
Val Gly Leu Met
        35

<210> 101
<211> 19
<212> PRT
<213> Homo sapiens

<400> 101
Gly His Gly Gln Ile Ser His Lys Arg His Lys Thr Asp Ser Phe Val
 1   5   10   15
Gly Leu Met

<210> 102
<211> 10
<212> PRT
<213> Homo sapiens

<400> 102
His Lys Thr Asp Ser Phe Val Gly Leu Met
 1   5   10

<210> 103
<211> 10
<212> PRT
<213> Rattus norvegicus

<400> 103
Asp Met His Asp Phe Phe Val Gly Leu Met
 1   5   10

<210> 104
<211> 10
<212> PRT
<213> Homo sapiens

<400> 104
Ser Arg Thr Arg Gln Phe Tyr Gly Leu Met
 1   5   10

<210> 105
<211> 10
<212> PRT
<213> Homo sapiens

<400> 105
Gly Lys Ala Ser Gln Phe Phe Gly Leu Met
 1   5   10

<210> 106
<211> 14
<212> PRT
<213> Homo sapiens

<400> 106
Lys Lys Ala Tyr Gln Leu Glu His Thr Phe Gln Gly Leu Leu
 1   5   10

<210> 107
<211> 14
<212> PRT
<213> Homo sapiens

<400> 107
Val Gly Ala Tyr Gln Leu Glu His Thr Phe Gln Gly Leu Leu
 1   5   10

<210> 108
<211> 36
<212> PRT
<213> Homo sapiens

<400> 108
Tyr Pro Ser Lys Pro Asp Asn Pro Gly Glu Asp Ala Pro Ala Glu Asp
 1   5   10   15
Met Ala Arg Tyr Tyr Ser Ala Leu Arg His Tyr Ile Asn Leu Ile Thr
            20   25   30
Arg Gln Arg Tyr
        35

<210> 109
<211> 36
<212> PRT
<213> Homo sapiens

<400> 109
Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu
 1   5   10   15
Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr
            20   25   30
Arg Gln Arg Tyr
        35

<210> 110
<211> 33
<212> PRT
<213> Homo sapiens

<400> 110
Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln Met Ala Gln
 1   5   10   15
Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr Arg Pro Arg
            20   25   30
Tyr

<210> 111
<211> 36
<212> PRT
<213> Homo sapiens

<400> 111
Ala Pro Leu Glu Pro Val Tyr Pro Gly Asp Asn Ala Thr Pro Glu Gln
 1   5   10   15
Met Ala Gln Tyr Ala Ala Asp Leu Arg Arg Tyr Ile Asn Met Leu Thr
            20   25   30
Arg Pro Arg Tyr
        35

<210> 112
<211> 20
<212> PRT
<213> Homo sapiens

<400> 112
His Lys Glu Asp Thr Leu Ala Phe Ser Glu Trp Gly Ser Pro His Ala
 1   5   10   15
Ala Val Pro Arg
            20

<210> 113
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Thrombin cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is S, T, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2, 6, 7, 8
<223> Position 1, 6, 7, and 8, are independently any amino acid

<221> VARIANT
<222> 3
<223> Position 3 is F, S, T, an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5
<223> Position 5 is S, T, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 113
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 114
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 114
Leu Val Pro Arg Gly Ser
1 5

<210> 115
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 115
Leu Val Pro Lys Gly Ser
1 5

<210> 116
<211> 6
<212> PRT

<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 116
Phe Ile Pro Arg Thr Phe
 1   5

<210> 117
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 117
Val Leu Pro Arg Ser Phe
 1   5

<210> 118
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 118
Ile Val Pro Arg Ser Phe
 1   5

<210> 119
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 119
Ile Val Pro Arg Gly Tyr
 1   5

<210> 120
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 120
Val Val Pro Arg Gly Val
 1   5

<210> 121

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 121
Val Leu Pro Arg Leu Ile
1 5

<210> 122
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 122
Val Met Pro Arg Ser Leu
1 5

<210> 123
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Thrombin cleavage site

<400> 123
Met Phe Pro Arg Ser Leu
1 5

<210> 124
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Coagulation Factor VIIa cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is an acidic amino acid like D and E, an amidic amino acid like N and Q, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 2 is Q, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3

<223> Position 3 is Q, S, T, or an aliphatic hydrophobic
amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5, 6, 7 8
<223> Position 5, 6, 7, and 8 are independently any
amino acid

<400> 124
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 125
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 125
Lys Leu Thr Arg Ala Glu Thr Val
1 5

<210> 126
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 126
Asp Phe Thr Arg Val Val Gly Gly
1 5

<210> 127
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 127
Leu Ser Pro Arg Thr Phe His Pro
1 5

<210> 128
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 128
Leu Ile Gln Arg Asn Leu Ser Pro
1 5

<210> 129
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 129
Met Ala Thr Arg Lys Met His Asp
1 5

<210> 130
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 130
Leu Gly Ile Arg Ser Phe Arg Asn
1 5

<210> 131
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 131
Pro Gln Gly Arg Ile Val Gly Gly
1 5

<210> 132
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 132
Asn Leu Thr Arg Ile Val Gly Gly
1 5

<210> 133
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor VIIa cleavage site

<400> 133
Gln Val Val Arg Ile Val Gly Gly
1 5

<210> 134
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consenus sequence for Coagulation Factor IXa cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 2 is an acidic amino acid like D and E, an amidic amino acid like N and Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3
<223> Position 3 is, S, T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5, 6, 7, 8
<223> Positions 5, 6, 7, and 8 are independently any amino acid

<400> 134
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 135
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor IXa cleavage site

<400> 135
Pro Gln Gly Arg Ile Val Gly Gly
1 5

<210> 136
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor IXa cleavage site

<400> 136
Pro Gln Leu Arg Met Lys Asn Asn
 1   5

<210> 137
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor IXa cleavage site

<400> 137
Asn Leu Thr Arg Ile Val Gly Gly
 1   5

<210> 138
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor IXa cleavage site

<400> 138
Gln Val Val Arg Ile Val Gly Gly
 1   5

<210> 139
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Coagulation Factor Xa
      cleavage site

<221> VARIANT
<222> 1, 6, 7, 8
<223> Positions 1, 6, 7, and 8 are independantly any
      amino acid

<221> VARIANT
<222> 2
<223> Position 2 is G, A, S, an acidic amino acid like D
      and E, an amidic amino acid like Q and N, or an
      aromatic hydrophobic amino acid like F, W and Y

<221> VARIANT
<222> 3
<223> Position 3 is an aromatic hydrophobic amino acid

like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5
<223> Position 5 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 139
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 140
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 140
Ile Asp Gly Arg
1

<210> 141
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 141
Ile Glu Gly Arg
1

<210> 142
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 142
Ile Asp Gly Arg Ser Val Gly Gly
1 5

<210> 143
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 143
Ile Asp Gly Arg Thr Val Gly Gly
 1   5

<210> 144
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 144
Ile Asp Gly Arg Ile Val Gly Gly
 1   5

<210> 145
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 145
Ile Glu Gly Arg Ser Val Gly Gly
 1   5

<210> 146
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 146
Ile Glu Gly Arg Thr Val Gly Gly
 1   5

<210> 147
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 147
Ile Glu Gly Arg Ile Val Gly Gly
 1   5

<210> 148
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 148
Pro Gln Gly Arg Ile Val Gly Gly
 1   5

<210> 149
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 149
Ile Glu Gly Arg Thr Ser Glu Asp
 1   5

<210> 150
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 150
Ile Glu Gly Arg Ile Val Glu Gly
 1   5

<210> 151
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 151
Ile Asp Gly Arg Ile Val Glu Gly
 1   5

<210> 152
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 152
Phe Asn Pro Arg Thr Phe Gly Ser
 1   5

<210> 153

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 153
Phe Asp Glu Arg Thr Phe Gly Leu
1 5

<210> 154
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 154
Ile Asp Glu Arg Ile Val Gly Gly
1 5

<210> 155
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor Xa cleavage site

<400> 155
Phe Asn Glu Lys Thr Phe Gly Leu
1 5

<210> 156
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Coagulation Factor XIa cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is an acidic amino acid like D or E, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 2 is an acidic amino acid like D or E, an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT

<222> 3
<223> Position 3 is H, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5, 8
<223> Positions 5 and 6 are independantly H, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> (6)...(6)
<223> Position 6 is an acidic amino acid like D and E, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> (7)...(7)
<223> Position 7 is an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 156
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 157
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 157
Ala Phe Trp Lys Thr Asp Ala Ser
1 5

<210> 158
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 158
Lys Leu Thr Arg Ala Glu Thr Val
1 5

<210> 159

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 159
Lys Leu Thr Arg Ala Glu Thr Ile
 1   5

<210> 160
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 160
Asp Phe Thr Arg Val Val Gly Gly
 1   5

<210> 161
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 161
Glu Phe Ser Arg Val Val Gly Gly
 1   5

<210> 162
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 162
Lys Leu Thr Arg Ala Glu Thr Val
 1   5

<210> 163
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 163
Asp Phe Thr Arg Val Val Gly Gly
 1   5

<210> 164
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 164
Ile Lys Pro Arg Ile Val Gly Gly
 1   5

<210> 165
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 165
Asp Leu His Arg His Ile Phe Trp
 1   5

<210> 166
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIa cleavage site

<400> 166
Lys Gln Leu Arg Val Val Asn Gly
 1   5

<210> 167
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Coagulation Factor XIIa
      cleavage site

<221> VARIANT
<222> 1, 6
<223> Positions 1 and 6 are independantly an uncharged
      amino acid like C, S, and T, or an aliphatic
      hydrophobic amino acid like, G, P, A, V, L, I, and
      M

<221> VARIANT
<222> 2
<223> Position 2 is an acidic amino acid like D and E, a
      basic amino acid like K and R, an uncharged amino
      acid like C, S, and T, or an aliphatic hydrophobic
      amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3
<223> Position 3 is a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 5, 7, 8
<223> Positions 5, 7 and 8 are independantly an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 167
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 168
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIIa cleavage site

<400> 168
Pro Gln Gly Arg Ile Val Gly Gly
1 5

<210> 169
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIIa cleavage site

<400> 169
Ile Lys Pro Arg Ile Val Gly Gly
1 5

<210> 170
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIIa cleavage site

<400> 170
Ser Met Thr Arg Val Val Gly Gly
1 5

<210> 171
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIIa cleavage site

<400> 171
Thr Ser Thr Arg Ile Val Gly Gly
 1   5

<210> 172
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Coagulation Factor XIIa cleavage site

<400> 172
Pro Met Lys Arg Leu Thr Leu Gly
 1   5

<210> 173
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Kallikrein 1 cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is an acidic amino acid like D and E, an amidic amino acid like Q and N, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2, 3, 6, 7, 8
<223> Positions 2, 3, 6, 7 and 8 are independently any amino acid

<221> VARIANT
<222> 4
<223> Position 4 is a positive amino acid like H, K, and R, a large non-polar amino acid like F, I, L, M and V, or an aromatic hydrophobic amino acid like F, W and Y

