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.