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(54) **Title**: CHOLIX TOXIN-DERIVED FUSION MOLECULES FOR ORAL DELIVERY OF BIOLOGICALLY ACTIVE CARGO

(57) **Abstract**: The present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery to a subject, and designed to provide for improved, effective therapies for treatment of, e.g., inflammatory diseases, autoimmune diseases, cancer, metabolic disorders, and growth deficiency disorders.

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CHOLIX TOXIN-DERIVED FUSION MOLECULES FOR ORAL DELIVERY OF BIOLOGICALLY ACTIVE CARGO

RELATED PATENT APPLICATIONS

[001] This application claims benefit of U.S. Provisional Application No. 61/990,054, filed on May 7, 2014, incorporated in its entirety by reference herein.

TECHNICAL FIELD

[002] Oral delivery of biologically active polypeptides (referring to a polymer composed of amino acid residues; typically also defined as proteins or peptides) has been a long-standing goal of the pharmaceutical industry. Unfortunately, the numerous physical, physiological, and biological barriers of the gastrointestinal (GI) tract are designed to inhibit uptake of proteins and peptides until they can be sufficiently degraded for absorption through amino acid and di- or tri-peptide transporters; and/or to traffic the proteins and peptides intracellularly to destructive lysosome compartments after endosomal uptake at the luminal surface. As such, the feasibility of polypeptide uptake from the intestine in a manner similar to that achievable with, e.g., small molecules, has been limited and low oral bioavailability continues to be a problem for most polypeptides and proteins.

[003] While there have been some promising results from clinical studies evaluating various biologically active polypeptides for the treatment of diseases such as cancer, inflammatory diseases, immune diseases, growth deficiency disorders, etc., and several DNA-based therapeutics have been FDA approved for such uses, these therapeutics often fail to really reach their optimum potential, as there is often marginal or inadequate overall efficacy due to inherent limitations such as short biological half-life which prevents the delivery of optimal therapeutically effective dosages, and/or detrimental side effects and toxicities observed at the therapeutically effective doses. Moreover, many such therapeutics require multiple dosing regimens, necessitating continuous administration intravenously or by frequent subcutaneous injections, which are burdensome on the patients and caregivers.

[004] Future clinical studies directed toward evaluating the promising biologically active polypeptides could benefit greatly from new methods and/or pharmaceutical compositions that could be used to orally administer such polypeptides to a human subject.

[004a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge in the field.

DISCLOSURE OF THE INVENTION

[005] The present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of, e.g., inflammatory diseases and/or autoimmune diseases and/or cancers.

[006] The present disclosure is based in part on the inventors’ unique insight that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a biologically active cargo may, among other things, provide the following advantages: a) in embodiments wherein the modified Cholix toxin is coupled to the biologically active cargo without a linker, or with a non-cleavable linker, the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of, e.g., immune cells that also express the receptor for the biologically active cargo, can allow for greater exposure of the biologically active cargo at the surface of the targeted cells and provide a synergistic effect by binding to both the Cholix receptor and the biologically active cargo receptor; b) in embodiments wherein the modified Cholix toxin is coupled to the biologically active cargo with a linker that is cleavable by an enzyme present at a basolateral membrane of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the biologically active cargo to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane c) the direct delivery of the biologically active cargo to the submucosal-GI space and hepatic-portal system may reduce the systemic toxicity observed when the cargo are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes; d) once transported across the GI epithelium, the fusion molecules of the disclosure will exhibit extended half-life in serum, that is, the biologically active cargo of the fusion molecules will exhibit an extended serum half-life compared to the biologically active cargo in its non-fused state; e) oral administration of the fusion molecule can deliver a higher effective concentration of the delivered biologically active cargo to the liver of the subject than is observed in the subject's plasma; and f) the ability to deliver the biologically active cargo to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith, in addition to improved patient/care-giver convenience and compliance.

[006a] According to a first aspect, the present invention provides a pharmaceutical composition comprising a non-naturally occurring, dimeric fusion molecule and one or more pharmaceutically acceptable carriers, wherein each monomer of the dimeric fusion molecule comprises

(i) a polypeptide consisting of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or a fragment thereof, wherein the fragment consists of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS: 3, 42, 52, 70, or 80, coupled to

(ii) a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

[006b] According to a second aspect, the present invention provides a method for delivering a biologically active cargo to a subject, the method comprising orally delivering to the subject a pharmaceutical composition comprising a non-naturally occurring dimeric fusion molecule and one or more pharmaceutically acceptable carriers, wherein each monomer of the dimeric fusion molecule comprises

(i) a polypeptide consisting of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or a fragment thereof, wherein the fragment consists of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS: 3, 42, 52, 70, or 80, coupled to

(ii) a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

[007] Thus, in one aspect, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a biologically active cargo to be delivered to a subject, wherein the Cholix toxin is non-toxic.

[008] In one aspect, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a biologically active cargo to be delivered to a subject, wherein the Cholix toxin is non-toxic, and wherein the fusion molecule has the ability to activate the receptor for the biologically active cargo, or to enable the catalytic process of a catalytically-active material.

[009] In various embodiments, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain II. In various embodiments, the fusion molecules comprise a truncated Cholix toxin having the amino acid sequence set forth in, *e.g.,* SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40 or SEQ ID NO: 41.

