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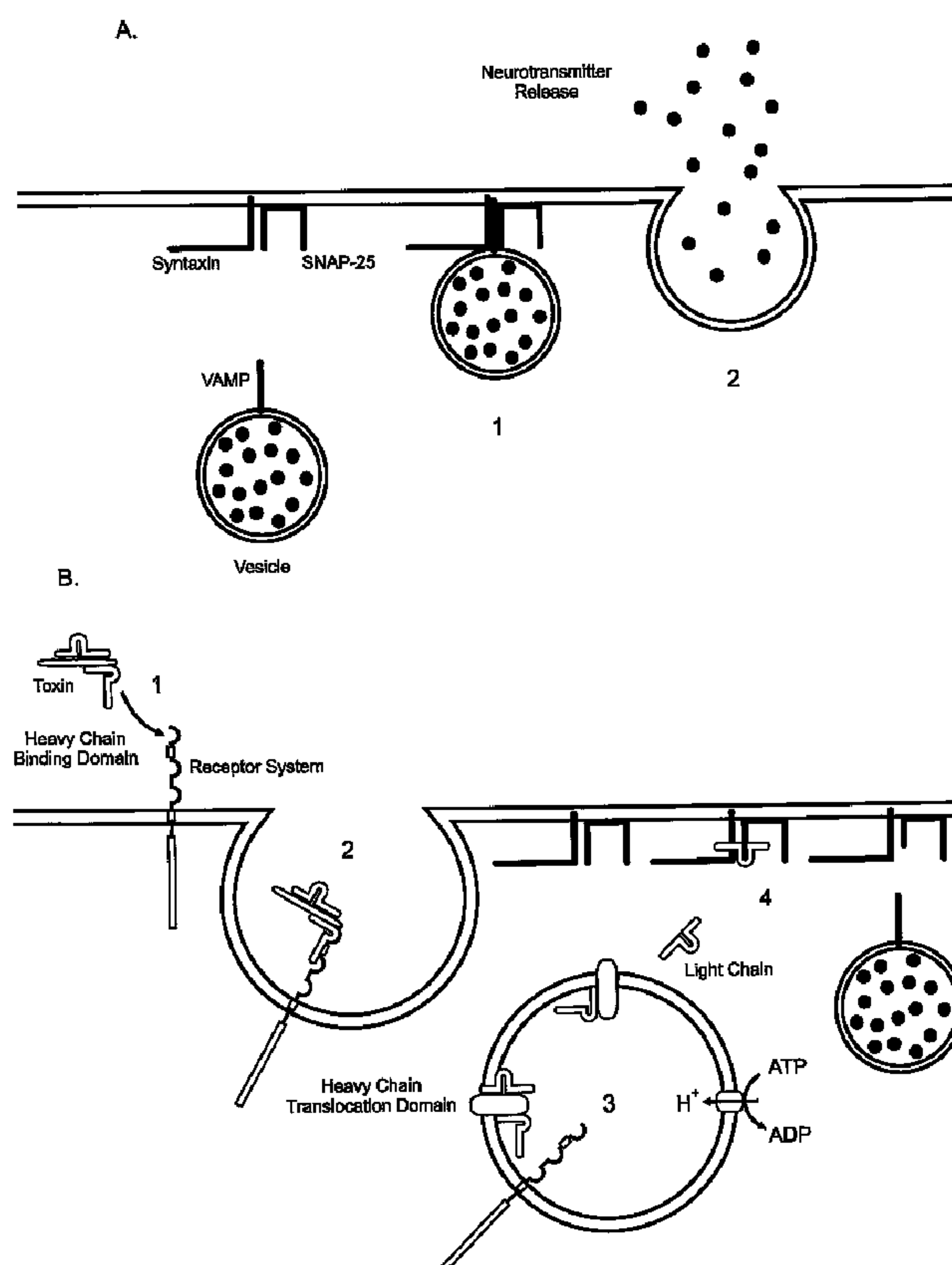
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(54) Titre : TOXINES CLOSTRIDIALES MODIFIEES PRESENTANT DES CAPACITES DE CIBLAGE MODIFIEES
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(54) Title: MODIFIED CLOSTRIDIAL TOXINS WITH ALTERED TARGETING CAPABILITIES FOR CLOSTRIDIAL TOXIN
TARGET CELLS



(57) Abrégé/Abstract:

The specification discloses modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced Clostridial toxin binding domain; polynucleotide molecules encoding such modified Clostridial toxins; and method of producing such modified Clostridial toxins.



Patent Application

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Steward, L.E. *et al.*, Modified Clostridial Toxins with Altered Targeting Capabilities
For Clostridial Toxin Target Cells

ABSTRACT

[0497] The specification discloses modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced Clostridial toxin binding domain; polynucleotide molecules encoding such modified Clostridial toxins; and method of producing such modified Clostridial toxins.

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JUMBO APPLICATIONS/PATENTS

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**Modified Clostridial Toxins with Altered Targeting Capabilities
For Clostridial Toxin Target Cells**

[01] This patent application claims priority pursuant to 35 U.S.C. § 365(c) to International Patent Application Serial No. 2006/009831 filed on March 14, 2006, which claims priority pursuant to 35 U.S.C. § 19(e) to United States Provisional Patent Application Serial No. 60/662,151 filed on March 15, 2005 and United States Provisional Patent Application Serial No. 60/661,953 filed on March 15, 2005, all three of which are hereby incorporated by reference in their entirety.

[02] All of the patents and publications cited in this application are hereby incorporated by reference in their entirety:

[03] The ability of Clostridial toxins, such as, e.g., Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see e.g., William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder. In addition, Clostridial toxin therapies are proposed for treating neuromuscular disorders, see e.g., Kei Roger Aoki et al., *Method for Treating Neuromuscular Disorders and Conditions with Botulinum Toxin Types A and B*, U.S. Patent No. 6,872,397 (Mar. 29, 2005); Rhett M. Schiffman, *Methods for Treating Uterine Disorders*, U.S. Patent Publication No. 2004/0175399 (Sep. 9, 2004); Richard L. Barron, *Methods for Treating Ulcers and Gastroesophageal Reflux Disease*, U.S. Patent Publication No. 2004/0086531 (May. 7, 2004); and Kei Roger Aoki, et al., *Method for Treating Dystonia with Botulinum Toxin C to G*, U.S. Patent No. 6,319,505 (Nov. 20, 2001); eye disorders, see e.g., Eric R. First, *Methods and Compositions for Treating Eye Disorders*, U.S. Patent Publication No. 2004/0234532 (Nov. 25, 2004); Kei Roger Aoki et al., *Botulinum Toxin Treatment for Blepharospasm*, U.S. Patent Publication No. 2004/0151740 (Aug. 5, 2004); and Kei Roger Aoki et al., *Botulinum Toxin Treatment for Strabismus*, U.S. Patent Publication No. 2004/0126396 (Jul. 1, 2004); pain, see e.g., Kei Roger Aoki et al., *Pain Treatment by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,869,610 (Mar. 22, 2005); Stephen Donovan, *Clostridial Toxin Derivatives and Methods to Treat Pain*, U.S. Patent No. 6,641,820 (Nov. 4, 2003); Kei Roger Aoki, et al., *Method for Treating Pain by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,464,986 (Oct. 15, 2002); Kei Roger Aoki and Minglei Cui, *Methods for Treating Pain*, U.S. Patent No. 6,113,915 (Sep. 5, 2000); Martin A. Voet, *Methods for Treating Fibromyalgia*, U.S. Patent 6,623,742 (Sep. 23, 2003); Martin A. Voet, *Botulinum Toxin Therapy for Fibromyalgia*, U.S. Patent Publication No. 2004/0062776 (Apr. 1, 2004); and Kei Roger Aoki et al., *Botulinum Toxin Therapy for Lower Back Pain*, U.S. Patent Publication

No. 2004/0037852 (Feb. 26, 2004); muscle injuries, see e.g., Gregory F. Brooks, *Methods for Treating Muscle Injuries*, U.S. Patent No. 6,423,319 (Jul. 23, 2002); headache, see e.g., Martin Voet, *Methods for Treating Sinus Headache*, U.S. Patent No. 6,838,434 (Jan. 4, 2005); Kei Roger Aoki et al., *Methods for Treating Tension Headache*, U.S. Patent No. 6,776,992 (Aug. 17, 2004); and Kei Roger Aoki et al., *Method for Treating Headache*, U.S. Patent No. 6,458,365 (Oct. 1, 2002); William J. Binder, *Method for Reduction of Migraine Headache Pain*, U.S. Patent 5,714,469 (Feb. 3, 1998); cardiovascular diseases, see e.g., Gregory F. Brooks and Stephen Donovan, *Methods for Treating Cardiovascular Diseases with Botulinum Toxin*, U.S. Patent No. 6,767,544 (Jul. 27, 2004); neurological disorders, see e.g., Stephen Donovan, *Parkinson's Disease Treatment*, U.S. Patent No. 6,620,415 (Sep. 16, 2003); and Stephen Donovan, *Method for Treating Parkinson's Disease with a Botulinum Toxin*, U.S. Patent No. 6,306,403 (Oct. 23, 2001); neuropsychiatric disorders, see e.g., Stephen Donovan, *Botulinum Toxin Therapy for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2004/0180061 (Sep. 16, 2004); and Steven Donovan, *Therapeutic Treatments for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2003/0211121 (Nov. 13, 2003); endocrine disorders, see e.g., Stephen Donovan, *Method for Treating Endocrine Disorders*, U.S. Patent No. 6,827,931 (Dec. 7, 2004); Stephen Donovan, *Method for Treating Thyroid Disorders with a Botulinum Toxin*, U.S. Patent No. 6,740,321 (May. 25, 2004); Kei Roger Aoki et al., *Method for Treating a Cholinergic Influenced Sweat Gland*, U.S. Patent No. 6,683,049 (Jan. 27, 2004); Stephen Donovan, *Neurotoxin Therapy for Diabetes*, U.S. Patent No. 6,416,765 (Jul. 9, 2002); Stephen Donovan, *Methods for Treating Diabetes*, U.S. Patent No. 6,337,075 (Jan. 8, 2002); Stephen Donovan, *Method for Treating a Pancreatic Disorder with a Neurotoxin*, U.S. Patent No. 6,261,572 (Jul. 17, 2001); Stephen Donovan, *Methods for Treating Pancreatic Disorders*, U.S. Patent No. 6,143,306 (Nov. 7, 2000); cancers, see e.g., Stephen Donovan, *Methods for Treating Bone Tumors*, U.S. Patent No. 6,565,870 (May 20, 2003); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin to Improve Patient Function*, U.S. Patent No. 6,368,605 (Apr. 9, 2002); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin*, U.S. Patent No. 6,139,845 (Oct. 31, 2000); and Mitchell F. Brin and Stephen Donovan, *Methods for Treating Diverse Cancers*, U.S. Patent Publication No. 2005/0031648 (Feb. 10, 2005); otic disorders, see e.g., Stephen Donovan, *Neurotoxin Therapy for Inner Ear Disorders*, U.S. Patent No. 6,358,926 (Mar. 19, 2002); and Stephen Donovan, *Method for Treating Otic Disorders*, U.S. Patent No. 6,265,379 (Jul. 24, 2001); autonomic disorders, see e.g., Pankaj J. Pasricha and Anthony N. Kalloo, *Method for Treating Gastrointestinal Muscle Disorders and Other Smooth Muscle Dysfunction*, U.S. Patent 5,437,291 (Aug. 1, 1995); as well as other disorders, see e.g., William J. Binder, *Method for Treatment of Skin Lesions Associated with Cutaneous Cell-proliferative Disorders*, U.S. Patent 5,670,484 (Sep. 23, 1997); Eric R. First, *Application of Botulinum Toxin to the Management of Neurogenic Inflammatory Disorders*, U.S. Patent 6,063,768 (May 16, 2000); Marvin Schwartz and Brian J. Freund, *Method to Reduce Hair Loss and Stimulate Hair Growth*, U.S. Patent 6,299,893 (Oct. 9, 2001); Jean D. A. Carruthers and Alastair Carruthers, *Cosmetic Use of Botulinum Toxin for Treatment of Downturned Mouth*, U.S. Patent 6,358,917 (Mar. 19, 2002); Stephen Donovan, *Use of a Clostridial Toxin to Reduce Appetite*, U.S. Patent Publication No. 2004/40253274 (Dec. 16, 2004); and Howard I. Katz and Andrew M. Blumenfeld, *Botulinum Toxin Dental Therapies and Procedures*, U.S. Patent Publication No.

2004/0115139 (Jun. 17, 2004); Kei Roger Aoki, et al., *Treatment of Neuromuscular Disorders and Conditions with Different Botulinum*, U.S. Patent Publication No. 2002/0010138 (Jan. 24, 2002); and Kei Roger Aoki, et al., *Use of Botulinum Toxins for Treating Various Disorders and Conditions and Associated Pain*, U.S. Patent Publication No. 2004/0013692 (Jan. 22, 2004). In addition, the expected use of Clostridial toxins, such as, *e.g.*, BoNTs and TeNT, in therapeutic and cosmetic treatments of humans and other mammals is anticipated to expand to an ever widening range of diseases and ailments that can benefit from the properties of these toxins.

[04] Clostridial toxin therapies are successfully used for many indications. Generally, administration of a Clostridial toxin treatment is well tolerated. However, toxin administration in some applications can be challenging because of the larger doses required to achieve a beneficial effect. Larger doses can increase the likelihood that the toxin may move through the interstitial fluids and the circulatory systems, such as, *e.g.*, the cardiovascular system and the lymphatic system, of the body, resulting in the undesirable dispersal of the toxin to areas not targeted for toxin treatment. Such dispersal can lead to undesirable side effects, such as, *e.g.*, inhibition of neurotransmitter release in neurons not targeted for treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. Thus, there remains a need for improved Clostridial toxins that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for a toxin treatment.

[05] The growing clinical, therapeutic and cosmetic use of Clostridial toxins in therapies requiring larger doses necessitates the pharmaceutical industry to develop modified Clostridial toxins that are effective at the target site of the application, but reduce or prevent the undesirable side-effects associated with the dispersal of the toxins to an unwanted location or locations. The present invention provides novel Clostridial toxins that reduce or prevent unwanted side-effects associated with toxin dispersal into non-targeted areas. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuromuscular disorders, neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders and hyperkinetic facial lines, as well as, other disorders where a Clostridial toxin administration to a mammal can produce a beneficial effect.

BRIEF DESCRIPTION OF THE DRAWINGS

[06] FIG. 1 shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma

membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, *e.g.*, changes in the internal pH of the vesicle, formation of a channel pore comprising the H_N domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, *e.g.*, SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[07] FIG. 2 shows the domain organization of naturally-occurring Clostridial toxins. The single chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, and a binding domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment, converts the single chain form of the toxin into the di-chain form. Above the single-chain form, the H_{CC} region of the Clostridial toxin binding domain is depicted. This region comprises the β -trefoil domain which comprises in a amino to carboxyl linear organization an α -fold, a β 4/ β 5 hairpin turn, a β -fold, a β 8/ β 9 hairpin turn and a γ -fold.

[08] FIG. 3 shows modified Clostridial toxins with an enhanced targeting domain located at the amino terminus of the modified toxin. FIG. 3A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enhanced targeting domain, an enzymatic domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation and enzymatic domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the targeting and translocation domains, the translocation and enzymatic domains or any combination thereof. FIG. 3B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enhanced targeting domain, an enzymatic domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the enzymatic and translocation domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease

cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the targeting and enzymatic domains, the enzymatic and translocation domains or any combination thereof.

[09]FIG. 4 shows modified Clostridial toxins with an enhanced targeting domain located between the other two domains. FIG. 4A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, an enhanced targeting domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the enzymatic and targeting domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the enzymatic and targeting domains, the targeting and translocation domains or any combination thereof. FIG. 4B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation domain, an enhanced targeting domain and an enzymatic domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation and targeting domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the translocation and targeting domains, the targeting and enzymatic domains or any combination thereof.

[010] FIG. 5 shows modified Clostridial toxins with an enhanced targeting domain located at the carboxyl terminus of the modified toxin. FIG. 5A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain and an enhanced targeting domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the enzymatic and translocation domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed between the enzymatic and translocation domains, the translocation and targeting domains or any combination thereof. FIG. 5B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation domain, an enzymatic domain and an enhanced targeting domain, with the di-chain loop region depicted by the double SS bracket. A proteolytic cleavage site (P) within a di-chain loop region is located between the translocation and enzymatic domains. Upon proteolytic cleavage with a P protease, the single chain form of the toxin is converted to the di-chain form. The P protease site can be a Clostridial toxin endogenous protease cleavage site or a non-Clostridial toxin exogenous protease cleavage site. Spacers can be placed

between the translocation and enzymatic domains, the enzymatic and targeting domains or any combination thereof.

DETAILED DESCRIPTION

[011] The present invention discloses modified Clostridial toxins that exhibit enhanced binding activity for cells targeted by naturally-occurring Clostridial toxins. Enhanced binding activity is achieved by replacing a naturally-occurring binding domain of a Clostridial toxin with a binding domain for a non-Clostridial toxin receptor system present on a Clostridial toxin target cell. This enhanced binding activity for a target cell should allow lower effective doses of a modified Clostridial toxin to be administered to an individual because more toxin will be delivered to a target cell. Thus modified Clostridial toxins with enhanced binding activity will reduce the undesirable dispersal of the toxin to areas not targeted for treatment, thereby reducing or preventing the undesirable side-effects associated with diffusion of a Clostridial toxin to an unwanted location.

[012] Aspects of the present invention provide modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain, wherein the modified Clostridial toxin exhibits a binding activity for a non-Clostridial toxin receptor system present on a Clostridial toxin target cell relative to a naturally-occurring Clostridial toxin. It is envisioned that the location of the enhanced targeting domain in the modified Clostridial toxins of the present specification can be located at the amino terminus of the toxin, between the enzymatic and translocation domains or at the carboxyl terminus of the toxin. Thus, a modified Clostridial toxins disclosed in the present specification can comprise an amino to carboxyl domain arrangement of, *e.g.*, an enhanced targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain; an enhanced targeting domain, a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain; a Clostridial toxin enzymatic domain, an enhanced targeting domain and a Clostridial toxin translocation domain; a Clostridial toxin translocation domain, an enhanced targeting domain and a Clostridial toxin enzymatic domain; a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain; and a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain and an enhanced targeting domain.

[013] Other aspects of the present invention provide polynucleotide molecules encoding modified Clostridial toxins comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain, wherein the modified Clostridial toxin exhibits an enhanced binding activity for a non-Clostridial toxin receptor system present on a Clostridial toxin target cell relative to a naturally-occurring Clostridial toxin. It is envisioned that the location of the enhanced targeting domain of the modified Clostridial toxins encoded by polynucleotide molecules of the present specification can be located at the amino terminus of the toxin, between the enzymatic and translocation domains or at the carboxyl terminus of the toxin. Thus, polynucleotide molecules disclosed in the present specification can

encoded modified Clostridial toxins comprising an amino to carboxyl domain arrangement of, *e.g.*, an enhanced targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain; an enhanced targeting domain, a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain; a Clostridial toxin enzymatic domain, an enhanced targeting domain and a Clostridial toxin translocation domain; a Clostridial toxin translocation domain, an enhanced targeting domain and a Clostridial toxin enzymatic domain; a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain; and a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain and an enhanced targeting domain.

[014] Other aspects of the present invention provide methods of producing a modified Clostridial toxin disclosed in the present specification, the method comprising the step of expressing in a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain, wherein the modified Clostridial toxin exhibits an enhanced targeting activity for a Clostridial toxin target cell relative to a naturally-occurring Clostridial toxin. Other aspects of the present invention provide methods of producing a modified Clostridial toxin disclosed in the present specification, the method comprising the steps of introducing in a cell an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain, wherein the modified Clostridial toxin exhibits an enhanced targeting activity for a Clostridial toxin target cell relative to a naturally-occurring Clostridial toxin and expressing the expression construct in the cell.

[015] The toxins produced by *Clostridium botulinum*, *Clostridium tetani*, *Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). While all seven botulinum toxins (BoNT) serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other species of Clostridia, *C. baratii* and *C. butyricum*, also produce toxins similar to BoNT/F and BoNT/E, respectively.

[016] Clostridia toxins possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring proteases produced in the environment (FIG. 2). This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulfide bond and

noncovalent interactions. Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus (Table 1); 2) a translocation domain contained within the amino-terminal half of the HC (H_N) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell (Table 1); and 3) a binding domain found within the carboxyl-terminal half of the HC (H_C) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell (Table 1).

Table 1. Clostridial Toxin Reference Sequences and Regions				
Toxin	SEQ ID NO:	LC	H_N	H_C
BoNT/A	1	M1-K448	A449-K871	N872-L1296
BoNT/B	2	M1-K441	A442-S858	E859-E1291
BoNT/C1	3	M1-K449	T450-N866	N867-E1291
BoNT/D	4	M1-R445	D446-N862	S863-E1276
BoNT/E	5	M1-R422	K423-K845	R846-K1252
BoNT/F	6	M1-K439	A440-K864	K865-E1274
BoNT/G	7	M1-K446	S447-S863	N864-E1297
TeNT	8	M1-A457	S458-V879	I880-D1315

[017] The binding, translocation and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of serotype or subtype. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. 1). The process is initiated when the H_C domain of a Clostridial toxin binds to a toxin-specific receptor system located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it appears to specifically targets one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are

necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble *N*-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo*. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, *e.g.*, Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) *Biochimie*. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) *Trends Biochem. Sci.* 552-558. (2002); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) *Trends Microbiol.* 431-437, (2003).

[018] Aspects of the present invention provide, in part, a modified Clostridial toxin. As used herein, the term “modified Clostridial toxin” means any polypeptide that can execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. A modified Clostridial toxin disclosed in the present specification is distinguished from a naturally-occurring Clostridial toxin by the fact that a modified Clostridial toxin lacks the cell binding activity of a naturally-occurring binding domain found in a Clostridial toxin. Instead, a modified Clostridial toxin disclosed in the present specification comprises an enhanced targeting domain that determines the binding activity of the modified Clostridial toxin to an endogenous Clostridial toxin receptor system located at the surface of the target cell. By definition, a naturally-occurring Clostridial toxin lacks an enhanced targeting domain.

[019] Aspects of the present invention provide, in part, a Clostridial toxin enzymatic domain. As used herein, the term “Clostridial toxin enzymatic domain” means any Clostridial toxin polypeptide that can execute the enzymatic target modification step of the intoxication process. Thus, a Clostridial toxin enzymatic domain specifically targets a Clostridial toxin substrate and encompasses the proteolytic cleavage of a Clostridial toxin substrate, such as, *e.g.*, SNARE proteins like a SNAP-25 substrate, a VAMP substrate and a Syntaxin substrate. Non-limiting examples of a Clostridial toxin enzymatic domain include, *e.g.*, a Clostridial toxin light chain region such as, *e.g.*, a BoNT/A light chain region, a BoNT/B light chain region, a BoNT/C1 light chain region, a BoNT/D light chain region, a BoNT/E light chain region, a BoNT/F light chain region, a BoNT/G light chain region, and a TeNT light chain region.

[020] A Clostridial toxin enzymatic domain includes, without limitation, naturally occurring Clostridial toxin light chain variants, such as, *e.g.*, Clostridial toxin light chain isoforms and Clostridial toxin light chain subtypes; non-naturally occurring Clostridial toxin light chain variants, such as, *e.g.*, conservative Clostridial toxin light chain variants, non-conservative Clostridial toxin light chain variants, Clostridial toxin light chain chimerics, active Clostridial toxin light chain fragments thereof, or any combination thereof.

[021] As used herein, the term "Clostridial toxin light chain variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin light chain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin light chain variants disclosed in the present specification are capable of executing the enzymatic target modification step of the intoxication process. As non-limiting examples, a BoNT/A light chain variant comprising amino acids 1-448 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-448 of SEQ ID NO: 1; a BoNT/B light chain variant comprising amino acids 1-441 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-441 of SEQ ID NO: 2; a BoNT/C1 light chain variant comprising amino acids 1-449 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-449 of SEQ ID NO: 3; a BoNT/D light chain variant comprising amino acids 1-445 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-445 of SEQ ID NO: 4; a BoNT/E light chain variant comprising amino acids 1-422 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-422 of SEQ ID NO: 5; a BoNT/F light chain variant comprising amino acids 1-439 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-439 of SEQ ID NO: 6; a BoNT/G light chain variant comprising amino acids 1-446 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-446 of SEQ ID NO: 7; and a TeNT light chain variant comprising amino acids 1-457 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-457 of SEQ ID NO: 8.

[022] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin light chain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific light chain subtypes showing approximately 95% amino acid identity when compared to another BoNT/A light chain subtype. As used herein, the term "naturally occurring Clostridial toxin light chain variant" means any Clostridial toxin light chain produced by a naturally-occurring process, including, without limitation, Clostridial toxin

light chain isoforms produced from alternatively-spliced transcripts, Clostridial toxin light chain isoforms produced by spontaneous mutation and Clostridial toxin light chain subtypes. A naturally occurring Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A naturally occurring Clostridial toxin light chain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based. A naturally occurring Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the naturally occurring Clostridial toxin light chain variant is based.

[023] A non-limiting examples of a naturally occurring Clostridial toxin light chain variant is a Clostridial toxin light chain isoform such as, *e.g.*, a BoNT/A light chain isoform, a BoNT/B light chain isoform, a BoNT/C1 light chain isoform, a BoNT/D light chain isoform, a BoNT/E light chain isoform, a BoNT/F light chain isoform, a BoNT/G light chain isoform, and a TeNT light chain isoform. A Clostridial toxin light chain isoform can function in substantially the same manner as the reference Clostridial toxin light chain on which the Clostridial toxin light chain isoform is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention.

[024] Another non-limiting examples of a naturally occurring Clostridial toxin light chain variant is a Clostridial toxin light chain subtype such as, *e.g.*, a light chain from subtype BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; a light chain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a light chain from subtype BoNT/C1-1 and BoNT/C1-2; a light chain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a light chain from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4.. A Clostridial toxin light chain subtype can function in substantially the same manner as the reference Clostridial toxin light chain on which the Clostridial toxin light chain subtype is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention.

[025] As used herein, the term "non-naturally occurring Clostridial toxin light chain variant" means any Clostridial toxin light chain produced with the aid of human manipulation, including, without limitation, Clostridial toxin light chains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin light chains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin light chain variants include, *e.g.*, conservative Clostridial toxin light

chain variants, non-conservative Clostridial toxin light chain variants, Clostridial toxin light chain chimeric variants and active Clostridial toxin light chain fragments.

[026] As used herein, the term “conservative Clostridial toxin light chain variant” means a Clostridial toxin light chain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin light chain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A conservative Clostridial toxin light chain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based. A conservative Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the conservative Clostridial toxin light chain variant is based. Non-limiting examples of a conservative Clostridial toxin light chain variant include, *e.g.*, conservative BoNT/A light chain variants, conservative BoNT/B light chain variants, conservative BoNT/C1 light chain variants, conservative BoNT/D light chain variants, conservative BoNT/E light chain variants, conservative BoNT/F light chain variants, conservative BoNT/G light chain variants, and conservative TeNT light chain variants.

[027] As used herein, the term “non-conservative Clostridial toxin light chain variant” means a Clostridial toxin light chain in which 1) at least one amino acid is deleted from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based; 2) at least one amino acid added to the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin light chain sequence (Table 1). A non-conservative Clostridial toxin light chain variant can function in substantially the same manner as the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based, and can be substituted for the reference Clostridial toxin light chain in any aspect of the present invention. A non-conservative Clostridial toxin

light chain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. A non-conservative Clostridial toxin light chain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin light chain on which the non-conservative Clostridial toxin light chain variant is based. Non-limiting examples of a non-conservative Clostridial toxin light chain variant include, *e.g.*, non-conservative BoNT/A light chain variants, non-conservative BoNT/B light chain variants, non-conservative BoNT/C1 light chain variants, non-conservative BoNT/D light chain variants, non-conservative BoNT/E light chain variants, non-conservative BoNT/F light chain variants, non-conservative BoNT/G light chain variants, and non-conservative TeNT light chain variants.

[028] As used herein, the term “Clostridial toxin light chain chimeric” means a polypeptide comprising at least a portion of a Clostridial toxin light chain and at least a portion of at least one other polypeptide to form a toxin light chain with at least one property different from the reference Clostridial toxin light chains of Table 1, with the proviso that this Clostridial toxin light chain chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Such Clostridial toxin light chain chimerics are described in, *e.g.*, Lance E. Steward et al., *Leucine-based Motif and Clostridial Toxins*, U.S. Patent Publication 2003/0027752 (Feb. 6, 2003); Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2003/0219462 (Nov. 27, 2003); and Lance E. Steward et al., *Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins*, U.S. Patent Publication 2004/0220386 (Nov. 4, 2004).

[029] As used herein, the term “active Clostridial toxin light chain fragment” means any of a variety of Clostridial toxin fragments comprising the light chain can be useful in aspects of the present invention with the proviso that these light chain fragments can specifically target the core components of the

neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The light chains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin light chain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A light chain (residues 1-8 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT light chain (residues 1-8 of SEQ ID NO: 8) are not required for enzymatic activity. Likewise, the carboxyl-terminus of the light chain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A light chain (residues 417-448 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT light chain (residues 427-457 of SEQ ID NO: 8) are not required for enzymatic activity. Thus, aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids, at least 425 amino acids and at least 450 amino acids. Other aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids, at most 425 amino acids and at most 450 amino acids.

[030] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin light chain variants and non-naturally-occurring Clostridial toxin light chain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[031] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.*, CLUSTAL W, see, *e.g.*, Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice*, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, *e.g.*, Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments*, 264(4) J. Mol. Biol. 823-838 (1996).

[032] Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.*, Match-box, see, *e.g.*, Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences*, 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.*, C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment*, 262(5131)

Science 208-214 (1993); Align-M, see, *e.g.*, Ivo Van Walle et al., *Align-M – A New Algorithm for Multiple Alignment of Highly Divergent Sequences*, 20(9) Bioinformatics, 1428-1435 (2004).

[033] Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.*, segment-to-segment comparison, see, *e.g.*, Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison*, 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, *e.g.*, Cédric Notredame et al., *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment*, 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, *e.g.*, Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput*, 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, *e.g.*, Amarendran R Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment*, 6(1) BMC Bioinformatics 66 (2005).

[034] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin light chain variant, such as, *e.g.*, a Clostridial toxin light chain isoform or a Clostridial toxin light chain subtype. In another aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin light chain variant, such as, *e.g.*, a conservative Clostridial toxin light chain variant, a non-conservative Clostridial toxin light chain variant, a Clostridial toxin chimeric light chain, an active Clostridial toxin light chain fragment, or any combination thereof.

[035] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/A light chain. In an aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A light chain comprises a naturally occurring BoNT/A light chain variant, such as, *e.g.*, a light chain from a BoNT/A isoform or a light chain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of a naturally occurring BoNT/A light chain variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 1-448 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 1-448 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A light chain comprises a non-naturally occurring BoNT/A light chain variant, such as, *e.g.*, a conservative BoNT/A light chain variant, a non-conservative BoNT/A light chain variant, a BoNT/A chimeric light chain, an active BoNT/A light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A light chain comprises amino acids 1-448 of a non-naturally occurring BoNT/A light chain variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 1-448 of a conservative BoNT/A light chain variant of SEQ ID NO: 1, amino acids 1-448 of a non-conservative BoNT/A light chain variant of SEQ ID NO: 1, amino acids 1-448 of an active BoNT/A light chain fragment of SEQ ID NO: 1, or any combination thereof.

[036] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 75% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 80% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 85% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at least 90% amino acid identity with amino acids 1-448 of SEQ ID NO: 1 or at least 95% amino acid identity with amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 75% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 80% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 85% amino acid identity with amino acids 1-448 of SEQ ID NO: 1, at most 90% amino acid identity with amino acids 1-448 of SEQ ID NO: 1 or at most 95% amino acid identity with amino acids 1-448 of SEQ ID NO: 1.

[037] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

[038] In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid

deletions relative to amino acids 1-448 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

[039] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/B light chain. In an aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B light chain comprises a naturally occurring BoNT/B light chain variant, such as, *e.g.*, a light chain from a BoNT/B isoform or a light chain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of a naturally occurring BoNT/B light chain variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 1-441 of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 1-441 of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B light chain comprises a non-naturally occurring BoNT/B light chain variant, such as, *e.g.*, a conservative BoNT/B light chain variant, a non-conservative BoNT/B light chain variant, a BoNT/B chimeric light chain, an active BoNT/B light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B light chain comprises amino acids 1-441 of a non-naturally occurring BoNT/B light chain variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 1-441 of a conservative BoNT/B light chain variant of SEQ ID NO: 2, amino acids 1-441 of a non-conservative BoNT/B light chain variant of SEQ ID NO: 2, amino acids 1-441 of an active BoNT/B light chain fragment of SEQ ID NO: 2, or any combination thereof.

