CLAIMS

1. A method for proteolytically processing a single-chain engineered clostridial neurotoxin into a corresponding di-chain engineered clostridial neurotoxin, the method comprising:

   a. providing the single-chain engineered clostridial neurotoxin in which an endogenous activation loop has been replaced by an exogenous activation loop; and

   b. contacting the single-chain engineered clostridial neurotoxin with enterokinase or factor Xa;

   wherein the single-chain engineered clostridial neurotoxin has an activation loop comprising the polypeptide sequence Cys-$(Xaa)_a$-Ile-Asp/Glu-Gly-Arg-$(Yaa)_b$-Cys (SEQ ID NO: 1), wherein a = 1-10 and b = 4-15, and wherein Xaa is any amino acid and Yaa is any amino acid;

   wherein the engineered clostridial neurotoxin is not botulinum neurotoxin C1 (BoNT/C1); and

   wherein enterokinase or factor Xa hydrolyses a peptide bond of the activation loop thereby producing the di-chain engineered clostridial neurotoxin.

2. A method for manufacturing an engineered clostridial neurotoxin, the method comprising:

   a. identifying an endogenous activation loop of a clostridial neurotoxin, wherein the clostridial neurotoxin is characterised in that:

      i. a peptide bond outside of the endogenous activation loop of the clostridial neurotoxin is hydrolysed by trypsin or Lys-C; and/or

      ii. the endogenous activation loop is inefficiently proteolytically processed by trypsin or Lys-C; and

   b. replacing the endogenous activation loop with an exogenous activation loop thereby providing an engineered clostridial neurotoxin, wherein the exogenous activation loop comprises the polypeptide sequence Cys-$(Xaa)_a$-Ile-Asp/Glu-Gly-Arg-$(Yaa)_b$-Cys (SEQ ID NO: 1), wherein a = 1-10 and b = 4-15, and wherein Xaa is any amino acid and Yaa is any amino acid;

   wherein the engineered clostridial neurotoxin is not botulinum neurotoxin C1 (BoNT/C1).

3. The method according to claim 2, wherein a clostridial neurotoxin is identified as being suitable for use in the method by contacting the clostridial neurotoxin with trypsin or Lys-C

and confirming hydrolysis of a peptide bond outside of the endogenous activation loop of the clostridial neurotoxin.

4. The method according to claim 2 or 3, further comprising contacting the engineered clostridial neurotoxin with enterokinase or factor Xa thereby producing a corresponding di-chain engineered clostridial neurotoxin.

5. An engineered clostridial neurotoxin, wherein an endogenous activation loop of a clostridial neurotoxin has been replaced by an exogenous activation loop thereby providing an engineered clostridial neurotoxin,

wherein the exogenous activation loop comprises the polypeptide sequence Cys-$(Xaa)_a$-Ile-Asp/Glu-Gly-Arg-$(Yaa)_b$-Cys (SEQ ID NO: 1), wherein a = 1-10 and b = 4-15, and wherein Xaa is any amino acid and Yaa is any amino acid,

wherein the clostridial neurotoxin is characterised in that: a peptide bond outside of the endogenous activation loop of the clostridial neurotoxin is hydrolysed by trypsin or Lys-C; and/or the endogenous activation loop is inefficiently proteolytically processed by trypsin or Lys-C, and

wherein the engineered clostridial neurotoxin is not botulinum neurotoxin C1 (BoNT/C1).

6. The method or engineered clostridial neurotoxin according to any one of claims 2-5, wherein the endogenous activation loop is one or more selected from SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, 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 or SEQ ID NO: 71.

7. The method or engineered clostridial neurotoxin according to any one of the preceding claims, wherein:

a. a = 2-4;
b. a = 3;
c. b = 6-10;
d. b = 8;
e. the (exogenous) activation loop comprises a polypeptide sequence having at least 70%, 80% or 90% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 3;

f. the (exogenous) activation loop comprises a polypeptide sequence having SEQ ID NO: 2 or SEQ ID NO: 3;
g. the (exogenous) activation loop comprises SEQ ID NO: 2; and/or
h. the (exogenous) activation loop consists of SEQ ID NO: 2.