[010] In various embodiments, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain Ib. In various embodiments, the fusion molecules comprise a truncated Cholix toxin having the amino acid sequence set forth in, *e.g.,* SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO:48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, 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, or SEQ ID NO: 80.

[011] In various embodiments, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin wherein domain III has been truncated or mutated. In various embodiments, the fusion molecules comprise a mutated Cholix toxin having the amino acid sequence set forth in SEQ ID NO: 81 wherein the amino acid residue E581 of SEQ ID NO: 1 has been deleted (designated herein as “Cholix ΔE581”).

[012] In various embodiments, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin wherein domain Ia has been mutated.

[013] In various embodiments, the biologically active cargo is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material.

[014] In various embodiments, the biologically active cargo is an enzyme selected from hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, or PGE-adenosine deaminase.

[015] In various embodiments, the biologically active cargo is a polypeptide that is a modulator of inflammation in the GI tract selected from, *e.g.,* interleukin-10, interleukin-19, interleukin-20, interleukin-22, interleukin-24, or interleukin-26. In various embodiments, the biologically active polypeptide is interleukin-10 having the amino acid sequence set forth is SEQ ID NO: 82. In various embodiments, the biologically active polypeptide is interleukin-19 having the amino acid sequence set forth is SEQ ID NO: 83. In various embodiments, the biologically active polypeptide is interleukin-20 having the amino acid sequence set forth is SEQ ID NO: 84. In various embodiments, the biologically active polypeptide is interleukin-22 having the amino acid sequence set forth is SEQ ID NO: 85. In various embodiments, the biologically active polypeptide is interleukin-24 having the amino acid sequence set forth is SEQ ID NO: 86. In various embodiments, the biologically active polypeptide is interleukin-26 having the amino acid sequence set forth is SEQ ID NO: 87. In various embodiments, the biologically active cargo is a modulator of inflammation in the GI tract that is a small molecule. In various embodiments, the biologically active cargo is a modulator of inflammation in the GI tract that is an antisense or siRNA molecule.

[016] In various embodiments, the biologically active cargo is a TNFSF inhibitor that is an antibody, or a fragment thereof, or an artificial construct comprising an antibody or fragment thereof, or an artificial construct designed to mimic the binding of an antibody or fragment thereof to its antigen. In various embodiments, the biologically active cargo is a TNFSF inhibitor that is a soluble TNFSF receptor fusion protein. In various embodiments, the biologically active cargo is a TNFSF inhibitor that is a small molecule. In various embodiments, the biologically active cargo is a TNFSF inhibitor that is an antisense or siRNA molecule.

[017] In various embodiments, the biologically active cargo is an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 88 and light chain variable region amino acid sequence set forth in SEQ ID NO: 89. In various embodiments, the biologically active cargo is an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 90 and light chain variable region amino acid sequences set forth in SEQ ID NO: 91. In various embodiments, the biologically active cargo is a soluble TNFSF receptor fusion protein dimer comprising the amino acid sequence set forth in SEQ ID NO: 92.

[018] In one aspect, the present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of metabolic disorders, e.g., Type 1 Diabetes and Type 2 Diabetes. Oral delivery of biologically active polypeptides (referring to a polymer composed of amino acid residues; typically also defined as proteins or peptides) has been a long-standing goal of the pharmaceutical industry. Unfortunately, the numerous physical, physiological, and biological barriers of the gastrointestinal (GI) tract are designed to inhibit uptake of proteins and peptides until they can be sufficiently degraded for absorption through amino acid and di- or tri-peptide transporters; and/or to traffic the proteins and peptides intracellularly to destructive lysosome compartments after endosomal uptake at the luminal surface. As such, the feasibility of polypeptide uptake from the intestine in a manner similar to that achievable with, e.g., small molecules, has been limited and low oral bioavailability continues to be a problem for most polypeptides and proteins.

[019] In various embodiments, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a glucose-lowering agent to be delivered to a subject.

[020] In various embodiments, the present disclosure is based in part on that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a glucose-lowering agent may, among other things, provide the following advantages: a) in embodiments wherein the modified Cholix toxin is coupled to the glucose-lowering agent without a linker, the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of cells that also express the receptor for the glucose-lowering agent, can allow for greater exposure of the glucose-lowering agent at the surface of the targeted cells;

b) in embodiments wherein the modified Cholix toxin is coupled to the glucose-lowering agent with a linker that is cleavable by an enzyme present at a basal-lateral membrane of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the glucose-lowering agent to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane; c) the direct delivery of the glucose-lowering agent to the submucosal-GI space and hepatic-portal system may reduce the systemic toxicity observed when the glucose-lowering agents are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes; d) the direct delivery of the glucose-lowering agent to the submucosal-GI space and hepatic-portal system may provide for improved dosing regimens, including less frequent insulin injections; and e) the ability to deliver the glucose-lowering agent to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith.

[021] In various embodiments, the glucose-lowering agent is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material. In various embodiments, the glucose-lowering agent is an incretin or incretin mimetic. In various embodiments, the glucose-lowering agent is a GLP-1. In various embodiments, the glucose-lowering agent is a GLP-1 agonist. In various embodiments, the glucose-lowering agent is an exendin. In various embodiments, the glucose-lowering agent is a glucose inhibitory protein receptor (GIPR) agonist.