[040] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 75% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 80% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 85% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at least 90% amino acid identity with amino acids 1-441 of SEQ ID NO: 2 or at least 95% amino acid identity with amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 75% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 80% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 85% amino acid identity with amino acids 1-441 of SEQ ID NO: 2, at most 90% amino acid identity with amino acids 1-441 of SEQ ID NO: 2 or at most 95% amino acid identity with amino acids 1-441 of SEQ ID NO: 2.

[041] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four,

five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

[042] In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

[043] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/C1 light chain. In an aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 light chain comprises a naturally occurring BoNT/C1 light chain variant, such as, *e.g.*, a light chain from a BoNT/C1 isoform or a light chain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of a naturally occurring BoNT/C1 light chain variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 1-449 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 1-449 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 light chain comprises a non-naturally occurring BoNT/C1 light chain variant, such as, *e.g.*, a conservative BoNT/C1 light chain variant, a non-conservative

BoNT/C1 light chain variant, a BoNT/C1 chimeric light chain, an active BoNT/C1 light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 light chain comprises amino acids 1-449 of a non-naturally occurring BoNT/C1 light chain variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 1-449 of a conservative BoNT/C1 light chain variant of SEQ ID NO: 3, amino acids 1-449 of a non-conservative BoNT/C1 light chain variant of SEQ ID NO: 3, amino acids 1-449 of an active BoNT/C1 light chain fragment of SEQ ID NO: 3, or any combination thereof.

[044] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 75% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 80% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 85% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at least 90% amino acid identity with amino acids 1-449 of SEQ ID NO: 3 or at least 95% amino acid identity with amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 75% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 80% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 85% amino acid identity with amino acids 1-449 of SEQ ID NO: 3, at most 90% amino acid identity with amino acids 1-449 of SEQ ID NO: 3 or at most 95% amino acid identity with amino acids 1-449 of SEQ ID NO: 3.

[045] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

[046] In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this

embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

[047] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/D light chain. In an aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D light chain comprises a naturally occurring BoNT/D light chain variant, such as, *e.g.*, a light chain from a BoNT/D isoform or a light chain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of a naturally occurring BoNT/D light chain variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 1-445 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 1-445 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D light chain comprises a non-naturally occurring BoNT/D light chain variant, such as, *e.g.*, a conservative BoNT/D light chain variant, a non-conservative BoNT/D light chain variant, a BoNT/D chimeric light chain, an active BoNT/D light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D light chain comprises amino acids 1-445 of a non-naturally occurring BoNT/D light chain variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 1-445 of a conservative BoNT/D light chain variant of SEQ ID NO: 4, amino acids 1-445 of a non-conservative BoNT/D light chain variant of SEQ ID NO: 4, amino acids 1-445 of an active BoNT/D light chain fragment of SEQ ID NO: 4, or any combination thereof.

[048] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 75% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 80% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 85% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at least 90% amino acid identity with amino acids 1-445 of SEQ ID NO: 4 or at least 95% amino acid identity with amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 75% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 80% amino acid identity with amino acids 1-445 of SEQ ID NO: 4, at most 85% amino acid identity with amino acids 1-445

of SEQ ID NO: 4, at most 90% amino acid identity with amino acids 1-445 of SEQ ID NO: 4 or at most 95% amino acid identity with amino acids 1-445 of SEQ ID NO: 4.

[049] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

[050] In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

[051] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/E light chain. In an aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of SEQ ID NO: 5. In

another aspect of this embodiment, a BoNT/E light chain comprises a naturally occurring BoNT/E light chain variant, such as, *e.g.*, a light chain from a BoNT/E isoform or a light chain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of a naturally occurring BoNT/E light chain variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 1-422 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 1-422 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E light chain comprises a non-naturally occurring BoNT/E light chain variant, such as, *e.g.*, a conservative BoNT/E light chain variant, a non-conservative BoNT/E light chain variant, a BoNT/E chimeric light chain, an active BoNT/E light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E light chain comprises amino acids 1-422 of a non-naturally occurring BoNT/E light chain variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 1-422 of a conservative BoNT/E light chain variant of SEQ ID NO: 5, amino acids 1-422 of a non-conservative BoNT/E light chain variant of SEQ ID NO: 5, amino acids 1-422 of an active BoNT/E light chain fragment of SEQ ID NO: 5, or any combination thereof.

[052] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 75% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 80% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 85% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at least 90% amino acid identity with amino acids 1-422 of SEQ ID NO: 5 or at least 95% amino acid identity with amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 75% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 80% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 85% amino acid identity with amino acids 1-422 of SEQ ID NO: 5, at most 90% amino acid identity with amino acids 1-422 of SEQ ID NO: 5 or at most 95% amino acid identity with amino acids 1-422 of SEQ ID NO: 5.

[053] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative

to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

[054] In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

[055] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/F light chain. In an aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F light chain comprises a naturally occurring BoNT/F light chain variant, such as, *e.g.*, a light chain from a BoNT/F isoform or a light chain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of a naturally occurring BoNT/F light chain variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 1-439 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 1-439 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F light chain comprises a non-naturally occurring BoNT/F light chain variant, such as, *e.g.*, a conservative BoNT/F light chain variant, a non-conservative BoNT/F light chain variant, a BoNT/F chimeric light chain, an active BoNT/F light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F light chain comprises amino acids 1-439 of a non-naturally occurring BoNT/F light chain variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 1-439 of a conservative BoNT/F light chain variant of SEQ ID NO: 6, amino acids 1-439 of a non-conservative BoNT/F light chain variant of SEQ ID NO: 6, amino acids 1-439 of an active BoNT/F light chain fragment of SEQ ID NO: 6, or any combination thereof.

[056] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 75% amino acid

identity with amino acids 1-439 of SEQ ID NO: 6, at least 80% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 85% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at least 90% amino acid identity with amino acids 1-439 of SEQ ID NO: 6 or at least 95% amino acid identity with amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 75% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 80% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 85% amino acid identity with amino acids 1-439 of SEQ ID NO: 6, at most 90% amino acid identity with amino acids 1-439 of SEQ ID NO: 6 or at most 95% amino acid identity with amino acids 1-439 of SEQ ID NO: 6.

[057] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

[058] In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven,

eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

[059] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/G light chain. In an aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G light chain comprises a naturally occurring BoNT/G light chain variant, such as, *e.g.*, a light chain from a BoNT/G isoform or a light chain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of a naturally occurring BoNT/G light chain variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 1-446 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 1-446 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G light chain comprises a non-naturally occurring BoNT/G light chain variant, such as, *e.g.*, a conservative BoNT/G light chain variant, a non-conservative BoNT/G light chain variant, a BoNT/G chimeric light chain, an active BoNT/G light chain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G light chain comprises amino acids 1-446 of a non-naturally occurring BoNT/G light chain variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 1-446 of a conservative BoNT/G light chain variant of SEQ ID NO: 7, amino acids 1-446 of a non-conservative BoNT/G light chain variant of SEQ ID NO: 7, amino acids 1-446 of an active BoNT/G light chain fragment of SEQ ID NO: 7, or any combination thereof.

[060] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 75% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 80% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 85% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at least 90% amino acid identity with amino acids 1-446 of SEQ ID NO: 7 or at least 95% amino acid identity with amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 75% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 80% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 85% amino acid identity with amino acids 1-446 of SEQ ID NO: 7, at most 90% amino acid identity with amino acids 1-446 of SEQ ID NO: 7 or at most 95% amino acid identity with amino acids 1-446 of SEQ ID NO: 7.

[061] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light

chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

[062] In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

[063] In another embodiment, a Clostridial toxin enzymatic domain comprises a TeNT light chain. In an aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT light chain comprises a naturally occurring TeNT light chain variant, such as, *e.g.*, a light chain from a TeNT isoform or a light chain from a TeNT subtype. In another aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of a naturally occurring TeNT light chain variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 1-457 of a TeNT isoform of SEQ ID NO: 8 or amino acids 1-457 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT light chain comprises a non-naturally occurring TeNT light chain variant, such as, *e.g.*, a conservative TeNT light chain variant, a non-conservative TeNT light chain variant, a TeNT chimeric light chain, an active TeNT light chain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT light chain comprises amino acids 1-457 of a non-naturally occurring TeNT light

chain variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 1-457 of a conservative TeNT light chain variant of SEQ ID NO: 8, amino acids 1-457 of a non-conservative TeNT light chain variant of SEQ ID NO: 8, amino acids 1-457 of an active TeNT light chain fragment of SEQ ID NO: 8, or any combination thereof.

[064] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 75% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 80% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 85% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at least 90% amino acid identity with amino acids 1-457 of SEQ ID NO: 8 or at least 95% amino acid identity with amino acids 1-457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 75% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 80% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 85% amino acid identity with amino acids 1-457 of SEQ ID NO: 8, at most 90% amino acid identity with amino acids 1-457 of SEQ ID NO: 8 or at most 95% amino acid identity with amino acids 1-457 of SEQ ID NO: 8.

[065] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

[066] In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-

457 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT light chain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

[067] Aspects of the present invention provide, in part, a Clostridial toxin translocation domain. As used herein, the term "Clostridial toxin translocation domain" means any Clostridial toxin polypeptide that can execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. Thus, a Clostridial toxin translocation domain facilitates the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridial toxin light chain through the membrane an intracellular vesicle into the cytoplasm of a cell. Non-limiting examples of a Clostridial toxin translocation domain include, *e.g.*, a Clostridial toxin H_N region such as, *e.g.*, a BoNT/A H_N region, a BoNT/B H_N region, a BoNT/C1 H_N region, a BoNT/D H_N region, a BoNT/E H_N region, a BoNT/F H_N region, a BoNT/G H_N region, and a TeNT H_N region.

[068] A Clostridial toxin translocation domain includes, without limitation, naturally occurring Clostridial toxin H_N region variants, such as, *e.g.*, Clostridial toxin H_N region isoforms and Clostridial toxin H_N region subtypes; non-naturally occurring Clostridial toxin H_N region variants, such as, *e.g.*, conservative Clostridial toxin H_N region variants, non-conservative Clostridial toxin H_N region variants, Clostridial toxin H_N region chimerics, active Clostridial toxin H_N region fragments thereof, or any combination thereof.

[069] As used herein, the term "Clostridial toxin H_N region variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin H_N region that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all Clostridial toxin H_N region variants disclosed in the present specification are capable of executing the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A H_N region variant comprising amino acids 449-871 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 449-871 of SEQ ID NO: 1; a BoNT/B H_N region variant comprising amino acids 442-858 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 442-858 of SEQ ID

NO: 2; a BoNT/C1 H_N region variant comprising amino acids 450-866 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 450-866 of SEQ-ID NO: 3; a BoNT/D H_N region variant comprising amino acids 446-862 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 446-862 of SEQ ID NO: 4; a BoNT/E H_N region variant comprising amino acids 423-845 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 423-845 of SEQ ID NO: 5; a BoNT/F H_N region variant comprising amino acids 440-864 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 440-864 of SEQ ID NO: 6; a BoNT/G H_N region variant comprising amino acids 447-863 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 447-863 of SEQ ID NO: 7; and a TeNT H_N region variant comprising amino acids 458-879 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 458-879 of SEQ ID NO: 8.

[070] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin H_N region variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific H_N region subtypes showing approximately 87% amino acid identity when compared to another BoNT/A H_N region subtype. As used herein, the term "naturally occurring Clostridial toxin H_N region variant" means any Clostridial toxin H_N region produced by a naturally-occurring process, including, without limitation, Clostridial toxin H_N region isoforms produced from alternatively-spliced transcripts, Clostridial toxin H_N region isoforms produced by spontaneous mutation and Clostridial toxin H_N region subtypes. A naturally occurring Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A naturally occurring Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based. A naturally occurring Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_N region on which the naturally occurring Clostridial toxin H_N region variant is based.

[071] A non-limiting examples of a naturally occurring Clostridial toxin H_N region variant is a Clostridial toxin H_N region isoform such as, *e.g.*, a BoNT/A H_N region isoform, a BoNT/B H_N region isoform, a BoNT/C1 H_N region isoform, a BoNT/D H_N region isoform, a BoNT/E H_N region isoform, a BoNT/F H_N region isoform, a BoNT/G H_N region isoform, and a TeNT H_N region isoform. A Clostridial toxin H_N region isoform can function in substantially the same manner as the reference Clostridial toxin H_N region on which the Clostridial toxin H_N region isoform is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention.

[072] Another non-limiting examples of a naturally occurring Clostridial toxin H_N region variant is a Clostridial toxin H_N region subtype such as, *e.g.*, a H_N region from subtype BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; a H_N region from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a H_N region from subtype BoNT/C1-1 and BoNT/C1-2; a H_N region from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a H_N region from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4. A Clostridial toxin H_N region subtype can function in substantially the same manner as the reference Clostridial toxin H_N region on which the Clostridial toxin H_N region subtype is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention.

[073] As used herein, the term “non-naturally occurring Clostridial toxin H_N region variant” means any Clostridial toxin H_N region produced with the aid of human manipulation, including, without limitation, Clostridial toxin H_N regions produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin H_N regions produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin H_N region variants include, *e.g.*, conservative Clostridial toxin H_N region variants, non-conservative Clostridial toxin H_N region variants, Clostridial toxin H_N region chimeric variants and active Clostridial toxin H_N region fragments.

[074] As used herein, the term “conservative Clostridial toxin H_N region variant” means a Clostridial toxin H_N region that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin H_N region sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A conservative Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino

acids, or 500 or more amino acids from the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based. A conservative Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_N region on which the conservative Clostridial toxin H_N region variant is based. Non-limiting examples of a conservative Clostridial toxin H_N region variant include, *e.g.*, conservative BoNT/A H_N region variants, conservative BoNT/B H_N region variants, conservative BoNT/C1 H_N region variants, conservative BoNT/D H_N region variants, conservative BoNT/E H_N region variants, conservative BoNT/F H_N region variants, conservative BoNT/G H_N region variants, and conservative TeNT H_N region variants.

[075] As used herein, the term “non-conservative Clostridial toxin H_N region variant” means a Clostridial toxin H_N region in which 1) at least one amino acid is deleted from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based; 2) at least one amino acid added to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin H_N region sequence (Table 1). A non-conservative Clostridial toxin H_N region variant can function in substantially the same manner as the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based, and can be substituted for the reference Clostridial toxin H_N region in any aspect of the present invention. A non-conservative Clostridial toxin H_N region variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. A non-conservative Clostridial toxin H_N region variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity,

85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin H_N region on which the non-conservative Clostridial toxin H_N region variant is based. Non-limiting examples of a non-conservative Clostridial toxin H_N region variant include, *e.g.*, non-conservative BoNT/A H_N region variants, non-conservative BoNT/B H_N region variants, non-conservative BoNT/C1 H_N region variants, non-conservative BoNT/D H_N region variants, non-conservative BoNT/E H_N region variants, non-conservative BoNT/F H_N region variants, non-conservative BoNT/G H_N region variants, and non-conservative TeNT H_N region variants.

[076] As used herein, the term “Clostridial toxin H_N region chimeric” means a polypeptide comprising at least a portion of a Clostridial toxin H_N region and at least a portion of at least one other polypeptide to form a toxin H_N region with at least one property different from the reference Clostridial toxin H_N regions of Table 1, with the proviso that this Clostridial toxin H_N region chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

[077] As used herein, the term “active Clostridial toxin H_N region fragment” means any of a variety of Clostridial toxin fragments comprising the H_N region can be useful in aspects of the present invention with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_N regions from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a H_N region from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include Clostridial toxin H_N regions comprising a translocation domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H_N regions comprising translocation domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

[078] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin H_N region variants and non-naturally-occurring Clostridial toxin H_N region variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[079] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation domain. In an aspect of this embodiment, a Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin H_N region variant, such as, *e.g.*, a Clostridial toxin H_N region isoform or a Clostridial toxin H_N region subtype. In another aspect of this

embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin H_N region variant, such as, *e.g.*, a conservative Clostridial toxin H_N region variant, a non-conservative Clostridial toxin H_N region variant, a Clostridial toxin chimeric H_N region, an active Clostridial toxin H_N region fragment, or any combination thereof.

[080] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/A H_N region. In an aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-871 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A H_N region comprises a naturally occurring BoNT/A H_N region variant, such as, *e.g.*, a H_N region from a BoNT/A isoform or a H_N region from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-871 of a naturally occurring BoNT/A H_N region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 449-871 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 449-871 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A H_N region comprises a non-naturally occurring BoNT/A H_N region variant, such as, *e.g.*, a conservative BoNT/A H_N region variant, a non-conservative BoNT/A H_N region variant, a BoNT/A chimeric H_N region, an active BoNT/A H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A H_N region comprises amino acids 449-871 of a non-naturally occurring BoNT/A H_N region variant of SEQ ID NO: 1, such as, *e.g.*, amino acids 449-871 of a conservative BoNT/A H_N region variant of SEQ ID NO: 1, amino acids 449-871 of a non-conservative BoNT/A H_N region variant of SEQ ID NO: 1, amino acids 449-871 of an active BoNT/A H_N region fragment of SEQ ID NO: 1, or any combination thereof.

[081] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at least 75% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at least 80% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at least 85% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at least 90% amino acid identity with amino acids 449-871 of SEQ ID NO: 1 or at least 95% amino acid identity with amino acids 449-871 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at most 75% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at most 80% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at most 85% amino acid identity with amino acids 449-871 of SEQ ID NO: 1, at most 90% amino acid identity with amino acids 449-871 of SEQ ID NO: 1 or at most 95% amino acid identity with amino acids 449-871 of SEQ ID NO: 1.

[082] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 449-871 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region

comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 449-871 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 449-871 of SEQ ID NO: 1.

[083] In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 449-871 of SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 449-871 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 449-871 of SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 449-871 of SEQ ID NO: 1.

[084] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/B H_N region. In an aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-858 of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B H_N region comprises a naturally occurring BoNT/B H_N region variant, such as, *e.g.*, a H_N region from a BoNT/B isoform or a H_N region from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-858 of a naturally occurring BoNT/B H_N region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 442-858 of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 442-858 of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B H_N region comprises a non-naturally occurring BoNT/B H_N region variant, such as, *e.g.*, a conservative BoNT/B H_N region variant, a non-conservative BoNT/B H_N region variant, a BoNT/B chimeric H_N region, an active BoNT/B H_N region fragment, or any combination thereof.

In still another aspect of this embodiment, a BoNT/B H_N region comprises amino acids 442-858 of a non-naturally occurring BoNT/B H_N region variant of SEQ ID NO: 2, such as, *e.g.*, amino acids 442-858 of a conservative BoNT/B H_N region variant of SEQ ID NO: 2, amino acids 442-858 of a non-conservative BoNT/B H_N region variant of SEQ ID NO: 2, amino acids 442-858 of an active BoNT/B H_N region fragment of SEQ ID NO: 2, or any combination thereof.

[085] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at least 75% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at least 80% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at least 85% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at least 90% amino acid identity with amino acids 442-858 of SEQ ID NO: 2 or at least 95% amino acid identity with amino acids 442-858 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at most 75% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at most 80% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at most 85% amino acid identity with amino acids 442-858 of SEQ ID NO: 2, at most 90% amino acid identity with amino acids 442-858 of SEQ ID NO: 2 or at most 95% amino acid identity with amino acids 442-858 of SEQ ID NO: 2.

[086] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 442-858 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 442-858 of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 442-858 of SEQ ID NO: 2.

[087] In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment,

a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 442-858 of SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 442-858 of SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 442-858 of SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 442-858 of SEQ ID NO: 2.

[088] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/C1 H_N region. In an aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-866 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 H_N region comprises a naturally occurring BoNT/C1 H_N region variant, such as, *e.g.*, a H_N region from a BoNT/C1 isoform or a H_N region from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-866 of a naturally occurring BoNT/C1 H_N region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 450-866 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 450-866 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 H_N region comprises a non-naturally occurring BoNT/C1 H_N region variant, such as, *e.g.*, a conservative BoNT/C1 H_N region variant, a non-conservative BoNT/C1 H_N region variant, a BoNT/C1 chimeric H_N region, an active BoNT/C1 H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 H_N region comprises amino acids 450-866 of a non-naturally occurring BoNT/C1 H_N region variant of SEQ ID NO: 3, such as, *e.g.*, amino acids 450-866 of a conservative BoNT/C1 H_N region variant of SEQ ID NO: 3, amino acids 450-866 of a non-conservative BoNT/C1 H_N region variant of SEQ ID NO: 3, amino acids 450-866 of an active BoNT/C1 H_N region fragment of SEQ ID NO: 3, or any combination thereof.

[089] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at least 75% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at least 80% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at least 85% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at least 90% amino acid identity with amino acids 450-866 of SEQ ID NO: 3 or at least 95% amino acid identity with amino acids 450-866 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at most 75% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at most 80% amino acid identity with amino acids 450-866 of SEQ ID NO: 3, at most 85% amino acid

identity with amino acids 450-866 of SEQ ID NO: 3, at most 90% amino acid identity with amino acids 450-866 of SEQ ID NO: 3 or at most 95% amino acid identity with amino acids 450-866 of SEQ ID NO: 3.

[090] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 450-866 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 450-866 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 450-866 of SEQ ID NO: 3.

[091] In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 450-866 of SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 450-866 of SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 450-866 of SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 450-866 of SEQ ID NO: 3.

[092] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/D H_N region. In an aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-862 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D H_N region comprises a naturally occurring BoNT/D H_N region variant, such as, *e.g.*, a H_N region from a BoNT/D isoform or a H_N region from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-862 of a naturally occurring BoNT/D H_N region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 446-862 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 446-862 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D H_N region comprises a non-naturally occurring BoNT/D H_N region variant, such as, *e.g.*, a conservative BoNT/D H_N region variant, a non-conservative BoNT/D H_N region variant, a BoNT/D chimeric H_N region, an active BoNT/D H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D H_N region comprises amino acids 446-862 of a non-naturally occurring BoNT/D H_N region variant of SEQ ID NO: 4, such as, *e.g.*, amino acids 446-862 of a conservative BoNT/D H_N region variant of SEQ ID NO: 4, amino acids 446-862 of a non-conservative BoNT/D H_N region variant of SEQ ID NO: 4, amino acids 446-862 of an active BoNT/D H_N region fragment of SEQ ID NO: 4, or any combination thereof.

[093] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at least 75% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at least 80% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at least 85% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at least 90% amino acid identity with amino acids 446-862 of SEQ ID NO: 4 or at least 95% amino acid identity with amino acids 446-862 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at most 75% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at most 80% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at most 85% amino acid identity with amino acids 446-862 of SEQ ID NO: 4, at most 90% amino acid identity with amino acids 446-862 of SEQ ID NO: 4 or at most 95% amino acid identity with amino acids 446-862 of SEQ ID NO: 4.

[094] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 446-862 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 446-862 of SEQ ID NO: 4. In still other aspects

of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 446-862 of SEQ ID NO: 4.

[095] In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 446-862 of SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 446-862 of SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 446-862 of SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 446-862 of SEQ ID NO: 4.

[096] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/E H_N region. In an aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-845 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E H_N region comprises a naturally occurring BoNT/E H_N region variant, such as, *e.g.*, a H_N region from a BoNT/E isoform or a H_N region from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-845 of a naturally occurring BoNT/E H_N region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 423-845 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 423-845 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E H_N region comprises a non-naturally occurring BoNT/E H_N region variant, such as, *e.g.*, a conservative BoNT/E H_N region variant, a non-conservative BoNT/E H_N region variant, a BoNT/E chimeric H_N region, an active BoNT/E H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E H_N region comprises amino acids 423-845 of a non-naturally occurring BoNT/E H_N region variant of SEQ ID NO: 5, such as, *e.g.*, amino acids 423-845 of a conservative BoNT/E H_N region variant of SEQ ID NO: 5, amino acids 423-845 of a non-conservative BoNT/E H_N region variant of SEQ ID NO: 5, amino acids 423-845 of an active BoNT/E H_N region fragment of SEQ ID NO: 5, or any combination thereof.

[097] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at least 75% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at least 80% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at least 85% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at least 90% amino acid identity with amino acids 423-845 of SEQ ID NO: 5 or at least 95% amino acid identity with amino acids 423-845 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at most 75% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at most 80% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at most 85% amino acid identity with amino acids 423-845 of SEQ ID NO: 5, at most 90% amino acid identity with amino acids 423-845 of SEQ ID NO: 5 or at most 95% amino acid identity with amino acids 423-845 of SEQ ID NO: 5.

[098] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 423-845 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 423-845 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 423-845 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 423-845 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 423-845 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 423-845 of SEQ ID NO: 5.

[099] In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 423-845 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 423-845 of SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 423-845 of SEQ ID NO: 5. In other

aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 423-845 of SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 423-845 of SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 423-845 of SEQ ID NO: 5.

[0100] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/F H_N region. In an aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-864 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F H_N region comprises a naturally occurring BoNT/F H_N region variant, such as, *e.g.*, a H_N region from a BoNT/F isoform or a H_N region from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-864 of a naturally occurring BoNT/F H_N region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 440-864 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 440-864 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F H_N region comprises a non-naturally occurring BoNT/F H_N region variant, such as, *e.g.*, a conservative BoNT/F H_N region variant, a non-conservative BoNT/F H_N region variant, a BoNT/F chimeric H_N region, an active BoNT/F H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F H_N region comprises amino acids 440-864 of a non-naturally occurring BoNT/F H_N region variant of SEQ ID NO: 6, such as, *e.g.*, amino acids 440-864 of a conservative BoNT/F H_N region variant of SEQ ID NO: 6, amino acids 440-864 of a non-conservative BoNT/F H_N region variant of SEQ ID NO: 6, amino acids 440-864 of an active BoNT/F H_N region fragment of SEQ ID NO: 6, or any combination thereof.

[0101] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at least 75% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at least 80% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at least 85% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at least 90% amino acid identity with amino acids 440-864 of SEQ ID NO: 6 or at least 95% amino acid identity with amino acids 440-864 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at most 75% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at most 80% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at most 85% amino acid identity with amino acids 440-864 of SEQ ID NO: 6, at most 90% amino acid identity with amino acids 440-864 of SEQ ID NO: 6 or at most 95% amino acid identity with amino acids 440-864 of SEQ ID NO: 6.

[0102] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous

amino acid substitutions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 440-864 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 440-864 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 440-864 of SEQ ID NO: 6.

[0103] In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 440-864 of SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 440-864 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 440-864 of SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 440-864 of SEQ ID NO: 6.

[0104] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/G H_N region. In an aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-863 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G H_N region comprises a naturally occurring BoNT/G H_N region variant, such as, *e.g.*, a H_N region from a BoNT/G isoform or a H_N region from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-863 of a naturally occurring BoNT/G H_N region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 447-863 of a BoNT/G

isoform of SEQ ID NO: 7 or amino acids 447-863 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G H_N region comprises a non-naturally occurring BoNT/G H_N region variant, such as, *e.g.*, a conservative BoNT/G H_N region variant, a non-conservative BoNT/G H_N region variant, a BoNT/G chimeric H_N region, an active BoNT/G H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G H_N region comprises amino acids 447-863 of a non-naturally occurring BoNT/G H_N region variant of SEQ ID NO: 7, such as, *e.g.*, amino acids 447-863 of a conservative BoNT/G H_N region variant of SEQ ID NO: 7, amino acids 447-863 of a non-conservative BoNT/G H_N region variant of SEQ ID NO: 7, amino acids 447-863 of an active BoNT/G H_N region fragment of SEQ ID NO: 7, or any combination thereof.

[0105] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at least 75% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at least 80% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at least 85% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at least 90% amino acid identity with amino acids 447-863 of SEQ ID NO: 7 or at least 95% amino acid identity with amino acids 447-863 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at most 75% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at most 80% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at most 85% amino acid identity with amino acids 447-863 of SEQ ID NO: 7, at most 90% amino acid identity with amino acids 447-863 of SEQ ID NO: 7 or at most 95% amino acid identity with amino acids 447-863 of SEQ ID NO: 7.

[0106] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 447-863 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 447-863 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 447-863 of SEQ ID NO: 7.

[0107] In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 447-863 of SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 447-863 of SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 447-863 of SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 447-863 of SEQ ID NO: 7.

[0108] In another embodiment, a Clostridial toxin translocation domain comprises a TeNT H_N region. In an aspect of this embodiment, a TeNT H_N region comprises amino acids 458-879 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT H_N region comprises a naturally occurring TeNT H_N region variant, such as, *e.g.*, a H_N region from a TeNT isoform or a H_N region from a TeNT subtype. In another aspect of this embodiment, a TeNT H_N region comprises amino acids 458-879 of a naturally occurring TeNT H_N region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 458-879 of a TeNT isoform of SEQ ID NO: 8 or amino acids 458-879 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT H_N region comprises a non-naturally occurring TeNT H_N region variant, such as, *e.g.*, a conservative TeNT H_N region variant, a non-conservative TeNT H_N region variant, a TeNT chimeric H_N region, an active TeNT H_N region fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT H_N region comprises amino acids 458-879 of a non-naturally occurring TeNT H_N region variant of SEQ ID NO: 8, such as, *e.g.*, amino acids 458-879 of a conservative TeNT H_N region variant of SEQ ID NO: 8, amino acids 458-879 of a non-conservative TeNT H_N region variant of SEQ ID NO: 8, amino acids 458-879 of an active TeNT H_N region fragment of SEQ ID NO: 8, or any combination thereof.

[0109] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at least 75% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at least 80% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at least 85% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at least 90% amino acid identity with amino acids 458-879 of SEQ ID NO: 8 or at least 95% amino acid

identity with amino acids 458-879 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at most 75% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at most 80% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at most 85% amino acid identity with amino acids 458-879 of SEQ ID NO: 8, at most 90% amino acid identity with amino acids 458-879 of SEQ ID NO: 8 or at most 95% amino acid identity with amino acids 458-879 of SEQ ID NO: 8.

[0110] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 458-879 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to amino acids 458-879 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to amino acids 458-879 of SEQ ID NO: 8.

[0111] In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 458-879 of SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 458-879 of SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 458-879 of SEQ ID NO: 8. In other aspects of this embodiment, a TeNT H_N region comprises a polypeptide having, *e.g.*, at

least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 458-879 of SEQ ID NO: 8.

[0112] Aspects of the present invention provide, in part, an enhanced targeting domain. As used herein, the term “enhanced targeting domain” means any polypeptide that can selectively bind to a non-Clostridial toxin receptor system present on a Clostridial toxin target cell and initiate the overall internalization mechanism whereby a Clostridial toxin intoxicates a target cell. As used herein, the term “selectively” means having a highly preferred activity or effect. As used herein, the term “selectively bind” means a molecule is able to bind its target receptor system under physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree relative to other, non-target receptor systems. Thus, with reference to an enhanced targeting domain of the present specification, there is a discriminatory binding of the enhanced targeting domain to a non-Clostridial toxin receptor system.

[0113] An enhanced targeting domain disclosed in the present specification facilitates the binding activity of the modified Clostridial toxins disclosed in the present specification to a non-Clostridial toxin receptor system located at the surface of a Clostridial toxin target cell. As used herein, the term “binding activity” means that one molecule is directly or indirectly contacting another molecule via at least one intermolecular or intramolecular force, including, without limitation, a covalent bond, an ionic bond, a metallic bond, a hydrogen bond, a hydrophobic interaction, a van der Waals interaction, and the like, or any combination thereof. “Bound” and “bind” are considered terms for binding.

[0114] As used herein, the term “binding affinity” means how strong a molecule’s binding activity is for a particular receptor system. In general, high binding affinity results from greater intermolecular force between a binding domain and its receptor system while low binding affinity involves less intermolecular force between the ligand and its receptor. High binding affinity involves a longer residence time for the binding domain at its receptor binding site than is the case for low binding affinity. As such, a molecule with a high binding affinity means a lower concentration of that molecule is required to maximally occupy the binding sites of a receptor system and trigger a physiological response. Conversely, low binding affinity means a relatively high concentration of a molecule is required before the receptor binding sites of a receptor system is maximally occupied and the maximum physiological response is achieved. Thus, modified Clostridial toxins with increased binding activity due to high binding affinity will allow administration of reduced doses of the toxin, thereby reducing or preventing unwanted side-effects associated with toxin dispersal into non-targeted areas.