<221> VARIANT
<222> 5
<223> Position 5 is S, K, or R

<400> 173
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1   5

<210> 174
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 174
Ser Met Thr Arg Val Val Gly Gly
 1   5

<210> 175
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 175
Ser Pro Phe Arg Ser Ser Asp Ile
 1   5

<210> 176
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 176
Ser Leu Met Lys Arg Pro Pro Gly
 1   5

<210> 177
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 177
Tyr Asp Trp Arg Thr Pro Tyr Leu
 1   5

<210> 178
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 178
Ser Pro Phe Arg Ser Val Gln Val
 1   5

<210> 179
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 179
Ser Pro Phe Arg Thr Pro Tyr Leu
 1   5

<210> 180
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 180
Thr Phe His Lys Ala Glu Tyr Arg
 1   5

<210> 181
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 181
Pro Arg Phe Lys Ile Ile Gly Gly
 1   5

<210> 182
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 182
Ile Ser Leu Met Lys Arg Pro Pro
 1   5

<210> 183
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 183
Leu Glu Ala Arg Ser Ala Tyr His
 1   5

<210> 184
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 184
Glu Ala Lys Arg Ser Tyr His Ser
1 5

<210> 185
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 185
Pro Asn Arg Trp Ser Thr Gly Ala
1 5

<210> 186
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 186
Glu Ala Phe Tyr Ser Gln Phe Gly
1 5

<210> 187
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 187
Asn Ala Ala Arg Ser Thr Gly Ala
1 5

<210> 188
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 188

Ser Ser Glu Trp Ser Met Pro Tyr
1 5

<210> 189
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 189
Gly Thr Leu Phe Arg Ser Gly Asn
1 5

<210> 190
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 190
Ala Arg Leu Tyr Ser Arg Gly Ala
1 5

<210> 191
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 191
Glu Ala Ser Arg Ser Ala Thr Leu
1 5

<210> 192
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 192
Glu Ala Ser Tyr Arg Arg Lys Gln
1 5

<210> 193
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 193
Thr Thr Phe Tyr Arg Arg Gly Ala
1 5

<210> 194
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 194
Ala Ala Trp Tyr Arg Thr Ser Arg
1 5

<210> 195
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 195
Ser Phe His Tyr Arg Met Val Gly
1 5

<210> 196
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 196
Ala Ser Ser Tyr Arg Thr Ser Arg
1 5

<210> 197
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 197
Thr Arg Phe Tyr Ser Arg Gly Arg
1 5

<210> 198
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Kallikrein 1 cleavage site

<400> 198
Ile Lys Phe Phe Ser Ala Gln Thr
 1   5

<210> 199
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Protein C cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 2 is an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3, 5
<223> Positions 3 and 5 is an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 6
<223> Position 6 is an amidic amino acid like Q and N, a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> (7)...(7)
<223> Position 7 is an amidic amino acid like Q and N, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aromatic hydrophobic amino acid like F, W and Y

<221> VARIANT
<222> (8)...(8)
<223> Position 8 is an acidic amino acid like D and E, an amidic amino acid like Q and N, a basic amino acid like K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 199
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 200
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 200
Lys Lys Thr Arg Asn Leu Lys Lys
1 5

<210> 201
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 201
Leu Asp Arg Arg Gly Leu Gln Arg
1 5

<210> 202
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 202
Met Ala Thr Arg Lys Met His Asp
1 5

<210> 203
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 203
Arg Leu Lys Lys Ser Gln Phe Leu
1 5

<210> 204
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 204
Pro Gln Leu Arg Met Lys Asn Asn
 1   5

<210> 205
<211> 8
<212> PRT
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<220>
<223> Protein C cleavage site

<400> 205
Val Asp Gln Arg Gly Asn Gln Ile
 1   5

<210> 206
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 206
Ile Glu Pro Arg Ser Pro Ser Gln
 1   5

<210> 207
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 207
Lys Lys Thr Arg Ser Pro Lys Thr
 1   5

<210> 208
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 208
Leu Asp Gln Arg Gly Val Gln Arg
 1   5

<210> 209
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> Protein C cleavage site

<400> 209
Pro Asp Pro Arg Ser Lys Asn Asn
1 5

<210> 210
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Plasminogen cleavage site

<221> VARIANT
<222> 1, 5, 6
<223> Positions 1, 5, and 6 are independantly a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 3 is an amidic amino acid like N and Q, a positive amino acid like H, K and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3
<223> Position 3 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 7
<223> Position 7 is any amino acid

<221> VARIANT
<222> (8)...(8)
<223> Position 8 is H, F, Y, R, an uncharged amino acid like C, S, and T, an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 211
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 211
Gly Glu Ala Arg Gly Ser Val Ile
1 5

<210> 212
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 212
Gly His Ala Arg Leu Val His Val
1 5

<210> 213
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 213
Ala Glu Phe Arg His Asp Ser Gly
1 5

<210> 214
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 214
His His Gln Lys Leu Val Phe Phe
1 5

<210> 215
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 215
Gly Ser Asn Lys Gly Ala Leu Leu
1 5

<210> 216

<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 216
Arg Ala Gln Arg Ser Ala Gly Ala
 1   5

<210> 217
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 217
Ala Phe Trp Lys Thr Asp Ala Ser
 1   5

<210> 218
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 218
Met Ser Met Arg Val Arg Arg His
 1   5

<210> 219
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 219
Arg Gly Val Arg Arg Thr Ala Ser
 1   5

<210> 220
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 220
Arg Ala Ala Arg Ser Gln Cys Thr
 1   5

<210> 221
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 221
Pro Gln Ser Arg Ser Val Pro Pro
 1   5

<210> 222
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 222
Pro Tyr Leu Lys Val Phe Asn Pro
 1   5

<210> 223
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

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Leu Ser Phe Arg Ala Arg Ala Tyr
 1   5

<210> 224
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 224
Pro Gln Leu Arg Arg Gly Trp Arg
 1   5

<210> 225
<211> 8
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<220>
<223> Plasminogen cleavage site

<400> 225
Glu Asp Asn Arg Asp Ser Ser Met

1 5

<210> 226
<211> 8
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<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 226
Leu Ser Phe Arg Ala Arg Ala Tyr
1 5

<210> 227
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 227
Phe Arg Ala Arg Ala Tyr Gly Phe
1 5

<210> 228
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 228
Tyr Gly Phe Arg Gly Pro Gly Pro
1 5

<210> 229
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 229
Ile Thr Phe Arg Met Asn Val Ala
1 5

<210> 230
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 230
Thr His Glu Lys Gly Arg Gln Ser
1 5

<210> 231
<211> 8
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<220>
<223> Plasminogen cleavage site

<400> 231
Pro Arg Leu Lys Ala Arg Ala Gly
1 5

<210> 232
<211> 8
<212> PRT
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<220>
<223> Plasminogen cleavage site

<400> 232
Pro Lys Ala Lys Ser His Ala Pro
1 5

<210> 233
<211> 8
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<220>
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<400> 233
Pro Ser His Lys Glu Gly Pro Gln
1 5

<210> 234
<211> 8
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<400> 234
Leu Phe Glu Lys Lys Val Tyr Leu
1 5

<210> 235
<211> 8
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<400> 235
Ala Asp Gly Lys Lys Pro Ser Ser
 1   5

<210> 236
<211> 8
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<220>
<223> Plasminogen cleavage site

<400> 236
Pro Arg Phe Lys Ile Ile Gly Gly
 1   5

<210> 237
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<223> Plasminogen cleavage site

<400> 237
Pro Gln Phe Arg Ile Lys Gly Gly
 1   5

<210> 238
<211> 8
<212> PRT
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<220>
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<400> 238
Pro Arg Cys Arg His Arg Pro His
 1   5

<210> 239
<211> 8
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<220>
<223> Plasminogen cleavage site

<400> 239
Lys Gly Tyr Arg Ser Gln Arg Gly
 1   5

<210> 240
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Plasminogen cleavage site

<400> 240
Asp Val Ala Gln Phe Val Leu Thr
 1   5

<210> 241
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for MMP-2 cleavage site

<221> VARIANT
<222> 1, 3, 4, 6, 7, 8
<223> Positions 1, 3, 4, 6, 7, 8 are independantly any
      amino acid

<221> VARIANT
<222> 2
<223> Position 2 is P, A, V, L, or I

<221> VARIANT
<222> 5
<223> Position 5 is V, L, I, F, or Q

<400> 241
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1   5

<210> 242
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 242
Gln Pro Val Ser Val Lys Val Gly
 1   5

<210> 243
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 243
Arg Gly Val Gly Ile Lys Ser Thr
 1   5

<210> 244
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 244
Phe Val Asp Cys Leu Ile Glu Gln
 1   5

<210> 245
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 245
Val Pro Ala Gly Asn Trp Val Leu
 1   5

<210> 246
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 246
Tyr His Ala Asp Ile Tyr Asp Lys
 1   5

<210> 247
<211> 8
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<220>
<223> MMP-2 cleavage site

<400> 247
Arg Ala Cys Arg Leu Ala Lys Ala
 1   5

<210> 248
<211> 8
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<220>
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<400> 248
Gln Gly Ala Tyr Gln Glu Ala Phe
 1   5

<210> 249
<211> 8
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<220>
<223> MMP-2 cleavage site

<400> 249
Asp Val Leu Ser Leu Leu Glu Lys
 1   5

<210> 250
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 250
Thr Leu Asp Asp Leu Ile Met Ala
 1   5

<210> 251
<211> 8
<212> PRT
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<220>
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<400> 251
His Ile Ser Ser Leu Ile Lys Leu
 1   5

<210> 252
<211> 8
<212> PRT
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<220>
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<400> 252
Asp Pro Asn Asn Leu Leu Asn Asp
 1   5

<210> 253
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 253
Pro Val Gln Pro Gln Gln Ser Pro
 1   5

<210> 254
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 254
Lys Pro Lys Thr Ile Thr Gly Pro
1 5

<210> 255
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 255
Val Val His Pro Leu Val Leu Leu
1 5

<210> 256
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 256
His Pro Leu Val Leu Leu Ser Val
1 5

<210> 257
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 257
Ala Val Ala Leu Leu Ile Gly Pro
1 5

<210> 258
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 258

Gln Pro Leu Gln Leu Leu Asp Ala
1 5

<210> 259
<211> 8
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<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 259
Tyr Ile Gln Gly Ile Asn Leu Val
1 5

<210> 260
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 260
Leu Pro Gln Glu Ile Lys Ala Asn
1 5

<210> 261
<211> 8
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<220>
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<400> 261
Asn Ile Ser Asp Leu Thr Ala Ala
1 5

<210> 262
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 262
Lys Pro Arg Ala Leu Thr Ala Leu
1 5

<210> 263
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 263
Ala Pro Ser Trp Leu Leu Thr Ala
1 5