[022] In various embodiments, the glucose-lowering agent is a GLP-1 agonist that is a peptide. In various embodiments, the glucose-lowering agent is a GLP-1 agonist that is a small molecule. In various embodiments, the glucose-lowering agent is a GLP-1 agonist that is an antisense or siRNA molecule. In various embodiments, the glucose-lowering agent is a GLP-1 agonist that is an antibody, or a fragment thereof, or an artificial construct comprising an antibody or fragment thereof, or an artificial construct designed to mimic the binding of an antibody or fragment thereof to its antigen.

[023] In various embodiments, the biologically active cargo is a glucose-lowering agent that is a GLP-1 agonist peptide comprising the amino acid sequence set forth in SEQ ID NO: 93. In various embodiments, the biologically active cargo is a glucose-lowering agent that is a GLP-1 agonist peptide comprising the amino acid sequence set forth in SEQ ID NO: 94.

[024] In one aspect, the present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of growth hormone deficiency, and like disorders.

[025] In various embodiments, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a growth hormone (GH) to be delivered to a subject.

[026] In various embodiments, the present disclosure is based in part on the inventors' unique insight that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a growth hormone may, among other things, provide the following advantages: a) in embodiments wherein the modified Cholix toxin is coupled to the growth hormone with a linker that is cleavable by an enzyme present at a basolateral membrane surface of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the growth hormone to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane; b) the direct delivery of the growth hormone to the submucosal-GI space and hepatic-portal system may reduce systemic toxicities observed when the growth hormones are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes (e,g, provide a more efficient induction of IGF-1 relative to systemic delivery via subcutaneous (sc) injection); c) the direct delivery of the growth hormone to the submucosal-GI space and hepatic-portal system may provide for improved dosing regimens; d) oral delivery will achieve a brief pulse of growth hormone to the liver that is more consistent with serum level observed in growing children, and this pulse profile is not achievable by sc injection; and e) the ability to deliver the growth hormone to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith, in addition to improved patient/care-giver convenience and compliance.

[027] In various embodiments, the growth hormone is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material. In various embodiments, the growth hormone is human growth hormone (or a variant thereof), growth hormone 2, or growth hormone-releasing hormone. In various embodiments, the growth hormone is human growth hormone (somatotropin) comprising the amino acid sequence set forth in SEQ ID NO: 95.

[028] In various embodiments, the fusion molecules comprise a modified Cholix toxin directly coupled to a biologically active cargo. In various embodiments, the biologically active cargo is directly coupled to the C-terminus of the Cholix toxin.

[029] In various embodiments, the fusion molecules comprise a modified Cholix toxin chemically coupled to a biologically active cargo.

[030] In various embodiments, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a non-cleavable linker. In various embodiments, the non-cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 or SEQ ID NO: 99.

[031] In various embodiments, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a cleavable linker. In various embodiments, the linker is cleavable by an enzyme that is present at a basolateral membrane of a polarized epithelial cell of the subject. In various embodiments, the linker is cleavable by an enzyme that is present in the plasma of said subject. In various embodiments, the cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, or SEQ ID NO: 120.

[032] In various embodiments, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a cleavable linker, wherein the cleavable linker comprises an amino acid sequence that is known to be a substrate for tobacco etch virus (TEV) protease. In various embodiments, the cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 121.

[033] In various embodiments, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 122. (this is Cholix^{415}–TEV-IL-10)

[034] In various embodiments, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 123. (this is Cholix^{415}–$(G_4S)_3$-IL-10)

[035] In another aspect, the present disclosure provides a method of treating an inflammatory disease in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various embodiments, the inflammatory disease is selected from an inflammatory bowel disease, psoriasis or bacterial sepsis. In various embodiments, the inflammatory bowel disease is Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome or indeterminate colitis.

[036] In another aspect, the present disclosure provides a method of treating an autoimmune disease in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various embodiments, the autoimmune disease is systemic lupus erythematosus (SLE), pemphigus vulgaris, myasthenia gravis, hemolytic anemia, thrombocytopenia purpura, Grave's disease, Sjogren's disease, dermatomyositis, Hashimoto's disease, polymyositis, inflammatory bowel disease, multiple sclerosis (MS), diabetes mellitus, rheumatoid arthritis, or scleroderma.

[037] In another aspect, the present disclosure provides a method of treating a cancer in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various embodiments, the cancer to be treated includes, but is not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, chronic lymphocytic leukemia, hairy cell leukemia, acute lymphoblastic leukemia, multiple myeloma, carcinomas of the bladder, kidney ovary, cervix, breast, lung, nasopharynx, malignant melanoma and rituximab resistant NHL and leukemia.

[038] In another aspect, the present disclosure provides a method of treating a subject having a metabolic disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said metabolic disorder is diabetes, obesity, diabetes as a consequence of obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, impaired glucose tolerance (IGT), diabetic dyslipidemia, or hyperlipidemia.

[039] In another aspect, the present disclosure provides a method of treating a subject having a fatty liver disease (e.g., nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH)), a gastrointestinal disease, or a neurodegenerative disease, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disease.

[040] In another aspect, the present disclosure provides a method of treating a subject having a GH deficient growth disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said disorder is growth hormone deficiency (GHD), Turner syndrome (TS), Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene (SHOX) deficiency, chronic renal insufficiency, and idiopathic short stature short bowel syndrome, GH deficiency due to rare pituitary tumors or their treatment, and muscle-wasting disease associated with HIV/AIDS.