[0115] As used herein, the term “binding specificity” means how specific a molecule’s binding activity is one particular receptor system. In general, high binding specificity results in a more exclusive interaction with one particular receptor system or subgroup of receptor systems while low binding specificity results in a more promiscuous interaction with a larger group of receptor systems. As such, a molecule with a

high binding specificity means that molecule will occupy the binding sites of a particular receptor system and trigger a physiological response. Conversely, low binding specificity means a molecule will occupy the binding sites of a many receptor systems and trigger a multitude of physiological responses. Thus, modified Clostridial toxins with increased binding activity due to high binding specificity will only target non-Clostridial toxin receptors present on a subgroup of Clostridial toxin target cells, thereby reducing the side effects associated with the targeting of all Clostridial toxin target cells.

[0116] As used herein, the term "Clostridial toxin target cell" means a cell that is a naturally occurring cell that a naturally occurring Clostridial toxin is capable of intoxicating, including, without limitation, motor neurons; sensory neurons; autonomic neurons; such as, *e.g.*, sympathetic neurons and parasympathetic neurons; non-peptidergic neurons, such as, *e.g.*, cholinergic neurons, adrenergic neurons, noradrenergic neurons, serotonergic neurons, GABAergic neurons; and peptidergic neurons, such as, *e.g.*, Substance P neurons, Calcitonin Gene Related Peptide neurons, vasoactive intestinal peptide neurons, Neuropeptide Y neurons, cholecystokinin neurons.

[0117] It is envisioned that any and all enhanced targeting domains that exhibits a binding activity for a non-Clostridial toxin receptor system present on a naturally-occurring Clostridial toxin target cell can be used to practice aspects of the present invention, including, without limitation, polypeptides that selectively bind to a receptor system present on a presynaptic membrane and polypeptides that selectively bind to a receptor system present on a postsynaptic membrane. Polypeptides that appear to bind to a receptor system present on a presynaptic membrane, include, without limitation, Glucagon like hormones, such as, *e.g.*, secretin, glucagon-like peptides, pituitary adenylate cyclase activating peptide (PACAP), glicentin, glicentin-related polypeptide (GRPP), oxyntomodulin (OXY), vasoactive intestinal peptides (VIPs), gastric inhibitory polypeptide (GIP), and calcitonin-related peptides; visceral gut peptides; neurohormones, such as, *e.g.*, corticotropin-releasing hormone (CRH) and parathyroid hormone (PTH); neuroregulatory cytokines, such as, *e.g.*, ciliary neurotrophic factor (CNTF), glycophorin-A (GPA), leukemia inhibitory factor (LIF), interleukins (ILs), onostatin M, cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), neuroleukins, VEGF, insulin-like growth factors (IGFs), epidermal growth factor (EGF); neurotrophins, such as, *e.g.*, nerve growth factors (NGFs), brain-derived growth factors (BDNFs), neurotrophin-3s (NT-3s) and neurotrophin-4/5s (NT-4/5s); growth factors, such as, *e.g.*, glial cell derived neurotrophic factor (GDNF), neurturin, persephrin, artemin, transformation growth factor betas (TGFβs), bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins; axon guidance signaling molecules, such as, *e.g.*, netrins, semaphorins and ephrins; sugar binding proteins, such as, *e.g.*, serum amyloid P, β-glucanase, sialidase, lectin, crya, insecticidal delta-endotoxin, agglutinin, abrin and ricin; ligands that selectively bind neurexins, such as, *e.g.*, ligands for neurexin-1α and neurexin-1β; ligands for neurexin-2α and neurexin-2β; and ligands for neurexin-3α and neurexin-3β; and WNTs. Ligands that appear to bind to a receptor system present on a postsynaptic membrane, include, without limitation, Ng-CAM(L1), NCAM, N-cadherin, Agrin-MUSK, basement membrane polypeptides, such as, *e.g.*, laminin β-2.

[0118] An enhanced targeting domain includes, without limitation, naturally occurring enhanced targeting domain variants, such as, *e.g.*, enhanced targeting domain isoforms; non-naturally occurring enhanced targeting domain variants, such as, *e.g.*, conservative enhanced targeting domain variants, non-conservative enhanced targeting domain variants, enhanced targeting domain chimerics, active enhanced targeting domain fragments thereof, or any combination thereof.

[0119] As used herein, the term “variant,” when used to describe an enhanced targeting domain variant, whether naturally-occurring or non-naturally-occurring, means an enhanced targeting domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, all enhanced targeting domain variants disclosed in the present specification are capable of selectively binding to a non-Clostridial toxin receptor system present on a Clostridial toxin target cell and initiate the overall internalization mechanism whereby a Clostridial toxin intoxicates a target cell. As non-limiting examples, a glycogen-like peptide variant derived from amino acids 21-50 of SEQ ID NO: 9 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 21-50 of SEQ ID NO: 9; a PACAP variant derived from amino acids 132-158 of SEQ ID NO: 10 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 132-158 of SEQ ID NO: 10; a CCRH variant derived from amino acids 159-193 of SEQ ID NO: 19 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 159-193 of SEQ ID NO: 19; a PTH variant derived from amino acids 35-70 of SEQ ID NO: 20 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 35-70 of SEQ ID NO: 20; an IL-6 variant derived from amino acids 57-210 of SEQ ID NO: 28 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 57-210 of SEQ ID NO: 28; a neuroleukin variant derived from amino acids 54-546 of SEQ ID NO: 31 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 54-546 of SEQ ID NO: 31; an IGF-1 variant derived from amino acids 52-109 of SEQ ID NO: 33 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 52-109 of SEQ ID NO: 33; and a NT-3 variant derived from amino acids 144-249 of SEQ ID NO: 38 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 144-249 of SEQ ID NO: 38.

[0120] It is recognized by those of skill in the art that there can be naturally occurring enhanced targeting domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. As used herein, the term “naturally occurring enhanced targeting domain variant” means any enhanced targeting domain produced by a naturally-occurring process, including, without limitation,

enhanced targeting domain isoforms produced from alternatively-spliced transcripts, enhanced targeting domain isoforms produced by spontaneous mutation and enhanced targeting domain subtypes. A naturally occurring enhanced targeting domain variant can function in substantially the same manner as the reference enhanced targeting domain on which the naturally occurring enhanced targeting domain variant is based, and can be substituted for the reference enhanced targeting domain in any aspect of the present invention. A naturally occurring enhanced targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference enhanced targeting domain on which the naturally occurring enhanced targeting domain variant is based. A naturally occurring enhanced targeting domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference enhanced targeting domain on which the naturally occurring enhanced targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enhanced targeting domain on which the naturally occurring enhanced targeting domain variant is based.

[0121] A non-limiting examples of a naturally occurring enhanced targeting domain variant is an enhanced targeting domain isoform such as, *e.g.*, a glucogen-like peptide isoform, a PACAP isoform, a CCRH isoform, a PTH isoform, an IL-6 isoform, a neuroleukin isoform, an IGF-1 isoform, and a NT-3 isoform. An enhanced targeting domain isoform can function in substantially the same manner as the reference enhanced targeting domain on which the enhanced targeting domain isoform is based, and can be substituted for the reference enhanced targeting domain in any aspect of the present invention.

[0122] As used herein, the term “non-naturally occurring enhanced targeting domain variant” means any enhanced targeting domain produced with the aid of human manipulation, including, without limitation, enhanced targeting domains produced by genetic engineering using random mutagenesis or rational design and enhanced targeting domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring enhanced targeting domain variants include, *e.g.*, conservative enhanced targeting domain variants, non-conservative enhanced targeting domain variants, enhanced targeting domain chimeric variants and active enhanced targeting domain fragments.

[0123] As used herein, the term “conservative enhanced targeting domain variant” means an enhanced targeting domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference enhanced targeting domain sequence. Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative enhanced targeting domain variant can function in substantially the same manner as the reference

enhanced targeting domain on which the conservative enhanced targeting domain variant is based, and can be substituted for the reference enhanced targeting domain in any aspect of the present invention. A conservative enhanced targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference enhanced targeting domain on which the conservative enhanced targeting domain variant is based. A conservative enhanced targeting domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference enhanced targeting domain on which the conservative enhanced targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enhanced targeting domain on which the conservative enhanced targeting domain variant is based. Non-limiting examples of a conservative enhanced targeting domain variant include, *e.g.*, conservative glucogen-like peptide variants, conservative PACAP region variants, conservative CCRH region variants, conservative PTH variants, conservative IL-6 variants, conservative neuroleukin variants, conservative IGF-1 variants, and conservative NT-3 region variants.

[0124] As used herein, the term “non-conservative enhanced targeting domain variant” means an enhanced targeting domain in which 1) at least one amino acid is deleted from the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based; 2) at least one amino acid added to the reference enhanced targeting domain on which the non-conservative enhanced targeting domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference enhanced targeting domain sequence. A non-conservative enhanced targeting domain variant can function in substantially the same manner as the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based, and can be substituted for the reference enhanced targeting domain in any aspect of the present invention. A non-conservative enhanced targeting domain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based. A non-conservative enhanced targeting domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based. A non-conservative enhanced targeting domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the

reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based. A non-conservative enhanced targeting domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference enhanced targeting domain on which the non-conservative enhanced targeting domain variant is based. Non-limiting examples of a non-conservative enhanced targeting domain variant include, *e.g.*, non-conservative glucagon-like peptide variants, non-conservative PACAP region variants, non-conservative CCRH region variants, non-conservative PTH variants, non-conservative IL-6 variants, non-conservative neuroleukin variants, non-conservative IGF-1 variants, and non-conservative NT-3 region variants.

[0125] As used herein, the term “enhanced targeting domain chimeric” means a polypeptide comprising at least a portion of an enhanced targeting domain and at least a portion of at least one other polypeptide to form an enhanced targeting domain with at least one property different from the reference enhanced targeting domain, with the proviso that this enhanced targeting domain chimeric is still capable of selectively binding to a non-Clostridial toxin receptor system present on a Clostridial toxin target cell and initiate the overall internalization mechanism whereby a Clostridial toxin intoxicates a target cell.

[0126] As used herein, the term “active enhanced targeting domain fragment” means any of a variety of enhanced targeting domain fragments can be useful in aspects of the present invention with the proviso that these active fragments are still capable of selectively binding to a non-Clostridial toxin receptor system present on a Clostridial toxin target cell and initiate the overall internalization mechanism whereby a Clostridial toxin intoxicates a target cell. Thus, aspects of this embodiment can include enhanced targeting domains comprising a length of, *e.g.*, at least 50 amino acids, at least 100 amino acids, at least 150 amino acids, at least 200 amino acids, at least 250 amino acids, at least 300 amino acids, at least 350 amino acids, at least 400 amino acids and at least 450 amino acids. Other aspects of this embodiment can include enhanced targeting domains comprising translocation domain having a length of, *e.g.*, at most 50 amino acids, at most 100 amino acids, at most 150 amino acids, at most 200 amino acids, at most 250 amino acids, at most 300 amino acids, at most 350 amino acids, at most 400 amino acids and at most 450 amino acids.

[0127] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring enhanced targeting domain variants and non-naturally-occurring enhanced targeting domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0128] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises an enhanced targeting domain. In an aspect of this embodiment, an enhanced targeting domain comprises a naturally occurring enhanced targeting domain variant, such as, *e.g.*, an enhanced targeting domain isoform or an enhanced targeting domain subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring enhanced targeting domain variant, such as, *e.g.*, a conservative enhanced targeting domain variant, a non-conservative enhanced targeting domain variant, a enhanced targeting domain chimeric, an active enhanced targeting domain fragment, or any combination thereof.

[0129] An example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, Glucagon like hormones, such as, *e.g.*, secretin, glucagon-like peptides, like GLP-1 and GLP-2, pituitary adenylate cyclase activating peptide (PACAP), glicentin, glicentin-related polypeptide (GRPP), oxyntomodulin (OXY), growth hormone-releasing hormone (GHRH), vasoactive intestinal peptides (VIPs), gastric inhibitory polypeptide (GIP), and calcitonin-related peptides visceral gut peptides, such as, *e.g.*, gastrin, gastrin-releasing peptide (GRP, bombesin) or cholecystokinin (CCK).

[0130] Thus, in an embodiment, an enhanced binding domain is derived from a glycogen-like peptide. In another embodiment, an enhanced binding domain is derived from a glycogen-like peptide of SEQ ID NO: 9. In aspects of this embodiment, an enhanced binding domain is derived from a glycogen-like peptide comprises a GRPP, a GLP-1, a GLP-2, a glucagon or an oxyntomodulin. In aspects of this embodiment, an enhanced binding domain is derived from a glycogen-like peptide comprises amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9.

[0131] In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at least 75% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at least 80% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at least 85% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at least 90% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9 or at least 95% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In yet other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at most 75% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at most 80% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at most 85% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at most 90% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, or at most 95% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9.

NO: 9, at most 85% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9, at most 90% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9 or at most 95% amino acid identity with amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9.

[0132] In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In yet other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In still other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9.

[0133] In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In yet other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino

acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In still other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9. In other aspects of this embodiment, a glycogen-like peptide comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-50, amino acids 53-81, amino acids 53-89, amino acids 98-124, or amino acids 146-178 of SEQ ID NO: 9.

[0134] In another embodiment, an enhanced binding domain is derived from a PACAP. In another embodiment, an enhanced binding domain is derived from a PACAP of SEQ ID NO: 10. In an aspect of this embodiment, an enhanced binding domain is derived from a PACAP comprises amino acids 132-158 of SEQ ID NO: 10.

[0135] In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at least 75% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at least 80% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at least 85% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at least 90% amino acid identity with amino acids 132-158 of SEQ ID NO: 10 or at least 95% amino acid identity with amino acids 132-158 of SEQ ID NO: 10. In yet other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at most 75% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at most 80% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at most 85% amino acid identity with amino acids 132-158 of SEQ ID NO: 10, at most 90% amino acid identity with amino acids 132-158 of SEQ ID NO: 10 or at most 95% amino acid identity with amino acids 132-158 of SEQ ID NO: 10.

[0136] In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 132-158 of SEQ ID NO: 10. In yet other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 132-158 of SEQ ID NO: 10. In still other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five,

six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 132-158 of SEQ ID NO: 10.

[0137] In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 132-158 of SEQ ID NO: 10. In yet other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 132-158 of SEQ ID NO: 10. In still other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 132-158 of SEQ ID NO: 10. In other aspects of this embodiment, a PACAP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 132-158 of SEQ ID NO: 10.

[0138] In another embodiment, an enhanced binding domain is derived from a GHRH. In another embodiment, an enhanced binding domain is derived from a GHRH of SEQ ID NO: 11. In aspects of this embodiment, an enhanced binding domain is derived from a GHRH comprises amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11.

[0139] In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at least 75% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at least 80% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at least 85% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at least 90% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11 or at least 95% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In yet other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at most 75% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at most 80% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at most 85% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11, at most 90% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11 or at most 95% amino acid identity with amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11.

[0140] In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In yet other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In still other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11.

[0141] In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In yet other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In still other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11. In other aspects of this embodiment, a GHRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 32-58 or amino acids 32-75 of SEQ ID NO: 11.

[0142] In another embodiment, an enhanced binding domain is derived from a VIP1. In another embodiment, an enhanced binding domain is derived from a VIP1 of SEQ ID NO: 12. In aspects of this embodiment, an enhanced binding domain is derived from a VIP1 comprises amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12.

[0143] In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at least 75% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at least 80% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at least 85% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at least 90% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12 or at least 95% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In yet other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at most 75% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at most 80% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at most 85% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12, at most 90% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12 or at most 95% amino acid identity with amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12.

[0144] In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In yet other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In still other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12.

[0145] In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In yet other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to

amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In still other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12. In other aspects of this embodiment, a VIP1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 81-107 or amino acids 125-151 of SEQ ID NO: 12.

[0146] In another embodiment, an enhanced binding domain is derived from a VIP2. In another embodiment, an enhanced binding domain is derived from a VIP2 of SEQ ID NO: 13. In aspects of this embodiment, an enhanced binding domain is derived from a VIP2 comprises amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13.

[0147] In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at least 75% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at least 80% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at least 85% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at least 90% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13 or at least 95% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In yet other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at most 75% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at most 80% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at most 85% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13, at most 90% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13 or at most 95% amino acid identity with amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13.

[0148] In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In yet other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven,

eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In still other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13.

[0149] In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In yet other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In still other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13. In other aspects of this embodiment, a VIP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 81-107 or amino acids 124-150 of SEQ ID NO: 13.

[0150] In another embodiment, an enhanced binding domain is derived from a GIP. In another embodiment, an enhanced binding domain is derived from a GIP of SEQ ID NO: 14. In aspects of this embodiment, an enhanced binding domain is derived from a GIP comprises amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14.

[0151] In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at least 75% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at least 80% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at least 85% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at least 90% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14 or at least 95% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In yet other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at most 75% amino acid identity with amino acids 52-78 or amino acids

52-93 of SEQ ID NO: 14, at most 80% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at most 85% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14, at most 90% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14 or at most 95% amino acid identity with amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14.

[0152] In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In yet other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In still other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14.

[0153] In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In yet other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In still other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14. In other aspects of this embodiment, a GIP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 52-78 or amino acids 52-93 of SEQ ID NO: 14.

[0154] In another embodiment, an enhanced binding domain is derived from a Secretin. In another embodiment, an enhanced binding domain is derived from a Secretin of SEQ ID NO: 15. In an aspect of this embodiment, an enhanced binding domain is derived from a Secretin comprises amino acids 28-54 of SEQ ID NO: 15.

[0155] In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at least 75% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at least 80% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at least 85% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at least 90% amino acid identity with amino acids 28-54 of SEQ ID NO: 15 or at least 95% amino acid identity with amino acids 28-54 of SEQ ID NO: 15. In yet other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at most 75% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at most 80% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at most 85% amino acid identity with amino acids 28-54 of SEQ ID NO: 15, at most 90% amino acid identity with amino acids 28-54 of SEQ ID NO: 15 or at most 95% amino acid identity with amino acids 28-54 of SEQ ID NO: 15.

[0156] In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 28-54 of SEQ ID NO: 15. In yet other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 28-54 of SEQ ID NO: 15. In still other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 28-54 of SEQ ID NO: 15.

[0157] In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 28-54 of SEQ ID NO: 15. In yet other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six,

seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 28-54 of SEQ ID NO: 15. In still other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 28-54 of SEQ ID NO: 15. In other aspects of this embodiment, a Secretin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 28-54 of SEQ ID NO: 15.

[0158] In another embodiment, an enhanced binding domain is derived from a Gastrin. In another embodiment, an enhanced binding domain is derived from a Gastrin of SEQ ID NO: 16. In aspects of this embodiment, an enhanced binding domain is derived from a Gastrin comprises amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16.

[0159] In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at least 75% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at least 80% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at least 85% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at least 90% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16 or at least 95% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In yet other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at most 75% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at most 80% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at most 85% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16, at most 90% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16 or at most 95% amino acid identity with amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16.

[0160] In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In yet other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 76-92 or amino

acids 59-92 of SEQ ID NO: 16. In still other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16.

[0161] In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In yet other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In still other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16. In other aspects of this embodiment, a Gastrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 76-92 or amino acids 59-92 of SEQ ID NO: 16.

[0162] In another embodiment, an enhanced binding domain is derived from a GRP. In another embodiment, an enhanced binding domain is derived from a GRP of SEQ ID NO: 17. In aspects of this embodiment, an enhanced binding domain is derived from a GRP comprises amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17.

[0163] In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at least 75% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at least 80% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at least 85% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at least 90% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17 or at least 95% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In yet other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at most 75% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at most 80% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at most 85% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17, at most 90% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17 or at most 95% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17.

NO: 17, at most 90% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17 or at most 95% amino acid identity with amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17.

[0164] In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In yet other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In still other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17.

[0165] In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In yet other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In still other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17. In other aspects of this embodiment, a GRP comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 41-50 or amino acids 24-50 of SEQ ID NO: 17.

[0166] In another embodiment, an enhanced binding domain is derived from a CCK. In another embodiment, an enhanced binding domain is derived from a CCK of SEQ ID NO: 18. In an aspect of this

embodiment, an enhanced binding domain is derived from a CCK comprises amino acids 99-112 of SEQ ID NO: 18.

[0167] In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at least 75% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at least 80% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at least 85% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at least 90% amino acid identity with amino acids 99-112 of SEQ ID NO: 18 or at least 95% amino acid identity with amino acids 99-112 of SEQ ID NO: 18. In yet other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at most 75% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at most 80% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at most 85% amino acid identity with amino acids 99-112 of SEQ ID NO: 18, at most 90% amino acid identity with amino acids 99-112 of SEQ ID NO: 18 or at most 95% amino acid identity with amino acids 99-112 of SEQ ID NO: 18.

[0168] In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 99-112 of SEQ ID NO: 18. In yet other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 99-112 of SEQ ID NO: 18. In still other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 99-112 of SEQ ID NO: 18.

[0169] In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 99-112 of SEQ ID NO: 18. In yet other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two,

three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 99-112 of SEQ ID NO: 18. In still other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 99-112 of SEQ ID NO: 18. In other aspects of this embodiment, a CCK comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 99-112 of SEQ ID NO: 18.

[0170] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, neurohormones, such as, *e.g.*, corticotropin-releasing hormone (CCRH) or parathyroid hormone (PTH).

[0171] Thus, in an embodiment, an enhanced binding domain is derived from a CCRH. In another embodiment, an enhanced binding domain is derived from a CCRH of SEQ ID NO: 19. In aspects of this embodiment, an enhanced binding domain is derived from a CCRH comprises amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19.

[0172] In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at least 75% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at least 80% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at least 85% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at least 90% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19 or at least 95% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In yet other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at most 75% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at most 80% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at most 85% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19, at most 90% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19 or at most 95% amino acid identity with amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19.

[0173] In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In yet other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven,

eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In still other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19.

[0174] In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In yet other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In still other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19. In other aspects of this embodiment, a CCRH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 159-193 or amino acids 154-194 of SEQ ID NO: 19.

[0175] In another embodiment, an enhanced binding domain is derived from a PTH. In another embodiment, an enhanced binding domain is derived from a PTH of SEQ ID NO: 20. In aspects of this embodiment, an enhanced binding domain is derived from a PTH comprises amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20.

[0176] In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at least 75% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at least 80% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at least 85% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at least 90% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20 or at least 95% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In yet other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at most 75% amino acid identity with

amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at most 80% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at most 85% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20, at most 90% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20 or at most 95% amino acid identity with amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20.

[0177] In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In yet other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In still other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20.

[0178] In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In yet other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In still other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20. In other aspects of this embodiment, a PTH comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 35-70 or amino acids 145-177 of SEQ ID NO: 20.

[0179] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, neuroregulatory cytokines, such as, *e.g.*, ciliary neurotrophic factor (CNTF), glycophorin-A (GPA), leukemia inhibitory factor (LIF), also known as cholinergic differentiation factor (CDF), interleukins (ILs), like IL1, IL2, IL6, IL8 and IL10, onostatin M, cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), or neuroleukin, also known as glucose phosphate isomerase (GPI), autocrine motility factor (AMF), maturation and differentiation factor (MF).

[0180] Thus, in an embodiment, an enhanced binding domain is derived from a CNTF. In another embodiment, an enhanced binding domain is derived from a CNTF of SEQ ID NO: 21. In aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 21 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 21. In yet other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 21, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 21 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 21.

[0181] In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 21. In yet other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 21. In still other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 21.

[0182] In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the

amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 21. In yet other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 21. In still other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 21. In other aspects of this embodiment, a CNTF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 21.

[0183] In another embodiment, an enhanced binding domain is derived from a GPA. In another embodiment, an enhanced binding domain is derived from a GPA of SEQ ID NO: 22. In aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 22 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 22. In yet other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 22, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 22 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 22.

[0184] In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 22. In yet other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 22. In still other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven,

eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 22.

[0185] In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 22. In yet other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 22. In still other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 22. In other aspects of this embodiment, a GPA comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 22.

[0186] In another embodiment, an enhanced binding domain is derived from a LIF. In another embodiment, an enhanced binding domain is derived from a LIF of SEQ ID NO: 23. In aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 23 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 23. In yet other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 23, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 23 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 23.

[0187] In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-

contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 23. In yet other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 23. In still other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 23.

[0188] In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 23. In yet other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 23. In still other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 23. In other aspects of this embodiment, a LIF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 23.

[0189] In another embodiment, an enhanced binding domain is derived from a CT-1. In another embodiment, an enhanced binding domain is derived from a CT-1 of SEQ ID NO: 24. In aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 24 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 24. In yet other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 24, at most 85% amino acid identity with the

amino acid sequence of SEQ ID NO: 24, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 24 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 24.

[0190] In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 24. In yet other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 24. In still other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 24.

[0191] In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 24. In yet other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 24. In still other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 24. In other aspects of this embodiment, a CT-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 24.

[0192] In another embodiment, an enhanced binding domain is derived from a CLC. In another embodiment, an enhanced binding domain is derived from a CLC of SEQ ID NO: 25. In aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at least 75% amino acid identity with the amino acid sequence of SEQ

ID NO: 25, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 25 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 25. In yet other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 25, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 25 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 25.

[0193] In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 25. In yet other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 25. In still other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 25.

[0194] In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 25. In yet other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 25. In still other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 25. In other aspects of this embodiment, a CLC comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven,

eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 25.

[0195] In another embodiment, an enhanced binding domain is derived from an IL-1. In another embodiment, an enhanced binding domain is derived from an IL-1 of SEQ ID NO: 26. In an aspect of this embodiment, an enhanced binding domain is derived from an IL-1 comprises amino acids 123-265 of SEQ ID NO: 26.

[0196] In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at least 75% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at least 80% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at least 85% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at least 90% amino acid identity with amino acids 123-265 of SEQ ID NO: 26 or at least 95% amino acid identity with amino acids 123-265 of SEQ ID NO: 26. In yet other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at most 75% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at most 80% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at most 85% amino acid identity with amino acids 123-265 of SEQ ID NO: 26, at most 90% amino acid identity with amino acids 123-265 of SEQ ID NO: 26 or at most 95% amino acid identity with amino acids 123-265 of SEQ ID NO: 26.

[0197] In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 123-265 of SEQ ID NO: 26. In yet other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 123-265 of SEQ ID NO: 26. In still other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 123-265 of SEQ ID NO: 26.

[0198] In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a

polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 123-265 of SEQ ID NO: 26. In yet other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 123-265 of SEQ ID NO: 26. In still other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 123-265 of SEQ ID NO: 26. In other aspects of this embodiment, an IL-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 123-265 of SEQ ID NO: 26.

[0199] In another embodiment, an enhanced binding domain is derived from an IL-2. In another embodiment, an enhanced binding domain is derived from an IL-2 of SEQ ID NO: 27. In an aspect of this embodiment, an enhanced binding domain is derived from an IL-2 comprises amino acids 21-153 of SEQ ID NO: 27.

[0200] In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at least 75% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at least 80% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at least 85% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at least 90% amino acid identity with amino acids 21-153 of SEQ ID NO: 27 or at least 95% amino acid identity with amino acids 21-153 of SEQ ID NO: 27. In yet other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at most 75% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at most 80% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at most 85% amino acid identity with amino acids 21-153 of SEQ ID NO: 27, at most 90% amino acid identity with amino acids 21-153 of SEQ ID NO: 27 or at most 95% amino acid identity with amino acids 21-153 of SEQ ID NO: 27.

[0201] In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-153 of SEQ ID NO: 27. In yet other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-153 of SEQ ID NO: 27. In still other aspects of this embodiment, an IL-2

comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-153 of SEQ ID NO: 27.

[0202] In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-153 of SEQ ID NO: 27. In yet other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 21-153 of SEQ ID NO: 27. In still other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-153 of SEQ ID NO: 27. In other aspects of this embodiment, an IL-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-153 of SEQ ID NO: 27.

[0203] In another embodiment, an enhanced binding domain is derived from an IL-6. In another embodiment, an enhanced binding domain is derived from an IL-6 of SEQ ID NO: 28. In an aspect of this embodiment, an enhanced binding domain is derived from an IL-6 comprises amino acids 57-210 of SEQ ID NO: 28.

[0204] In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at least 75% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at least 80% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at least 85% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at least 90% amino acid identity with amino acids 57-210 of SEQ ID NO: 28 or at least 95% amino acid identity with amino acids 57-210 of SEQ ID NO: 28. In yet other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at most 75% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at most 80% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at most 85% amino acid identity with amino acids 57-210 of SEQ ID NO: 28, at most 90% amino acid identity with amino acids 57-210 of SEQ ID NO: 28 or at most 95% amino acid identity with amino acids 57-210 of SEQ ID NO: 28.

[0205] In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 57-210 of SEQ ID NO: 28. In yet other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 57-210 of SEQ ID NO: 28. In still other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 57-210 of SEQ ID NO: 28.

[0206] In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 57-210 of SEQ ID NO: 28. In yet other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 57-210 of SEQ ID NO: 28. In still other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 57-210 of SEQ ID NO: 28. In other aspects of this embodiment, an IL-6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 57-210 of SEQ ID NO: 28.

[0207] In another embodiment, an enhanced binding domain is derived from an IL-8. In another embodiment, an enhanced binding domain is derived from an IL-8 of SEQ ID NO: 29. In an aspect of this embodiment, an enhanced binding domain is derived from an IL-8 comprises amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29.

[0208] In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at least 75% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at least 80% amino acid

identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at least 85% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at least 90% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29 or at least 95% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In yet other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at most 75% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at most 80% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at most 85% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29, at most 90% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29 or at most 95% amino acid identity with amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29.

[0209] In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In yet other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In still other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29.

[0210] In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In yet other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In still other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at most one, two,

three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29. In other aspects of this embodiment, an IL-8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 29.

[0211] In another embodiment, an enhanced binding domain is derived from an IL-10. In another embodiment, an enhanced binding domain is derived from an IL-10 of SEQ ID NO: 30. In an aspect of this embodiment, an enhanced binding domain is derived from an IL-10 comprises amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30.

[0212] In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at least 75% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at least 80% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at least 85% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at least 90% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30 or at least 95% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In yet other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at most 75% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at most 80% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at most 85% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30, at most 90% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30 or at most 95% amino acid identity with amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30.

[0213] In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In yet other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In still other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six,

seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30.

[0214] In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In yet other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In still other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30. In other aspects of this embodiment, an IL-10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 30.

[0215] In another embodiment, an enhanced binding domain is derived from a neuroleukin. In another embodiment, an enhanced binding domain is derived from a neuroleukin of SEQ ID NO: 31. In aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 31 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 31. In yet other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 31, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 31 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 31.

[0216] In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight,

nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 31. In yet other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 31. In still other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 31.

[0217] In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 31. In yet other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 31. In still other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 31. In other aspects of this embodiment, a neuroleukin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 31.

[0218] In another embodiment, an enhanced binding domain is derived from a VEGF. In another embodiment, an enhanced binding domain is derived from a VEGF of SEQ ID NO: 32. In aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 32 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 32. In yet other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 32, at most 85% amino acid identity with the

amino acid sequence of SEQ ID NO: 32, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 32 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 32.

[0219] In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 32. In yet other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 32. In still other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 32.

[0220] In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 32. In yet other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 32. In still other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 32. In other aspects of this embodiment, a VEGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 32.

[0221] In another embodiment, an enhanced binding domain is derived from an IGF-1. In another embodiment, an enhanced binding domain is derived from an IGF-1 of SEQ ID NO: 33. In an aspect of this embodiment, an enhanced binding domain is derived from an IGF-1 comprises amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33.

[0222] In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at least 75% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at least 80% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at least 85% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at least 90% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33 or at least 95% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In yet other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at most 75% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at most 80% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at most 85% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33, at most 90% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33 or at most 95% amino acid identity with amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33.

[0223] In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In yet other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In still other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33.

[0224] In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In yet other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at

most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In still other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33. In other aspects of this embodiment, an IGF-1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 33.

[0225] In another embodiment, an enhanced binding domain is derived from an IGF-2. In another embodiment, an enhanced binding domain is derived from an IGF-2 of SEQ ID NO: 34. In an aspect of this embodiment, an enhanced binding domain is derived from an IGF-2 comprises amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34.

[0226] In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at least 75% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at least 80% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at least 85% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at least 90% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34 or at least 95% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In yet other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at most 75% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at most 80% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at most 85% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34, at most 90% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34 or at most 95% amino acid identity with amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34.

[0227] In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In yet other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this

embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In still other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34.

[0228] In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In yet other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In still other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34. In other aspects of this embodiment, an IGF-2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 34.

[0229] In another embodiment, an enhanced binding domain is derived from an EGF. In another embodiment, an enhanced binding domain is derived from an EGF of SEQ ID NO: 35. In aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at least 75% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at least 80% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at least 85% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at least 90% amino acid identity with the amino acid sequence of SEQ ID NO: 35 or at least 95% amino acid identity with the amino acid sequence of SEQ ID NO: 35. In yet other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at most 75% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at most 80% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at most 85% amino acid identity with the amino acid sequence of SEQ ID NO: 35, at most 90% amino acid identity with the amino acid sequence of SEQ ID NO: 35 or at most 95% amino acid identity with the amino acid sequence of SEQ ID NO: 35.

