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.

11. A composition comprising an active di-chain BoNT/A protein obtainable by the method according to any one of Claims 1 to 10, wherein said active di-chain BoNT/A protein is separated from Lys-C by hydrophobic interaction chromatography; and wherein said composition comprises less than 2% single-chain BoNT/A.

12. The composition according to claim 11, wherein said composition comprises less than 1 % single-chain BoNT/A.

13. The composition according to Claim 11 or 12, wherein said composition contains endoproteinase Lys-C (Lys-C) at a concentration of less than 400 pg Lys-C per 100 ng BoNT/A protein, or less than 300 pg Lys-C per 100 ng BoNT/A protein, or less than 200 pg Lys-C per 100 ng BoNT/A protein, or less than 100 pg Lys-C per 100ng BoNT/A protein, or less than 50 pg Lys-C per 100ng BoNT/A protein, or less than 20 pg Lys-C per 100 ng of BoNT/A protein, or less than 15 pg Lys-C per 100 ng of BoNT/A protein, or less than 10 pg Lys-C per 100 ng of BoNT/A protein.

14. A liquid pharmaceutical composition comprising:

a) an active di-chain BoNT/A protein obtainable by the method according to any one of Claims 1 to 10, wherein said active di-chain BoNT/A protein is separated from Lys-C by hydrophobic interaction chromatography;
b) a non-protein stabilising agent that is a surfactant; and
c) water;
wherein said liquid pharmaceutical composition: (i) does not comprise a protein stabilising agent; and
(ii) comprises less than 2% single-chain BoNT/A.

15. The liquid pharmaceutical composition according to Claim 14, wherein said liquid pharmaceutical composition contains endoproteinase Lys-C (Lys-C) at a concentration of less than 400 pg Lys-C per 100 ng BoNT/A protein, or less than 300 pg Lys-C per 100 ng BoNT/A protein, or less than 200 pg Lys-C per 100 ng BoNT/A protein, or less than 100 pg Lys-C per 100ng BoNT/A protein, or less than 50 pg Lys-C per 100ng BoNT/A protein, or less than 20 pg Lys-C per 100 ng of BoNT/A protein, or less than 15 pg Lys-C per 100 ng of BoNT/A protein, or less than 10 pg Lys-C per 100 ng of BoNT/A protein.

16. The liquid pharmaceutical composition according to Claim 14 or 15, wherein said liquid pharmaceutical composition further comprises:

sodium chloride,

a buffer to maintain pH between 5.5 and 7.5, and

a disaccharide;

wherein the water is sterile water.

17. A composition according to any one of Claims 11 to 13, or a liquid pharmaceutical composition according to any one of Claims 14 to 16, for use in therapy.

18. The use of a composition according to any one of Claims 11 to 13, or a liquid pharmaceutical composition according to any one of Claims 14 to 16 in the manufacture of a medicament for prevention and/or treatment of at least one of the following diseases and disorders:

voluntary muscle strength, focal dystonia, including cervical, cranial dystonia, and benign essential blepharospasm, hemifacial spasm, and focal spasticity, gastrointestinal disorders,

hyperhidrosis, and cosmetic wrinkle correction, in a further aspect also blepharospasm, oromandibular dystonia, jaw opening type, jaw closing type, bruxism, Meige syndrome, lingual dystonia, apraxia of eyelid, opening cervical dystonia, antecollis, retrocollis, laterocollis, torticollis, pharyngeal dystonia, laryngeal dystonia, spasmodic dysphonia/adductor type, spasmodic dysphonia/abductor type, spasmodic dyspnea, limb dystonia, arm dystonia, task specific dystonia, writer's cramp, musician's cramp, golfer's cramp, leg dystonia, thigh adduction, thigh abduction knee flexion, knee extension, ankle flexion, ankle extension, equinovarus, deformity foot dystonia, striatal toe, toe flexion, toe extension, axial dystonia, pisa syndrome, belly dancer dystonia, segmental dystonia, hemidystonia, generalised dystonia, dystonia in lubag, dystonia in corticobasal degeneration, dystonia in lubag, tardive dystonia, dystonia in spinocerebellar ataxia, dystonia in Parkinson's disease, dystonia in Huntington's disease, dystonia in Hallervorden Spatz disease, dopainduced dyskinesias/dopa-induced dystonia, tardive dyskinesias/tardive dystonia, paroxysmal dyskinesias/dystonias, kinesiogenic non- kinesiogenic action-induced palatal myoclonus, myoclonus myokymia, rigidity, benign muscle cramps, hereditary chin trembling, paradoxic jaw muscle activity, hemimasticatory spasms, hypertrophic branchial myopathy, maseteric hypertrophy, tibialis anterior hypertrophy, nystagmus, oscillopsia supranuclear gaze palsy, epilepsia, partialis continua, planning of spasmodic torticollis operation, abductor vocal cord paralysis, recalcitant mutational dysphoria, upper oesophageal sphincter dysfunction, vocal fold granuloma, stuttering Gilles de la Tourette syndrome, middle ear myoclonus, protective larynx closure, postlaryngectomy, speech failure, protective ptosis, entropion sphincter Odii dysfunction, pseudoachalasia, nonachalsia, oesophageal motor disorders, vaginismus, postoperative immobilisation tremor, bladder dysfunction, detrusor sphincter dyssynergia ,bladder sphincter spasm, hemifacial spasm, reinnervation dyskinesias, cosmetic use craw's feet, frowning facial asymmetries, mentalis dimples, stiff person syndrome, tetanus prostate hyperplasia, adipositas, treatment infantile cerebral palsy strabismus, mixed paralytic concomitant, after retinal detachment surgery, after cataract surgery, in aphakia myositic strabismus, myopathic strabismus, dissociated vertical deviation, as an adjunct to strabismus surgery, esotropia, exotropia, achalasia, anal fissures, exocrine gland hyperactivity, Frey syndrome, Crocodile Tears syndrome, hyperhidrosis, axillar palmar plantar rhinorrhea, relative hypersalivation in stroke, in Parkinson's, in amyotrophic lateral sclerosis spastic conditions, in encephalitis and myelitis autoimmune processes, multiple sclerosis, transverse myelitis, Devic syndrome, viral infections, bacterial infections, parasitic infections, fungal infections, in hereditary spastic paraparesis postapoplectic syndrome hemispheric infarction, brainstem infarction, myelon infarction, migraine, in central nervous system trauma, hemispheric lesions, brainstem lesions, myelon lesion, in central nervous system hemorrhage, intracerebral hemorrhage, sub arachnoidal hemorrhage, subdural hemorrhage, intraspinal hemorrhage, in neoplasias, hemispheric tumors, brainstem tumors, myelon tumors, or snoring.