CLAIMS

1. A molecule comprising a non-toxic non-hemagglutinin (NTNHA) polypeptide covalently linked to a heterologous affinity moiety for use in isolation and purification of a botulinum neurotoxin (BoNT) protein, or a polypeptide comprising a receptor binding domain of BoNT, from a solution, in which:

the molecule is further bound to a binding target through the affinity moiety; and

the NTNHA polypeptide is in a complex with a compatible Botulinum neurotoxin (BoNT) or a polypeptide comprising a receptor binding domain thereof.

2. A molecule according to Claim 1, in which the complex is intact in solution at pH 6 and disrupted in solution at pH 8.

3. A molecule according to Claim 1, in which the compatible BoNT, or a polypeptide comprising a receptor binding domain of BoNT, can be activated upon exposure to a protease while remaining in complex with the NTNHA polypeptide.

4. A molecule according to Claim 1, in which the NTNHA and the affinity moiety are expressed as a fusion protein.

5. A molecule according to any preceding claim, in which:

(i) the affinity moiety is located at a position selected from the group consisting of the N-terminus of NTNHA amino acid sequence, the C-terminus of NTNHA amino acid sequence, and internal to the NTNHA amino acid sequence;

(ii) the affinity moiety effectively binds a binding target under conditions of about pH 6 to about pH 8; and/or

(iii) the affinity moiety is selected from the group consisting of glutathione -S-transferase (GST), C-myc tag, Chitin-binding domain, Streptavidin binding protein (SBP), Cellulose-binding domain, Calmodulin-binding peptide, S-tag, Strep-tag II, FLA, Protein A, Protein G, histidine affinity tag (HAT), Poly-His, and maltose binding protein (MBP).

6. A molecule according to any preceding claim, in which:

(i) the NTNHA is from serotype B, A, C1, D, E, F, or G; and/or

(ii) the NTNHA is from serotype B.

7. A molecule according to any preceding claim, in which the binding target is stably attached to a matrix.

8. An aqueous solution comprising a molecule according to any preceding claim.

9. A host cell that comprises a nucleic acid encoding a functional NTNHA and affinity moiety fusion protein according to any of Claims 4 to 7 and that further expresses a compatible Botulinum neurotoxin (BoNT); which cell

(i) is prokaryotic or eukaryotic; or

(ii) is a bacterial cell, a yeast cell, a mammalian cell, an insect cell, a plant cell, or an amphibian cell.

10. A method of purifying Botulinum neurotoxin (BoNT), in which the BoNT is in solution, comprising the steps of:

(a) providing a molecule comprising a compatible non-toxic non-hemagglutinin (NTNHA) polypeptide covalently linked to a heterologous affinity moiety, in which the molecule is attached to a matrix via the affinity moiety;

(b) contacting a solution comprising BoNT with the molecule under conditions appropriate for binding of the NTNHA to the BoNT to thereby form a NTNHA-BoNT complex;

(c) washing the matrix to thereby remove unbound materials; and

(d) eluting the BoNT from the matrix by contacting the matrix with an aqueous solution that dissociates the BoNT from the NTNHA-BoNT complex.

11. A method of purifying Botulinum neurotoxin (BoNT), in which the BoNT is in solution, comprising the steps of:

(a) providing a molecule comprising a compatible non-toxic non-hemagglutinin (NTNHA) polypeptide covalently linked to a heterologous affinity moiety, in which the molecule is attached to a matrix via the affinity moiety;

(b) contacting a solution comprising BoNT with the molecule under conditions appropriate for binding of the NTNHA to the BoNT to thereby form a NTNHA-BoNT complex;

(c) washing the matrix to thereby remove unbound materials;

(d) contacting the matrix with a protease under conditions that preserve the NTNHA-BoNT complex and are appropriate for cleavage of the BoNT within the NTNHA-BoNT complex;

(e) washing the matrix to thereby remove the protease and unbound materials; and

(f) eluting the cleaved BoNT from the matrix by contacting the matrix with an aqueous solution that dissociates the cleaved BoNT from the NTNHA-BoNT complex.

12. A method according to Claim 10 or 11, in which the matrix is linked to a binding target of the affinity moiety, and the molecule is non-covalently bound to the matrix through interactions of the affinity moiety and the binding target.

13. A method according to Claim 10, 11 or 12, in which the aqueous solution has a pH of ≥ 7.5.

14. A method according to any of Claims 10 to 13, in which:

(i) the solution comprising the BoNT is a cleared cell extract from BoNT expressing cells; or

(ii) the solution comprising the BoNT is a cleared cell extract from BoNT expressing cells and the cleared cell extract further comprises 1 mM of phenylmethylsulfonyl fluoride (PMSF).

15. A method according to any of Claims 10 to 14, in which the conditions appropriate for binding comprise contacting the BoNT in the context of a binding buffer which has a physiological ionic strength and a pH of < 7.5.

16. A method according to any of Claims 10 to 15, in which the washing is with a wash buffer that is of physiological ionic strength with a pH of < 7.5.