[0230] In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 35. In yet other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 35. In still other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 35.

[0231] In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 35. In yet other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to the amino acid sequence of SEQ ID NO: 35. In still other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 35. In other aspects of this embodiment, an EGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to the amino acid sequence of SEQ ID NO: 35.

[0232] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, a neurotrophin, such as, *e.g.*, a NGF, a BDNF, a NT-3 or a NT-5.

[0233] Thus, in an embodiment, an enhanced binding domain is derived from a NGF. In another embodiment, an enhanced binding domain is derived from a NGF of SEQ ID NO: 36. In an aspect of this

embodiment, an enhanced binding domain is derived from a NGF comprises amino acids 139-257 of SEQ ID NO: 36.

[0234] In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at least 75% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at least 80% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at least 85% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at least 90% amino acid identity with amino acids 139-257 of SEQ ID NO: 36 or at least 95% amino acid identity with amino acids 139-257 of SEQ ID NO: 36. In yet other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at most 75% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at most 80% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at most 85% amino acid identity with amino acids 139-257 of SEQ ID NO: 36, at most 90% amino acid identity with amino acids 139-257 of SEQ ID NO: 36 or at most 95% amino acid identity with amino acids 139-257 of SEQ ID NO: 36.

[0235] In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 139-257 of SEQ ID NO: 36. In yet other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 139-257 of SEQ ID NO: 36. In still other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 139-257 of SEQ ID NO: 36.

[0236] In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 139-257 of SEQ ID NO: 36. In yet other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two,

three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 139-257 of SEQ ID NO: 36. In still other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 139-257 of SEQ ID NO: 36. In other aspects of this embodiment, a NGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 139-257 of SEQ ID NO: 36.

[0237] In another embodiment, an enhanced binding domain is derived from a BDGF. In another embodiment, an enhanced binding domain is derived from a BDGF of SEQ ID NO: 37. In an aspect of this embodiment, an enhanced binding domain is derived from a BDGF comprises amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37.

[0238] In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at least 75% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at least 80% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at least 85% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at least 90% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37 or at least 95% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In yet other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at most 75% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at most 80% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at most 85% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37, at most 90% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37 or at most 95% amino acid identity with amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37.

[0239] In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In yet other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In still other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino

acid additions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37.

[0240] In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In yet other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In still other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37. In other aspects of this embodiment, a BDGF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 133-240 or amino acids 129-247 of SEQ ID NO: 37.

[0241] In another embodiment, an enhanced binding domain is derived from a NT-3. In another embodiment, an enhanced binding domain is derived from a NT-3 of SEQ ID NO: 38. In an aspect of this embodiment, an enhanced binding domain is derived from a NT-3 comprises amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38.

[0242] In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at least 75% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at least 80% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at least 85% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at least 90% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38 or at least 95% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In yet other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at most 75% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at most 80% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at most 85% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38, at most 90% amino acid

identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38 or at most 95% amino acid identity with amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38.

[0243] In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In yet other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In still other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38.

[0244] In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In yet other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In still other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38. In other aspects of this embodiment, a NT-3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 144-249 or amino acids 19-257 of SEQ ID NO: 38.

[0245] In another embodiment, an enhanced binding domain is derived from a NT-4/5. In another embodiment, an enhanced binding domain is derived from a NT-4/5 of SEQ ID NO: 39. In an aspect of

this embodiment, an enhanced binding domain is derived from a NT-4/5 comprises amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39.

[0246] In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at least 75% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at least 80% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at least 85% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at least 90% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39 or at least 95% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In yet other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at most 75% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at most 80% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at most 85% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39, at most 90% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39 or at most 95% amino acid identity with amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39.

[0247] In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In yet other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In still other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39.

[0248] In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10

or 20 contiguous amino acid substitutions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In yet other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In still other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39. In other aspects of this embodiment, a NT-4/5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 89-202 or amino acids 81-210 of SEQ ID NO: 39.

[0249] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, a GDNF, a neurturin, a persephrin or an artemin.

[0250] Thus, in an embodiment, an enhanced binding domain is derived from a GDNF. In another embodiment, an enhanced binding domain is derived from a GDNF of SEQ ID NO: 40. In an aspect of this embodiment, an enhanced binding domain is derived from a GDNF comprises amino acids 118-211 of SEQ ID NO: 40.

[0251] In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at least 75% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at least 80% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at least 85% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at least 90% amino acid identity with amino acids 118-211 of SEQ ID NO: 40 or at least 95% amino acid identity with amino acids 118-211 of SEQ ID NO: 40. In yet other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at most 75% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at most 80% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at most 85% amino acid identity with amino acids 118-211 of SEQ ID NO: 40, at most 90% amino acid identity with amino acids 118-211 of SEQ ID NO: 40 or at most 95% amino acid identity with amino acids 118-211 of SEQ ID NO: 40.

[0252] In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 118-211 of SEQ ID NO: 40. In yet other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four,

five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 118-211 of SEQ ID NO: 40. In still other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 118-211 of SEQ ID NO: 40.

[0253] In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 118-211 of SEQ ID NO: 40. In yet other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 118-211 of SEQ ID NO: 40. In still other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 118-211 of SEQ ID NO: 40. In other aspects of this embodiment, a GDNF comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 118-211 of SEQ ID NO: 40.

[0254] In another embodiment, an enhanced binding domain is derived from a Neurturin. In another embodiment, an enhanced binding domain is derived from a Neurturin of SEQ ID NO: 41. In an aspect of this embodiment, an enhanced binding domain is derived from a Neurturin comprises amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41.

[0255] In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at least 75% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at least 80% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at least 85% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at least 90% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41 or at least 95% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In yet other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at most 75% amino acid

identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at most 80% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at most 85% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41, at most 90% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41 or at most 95% amino acid identity with amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41.

[0256] In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In yet other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In still other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41.

[0257] In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In yet other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In still other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41. In other aspects of this embodiment, a Neurturin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 107-196 or amino acids 96-197 of SEQ ID NO: 41.

[0258] In another embodiment, an enhanced binding domain is derived from a Persephrin. In another embodiment, an enhanced binding domain is derived from a Persephrin of SEQ ID NO: 42. In an aspect of this embodiment, an enhanced binding domain is derived from a Persephrin comprises amino acids 66-155 of SEQ ID NO: 42.

[0259] In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at least 75% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at least 80% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at least 85% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at least 90% amino acid identity with amino acids 66-155 of SEQ ID NO: 42 or at least 95% amino acid identity with amino acids 66-155 of SEQ ID NO: 42. In yet other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at most 75% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at most 80% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at most 85% amino acid identity with amino acids 66-155 of SEQ ID NO: 42, at most 90% amino acid identity with amino acids 66-155 of SEQ ID NO: 42 or at most 95% amino acid identity with amino acids 66-155 of SEQ ID NO: 42.

[0260] In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 66-155 of SEQ ID NO: 42. In yet other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 66-155 of SEQ ID NO: 42. In still other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 66-155 of SEQ ID NO: 42.

[0261] In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 66-155 of SEQ ID NO: 42. In yet other aspects of this

embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 66-155 of SEQ ID NO: 42. In still other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 66-155 of SEQ ID NO: 42. In other aspects of this embodiment, a Persephrin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 66-155 of SEQ ID NO: 42.

[0262] In another embodiment, an enhanced binding domain is derived from an Artemin. In another embodiment, an enhanced binding domain is derived from an Artemin of SEQ ID NO: 43. In an aspect of this embodiment, an enhanced binding domain is derived from an Artemin comprises amino acids 123-218 of SEQ ID NO: 43.

[0263] In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at least 75% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at least 80% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at least 85% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at least 90% amino acid identity with amino acids 123-218 of SEQ ID NO: 43 or at least 95% amino acid identity with amino acids 123-218 of SEQ ID NO: 43. In yet other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at most 75% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at most 80% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at most 85% amino acid identity with amino acids 123-218 of SEQ ID NO: 43, at most 90% amino acid identity with amino acids 123-218 of SEQ ID NO: 43 or at most 95% amino acid identity with amino acids 123-218 of SEQ ID NO: 43.

[0264] In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 123-218 of SEQ ID NO: 43. In yet other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 123-218 of SEQ ID NO: 43. In still other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six,

seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 123-218 of SEQ ID NO: 43.

[0265] In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 123-218 of SEQ ID NO: 43. In yet other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 123-218 of SEQ ID NO: 43. In still other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 123-218 of SEQ ID NO: 43. In other aspects of this embodiment, an Artemin comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 123-218 of SEQ ID NO: 43.

[0266] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, a TGF β s, such as, *e.g.*, TGF β 1, TGF β 2, TGF β 3 or TGF β 4.

[0267] Thus, in an embodiment, an enhanced binding domain is derived from a TGF β 1. In another embodiment, an enhanced binding domain is derived from a TGF β 1 of SEQ ID NO: 44. In an aspect of this embodiment, an enhanced binding domain is derived from a TGF β 1 comprises amino acids 293-390 of SEQ ID NO: 44.

[0268] In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at least 75% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at least 80% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at least 85% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at least 90% amino acid identity with amino acids 293-390 of SEQ ID NO: 44 or at least 95% amino acid identity with amino acids 293-390 of SEQ ID NO: 44. In yet other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at most 75% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at most 80% amino acid identity with amino acids 293-390 of SEQ ID NO: 44, at most 85% amino acid identity with

amino acids 293-390 of SEQ ID NO: 44, at most 90% amino acid identity with amino acids 293-390 of SEQ ID NO: 44 or at most 95% amino acid identity with amino acids 293-390 of SEQ ID NO: 44.

[0269] In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 293-390 of SEQ ID NO: 44. In yet other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 293-390 of SEQ ID NO: 44. In still other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 293-390 of SEQ ID NO: 44.

[0270] In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 293-390 of SEQ ID NO: 44. In yet other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 293-390 of SEQ ID NO: 44. In still other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 293-390 of SEQ ID NO: 44. In other aspects of this embodiment, a TGF β 1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 293-390 of SEQ ID NO: 44.

[0271] In another embodiment, an enhanced binding domain is derived from a TGF β 2. In another embodiment, an enhanced binding domain is derived from a TGF β 2 of SEQ ID NO: 44. In an aspect of this embodiment, an enhanced binding domain is derived from a TGF β 2 comprises amino acids 317-414 of SEQ ID NO: 45.

[0272] In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at least 75% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at least 80% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at least 85% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at least 90% amino acid identity with amino acids 317-414 of SEQ ID NO: 45 or at least 95% amino acid identity with amino acids 317-414 of SEQ ID NO: 45. In yet other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at most 75% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at most 80% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at most 85% amino acid identity with amino acids 317-414 of SEQ ID NO: 45, at most 90% amino acid identity with amino acids 317-414 of SEQ ID NO: 45 or at most 95% amino acid identity with amino acids 317-414 of SEQ ID NO: 45.

[0273] In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 317-414 of SEQ ID NO: 45. In yet other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 317-414 of SEQ ID NO: 45. In still other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 317-414 of SEQ ID NO: 45.

[0274] In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 317-414 of SEQ ID NO: 45. In yet other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 317-414 of SEQ ID NO: 45. In still other aspects of this embodiment, a TGF β 2 comprises a

polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 317-414 of SEQ ID NO: 45. In other aspects of this embodiment, a TGF β 2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 317-414 of SEQ ID NO: 45.

[0275] In another embodiment, an enhanced binding domain is derived from a TGF β 3. In another embodiment, an enhanced binding domain is derived from a TGF β 3 of SEQ ID NO: 44. In an aspect of this embodiment, an enhanced binding domain is derived from a TGF β 3 comprises amino acids 315-412 of SEQ ID NO: 46.

[0276] In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at least 75% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at least 80% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at least 85% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at least 90% amino acid identity with amino acids 315-412 of SEQ ID NO: 46 or at least 95% amino acid identity with amino acids 315-412 of SEQ ID NO: 46. In yet other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at most 75% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at most 80% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at most 85% amino acid identity with amino acids 315-412 of SEQ ID NO: 46, at most 90% amino acid identity with amino acids 315-412 of SEQ ID NO: 46 or at most 95% amino acid identity with amino acids 315-412 of SEQ ID NO: 46.

[0277] In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 315-412 of SEQ ID NO: 46. In yet other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 315-412 of SEQ ID NO: 46. In still other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 315-412 of SEQ ID NO: 46.

[0278] In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 315-412 of SEQ ID NO: 46. In yet other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 315-412 of SEQ ID NO: 46. In still other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 315-412 of SEQ ID NO: 46. In other aspects of this embodiment, a TGF β 3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 315-412 of SEQ ID NO: 46.

[0279] In another embodiment, an enhanced binding domain is derived from a TGF β 4. In another embodiment, an enhanced binding domain is derived from a TGF β 4 of SEQ ID NO: 44. In an aspect of this embodiment, an enhanced binding domain is derived from a TGF β 4 comprises amino acids 276-373 of SEQ ID NO: 47.

[0280] In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at least 75% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at least 80% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at least 85% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at least 90% amino acid identity with amino acids 276-373 of SEQ ID NO: 47 or at least 95% amino acid identity with amino acids 276-373 of SEQ ID NO: 47. In yet other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at most 75% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at most 80% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at most 85% amino acid identity with amino acids 276-373 of SEQ ID NO: 47, at most 90% amino acid identity with amino acids 276-373 of SEQ ID NO: 47 or at most 95% amino acid identity with amino acids 276-373 of SEQ ID NO: 47.

[0281] In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 276-373 of SEQ ID NO: 47. In yet other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four,

five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 276-373 of SEQ ID NO: 47. In still other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 276-373 of SEQ ID NO: 47.

[0282] In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 276-373 of SEQ ID NO: 47. In yet other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 276-373 of SEQ ID NO: 47. In still other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 276-373 of SEQ ID NO: 47. In other aspects of this embodiment, a TGF β 4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 276-373 of SEQ ID NO: 47.

[0283] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, a BMPs, such as, *e.g.*, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8 or BMP10.

[0284] Thus, in an embodiment, an enhanced binding domain is derived from a BMP2. In another embodiment, an enhanced binding domain is derived from a BMP2 of SEQ ID NO: 48. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP2 comprises amino acids 296-396 of SEQ ID NO: 48.

[0285] In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at least 75% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at least 80% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at least 85% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at least 90% amino acid identity with amino acids 296-396 of SEQ ID NO: 48 or at least 95% amino acid identity

with amino acids 296-396 of SEQ ID NO: 48. In yet other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at most 75% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at most 80% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at most 85% amino acid identity with amino acids 296-396 of SEQ ID NO: 48, at most 90% amino acid identity with amino acids 296-396 of SEQ ID NO: 48 or at most 95% amino acid identity with amino acids 296-396 of SEQ ID NO: 48.

[0286] In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 296-396 of SEQ ID NO: 48. In yet other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 296-396 of SEQ ID NO: 48. In still other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 296-396 of SEQ ID NO: 48.

[0287] In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 296-396 of SEQ ID NO: 48. In yet other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 296-396 of SEQ ID NO: 48. In still other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 296-396 of SEQ ID NO: 48. In other aspects of this embodiment, a BMP2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 296-396 of SEQ ID NO: 48.

[0288] In another embodiment, an enhanced binding domain is derived from a BMP3. In another embodiment, an enhanced binding domain is derived from a BMP3 of SEQ ID NO: 49. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP3 comprises amino acids 370-472 of SEQ ID NO: 49.

[0289] In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at least 75% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at least 80% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at least 85% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at least 90% amino acid identity with amino acids 370-472 of SEQ ID NO: 49 or at least 95% amino acid identity with amino acids 370-472 of SEQ ID NO: 49. In yet other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at most 75% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at most 80% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at most 85% amino acid identity with amino acids 370-472 of SEQ ID NO: 49, at most 90% amino acid identity with amino acids 370-472 of SEQ ID NO: 49 or at most 95% amino acid identity with amino acids 370-472 of SEQ ID NO: 49.

[0290] In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 370-472 of SEQ ID NO: 49. In yet other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 370-472 of SEQ ID NO: 49. In still other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 370-472 of SEQ ID NO: 49.

[0291] In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 370-472 of SEQ ID NO: 49. In yet other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven,

eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 370-472 of SEQ ID NO: 49. In still other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 370-472 of SEQ ID NO: 49. In other aspects of this embodiment, a BMP3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 370-472 of SEQ ID NO: 49.

[0292] In another embodiment, an enhanced binding domain is derived from a BMP4. In another embodiment, an enhanced binding domain is derived from a BMP4 of SEQ ID NO: 50. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP4 comprises amino acids 309-409 of SEQ ID NO: 50.

[0293] In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at least 75% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at least 80% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at least 85% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at least 90% amino acid identity with amino acids 309-409 of SEQ ID NO: 50 or at least 95% amino acid identity with amino acids 309-409 of SEQ ID NO: 50. In yet other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at most 75% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at most 80% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at most 85% amino acid identity with amino acids 309-409 of SEQ ID NO: 50, at most 90% amino acid identity with amino acids 309-409 of SEQ ID NO: 50 or at most 95% amino acid identity with amino acids 309-409 of SEQ ID NO: 50.

[0294] In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 309-409 of SEQ ID NO: 50. In yet other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 309-409 of SEQ ID NO: 50. In still other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six,

seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 309-409 of SEQ ID NO: 50.

[0295] In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 309-409 of SEQ ID NO: 50. In yet other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 309-409 of SEQ ID NO: 50. In still other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 309-409 of SEQ ID NO: 50. In other aspects of this embodiment, a BMP4 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 309-409 of SEQ ID NO: 50.

[0296] In another embodiment, an enhanced binding domain is derived from a BMP5. In another embodiment, an enhanced binding domain is derived from a BMP5 of SEQ ID NO: 51. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP5 comprises amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51.

[0297] In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at least 75% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at least 80% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at least 85% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at least 90% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51 or at least 95% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In yet other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at most 75% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at most 80% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at most 85% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51, at most 90% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51 or at most 95% amino acid identity with amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51.

[0298] In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In yet other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In still other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51.

[0299] In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In yet other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In still other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51. In other aspects of this embodiment, a BMP5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 51.

[0300] In another embodiment, an enhanced binding domain is derived from a BMP6. In another embodiment, an enhanced binding domain is derived from a BMP6 of SEQ ID NO: 52. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP6 comprises amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52.

[0301] In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at least 75% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at least 80% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at least 85% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at least 90% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52 or at least 95% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In yet other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at most 75% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at most 80% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at most 85% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52, at most 90% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52 or at most 95% amino acid identity with amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52.

[0302] In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In yet other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In still other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52.

[0303] In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In yet other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative

to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In still other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52. In other aspects of this embodiment, a BMP6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 52.

[0304] In another embodiment, an enhanced binding domain is derived from a BMP7. In another embodiment, an enhanced binding domain is derived from a BMP7 of SEQ ID NO: 53. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP7 comprises amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53.

[0305] In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at least 75% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at least 80% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at least 85% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at least 90% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53 or at least 95% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In yet other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at most 75% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at most 80% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at most 85% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53, at most 90% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53 or at most 95% amino acid identity with amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53.

[0306] In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In yet other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven,

eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In still other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53.

[0307] In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In yet other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In still other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53. In other aspects of this embodiment, a BMP7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 53.

[0308] In another embodiment, an enhanced binding domain is derived from a BMP8. In another embodiment, an enhanced binding domain is derived from a BMP8 of SEQ ID NO: 54. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP8 comprises amino acids 301-402 of SEQ ID NO: 54.

[0309] In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at least 75% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at least 80% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at least 85% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at least 90% amino acid identity with amino acids 301-402 of SEQ ID NO: 54 or at least 95% amino acid identity with amino acids 301-402 of SEQ ID NO: 54. In yet other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at most 75% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at most 80% amino acid identity with amino acids 301-402 of SEQ ID NO: 54, at most 85% amino acid identity with

amino acids 301-402 of SEQ ID NO: 54, at most 90% amino acid identity with amino acids 301-402 of SEQ ID NO: 54 or at most 95% amino acid identity with amino acids 301-402 of SEQ ID NO: 54.

[0310] In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 301-402 of SEQ ID NO: 54. In yet other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 301-402 of SEQ ID NO: 54. In still other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 301-402 of SEQ ID NO: 54.

[0311] In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 301-402 of SEQ ID NO: 54. In yet other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 301-402 of SEQ ID NO: 54. In still other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 301-402 of SEQ ID NO: 54. In other aspects of this embodiment, a BMP8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 301-402 of SEQ ID NO: 54.

[0312] In another embodiment, an enhanced binding domain is derived from a BMP10. In another embodiment, an enhanced binding domain is derived from a BMP10 of SEQ ID NO: 55. In an aspect of this embodiment, an enhanced binding domain is derived from a BMP10 comprises amino acids 323-424 of SEQ ID NO: 55.

[0313] In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at least 75% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at least 80% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at least 85% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at least 90% amino acid identity with amino acids 323-424 of SEQ ID NO: 55 or at least 95% amino acid identity with amino acids 323-424 of SEQ ID NO: 55. In yet other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at most 75% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at most 80% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at most 85% amino acid identity with amino acids 323-424 of SEQ ID NO: 55, at most 90% amino acid identity with amino acids 323-424 of SEQ ID NO: 55 or at most 95% amino acid identity with amino acids 323-424 of SEQ ID NO: 55.

[0314] In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 323-424 of SEQ ID NO: 55. In yet other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 323-424 of SEQ ID NO: 55. In still other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 323-424 of SEQ ID NO: 55.

[0315] In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 323-424 of SEQ ID NO: 55. In yet other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 323-424 of SEQ ID NO: 55. In still other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous

amino acid additions relative to amino acids 323-424 of SEQ ID NO: 55. In other aspects of this embodiment, a BMP10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 323-424 of SEQ ID NO: 55.

[0316] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, a GFPs, such as, *e.g.*, GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF10, GDF11 or GDF15.

[0317] Thus, in an embodiment, an enhanced binding domain is derived from a GDF1. In another embodiment, an enhanced binding domain is derived from a GDF1 of SEQ ID NO: 56. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF1 comprises amino acids 267-372 of SEQ ID NO: 56.

[0318] In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at least 75% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at least 80% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at least 85% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at least 90% amino acid identity with amino acids 267-372 of SEQ ID NO: 56 or at least 95% amino acid identity with amino acids 267-372 of SEQ ID NO: 56. In yet other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at most 75% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at most 80% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at most 85% amino acid identity with amino acids 267-372 of SEQ ID NO: 56, at most 90% amino acid identity with amino acids 267-372 of SEQ ID NO: 56 or at most 95% amino acid identity with amino acids 267-372 of SEQ ID NO: 56.

[0319] In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 267-372 of SEQ ID NO: 56. In yet other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 267-372 of SEQ ID NO: 56. In still other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six,

seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 267-372 of SEQ ID NO: 56.

[0320] In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 267-372 of SEQ ID NO: 56. In yet other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 267-372 of SEQ ID NO: 56. In still other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 267-372 of SEQ ID NO: 56. In other aspects of this embodiment, a GDF1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 267-372 of SEQ ID NO: 56.

[0321] In another embodiment, an enhanced binding domain is derived from a GDF2. In another embodiment, an enhanced binding domain is derived from a GDF2 of SEQ ID NO: 57. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF2 comprises amino acids 327-429 of SEQ ID NO: 57.

[0322] In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at least 75% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at least 80% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at least 85% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at least 90% amino acid identity with amino acids 327-429 of SEQ ID NO: 57 or at least 95% amino acid identity with amino acids 327-429 of SEQ ID NO: 57. In yet other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at most 75% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at most 80% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at most 85% amino acid identity with amino acids 327-429 of SEQ ID NO: 57, at most 90% amino acid identity with amino acids 327-429 of SEQ ID NO: 57 or at most 95% amino acid identity with amino acids 327-429 of SEQ ID NO: 57.

[0323] In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-

contiguous amino acid substitutions relative to amino acids 327-429 of SEQ ID NO: 57. In yet other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 327-429 of SEQ ID NO: 57. In still other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 327-429 of SEQ ID NO: 57.

[0324] In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 327-429 of SEQ ID NO: 57. In yet other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 327-429 of SEQ ID NO: 57. In still other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 327-429 of SEQ ID NO: 57. In other aspects of this embodiment, a GDF2 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 327-429 of SEQ ID NO: 57.

[0325] In another embodiment, an enhanced binding domain is derived from a GDF3. In another embodiment, an enhanced binding domain is derived from a GDF3 of SEQ ID NO: 58. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF3 comprises amino acids 264-364 of SEQ ID NO: 58.

[0326] In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at least 75% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at least 80% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at least 85% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at least 90% amino acid identity with amino acids 264-364 of SEQ ID NO: 58 or at least 95% amino acid identity with amino acids 264-364 of SEQ ID NO: 58. In yet other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 264-364 of SEQ ID NO: 58,

at most 75% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at most 80% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at most 85% amino acid identity with amino acids 264-364 of SEQ ID NO: 58, at most 90% amino acid identity with amino acids 264-364 of SEQ ID NO: 58 or at most 95% amino acid identity with amino acids 264-364 of SEQ ID NO: 58.

[0327] In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 264-364 of SEQ ID NO: 58. In yet other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 264-364 of SEQ ID NO: 58. In still other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 264-364 of SEQ ID NO: 58.

[0328] In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 264-364 of SEQ ID NO: 58. In yet other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 264-364 of SEQ ID NO: 58. In still other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 264-364 of SEQ ID NO: 58. In other aspects of this embodiment, a GDF3 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 264-364 of SEQ ID NO: 58.

[0329] In another embodiment, an enhanced binding domain is derived from a GDF5. In another embodiment, an enhanced binding domain is derived from a GDF5 of SEQ ID NO: 59. In an aspect of

this embodiment, an enhanced binding domain is derived from a GDF5 comprises amino acids 400-501 of SEQ ID NO: 59.

[0330] In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at least 75% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at least 80% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at least 85% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at least 90% amino acid identity with amino acids 400-501 of SEQ ID NO: 59 or at least 95% amino acid identity with amino acids 400-501 of SEQ ID NO: 59. In yet other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at most 75% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at most 80% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at most 85% amino acid identity with amino acids 400-501 of SEQ ID NO: 59, at most 90% amino acid identity with amino acids 400-501 of SEQ ID NO: 59 or at most 95% amino acid identity with amino acids 400-501 of SEQ ID NO: 59.

[0331] In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 400-501 of SEQ ID NO: 59. In yet other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 400-501 of SEQ ID NO: 59. In still other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 400-501 of SEQ ID NO: 59.

[0332] In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 400-501 of SEQ ID NO: 59. In yet other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two,

three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 400-501 of SEQ ID NO: 59. In still other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 400-501 of SEQ ID NO: 59. In other aspects of this embodiment, a GDF5 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 400-501 of SEQ ID NO: 59.

[0333] In another embodiment, an enhanced binding domain is derived from a GDF6. In another embodiment, an enhanced binding domain is derived from a GDF6 of SEQ ID NO: 60. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF6 comprises amino acids 354-455 of SEQ ID NO: 60.

[0334] In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at least 75% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at least 80% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at least 85% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at least 90% amino acid identity with amino acids 354-455 of SEQ ID NO: 60 or at least 95% amino acid identity with amino acids 354-455 of SEQ ID NO: 60. In yet other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at most 75% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at most 80% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at most 85% amino acid identity with amino acids 354-455 of SEQ ID NO: 60, at most 90% amino acid identity with amino acids 354-455 of SEQ ID NO: 60 or at most 95% amino acid identity with amino acids 354-455 of SEQ ID NO: 60.

[0335] In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 354-455 of SEQ ID NO: 60. In yet other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 354-455 of SEQ ID NO: 60. In still other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 354-455 of SEQ ID NO: 60.

[0336] In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 354-455 of SEQ ID NO: 60. In yet other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 354-455 of SEQ ID NO: 60. In still other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 354-455 of SEQ ID NO: 60. In other aspects of this embodiment, a GDF6 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 354-455 of SEQ ID NO: 60.

[0337] In another embodiment, an enhanced binding domain is derived from a GDF7. In another embodiment, an enhanced binding domain is derived from a GDF7 of SEQ ID NO: 61. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF7 comprises amino acids 352-450 of SEQ ID NO: 61.

[0338] In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at least 75% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at least 80% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at least 85% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at least 90% amino acid identity with amino acids 352-450 of SEQ ID NO: 61 or at least 95% amino acid identity with amino acids 352-450 of SEQ ID NO: 61. In yet other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at most 75% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at most 80% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at most 85% amino acid identity with amino acids 352-450 of SEQ ID NO: 61, at most 90% amino acid identity with amino acids 352-450 of SEQ ID NO: 61 or at most 95% amino acid identity with amino acids 352-450 of SEQ ID NO: 61.

[0339] In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 352-450 of SEQ ID NO: 61. In yet other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four,

five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 352-450 of SEQ ID NO: 61. In still other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 352-450 of SEQ ID NO: 61.

[0340] In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 352-450 of SEQ ID NO: 61. In yet other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 352-450 of SEQ ID NO: 61. In still other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 352-450 of SEQ ID NO: 61. In other aspects of this embodiment, a GDF7 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 352-450 of SEQ ID NO: 61.

[0341] In another embodiment, an enhanced binding domain is derived from a GDF8. In another embodiment, an enhanced binding domain is derived from a GDF8 of SEQ ID NO: 62. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF8 comprises amino acids 281-375 of SEQ ID NO: 62.

[0342] In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at least 75% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at least 80% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at least 85% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at least 90% amino acid identity with amino acids 281-375 of SEQ ID NO: 62 or at least 95% amino acid identity with amino acids 281-375 of SEQ ID NO: 62. In yet other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at most 75% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at most 80% amino acid identity with amino acids 281-375 of SEQ ID NO: 62, at most 85% amino acid identity with amino acids

281-375 of SEQ ID NO: 62, at most 90% amino acid identity with amino acids 281-375 of SEQ ID NO: 62 or at most 95% amino acid identity with amino acids 281-375 of SEQ ID NO: 62.

[0343] In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 281-375 of SEQ ID NO: 62. In yet other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 281-375 of SEQ ID NO: 62. In still other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 281-375 of SEQ ID NO: 62.

[0344] In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 281-375 of SEQ ID NO: 62. In yet other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 281-375 of SEQ ID NO: 62. In still other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 281-375 of SEQ ID NO: 62. In other aspects of this embodiment, a GDF8 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 281-375 of SEQ ID NO: 62.

[0345] In another embodiment, an enhanced binding domain is derived from a GDF10. In another embodiment, an enhanced binding domain is derived from a GDF10 of SEQ ID NO: 63. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF10 comprises amino acids 376-478 of SEQ ID NO: 63.

[0346] In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at least 75% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at least 80% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at least 85% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at least 90% amino acid identity with amino acids 376-478 of SEQ ID NO: 63 or at least 95% amino acid identity with amino acids 376-478 of SEQ ID NO: 63. In yet other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at most 75% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at most 80% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at most 85% amino acid identity with amino acids 376-478 of SEQ ID NO: 63, at most 90% amino acid identity with amino acids 376-478 of SEQ ID NO: 63 or at most 95% amino acid identity with amino acids 376-478 of SEQ ID NO: 63.

[0347] In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 376-478 of SEQ ID NO: 63. In yet other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 376-478 of SEQ ID NO: 63. In still other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 376-478 of SEQ ID NO: 63.

[0348] In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 376-478 of SEQ ID NO: 63. In yet other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 376-478 of SEQ ID NO: 63. In still other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous

amino acid additions relative to amino acids 376-478 of SEQ ID NO: 63. In other aspects of this embodiment, a GDF10 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 376-478 of SEQ ID NO: 63.

[0349] In another embodiment, an enhanced binding domain is derived from a GDF11. In another embodiment, an enhanced binding domain is derived from a GDF11 of SEQ ID NO: 64. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF11 comprises amino acids 313-407 of SEQ ID NO: 64.

[0350] In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at least 75% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at least 80% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at least 85% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at least 90% amino acid identity with amino acids 313-407 of SEQ ID NO: 64 or at least 95% amino acid identity with amino acids 313-407 of SEQ ID NO: 64. In yet other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at most 75% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at most 80% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at most 85% amino acid identity with amino acids 313-407 of SEQ ID NO: 64, at most 90% amino acid identity with amino acids 313-407 of SEQ ID NO: 64 or at most 95% amino acid identity with amino acids 313-407 of SEQ ID NO: 64.

[0351] In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 313-407 of SEQ ID NO: 64. In yet other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 313-407 of SEQ ID NO: 64. In still other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 313-407 of SEQ ID NO: 64.