[041] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of an inflammatory disease in a subject in need thereof.

[042] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of an autoimmune disease in a subject in need thereof.

[043] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of a cancer in a subject in need thereof.

[044] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of a metabolic disorder in a subject in need thereof.

[045] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of a fatty liver disease in a subject in need thereof.

[046] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present invention for the preparation of a medicament for treatment, prophylaxis and/or prevention of GH deficient growth disorder in a subject in need thereof.

[047] In other aspects, the present disclosure provides polynucleotides that encode the non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the present disclosure; vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the disclosure; optionally, operably-linked to control sequences recognized by a host cell transformed with the vector; host cells comprising vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the disclosure; a process for producing a non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecule of the disclosure comprising culturing host cells comprising vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the disclosure such that the polynucleotide is expressed; and, optionally, recovering the non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecule from the host cell culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[048] FIG. 1 depicts the genetic constructions of two exemplary Cholix toxin-IL-10 fusion molecules evaluated herein. The N-terminus of a human IL-10 monomer sequence was genetically attached to the C-terminus of a modified Cholix toxin (Cholix^{415}) using a stable non-cleavable linker sequence ($(G_4S)_3$) or a linker sequence that is a known substrate for the tobacco etch virus (TEV) protease. Each construct also contains an N-terminal Methionine (M).

[049] FIG. 2 is a ribbon diagram representation of an exemplary “dimer Cholix toxin-IL-10” fusion molecule after refolding that would be driven by IL-10 dimerization. The first 415 amino acids of Cholix toxin (SEQ ID NO: 1) are connected through a 16 amino acid linker (not shown) to connect with the human IL-10 sequence. IL-10 dimerization is envisaged to result in purple Cholix^{415} /blue hIL-10 and orange Cholix^{415} /green organization shown.

[050] FIG. 3 is a coomassie stained SDS PAGE of Cholix^{415}-TEV-IL-10 (depicted as “C”) and Cholix^{415}-$(G_4S)_3$-IL-10 (depicted as “N”) following induction and expression from inclusion bodies. The expressed fusion molecules demonstrate the anticipated molecular size of ~ 66 kDa that was comparable to the calculated mass of 66380.78 and 65958.25 Daltons, respectively. SeeBlue® Plus2 Prestained MW standards are shown.

[051] FIG. 4 is bar graph depicting the results of a flow cytometry assay using a mouse macrophage-derived J774.2 cell line treated with an exemplary Cholix toxin-IL-10 fusion molecules of the present disclosure at two concentrations. % proliferation was measured at 48 hours post treatment. Values represent n=4 ± standard deviation. The data shows that “dimer Cholix^{415}-$(G_4S)_3$-IL-10” fusion molecule demonstrates biologically active IL-10.

[052] FIG. 5 is a line graph depicting the results of an assay wherein the dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule was tested for effects on the barrier properties of Caco-2 cell monolayers *in vitro*. Fluorescein-labeled 70 kDa dextran and varying concentrations of dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule was added to the apical surface of these monolayers and the cumulative amount of florescence detected in the basal compartment monitored over time by collecting 150 µL volumes with replacement. Cumulative Basal Dextran levels (pmol) are plotted vs time. Each line represents the average (n=4) of basal fluorescence values measured at 0, 15, 30, 45, 60, 90, 120, 180, and 240 min.

[053] FIG. 6 is a line graph depicting the results of an assay wherein the dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule was tested for effects on the barrier properties of Caco-2 cell monolayers *in vitro*. Fluorescein-labeled 70 kDa dextran and varying concentrations of dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule was added to the apical surface of these monolayers and the cumulative amount of florescence detected in the basal compartment monitored over time.

[054] FIG. 7A and 7B are line graphs depicting the results an ELISA assay evaluating the ability of the dimer Cholix^{415}-(G_4S)$_3$-IL-10 fusion molecule to move across Caco-2 cell monolayers. The cumulative amount of dimer Cholix^{415}-(G_4S)$_3$-IL-10 fusion molecule reaching the basal compartment over time following an apical addition at various concentrations denoted in the legend. Each line represents the average (n=4) of basal IL-10 levels measured at 0, 15, 30, 45, 60, 90, 120, 180, and 240 min. Cumulative IL-10 transported over time graphed over a range of 6A = 8000 fmol IL-10 expanded and 6B = 1000 fmol IL-10.

MODE(S) FOR CARRYING OUT THE INVENTION

[055] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those commonly used and well known in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those commonly used and well known in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Definitions

[055a] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

[056] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. In various embodiments, "peptides", "polypeptides", and "proteins" are chains of amino acids whose alpha carbons are linked through peptide bonds. The terminal amino acid at one end of the chain (amino terminal) therefore has a free amino group, while the terminal amino acid at the other end of the chain (carboxy terminal) has a free carboxyl group. As used herein, the term "amino terminus" (abbreviated N-terminus) refers to the free α-amino group on an amino acid at the amino terminal of a peptide or to the α-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" refers to the free carboxyl group on the carboxy terminus of a peptide or the carboxyl group of an amino acid at any other location within the peptide. Peptides also include essentially any polyamino acid including, but not limited to, peptide mimetics such as amino acids joined by an ether as opposed to an amide bond.