17. A method according to Claim 15 or 16, in which:

(i) the binding buffer and/or wash buffer is between 100-200 mM KCl or NaCl;

(ii) the binding buffer and/or wash buffer has a pH of about 6;

(iii) the binding buffer and/or wash buffer comprises 50 mM MES, 150 mM NaCl, pH 6;

(iv) the aqueous solution is an elution buffer of about 50 mM Tris, 150 mM NaCl; and/or

(v) the aqueous solution is an elution buffer of about pH 8.

18. A method according to any of Claims 16 to 17, in which:

(i) the affinity moiety is selected from the group consisting of glutathione -S-transferase (GST), C-myc tag, Chitin-binding domain, Streptavidin binding protein (SBP), Cellulose-binding domain, Calmodulin-binding peptide, S-tag, Strep-tag II, FLA, Protein A, Protein G, histidine affinity tag (HAT), Poly-His, and maltose binding protein (MBP); and/or

(ii) the affinity moiety is GST, and the binding target is glutathione.

19. A method according to Claim 11 or any of Claims 12 to 18 as dependent on Claim 11, in which:

(i) the protease is trypsin or Lys-C endoproteinase;

(ii) the protease is added at a molar ratio of from about 1:2 to about 1:1000 to the NTNHA;

(iii) the protease is contacted to the matrix at room temperature; and/or

(iv) the protease is contacted to the matrix for about 10 minutes to 18 hours.

20. A method according to Claim 10, in which:

the molecule is attached to a glutathione coated matrix and the affinity moiety is glutathione-S-transferase;

step (a) of contacting comprises contacting a cleared cell extract comprising the BoNT to the matrix in a binding buffer with a pH of about 6 to thereby form the NTNHA-BoNT complex;

step (b) of washing comprises washing the matrix with a wash buffer with a pH of about 6 to thereby remove unbound materials; and

step (c) of eluting comprises eluting the BoNT from the matrix by contacting the matrix with an elution buffer that has a pH of ≥ 7.5 to thereby dissociate the BoNT from the NTNHA-BoNT complex.

21. A method according to Claim 10, in which:

the molecule is attached to a glutathione coated matrix and the affinity moiety is glutathione-S-transferase;

step (a) of contacting comprises contacting a cleared cell extract comprising the BoNT to the matrix in a binding buffer with a pH of about 6 to thereby form a NTNHA-BoNT complex;

step (b) of washing comprises washing the matrix with a wash buffer with a pH of about 6 to thereby remove unbound materials; and

step (c) of eluting comprises each of:

(i) contacting the matrix with a protease in a buffer with a pH of about 6 to thereby cleave the BoNT within the NTNHA-BoNT complex;

(ii) washing the matrix with a wash buffer with a pH of about 6 to thereby remove the protease and unbound materials; and

(iii) eluting the BoNT from the matrix by contacting the matrix with an elution buffer that has a pH of ≥ 7.5 to thereby dissociate the BoNT from the NTNHA-BoNT complex.

22. A method according to any of Claims 20 or 21, in which:

(i) the binding buffer and/or wash buffer comprises 50 mM MES, 150 mM NaCl;

(ii) the binding buffer and/or wash buffer comprises 50 mM MES, 150 mM NaCl and the binding buffer further comprises 1 mM phenylmethylsulfonyl fluoride (PMSF);

(iii) the elution buffer comprises 50 mM Tris, 150 mM NaCl, and has a pH of about 8;

(iv) the glutathione coated matrix is glutathione-linked agarose beads or the glutathione coated matrix is a column;

(v) the glutathione coated matrix has about 5 mg/ml bound NTNHA; and/or

(vi) the protease is trypsin or Lys-C endoproteinase.

23. A method of purifying a polypeptide comprising a receptor binding domain (Hc polypeptide) of Botulinum neurotoxin, comprising the steps of:

(a) contacting a solution comprising the Hc polypeptide to a matrix which has attached thereto compatible non-toxic non-hemagglutinin (NTNHA), under conditions appropriate for binding of the NTNHA to the Hc-polypeptide to thereby form a NTNHA-Hc polypeptide complex;

(b) washing the matrix to thereby remove unbound materials;

(c) eluting the Hc polypeptide from the matrix by contacting the matrix with an aqueous solution that dissociates the Hc polypeptide from the NTNHA-Hc polypeptide complex.

24. A method according to Claim 23, in which the Hc polypeptide is a Botulinum neurotoxin (BoNT) polypeptide or the Hc polypeptide is a chimeric Botulinum neurotoxin (BoNT) polypeptide.

25. A molecule according to any of Claims 1 to 7, a host cell according to Claim 9, or a method according to any of Claims 12 to 24, in which the BoNT or the polypeptide comprises a modified receptor binding domain of Clostridial botulinum serotype B (B-H_c).

26. Use of a molecule according to any of Claims 1 to 7 in a method of purifying a Botulinum neurotoxin (BoNT) polypeptide.