fashion is the spread of toxin away from the administration site and into surrounding tissue or systemic circulation. The diffusion of toxin away from the target tissue is believed to be responsible for undesirable side effects that in extreme cases may be life threatening. This can be a particular concern when using clostridial toxin therapeutics (such as BoNT therapeutics) at high doses, concentrations and injection volumes. Adverse effects associated with this problem that have been reported for commercial BoNT/A therapeutics include asthenia, generalised muscle weakness, diplopia, ptosis, dysphagia, dysphonia, dysarthria, urinary incontinence, and breathing difficulties. Swallowing and breathing difficulties can be life threatening and there have been reported deaths related to the spread of toxin effects.

There is therefore a need in the art for clostridial toxins which have properties of increased tissue retention at the site of administration, and which accordingly exhibit a reduction in diffusion away from the administration site, as compared to known clostridial toxins.

Summary

The present disclosure solves the above problem by providing engineered clostridial toxins, as specified in the claims.

In one aspect, the present invention refers to An engineered clostridial toxin, wherein said engineered clostridial toxin is a) a botulinum neurotoxin A (BoNT/A) corresponding to SEQ ID NO: 2 but comprising a modification of at least one amino acid selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN 954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032, ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083, ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274, and THR 1277; or b) a BoNT/B comprising a modification of at least one amino acid selected from: ASN 873, ASN 874, GLU 892, ASP 895, ASN 906, ASP 940, ASN 948, GLU 949, ASN 958, ASN 959, ASN 979, ASN 990, GLU 993, ASP 994, GLU 997, ASN 1012, ASN 1019, ASP 1030, ASP 1047, ASP 1049, GLU 1065, GLU 1072, GLN 1176, GLU 1189, GLU 1252, and ASN 1273; or c) a BoNT/C1 comprising a modification of at least one amino acid selected from: ASN 881, ASP 898, GLU 916, GLU 927, ASN 952, ASN 964, ASN 965, ASN 984, GLU 985, ASP 986, ASP 996, ASN 1000, GLU 1036, ASN 1041, ASP 1062, ASP 1064, GLU 1079, and ASP 1081; or d) a BoNT/D comprising a modification of at least one amino acid selected from: ASN 877, ASP 893, ASN 894, ASN 898, ASN 920, ASN 945, ASN 948, GLU 957, GLN 958, ASN 959, ASN 968, ASN 979, GLU 1030, ASP 1031, ASP 1033, GLU 1047, GLU 1051, ASN 1052, GLU 1066, and GLN 1122; or e) a BoNT/E comprising a modification of at least one amino acid selected from: ASN 859, ASP 860, ASN 892, ASP 893, ASP 904, ASP 909, ASN 928, ASN 932, ASN 934, ASN 935, GLU 936, ASP 945, ASN 946, ASN 947, ASN 966, ASN 976, ASN 979, ASN 981, ASP 985, GLN 1014, ASN 1019, ASN 1022, ASP 1027, ASN 1035, and ASN 1140; or f) a BoNT/F comprising a modification of at least one amino acid selected from: ASN 879, ASP 896, ASN 922, ASN 923, ASN 928, ASN 947, ASN 950, ASN 952, ASN 953, GLU 954, ASN 963, ASN 964, ASN 965, ASN 987, GLN 997, ASN 1037, ASP 1040, ASP 1045, ASN 1055, and ASP 1056; or g) a BoNT/G comprising a modification of at least one amino acid selected from: ASP 900, ASN 909, ASN 910, GLU 912, ASN 913, ASN 945, ASN 947, GLU 956, ASN 965, ASP 966, ASN 986, ASN 1001, ASN 1038, ASP 1040, ASN 1046, ASP 1057, GLU 1073, ASN 1075, and ASN 1090; or h) a tetanus neurotoxin (TeNT) comprising a modification of at least one amino acid selected from: ASN 893, ASP 894, ASP 911, ASN 919, ASN 927, ASN 928, GLU 929, GLN 968, ASN 972, GLU 973, GLU 1010, ASP 1018, ASN 1079, ASN 1080, ASN 1081, and ASN 1097; wherein said modification increases the calculated isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said modification; wherein said modification is located in the clostridial toxin receptor binding domain (H_C domain); wherein said engineered clostridial toxin demonstrates increased tissue retention, potency and/or duration of action when compared to an otherwise identical clostridial toxin lacking said modification; wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s); wherein said modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue; substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue. In one example, there is disclosed