[057] Polypeptides of the disclosure include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (5) confer or modify other physicochemical or functional properties. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A "conservative amino acid substitution" refers to the substitution in a polypeptide of an amino acid with a functionally similar amino acid. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), and Threonine (T)
2) Aspartic acid (D) and Glutamic acid (E)
3) Asparagine (N) and Glutamine (Q)
4) Arginine (R) and Lysine (K)
5) Isoleucine (I), Leucine (L), Methionine (M), and Valine (V)
6) Phenylalanine (F), Tyrosine (Y), and Tryptophan (W)

[058] A “non-conservative amino acid substitution” refers to the substitution of a member of one of these classes for a member from another class. In making such changes, according to various embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[059] The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, for example, Kyte et al., 1982, J. Mol. Biol. 157:105-131). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in various embodiments, the substitution of amino acids whose hydropathic indices are within ± 2 is included. In various embodiments, those that are within ± 1 are included, and in various embodiments, those within ± 0.5 are included.

[060] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In various embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

[061] The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0.+-.1); glutamate (+3.0.+-.1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5.+-.1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in various embodiments, the substitution of amino acids whose hydrophilicity values are within ± 2 is included, in various embodiments, those that are within ± 1 are included, and in various embodiments, those within ± 0.5 are included.

[062] Exemplary amino acid substitutions are set forth in Table 1.

Table 1

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[063] A skilled artisan will be able to determine suitable variants of polypeptides as set forth herein using well-known techniques. In various embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In other embodiments, the skilled artisan can identify residues and portions of the molecules that are conserved among similar polypeptides. In further embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[064] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, the skilled artisan can predict the importance of amino acid residues in a polypeptide that correspond to amino acid residues important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[065] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of a polypeptide with respect to its three-dimensional structure. In various embodiments, one skilled in the art may choose to not make radical changes to amino acid residues predicted to be on the surface of the polypeptide, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[066] The term "polypeptide fragment" and “truncated polypeptide” as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. In various embodiments, fragments can be, *e.g.,* at least 5, at least 10, at least 25, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 600, at least 700, at least 800, at least 900 or at least 1000 amino acids in length. In various embodiments, fragments can also be, *e.g.,* at most 1000, at most 900, at most 800, at most 700, at most 600, at most 500, at most 450, at most 400, at most 350, at most 300, at most 250, at most 200, at most 150, at most 100, at most 50, at most 25, at most 10, or at most 5 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (*e.g.,* an Fc or leucine zipper domain) or an artificial amino acid sequence (*e.g.*, an artificial linker sequence).

[067] The terms "polypeptide variant" and “polypeptide mutant” as used herein refers to a polypeptide that comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. In various embodiments, the number of amino acid residues to be inserted, deleted, or substituted can be, *e.g.,* at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 350, at least 400, at least 450 or at least 500 amino acids in length. Variants of the present disclosure include fusion proteins.

[068] A "derivative" of a polypeptide is a polypeptide that has been chemically modified, *e.g.,* conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (*e.g.,* human serum albumin), phosphorylation, and glycosylation.

[069] The term "% sequence identity" is used interchangeably herein with the term "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences or the level of nucleotide sequence identity between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% identity means the same thing as 80% sequence identity determined by a defined algorithm, and means that a given sequence is at least 80% identical to another length of another sequence. In various embodiments, the % identity is selected from, *e.g.,* at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence identity to a given sequence. In various embodiments, the % identity is in the range of, *e.g.,* about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[070] The term "% sequence homology" is used interchangeably herein with the term "% homology" and refers to the level of amino acid sequence homology between two or more peptide sequences or the level of nucleotide sequence homology between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence homology determined by a defined algorithm, and accordingly a homologue of a given sequence has greater than 80% sequence homology over a length of the given sequence. In various embodiments, the % homology is selected from, *e.g.,* at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence homology to a given sequence. In various embodiments, the % homology is in the range of, *e.g.,* about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[071] Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, e.g., BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at the NCBI website. See also Altschul et al., 1990, J. Mol. Biol. 215:403-10 (with special reference to the published default setting, i.e., parameters w=4, t=17) and Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402. Sequence searches are typically carried out using the BLASTP program when evaluating a given amino acid sequence relative to amino acid sequences in the GenBank Protein Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTP and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. See id.

[072] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA, 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is, *e.g.,* at most 0.1, at most 0.01, or at most 0.001.

[073] "Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. Nucleic acid analogs include those which include non-naturally occurring bases, nucleotides that engage in linkages with other nucleotides other than the naturally occurring phosphodiester bond or which include bases attached through linkages other than phosphodiester bonds. Thus, nucleotide analogs include, for example and without limitation, phosphorothioates, phosphorodithioates, phosphorotriesters, phosphoramidates, boranophosphates, methylphosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

[074] Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences"; sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

[075] "Complementary" refers to the topological compatibility or matching together of interacting surfaces of two polynucleotides. Thus, the two molecules can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other. A first polynucleotide is complementary to a second polynucleotide if the nucleotide sequence of the first polynucleotide is substantially identical to the nucleotide sequence of the polynucleotide binding partner of the second polynucleotide, or if the first polynucleotide can hybridize to the second polynucleotide under stringent hybridization conditions.