<210> 264
<211> 8
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<220>
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<400> 264
Ala Val Arg Trp Leu Leu Thr Ala
1 5

<210> 265
<211> 8
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<220>
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<400> 265
Ala Val Ser Trp Leu Leu Thr Ala
1 5

<210> 266
<211> 8
<212> PRT
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<220>
<223> MMP-2 cleavage site

<400> 266
Ser Leu Arg Arg Leu Thr Ala Ala
1 5

<210> 267
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 267
Ser Leu Ser Arg Leu Thr Ala Leu
1 5

<210> 268
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 268
Arg Tyr Ser Ser Leu Thr Ala Ala
 1   5

<210> 269
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 269
Ser Leu Ala Tyr Tyr Thr Ala Leu
 1   5

<210> 270
<211> 8
<212> PRT
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<220>
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<400> 270
Ser Leu Arg Tyr Tyr Thr Ala Ala
 1   5

<210> 271
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 271
Ser Pro Ala Tyr Tyr Thr Ala Leu
 1   5

<210> 272
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 272
Met His Lys Ala Leu Thr Ala Ala
 1   5

<210> 273
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> MMP-2 cleavage site

<400> 273
Leu Arg Leu Ala Ile Thr Ala Leu
1 5

<210> 274
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for MMP-9 cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is F, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2
<223> Position 2 is F, Y, S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3, 8
<223> Positions 3 and 8 are independantly F, Y, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4, 6, 7
<223> Positions 4, 6 and 7 are independantly any amino acid

<221> VARIANT
<222> 5
<223> Position 5 is S, T, an acidic amino acid like D and E, an amidic amino acid like N and Q, a positive amino acid like H, K, and R, an aromatic hydrophobic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 274
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 275

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 275
Ile Pro Glu Asn Phe Phe Gly Val
1 5

<210> 276
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 276
Met Asp Ile Ala Ile His His Pro
1 5

<210> 277
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 277
Ser Pro Ser Arg Leu Phe Asp Gln
1 5

<210> 278
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 278
Ser Glu Met Arg Leu Glu Lys Asp
1 5

<210> 279
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 279
Phe Ser Val Asn Leu Asp Val Lys
1 5

<210> 280
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 280
Arg Leu Phe Asp Gln Phe Phe Gly
 1   5

<210> 281
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 281
Phe Phe Gly Glu His Leu Leu Glu
 1   5

<210> 282
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 282
Gly Leu Ser Glu Met Arg Leu Glu
 1   5

<210> 283
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 283
Ser Pro Glu Glu Leu Lys Val Lys
 1   5

<210> 284
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 284
Asp Val Ile Glu Val His Gly Lys

1 5

<210> 285
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 285
Glu Val His Gly Lys His Glu Glu
1 5

<210> 286
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 286
Asp Glu His Gly Phe Ile Ser Arg
1 5

<210> 287
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 287
Gly Glu His Leu Leu Glu Ser Asp
1 5

<210> 288
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 288
Phe His Arg Lys Tyr Arg Ile Pro
1 5

<210> 289
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 289
Gly Pro Arg Lys Gln Val Ser Gly
1 5

<210> 290
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 290
Leu Ser Pro Phe Tyr Leu Arg Pro
1 5

<210> 291
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 291
Pro Pro Ser Phe Leu Arg Ala Pro
1 5

<210> 292
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 292
Asn Pro Leu Glu Asn Ser Gly Phe
1 5

<210> 293
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 293
Val Pro Tyr Gly Leu Gly Ser Pro
1 5

<210> 294
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> MMP-9 cleavage site

<400> 294
Pro Pro Leu Lys Leu Met His Ser
1 5

<210> 295
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 295
Gly Pro Glu Gly Leu Arg Val Gly
1 5

<210> 296
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 296
Phe Met Lys Gly Leu Ser Lys Ala
1 5

<210> 297
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 297
Val Val Thr Gly Val Thr Ala Val
1 5

<210> 298
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 298
Ala Ile Ile Gly Leu Met Val Gly
1 5

<210> 299
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 299
Ser Asp Leu Gly Leu Thr Gly Ile
1 5

<210> 300
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 300
Val Pro Tyr Gly Leu Gly Ser Pro
1 5

<210> 301
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 301
Gly Ala Ala Gly Val Lys Gly Asp
1 5

<210> 302
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 302
Gly Pro Thr Gly Lys Gln Gly Asp
1 5

<210> 303
<211> 8
<212> PRT
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<220>
<223> MMP-9 cleavage site

<400> 303
Gly Pro Ser Gly Asp Gln Gly Ala
1 5

<210> 304
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> MMP-9 cleavage site

<400> 304
Gly Pro Ser Gly Phe Pro Phe Pro
 1   5

<210> 305
<211> 8
<212> PRT
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<220>
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<400> 305
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<210> 306
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<220>
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<400> 306
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<210> 307
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<220>
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<400> 307
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<210> 308
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<220>
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1 5

<210> 310
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<220>
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<400> 310
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1 5

<210> 311
<211> 8
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<220>
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<210> 312
<211> 8
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<400> 312
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1 5

<210> 313
<211> 8
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1 5

<210> 314
<211> 8
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Gly Pro Leu Gly Met Leu Ser Gln
1 5

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1 5

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1 5

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1 5

<210> 318
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<220>
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<400> 318

Asn Ser Phe Gly Leu Arg Phe Gly
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<211> 8
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<220>
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<400> 319
Arg Ala Ile His Ile Asn Ala Glu
1 5

<210> 320
<211> 8
<212> PRT
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<220>
<223> Consensus sequence for Furin cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is R, I, or A

<221> VARIANT
<222> 2, 5, 6, 7, 8
<223> Positions 2, 5, 6, 7, and 8 are independantly any amino acid

<221> VARIANT
<222> 3
<223> Position 3 is R, K, A, or P

<221> VARIANT
<222> (0)...(0)

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1 5

<210> 322
<211> 8
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<220>
<223> Furin cleavage site

<400> 322
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1 5

<210> 323
<211> 8
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<220>
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<400> 323
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1 5

<210> 324
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<220>
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1 5

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<220>
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1 5

<210> 326
<211> 8
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<220>
<223> Furin cleavage site

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1 5

<210> 327
<211> 8

<212> PRT
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<220>
<223> Furin cleavage site

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 1   5

<210> 328
<211> 8
<212> PRT
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<220>
<223> Furin cleavage site

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<210> 329
<211> 8
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<220>
<223> Furin cleavage site

<400> 329
Arg Ala Lys Arg Phe Ala Ser Leu
 1   5

<210> 330
<211> 8
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<220>
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<400> 330
Arg Lys Lys Arg Phe Val Ser Ser
 1   5

<210> 331
<211> 8
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<220>
<223> Furin cleavage site

<400> 331
Arg Thr Lys Arg Phe Leu Ser Tyr
 1   5

<210> 332
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<210> 333
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<220>
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1 5

<210> 334
<211> 8
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<220>
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<400> 334
Val Phe Arg Arg Glu Ala His Lys
1 5

<210> 335
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<220>
<223> Furin cleavage site

<400> 335
Arg Val Ala Arg Asp Ile Thr Met
1 5

<210> 336
<211> 8
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<220>
<223> Furin cleavage site

<400> 336
Arg Ile Ser Arg Ser Leu Pro Gln
1 5

<210> 337
<211> 8
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<220>
<223> Furin cleavage site

<400> 337
Arg Ser Arg Arg Ala Ala Thr Ser
1 5

<210> 338
<211> 8
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<220>
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<400> 338
Arg Ala Lys Arg Ser Pro Lys His
1 5

<210> 339
<211> 8
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<220>
<223> Furin cleavage site

<400> 339
Phe Trp His Arg Gly Val Thr Lys
1 5

<210> 340
<211> 8
<212> PRT
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<220>
<223> Furin cleavage site

<400> 340
Ala Lys Arg Arg Thr Lys Arg Asp
1 5

<210> 341
<211> 8
<212> PRT
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<220>
<223> Furin cleavage site

<400> 341

Ala Lys Arg Arg Ala Lys Arg Asp
1 5

<210> 342
<211> 8
<212> PRT
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<220>
<223> Furin cleavage site

<400> 342
Ala Lys Gln Arg Ala Lys Arg Asp
1 5

<210> 343
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 343
Arg Asp Val Arg Gly Phe Ala Ser
1 5

<210> 344
<211> 8
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<220>
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<400> 344
Arg Lys Arg Arg Ser Val Asn Pro
1 5

<210> 345
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Furin cleavage site

<400> 345
Arg Gln Lys Arg Phe Val Leu Ser
1 5

<210> 346
<211> 8
<212> PRT
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<220>
<223> Furin cleavage site

<400> 346
Arg Ser Lys Arg Ser Leu Ser Cys
1 5

<210> 347
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for u-PA cleavage site

<221> VARIANT
<222> 1, 5, 8
<223> Positions 1, 5, and 8 are independantly any amino acid

<221> VARIANT
<222> 2, 7
<223> Positions 2 and 7 are independantly an uncharged amino acid like C, S, and T, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3
<223> Position 3 is an amidic amino acid like N and Q, an uncharged amino acid like C, S, and T, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

<221> VARIANT
<222> 6
<223> Position 6 is a basic amino acid like K and R, an aromatic amino acid like F, W, and Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

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1 5

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<211> 8
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<220>
<223> u-PA cleavage site

<400> 348
Gly Ser Gly Lys Ser Ala Thr Leu
1 5

<210> 349

<211> 8
<212> PRT
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<220>
<223> u-PA cleavage site

<400> 349
Gln Arg Gly Arg Ser Ala Thr Leu
1 5

<210> 350
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> u-PA cleavage site

<400> 350
Arg Gly Ser Val Ile Leu Thr Val
1 5

<210> 351
<211> 8
<212> PRT
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<220>
<223> u-PA cleavage site

<400> 351
Pro Ser Ser Arg Arg Arg Val Asn
1 5

<210> 352
<211> 8
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<220>
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<400> 352
Cys Pro Gly Arg Val Val Gly Gly
1 5

<210> 353
<211> 8
<212> PRT
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<220>
<223> u-PA cleavage site

<400> 353
Pro Gly Ala Arg Gly Arg Ala Phe
1 5

<210> 354
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> u-PA cleavage site

<400> 354
Ser Ser Ser Arg Gly Pro Thr His
1 5

<210> 355
<211> 8
<212> PRT
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<220>
<223> u-PA cleavage site

<400> 355
Val Ser Asn Lys Tyr Phe Ser Asn
1 5

<210> 356
<211> 8
<212> PRT
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<220>
<223> u-PA cleavage site

<400> 356
Asn Ser Gly Arg Ala Val Thr Tyr
1 5

<210> 357
<211> 8
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<220>
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<400> 357
Thr Tyr Ser Arg Ser Arg Tyr Leu
1 5

<210> 358
<211> 8
<212> PRT
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<220>
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Asn Ser Gly Arg Ala Val Thr Tyr

1 5

<210> 359
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<400> 359
Pro Ser Gly Arg Gly Arg Thr Leu
1 5

