CLAIMS

1. A method for producing soluble di-chain BoNT/A protein, said method comprising:

   a) providing a soluble single-chain BoNT/A protein;
   b) contacting said BoNT/A protein with endoproteinase Lys-C (Lys-C) in solution; and
   c) separating the soluble BoNT/A protein from the Lys-C by contacting the solution containing soluble BoNT/A protein and Lys-C with a hydrophobic surface, wherein the soluble BoNT/A protein binds with preference to the hydrophobic surface.

2. The method according to Claim 1, wherein the soluble single-chain BoNT/A protein is produced (i) in a host cell, by expressing a nucleic acid encoding said single-chain BoNT/A protein in an expression system; or (ii) in an *in vitro* expression system.

3. The method according to Claim 2, wherein the host cell is a bacterial host cell and the expression system is a bacterial expression system.

4. The method according to Claim 3, wherein the bacterial expression system is an *E. coli* expression system and the host cell is an *E. coli* cell.

5. The method according to any one of Claims 2 to 4, wherein said soluble single-chain BoNT/A protein is expressed in the cytoplasm of said host cell.

6. The method according to any one of Claims 2 to 5, wherein said soluble single-chain BoNT/A protein is expressed at a level of at least 5 mg/L.

7. The method according to any of one of Claims 2 to 6, comprising lysis of the bacterial or *E. coli* host cell to provide a bacterial or *E. coli* host cell homogenate containing said soluble single-chain BoNT/A protein.

8. The method according to any one of Claims 1 to 7, wherein the hydrophobic surface is an inert matrix to which a ligand consisting of aryl or alkyl groups is attached.

9. The method according to Claim 8, wherein the ligand is selected from the group consisting of: butyl, phenyl or octyl ligands.

10. The method according to Claim 9, wherein a high performance hydrophobic surface is used.