[076] "Hybridizing specifically to" or "specific hybridization" or "selectively hybridize to", refers to the binding, duplexing, or hybridizing of a nucleic acid molecule preferentially to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence-dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids can be found in Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, N.Y.; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 3.sup.rd ed., NY; and Ausubel et al., eds., Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY.

[077] Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than about 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2 x SSC wash at 65°C for 15 minutes. See Sambrook et al. for a description of SSC buffer. A high stringency wash can be preceded by a low stringency wash to remove background probe signal. An exemplary medium stringency wash for a duplex of, e.g., more than about 100 nucleotides, is 1 x SSC at 45°C for 15 minutes. An exemplary low stringency wash for a duplex of, e.g., more than about 100 nucleotides, is 4-6 x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2 x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

[078] "Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

[079] "Probe," when used in reference to a polynucleotide, refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties. In instances where a probe provides a point of initiation for synthesis of a complementary polynucleotide, a probe can also be a primer.

[080] A "vector" is a polynucleotide that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

[081] A "regulatory sequence" is a nucleic acid that affects the expression (e.g., the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. and Baron et al., 1995, Nucleic Acids Res. 23:3605-06. A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the nucleotide sequence.

[082] A "host cell" is a cell that can be used to express a polynucleotide of the disclosure. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[083] The term "isolated molecule" (where the molecule is, for example, a polypeptide or a polynucleotide) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[084] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60% to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and e.g., will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[085] "Linker" refers to a molecule that joins two other molecules, either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., a nucleic acid molecule that hybridizes to one complementary sequence at the 5' end and to another complementary sequence at the 3' end, thus joining two non-complementary sequences. A "cleavable linker" refers to a linker that can be degraded or otherwise severed to separate the two components connected by the cleavable linker. Cleavable linkers are generally cleaved by enzymes, typically peptidases, proteases, nucleases, lipases, and the like. Cleavable linkers may also be cleaved by environmental cues, such as, for example, specific enzymatic activities, changes in temperature, pH, salt concentration, etc. when there is such a change in environment following transcytosis of the fusion molecules across a polarized epithelial membrane.

[086] "Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in an animal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and a pharmaceutically acceptable carrier. "Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result

[087] "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, vehicles, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 21st Ed. 2005, Mack Publishing Co, Easton. A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use including, e.g., metal salts (sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

[088] The terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a biological disorder and/or at least one of its attendant symptoms. As used herein, to "alleviate" a disease, disorder or condition means reducing the severity and/or occurrence frequency of the symptoms of the disease, disorder, or condition. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

Modified Cholix Toxin Polypeptides

[089] Mature Cholix toxin (Jorgensen, R. et al., J Biol Chem 283(16):10671-10678 (2008)) as used herein is a 70.7 kD, 634 residue protein, whose sequence is set forth in SEQ ID NO: 1:

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDK
GESIITIGEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGE

DSPASIKISVDELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPS
VSYKAAQKEGSRHKRWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYET
VAGTPKVITVKQGIEQKPVEQRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDD
LSCAYQAQNIVSLFVATRILFSHLDSVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQV
LTVARQIYNDYVTHHPGLTPEQTSAGAQAADILSLFCPDADKSCVASNNDQANINIESR
SGRSYLPENRAVITPQGVTNWTYQELEATHQALTREGYVFVGYHGTNHVAAQTIVNRI
APVPRGNNTENEEKWGGLYVATHAEVAHGYARIKEGTGEYGLPTRAERDARGVMLRV
YIPRASLERFYRTNTPLENAEEHITQVIGHSLPLRNEAFTGPESAGGEDETVIGWDMAIH
AVAIPSTIPGNAYEELAIDEEAVAKEQSISTKPPYKERKDELK (SEQ ID NO: 1)

[090] In various embodiments, the Cholix toxin has an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 1.

[091] An exemplary nucleic acid encoding the mature Cholix toxin is set forth in SEQ ID NO: 2:

ATGGTCGAAGAAGCTTTAAACATCTTTGATGAATGCCGTTCGCCATGTTCGTTGACCCCGGA
ACCGGGTAAGCCGATTCAATCAAAACTGTCTATCCCTAGTGATGTTGTTCTGGATGAAGGTG
TTCTGTATTACTCGATGACGATTAATGATGAGCAGAATGATATTAAGGATGAGGACAAAGGC
GAGTCCATTATCACTATTGGTGAATTTGCCACAGTACGCGCGACTAGACATTATGTTAATCAA
GATGCGCCTTTTGGTGTCATCCATTTAGATATTACGACAGAAAATGGTACAAAAACGTACTCT
TATAACCGCAAAGAGGGTGAATTTGCAATCAATTGGTTAGTGCCTATTGGTGAAGATTCTCCT
GCAAGCATCAAAATCTCCGTTGATGAGCTCGATCAGCAACGCAATATCATCGAGGTGCCTAA
ACTGTATAGTATTGATCTCGATAACCAAACGTTAGAGCAGTGGAAAACCCAAGGTAATGTTTC
TTTTTCGGTAACGCGTCCTGAACATAATATCGCTATCTCTTGGCCAAGCGTGAGTTACAAAG
CAGCGCAGAAAGAGGGTTCACGCCATAAGCGTTGGGCTCATTGGCATACAGGCTTAGCACT
GTGTTGGCTTGTGCCAATGGATGCTATCTATAACTATATCACCCAGCAAAATTGTACTTTAGG
GGATAATTGGTTTGGTGGCTCTTATGAGACTGTTGCAGGCACTCCGAAGGTGATTACGGTTA
AGCAAGGGATTGAACAAAAGCCAGTTGAGCAGCGCATCCATTTCTCCAAGGGGAATGCGAT
GAGCGCACTTGCTGCTCATCGCGTCTGTGGTGTGCCATTAGAAACTTTGGCGCGCAGTCGC
AAACCTCGTGATCTGACGGATGATTTATCATGTGCCTATCAAGCGCAGAATATCGTGAGTTTA
TTTGTCGCGACGCGTATCCTGTTCTCTCATCTGGATAGCGTATTTACTCTGAATCTTGACGAA
CAAGAACCAGAGGTGGCTGAACGTCTAAGTGATCTTCGCCGTATCAATGAAAATAACCCGG
GCATGGTTACACAGGTTTTAACCGTTGCTCGTCAGATCTATAACGATTATGTCACTCACCATC
CGGGCTTAACTCCTGAGCAAACCAGTGCGGGTGCACAAGCTGCCGATATCCTCTCTTTATTT
TGCCCAGATGCTGATAAGTCTTGTGTGGCTTCAAACAACGATCAAGCCAATATCAACATCGA
GTCTCGTTCTGGCCGTTCATATTTGCCTGAAAACCGTGCGGTAATCACCCCTCAAGGCGTCA
CAAATTGGACTTACCAGGAACTCGAAGCAACACATCAAGCTCTGACTCGTGAGGGTTATGTG
TTCGTGGGTTACCATGGTACGAATCATGTCGCTGCGCAAACCATCGTGAATCGCATTGCCCC
TGTTCCGCGCGGCAACAACACTGAAAACGAGGAAAAGTGGGGCGGGTTATATGTTGCAACT
CACGCTGAAGTTGCCCATGGTTATGCTCGCATCAAAGAAGGGACAGGGGAGTATGGCCTTC
CGACCCGTGCTGAGCGCGACGCTCGTGGGGTAATGCTGCGCGTGTATATCCCTCGTGCTTC
ATTAGAACGTTTTTATCGCACGAATACACCTTTGGAAAATGCTGAGGAGCATATCACGCAAGT
GATTGGTCATTCTTTGCCATTACGCAATGAAGCATTTACTGGTCCAGAAAGTGCGGGCGGGG
AAGACGAAACTGTCATTGGCTGGGATATGGCGATTCATGCAGTTGCGATCCCTTCGACTATC
CCAGGGAACGCTTACGAAGAATTGGCGATTGATGAGGAGGCTGTTGCAAAAGAGCAATCGA
TTAGCACAAAACCACCTTATAAAGAGCGCAAAGATGAACTTAAG (SEQ ID NO: 2)

[092] In various embodiments, the Cholix toxin contains an nucleic acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 2.

[093] In various embodiments, the modified Cholix toxin used in the preparation of the fusion molecules is a truncated Cholix toxin, wherein the fusion molecule has the ability to activate the receptor for the biologically active cargo. A truncated Cholix toxin as described herein will be identified by reference to the amino acid residues comprising the truncated Cholix toxin, e.g., a truncated Cholix toxin consisting of amino acid residues 1-386 of SEQ ID NO: 1 will be identified as Cholix^{386}.

[094] In various embodiments, the modified Cholix toxin used in the preparation of the fusion molecule is mutated Cholix toxin. As described herein, a mutated Cholix toxin wherein the mutation involves an amino acid residue deletion will be identified by reference to the amino acid residue being deleted, e.g., a mutated Cholix toxin wherein amino acid E581 of SEQ ID NO: 1 has been deleted, the will be identified as “Cholix ΔE581”. A mutated Cholix toxin wherein the mutation involves an amino acid residue substitution will be identified by reference to the particular amino acid substitution at a specific amino acid residue. Thus, e.g., the term "S30A" indicates that the "S" (serine, in standard single letter code) residue at position 30 in SEQ ID NO: 1 has been substituted with an "A" (alanine, in standard single letter code) even if the residue appears in a truncated Cholix toxin, and the modified toxin will be identified as “Cholix^{S30A}”.

[095] Cholix toxin Domain Ia (amino acids 1-265 of SEQ ID NO: 1) is a "receptor binding domain" that functions as a ligand for a cell surface receptor and mediates binding of the fusion molecule to a cell, e.g., Domain Ia will bind to a cell surface receptor that is present on the apical membrane of an epithelial cell, with sufficient affinity to allow endocytosis of the fusion molecule. Domain 1a can bind to any receptor known to be present on the apical membrane of an epithelial cell by one of skill in the art without limitation. For example, the receptor binding domain can bind to α2-MR. Conservative or nonconservative substitutions can be made to the amino acid sequence of domain Ia, as long as the ability to mediate binding of the fusion molecule to a cell is not substantially eliminated. In various embodiments, the fusion molecules comprise a Cholix toxin comprising a mutated domain Ia.

[096] In various embodiments, domain Ia comprises an antigen presenting cell (APC) receptor binding domain. In various embodiments, the APC receptor binding domain is the cell recognition domain of Cholix domain Ia or a portion of Cholix domain Ia sufficient to engage with a cell surface receptor on APCs.