<210> 360
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<220>
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<400> 360
Ala Gly Ser Arg Ala Val Tyr Tyr
1 5

<210> 361
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<220>
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<400> 361
Thr Tyr Gly Arg Ser Arg Thr Asn
1 5

<210> 362
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<220>
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<400> 362
Asn Ser Ser Arg Gly Val Tyr Leu
1 5

<210> 363
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<210> 364
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Ala Ser Gly Arg Gly Arg Thr Tyr
1 5

<210> 365
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<220>
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<400> 365
Thr Ser Ser Arg Ala Val Tyr Leu
1 5

<210> 366
<211> 8
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<400> 366
Asn Ser Gly Arg Ser Arg Thr Leu
1 5

<210> 367
<211> 8
<212> PRT
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<220>
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Val Ser Gly Arg Ile Arg Thr Gly
1 5

<210> 368
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> u-PA cleavage site

<400> 368
Ser Ser Gly Arg Ile Arg Thr Val
1 5

<210> 369
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for t-PA cleavage site

<221> VARIANT
<222> 1, 2, 3, 5, 6, 7, 8
<223> Positions 1, 2, 3, 5, 6, 7, and 8 are independantly any amino acid

<221> VARIANT
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<223> Position 4 is K or R

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<210> 370
<211> 8
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<220>
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<400> 370
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<210> 371
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<220>
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<400> 371
Cys Pro Gly Arg Val Val Gly Gly
1 5

<210> 372
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<220>
<223> t-PA cleavage site

<400> 372
Pro Gln Phe Arg Ile Lys Gly Gly
1 5

<210> 373
<211> 8
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<400> 373
Ala Leu Ser Arg Met Ala Val Leu
1 5

<210> 374
<211> 6
<212> PRT
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<220>
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<221> VARIANT
<222> 1
<223> Position 1 is K or R

<221> VARIANT
<222> 2, 3, 4, 5
<223> Positions 2, 3, 4, and 5 are independantly an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 6
<223> Position 6 is D or E

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<210> 375
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<212> PRT
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<220>
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1 5

<210> 376
<211> 6
<212> PRT
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<220>
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<400> 376
Arg Ile Val Gly Gly Glu
 1   5

<210> 377
<211> 6
<212> PRT
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<220>
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<400> 377
Arg Ile Ile Gly Gly Glu
 1   5

<210> 378
<211> 6
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<220>
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<400> 378
Arg Val Val Gly Gly Asp
 1   5

<210> 379
<211> 6
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<220>
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<400> 379
Arg Ile Val Gly Gly Asp
 1   5

<210> 380
<211> 6
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<220>
<223> Tryptase-epsilon cleavage site

<400> 380
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 1   5

<210> 381
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<220>
<223> Tryptase-epsilon cleavage site

<400> 381
Lys Val Val Gly Gly Glu
 1   5

<210> 382
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Tryptase-epsilon cleavage site

<400> 382
Lys Ile Val Gly Gly Glu
 1   5

<210> 383
<211> 6
<212> PRT
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<220>
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<400> 383
Lys Ile Ile Gly Gly Glu
 1   5

<210> 384
<211> 6
<212> PRT
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<220>
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<400> 384
Lys Val Val Gly Gly Asp
 1   5

<210> 385
<211> 6
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<220>
<223> Tryptase-epsilon cleavage site

<400> 385
Lys Ile Val Gly Gly Asp
 1   5

<210> 386
<211> 6
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<220>
<223> Tryptase-epsilon cleavage site

<400> 386
Lys Ile Ile Gly Gly Asp
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<210> 387
<211> 8
<212> PRT
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<220>
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<221> VARIANT
<222> 1, 5, 6, 7, 8
<223> Positions 1, 5, 6, 7, and 8 are independently any amino acid

<221> VARIANT
<222> 2
<223> Potition 2 is an amidic amino acid like N or Q, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3
<223> Position 3 is an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is K or R

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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<210> 388
<211> 8
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<220>
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Leu Ser Ser Arg Gln Ser Pro Gly
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<210> 389
<211> 8
<212> PRT
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<220>
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<400> 389
Leu Gln Ala Arg Gly Ala Ser Leu
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<210> 390
<211> 8
<212> PRT
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<220>
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<400> 390
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 1   5

<210> 391
<211> 8
<212> PRT
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<220>
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Leu Gly Pro Arg Ser Ala Val Tyr
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<210> 392
<211> 8
<212> PRT
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<220>
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<221> VARIANT
<222> 1, 2, 3, 4, 6, 7, 8
<223> Positions 1, 2, 3, 4, 6, 7, and 8 are
      independantly any amino acid

<221> VARIANT
<222> 5
<223> Position 5 is F, L, I, V, or Y

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<210> 393
<211> 8
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<220>

<223> ECE-1 cleavage site

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His Gln Lys Leu Val Phe Phe Ala
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<210> 394
<211> 8
<212> PRT
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<220>
<223> ECE-1 cleavage site

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His His Gln Lys Leu Val Phe Phe
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<210> 395
<211> 8
<212> PRT
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<220>
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<400> 395
Lys Leu Val Phe Phe Ala Glu Asp
 1   5

<210> 396
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 396
Asp Arg Val Tyr Ile His Pro Phe
 1   5

<210> 397
<211> 8
<212> PRT
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<220>
<223> ECE-1 cleavage site

<400> 397
Tyr Ile His Pro Phe His Leu Val
 1   5

<210> 398
<211> 8
<212> PRT
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<220>
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<400> 398
Tyr Gly Leu Gly Ser Pro Arg Ser
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<210> 399
<211> 8
<212> PRT
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<220>
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<400> 399
Thr Pro Glu His Val Val Pro Tyr
1 5

<210> 400
<211> 8
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<220>
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<400> 400
Asp Ile Ile Trp Val Asn Thr Pro
1 5

<210> 401
<211> 8
<212> PRT
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<220>
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<400> 401
Asp Ile Ile Trp Ile Asn Thr Pro
1 5

<210> 402
<211> 8
<212> PRT
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<220>
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<400> 402
Cys His Leu Asp Ile Ile Trp Val
1 5

<210> 403
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 403
His Leu Asp Ile Ile Trp Val Asn
 1   5

<210> 404
<211> 8
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<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 404
Cys Val Tyr Phe Cys His Leu Asp
 1   5

<210> 405
<211> 8
<212> PRT
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<220>
<223> ECE-1 cleavage site

<400> 405
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 1   5

<210> 406
<211> 8
<212> PRT
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<220>
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<400> 406
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 1   5

<210> 407
<211> 8
<212> PRT
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<220>
<223> ECE-1 cleavage site

<400> 407
Arg Ser Lys Arg Cys Ser Cys Ser
 1   5

<210> 408
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 408
Arg Ser Lys Arg Ala Leu Glu Asn
 1   5

<210> 409
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 409
Gly Phe Ser Pro Phe Arg Ser Ser
 1   5

<210> 410
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 410
Pro Arg Arg Pro Tyr Ile Leu Pro
 1   5

<210> 411
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> ECE-1 cleavage site

<400> 411
Lys Pro Gln Gln Phe Phe Gly Leu
 1   5

<210> 412
<211> 8
<212> PRT
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<220>
<223> ECE-1 cleavage site

<400> 412
Pro Gln Gln Phe Phe Gly Leu Met
 1   5

<210> 413
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for KBGP cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is an acidic amino acid like D and E

<221> VARIANT
<222> 2, 5
<223> Positions 2 and 5 are independantly T or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 3, 8
<223> Positions 3 and 8 are independantly an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 4
<223> Position 4 is an aromatic amino acid like F, W

<221> VARIANT
<222> 6
<223> Position 6 is an amidic amino acid like N and Q

<221> VARIANT
<222> (7)...(7)
<223> Position 7 is an uncharged amino acid like C, S, and T, or a C-beta branched amino acid like I, V, or T

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 414
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KBGP cleavage site

<400> 414
Asp Ile Ile Trp Val Asn Thr Pro
1 5

<210> 415
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KBGP cleavage site

<400> 415
Asp Ile Ile Trp Ile Asn Thr Pro
 1   5

<210> 416
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Cathepsin L cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is W, an acidic amino acid like D and
      E, an amidic amino acid like N and Q, a positive
      amino acid like H, K, and R, an uncharged amino
      acid like C, S, and T, or an aliphatic hydrophobic
      amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 2, 4, 5, 6, 7, 8
<223> Positions 2, 4, 5, 6, 7, 8 are independantly any
      amino acid

<221> VARIANT
<222> 3
<223> Position 3 is L, V, F or Y

<400> 416
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1   5

<210> 417
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 417
Met Phe Leu Glu Ala Ile Pro Met
 1   5

<210> 418
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 418
Lys Val Phe Gln Glu Pro Leu Phe
 1   5

<210> 419
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 419
Ala Thr Leu Thr Phe Asp His Ser
 1   5

<210> 420
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 420
Pro Leu Phe Tyr Glu Ala Pro Arg
 1   5

<210> 421
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 421
Thr Gly Leu Arg Asp Pro Phe Asn
 1   5

<210> 422
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 422
Lys Ile Leu His Leu Pro Thr Ser
 1   5

<210> 423
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 423
Ala His Leu Lys Asn Ser Gln Glu

1 5

<210> 424
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 424
Ala Pro Leu Thr Ala Glu Ile Gln
1 5

<210> 425
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 425
Glu Ala Leu Phe Ala Glu Arg Lys
1 5

<210> 426
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 426
Glu Pro Leu Ala Ala Glu Arg Lys
1 5

<210> 427
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 427
Gly Thr Phe Thr Ser Asp Tyr Ser
1 5

<210> 428
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 428
Lys Tyr Leu Asp Ser Arg Arg Ala
1 5

<210> 429
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 429
Gln Asp Phe Val Gln Trp Leu Met
1 5

<210> 430
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 430
Lys Gln Leu Ala Thr Lys Ala Ala
1 5

<210> 431
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 431
Ser Thr Phe Glu Glu Arg Ser Tyr
1 5

<210> 432
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 432
Leu Arg Leu Glu Trp Pro Tyr Gln
1 5

<210> 433
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> Cathepsin L cleavage site

<400> 433
Arg Gly Phe Phe Tyr Thr Pro Lys
 1   5

<210> 434
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 434
Gly Phe Phe Tyr Thr Pro Lys Ala
 1   5

<210> 435
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 435
His Phe Phe Lys Asn Ile Val Thr
 1   5

<210> 436
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 436
Arg Gly Leu Ser Leu Ser Arg Phe
 1   5

<210> 437
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 437
Gln Trp Leu Gly Ala Pro Val Pro
 1   5

<210> 438
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 438
Asn Met Leu Lys Arg Gly Leu Pro
 1   5

<210> 439
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 439
Leu Ser Leu Ala His Thr His Gln
 1   5

<210> 440
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 440
Thr Pro Phe Ala Ala Thr Ser Ser
 1   5