[0352] In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 313-407 of SEQ ID NO: 64. In yet other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 313-407 of SEQ ID NO: 64. In still other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 313-407 of SEQ ID NO: 64. In other aspects of this embodiment, a GDF11 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 313-407 of SEQ ID NO: 64.

[0353] In another embodiment, an enhanced binding domain is derived from a GDF15. In another embodiment, an enhanced binding domain is derived from a GDF15 of SEQ ID NO: 65. In an aspect of this embodiment, an enhanced binding domain is derived from a GDF15 comprises amino acids 211-308 of SEQ ID NO: 65.

[0354] In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at least 75% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at least 80% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at least 85% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at least 90% amino acid identity with amino acids 211-308 of SEQ ID NO: 65 or at least 95% amino acid identity with amino acids 211-308 of SEQ ID NO: 65. In yet other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at most 75% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at most 80% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at most 85% amino acid identity with amino acids 211-308 of SEQ ID NO: 65, at most 90% amino acid identity with amino acids 211-308 of SEQ ID NO: 65 or at most 95% amino acid identity with amino acids 211-308 of SEQ ID NO: 65.

[0355] In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 211-308 of SEQ ID NO: 65. In yet other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four,

five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 211-308 of SEQ ID NO: 65. In still other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 211-308 of SEQ ID NO: 65.

[0356] In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 211-308 of SEQ ID NO: 65. In yet other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 211-308 of SEQ ID NO: 65. In still other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 211-308 of SEQ ID NO: 65. In other aspects of this embodiment, a GDF15 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 211-308 of SEQ ID NO: 65.

[0357] Another example of an enhanced targeting domain disclosed in the present specification is, *e.g.*, an activin A, an activin B, an activin C, an activin E or an inhibin A.

[0358] In another embodiment, an enhanced binding domain is derived from an Activin A. In another embodiment, an enhanced binding domain is derived from an Activin A of SEQ ID NO: 66. In an aspect of this embodiment, an enhanced binding domain is derived from an Activin A comprises amino acids 321-426 of SEQ ID NO: 66.

[0359] In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at least 75% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at least 80% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at least 85% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at least 90% amino acid identity with amino acids 321-426 of SEQ ID NO: 66 or at least 95% amino acid identity

with amino acids 321-426 of SEQ ID NO: 66. In yet other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at most 75% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at most 80% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at most 85% amino acid identity with amino acids 321-426 of SEQ ID NO: 66, at most 90% amino acid identity with amino acids 321-426 of SEQ ID NO: 66 or at most 95% amino acid identity with amino acids 321-426 of SEQ ID NO: 66.

[0360] In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 321-426 of SEQ ID NO: 66. In yet other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 321-426 of SEQ ID NO: 66. In still other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 321-426 of SEQ ID NO: 66.

[0361] In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 321-426 of SEQ ID NO: 66. In yet other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 321-426 of SEQ ID NO: 66. In still other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 321-426 of SEQ ID NO: 66. In other aspects of this embodiment, an Activin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 321-426 of SEQ ID NO: 66.

[0362] In another embodiment, an enhanced binding domain is derived from an Activin B. In another embodiment, an enhanced binding domain is derived from an Activin B of SEQ ID NO: 67. In an aspect of this embodiment, an enhanced binding domain is derived from an Activin B comprises amino acids 303-406 of SEQ ID NO: 67.

[0363] In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at least 75% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at least 80% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at least 85% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at least 90% amino acid identity with amino acids 303-406 of SEQ ID NO: 67 or at least 95% amino acid identity with amino acids 303-406 of SEQ ID NO: 67. In yet other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at most 75% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at most 80% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at most 85% amino acid identity with amino acids 303-406 of SEQ ID NO: 67, at most 90% amino acid identity with amino acids 303-406 of SEQ ID NO: 67 or at most 95% amino acid identity with amino acids 303-406 of SEQ ID NO: 67.

[0364] In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 303-406 of SEQ ID NO: 67. In yet other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 303-406 of SEQ ID NO: 67. In still other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 303-406 of SEQ ID NO: 67.

[0365] In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 303-406 of SEQ ID NO: 67. In yet other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six,

seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 303-406 of SEQ ID NO: 67. In still other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 303-406 of SEQ ID NO: 67. In other aspects of this embodiment, an Activin B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 303-406 of SEQ ID NO: 67.

[0366] In another embodiment, an enhanced binding domain is derived from an Activin C. In another embodiment, an enhanced binding domain is derived from an Activin C of SEQ ID NO: 68. In an aspect of this embodiment, an enhanced binding domain is derived from an Activin C comprises amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68.

[0367] In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at least 75% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at least 80% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at least 85% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at least 90% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68 or at least 95% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In yet other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at most 75% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at most 80% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at most 85% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68, at most 90% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68 or at most 95% amino acid identity with amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68.

[0368] In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In yet other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one,

two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In still other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68.

[0369] In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In yet other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In still other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68. In other aspects of this embodiment, an Activin C comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 68.

[0370] In another embodiment, an enhanced binding domain is derived from an Activin E. In another embodiment, an enhanced binding domain is derived from an Activin E of SEQ ID NO: 69. In an aspect of this embodiment, an enhanced binding domain is derived from an Activin E comprises amino acids 247-350 of SEQ ID NO: 69.

[0371] In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at least 75% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at least 80% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at least 85% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at least 90% amino acid identity with amino acids 247-350 of SEQ ID NO: 69 or at least 95% amino acid identity with amino acids 247-350 of SEQ ID NO: 69. In yet other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at most 75% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at most 80%

amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at most 85% amino acid identity with amino acids 247-350 of SEQ ID NO: 69, at most 90% amino acid identity with amino acids 247-350 of SEQ ID NO: 69 or at most 95% amino acid identity with amino acids 247-350 of SEQ ID NO: 69.

[0372] In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 247-350 of SEQ ID NO: 69. In yet other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 247-350 of SEQ ID NO: 69. In still other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 247-350 of SEQ ID NO: 69.

[0373] In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 247-350 of SEQ ID NO: 69. In yet other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 247-350 of SEQ ID NO: 69. In still other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 247-350 of SEQ ID NO: 69. In other aspects of this embodiment, an Activin E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 247-350 of SEQ ID NO: 69.

[0374] In another embodiment, an enhanced binding domain is derived from an Inhibin A. In another embodiment, an enhanced binding domain is derived from an Inhibin A of SEQ ID NO: 70. In an aspect

of this embodiment, an enhanced binding domain is derived from an Inhibin A comprises amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70.

[0375] In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at least 75% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at least 80% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at least 85% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at least 90% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70 or at least 95% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In yet other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at most 75% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at most 80% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at most 85% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70, at most 90% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70 or at most 95% amino acid identity with amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70.

[0376] In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid substitutions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In yet other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid deletions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In still other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 non-contiguous amino acid additions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70.

[0377] In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an

Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid substitutions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In yet other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid deletions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In still other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70. In other aspects of this embodiment, an Inhibin A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10 or 20 contiguous amino acid additions relative to amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 70.

[0378] An enhanced targeting domain disclosed in the present specification replaces the binding activity of the Clostridial toxin binding domain found in naturally occurring Clostridial toxins. As used herein, the term "Clostridial toxin binding domain" is synonymous with "Clostridial toxin H_C region" and means any naturally occurring Clostridial toxin polypeptide that can execute the cell binding step of the intoxication process, including, *e.g.*, the binding of the Clostridial toxin to a Clostridial toxin-specific receptor system located on the plasma membrane surface of a target cell. It is envisioned that replacement of the binding activity can be achieved by, *e.g.*, replacing the entire Clostridial toxin binding domain with an enhanced targeting domain; replacing a portion of a Clostridial toxin binding domain with an enhanced targeting domain, with the proviso that the portion of a Clostridial toxin binding domain remaining cannot selectively bind to its Clostridial toxin receptor system; and operationally-linking an enhanced targeting domain to a Clostridial toxin comprising a Clostridial toxin binding domain, with the proviso that the a Clostridial toxin binding domain is altered so that it cannot selectively bind to its Clostridial toxin receptor system.

[0379] The three-dimensional crystal structures of BoNT/A, BoNT/B and the H_C domain of TeNT indicate that the three functional domains of Clostridial neurotoxins are structurally distinct. The HEXXH consensus motif of the light chain forms the tetrahedral zinc binding pocket of the catalytic site located in a deep cleft on the protein surface that is accessible by a channel. The structure of the H_N and H_C domains consists primarily of β -sheet topologies that are linked by a single α -helix. The cylindrical-shaped H_N domain comprises two long amphipathic α -helices that resemble the coiled-coil motif found in some viral proteins. The H_N domain also forms a long unstructured loop called the 'translocation belt,' which wraps around a large negatively charged cleft of the light chain that blocks access of the zinc atom to the catalytic-binding pocket of active site. The H_C domain comprises two distinct structural features of roughly equal size that indicate function. The first, designated the H_{CN} subdomain, is located in the amino half of the H_C domain. The H_{CN} subdomain forms a β -barrel, jelly-roll fold. The H_{CC} subdomain is the second subdomain that comprises the H_C domain. This carboxy-terminal subdomain comprises a

modified β -trefoil domain which forms three distinct carbohydrate binding regions that resembles the carbohydrate binding moiety found in many sugar-binding proteins, such as, *e.g.*, serum amyloid P, sialidase, crya, insecticidal δ -endotoxin and lectins. Biochemical studies indicate that the β -trefoil domain structure of the H_{CC} subdomain appears to mediate the binding to specific carbohydrate containing components of the Clostridial toxin receptor system on the cell surface, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000). The H_C domain tilts away from the H_N domain exposing the surface loops and making them accessible for binding. No contacts occur between the light chain and the H_C domain.

[0380] Proteins containing the structural β -trefoil domain represents a diverse group of proteins, see, *e.g.*, C. A. Orengo et al., *Protein Superfamilies and Domain Superfolds*, 372 Nature 631-634 (1994). The β -trefoil domain comprises a six-stranded β -barrel closed off at one end by three β -hairpin structures that exhibits a characteristic pseudo-threefold axis symmetry. The monomeric structural unit of this three-fold symmetry is referred to as the β -trefoil fold that contains four β -sheets organized as a pair of antiparallel β -sheets. Dividing each of these β -trefoil folds is a β -hairpin turn. Therefore, in a linear fashion, a β -trefoil domain comprises four β -sheets of the first β -trefoil fold, a β -hairpin turn, four β -sheets of the second β -trefoil fold, a second β -hairpin turn four β -sheets of the third β -trefoil fold. Because the first hairpin turn is located between the fourth and fifth β -sheets of the β -trefoil domain, it is designated the β_4/β_5 β -hairpin turn. Likewise, since the second hairpin turn is located between the eight and ninth β -sheets of the β -trefoil domain, it is designated the β_8/β_9 β -hairpin turn.

[0381] As is typical for proteins containing a β -trefoil fold, the overall amino acid sequence identity of the H_{CC} subdomain between Clostridial toxins is low. However, key residues essential for binding activity have been identified by structural analysis and mutagenesis experiments, see, *e.g.*, Krzysztof Ginalski et al., *Structure-based Sequence Alignment for the Beta-Trefoil Subdomain of the Clostridial Neurotoxin Family Provides Residue Level Information About the Putative Ganglioside Binding Site*, 482(1-2) FEBS Lett. 119-124 (2000); and Andreas Rummel et al., *The H_{CC} -Domain of Botulinum Neurotoxins A and B Exhibits a Singular Ganglioside Binding Site Displaying Serotype Specific Carbohydrate Interaction*, 51(3) Mol. Microbiol. 631-643 (2004). Additionally, research has elucidated that β_4/β_5 and β_8/β_9 β -hairpin turns are important in conferring the proper pseudo-threefold axis symmetry observed in the β -trefoil domain and that these turns are important for β -trefoil domain stability, see, *e.g.*, Stephen R. Brych et al., *Structure and Stability Effects of Mutations Designed to Increase the Primary Sequence Symmetry Within the Core Region of a β -trefoil*, 10 Protein Sci. 2587-2599 (2001); Jaewon Kim et al., *Alternative Type I and I' Turn Conformations in the β_8/β_9 β -hairpin of Human Acidic Fibroblast Growth Factor*, 11 Protein Sci. 459-466 (2002); Jaewon Kim et al., *Sequence swapping Does Not Result in Conformation Swapping for the β_4/β_5 and β_8/β_9 β -hairpin Turns in Human Acidic Fibroblast Growth Factor*, 14 Protein Sci. 351-359 (2005). The amino acid sequences comprising the β -trefoil domains found in various Clostridial toxins are shown in Table 2.

Table 2. β -trefoil Domains of Clostridial Toxins						
Protein	SEQ ID NO:	Amino Acid Sequence Region of Carbohydrate Binding Moieties				
		α -fold	β 4/ β 5 β -hairpin turn	β -fold	β 8/ β 9 β -hairpin turn	γ -fold
BoNT/A	1	1111-1162	1163-1178	1179-1223	1224-1236	1237-1296
BoNT/B	2	1098-1147	1148-1165	1166-1210	1211-1222	1223-1291
BoNT/C1	3	1112-1150	1151-1166	1167-1218	1219-1229	1230-1291
BoNT/D	4	1099-1137	1138-1153	1154-1207	1208-1218	1219-1276
BoNT/E	5	1086-1129	1130-1146	1147-1190	1191-1198	1199-1252
BoNT/F	6	1106-1152	1153-1171	1172-1213	1214-1221	1222-1274
BoNT/G	7	1106-1153	1154-1172	1173-1218	1219-1230	1231-1297
TeNT	8	1128-1177	1178-1194	1195-1240	1241-1254	1255-1315

[0382] Thus, in one embodiment, a Clostridial toxin binding domain comprising an H_C region can be replaced with an enhance binding domain disclosed in the present specification. In aspects of this embodiment, a BoNT/A H_C region can be replaced with an enhanced targeting domain, a BoNT/B H_C region can be replaced with an enhanced targeting domain, a BoNT/C1 H_C region can be replaced with an enhanced targeting domain, a BoNT/D H_C region can be replaced with an enhanced targeting domain, a BoNT/E H_C region can be replaced with an enhanced targeting domain, a BoNT/F H_C region can be replaced with an enhanced targeting domain, a BoNT/G H_C region can be replaced with an enhanced targeting domain and a TeNT H_C region can be replaced with an enhanced targeting domain. In other aspects of this embodiment, a BoNT/A H_C region comprising amino acids 872-1296 of SEQ ID NO: 1 can be replaced with an enhanced targeting domain, a BoNT/B H_C region comprising amino acids 859-1291 of SEQ ID NO: 2 can be replaced with an enhanced targeting domain, a BoNT/C1 H_C region comprising amino acids 867-1291 of SEQ ID NO: 3 can be replaced with an enhanced targeting domain, a BoNT/D H_C region comprising amino acids 863-1276 of SEQ ID NO: 4 can be replaced with an enhanced targeting domain, a BoNT/E H_C region comprising amino acids 846-1252 of SEQ ID NO: 5 can be replaced with an enhanced targeting domain, a BoNT/F H_C region comprising amino acids 865-1274 of SEQ ID NO: 6 can be replaced with an enhanced targeting domain, a BoNT/G H_C region comprising amino acids 864-1297 of SEQ ID NO: 7 can be replaced with an enhanced targeting domain and a TeNT H_C region comprising amino acids 880-1315 of SEQ ID NO: 8 can be replaced with an enhanced targeting domain.

[0383] In another embodiment, a Clostridial toxin binding domain comprising an H_{CC} region can be replaced with an enhance binding domain disclosed in the present specification. In aspects of this embodiment, a BoNT/A H_{CC} region can be replaced with an enhanced targeting domain, a BoNT/B H_{CC} region can be replaced with an enhanced targeting domain, a BoNT/C1 H_{CC} region can be replaced with an enhanced targeting domain, a BoNT/D H_{CC} region can be replaced with an enhanced targeting

domain, a BoNT/E H_{CC} region can be replaced with an enhanced targeting domain, a BoNT/F H_{CC} region can be replaced with an enhanced targeting domain, a BoNT/G H_{CC} region can be replaced with an enhanced targeting domain and a TeNT H_{CC} region can be replaced with an enhanced targeting domain. In other aspects of this embodiment, a BoNT/A H_{CC} region comprising amino acids 1092-1296 of SEQ ID NO: 1 can be replaced with an enhanced targeting domain, a BoNT/B H_{CC} region comprising amino acids 1079-1291 of SEQ ID NO: 2 can be replaced with an enhanced targeting domain, a BoNT/C1 H_{CC} region comprising amino acids 1093-1291 of SEQ ID NO: 3 can be replaced with an enhanced targeting domain, a BoNT/D H_{CC} region comprising amino acids 1080-1276 of SEQ ID NO: 4 can be replaced with an enhanced targeting domain, a BoNT/E H_{CC} region comprising amino acids 1067-1252 of SEQ ID NO: 5 can be replaced with an enhanced targeting domain, a BoNT/F H_{CC} region comprising amino acids 1087-1274 of SEQ ID NO: 6 can be replaced with an enhanced targeting domain, a BoNT/G H_{CC} region comprising amino acids 1087-1297 of SEQ ID NO: 7 can be replaced with an enhanced targeting domain and a TeNT H_{CC} region comprising amino acids 1109-1315 of SEQ ID NO: 8 can be replaced with an enhanced targeting domain.

[0384] In another embodiment, an enhance binding domain disclosed in the present specification is operationally-linked to a Clostridial toxin comprising a Clostridial toxin binding domain altered so that it cannot selectively bind to its Clostridial toxin receptor system. As used herein, the term “altered,” when referring to a Clostridial toxin binding domain, means a naturally occurring Clostridial toxin binding domain modified to eliminate or reduce the binding activity of the Clostridial toxin binding domain so that the domain can no longer selectively bind to a Clostridial toxin receptor system. By definition, an altered Clostridial toxin binding domain has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. As non-limiting examples, a modified BoNT/A H_C region comprising amino acids 872-1296 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 872-1296 of SEQ ID NO: 1; a modified BoNT/B H_C region comprising amino acids 859-1291 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 859-1291 of SEQ ID NO: 2; a modified BoNT/C1 H_C region comprising amino acids 867-1291 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 867-1291 of SEQ ID NO: 3; a modified BoNT/D H_C region comprising amino acids 863-1276 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 863-1276 of SEQ ID NO: 4; a modified BoNT/E H_C region comprising amino acids 846-1252 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 846-1252 of SEQ ID NO: 5; a modified BoNT/F H_C region comprising amino acids 865-1274 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 865-1274 of SEQ ID NO: 6; a modified BoNT/G H_C region comprising amino acids

864-1297 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 864-1297 of SEQ ID NO: 7; and a modified TeNT H_C region comprising amino acids 880-1315 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 880-1315 of SEQ ID NO: 8.

[0385] In aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with its reference sequence, at least 75% amino acid identity with its reference sequence, at least 80% amino acid identity with its reference sequence, at least 85% amino acid identity with its reference sequence, at least 90% amino acid identity with its reference sequence or at least 95% amino acid identity with its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with its reference sequence, at most 75% amino acid identity with its reference sequence, at most 80% amino acid identity with its reference sequence, at most 85% amino acid identity with its reference sequence, at most 90% amino acid identity with its reference sequence or at most 95% amino acid identity with its reference sequence.

[0386] In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, or 200 non-contiguous amino acid substitutions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid deletions relative to its reference sequence. In still other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid additions relative to its reference sequence.

[0387] In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two,

three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to its reference sequence. In yet other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to its reference sequence. In still other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to its reference sequence. In other aspects of this embodiment, an altered Clostridial toxin H_C region comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to its reference sequence.

[0388] In another aspect of the invention, a modified Clostridial toxin with enhanced binding activity comprises, in part, a protease cleavage site is located within a di-chain loop region. As used herein, the term “di-chain loop region” means the amino acid sequence of a Clostridial toxin containing a protease cleavage site used to convert the single-chain form of a Clostridial toxin into the di-chain form. Non-limiting examples of a Clostridial toxin di-chain loop region, include, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; and a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8 (Table 8).

Table 8. Di-chain Loop Region of Clostridial Toxins				
Toxin	SEQ ID NO:	Light Chain Region	Di-chain Loop Region Containing the Naturally-occurring Protease Cleavage Site	Heavy Chain Region
BoNT/A	1	NMNFTKLKNFTGLFEFYKLL	CVRGIITSKTKSLDKGYNK*----ALNDLC	IKVNNWDL
BoNT/B	2	KQAYEEISKEHLAVYKIQM	CKSVK*-----APGIC	IDVDNEDL
BoNT/C1	3	PALRKVNPENMLYLFTKF	CHKAIDGRSLYNK*-----TLDC	RELLVKNTDL
BoNT/D	4	PALQKLSSSESVVDLFTKV	CLRLTKNSR*-----DDSTC	IKVKNNRL
BoNT/E	5	IITPITGRGLVKKIIRF	CKNIVSVKGIR*-----KSIC	IEINNGEL
BoNT/F	6	IIDSIPDKGLVEKIVKF	CKSVIPRKGTK*-----APPRLC	IRVNNSEL
BoNT/G	7	KEAYEEISLEHLVIYRIAM	CKPVMYKNTGK*-----SEQC	IIVNNEDL
TeNT	8	TNAFRNVDGSGLVSKLIGL	CKKIIPPTNIRENLYNRTA*SLTDLGGELC	IKIKNEDL
The amino acid sequence displayed are as follows: BoNT/A, residues 325-462 of SEQ ID No: 1; BoNT/B, residues 332-454 of SEQ ID No: 2; BoNT/C1, residues 334-463 of SEQ ID No: 3; BoNT/D,				

residues 334-458 of SEQ ID No: 4; BoNT/E, residues 311-434 of SEQ ID No: 5; BoNT/F, residues 328-453 of SEQ ID No: 6; BoNT/G, residues 331-458 of SEQ ID No: 7; and TeNT, residues 334-474 of SEQ ID No: 8. An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0389] It is envisioned that any and all protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form, including, without limitation, endogenous di-chain loop protease cleavage sites and exogenous protease cleavage sites.

[0390] As used herein, the term “endogenous di-chain loop protease cleavage site” is synonymous with a “naturally occurring di-chain loop protease cleavage site” and means a naturally occurring protease cleavage site found within the di-chain loop region of a naturally occurring Clostridial toxin and includes, without limitation, naturally occurring Clostridial toxin di-chain loop protease cleavage site variants, such as, *e.g.*, Clostridial toxin di-chain loop protease cleavage site isoforms and Clostridial toxin di-chain loop protease cleavage site subtypes. Non-limiting examples of an endogenous protease cleavage site, include, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

[0391] As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleavage ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

[0392] Thus, in an embodiment, a protease cleavage site comprising an endogenous Clostridial toxin di-

chain loop protease cleavage site is used to convert the single-chain toxin into the di-chain form. In aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site.

[0393] In other aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.*, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; or a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8.

[0394] It is also envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. As used herein, the term “exogenous protease cleavage site” is synonymous with a “non-naturally occurring protease cleavage site” and means a protease cleavage site that is not normally present in a di-chain loop region from a naturally occurring Clostridial toxin. Non-limiting examples of exogenous protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 5); a Thrombin cleavage site (Table 9); a Factor Xa cleavage site (Table 9); a human rhinovirus 3C protease cleavage site (Table 9); a tobacco etch virus (TEV) protease cleavage site (Table 9); a dipeptidyl aminopeptidase cleavage site; a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQ GK EMDSLRFLYDGI RIQADQTPEDLDMEDNDIIEAHREQIGG (SEQ ID. NO: 57); and a Clostridial toxin substrate cleavage site.

Table 9. Exogenous Protease Cleavage Sites			
Protease Cleavage Site	Consensus Sequence	Non-limiting Examples	SEQ ID NO:
Bovine enterokinase	DDDDK*	DDDDK*	71
Tobacco Etch Virus (TEV)	E P ⁵ P ⁴ YP ² Q*(G/S), where P ² , P ⁴ and P ⁵ can be any amino acid	ENLYFQ*G ENLYFQ*S ENIYTQ*G ENIYTQ*S ENIYLQ*G ENIYLQ*S ENVYFQ*G ENVYSQ*S	72 73 74 75 76 77 78 79

		ENVYSQ*G	80
		ENVYSQ*S	81
Human Rhinovirus 3C	P ⁵ P ⁴ LFQ*GP where P ⁴ is G, A, V, L, I, M, S or T and P ⁵ can any amino acid, with D or E preferred.	EALFQ*GP EVLfQ*GP ELLFQ*GP DALFQ*GP DVLfQ*GP DLLFQ*GP	82 83 84 85 86 87
SUMO/ULP-1	Tertiary structure	polypeptide-G*	88
Thrombin	P ³ P ² (R/K)*P ¹ , where P ³ is any amino acid and P ² or P ¹ is G with the other position being any amino acid	GVR*G SAR*G SLR*G DGR*I QGK*I	89 90 91 92 93
Thrombin	P ⁴ P ³ P(R/K)*P ¹ P ² where P ¹ and P ² can be any amino acid except for acidic amino acids like D or E; and P ³ and P ⁴ are hydrophobic amino acids like F, L, I, Y, W, V, M, P, C or A	LVPR*GS LVPR*GS FIPR*TF VLPR*SF IVPR*SF IVPR*GY VVPR*GV VLPR*LI VMPR*SL MFPR*SL	94 95 96 97 98 99 100 101 102 103
Coagulation Factor Xa	I(E/D)GR*	IDGR* IEGR*	104 105
An asterisks (*) indicates the peptide bond that is cleaved by the indicated protease.			

[0395] As mentioned above, a Clostridial toxin is converted from a single polypeptide form into a di-chain molecule by proteolytic cleavage. While the naturally-occurring protease is currently not known, cleavage occurs within the di-chain loop region between the two cysteine residues that form the disulfide bridge (see Table 8). Replacement of an endogenous protease cleavage site with an exogenous protease cleavage site will enable cleavage of a modified Clostridial toxin disclosed in the present specification when expressed in an organism that does not produce the naturally-occurring protease used to cleave the di-chain loop region of a toxin. Similarly, an addition of an exogenous protease cleavage site in the di-chain loop region will also enable cleavage of a modified Clostridial toxin disclosed in the present specification when expressed in an organism that does not produce the naturally-occurring protease used to cleave the di-chain loop region of a toxin.

[0396] It is envisioned that an exogenous protease cleavage site of any and all lengths can be useful in aspects of the present invention with the proviso that the exogenous protease cleavage site is capable of being cleaved by its respective protease. Thus, in aspects of this embodiment, an exogenous protease cleavage site can be, *e.g.*, at least 6 amino acids in length, at least 7 amino acids in length, at least 8 amino acids in length, at least 9 amino acids in length, at least 10 amino acids in length, at least 15 amino acids in length, at least 20 amino acids in length, at least 25 amino acids in length, at least 30 amino acids in length, at least 40 amino acids in length, at least 50 amino acids in length or at least 60 amino

acids in length. In other aspects of this embodiment, an exogenous protease cleavage site can be, *e.g.*, at most 6 amino acids in length, at most 7 amino acids in length, at most 8 amino acids in length, at most 9 amino acids in length, at most 10 amino acids in length, at most 15 amino acids in length, at most 20 amino acids in length, at most 25 amino acids in length, at most 30 amino acids in length, at most 40 amino acids in length, at most 50 amino acids in length or at most 60 amino acids in length.

[0397] In aspects of this embodiment, a di-chain loop region can be modified to substitute a naturally-occurring protease cleavage site for an exogenous protease cleavage site. In this type of modification, the naturally-occurring protease cleavage site is made inoperable and thus can not be cleaved by its protease. Only the exogenous protease cleavage site can be cleaved by its corresponding exogenous protease. In this type of modification, the exogenous protease site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein and the site can be cleaved by its respective exogenous protease. As a non-limiting example, a single-chain modified BoNT/A comprising an exogenous protease cleavage site in the di-chain loop region can be cleaved by its respective exogenous protease to produce the di-chain form of the toxin.

[0398] In other aspects of this embodiment, a di-chain loop region can be modified to include an exogenous protease cleavage site in addition to the naturally-occurring protease cleavage site. In this type of modification, both cleavage sites are operably-linked in-frame to a modified Clostridial toxin as a fusion protein and both sites can be cleaved by their respective proteases. As a non-limiting example, a single-chain modified BoNT/A that comprises a di-chain loop containing both the naturally-occurring BoNT/A di-chain loop protease cleavage site and an exogenous protease cleavage site can be cleaved by either the naturally occurring di-chain loop protease or by the appropriate exogenous protease to produce the di-chain form of the toxin.

[0399] A naturally-occurring protease cleavage site can be made inoperable by altering at least the two amino acids flanking the peptide bond cleaved by the naturally-occurring di-chain loop protease. More extensive alterations can be made, with the proviso that the two cysteine residues of the di-chain loop region remain intact and can still form the disulfide bridge. Non-limiting examples of an amino acid alteration include deletion of an amino acid or replacement of the original amino acid with a different amino acid. Thus, in one embodiment, a naturally-occurring protease cleavage site is made inoperable by altering the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease. In other aspects of this embodiment, a naturally-occurring protease cleavage site is made inoperable by altering, *e.g.*, at least three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-

occurring protease; at least eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at least 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0400] In still other aspects of this embodiment, a naturally-occurring di-chain protease cleavage site is made inoperable by altering, *e.g.*, at most three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at most 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0401] In an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a modified Clostridial toxin comprises an exogenous protease cleavage site comprises, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, an exogenous protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0402] In an aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a bovine enterokinase cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 71. In still other aspects of this embodiment, a bovine enterokinase protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0403] In another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80 or SEQ ID NO: 81. In still other aspects of this embodiment, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0404] In still another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Human Rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86 or SEQ ID NO: 87. In still other aspects of this embodiment, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0405] In yet another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 88. In still other aspects of this embodiment, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0406] In a further aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Thrombin protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Thrombin protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102 or SEQ ID NO: 103. In still other aspects of this embodiment, a Thrombin protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0407] In another aspect of this embodiment, an exogenous protease cleavage site can be, *e.g.*, a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can be, *e.g.*, a Coagulation Factor Xa protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 104 or SEQ ID NO: 105. In still other aspects of this embodiment, a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0408] In another embodiment, an exogenous protease site comprises a Clostridial toxin substrate cleavage site. As used herein, the term "Clostridial toxin substrate cleavage site" means a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by a Clostridial toxin under conditions suitable for Clostridial toxin protease activity. By definition, a Clostridial toxin substrate cleavage site is susceptible to cleavage by at least one Clostridial toxin under conditions suitable for Clostridial toxin protease activity. Non-limiting examples of Clostridial toxin substrate cleavage site are disclosed in, *e.g.*, Steward, L.E. et al., Self-Activating Clostridial Toxins, U.S. Patent Application 60/718,616 (Sep, 19, 2005).

[0409] It is understood that a modified Clostridial toxin disclosed in the present specification can optionally include one or more additional components. As a non-limiting example of an optional component, a modified Clostridial toxin can further comprise a flexible region comprising a flexible spacer. Non-limiting examples of a flexible spacer include, *e.g.*, a G-spacer GGGGS (SEQ ID NO: 106) or an A-spacer EAAAK (SEQ ID NO: 107). A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better present an enhanced targeting domain, thereby facilitating the binding of that enhanced targeting domain to its receptor system.

[0410] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least

1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a modified Clostridial toxin can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0411] In aspects of this embodiment, a modified Clostridial toxin comprising a flexible spacer can be, *e.g.*, a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G or a modified TeNT.

[0412] It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise a flexible spacer in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. In aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an enhanced targeting domain, an enzymatic domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an enhanced targeting domain, an enzymatic domain and a protease cleavage site. In other aspects of this embodiment, a A-spacer is positioned between, *e.g.*, an enzymatic domain and a translocation domain, an enzymatic domain and an enhanced targeting domain, an enzymatic domain and a protease cleavage site.

[0413] In other aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, an enhanced targeting domain and a translocation domain, an enhanced targeting domain and an enzymatic domain, an enhanced targeting domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, an enhanced targeting domain and a translocation domain, an enhanced targeting domain and an enzymatic domain, an enhanced targeting domain and a protease cleavage site. In other aspects of this embodiment, a A-spacer is positioned between, *e.g.*, an enhanced targeting domain and a translocation domain, an enhanced targeting domain and an enzymatic domain, an enhanced targeting domain and a protease cleavage site.

[0414] In yet other aspects of this embodiment, a flexible spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, a translocation domain and an enhanced targeting domain, a translocation domain and a protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, a translocation domain and an enhanced targeting domain, a translocation domain and a protease cleavage site. In other aspects of this embodiment, a A-spacer is positioned between, *e.g.*, a translocation domain and an enzymatic domain, a translocation domain and an enhanced targeting domain, a translocation domain and a protease cleavage site.