an engineered clostridial toxin comprising at least one amino acid modification, wherein said at least one amino acid modification increases the calculated isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification; wherein said at least one amino acid modification is located in the clostridial toxin receptor binding domain (H_C domain); wherein said engineered clostridial toxin demonstrates increased tissue retention, potency and/or duration of action when compared to an otherwise identical clostridial toxin lacking said at least one amino acid modification; and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s); wherein said at least one amino acid modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue; substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue; and wherein the amino acid modified is: (i) a surface exposed amino acid; (ii) located outside of a clostridial toxin protein secondary structure; (iii) located in a clostridial toxin protein region that is non-essential for protein function; (iv) an amino acid whose identity is not conserved between clostridial toxin types, subtypes, or serotypes; and (v) an amino acid whose modification does not create a predicted ubiquitination site.

In another aspect, the present invention refers to Use of an engineered clostridial toxin receptor binding domain (HC domain) for increasing tissue retention, potency and/or duration of action of an engineered clostridial toxin comprising said engineered clostridial toxin receptor binding domain (HC domain), wherein said engineered domain (HC domain) comprises at least one amino acid modification which increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification, and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s); wherein said at least one amino acid modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue; substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue; and wherein the engineered clostridial toxin is a) a botulinum neurotoxin A (BoNT/A) comprising a modification of at least one amino acid selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN 954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032, ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083, ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274, and THR 1277; or b) a BoNT/B comprising a modification of at least one amino acid selected from: ASN 873, ASN 874, GLU 892, ASP 895, ASN 906, ASP 940, ASN 948, GLU 949, ASN 958, ASN 959, ASN 979, ASN 990, GLU 993, ASP 994, GLU 997, ASN 1012, ASN 1019, ASP 1030, ASP 1047, ASP 1049, GLU 1065, GLU 1072, GLN 1176, GLU 1189, GLU 1252, and ASN 1273; or c) a BoNT/C1 comprising a modification of at least one amino acid selected from: ASN 881, ASP 898, GLU 916, GLU 927, ASN 952, ASN 964, ASN 965, ASN 984, GLU 985, ASP 986, ASP 996, ASN 1000, GLU 1036, ASN 1041, ASP 1062, ASP 1064, GLU 1079, and ASP 1081; or d) a BoNT/D comprising a modification of at least one amino acid selected from: ASN 877, ASP 893, ASN 894, ASN 898, ASN 920, ASN 945, ASN 948, GLU 957, GLN 958, ASN 959, ASN 968, ASN 979, GLU 1030, ASP 1031, ASP 1033, GLU 1047, GLU 1051, ASN 1052, GLU 1066, and GLN 1122; or e) a BoNT/E comprising a modification of at least one amino acid selected from: ASN 859, ASP 860, ASN 892, ASP 893, ASP 904, ASP 909, ASN 928, ASN 932, ASN 934, ASN 935, GLU 936, ASP 945, ASN 946, ASN 947, ASN 966, ASN 976, ASN 979, ASN 981, ASP 985, GLN 1014, ASN 1019, ASN 1022, ASP 1027, ASN 1035, and ASN 1140; or f) a BoNT/F comprising a modification of at least one amino acid selected from: ASN 879, ASP 896, ASN 922, ASN 923, ASN 928, ASN 947, ASN 950, ASN 952, ASN 953, GLU 954, ASN 963, ASN 964, ASN 965, ASN 987, GLN 997, ASN 1037, ASP 1040, ASP 1045, ASN 1055, and ASP 1056; or g) a BoNT/G comprising a modification of at least one amino acid selected from: ASP 900, ASN 909, ASN 910, GLU 912, ASN 913, ASN 945, ASN 947, GLU 956, ASN 965, ASP 966, ASN 986, ASN 1001, ASN 1038, ASP 1040, ASN 1046, ASP 1057, GLU 1073, ASN 1075, and ASN 1090; or h) a tetanus neurotoxin (TeNT) comprising a modification of at least one amino acid selected from: ASN 893, ASP 894, ASP 911, ASN 919, ASN 927, ASN 928, GLU 929, GLN 968, ASN 972, GLU 973, GLU 1010, ASP 1018, ASN 1079, ASN 1080, ASN 1081, and ASN 1097. In one example, there is disclosed the use of an engineered clostridial toxin receptor binding domain (H_C domain) for increasing tissue retention, potency and/or duration of action of an engineered clostridial toxin comprising said engineered clostridial toxin receptor binding domain (HC domain), wherein said engineered domain