<210> 441
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 441
Lys Leu Leu Ala Val Ser Gly Pro
 1   5

<210> 442
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 442
Gln Leu Phe Arg Arg Ala Val Leu
 1   5

<210> 443
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> Cathepsin L cleavage site

<400> 443
Pro Arg Phe Lys Ile Ile Gly Gly
 1   5

<210> 444
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for PAR1 cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is a small non-polar amino acid like A,
      C G, S, and T

<221> VARIANT
<222> 2, 3
<223> Positions 2 and 3 are independantly a large
      non-polar amino acid like F, I, L, M, V, or an
      aromatic amino acid like F, H, W, or Y

<221> VARIANT
<222> 4
<223> Position 4 is an aliphatic hydrophobic amino acid
      like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 5
<223> Position 5 is K or R

<221> VARIANT
<222> 6
<223> Position 6 is an amidic amino acid like N and Q,
      or an aromatic hydrophobic amino acid like F, W,
      or Y

<400> 444
Xaa Xaa Xaa Xaa Xaa Xaa
 1   5

<210> 445
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 445
Ser Phe Leu Leu Arg Asn
 1   5

<210> 446
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 446
Ser Phe Phe Leu Arg Asn
 1   5

<210> 447
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 447
Ser Phe Phe Leu Lys Asn
 1   5

<210> 448
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 448
Thr Phe Leu Leu Arg Asn
 1   5

<210> 449
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 449
Gly Phe Pro Gly Lys Phe
 1   5

<210> 450
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 450
Gly Tyr Pro Ala Lys Phe
 1   5

<210> 451
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 451
Gly Tyr Pro Leu Lys Phe
 1   5

<210> 452
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR1 cleavage site

<400> 452
Gly Tyr Pro Ile Lys Phe
 1   5

<210> 453
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for PAR2 cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is a small non-polar amino acid like A,
      C G, S, and T

<221> VARIANT
<222> 2, 3, 6
<223> Positions 2, 3, and 6 are independantly a large
      non-polar amino acid like F, I, L, M, V

<221> VARIANT
<222> 4
<223> Position 4 is an aliphatic hydrophobic amino acid
      like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 5
<223> Position 5 is K or R

<400> 453
Xaa Xaa Xaa Xaa Xaa Xaa
 1   5

<210> 454
<211> 6
<212> PRT

<213> Artificial Sequence

<220>
<223> PAR2 cleavage site

<400> 454
Ser Leu Ile Gly Lys Val
1 5

<210> 455
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR2 cleavage site

<400> 455
Ser Leu Ile Gly Arg Leu
1 5

<210> 456
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for PAR3 cleavage site

<221> VARIANT
<222> 1, 4
<223> Positions 1 and 4 are independantly a small non-polar amino acid like A, C G, S, and T

<221> VARIANT
<222> 2
<223> Position 2 is a large non-polar amino acid like F, I, L, M, V

<221> VARIANT
<222> 3
<223> Position 3 is an amidic amino acid like N and Q, or a basic amino acid like K and R

<221> VARIANT
<222> 5
<223> Position 5 is a small non-polar amino acid like A, C G, S, and T, or a small polar amino acid like D, N, or P

<221> VARIANT
<222> 6
<223> Position 6 is an acidic amino acid like D and E, or a small polar amino acid like D, N, or P

<400> 456
Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 457

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR3 cleavage site

<400> 457
Thr Phe Arg Gly Ala Pro
1 5

<210> 458
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR3 cleavage site

<400> 458
Ser Phe Asn Gly Gly Pro
1 5

<210> 459
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR3 cleavage site

<400> 459
Ser Phe Asn Gly Asn Glu
1 5

<210> 460
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for PAR4 cleavage site

<221> VARIANT
<222> 1
<223> Position 1 is a small non-polar amino acid like A, C G, S, and T

<221> VARIANT
<222> 2
<223> Position 2 is a large non-polar amino acid like F, I, L, M, V, or an aromatic amino acid like F, H, W, or Y

<221> VARIANT
<222> 3, 4
<223> Positions 3 and 4 are independantly an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<221> VARIANT
<222> 5
<223> Position 5 is K or R

<221> VARIANT
<222> 6
<223> Position 6 is a basic amino acid like K and R, an aromatic hydrophobic amino acid like F, W, or Y, or an aliphatic hydrophobic amino acid like, G, P, A, V, L, I, and M

<400> 460
Xaa Xaa Xaa Xaa Xaa Xaa
1 5

<210> 461
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 461
Gly Tyr Pro Gly Gln Val
1 5

<210> 462
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 462
Ala Tyr Pro Gly Lys Phe
1 5

<210> 463
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 463
Thr Tyr Pro Gly Lys Phe
1 5

<210> 464
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 464
Gly Tyr Pro Gly Lys Tyr
1 5

<210> 465
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 465
Gly Tyr Pro Gly Lys Trp
1 5

<210> 466
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 466
Gly Tyr Pro Gly Lys Lys
1 5

<210> 467
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 467
Gly Tyr Pro Gly Lys Phe
1 5

<210> 468
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 468
Gly Tyr Pro Gly Arg Phe
1 5

<210> 469
<211> 6
<212> PRT
<213> Artificial Sequence

<220>

<223> PAR4 cleavage site

<400> 469
Gly Tyr Pro Gly Phe Lys
1 5

<210> 470
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 470
Gly Tyr Pro Ala Lys Phe
1 5

<210> 471
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 471
Gly Phe Pro Gly Lys Phe
1 5

<210> 472
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 472
Gly Phe Pro Gly Lys Pro
1 5

<210> 473
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 473
Ser Tyr Pro Gly Lys Phe
1 5

<210> 474
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 474
Ser Tyr Pro Ala Lys Phe
1 5

<210> 475
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 475
Ser Tyr Pro Gly Arg Phe
1 5

<210> 476
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 476
Ser Tyr Ala Gly Lys Phe
1 5

<210> 477
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 477
Ser Phe Pro Gly Gln Pro
1 5

<210> 478
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> PAR4 cleavage site

<400> 478
Ser Phe Pro Gly Gln Ala
1 5

<210> 479
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> ADAMTS13 cleavage site

<400> 479
Asn Leu Val Tyr Met Val Thr Gly
 1   5

<210> 480
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Bovine enterokinase protease cleavage site

<400> 480
Asp Asp Asp Asp Lys
 1   5

<210> 481
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for TEV protease cleavage site

<221> VARIANT
<222> 2, 3, 5
<223> Positions 2, 3, and 5 are independantly any amino
      amino acid

<400> 481
Glu Xaa Xaa Tyr Xaa Gln Gly
 1   5

<210> 482
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for TEV protease cleavage site

<221> VARIANT
<222> 2, 3, 5
<223> Positions 2, 3, and 5 are independantly any amino
      acid

<400> 482
Glu Xaa Xaa Tyr Xaa Gln Ser
 1   5

<210> 483
<211> 7
<212> PRT

<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 483
Glu Asn Leu Tyr Phe Gln Gly
1 5

<210> 484
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 484
Glu Asn Leu Tyr Phe Gln Ser
1 5

<210> 485
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 485
Glu Asn Ile Tyr Thr Gln Gly
1 5

<210> 486
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 486
Glu Asn Ile Tyr Thr Gln Ser
1 5

<210> 487
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 487
Glu Asn Ile Tyr Leu Gln Gly
1 5

<210> 488

<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 488
Glu Asn Ile Tyr Leu Gln Ser
 1   5

<210> 489
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 489
Glu Asn Val Tyr Phe Gln Gly
 1   5

<210> 490
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 490
Glu Asn Val Tyr Ser Gln Ser
 1   5

<210> 491
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 491
Glu Asn Val Tyr Ser Gln Gly
 1   5

<210> 492
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TEV protease cleavage site

<400> 492
Glu Asn Val Tyr Ser Gln Ser
 1   5

<210> 493
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for TVMV protease cleavage site

<221> VARIANT
<222> 1, 2
<223> Positions 1 and 2 are independantly any amino acid

<400> 493
Xaa Xaa Val Arg Phe Gln Gly
 1   5

<210> 494
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for TVMV protease cleavage site

<221> VARIANT
<222> 1, 2
<223> Positions 1 and 2 are independantly any amino acid

<400> 494
Xaa Xaa Val Arg Phe Gln Ser
 1   5

<210> 495
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TVMV protease cleavage site

<400> 495
Glu Thr Val Arg Phe Gln Gly
 1   5

<210> 496
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TVMV protease cleavage site

<400> 496
Glu Thr Val Arg Phe Gln Ser
 1   5

<210> 497
<211> 7

<212> PRT
<213> Artificial Sequence

<220>
<223> TVMV protease cleavage site

<400> 497
Asn Asn Val Arg Phe Gln Gly
 1   5

<210> 498
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> TVMV protease cleavage site

<400> 498
Asn Asn Val Arg Phe Gln Ser
 1   5

<210> 499
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for HR3C protease cleavage site

<221> VARIANT
<222> 1
<223> Position 1 can be amino acid, with D or E
      preferred

<221> VARIANT
<222> 2
<223> Position 2 is S, T, and an aliphatic hydrophobic
      amino acid like G, P, A, V, L, I, and M

<400> 499
Xaa Xaa Leu Phe Gln Gly Pro
 1   5

<210> 500
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 500
Glu Ala Leu Phe Gln Gly Pro
 1   5

<210> 501
<211> 7
<212> PRT

<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 501
Glu Val Leu Phe Gln Gly Pro
 1   5

<210> 502
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 502
Glu Leu Leu Phe Gln Gly Pro
 1   5

<210> 503
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 503
Asp Ala Leu Phe Gln Gly Pro
 1   5

<210> 504
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 504
Asp Val Leu Phe Gln Gly Pro
 1   5

<210> 505
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> HR3C protease cleavage site

<400> 505
Asp Leu Leu Phe Gln Gly Pro
 1   5

<210> 506

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Subtilisin protease cleavage site

<221> VARIANT
<222> 1, 2, 3, 4
<223> Positions 1, 2, 3, and 4 are independantly any amino acid

<400> 506
Xaa Xaa Xaa Xaa His Tyr
1 5

<210> 507
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Subtilisin protease cleavage site

<221> VARIANT
<222> 1, 2, 3, 4
<223> Positions 1, 2, 3, and 4 are independantly any amino acid

<400> 507
Xaa Xaa Xaa Xaa Tyr His
1 5

<210> 508
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Subtilisin protease cleavage site

<400> 508
His Tyr
1

<210> 509
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Subtilisin protease cleavage site

<400> 509
Tyr His
1

<210> 510
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Subtilisin protease cleavage site

<400> 510
Pro Gly Ala Ala His Tyr
1 5

<210> 511
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Hydroxylamine cleavage site

<400> 511
Asn Gly Asn Gly Asn Gly
1 5

<210> 512
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Hydroxylamine cleavage site

<400> 512
Asn Gly
1

<210> 513
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for SUMO/ULP-1 protease cleavage site