[0415] As another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™ (SEQ ID NO: 108), human Influenza virus hemagglutinin (HA) (SEQ ID NO: 109), human p62^{c-Myc} protein (c-MYC) (SEQ ID NO: 110), Vesicular Stomatitis Virus Glycoprotein (VSV-G) (SEQ ID NO: 111), Substance P (SEQ ID NO: 112), glycoprotein-D precursor of Herpes simplex virus (HSV) (SEQ ID NO: 113), V5 (SEQ ID NO: 114), AU1 (SEQ ID NO: 115) and AU5 (SEQ ID NO: 116); affinity-binding, such as, *e.g.*, polyhistidine (HIS) (SEQ ID NO: 117), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as, *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001); Antibodies: A Laboratory Manual (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and Using Antibodies: A Laboratory Manual: Portable Protocol No. I (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0416] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 epitope-binding region, at least 2 epitope-binding regions, at least 3 epitope-binding regions, at least 4 epitope-binding regions or at least 5 epitope-binding regions. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 epitope-binding region, at most 2 epitope-binding regions, at most 3 epitope-binding regions, at most 4 epitope-binding regions or at most 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof.

[0417] The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin. Thus, in an embodiment, an epitope-binding region is located at the amino-terminus of a modified Clostridial toxin. In such a location, a start methionine should be placed

in front of the epitope-binding region. In addition, it is known in the art that when adding a polypeptide that is operationally-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted. This is due to the fact that the added polypeptide will contain a new start methionine and that the original start methionine may reduce optimal expression of the fusion protein. In aspects of this embodiment, an epitope-binding region located at the amino-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, Express™ epitope-binding region, a human Influenza virus hemagglutinin (HA) epitope-binding region, a human p62^{c-Myc} protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0418] In another embodiment, an epitope-binding region is located at the carboxyl-terminus of a modified Clostridial toxin. In aspects of this embodiment, an epitope-binding region located at the carboxyl-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, Express™ epitope-binding region, a human Influenza virus hemagglutinin (HA) epitope-binding region, a human p62^{c-Myc} protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0419] As another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™ (SEQ ID NO: 108), human Influenza virus hemagglutinin (HA) (SEQ ID NO: 109), human p62^{c-Myc} protein (c-MYC) (SEQ ID NO: 110), Vesicular Stomatitis Virus Glycoprotein (VSV-G) (SEQ ID NO: 111), Substance P (SEQ ID NO: 112), glycoprotein-D precursor of Herpes simplex virus (HSV) (SEQ ID NO: 113), V5 (SEQ ID NO: 114), AU1 (SEQ ID NO: 115) and AU5 (SEQ ID NO: 116); affinity-binding, such as, *e.g.*, polyhistidine (HIS) (SEQ ID NO: 117), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as, *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-

limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001); Antibodies: A Laboratory Manual (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and Using Antibodies: A Laboratory Manual: Portable Protocol No. I (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0420] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 epitope-binding region, at least 2 epitope-binding regions, at least 3 epitope-binding regions, at least 4 epitope-binding regions or at least 5 epitope-binding regions. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 epitope-binding region, at most 2 epitope-binding regions, at most 3 epitope-binding regions, at most 4 epitope-binding regions or at most 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof.

[0421] The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin. Thus, in an embodiment, an epitope-binding region is located at the amino-terminus of a modified Clostridial toxin. In such a location, a start methionine should be placed in front of the epitope-binding region. In addition, it is known in the art that when adding a polypeptide that is operationally-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted. This is due to the fact that the added polypeptide will contain a new start methionine and that the original start methionine may reduce optimal expression of the fusion protein. In aspects of this embodiment, an epitope-binding region located at the amino-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, ExpressTM epitope-binding region, a human Influenza virus hemagglutinin (HA) epitope-binding region, a human p62^{c-Myc} protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione

binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0422] In another embodiment, an epitope-binding region is located at the carboxyl-terminus of a modified Clostridial toxin. In aspects of this embodiment, an epitope-binding region located at the carboxyl-terminus of a modified Clostridial toxin disclosed in the present specification can be, *e.g.*, a FLAG, Express™ epitope-binding region, a human Influenza virus hemagglutinin (HA) epitope-binding region, a human p62^{c-Myc} protein (c-MYC) epitope-binding region, a Vesicular Stomatitis Virus Glycoprotein (VSV-G) epitope-binding region, a Substance P epitope-binding region, a glycoprotein-D precursor of Herpes simplex virus (HSV) epitope-binding region, a V5 epitope-binding region, a AU1 epitope-binding region, a AU5 epitope-binding region, a polyhistidine epitope-binding region, a streptavidin binding peptide epitope-binding region, a biotin epitope-binding region, a biotinylation epitope-binding region, a glutathione binding domain of glutathione-S-transferase, a calmodulin binding domain of the calmodulin binding protein or a maltose binding domain of the maltose binding protein.

[0423] It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise an enhance binding domain in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. Non-limiting examples include, locating an enhance binding domain at the amino terminus of a modified Clostridial toxin (FIG. 3); locating an enhance binding domain between a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain of a modified Clostridial toxin (FIG. 4); and locating an enhance binding domain at the carboxyl terminus of a modified Clostridial toxin (FIG. 5).

[0424] Thus, in an embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and an enhanced targeting domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a protease cleavage site, a Clostridial toxin translocation domain and an enhanced targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an endogenous protease cleavage site, a Clostridial toxin translocation domain and an enhanced targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain and an enhanced targeting domain.

[0425] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an enhanced targeting domain and a Clostridial toxin translocation domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin

enzymatic domain, a protease cleavage site, an enhanced targeting domain and a Clostridial toxin translocation domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an endogenous protease cleavage site, an enhanced targeting domain and a Clostridial toxin translocation domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, an enhanced targeting domain and a Clostridial toxin translocation domain.

[0426] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin translocation domain, and a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin translocation domain, a protease cleavage site and a Clostridial toxin enzymatic domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin translocation domain, an endogenous protease cleavage site and a Clostridial toxin enzymatic domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin translocation domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0427] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin enzymatic domain, a protease cleavage site and a Clostridial toxin translocation domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin enzymatic domain, an endogenous protease cleavage site and a Clostridial toxin translocation domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enhanced targeting domain, a Clostridial toxin enzymatic domain, an exogenous protease cleavage site and a Clostridial toxin translocation domain.

[0428] In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain and an enhanced targeting domain. In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a protease cleavage site, a Clostridial toxin enzymatic domain and an enhanced targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, an

endogenous protease cleavage site, a Clostridial toxin enzymatic domain and an enhanced targeting domain. In another aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, an exogenous protease cleavage site, a Clostridial toxin enzymatic domain and an enhanced targeting domain.

[0429] Aspects of the present invention provide, in part modified Clostridial toxins. Non-limiting examples of Clostridial toxin modifications disclosed in the present specification include, *e.g.*, addition of an enhanced targeting domain, addition of a protease cleavage site, rearrangement of the enzymatic, translocation and binding domains and addition of a spacer region. It is understood that all such modifications do not substantially affect the ability of a modified Clostridial toxin to intoxicate a cell. As used herein, the term "do not substantially affect" means a modified Clostridial toxin can still execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at least 10% as toxic as a naturally-occurring Clostridial toxin, at least 20% as toxic as a naturally-occurring Clostridial toxin, at least 30% as toxic as a naturally-occurring Clostridial toxin, at least 40% as toxic as a naturally-occurring Clostridial toxin, at least 50% as toxic as a naturally-occurring Clostridial toxin, at least 60% as toxic as a naturally-occurring Clostridial toxin, at least 70% as toxic as a naturally-occurring Clostridial toxin, at least 80% as toxic as a naturally-occurring Clostridial toxin, at least 90% as toxic as a naturally-occurring Clostridial toxin or at least 95% as toxic as a naturally-occurring Clostridial toxin. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at most 10% as toxic as a naturally-occurring Clostridial toxin, at most 20% as toxic as a naturally-occurring Clostridial toxin, at most 30% as toxic as a naturally-occurring Clostridial toxin, at most 40% as toxic as a naturally-occurring Clostridial toxin, at most 50% as toxic as a naturally-occurring Clostridial toxin, at most 60% as toxic as a naturally-occurring Clostridial toxin, at most 70% as toxic as a naturally-occurring Clostridial toxin, at most 80% as toxic as a naturally-occurring Clostridial toxin, at most 90% as toxic as a naturally-occurring Clostridial toxin or at most 95% as toxic as a naturally-occurring Clostridial toxin.

[0430] Another aspect of the present invention provides polynucleotide molecules encoding modified Clostridial toxins disclosed in the present specification. It is envisioned that any and all modified Clostridial toxin disclosed in the present specification can be encoded by a polynucleotide molecule.

[0431] Aspects of the present invention provide, in part polynucleotide molecules. As used herein, the term "polynucleotide molecule" is synonymous with "nucleic acid molecule" and means a polymeric form of nucleotides, such as, *e.g.*, ribonucleotides and deoxyribonucleotides, of any length. It is envisioned that any and all polynucleotide molecules that can encode a modified Clostridial toxin disclosed in the present specification can be useful, including, without limitation naturally-occurring and non-naturally-

occurring DNA molecules and naturally-occurring and non-naturally-occurring RNA molecules. Non-limiting examples of naturally-occurring and non-naturally-occurring DNA molecules include single-stranded DNA molecules, double-stranded DNA molecules, genomic DNA molecules, cDNA molecules, vector constructs, such as, *e.g.*, plasmid constructs, phagmid constructs, bacteriophage constructs, retroviral constructs and artificial chromosome constructs. Non-limiting examples of naturally-occurring and non-naturally-occurring RNA molecules include single-stranded RNA, double stranded RNA and mRNA.

[0432] Thus, in an embodiment, a polynucleotide molecule encodes a modified Clostridial toxin disclosed in the present specification.

[0433] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a Clostridial toxin enzymatic domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a Clostridial toxin enzymatic domain isoform or a Clostridial toxin enzymatic domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.*, a conservative Clostridial toxin enzymatic domain variant, a non-conservative Clostridial toxin enzymatic domain variant or an active Clostridial toxin enzymatic domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, or active fragment thereof.

[0434] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a Clostridial toxin translocation domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.*, a Clostridial toxin translocation domain isoform or a Clostridial toxin translocation domain subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.*, a conservative Clostridial toxin translocation domain variant, a non-conservative Clostridial toxin translocation domain variant or an active Clostridial toxin translocation domain fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a Clostridial toxin translocation domain comprises a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, or active fragment thereof.

[0435] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising an enhanced targeting domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding an enhanced targeting domain comprises a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a presynaptic membrane of a Clostridial toxin target cell. In an aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a presynaptic membrane of a Clostridial toxin target cell comprises a naturally occurring variant, such as, *e.g.*, an isoform or a subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a presynaptic membrane of a Clostridial toxin target cell comprises a non-naturally occurring variant, such as, *e.g.*, a conservative variant, a non-conservative variant or an active fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a presynaptic membrane of a Clostridial toxin target cell comprises a TGF β superfamily polypeptide, such as, *e.g.*, a TGF β , a BMPs, a GDF and an activin; a neurotrophin, such as, *e.g.*, a NGF, a BDNF, a NT-3 and a NT-4/5; an axon guidance signaling molecule, such as, *e.g.*, a netrin, a semaphoring and an ephrin; a neuroregulatory cytokine, such as, *e.g.*, a CNTF, a GPA, a LIF, an interleukin, an onostatin M, a CT-1 and a CLC; a sugar binding protein, such as, *e.g.*, a serum amyloid P, a β -glucanase, a sialidase, a lectin, a cryia, an insecticidal delta-endotoxin, an agglutinin, an abrin and a ricin; an IGF, such as, *e.g.*, a IGF-1 and a IGF-2; a neurexin; a neuroleukin/AMF/MF; a TrkB; an EGF; a visceral gut peptide such as, *e.g.*, a gastrin, a VIP, a bombesin; and a WNT, such as, *e.g.*, a Frizzled.

[0436] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising an enhanced targeting domain disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding an enhanced targeting domain comprises a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a postsynaptic membrane of a Clostridial toxin target cell. In an aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a postsynaptic membrane of a Clostridial toxin target cell comprises a naturally occurring variant, such as, *e.g.*, an isoform or a subtype. In another aspect of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a postsynaptic membrane of a Clostridial toxin target cell comprises a non-naturally occurring variant, such as, *e.g.*, a conservative variant, a non-conservative variant or an active fragment, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encoding a polypeptide that selectively binds to a non-Clostridial toxin receptor system present on a postsynaptic membrane of a Clostridial toxin target cell comprises a Ng-CAM(L1), a N-CAM, a N-cadherin, an agrin-MUSK, and a basement membrane polypeptide, such as, *e.g.*, laminin β -2.

[0437] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a protease cleavage site disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a protease cleavage site comprises an endogenous Clostridial toxin protease site. In aspects of this embodiment, a polynucleotide molecule encoding an endogenous Clostridial toxin protease site can be, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site. In another aspect of this embodiment, a polynucleotide molecule encoding a protease cleavage site comprises an exogenous Clostridial toxin protease site. In aspects of this embodiment, a polynucleotide molecule encoding an exogenous Clostridial toxin protease site can be, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site, a Coagulation Factor Xa protease cleavage site or a Clostridial toxin substrate cleavage site, such as, *e.g.*, a BoNT/A substrate cleavage site, a BoNT/B substrate cleavage site, a BoNT/C1 substrate cleavage site, a BoNT/D substrate cleavage site, a BoNT/E substrate cleavage site, a BoNT/F substrate cleavage site, a BoNT/G substrate cleavage site or a TeNT substrate cleavage site.

[0438] In another embodiment, a polynucleotide molecule encodes, in part, a modified Clostridial toxin comprising a flexible spacer disclosed in the present specification. In an aspect of this embodiment, a polynucleotide molecule encoding a flexible spacer a G-spacer, a A-spacer of any combination thereof.

[0439] Well-established molecular biology techniques that may be necessary to make a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification including, but not limited to, procedures involving polymerase chain reaction (PCR) amplification, restriction enzyme reactions, agarose gel electrophoresis, nucleic acid ligation, bacterial transformation, nucleic acid purification, nucleic acid sequencing and recombination-based techniques are routine procedures well within the scope of one skilled in the art and from the teaching herein. Non-limiting examples of specific protocols necessary to make a polynucleotide molecule encoding a modified Clostridial toxin are described in *e.g.*, MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Frederick M. Ausubel et al., eds. John Wiley & Sons, 2004). Additionally, a variety of commercially available products useful for making a polynucleotide molecule encoding a modified Clostridial toxin are widely available. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0440] Another aspect of the present invention provides a method of producing a modified Clostridial toxin disclosed in the present specification, such method comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell. Another aspect of the present invention provides a method of producing a modified Clostridial toxin disclosed in the present

specification, such method comprising the steps of introducing an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin into a cell and expressing the expression construct in the cell.

[0441] The methods disclosed in the present specification include, in part, a modified Clostridial toxin. It is envisioned that any and all modified Clostridial toxins disclosed in the present specification can be produced using the methods disclosed in the present specification. It is also envisioned that any and all polynucleotide molecules encoding a modified Clostridial toxins disclosed in the present specification can be useful in producing a modified Clostridial toxins disclosed in the present specification using the methods disclosed in the present specification.

[0442] The methods disclosed in the present specification include, in part, an expression construct. An expression construct comprises a polynucleotide molecule disclosed in the present specification operably-linked to an expression vector useful for expressing the polynucleotide molecule in a cell or cell-free extract. A wide variety of expression vectors can be employed for expressing a polynucleotide molecule encoding a modified Clostridial toxin, including, without limitation, a viral expression vector; a prokaryotic expression vector; eukaryotic expression vectors, such as, *e.g.*, a yeast expression vector, an insect expression vector and a mammalian expression vector; and a cell-free extract expression vector. It is further understood that expression vectors useful to practice aspects of these methods may include those which express a modified Clostridial toxin under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both. Non-limiting examples of expression vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

[0443] Thus, aspects of this embodiment include, without limitation, a viral expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a prokaryotic expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a yeast expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; an insect expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; and a mammalian expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs suitable for expressing a modified Clostridial toxin disclosed in the present specification using a cell-free extract comprising a cell-free extract expression vector operably linked to a polynucleotide molecule encoding a modified Clostridial toxin.

[0444] The methods disclosed in the present specification include, in part, a cell. It is envisioned that any and all cells can be used. Thus, aspects of this embodiment include, without limitation, prokaryotic cells including, without limitation, strains of aerobic, microaerophilic, capnophilic, facultative, anaerobic, gram-negative and gram-positive bacterial cells such as those derived from, *e.g.*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacteroides fragilis*, *Clostridia perfringens*, *Clostridia difficile*, *Caulobacter crescentus*, *Lactococcus lactis*, *Methylobacterium extorquens*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Pseudomonas fluorescens* and *Salmonella typhimurium*; and eukaryotic cells including, without limitation, yeast strains, such as, *e.g.*, those derived from *Pichia pastoris*, *Pichia methanolica*, *Pichia angusta*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; insect cells and cell lines derived from insects, such as, *e.g.*, those derived from *Spodoptera frugiperda*, *Trichoplusia ni*, *Drosophila melanogaster* and *Manduca sexta*; and mammalian cells and cell lines derived from mammalian cells, such as, *e.g.*, those derived from mouse, rat, hamster, porcine, bovine, equine, primate and human. Cell lines may be obtained from the American Type Culture Collection, European Collection of Cell Cultures and the German Collection of Microorganisms and Cell Cultures. Non-limiting examples of specific protocols for selecting, making and using an appropriate cell line are described in *e.g.*, INSECT CELL CULTURE ENGINEERING (Mattheus F. A. Goosen et al. eds., Marcel Dekker, 1993); INSECT CELL CULTURES: FUNDAMENTAL AND APPLIED ASPECTS (J. M. Vlak et al. eds., Kluwer Academic Publishers, 1996); Maureen A. Harrison & Ian F. Rae, GENERAL TECHNIQUES OF CELL CULTURE (Cambridge University Press, 1997); CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Alan Doyle et al eds., John Wiley and Sons, 1998); R. Ian Freshney, CULTURE OF ANIMAL CELLS: A MANUAL OF BASIC TECHNIQUE (Wiley-Liss, 4th ed. 2000); ANIMAL CELL CULTURE: A PRACTICAL APPROACH (John R. W. Masters ed., Oxford University Press, 3rd ed. 2000); MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); BASIC CELL CULTURE: A PRACTICAL APPROACH (John M. Davis, Oxford Press, 2nd ed. 2002); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, *supra*, (2004). These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0445] The methods disclosed in the present specification include, in part, introducing into a cell a polynucleotide molecule. A polynucleotide molecule introduced into a cell can be transiently or stably maintained by that cell. Stably-maintained polynucleotide molecules may be extra-chromosomal and replicate autonomously, or they may be integrated into the chromosomal material of the cell and replicate non-autonomously. It is envisioned that any and all methods for introducing a polynucleotide molecule disclosed in the present specification into a cell can be used. Methods useful for introducing a nucleic acid molecule into a cell include, without limitation, chemical-mediated transfection such as, *e.g.*, calcium phosphate-mediated, diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polylysine-mediated and polybrene-mediated; physical-mediated transfection, such as, *e.g.*, biolistic particle delivery, microinjection, protoplast fusion and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection, see, *e.g.*, Introducing Cloned Genes into Cultured Mammalian Cells, pp. 16.1-16.62 (Sambrook & Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001). One skilled in the art understands that selection of a specific method to

introduce an expression construct into a cell will depend, in part, on whether the cell will transiently contain an expression construct or whether the cell will stably contain an expression construct. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0446] In an aspect of this embodiment, a chemical-mediated method, termed transfection, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In chemical-mediated methods of transfection the chemical reagent forms a complex with the nucleic acid that facilitates its uptake into the cells. Such chemical reagents include, without limitation, calcium phosphate-mediated, see, *e.g.*, Martin Jordan & Florian Worm, Transfection of adherent and suspended cells by calcium phosphate, 33(2) Methods 136-143 (2004); diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, cationic polymer-mediated like polyethyleneimine (PEI)-mediated and polylysine-mediated and polybrene-mediated, see, *e.g.*, Chun Zhang et al., Polyethylenimine strategies for plasmid delivery to brain-derived cells, 33(2) Methods 144-150 (2004). Such chemical-mediated delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, CellPfect Transfection Kit (Amersham Biosciences, Piscataway, NJ); Mammalian Transfection Kit, Calcium phosphate and DEAE Dextran, (Stratagene, Inc., La Jolla, CA); Lipofectamine™ Transfection Reagent (Invitrogen, Inc., Carlsbad, CA); ExGen 500 Transfection kit (Fermentas, Inc., Hanover, MD), and SuperFect and Effectene Transfection Kits (Qiagen, Inc., Valencia, CA).

[0447] In another aspect of this embodiment, a physical-mediated method is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. Physical techniques include, without limitation, electroporation, biolistic and microinjection. Biolistics and microinjection techniques perforate the cell wall in order to introduce the nucleic acid molecule into the cell, see, *e.g.*, Jeike E. Biewenga et al., Plasmid-mediated gene transfer in neurons using the biolistics technique, 71(1) J. Neurosci. Methods. 67-75 (1997); and John O'Brien & Sarah C. R. Lummis, Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells, 33(2) Methods 121-125 (2004). Electroporation, also termed electroporabilization, uses brief, high-voltage, electrical pulses to create transient pores in the membrane through which the nucleic acid molecules enter and can be used effectively for stable and transient transfections of all cell types, see, *e.g.*, M. Golzio et al., In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression, 33(2) Methods 126-135 (2004); and Oliver Greschet al., New non-viral method for gene transfer into primary cells, 33(2) Methods 151-163 (2004).

[0448] In another aspect of this embodiment, a viral-mediated method, termed transduction, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In viral-mediated methods of transient transduction, the process by which viral particles infect and replicate in a host cell has been manipulated in order to use this mechanism to introduce a nucleic acid molecule into the cell. Viral-mediated methods have been developed from a wide variety of viruses including, without limitation, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, picornaviruses,

alphaviruses and baculoviruses, see, *e.g.*, Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) *Methods* 164-172 (2004); and Maurizio Federico, From lentiviruses to lentivirus vectors, 229 *Methods Mol. Biol.* 3-15 (2003); E. M. Poeschla, Non-primate lentiviral vectors, 5(5) *Curr. Opin. Mol. Ther.* 529-540 (2003); Karim Benihoud et al, Adenovirus vectors for gene delivery, 10(5) *Curr. Opin. Biotechnol.* 440-447 (1999); H. Bueler, Adeno-associated viral vectors for gene transfer and gene therapy, 380(6) *Biol. Chem.* 613-622 (1999); Chooi M. Lai et al., Adenovirus and adeno-associated virus vectors, 21(12) *DNA Cell Biol.* 895-913 (2002); Edward A. Burton et al., Gene delivery using herpes simplex virus vectors, 21(12) *DNA Cell Biol.* 915-936 (2002); Paola Grandi et al., Targeting HSV amplicon vectors, 33(2) *Methods* 179-186 (2004); Ilya Frolov et al., Alphavirus-based expression vectors: strategies and applications, 93(21) *Proc. Natl. Acad. Sci. U. S. A.* 11371-11377 (1996); Markus U. Ehrenguber, Alphaviral gene transfer in neurobiology, 59(1) *Brain Res. Bull.* 13-22 (2002); Thomas A. Kost & J. Patrick Condreay, Recombinant baculoviruses as mammalian cell gene-delivery vectors, 20(4) *Trends Biotechnol.* 173-180 (2002); and A. Huser & C. Hofmann, Baculovirus vectors: novel mammalian cell gene-delivery vehicles and their applications, 3(1) *Am. J. Pharmacogenomics* 53-63 (2003).

[0449] Adenoviruses, which are non-enveloped, double-stranded DNA viruses, are often selected for mammalian cell transduction because adenoviruses handle relatively large polynucleotide molecules of about 36 kb, are produced at high titer, and can efficiently infect a wide variety of both dividing and non-dividing cells, see, *e.g.*, Wim T. J. M. C. Hermens et al., Transient gene transfer to neurons and glia: analysis of adenoviral vector performance in the CNS and PNS, 71(1) *J. Neurosci. Methods* 85-98 (1997); and Hiroyuki Mizuguchi et al., Approaches for generating recombinant adenovirus vectors, 52(3) *Adv. Drug Deliv. Rev.* 165-176 (2001). Transduction using adenoviral-based system do not support prolonged protein expression because the nucleic acid molecule is carried from an episome in the cell nucleus, rather than being integrated into the host cell chromosome. Adenoviral vector systems and specific protocols for how to use such vectors are disclosed in, *e.g.*, ViraPower™ Adenoviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Adenoviral Expression System Instruction Manual 25-0543 version A, Invitrogen, Inc., (Jul. 15, 2002); and AdEasy™ Adenoviral Vector System (Stratagene, Inc., La Jolla, CA) and AdEasy™ Adenoviral Vector System Instruction Manual 064004f, Stratagene, Inc..

[0450] Nucleic acid molecule delivery can also use single-stranded RNA retroviruses, such as, *e.g.*, oncoretroviruses and lentiviruses. Retroviral-mediated transduction often produce transduction efficiencies close to 100%, can easily control the proviral copy number by varying the multiplicity of infection (MOI), and can be used to either transiently or stably transduce cells, see, *e.g.*, Tiziana Tonini et al., Transient production of retroviral- and lentiviral-based vectors for the transduction of Mammalian cells, 285 *Methods Mol. Biol.* 141-148 (2004); Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) *Methods* 164-172 (2004); Félix Recillas-Targa, Gene transfer and expression in mammalian cell lines and transgenic animals, 267 *Methods Mol. Biol.* 417-433 (2004); and Roland Wolkowicz et al., Lentiviral vectors for the delivery of DNA into mammalian cells, 246 *Methods*

Mol. Biol. 391-411 (2004). Retroviral particles consist of an RNA genome packaged in a protein capsid, surrounded by a lipid envelope. The retrovirus infects a host cell by injecting its RNA into the cytoplasm along with the reverse transcriptase enzyme. The RNA template is then reverse transcribed into a linear, double stranded cDNA that replicates itself by integrating into the host cell genome. Viral particles are spread both vertically (from parent cell to daughter cells via the provirus) as well as horizontally (from cell to cell via virions). This replication strategy enables long-term persistent expression since the nucleic acid molecules of interest are stably integrated into a chromosome of the host cell, thereby enabling long-term expression of the protein. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year, see, *e.g.*, Luigi Naldini et al., In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector, 272(5259) Science 263-267 (1996). The Oncoretroviruses-derived vector systems, such as, *e.g.*, Moloney murine leukemia virus (MoMLV), are widely used and infect many different non-dividing cells. Lentiviruses can also infect many different cell types, including dividing and non-dividing cells and possess complex envelope proteins, which allows for highly specific cellular targeting.

[0451] Retroviral vectors and specific protocols for how to use such vectors are disclosed in, *e.g.*, U.S. Patent Nos. Manfred Gossen & Hermann Bujard, Tight control of gene expression in eukaryotic cells by tetracycline-responsive promoters, U.S. Patent No. 5,464,758 (Nov. 7, 1995) and Hermann Bujard & Manfred Gossen, Methods for regulating gene expression, U.S. Patent No. 5,814,618 (Sep. 29, 1998) David S. Hogness, Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same, U.S. Patent No. 5,514,578 (May 7, 1996) and David S. Hogness, Polynucleotide encoding insect ecdysone receptor, U.S. Patent 6,245,531 (Jun. 12, 2001); Elisabetta Vegeto et al., Progesterone receptor having C. terminal hormone binding domain truncations, U.S. Patent No. 5,364,791 (Nov. 15, 1994), Elisabetta Vegeto et al., Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,874,534 (Feb. 23, 1999) and Elisabetta Vegeto et al., Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,935,934 (Aug. 10, 1999). Furthermore, such viral delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, BD™ Tet-Off and Tet-On Gene Expression Systems (BD Biosciences-Clontech, Palo Alto, CA) and BD™ Tet-Off and Tet-On Gene Expression Systems User Manual, PT3001-1, BD Biosciences Clontech, (Mar. 14, 2003), GeneSwitch™ System (Invitrogen, Inc., Carlsbad, CA) and GeneSwitch™ System A Mifepristone-Regulated Expression System for Mammalian Cells version D, 25-0313, Invitrogen, Inc., (Nov. 4, 2002); ViraPower™ Lentiviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Lentiviral Expression System Instruction Manual 25-0501 version E, Invitrogen, Inc., (Dec. 8, 2003); and Complete Control® Retroviral Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and Complete Control® Retroviral Inducible Mammalian Expression System Instruction Manual, 064005e.

[0452] The methods disclosed in the present specification include, in part, expressing a modified Clostridial toxin from a polynucleotide molecule. It is envisioned that any of a variety of expression

systems may be useful for expressing a modified Clostridial toxin from a polynucleotide molecule disclosed in the present specification, including, without limitation, cell-based systems and cell-free expression systems. Cell-based systems include, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts and *E. coli* extracts and generally are equivalent to the method disclosed herein. Expression of a polynucleotide molecule using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in e.g., PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0453] A variety of cell-based expression procedures are useful for expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification. Examples included, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Viral expression systems include, without limitation, the ViraPower™ Lentiviral (Invitrogen, Inc., Carlsbad, CA), the Adenoviral Expression Systems (Invitrogen, Inc., Carlsbad, CA), the AdEasy™ XL Adenoviral Vector System (Stratagene, La Jolla, CA) and the ViraPort® Retroviral Gene Expression System (Stratagene, La Jolla, CA). Non-limiting examples of prokaryotic expression systems include the Champion™ pET Expression System (EMD Biosciences-Novagen, Madison, WI), the TriEx™ Bacterial Expression System (EMD Biosciences-Novagen, Madison, WI), the QIAexpress® Expression System (QIAGEN, Inc.), and the Affinity® Protein Expression and Purification System (Stratagene, La Jolla, CA). Yeast expression systems include, without limitation, the EasySelect™ *Pichia* Expression Kit (Invitrogen, Inc., Carlsbad, CA), the YES-Echo™ Expression Vector Kits (Invitrogen, Inc., Carlsbad, CA) and the SpECTRA™ *S. pombe* Expression System (Invitrogen, Inc., Carlsbad, CA). Non-limiting examples of baculoviral expression systems include the BaculoDirect™ (Invitrogen, Inc., Carlsbad, CA), the Bac-to-Bac® (Invitrogen, Inc., Carlsbad, CA), and the BD BaculoGold™ (BD Biosciences-Pharmingen, San Diego, CA). Insect expression systems include, without limitation, the *Drosophila* Expression System (DES®) (Invitrogen, Inc., Carlsbad, CA), InsectSelect™ System (Invitrogen, Inc., Carlsbad, CA) and

InsectDirect™ System (EMD Biosciences-Novagen, Madison, WI). Non-limiting examples of mammalian expression systems include the T-REx™ (Tetracycline-Regulated Expression) System (Invitrogen, Inc., Carlsbad, CA), the Flp-In™ T-REx™ System (Invitrogen, Inc., Carlsbad, CA), the pcDNA™ system (Invitrogen, Inc., Carlsbad, CA), the pSecTag2 system (Invitrogen, Inc., Carlsbad, CA), the Exchanger® System, InterPlay™ Mammalian TAP System (Stratagene, La Jolla, CA), Complete Control® Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and LacSwitch® II Inducible Mammalian Expression System (Stratagene, La Jolla, CA).

[0454] Another procedure of expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification employs a cell-free expression system such as, without limitation, prokaryotic extracts and eukaryotic extracts. Non-limiting examples of prokaryotic cell extracts include the RTS 100 *E. coli* HY Kit (Roche Applied Science, Indianapolis, IN), the ActivePro In Vitro Translation Kit (Ambion, Inc., Austin, TX), the EcoPro™ System (EMD Biosciences-Novagen, Madison, WI) and the Expressway™ Plus Expression System (Invitrogen, Inc., Carlsbad, CA). Eukaryotic cell extract include, without limitation, the RTS 100 Wheat Germ CECF Kit (Roche Applied Science, Indianapolis, IN), the TnT® Coupled Wheat Germ Extract Systems (Promega Corp., Madison, WI), the Wheat Germ IVT™ Kit (Ambion, Inc., Austin, TX), the Retic Lysate IVT™ Kit (Ambion, Inc., Austin, TX), the PROTEINscript® II System (Ambion, Inc., Austin, TX) and the TnT® Coupled Reticulocyte Lysate Systems (Promega Corp., Madison, WI).

EXAMPLES

[0455] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of disclosed embodiments and are in no way intended to limit any of the embodiments disclosed in the present specification.

Example 1

Construction of a modified Clostridial toxin comprising an amino-terminally presented enhanced targeting domain

[0456] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising an enhanced targeting domain located at the amino terminus of the modified toxin.

1a. A targeting-translocation-enzymatic domain organization.

