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.

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

In one aspect, the present 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 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification; and wherein said at least one amino acid modification is located in the clostridial toxin receptor binding domain (H_C domain).

In another aspect, the present 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 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification for use in increasing tissue retention and reducing diffusion away from a site of administration.

In another aspect, the present disclosure provides a nucleic acid comprising a nucleic acid sequence encoding an engineered clostridial toxin or the engineered clostridial toxin for use 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 disclosure provides a method of activating an engineered clostridial toxin, the method comprising providing a single-chain engineered clostridial toxin protein obtainable by 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 a method of activating an engineered clostridial toxin, the method comprising providing a single-chain engineered clostridial toxin protein obtainable by 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 an engineered clostridial toxin as disclosed above, for use in the prevention or treatment of a disease or condition selected from: 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), beauty therapy (cosmetic) applications benefiting from cell/muscle incapacitation (via SNARE down-regulation or inactivation), 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, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain (e.g. pain from muscle spasms), headache pain (e.g. tension headache), brow furrows, skin wrinkles, cancer, uterine disorders, uro-genital disorders, urogenital-neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

~~In one aspect,~~ T~~t~~he ~~invention~~ 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

least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.8 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 1 pi unit higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

~~In one aspect, t~~The disclosure ~~invention~~ 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 one pi unit higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

In certain embodiments, the engineered clostridial toxin comprises at least 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 amino acid modifications.

In certain embodiments, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 2, 3, 4 or 5 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

The present inventors have found that by increasing the pi of a clostridial toxin, for example, by at least 0.2 pi units, or 0.5 pi units, or one pi unit (through the introduction into the clostridial toxin protein of at least one amino acid modification), the resultant engineered clostridial toxin advantageously demonstrates properties of increased tissue retention and reduced diffusion away from sites of administration, while retaining abilities of target cell binding, translocation, and cleavage of target SNARE protein(s). Thus, the spread of clostridial toxin from the site of administration is significantly reduced, as compared to an otherwise identical clostridial toxin lacking said at least one amino acid modification.

The engineered clostridial toxins of the invention are suitable for use in any of the therapies described above, and advantageously may demonstrate a reduction in, or absence of, side effects compared to the use of known clostridial toxin therapeutics.

~~23. An engineered clostridial toxin substantially as described herein and with reference to the accompanying drawings.~~