<221> VARIANT
<222> 3, 4, 5
<223> Positions 3, 4, and 5 are independantly any amino acid

<400> 513
Gly Gly Xaa Xaa Xaa
1 5

<210> 514
<211> 98
<212> PRT
<213> Artificial Sequence

<220>
<223> SUMO/ULP-1 protease cleavage site

<400> 514
Met Ala Asp Ser Glu Val Asn Gln Glu Ala Lys Pro Glu Val Lys Pro
 1   5   10   15
Glu Val Lys Pro Glu Thr His Ile Asn Leu Lys Val Ser Asp Gly Ser
            20   25   30
Ser Glu Ile Phe Phe Lys Ile Lys Lys Thr Thr Pro Leu Arg Arg Leu
        35   40   45
Met Glu Ala Phe Ala Lys Arg Gln Gly Lys Glu Met Asp Ser Leu Arg
    50   55   60
Phe Leu Tyr Asp Gly Ile Arg Ile Gln Ala Asp Gln Thr Pro Glu Asp
65   70   75   80
Leu Asp Met Glu Asp Asn Asp Ile Ile Glu Ala His Arg Glu Gln Ile
                85   90   95
Gly Gly

<210> 515
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus sequence for Caspase 3 protease cleavage
      site

<221> VARIANT
<222> 2
<223> Position 2 can be any amino acid with E preferred

<221> VARIANT
<222> 3
<223> Position 3 is any amino acid

<221> VARIANT
<222> 5
<223> Position 5 can be any amino acid with G or S
      preferred

<400> 515
Asp Xaa Xaa Asp Xaa
 1   5

<210> 516
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 516
Asp Glu Val Asp Gly
 1   5

<210> 517
<211> 5
<212> PRT

<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 517
Asp Glu Val Asp Ser
 1   5

<210> 518
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 518
Asp Glu Pro Asp Gly
 1   5

<210> 519
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 519
Asp Glu Pro Asp Ser
 1   5

<210> 520
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 520
Asp Glu Leu Asp Gly
 1   5

<210> 521
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Caspase 3 protease cleavage site

<400> 521
Asp Glu Leu Asp Ser
 1   5

<210> 522

<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible G-spacer

<400> 522
Gly Gly Gly Gly
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<210> 523
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible G-spacer

<400> 523
Gly Gly Gly Gly Ser
 1   5

<210> 524
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible A-spacer

<400> 524
Ala Ala Ala Ala
 1

<210> 525
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible A-spacer

<400> 525
Ala Ala Ala Ala Val
 1   5

<210> 526
<211> 3939
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A with a HIS tag

<400> 526
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aaaatttggg tgatcccgga gcgcgatacg ttcacgaacc cggaagaagg agatttaaac 180

ccaccgcctg aggctaaaca ggtcccggtg tcttactatg atagcacata cctgagtacc 240
gacaatgaaa aggacaacta cctgaaaggt gttaccaaac tgttcgagcg catttattcg 300
acagatctcg gtcgcatgtt gctgacttct attgtgcgcg gcattccgtt ttggggtggt 360
agcaccatcg atacagaact caaagtgatt gacaccaact gcatcaatgt gattcagcct 420
gatgggagct accggtccga agagcttaac ctcgtaatca ttggcccgag cgcggatatt 480
atccaattcg aatgtaaatc ttttgggcat gaagtcctga atctgacgcg gaatggctat 540
ggatcgacgc agtatattcg tttttctcca gatttcacat ttggatttga agaaagcctc 600
gaagttgata cgaaccctct tttaggcgcg ggaaaattcg cgacggaccc agcggtgacc 660
ttggcacatg aacttattca tgccgggcat cgcttgtatg gaatcgccat taacccgaac 720
cgtgttttca aggtgaatac gaacgcgtat tacgagatgt cgggcttaga agtgtccttt 780
gaagaactgc gcacgtttgg cggtcatgat gcaaaattta ttgatagtct gcaagaaaac 840
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ctgtataaaa tgctcaccga gatctacaca gaggataact ttgtcaaatt cttcaaggtc 1080
ttgaatcgga aaacctatct gaacttcgat aaagccgtct ttaagatcaa catcgtaccg 1140
aaagttaact acaccatcta tgatggcttt aatctgcgca atacgaatct ggcggcgaac 1200
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tccttggata agggctataa caaggcgctc aatgatttat gcatcaaggt gaacaactgg 1380
gacttgtttt tctctccatc tgaagataat tttactaacg acttgaacaa aggagaggaa 1440
attacttccg ataccaacat cgaagcagcg gaagagaata ttagcctgga tcttattcaa 1500
caatattacc tgacctttaa ttttgataac gagcctgaga acatttccat tgagaatctc 1560
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gaaaaggaga ttaaggatct ttatgacaat cagtctaatt cgggtattct gaaagacttt 3300
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gataacaacg gcaacgacat tggttttatc ggcttccacc aattcaataa tatcgcgaaa 3780
ctggtggcct caaattggta caaccgtcag attgagcgca gctcccgcac tttaggctgt 3840
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cttgcggccg cactcgagca ccaccaccac caccactga 3939

<210> 527
<211> 1312
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A with a HIS tag

<400> 527
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1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
180 185 190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
405 410 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg

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                420                 425                 430
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
        435                 440                 445
Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460
Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480
Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495
Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510
Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525
Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540
Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560
His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575
Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590
Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605
Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620
Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640
Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655
Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670
Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685
Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700
Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720
Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735
Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750
Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
        755                 760                 765
Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
    770                 775                 780
Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785                 790                 795                 800
Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
                805                 810                 815
Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
            820                 825                 830
Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
        835                 840                 845
Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
    850                 855                 860
Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865                 870                 875                 880
Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
                885                 890                 895
Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
            900                 905                 910
Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
        915                 920                 925
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Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940
Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
945 950 955 960
Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
965 970 975
Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
980 985 990
Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
995 1000 1005
Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu
1010 1015 1020
Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro
1025 1030 1035 1040
Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys
1045 1050 1055
Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe
1060 1065 1070
Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr
1075 1080 1085
Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr
1090 1095 1100
Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn
1105 1110 1115 1120
Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu
1125 1130 1135
Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser
1140 1145 1150
Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly
1155 1160 1165
Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val
1170 1175 1180
Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala
1185 1190 1195 1200
Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn
1205 1210 1215
Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr
1220 1225 1230
Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245
Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser
1250 1255 1260
Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys
1265 1270 1275 1280
Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
1285 1290 1295
Lys Val Asp Lys Leu Ala Ala Ala Leu Glu His His His His His His
1300 1305 1310

<210> 528
<211> 3921
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A-TEV with a HIS tag

<400> 528
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aaaatttggg tgatcccgga gcgcgatacg ttcacgaacc cggaagaagg agatttaaac 180
ccaccgcctg aggctaaaca ggtcccggtg tcttactatg atagcacata cctgagtacc 240

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ggatcgacgc agtatattcg tttttctcca gatttcacat ttggatttga agaaagcctc 600
gaagttgata cgaaccctct tttaggcgcg ggaaaattcg cgacggaccc agcggtgacc 660
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tggggagaac gcccattgta a                                           3921
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<210> 529
<211> 1306
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A-TEV with a HIS tag

<400> 529

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 1               5                  10                  15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
            20                  25                  30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
        35                  40                  45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
    50                  55                  60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65                  70                  75                  80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85                  90                  95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
            100                 105                 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
        115                 120                 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
    130                 135                 140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145                 150                 155                 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165                 170                 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210                 215                 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275                 280                 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290                 295                 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305                 310                 315                 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325                 330                 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340                 345                 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355                 360                 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430
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Gly Ile Ile Thr Ser Lys Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu
        435                 440                 445
Tyr Phe Gln Gly Gly Gly Ser Gly Gly Gly Ala Leu Asn Asp Leu Cys
    450                 455                 460
Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
465                 470                 475                 480
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
                485                 490                 495
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
            500                 505                 510
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
        515                 520                 525
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
    530                 535                 540
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
545                 550                 555                 560
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
                565                 570                 575
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
            580                 585                 590
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
        595                 600                 605
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
    610                 615                 620
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
625                 630                 635                 640
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
                645                 650                 655
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
            660                 665                 670
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
        675                 680                 685
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
    690                 695                 700
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
705                 710                 715                 720
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
                725                 730                 735
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
            740                 745                 750
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
        755                 760                 765
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
    770                 775                 780
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
785                 790                 795                 800
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
                805                 810                 815
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
            820                 825                 830
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
        835                 840                 845
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
    850                 855                 860
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
865                 870                 875                 880
Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn
                885                 890                 895
His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser
            900                 905                 910
Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn
        915                 920                 925
Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr
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930 935 940
Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro
945 950 955 960
Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn
965 970 975
Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu
980 985 990
Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val
995 1000 1005
Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp
1010 1015 1020
Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr
1025 1030 1035 1040
Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn
1045 1050 1055
Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp
1060 1065 1070
Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
1075 1080 1085
Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
1090 1095 1100
Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
1105 1110 1115 1120
Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
1125 1130 1135
Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
1140 1145 1150
Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
1155 1160 1165
Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
1170 1175 1180
Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
1185 1190 1195 1200
Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
1205 1210 1215
Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
1220 1225 1230
Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
1235 1240 1245
Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
1250 1255 1260
Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
1265 1270 1275 1280
Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
1285 1290 1295
Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
1300 1305

<210> 530
<211> 3945
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A comprising Thrombin (N880)
and Thrombin (D890)

<400> 530
atgccgttcg taaacaaaca gttcaactat aaagacccag tcaacggcgt ggacattgcc 60
tatatcaaaa tcccgaatgc gggtcaaatg cagcccgtga agcatttaa aatccataac 120
aaaatttggg tgatcccgga gcgcgatacg ttcacgaacc cggaagaagg agatttaaac 180
ccaccgcctg aggctaaaca ggtcccggtg tcttactatg atagcacata cctgagtacc 240