(HC domain) comprises at least one amino acid modification which increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification, and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s); wherein said at least one amino acid modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue; substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue; and wherein the amino acid modified is: (i) a surface exposed amino acid; (ii) located outside of a clostridial toxin protein secondary structure; (iii) located in a clostridial toxin protein region that is non-essential for protein function; (iv) an amino acid whose identity is not conserved between clostridial toxin types, subtypes, or serotypes; and (v) an amino acid whose modification does not create a predicted ubiquitination site.

In another aspect, the present disclosure provides a nucleic acid comprising a nucleic acid sequence encoding an engineered clostridial toxin as disclosed above.

In another aspect, the present disclosure provides a method of producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain, the method comprising expressing a nucleic acid as disclosed above in a suitable host cell, lysing the host cell to provide a host cell homogenate containing the single-chain engineered clostridial toxin protein, and isolating the single-chain engineered clostridial toxin protein.

In another aspect, the present invention refers to a method of activating an engineered clostridial toxin, the method comprising - producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain as disclosed herein, - contacting the single-chain engineered clostridial toxin protein with a protease that cleaves the single-chain engineered clostridial toxin protein at a recognition site (cleavage site) located between the light chain and heavy chain, and - converting the single-chain engineered clostridial toxin protein into a di-chain polypeptide wherein the light chain and heavy chain are joined together by a disulphide bond. In another example, there is disclosed a method of activating an engineered clostridial toxin, the method comprising producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain according to the method as disclosed above, contacting the polypeptide with a protease that cleaves the polypeptide at a recognition site (cleavage site) located between the light chain and heavy chain, and converting the polypeptide into a di-chain polypeptide wherein the light chain and heavy chain are joined together by a disulphide bond.

In another aspect, the present disclosure provides an engineered clostridial toxin as disclosed above, for use in medicine.

In another aspect, the present disclosure provides use of an engineered clostridial toxin as disclosed above in the manufacture of a medicament for the prevention or treatment of a disease or condition selected from: strabismus, blepharospasm, squint, dystonia, torticollis, neuromuscular disorder or condition of ocular motility, writer's cramp, blepharospasm, bruxism, Wilson's disease, tremor, tics, segmental myoclonus, spasms, spasticity due to chronic multiple sclerosis, spasticity resulting in abnormal bladder control, back spasm, charley horse, tension headaches, levator pelvic syndrome, spina bifida, tardive dyskinesia, Parkinson's disease, stuttering, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain, headache pain, cancer, uterine disorders, uro-genital disorders, urogenital-neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

In another aspect, there is provided a method for cosmetic treatment, wherein said cosmetic treatment benefits from cell/muscle incapacitation, said method comprising administration of an engineered clostridial toxin as disclosed above. In a further aspect, there is provided a pharmaceutical

composition comprising: an engineered clostridial toxin as described herein, and at least one component selected from a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and/or salt.