8. The method or engineered clostridial neurotoxin according to any one of the preceding claims, wherein:
a. the clostridial neurotoxin lacks a functional H_C domain of a clostridial neurotoxin;
b. the clostridial neurotoxin is a retargeted clostridial neurotoxin comprising a non-clostridial Targeting Moiety (TM);
c. the clostridial neurotoxin is a re-targeted clostridial neurotoxin comprising a targeting moiety (TM), wherein the TM comprises an anthrax toxin protective antigen (PA) or a fragment thereof; and/or
d. the clostridial neurotoxin has a polypeptide sequence with at least 70% sequence identity to a polypeptide comprising:
i. SEQ ID NO: 52 or a fragment thereof; and
ii. 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 or SEQ ID NO: 60.

9. The method or engineered clostridial neurotoxin according to any one of claims 1-7, wherein:
a. the clostridial neurotoxin is selected from: BoNT/A, BoNT/B, BoNT/D, BoNT/E, BoNT/F, BoNT/G, BoNT/X, and TeNT;
b. the clostridial neurotoxin is BoNT/X, BoNT/E, a chimeric BoNT or a hybrid BoNT; and/or
c. the clostridial neurotoxin:
i. is encoded by a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 or SEQ ID NO: 12; or
ii. comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13.

10. A method for proteolytically processing an engineered clostridial neurotoxin according to any one of claims 4-9 into a corresponding di-chain engineered clostridial neurotoxin,

the method comprising contacting the engineered clostridial neurotoxin with enterokinase or factor Xa thereby producing the di-chain engineered clostridial neurotoxin.

11. A di-chain engineered clostridial neurotoxin obtainable by the method according to any one of claims 1, 4 or 6-10.

12. A pharmaceutical composition comprising the engineered clostridial neurotoxin according to any one of claims 5-9 or the di-chain engineered clostridial neurotoxin according to claim 11, and a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and/or salt.

13. Use of the engineered clostridial neurotoxin according to any one of claims 5-9, the di-chain engineered clostridial neurotoxin according to claim 11 or the pharmaceutical composition according to claim 12 in the manufacture of a medicament for the treatment of one or more of: a condition associated with unwanted immune secretion, strabismus, blepharospasm, squint, dystonia (e.g. spasmodic dystonia, oromandibular dystonia, focal dystonia, tardive dystonia, laryngeal dystonia, limb dystonia, cervical dystonia), torticollis (e.g. spasmodic torticollis), neuromuscular disorder or condition of ocular motility (e.g. concomitant strabismus, vertical strabismus, lateral rectus palsy, nystagmus, dysthyroid myopathy), 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, animus, 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, tics, tremors, bruxism, anal fissure, achalasia, dysphagia, lacrimation, hyperhidrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain (e.g. pain from muscle spasms), headache pain (e.g. tension headache), cancer, uterine disorders, uro-genital disorders, urogenital-neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

14. Use of enterokinase for hydrolysing a peptide bond of a polypeptide comprising a polypeptide sequence shown as SEQ ID NO: 18 or SEQ ID NO: 19.

15. A nucleotide sequence encoding an engineered clostridial neurotoxin comprising a sequence having at least 80% sequence identity to SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 or SEQ ID NO: 12.

16. Use of the engineered clostridial neurotoxin according to any one of claims 5-9, the di-chain engineered clostridial neurotoxin according to claim 11 or the pharmaceutical composition according to claim 12 in the cosmetic treatment of one or more of: beauty therapy (cosmetic) applications benefiting from cell/muscle incapacitation (via SNARE down-regulation or inactivation), brow furrows, and skin wrinkles.