gacaatgaaa aggacaacta cctgaaaggt gttaccaaac tgttcgagcg catttattcg 300
acagatctcg gtcgcatgtt gctgacttct attgtgcgcg gcattccgtt ttggggtggt 360
agcaccatcg atacagaact caaagtgatt gacaccaact gcatcaatgt gattcagcct 420
gatgggagct accggtccga agagcttaac ctcgtaatca ttggcccgag cgcggatatt 480
atccaattcg aatgtaaatc ttttgggcat gaagtcctga atctgacgcg gaatggctat 540
ggatcgacgc agtatattcg tttttctcca gatttcacat ttggatttga agaaagcctc 600
gaagttgata cgaaccctct tttaggcgcg ggaaaattcg cgacggaccc agcggtgacc 660
ttggcacatg aacttattca tgccgggcat cgcttgtatg gaatcgccat taacccgaac 720
cgtgttttca aggtgaatac gaacgcgtat tacgagatgt cgggcttaga agtgtccttt 780
gaagaactgc gcacgtttgg cggtcatgat gcaaaattta ttgatagtct gcaagaaaac 840
gaatttcggc tgtactatta caataaattc aaagacattg catcaacctt aaacaaggcg 900
aaaagcattg tgggtaccac ggctagctta caatatatga aaaacgtttt caaagaaaaa 960
tacctcctta gcgaagacac ttccggcaaa ttctctgtcg ataaactgaa atttgataaa 1020
ctgtataaaa tgctcaccga gatctacaca gaggataact ttgtcaaatt cttcaaggtc 1080
ttgaatcgga aaacctatct gaacttcgat aaagccgtct ttaagatcaa catcgtaccg 1140
aaagttaact acaccatcta tgatggcttt aatctgcgca atacgaatct ggcggcgaac 1200
tttaacggcc agaacaccga aatcaacaac atgaacttta ctaaactgaa aaattttacc 1260
ggcttgtttg aattctataa gctcctgtgt gtccgcggta ttatcaccag caaaggcggt 1320
ggttctggcg gtggtgaaaa cctgtacttc cagggcggtg gctccggtgg tggtgcgctc 1380
aatgatttat gcatcaaggt gaacaactgg gacttgtttt tctctccatc tgaagataat 1440
tttactaacg acttgaacaa aggagaggaa attacttccg ataccaacat cgaagcagcg 1500
gaagagaata ttagtctaga tcttattcaa caatattacc tgacctttaa ttttgataac 1560
gagcctgaga acatttccat tgagaatctc agctctgaca tcatcggcca gctggaactg 1620
atgccgaata tcgaacgctt tcctaatgga aagaaatatg aattggacaa atacaccatg 1680
ttccactatc tccgcgcgca ggagtttgag cacggcaagt ctcgtattgc tctgaccaat 1740
tcggtaaacg aagccctttt aaatccttcg cgtgtgtaca ccttttttctc aagcgattat 1800
gttaaaaaag tgaacaaggc gaccgaagcg gcgatgtttt tgggatgggt ggaacaactg 1860
gtatatgact ttacggatga aacttctgaa gtctcgacca ccgacaaaat tgccgatatt 1920
accattatca ttccctatat tggccctgca ctgaacattg gtaacatgct gtataaagat 1980
gattttgtgg gcgccctgat cttttcaggc gctgttatcc tgctggaatt tatcccggaa 2040
atcgccattc cagtactcgg tacctttgcg ctggtgtcct atatcgcaaa caaagttttg 2100
actgtccaga cgatcgacaa cgcgctcagt aaacgtaacg aaaaatggga tgaggtgtat 2160
aagtatattg ttaccaactg gctcgctaaa gtaaacaccc agattgacct gattcgcaag 2220
aagatgaaag aagcgctgga aaaccaagca gaagcgacca aagctattat caactatcaa 2280
tataaccagt acacagagga agaaaagaat aacatcaact tcaacatcga cgacttatct 2340
tcaaagctga atgaatctat taacaaagcg atgattaata ttaacaagtt cttgaaccaa 2400
tgtagtgtca gctatctgat gaactcgatg atcccttacg gtgtgaaacg tctggaagac 2460
ttcgatgcaa gccttaaaga tgcccttctg aagtatattt acgataatcg cggaactctt 2520
attggccaag tggatcgctt aaaagataaa gtcaacaaca cgctgagtac agacatccct 2580
tttcagctgt ctaaatatgt ggacaatcag cgcctgctgt ccacgtttac ggaatacatc 2640
aaaaacatca tcaacactag tattctgaac ctggtgccgc gtggctccta cgagagtaac 2700
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aaggtgaact tcgatcctat cgacaaaaac cagattcaat tgttcaactt agaatcgtca 2820
aagattgaag ttatcttaaa aaatgcgatt gtatataatt caatgtacga aaatttctct 2880
acgagctttt ggattcgtat tccgaaatat ttcaacagta tctctttaaa caacgagtat 2940
actatcatca attgtatgga gaataacagc gggtggaaag tgagccttaa ctatggtgaa 3000
atcatctgga ctctgcagga cactcaagaa attaaacaac gcgtggtgtt taaatactca 3060
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cggctgaaca acagcaaaat ttacattaac ggtcgcctga tcgatcagaa accaatcagt 3180
aatctcggta acattcacgc atcgaataat atcatgttca aactggatgg ttgtcgcgac 3240
acgcaccgtt acatttggat caaatacttc aatttattcg acaaagaact caacgaaaag 3300
gagattaagg atctttatga caatcagtct aattcgggta ttctgaaaga cttttggggt 3360
gattaccttc agtacgataa accgtattat atgttaaact tatatgatcc gaataaatac 3420
gttgacgtca acaacgttgg cattcgcggc tatatgtatc tgaaagggcc gcgtggcagc 3480
gtgatgacca ctaacattta cttaaactcc tccctctatc gcggtactaa atttattatc 3540
aagaaatatg cctctggcaa caaggacaat atcgtacgca ataacgatcg cgtctacatt 3600
aacgtggtgg tgaagaataa agaatatcgt ctggcgacca atgctagtca ggcgggcgtg 3660
gagaaaattc tgtctgcact tgaaatcccg gatgtgggta atttatccca ggtggttgtg 3720
atgaaaagta aaaatgacca agggatcacc aataaatgca aaatgaatct gcaagataac 3780
aacggcaacg acattggttt tatcggcttc caccaattca ataatatcgc gaagcttgtg 3840
gcctcaaatt ggtacaaccg tcagattgag cgcagctccc gcactttagg ctgtagctgg 3900
gagttcattc cggtagatga cggttgggga gaacgcccat tgtaa 3945

<210> 531
<211> 1314
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A comprising Thrombin (N880) and Thrombin (D890)

<400> 531

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1               5                  10                  15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
            20                  25                  30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
        35                  40                  45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
    50                  55                  60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65                  70                  75                  80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85                  90                  95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
            100                 105                 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
        115                 120                 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
    130                 135                 140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145                 150                 155                 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165                 170                 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210                 215                 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275                 280                 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290                 295                 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305                 310                 315                 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325                 330                 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340                 345                 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355                 360                 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
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                420                 425                 430
Gly Ile Ile Thr Ser Lys Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu
        435                 440                 445
Tyr Phe Gln Gly Gly Gly Ser Gly Gly Gly Ala Leu Asn Asp Leu Cys
    450                 455                 460
Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
465                 470                 475                 480
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
                485                 490                 495
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
            500                 505                 510
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
        515                 520                 525
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
    530                 535                 540
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
545                 550                 555                 560
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
                565                 570                 575
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
            580                 585                 590
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
        595                 600                 605
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
    610                 615                 620
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
625                 630                 635                 640
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
                645                 650                 655
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
            660                 665                 670
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
        675                 680                 685
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
    690                 695                 700
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
705                 710                 715                 720
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
                725                 730                 735
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
            740                 745                 750
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
        755                 760                 765
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
    770                 775                 780
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
785                 790                 795                 800
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
                805                 810                 815
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
            820                 825                 830
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
        835                 840                 845
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
    850                 855                 860
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
865                 870                 875                 880
Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Val Pro Arg Gly Ser
                885                 890                 895
Tyr Glu Ser Asn His Leu Ile Asp Leu Val Pro Arg Gly Ser Arg Tyr
            900                 905                 910
Ala Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp
        915                 920                 925
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Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val
930 935 940
Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser
945 950 955 960
Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu
965 970 975
Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp
980 985 990
Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr
995 1000 1005
Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn
1010 1015 1020
Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn
1025 1030 1035 1040
Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
1045 1050 1055
Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met
1060 1065 1070
Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys
1075 1080 1085
Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp
1090 1095 1100
Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly
1105 1110 1115 1120
Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp
1125 1130 1135
Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met
1140 1145 1150
Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu
1155 1160 1165
Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala
1170 1175 1180
Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile
1185 1190 1195 1200
Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser
1205 1210 1215
Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val
1220 1225 1230
Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly
1235 1240 1245
Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp
1250 1255 1260
Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val
1265 1270 1275 1280
Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu
1285 1290 1295
Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg
1300 1305 1310
Pro Leu

<210> 532
<211> 3945
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A comprising FXa (N872) and
Thrombin (E884)

<400> 532
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tatatcaaaa tcccgaatgc gggtcaaatg cagcccgtga aagcatttaa aatccataac 120
aaaatttggg tgatcccgga gcgcgatacg ttcacgaacc cggaagaagg agatttaaac 180
ccaccgcctg aggctaaaca ggtcccggtg tcttactatg atagcacata cctgagtacc 240
gacaatgaaa aggacaacta cctgaaaggt gttaccaaac tgttcgagcg catttattcg 300
acagatctcg gtcgcatgtt gctgacttct attgtgcgcg gcattccgtt ttggggtggt 360
agcaccatcg atacagaact caaagtgatt gacaccaact gcatcaatgt gattcagcct 420
gatgggagct accggtccga agagcttaac ctcgtaatca ttggcccgag cgcggatatt 480
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<210> 533
<211> 1314
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A comprising FXa (N872) and Thrombin (E884)

<400> 533
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Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
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Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
180 185 190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu

405 410 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430
Gly Ile Ile Thr Ser Lys Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu
435 440 445
Tyr Phe Gln Gly Gly Gly Ser Gly Gly Gly Ala Leu Asn Asp Leu Cys
450 455 460
Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
465 470 475 480
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
485 490 495
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
500 505 510
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
515 520 525
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
530 535 540
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
545 550 555 560
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
565 570 575
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
580 585 590
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
595 600 605
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
610 615 620
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
625 630 635 640
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
645 650 655
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
660 665 670
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
675 680 685
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
690 695 700
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
705 710 715 720
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
725 730 735
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
740 745 750
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
755 760 765
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
770 775 780
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
785 790 795 800
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
805 810 815
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
820 825 830
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
835 840 845
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
850 855 860
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
865 870 875 880
Lys Asn Ile Glu Gly Arg Ile Asn Thr Ser Ile Leu Asn Leu Arg Tyr
885 890 895
Glu Leu Val Pro Arg Gly Ser Asn His Leu Ile Asp Leu Ser Arg Tyr
900 905 910

Ala Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp
915 920 925
Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val
930 935 940
Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser
945 950 955 960
Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu
965 970 975
Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp
980 985 990
Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr
995 1000 1005
Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn
1010 1015 1020
Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn
1025 1030 1035 1040
Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
1045 1050 1055
Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met
1060 1065 1070
Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys
1075 1080 1085
Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp
1090 1095 1100
Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly
1105 1110 1115 1120
Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp
1125 1130 1135
Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met
1140 1145 1150
Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu
1155 1160 1165
Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala
1170 1175 1180
Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile
1185 1190 1195 1200
Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser
1205 1210 1215
Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val
1220 1225 1230
Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly
1235 1240 1245
Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp
1250 1255 1260
Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val
1265 1270 1275 1280
Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu
1285 1290 1295
Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg
1300 1305 1310
Pro Leu

<210> 534
<211> 3921
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A comprising MMP-9 (I870)

<400> 534

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tggggagaac gcccattgta a 3921

<210> 535
<211> 1306
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A comprising MMP-9 (I870)