Detailed Description

The disclosure provides an engineered clostridial toxin comprising at least one amino acid modification, wherein said at least one amino acid modification increases the isoelectric point (pi) of the engineered clostridial toxin to a value that is at least 0.2 (for example, at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1) pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.4 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.5 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.6 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at

CLAIMS

1. An engineered clostridial toxin, wherein said engineered clostridial toxin is:

a) a botulinum neurotoxin A (BoNT/A) corresponding to SEQ ID NO: 2 but comprising a modification of at least one amino acid selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN 954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032, ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083, ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274, and THR 1277; or b) a BoNT/B comprising a modification of at least one amino acid selected from: ASN 873, ASN 874, GLU 892, ASP 895, ASN 906, ASP 940, ASN 948, GLU 949, ASN 958, ASN 959, ASN 979, ASN 990, GLU 993, ASP 994, GLU 997, ASN 1012, ASN 1019, ASP 1030, ASP 1047, ASP 1049, GLU 1065, GLU 1072, GLN 1176, GLU 1189, GLU 1252, and ASN 1273; or

c) a BoNT/C1 comprising a modification of at least one amino acid selected from: ASN 881, ASP 898, GLU 916, GLU 927, ASN 952, ASN 964, ASN 965, ASN 984, GLU 985, ASP 986, ASP 996, ASN 1000, GLU 1036, ASN 1041, ASP 1062, ASP 1064, GLU 1079, and ASP 1081; or

d) a BoNT/D comprising a modification of at least one amino acid selected from: ASN 877, ASP 893, ASN 894, ASN 898, ASN 920, ASN 945, ASN 948, GLU 957, GLN 958, ASN 959, ASN 968, ASN 979, GLU 1030, ASP 1031, ASP 1033, GLU 1047, GLU 1051, ASN 1052, GLU 1066, and GLN 1122; or

e) a BoNT/E comprising a modification of at least one amino acid selected from: ASN 859, ASP 860, ASN 892, ASP 893, ASP 904, ASP 909, ASN 928, ASN 932, ASN 934, ASN 935, GLU 936, ASP 945, ASN 946, ASN 947, ASN 966, ASN 976, ASN 979, ASN 981, ASP 985, GLN 1014, ASN 1019, ASN 1022, ASP 1027, ASN 1035, and ASN 1140; or

f) a BoNT/F comprising a modification of at least one amino acid selected from: ASN 879, ASP 896, ASN 922, ASN 923, ASN 928, ASN 947, ASN 950, ASN 952, ASN 953, GLU 954, ASN 963, ASN 964, ASN 965, ASN 987, GLN 997, ASN 1037, ASP 1040, ASP 1045, ASN 1055, and ASP 1056; or

g) a BoNT/G comprising a modification of at least one amino acid selected from: ASP 900, ASN 909, ASN 910, GLU 912, ASN 913, ASN 945, ASN 947, GLU

956, ASN 965, ASP 966, ASN 986, ASN 1001, ASN 1038, ASP 1040, ASN 1046, ASP 1057, GLU 1073, ASN 1075, and ASN 1090; or

h) a tetanus neurotoxin (TeNT) comprising a modification of at least one amino acid selected from: ASN 893, ASP 894, ASP 911, ASN 919, ASN 927, ASN 928, GLU 929, GLN 968, ASN 972, GLU 973, GLU 1010, ASP 1018, ASN 1079, ASN 1080, ASN 1081, and ASN 1097;

wherein said modification increases the calculated isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said modification;

wherein said modification is located in the clostridial toxin receptor binding domain (H_C domain);

wherein said engineered clostridial toxin demonstrates increased tissue retention, potency and/or duration of action when compared to an otherwise identical clostridial toxin lacking said modification;

wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s);

wherein said modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue; substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue.