[0457] A polynucleotide molecule based on BoNT/A-AP4A-GRPP (SEQ ID NO: 118) will be synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting

domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 4A. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-AP4A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0458] If desired, an expression optimized polynucleotide molecule based on BoNT/A-AP4A-GRPP (SEQ ID NO: 118) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-AP4A-GRPP will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, e.g., Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type A*, International Patent Publication WO 2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-AP4A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, e.g., a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, e.g., Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0459] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-AP4A-GRPP, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/C1-AP4A-GRPP, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/D-AP4A-GRPP, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/E-AP4A-GRPP, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/F-AP4A-GRPP, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/G-AP4A-GRPP, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced

with amino acids 21-50 of SEQ ID NO: 9; and TeNT-AP4A-GRPP, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with amino acids 21-50 of SEQ ID NO: 9.

[0460] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4A that will replace the H_{CC} targeting domain from a Clostridial toxin the with an enhanced targeting domain comprising, e.g, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0461] To construct pET29/BoNT/A-AP4A-GRPP, a pUCBHB1/BoNT/A-AP4A-GRPP construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-AP4A-GRPP; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-AP4A-GRPP. The ligation mixture will be transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-AP4A-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0462] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-AP4A-GRPP toxins, such as, *e.g.*, BoNT/B-AP4A-GRPP, BoNT/C1-AP4A-GRPP, BoNT/D-AP4A-GRPP, BoNT/E-AP4A-GRPP, BoNT/F-AP4A-GRPP, BoNT/G-AP4A-GRPP or TeNT-AP4A-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4A comprising an enhanced targeting domain such as, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino

acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

1b. A targeting-enzymatic-translocation domain organization.

[0463] A polynucleotide molecule based on BoNT/A-AP4B-GRPP (SEQ ID NO: 119) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 4B. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0464] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4B that will replace the H_{CC} targeting domain from a Clostridial toxin the with an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO:

31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0465] In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-AP4B, such as, *e.g.*, BoNT/B-AP4B, BoNT/C1-AP4B, BoNT/D-AP4B, BoNT/E-AP4B, BoNT/F-AP4B, BoNT/G-AP4B or TeNT-AP4B, to comprise an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids

96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0466] To construct pET29/BoNT/A-AP4B-GRPP, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-AP4B-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-AP4B-GRPP toxins, such as, *e.g.*, BoNT/B-AP4B-GRPP, BoNT/C1-AP4B-GRPP, BoNT/D-AP4B-GRPP, BoNT/E-AP4B-GRPP, BoNT/F-AP4B-GRPP, BoNT/G-AP4B-GRPP or TeNT-AP4B-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-AP4B comprising an enhanced targeting domain such as, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257

of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

Example 2

Construction of a modified Clostridial toxin comprising a centrally presented enhanced targeting domain

[0467] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising an enhanced targeting domain located between two other domains of the modified toxin.

2a. An enzymatic-targeting-translocation domain organization.

[0468] A polynucleotide molecule based on BoNT/A-CP5A-GRPP (SEQ ID NO: 196) will be synthesized using standard procedures (BlueHeron[®] Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 5A. Cleavage of an enterokinase cleavage site used to form the di-chain toxin also exposes the first amino acid of the GRPP targeting domain. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-CP5A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator[™] Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0469] If desired, an expression optimized polynucleotide molecule based on BoNT/A-CP5A-GRPP (SEQ ID NO: 196) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-CP5A-GRPP will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, e.g., Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type A*, International Patent Publication WO 2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-CP5A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (CP5Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (CP5Applied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, e.g., a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, e.g., Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0470] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-CP5A-GRPP, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/C1-CP5A-GRPP, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/D-CP5A-GRPP, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/E-CP5A-GRPP, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/F-CP5A-GRPP, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/G-CP5A-GRPP, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced with amino acids 21-50 of SEQ ID NO: 9; and TeNT-CP5A-GRPP, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with amino acids 21-50 of SEQ ID NO: 9.

[0471] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5A that will replace the H_{CC} targeting domain from a Clostridial toxin with an enhanced targeting domain comprising, e.g., amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino

acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0472] To construct pET29/BoNT/A-CP5A-GRPP, a pUCBHB1/BoNT/A-CP5A-GRPP construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-CP5A-GRPP; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-CP5A-GRPP. The ligation mixture will be transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to

determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-CP5A-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0473] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-CP5A-GRPP toxins, such as, *e.g.*, BoNT/B-CP5A-GRPP, BoNT/C1-CP5A-GRPP, BoNT/D-CP5A-GRPP, BoNT/E-CP5A-GRPP, BoNT/F-CP5A-GRPP, BoNT/G-CP5A-GRPP or TeNT-CP5A-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5A comprising an enhanced targeting domain such as, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO:

68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

2b. A translocation-targeting-enzymatic domain organization.

[0474] A polynucleotide molecule based on BoNT/A-CP5B-GRPP (SEQ ID NO: 120) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 5B. Cleavage of an enterokinase cleavage site used to form the di-chain toxin also exposes the first amino acid of the GRPP targeting domain. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0475] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5B that will replace the H_{CC} targeting domain from a Clostridial toxin the with an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of

SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0476] In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-XP6B, such as, *e.g.*, BoNT/B-CP5B, BoNT/C1-CP5B, BoNT/D-CP5B, BoNT/E-CP5B, BoNT/F-CP5B, BoNT/G-CP5B or TeNT-CP5B, to comprise an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO:

59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0477] To construct pET29/BoNT/A-CP5B-GRPP, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-CP5B-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-CP5B-GRPP toxins, such as, *e.g.*, BoNT/B-CP5B-GRPP, BoNT/C1-CP5B-GRPP, BoNT/D-CP5B-GRPP, BoNT/E-CP5B-GRPP, BoNT/F-CP5B-GRPP, BoNT/G-CP5B-GRPP or TeNT-CP5B-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-CP5B with an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55;

amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

Example 3

Construction of a modified Clostridial toxin comprising a carboxyl-terminally presented enhanced targeting domain

[0478] This example illustrates how to make a modified Clostridial toxin disclosed in the present specification comprising an enhanced targeting domain located at the carboxyl terminus of the modified toxin.

3a. An enzymatic-translocation-targeting domain organization.

[0479] A polynucleotide molecule based on BoNT/A-XP6A-GRPP (SEQ ID NO: 121) will be synthesized using standard procedures (BlueHeron[®] Biotechnology, Bothell, WA). This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 6A. Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides will be hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule will be cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-XP6A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator[™] Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0480] If desired, an expression optimized polynucleotide molecule based on BoNT/A-XP6A-GRPP (SEQ ID NO: 121) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-XP6A-GRPP will be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, e.g., Lance E. Steward *et al.*, *Optimizing Expression of Active Botulinum Toxin Type E*, International Patent Publication WO 2006/011966 (Feb. 2, 2006); Lance E. Steward *et al.*, *Optimizing*

Expression of Active Botulinum Toxin Type A, International Patent Publication WO 2006/017749 (Feb. 16, 2006). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-XP6A-GRPP. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, expression optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0481] A similar cloning strategy will be used to make pUCBHB1 cloning constructs for BoNT/B-XP6A-GRPP, a modified BoNT/B where amino acids 1098-1291 of SEQ ID NO: 2 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/C1-XP6A-GRPP, a modified BoNT/C1 where amino acids 1112-1291 of SEQ ID NO: 3 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/D-XP6A-GRPP, a modified BoNT/D where amino acids 1099-1276 of SEQ ID NO: 4 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/E-XP6A-GRPP, a modified BoNT/E where amino acids 1086-1252 of SEQ ID NO: 5 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/F-XP6A-GRPP, a modified BoNT/F where amino acids 1106-1274 of SEQ ID NO: 6 are replaced with amino acids 21-50 of SEQ ID NO: 9; BoNT/G-XP6A-GRPP, a modified BoNT/G where amino acids 1106-1297 of SEQ ID NO: 7 are replaced with amino acids 21-50 of SEQ ID NO: 9; and TeNT-XP6A-GRPP, a modified TeNT where amino acids 1128-1315 of SEQ ID NO: 8 are replaced with amino acids 21-50 of SEQ ID NO: 9. Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6A with an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino

acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0482] To construct pET29/BoNT/A-XP6A-GRPP, a pUCBHB1/BoNT/A-XP6A-GRPP construct will be digested with restriction endonucleases that 1) will excise the polynucleotide molecule encoding the open reading frame of BoNT/A-XP6A-GRPP; and 2) will enable this polynucleotide molecule to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert will be subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-XP6A-GRPP. The ligation mixture will be transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and will be placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs will be identified as Kanamycin resistant colonies. Candidate constructs will be isolated using an alkaline lysis plasmid mini-preparation procedure and will be analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-XP6A-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide.

[0483] A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-XP6A-GRPP toxins, such as, *e.g.*, BoNT/B-XP6A-GRPP, BoNT/C1-XP6A-GRPP, BoNT/D-XP6A-GRPP, BoNT/E-XP6A-GRPP, BoNT/F-XP6A-GRPP, BoNT/G-XP6A-GRPP or TeNT-XP6A-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6A with an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of

SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

3b. A translocation-enzymatic-targeting domain organization.

[0484] A polynucleotide molecule based on BoNT/A-XP6B-GRPP (SEQ ID NO: 122) will be synthesized and cloned into a pUCBHB1 vector as described in Example 1a. This polynucleotide molecule encodes a BoNT/A modified to replace amino acids 1111-1296 of SEQ ID NO: 1, a BoNT/A H_{CC} targeting domain, with amino acids 21-50 of SEQ ID NO: 9, a GRPP targeting domain, and has the general domain arrangement of FIG. 6B. If so desired, expression optimization to a different organism, such as, *e.g.*, a bacteria, a yeast strain, an insect cell-line or a mammalian cell line, can be done as described above, see, *e.g.*, Steward, *supra*, (Feb. 2, 2006); and Steward, *supra*, (Feb. 16, 2006).

[0485] Likewise, a similar cloning strategy will be used to make pUCBHB1 cloning constructs comprising a polynucleotide molecule encoding a modified BoNT/A-XP6B comprising an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0486] In addition, similar cloning strategy will be used to produce a modified Clostridial toxin-XP6B, such as, *e.g.*, BoNT/B-XP6B, BoNT/C1-XP6B, BoNT/D-XP6B, BoNT/E-XP6B, BoNT/F-XP6B, BoNT/G-XP6B or TeNT-XP6B, to comprise an enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids

146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

[0487] To construct pET29/BoNT/A-XP6B-GRPP, a similar cloning strategy will be used as described in Example 1a. This cloning strategy will yield a pET29 expression construct comprising the polynucleotide molecule encoding the BoNT/A-XP6B-GRPP operably-linked to a carboxyl terminal polyhistidine affinity binding peptide. A similar cloning strategy will be used to make pET29 expression constructs for other modified Clostridial toxin-XP6B-GRPP toxins, such as, *e.g.*, BoNT/B-XP6B-GRPP, BoNT/C1-XP6B-GRPP, BoNT/D-XP6B-GRPP, BoNT/E-XP6B-GRPP, BoNT/F-XP6B-GRPP, BoNT/G-XP6B-GRPP or TeNT-XP6B-GRPP. Likewise, a similar cloning strategy will be used to make pET29 expression constructs comprising a polynucleotide molecule encoding a modified Clostridial toxin-XP6B with an

enhanced targeting domain comprising, *e.g.*, amino acids 53-81 of SEQ ID NO: 9; amino acids 53-89 of SEQ ID NO: 9; amino acids 98-124 of SEQ ID NO: 9; amino acids 146-178 of SEQ ID NO: 9; amino acids 132-158 of SEQ ID NO: 10; amino acids 32-58 of SEQ ID NO: 11; amino acids 32-75 of SEQ ID NO: 11; amino acids 81-107 of SEQ ID NO: 12; amino acids 125-151 of SEQ ID NO: 12; amino acids 81-107 of SEQ ID NO: 13; amino acids 124-150 of SEQ ID NO: 13; amino acids 52-78 of SEQ ID NO: 14; amino acids 52-93 of SEQ ID NO: 14; amino acids 28-54 of SEQ ID NO: 15; amino acids 76-92 of SEQ ID NO: 16; amino acids 59-92 of SEQ ID NO: 16; amino acids 41-50 of SEQ ID NO: 17; amino acids 24-50 of SEQ ID NO: 17; amino acids 99-112 of SEQ ID NO: 18; amino acids 159-193 of SEQ ID NO: 19; amino acids 154-194 of SEQ ID NO: 19; amino acids 35-70 of SEQ ID NO: 20; amino acids 145-177 of SEQ ID NO: 20; amino acids 1-200 of SEQ ID NO: 21; amino acids 1-150 of SEQ ID NO: 22; amino acids 1-202 of SEQ ID NO: 23; amino acids 1-201 of SEQ ID NO: 24; amino acids 1-225 of SEQ ID NO: 25; amino acids 123-265 of SEQ ID NO: 26; amino acids 21-153 of SEQ ID NO: 27; amino acids 57-210 of SEQ ID NO: 28; amino acids 21-99 of SEQ ID NO: 29; amino acids 31-94 of SEQ ID NO: 29; amino acids 19-178 of SEQ ID NO: 30; amino acids 1-558 of SEQ ID NO: 31; amino acids 1-371 of SEQ ID NO: 32; amino acids 49-118 of SEQ ID NO: 33; amino acids 25-180 of SEQ ID NO: 34; amino acids 1-54 of SEQ ID NO: 35; amino acids 139-257 of SEQ ID NO: 36; amino acids 129-247 of SEQ ID NO: 37; amino acids 19-257 of SEQ ID NO: 38; amino acids 81-210 of SEQ ID NO: 39; amino acids 118-211 of SEQ ID NO: 40; amino acids 107-196 of SEQ ID NO: 41; amino acids 96-197 of SEQ ID NO: 41; amino acids 66-155 of SEQ ID NO: 42; amino acids 123-218 of SEQ ID NO: 43; amino acids 293-390 of SEQ ID NO: 44; amino acids 317-414 of SEQ ID NO: 45; amino acids 315-412 of SEQ ID NO: 46; amino acids 276-373 of SEQ ID NO: 47; amino acids 296-396 of SEQ ID NO: 48; amino acids 370-472 of SEQ ID NO: 49; amino acids 309-409 of SEQ ID NO: 50; amino acids 323-454 of SEQ ID NO: 51; amino acids 412-513 of SEQ ID NO: 52; amino acids 374-513 of SEQ ID NO: 52; amino acids 330-431 of SEQ ID NO: 53; amino acids 293-431 of SEQ ID NO: 53; amino acids 301-402 of SEQ ID NO: 54; amino acids 323-424 of SEQ ID NO: 55; amino acids 267-372 of SEQ ID NO: 56; amino acids 327-429 of SEQ ID NO: 57; amino acids 264-364 of SEQ ID NO: 58; amino acids 400-501 of SEQ ID NO: 59; amino acids 354-455 of SEQ ID NO: 60; amino acids 352-450 of SEQ ID NO: 61; amino acids 281-375 of SEQ ID NO: 62; amino acids 376-478 of SEQ ID NO: 63; amino acids 313-407 of SEQ ID NO: 64; amino acids 211-308 of SEQ ID NO: 65; amino acids 321-426 of SEQ ID NO: 66; amino acids 303-406 of SEQ ID NO: 67; amino acids 247-352 of SEQ ID NO: 68; amino acids 237-352 of SEQ ID NO: 68; amino acids 247-350 of SEQ ID NO: 69; amino acids 262-366 of SEQ ID NO: 70; or amino acids 233-366 of SEQ ID NO: 70.

Example 4

Expression of Modified Clostridial Toxins in a Bacterial Cell

[0488] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a bacterial cell.

[0489] An expression construct, such as, e.g., any of the expression constructs in Examples 1-5, is introduced into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen, Inc, Carlsbad, CA) using a heat-shock transformation protocol. The heat-shock reaction is plated onto 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin and is placed in a 37 °C incubator for overnight growth. Kanamycin-resistant colonies of transformed *E. coli* containing the expression construct are used to inoculate a baffled flask containing 3.0 mL of PA-0.5G media containing 50 µg/mL of Kanamycin which is then placed in a 37 °C incubator, shaking at 250 rpm, for overnight growth. The resulting overnight starter culture is in turn used to inoculate a 3 L baffled flask containing ZYP-5052 autoinducing media containing 50 µg/mL of Kanamycin at a dilution of 1:1000. Culture volumes ranged from about 600 mL (20% flask volume) to about 750 mL (25% flask volume). These cultures are grown in a 37 °C incubator shaking at 250 rpm for approximately 5.5 hours and are then transferred to a 16 °C incubator shaking at 250 rpm for overnight expression. Cells are harvested by centrifugation (4,000 rpm at 4 °C for 20-30 minutes) and are used immediately, or stored dry at -80 °C until needed.

Example 5

Purification and Quantification of Modified Clostridial Toxins

[0490] The following example illustrates methods useful for purification and quantification of any modified Clostridial toxins disclosed in the present specification.

[0491] For immobilized metal affinity chromatography (IMAC) protein purification, *E. coli* BL21 (DE3) cell pellets used to express a modified Clostridial toxin, as described in Example 7, are resuspended in Column Binding Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 2x Protease Inhibitor Cocktail Set III (EMD Biosciences-Calbiochem, San Diego CA); 5 units/mL of Benzonase (EMD Biosciences-Novagen, Madison, WI); 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol), and then are transferred to a cold Oakridge centrifuge tube. The cell suspension is sonicated on ice (10-12 pulses of 10 seconds at 40% amplitude with 60 seconds cooling intervals on a Branson Digital Sonifier) in order to lyse the cells and then is centrifuged (16,000 rpm at 4 °C for 20 minutes) to clarify the lysate. An immobilized metal affinity chromatography column is prepared using a 20 mL Econo-Pac column support (Bio-Rad Laboratories, Hercules, CA) packed with 2.5-5.0 mL of TALON[™] SuperFlow Co²⁺ affinity resin (BD Biosciences-Clontech, Palo Alto, CA), which is then equilibrated by rinsing with 5 column volumes of deionized, distilled water, followed by 5 column volumes of Column Binding Buffer. The clarified lysate is applied slowly to the equilibrated column by gravity flow (approximately 0.25-0.3 mL/minute). The column is then washed with 5 column volumes of Column Wash Buffer (*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol). The modified Clostridial toxin is eluted with 20-30 mL of Column Elution Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES),

pH 7.8; 500 mM sodium chloride; 500 mM imidazole; 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol) and is collected in approximately twelve 1 mL fractions. The amount of modified Clostridial toxin contained in each elution fraction is determined by a Bradford dye assay. In this procedure, 20 μ L aliquots of each 1.0 mL fraction is combined with 200 μ L of Bio-Rad Protein Reagent (Bio-Rad Laboratories, Hercules, CA), diluted 1 to 4 with deionized, distilled water, and then the intensity of the colorimetric signal is measured using a spectrophotometer. The five fractions with the strongest signal are considered the elution peak and are combined together. Total protein yield is determined by estimating the total protein concentration of the pooled peak elution fractions using bovine gamma globulin as a standard (Bio-Rad Laboratories, Hercules, CA).

[0492] For purification of a modified Clostridial toxin using a FPLC desalting column, a HiPrep[™] 26/10 size exclusion column (Amersham Biosciences, Piscataway, NJ) is pre-equilibrated with 80 mL of 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5). After the column is equilibrated, a modified Clostridial toxin sample is applied to the size exclusion column with an isocratic mobile phase of 4 °C Column Buffer and at a flow rate of 10 mL/minute using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The desalted modified Clostridial toxin sample is collected as a single fraction of approximately 7-12 mL.

[0493] For purification of a modified Clostridial toxin using a FPLC ion exchange column, a modified Clostridial toxin sample that has been desalted following elution from an IMAC column is applied to a 1 mL Q1[™] anion exchange column (Bio-Rad Laboratories, Hercules, CA) using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The sample is applied to the column in 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5) and is eluted by linear gradient with 4 °C Elution Buffer (50 mM sodium phosphate, 1 M sodium chloride, pH 6.5) as follows: step 1, 5.0 mL of 5% Elution Buffer at a flow rate of 1 mL/minute; step 2, 20.0 mL of 5-30% Elution Buffer at a flow rate of 1 mL/minute; step 3, 2.0 mL of 50% Elution Buffer at a flow rate of 1.0 mL/minute; step 4, 4.0 mL of 100% Elution Buffer at a flow rate of 1.0 mL/minute; and step 5, 5.0 mL of 0% Elution Buffer at a flow rate of 1.0 mL/minute. Elution of modified Clostridial toxin from the column is monitored at 280, 260, and 214 nm, and peaks absorbing above a minimum threshold (0.01 au) at 280 nm are collected. Most of the modified Clostridial toxin will elute at a sodium chloride concentration of approximately 100 to 200 mM. Average total yields of modified Clostridial toxin will be determined by a Bradford assay.

[0494] Expression of a modified Clostridial toxin is analyzed by polyacrylamide gel electrophoresis. Samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE[®] Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Gels are stained with SYPRO[®] Ruby (Bio-Rad Laboratories, Hercules, CA) and the separated polypeptides are imaged using a Fluor-S MAX Multilmager (Bio-Rad Laboratories, Hercules, CA) for quantification of modified Clostridial toxin expression levels. The size and amount of modified

Clostridial toxin is determined by comparison to MagicMark™ protein molecular weight standards (Invitrogen, Inc, Carlsbad, CA).

[0495] Expression of modified Clostridial toxin is also analyzed by Western blot analysis. Protein samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Separated polypeptides are transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a Trans-Blot® SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes are blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes are incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20® (25 mM Tris-Buffered Saline, 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate) containing appropriate primary antibodies as a probe. Primary antibody probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Washed membranes are incubated at room temperature for 2 hours in Tris-Buffered Saline TWEEN-20® containing an appropriate immunoglobulin G antibody conjugated to horseradish peroxidase as a secondary antibody. Secondary antibody-probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Signal detection of the labeled modified Clostridial toxin are visualized using the ECL Plus™ Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and are imaged with a Typhoon 9410 Variable Mode Imager (Amersham Biosciences, Piscataway, NJ) for quantification of modified Clostridial toxin expression levels.

[0496] Although aspects of the present invention have been described with reference to the disclosed embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of these aspects and in no way limit the present invention. Various modifications can be made without departing from the spirit of the present invention.

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THIS IS VOLUME 1 OF 2
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What is claimed is

1. A modified Clostridial toxin comprising:

- a) a Clostridial toxin enzymatic domain capable of executing an enzymatic target modification step of a Clostridial toxin intoxication process;
- b) a Clostridial toxin translocation domain capable of executing a translocation step of a Clostridial toxin intoxication process;
- c) a targeting domain comprising a polypeptide selected from the group consisting of a glucagon like hormone, a neurohormone, a neuroregulatory cytokine, a neurotrophin, a growth factor, an axon guidance signaling molecule, a sugar binding protein, a ligand that selectively binds a neurexin, a ligand for neurexin-2 α , a ligand for neurexin-2 β , a ligand for neurexin-3 α , a ligand for neurexin-3 β , a WNT, Ng-CAM(L1), NCAM, N-cadherin, Agrin-MUSK and a basement membrane polypeptide, such that the targeting domain is capable of executing a cell binding step of a Clostridial toxin intoxication process; and
- d) a protease cleavage site,

wherein cleavage of the protease cleavage site converts the single-chain form of the modified Clostridial toxin into the di-chain form.

- 2. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin enzymatic domain, the protease cleavage site, the Clostridial toxin translocation domain and the targeting domain.
- 3. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin enzymatic domain, the protease cleavage site, the targeting domain and the Clostridial toxin translocation domain.
- 4. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the targeting domain, the Clostridial toxin translocation domain, the protease cleavage site and the Clostridial toxin enzymatic domain.
- 5. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the targeting domain, the Clostridial toxin enzymatic domain, the protease cleavage site and the Clostridial toxin translocation domain.

6. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin translocation domain, the protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain.
7. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin comprises, in a linear amino-to-carboxyl single polypeptide order, the Clostridial toxin translocation domain, the protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.
8. The modified Clostridial toxin according to Claim 1, wherein the Clostridial toxin enzymatic domain is selected from the group consisting of a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain and a TeNT enzymatic domain.
9. The modified Clostridial toxin according to Claim 1, wherein the Clostridial toxin translocation domain is selected from the group consisting of a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain and a TeNT translocation domain.
10. The modified Clostridial toxin according to Claim 1, wherein the protease cleavage site is an endogenous Clostridial toxin di-chain loop protease cleavage site or an exogenous cleavage site.
11. The modified Clostridial toxin according to Claim 10, wherein the endogenous Clostridial toxin di-chain loop protease cleavage site is selected from the group consisting of a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.
12. The modified Clostridial toxin according to Claim 10, wherein the exogenous protease cleavage site is selected from the group consisting of an enterokinase cleavage site, a Thrombin cleavage site, a Factor Xa cleavage site, a human rhinovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a dipeptidyl aminopeptidase cleavage site, a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, and a Clostridial toxin substrate cleavage site.
13. The modified Clostridial toxin according to Claim 12, wherein the Clostridial toxin substrate cleavage site is selected from the group consisting of a BoNT/A substrate cleavage site, a BoNT/B substrate cleavage site, a BoNT/C1 substrate cleavage site, a BoNT/D substrate cleavage site, a BoNT/E

substrate cleavage site, a BoNT/F substrate cleavage site, a BoNT/G substrate cleavage site and a TeNT substrate cleavage site.

14. A modified Clostridial toxin according to any one of the preceding claims, wherein the targeting domain comprises a glucagon like hormone selected from the group consisting of a secretin, a glucagon-like peptide, a pituitary adenylate cyclase activating peptide, a glicentin, a glicentin-related polypeptide, an oxyntomodulin, a vasoactive intestinal peptides, a gastric inhibitory polypeptide and a calcitonin-related peptides visceral gut peptide.
15. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a neurohormone selected from the group consisting of corticotropin-releasing hormone (CCRH) and parathyroid hormone (PTH).
16. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a neuroregulatory cytokine selected from the group consisting of ciliary neurotrophic factor (CNTF), glycoporphin-A (GPA), leukemia inhibitory factor (LIF), an interleukin (IL), onostatin M, cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), a neuroleukin, VEGF, an insulin-like growth factors (IGF) and an epidermal growth factor (EGF).
17. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a neurotrophin selected from the group consisting of a nerve growth factor (NGF), a brain-derived growth factor (BDNF), a neurotrophin-3 (NT-3) and a neurotrophin-4/5 (NT-4/5).
18. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a growth factor selected from the group consisting of glial cell derived neurotrophic factor (GDNF), neurturin, persephrin, artemin, a transformation growth factor beta (TGF β) polypeptide, a bone morphogenetic protein (BMP), a growth and differentiation factor (GDF) and an activin.
19. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a TGF β polypeptide selected from the group consisting of TGF β 1, TGF β 2, TGF β 3 and TGF β 4.
20. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a BMP selected from the group consisting of BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8 and BMP10.
21. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a GDF selected from the group consisting of GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF10, GDF11 and GDF15.

22. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a polypeptide selected from the group consisting of an activin A, an activin B, an activin C, an activin E and an inhibin A.
23. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a sugar binding protein selected from the group consisting of serum amyloid P, β -glucanase, sialidase, lectin, cryia, insecticidal delta-endotoxin, agglutinin, abrin and ricin.
24. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a ligand that binds a polypeptide selected from the group consisting of neurexin-1 α , neurexin-1 β , neurexin-2 α , neurexin-2 β , neurexin-3 α and neurexin-3 β .
25. A modified Clostridial toxin according to any one of claims 1-13, wherein the targeting domain comprises a laminin β -2.
26. A polynucleotide molecule encoding the polypeptide of any one of the preceding claims.
27. A method of producing a modified Clostridial toxin comprising the step of expressing in a cell the polynucleotide of claim 26.

FIG. 1A.

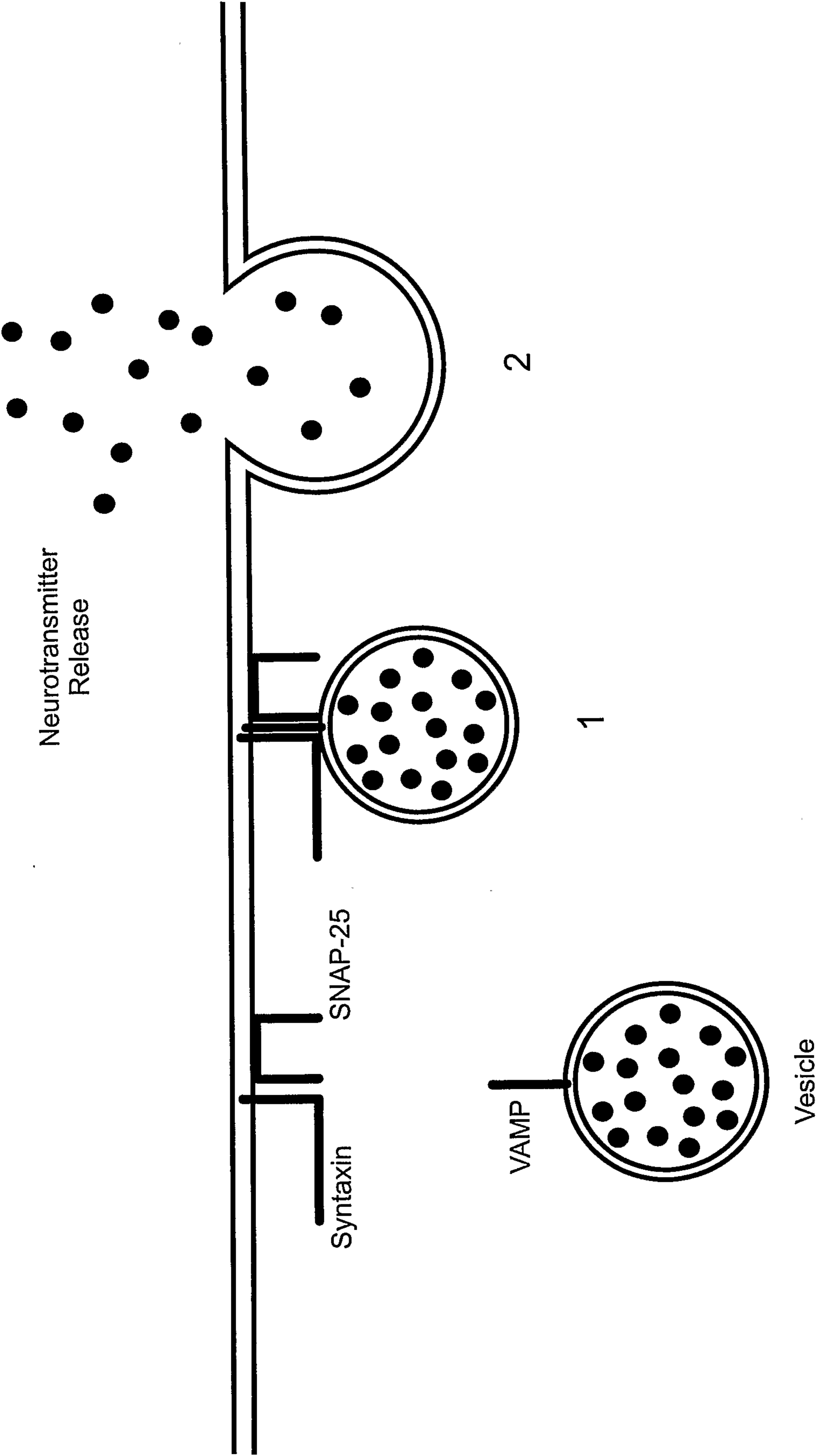


FIG. 1B.