<400> 535

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Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
405 410 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430
Gly Ile Ile Thr Ser Lys Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu
435 440 445
Tyr Phe Gln Gly Gly Gly Ser Gly Gly Gly Ala Leu Asn Asp Leu Cys
450 455 460
Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
465 470 475 480
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
485 490 495
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
500 505 510
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
515 520 525
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
530 535 540
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
545 550 555 560
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
565 570 575
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
580 585 590
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
595 600 605
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
610 615 620
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
625 630 635 640
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
645 650 655
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
660 665 670
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
675 680 685
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
690 695 700
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
705 710 715 720
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
725 730 735
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
740 745 750
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
755 760 765
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
770 775 780
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
785 790 795 800
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
805 810 815
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
820 825 830
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
835 840 845
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
850 855 860
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
865 870 875 880
Gly Pro Leu Gly Leu Trp Ala Gln Leu Asn Leu Arg Tyr Glu Ser Asn
885 890 895
His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser

900 905 910
Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn
915 920 925
Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr
930 935 940
Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro
945 950 955 960
Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn
965 970 975
Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu
980 985 990
Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val
995 1000 1005
Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp
1010 1015 1020
Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr
1025 1030 1035 1040
Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn
1045 1050 1055
Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp
1060 1065 1070
Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
1075 1080 1085
Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
1090 1095 1100
Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
1105 1110 1115 1120
Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
1125 1130 1135
Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
1140 1145 1150
Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
1155 1160 1165
Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
1170 1175 1180
Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
1185 1190 1195 1200
Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
1205 1210 1215
Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
1220 1225 1230
Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
1235 1240 1245
Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
1250 1255 1260
Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
1265 1270 1275 1280
Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
1285 1290 1295
Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
1300 1305

<210> 536
<211> 3916
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding a BoNT/A comprising Fxa (N872)

<400> 536
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tatatcaaaa tcccgaatgc gggtcaaatg cagcccgtga aagcatttaa aatccataac 120
aaaatttggg tgatcccgga gcgcgatacg ttcacgaacc cggaagaagg agatttaaac 180
ccaccgcctg aggctaaaca ggtcccggtg tcttactatg atagcacata cctgagtacc 240
gacaatgaaa aggacaacta cctgaaaggt gttaccaaac tgttcgagcg catttattcg 300
acagatctcg gtcgcatgtt gctgacttct attgtgcgcg gcattccgtt ttggggtggt 360
agcaccatcg atacagaact caaagtgatt gacaccaact gcatcaatgt gattcagcct 420
gatgggagct accggtccga agagcttaac ctcgtaatca ttggcccgag cgcggatatt 480
atccaattcg aatgtaaatc ttttgggcat gaagtcctga atctgacgcg gaatggctat 540
ggatcgacgc agtatattcg tttttctcca gatttcacat ttggatttga agaaagcctc 600
gaagttgata cgaaccctct tttaggcgcg ggaaaattcg cgacggaccc agcggtgacc 660
ttggcacatg aacttattca tgccgggcat cgcttgtatg gaatcgccat taacccgaac 720
cgtgttttca aggtgaatac gaacgcgtat tacgagatgt cgggcttaga agtgtccttt 780
gaagaactgc gcacgtttgg cggtcatgat gcaaaattta ttgatagtct gcaagaaaac 840
gaatttcggc tgtactatta caataaattc aaagacattg catcaacctt aaacaaggcg 900
aaaagcattg tgggtaccac ggctagctta caatatatga aaaacgtttt caaagaaaaa 960
tacctcctta gcgaagacac ttccggcaaa ttctctgtcg ataaactgaa atttgataaa 1020
ctgtataaaa tgctcaccga gatctacaca gaggataact ttgtcaaatt cttcaaggtc 1080
ttgaatcgga aaacctatct gaacttcgat aaagccgtct ttaagatcaa catcgtaccg 1140
aaagttaact acaccatcta tgatggcttt aatctgcgca atacgaatct ggcggcgaac 1200
tttaacggcc agaacaccga aatcaacaac atgaacttta ctaaactgaa aaattttacc 1260
ggcttgtttg aattctataa gctcctgtgt gtccgcggta ttatcaccag caaaggcggt 1320
ggttctggcg gtggtgaaaa cctgtacttc cagggcggtg gctccggtgg tggtgcgctc 1380
aatgatttat gcatcaaggt gaacaactgg gacttgtttt tctctccatc tgaagataat 1440
tttactaacg acttgaacaa aggagaggaa attacttccg ataccaacat cgaagcagcg 1500
gaagagaata ttagtctaga tcttattcaa caatattacc tgacctttaa ttttgataac 1560
gagcctgaga acatttccat tgagaatctc agctctgaca tcatcggcca gctggaactg 1620
atgccgaata tcgaacgctt tcctaatgga aagaaatatg aattggacaa atacaccatg 1680
ttccactatc tccgcgcgca ggagtttgag cacggcaagt ctcgtattgc tctgaccaat 1740
tcggtaaacg aagccctttt aaatccttcg cgtgtgtaca ccttttttctc aagcgattat 1800
gttaaaaaag tgaacaaggc gaccgaagcg gcgatgtttt tgggatgggt ggaacaactg 1860
gtatatgact ttacggatga aacttctgaa gtctcgacca ccgacaaaat tgccgatatt 1920
accattatca ttccctatat tggccctgca ctgaacattg gtaacatgct gtataaagat 1980
gattttgtgg gcgccctgat cttttcaggc gctgttatcc tgctggaatt tatcccggaa 2040
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actgtccaga cgatcgacaa cgcgctcagt aaacgtaacg aaaaatggga tgaggtgtat 2160
aagtatattg ttaccaactg gctcgctaaa gtaaacaccc agattgacct gattcgcaag 2220
aagatgaaag aagcgctgga aaaccaagca gaagcgacca aagctattat caactatcaa 2280
tataaccagt acacagagga agaaaagaat aacatcaact tcaacatcga cgacttatct 2340
tcaaagctga atgaatctat taacaaagcg atgattaata ttaacaagtt cttgaaccaa 2400
tgtagtgtca gctatctgat gaactcgatg atcccttacg gtgtgaaacg tctggaagac 2460
ttcgatgcaa gccttaaaga tgcccttctg aagtatattt acgataatcg cggaactctt 2520
attggccaag tggatcgctt aaaagataaa gtcaacaaca cgctgagtac agacatccct 2580
tttcagctgt ctaaatatgt ggacaatcag cgcctgctgt ccacgtttac ggaatacatc 2640
aaaaacattg agggccgtat caacactagt attctgatct aaccatctga ttgatctgag 2700
ccgttacgca tctaaaatca acatcggatc caaggtgaac ttcgatccta tcgacaaaaa 2760
ccagattcaa ttgttcaact tagaatcgtc aaagattgaa gttatcttaa aaaatgcgat 2820
tgtatataat tcaatgtacg aaaatttctc tacgagcttt tggattcgta ttccgaaata 2880
tttcaacagt atctctttaa acaacgagta tactatcatc aattgtatgg agaataacag 2940
cgggtggaaa gtgagcctta actatggtga aatcatctgg actctgcagg acactcaaga 3000
aattaaacaa cgcgtggtgt ttaaatactc acagatgatt aacatctcgg attatattaa 3060
tcgctggatt tttgtgacaa ttactaacaa ccggctgaac aacagcaaaa tttacattaa 3120
cggtcgcctg atcgatcaga aaccaatcag taatctcggt aacattcacg catcgaataa 3180
tatcatgttc aaactggatg gttgtcgcga cacgcaccgt tacatttgga tcaaatactt 3240
caatttattc gacaaagaac tcaacgaaaa ggagattaag gatctttatg acaatcagtc 3300
taattcgggt attctgaaag acttttgggg tgattacctt cagtacgata aaccgtatta 3360
tatgttaaac ttatatgatc cgaataaata cgttgacgtc aacaacgttg gcattcgcgg 3420
ctatatgtat ctgaaagggc cgcgtggcag cgtgatgacc actaacattt acttaaactc 3480
ctccctctat cgcggtacta aatttattat caagaaatat gcctctggca acaaggacaa 3540
tatcgtacgc aataacgatc gcgtctacat taacgtggtg gtgaagaata aagaatatcg 3600
tctggcgacc aatgctagtc aggcgggcgt ggagaaaatt ctgtctgcac ttgaaatccc 3660
ggatgtgggt aatttatccc aggtggttgt gatgaaaagt aaaaatgacc aagggatcac 3720
caataaatgc aaaatgaatc tgcaagataa caacggcaac gacattggtt ttatcggctt 3780
ccaccaattc aataatatcg cgaagcttgt ggcctcaaat tggtacaacc gtcagattga 3840

gcgcagctcc cgcactttag gctgtagctg ggagttcatt ccggtagatg acggttgggg 3900
agaacgccca ttgtaa 3916

<210> 537
<211> 1309
<212> PRT
<213> Artificial Sequence

<220>
<223> BoNT/A comprising Fxa (N872)

<400> 537
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Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
            20   25   30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
        35   40   45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
    50   55   60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65   70   75   80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85   90   95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
            100   105   110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
        115   120   125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
    130   135   140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145   150   155   160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165   170   175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180   185   190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195   200   205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210   215   220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225   230   235   240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245   250   255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260   265   270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275   280   285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290   295   300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305   310   315   320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325   330   335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340   345   350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355   360   365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370   375   380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385   390   395   400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu

405 410 415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430
Gly Ile Ile Thr Ser Lys Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu
435 440 445
Tyr Phe Gln Gly Gly Gly Ser Gly Gly Gly Ala Leu Asn Asp Leu Cys
450 455 460
Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
465 470 475 480
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
485 490 495
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
500 505 510
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
515 520 525
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
530 535 540
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
545 550 555 560
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
565 570 575
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
580 585 590
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
595 600 605
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
610 615 620
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
625 630 635 640
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
645 650 655
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
660 665 670
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
675 680 685
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
690 695 700
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
705 710 715 720
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
725 730 735
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
740 745 750
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
755 760 765
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
770 775 780
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
785 790 795 800
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
805 810 815
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
820 825 830
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
835 840 845
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
850 855 860
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
865 870 875 880
Lys Asn Ile Glu Gly Arg Ile Asn Thr Ser Ile Leu Asn Leu Arg Tyr
885 890 895
Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile Asn
900 905 910

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Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile Gln
        915                 920                 925
Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn Ala
    930                 935                 940
Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile
945                 950                 955                 960
Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr Thr
                965                 970                 975
Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn
            980                 985                 990
Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys Gln
        995                 1000                1005
Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile
    1010                1015                1020
Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser
1025                1030                1035                1040
Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn
                1045                1050                1055
Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly
            1060                1065                1070
Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe
        1075                1080                1085
Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln
    1090                1095                1100
Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr
1105                1110                1115                1120
Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val
                1125                1130                1135
Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro
            1140                1145                1150
Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr
        1155                1160                1165
Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp
    1170                1175                1180
Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys
1185                1190                1195                1200
Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu
                1205                1210                1215
Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln
            1220                1225                1230
Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys
        1235                1240                1245
Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly
    1250                1255                1260
Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr
1265                1270                1275                1280
Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu
                1285                1290                1295
Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
            1300                1305
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