2. Use of an engineered clostridial toxin receptor binding domain (H_C domain) for increasing tissue retention, potency and/or duration of action of an engineered clostridial toxin comprising said engineered clostridial toxin receptor binding domain (H_C domain), wherein said engineered domain (HC domain) comprises at least one amino acid modification which increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.4 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification, and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s);

wherein said at least one amino acid modification is selected from: substitution of an acidic amino acid residue with a basic amino acid residue; substitution of an acidic amino acid residue with an uncharged amino acid residue;

substitution of an uncharged amino acid residue with a basic amino acid residue; insertion of a basic amino acid residue; and deletion of an acidic amino acid residue;

and

wherein the engineered clostridial toxin is:

a) a botulinum neurotoxin A (BoNT/A) comprising a modification of at least one amino acid selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN 954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032, ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083, ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274, and THR 1277; or

b) a BoNT/B comprising a modification of at least one amino acid selected from: ASN 873, ASN 874, GLU 892, ASP 895, ASN 906, ASP 940, ASN 948, GLU 949, ASN 958, ASN 959, ASN 979, ASN 990, GLU 993, ASP 994, GLU 997, ASN 1012, ASN 1019, ASP 1030, ASP 1047, ASP 1049, GLU 1065, GLU 1072, GLN 1176, GLU 1189, GLU 1252, and ASN 1273; or

c) a BoNT/C1 comprising a modification of at least one amino acid selected from: ASN 881, ASP 898, GLU 916, GLU 927, ASN 952, ASN 964, ASN 965, ASN 984, GLU 985, ASP 986, ASP 996, ASN 1000, GLU 1036, ASN 1041, ASP 1062, ASP 1064, GLU 1079, and ASP 1081; or

d) a BoNT/D comprising a modification of at least one amino acid selected from: ASN 877, ASP 893, ASN 894, ASN 898, ASN 920, ASN 945, ASN 948, GLU 957, GLN 958, ASN 959, ASN 968, ASN 979, GLU 1030, ASP 1031, ASP 1033, GLU 1047, GLU 1051, ASN 1052, GLU 1066, and GLN 1122; or

e) a BoNT/E comprising a modification of at least one amino acid selected from: ASN 859, ASP 860, ASN 892, ASP 893, ASP 904, ASP 909, ASN 928, ASN 932, ASN 934, ASN 935, GLU 936, ASP 945, ASN 946, ASN 947, ASN 966, ASN 976, ASN 979, ASN 981, ASP 985, GLN 1014, ASN 1019, ASN 1022, ASP 1027, ASN 1035, and ASN 1140; or

f) a BoNT/F comprising a modification of at least one amino acid selected from: ASN 879, ASP 896, ASN 922, ASN 923, ASN 928, ASN 947, ASN 950, ASN 952, ASN 953, GLU 954, ASN 963, ASN 964, ASN 965, ASN 987, GLN 997, ASN 1037, ASP 1040, ASP 1045, ASN 1055, and ASP 1056; or

g) a BoNT/G comprising a modification of at least one amino acid selected from: ASP 900, ASN 909, ASN 910, GLU 912, ASN 913, ASN 945, ASN 947, GLU

956, ASN 965, ASP 966, ASN 986, ASN 1001, ASN 1038, ASP 1040, ASN 1046, ASP 1057, GLU 1073, ASN 1075, and ASN 1090; or

h) a tetanus neurotoxin (TeNT) comprising a modification of at least one amino acid selected from: ASN 893, ASP 894, ASP 911, ASN 919, ASN 927, ASN 928, GLU 929, GLN 968, ASN 972, GLU 973, GLU 1010, ASP 1018, ASN 1079, ASN 1080, ASN 1081, and ASN 1097.

3. The engineered clostridial toxin of claim 1 or the use according to claim 2, wherein the engineered clostridial toxin has a Safety Ratio of at least 8, wherein the Safety Ratio is calculated as: dose of toxin required for -10% bodyweight change measured as pg/mouse divided by DAS ED_{50} measured as pg/mouse, wherein ED_{50} = dose required to produce a DAS score of 2.

4. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.5 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

5. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least one pI unit higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

6. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least two pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

7. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is between 2

and 5 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

8. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin has a pI of at least 6.

9. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin has a pI of between 6 and 10.

10. The engineered clostridial toxin or the use according to claim 9, wherein the engineered clostridial toxin has a pI of between 7 and 9, preferably between 8 and 9.

11. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin comprises between 1 and 80 amino acid modifications.

12. The engineered clostridial toxin or the use according to claim 11, wherein the engineered clostridial toxin comprises between 4 and 40 amino acid modifications.

13. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification is located in the clostridial toxin H_{CN} domain.

14. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification comprises modification of an amino acid residue selected from: an aspartic acid residue, a glutamic acid residue, a histidine residue, a serine residue, a threonine residue, an asparagine residue, a glutamine residue, a cysteine residue, or a tyrosine residue.

15. The engineered clostridial toxin or the use according to claim 14, wherein the amino acid residue is substituted with a lysine residue or an arginine residue.

16. A nucleic acid comprising a nucleic acid sequence encoding an engineered clostridial toxin according to any one of claims 1 and 3 to 15.

17. A method of producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain, the method comprising expressing a nucleic acid according to claim 16 in a suitable host cell, lysing the host cell to provide a host cell homogenate containing the single-chain engineered clostridial toxin protein, and isolating the single-chain engineered clostridial toxin protein.

18. A method of activating an engineered clostridial toxin, the method comprising
- producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain according to the method of claim 17,
- contacting the single-chain engineered clostridial toxin protein with a protease that cleaves the single-chain engineered clostridial toxin protein at a recognition site (cleavage site) located between the light chain and heavy chain, and
- converting the single-chain engineered clostridial toxin protein into a di-chain polypeptide wherein the light chain and heavy chain are joined together by a disulphide bond.

19. An engineered clostridial toxin according to any one of claims 1 and 3-15, for use in medicine.

20. Use of an engineered clostridial toxin according to any one of claims 1 and 3-15 in the manufacture of a medicament for the prevention or treatment of a disease or condition selected from: strabismus, blepharospasm, squint, dystonia, torticollis, neuromuscular disorder or condition of ocular motility, writer's cramp, blepharospasm, bruxism, Wilson's disease, tremor, tics, segmental myoclonus, spasms, spasticity due to chronic multiple sclerosis, spasticity resulting in abnormal bladder control, back spasm, charley horse, tension headaches, levator pelvic syndrome, spina bifida, tardive dyskinesia, Parkinson's disease, stuttering, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain, headache pain, cancer, uterine disorders, uro-genital disorders, urogenital-

neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

21. Use according to claim 20, wherein said dystonia is selected from: spasmodic dystonia, oromandibular dystonia, focal dystonia, tardive dystonia, laryngeal dystonia, limb dystonia and cervical dystonia.

22. Use according to claim 20, wherein said torticollis a spasmodic torticollis.

23. Use according to claim 20, wherein said neuromuscular disorder or condition of ocular motility is selected from: concomitant strabismus, vertical strabismus, lateral rectus palsy, nystagmus, dysthyroid myopathy.

24. Use according to claim 20, wherein said muscle pain is pain from muscle spasms.

25. Use according to claim 20, wherein said headache pain is tension headache.

26. A method for cosmetic treatment, wherein said cosmetic treatment benefits from cell/muscle incapacitation, said method comprising administration of an engineered clostridial toxin according to any one of claims 1 and 3-15.

27. A method according to claim 26, wherein the cosmetic treatment is the treatment of brow furrows or skin wrinkles.

28. A pharmaceutical composition comprising:
- an engineered clostridial toxin according to any one of claims 1 and 3 to 15, and
- at least one component selected from a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and/or salt.

29. The pharmaceutical composition according to claim 28, wherein said composition is suitable for injection.

30. The pharmaceutical composition according to claim 29, wherein said composition is in the form of a solution, or of a dry powder.