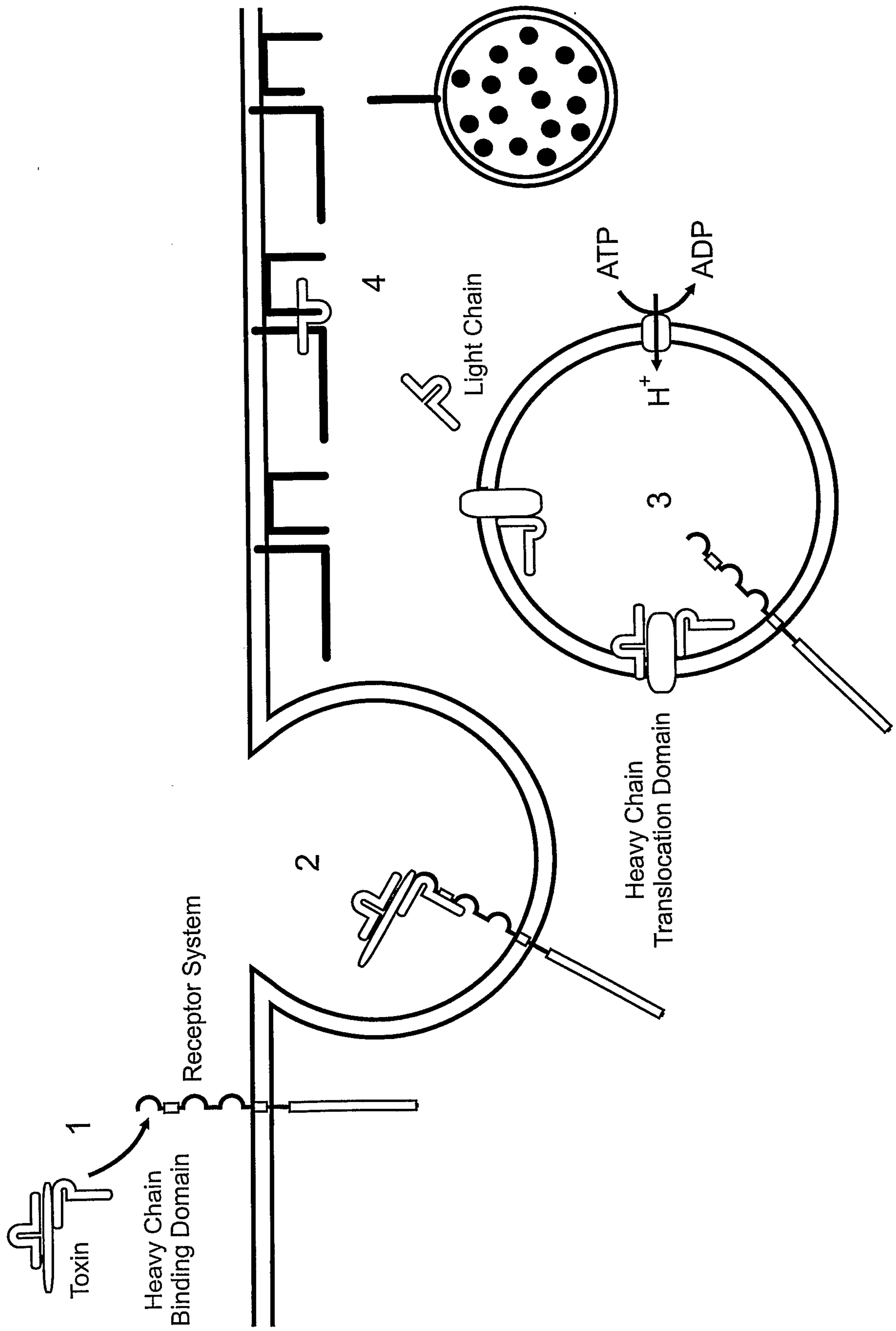


FIG. 2.

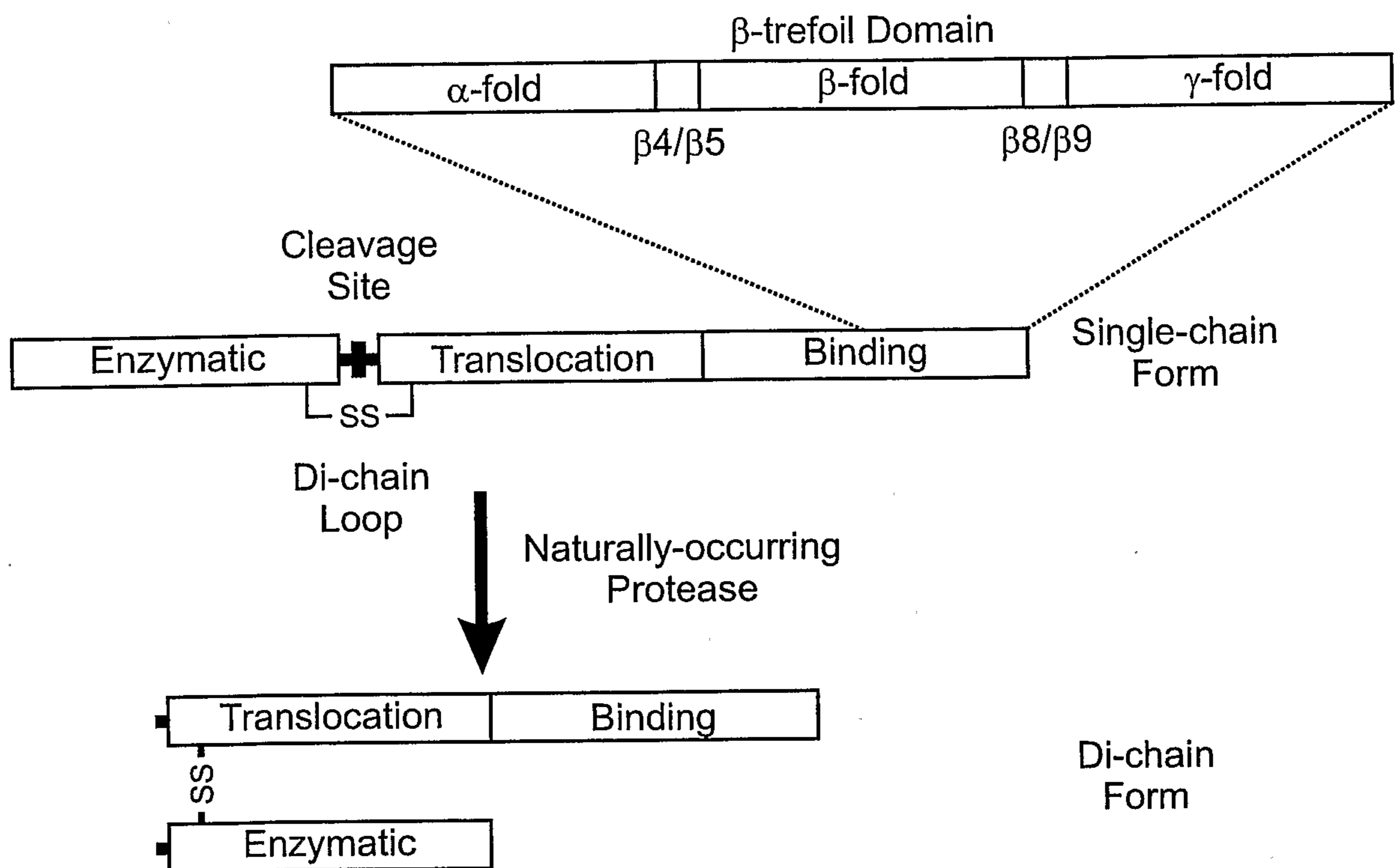


FIG. 3A.

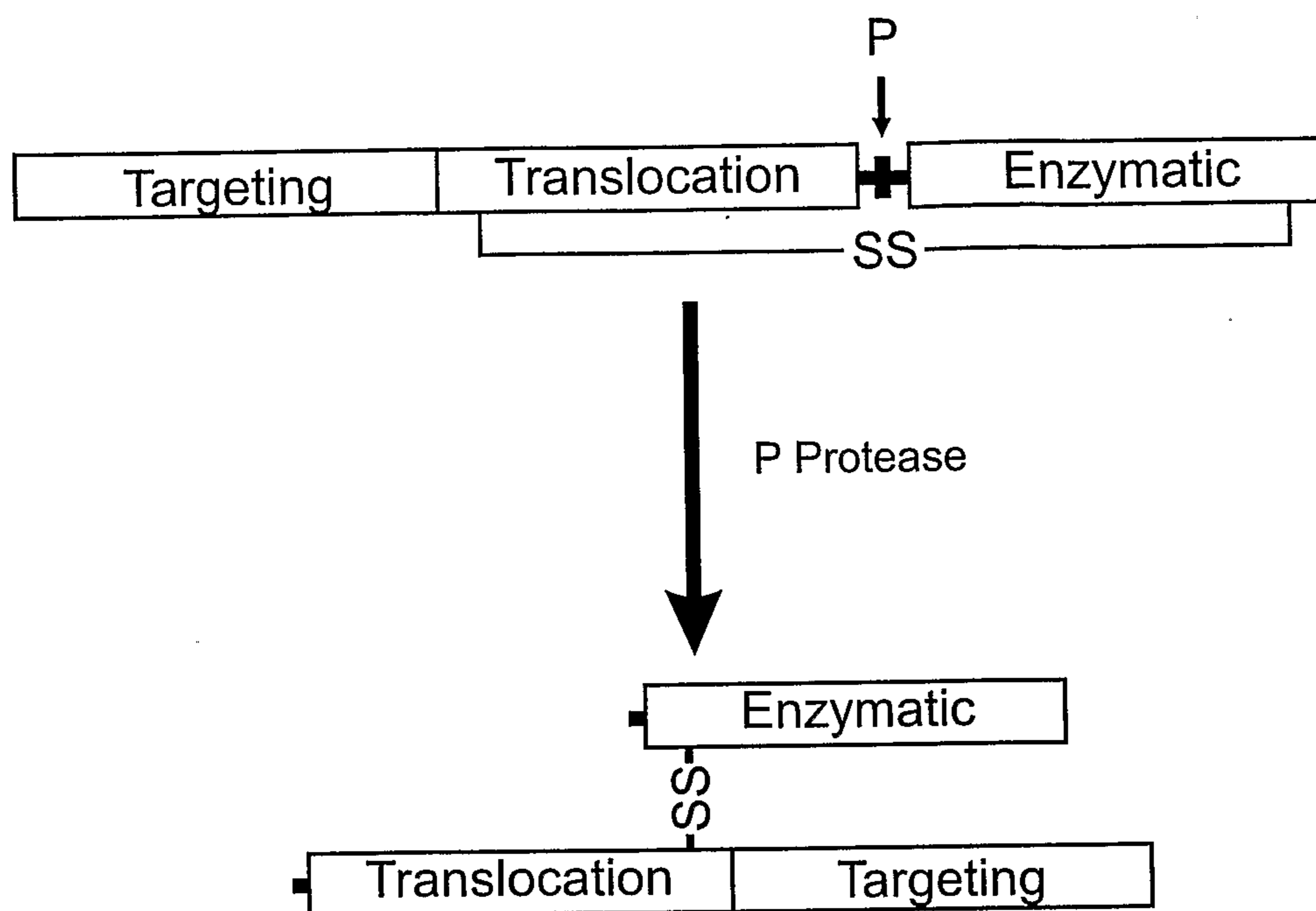


FIG. 3B.

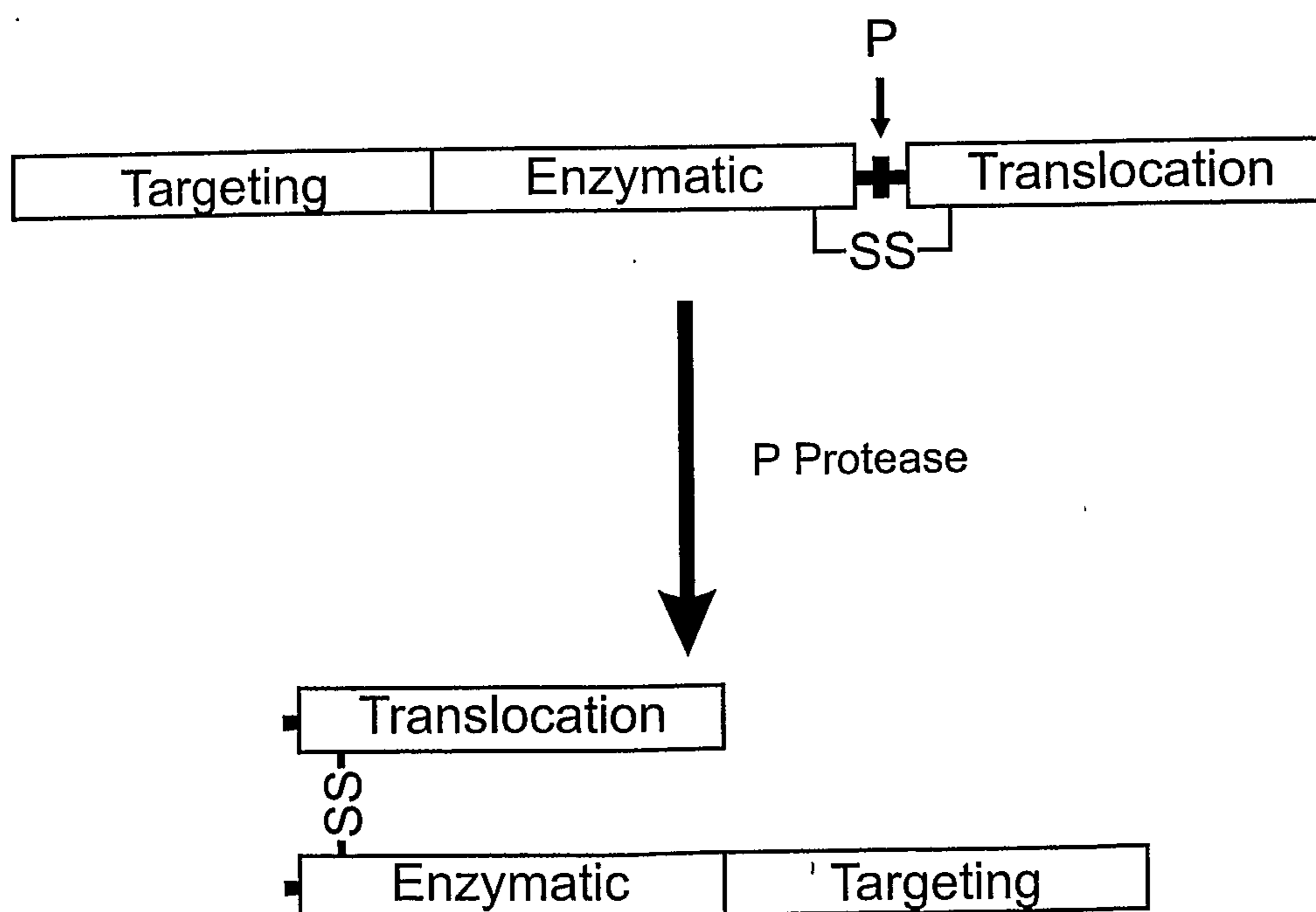


FIG. 4A.

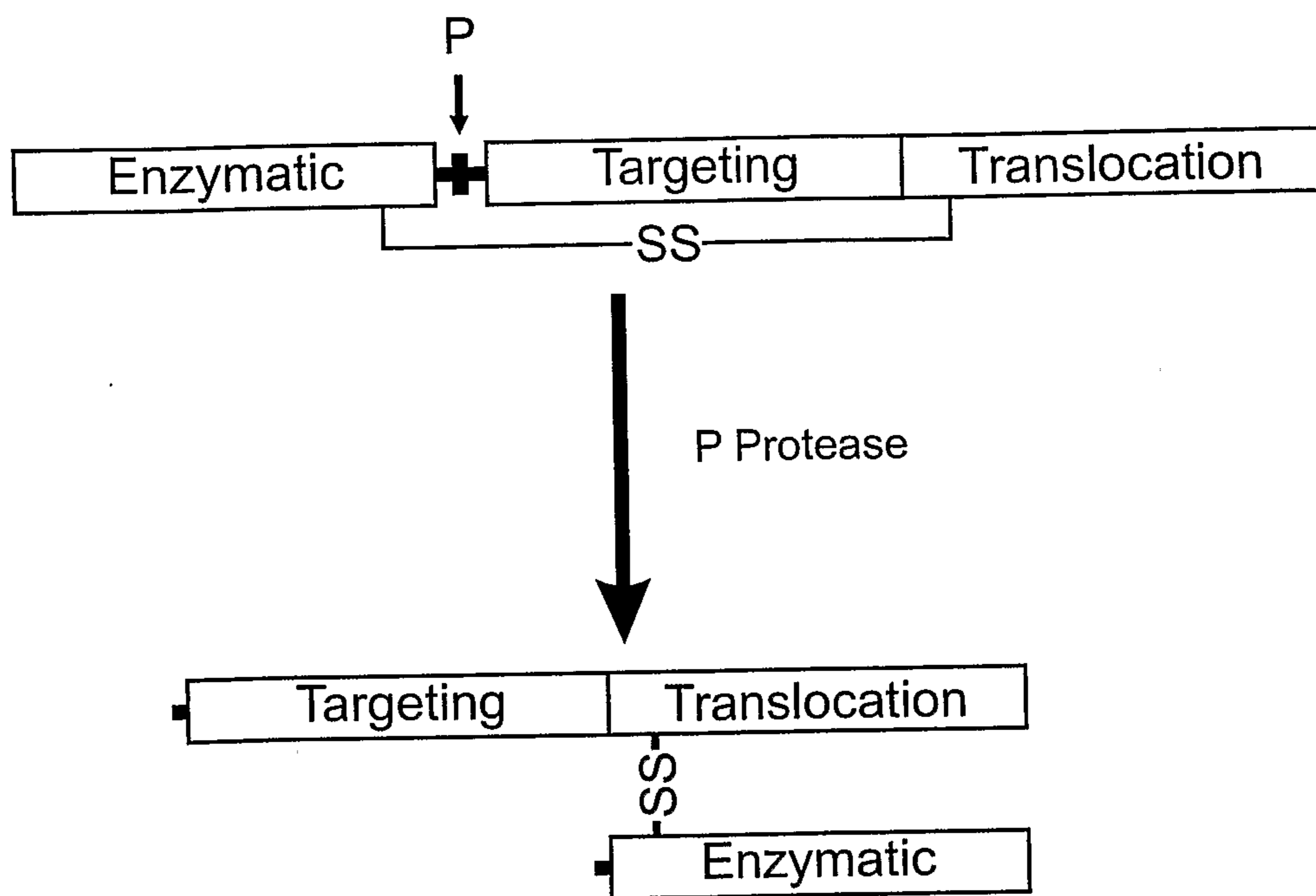


FIG. 4B.

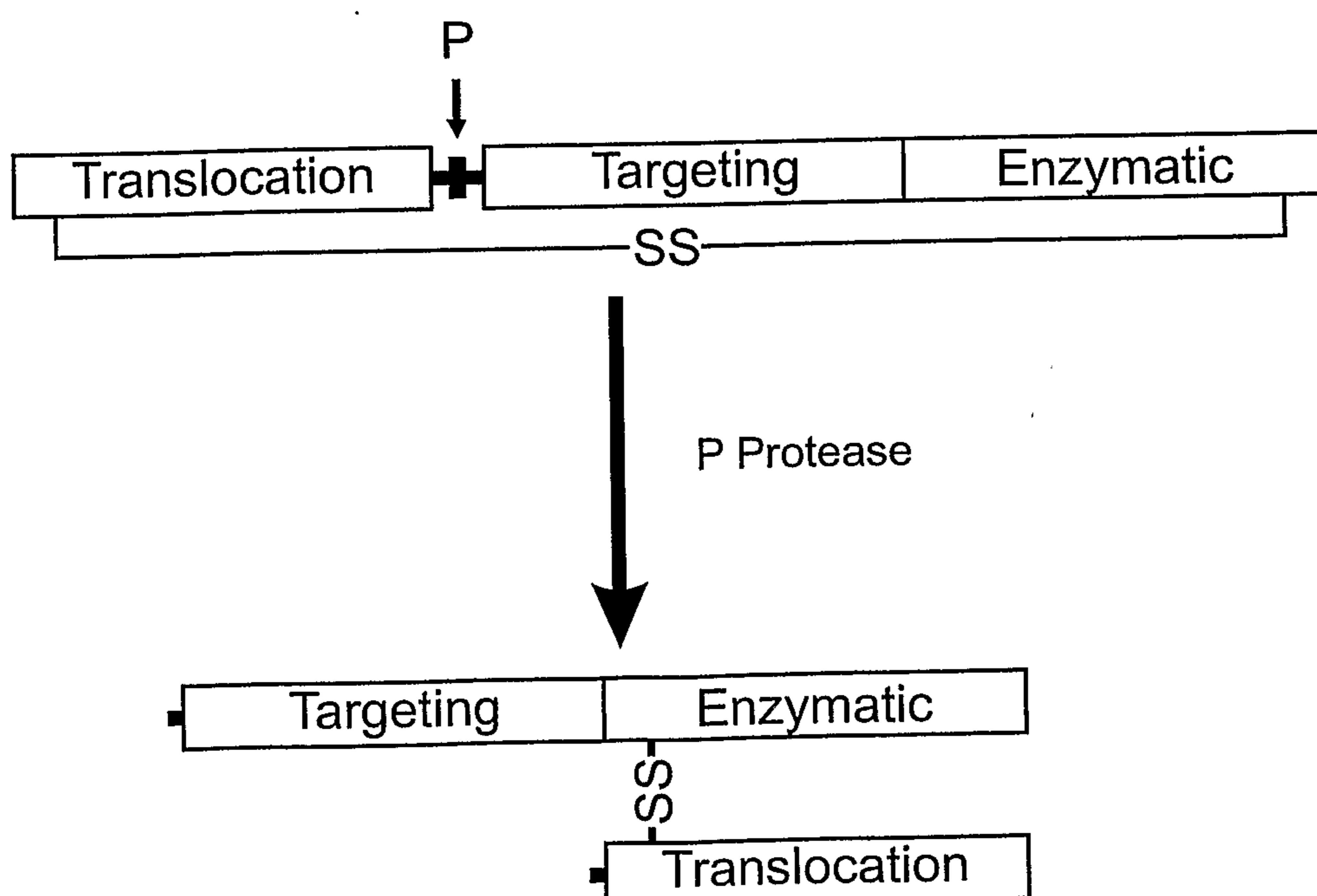


FIG. 5A.

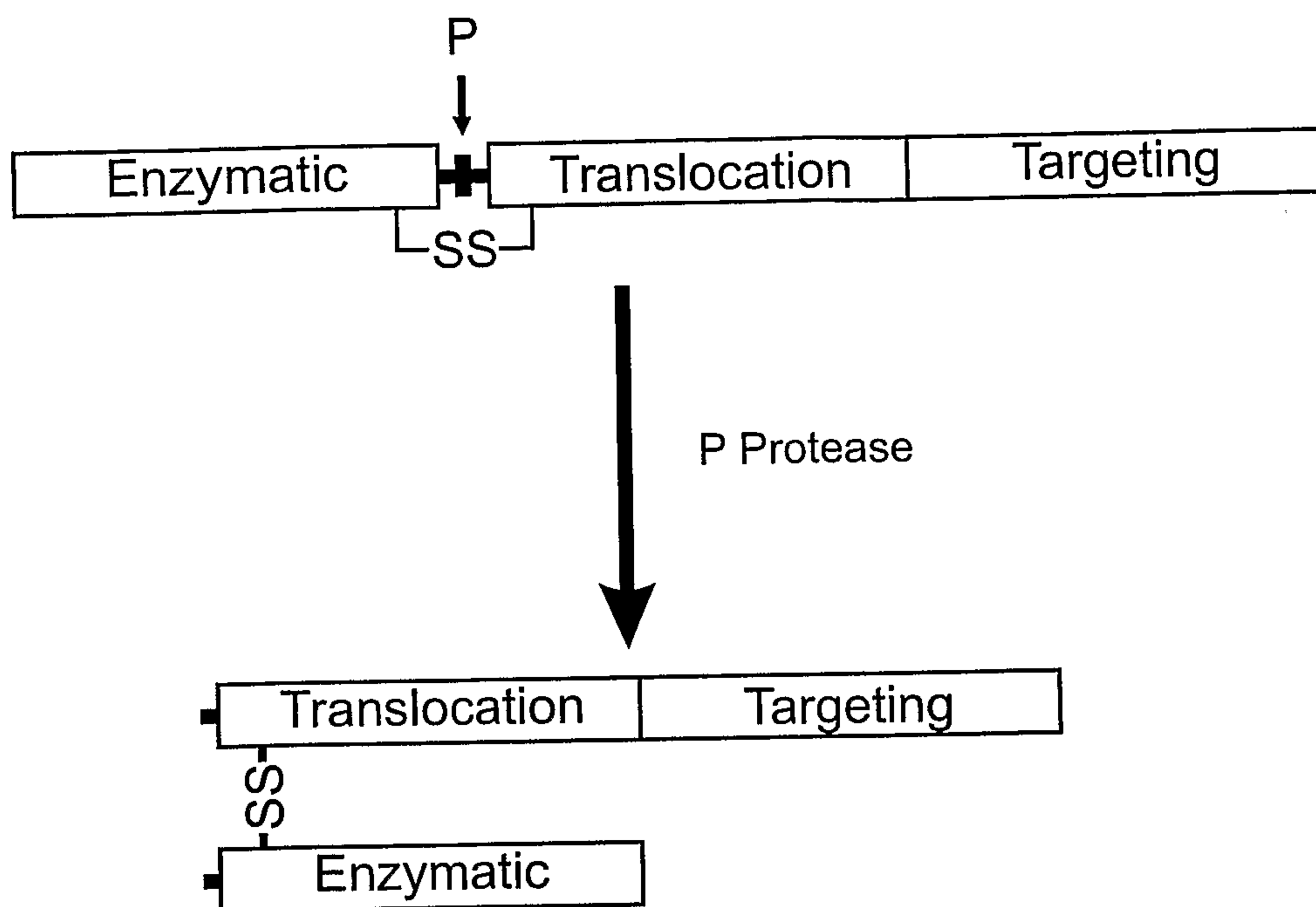
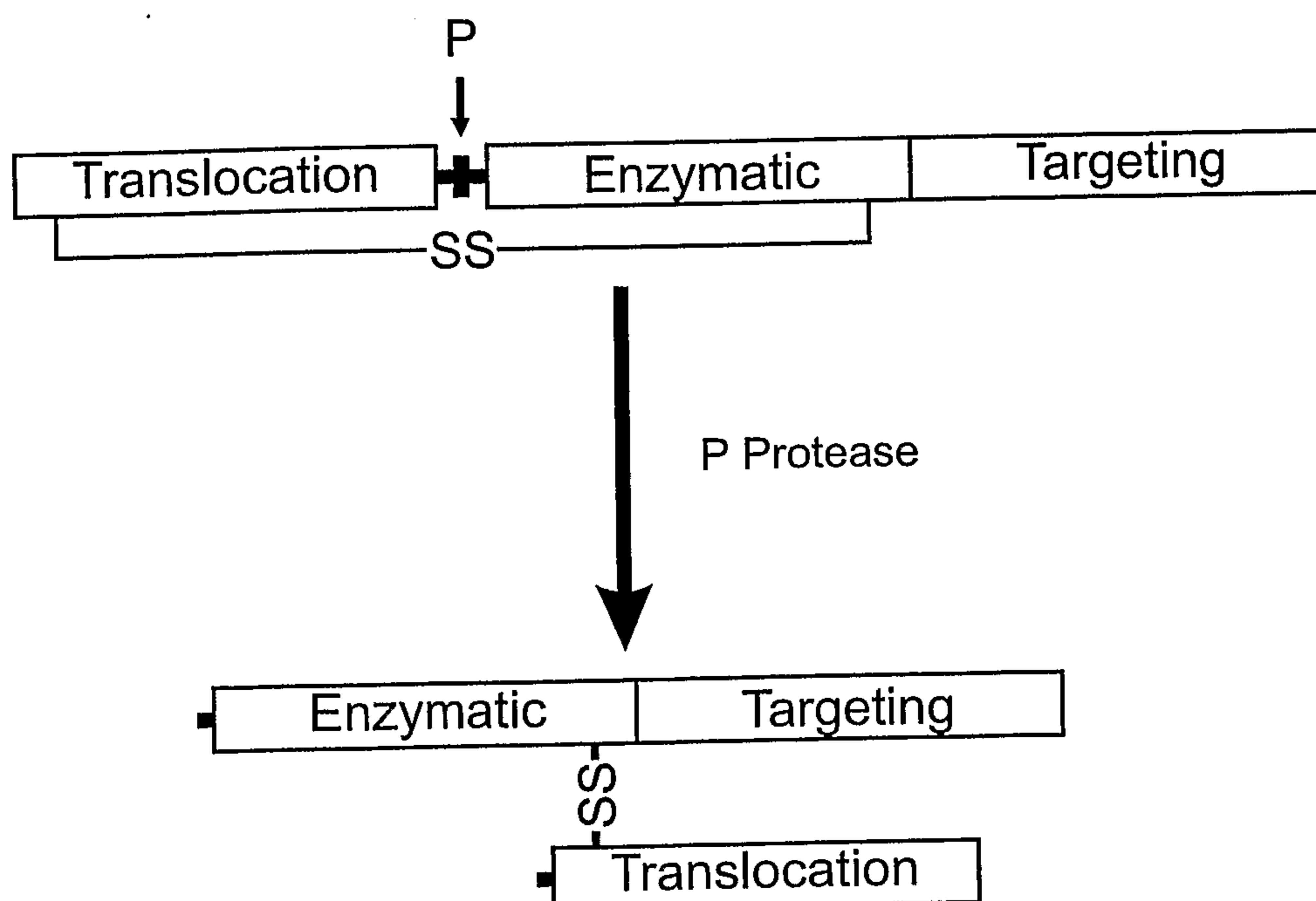
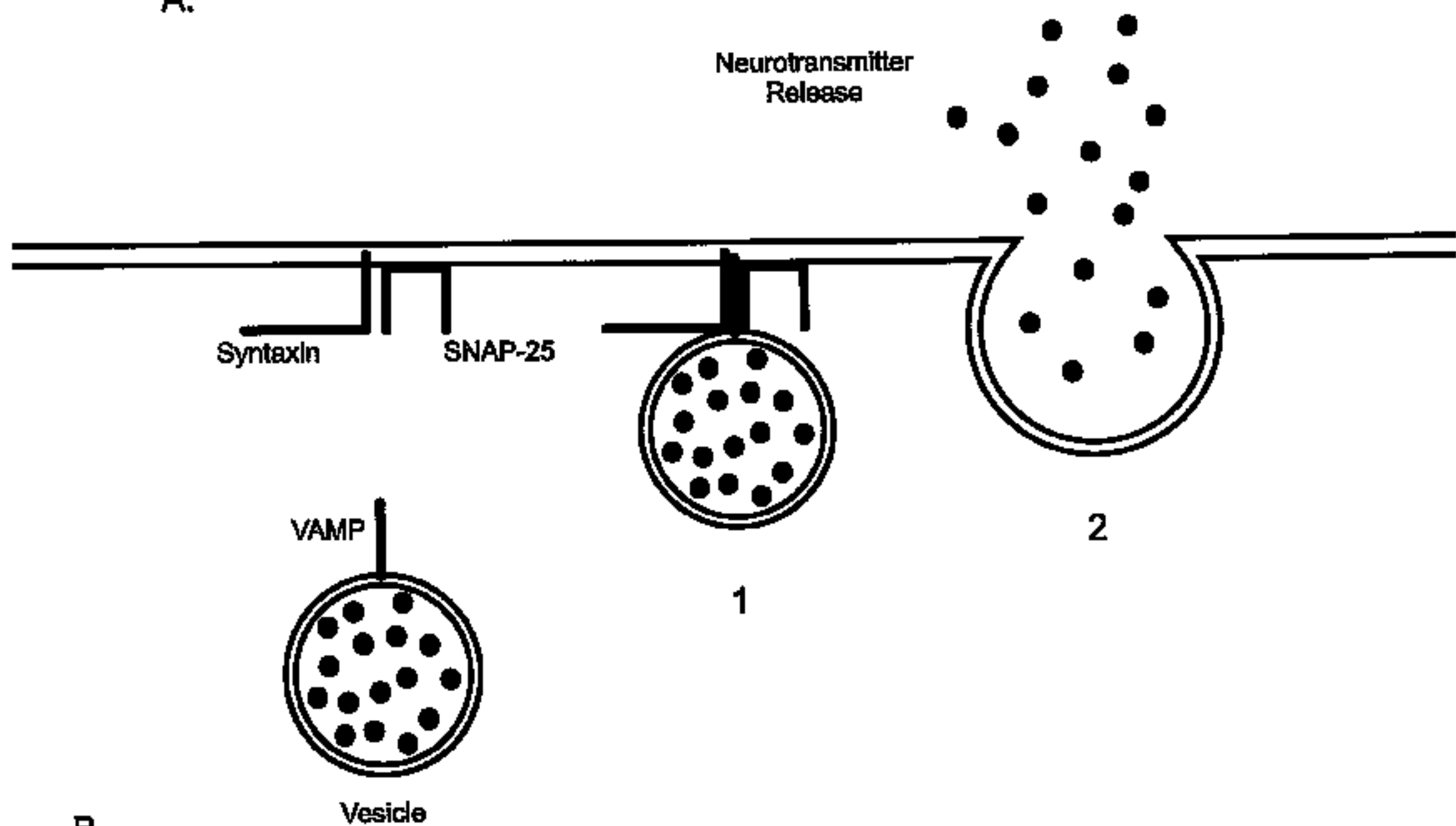


FIG. 5B.



A.



B.