[097] In various embodiments, the APC receptor binding domain binds to a receptor identified as present on a dendritic cell or other APC. Examples of cell surface receptors on APCs can include, but are not limited to, DEC-205 (CD205), CD207, CD209, CD11a, CD11b, CD11c, CD36, CD14, CD50, CD54, CD58, CD68, CD80, CD83, CD86, CD102, CD3, CD14, CD19, Clec9a, CMFR-44, dectin-1,dectin-2, FLT3, HLA-DR, LOX-1, MHC II, BDCA-1, DC-SIGN, Toll-like receptors (TLR)-2, -3, -4, and -7, and α2-macroglobulin receptor (“α2-MR”). In various embodiments, the APC receptor binding domain is α2-MR.

[098] Cholix toxin Domain II (amino acids 266-386 of SEQ ID NO: 1) is a "transcytosis domain" that mediates transcytosis from a lumen bordering the apical surface of a mucous membrane to the basolateral side of a mucous membrane. As referred to herein, “transcytosis” refers to the trafficking of the fusion molecule through a polarized epithelial cell. Such trafficking permits the release of the biologically active cargo from the basolateral membrane of the polarized epithelial cell. The fusion molecules of the present disclosure may comprise a modified Cholix toxin comprising the entire amino acid sequence of Domain II, or may comprise portions of Domain II, so long as transcytosis acitivity is not substantially eliminated. Further, conservative or nonconservative substitutions can be made to the amino acid sequence of the transcytosis domain, as long as transcytosis activity is not substantially eliminated. A representative assay that can routinely be used by one of skill in the art to determine whether a transcytosis domain has transcytosis activity is described herein. As used herein, the transcytosis activity is not substantially eliminated so long as the activity is, *e.g.,* at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% as compared to a modified Cholix toxin comprising the entire amino acid sequence of Domain II.

[099] In various embodiments, the non-naturally occurring fusion molecules comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain II, wherein the fusion molecule has the ability to activate the receptor for the biologically active cargo. In one embodiment, the truncated Cholix toxin is Cholix^{386} (SEQ ID NO: 3). In one embodiment, the truncated Cholix toxin is Cholix^{385} (SEQ ID NO: 4). In one embodiment, the truncated Cholix toxin is Cholix^{384} (SEQ ID NO: 5). In one embodiment, the truncated Cholix toxin is Cholix^{383} (SEQ ID NO: 6). In one embodiment, the truncated Cholix toxin is Cholix^{382} (SEQ ID NO: 7). In one embodiment, the truncated Cholix toxin is Cholix^{381} (SEQ ID NO: 8). In one embodiment, the truncated Cholix toxin is Cholix^{380} (SEQ ID NO: 9). In one embodiment, the truncated Cholix toxin is Cholix^{379} (SEQ ID NO: 10). In one embodiment, the truncated Cholix toxin is Cholix^{378} (SEQ ID NO: 11). In one embodiment, the truncated Cholix toxin is Cholix^{377} (SEQ ID NO: 12). In one embodiment, the truncated Cholix toxin is Cholix^{376} (SEQ ID NO: 13). In one embodiment, the truncated Cholix toxin is Cholix^{375} (SEQ ID NO: 14). In one embodiment, the truncated Cholix toxin is Cholix^{374} (SEQ ID NO: 15). In one embodiment, the truncated Cholix toxin is Cholix^{373} (SEQ ID NO: 16). In one embodiment, the truncated Cholix toxin is Cholix^{372} (SEQ ID NO: 17). In one embodiment, the truncated Cholix toxin is Cholix^{371} (SEQ ID NO: 18). In one embodiment, the truncated Cholix toxin is Cholix^{370} (SEQ ID NO: 19). In one embodiment, the truncated Cholix toxin is Cholix^{369} (SEQ ID NO: 20). In one embodiment, the truncated Cholix toxin is Cholix^{368} (SEQ ID NO: 21). In one embodiment, the truncated Cholix toxin is Cholix^{367} (SEQ ID NO: 22). In one embodiment, the truncated Cholix toxin is Cholix^{366} (SEQ ID NO: 23). In one embodiment, the truncated Cholix toxin is Cholix^{365} (SEQ ID NO: 24). In one embodiment, the truncated Cholix toxin is Cholix^{364} (SEQ ID NO: 25). In one embodiment, the truncated Cholix toxin is Cholix^{363} (SEQ ID NO: 26). In one embodiment, the truncated Cholix toxin is Cholix^{362} (SEQ ID NO: 27). In one embodiment, the truncated Cholix toxin is Cholix^{361} (SEQ ID NO: 28). In one embodiment, the truncated Cholix toxin is Cholix^{360} (SEQ ID NO: 29). In one embodiment, the truncated Cholix toxin is Cholix^{359} (SEQ ID NO: 30). In one embodiment, the truncated Cholix toxin is Cholix^{358} (SEQ ID NO: 31). In one embodiment, the truncated Cholix toxin is Cholix^{357} (SEQ ID NO: 32). In one embodiment, the truncated Cholix toxin is Cholix^{356} (SEQ ID NO: 33). In one embodiment, the truncated Cholix toxin is Cholix^{355} (SEQ ID NO: 34). In one embodiment, the truncated Cholix toxin is Cholix^{354} (SEQ ID NO: 35). In one embodiment, the truncated Cholix toxin is Cholix^{353} (SEQ ID NO: 36). In one embodiment, the truncated Cholix toxin is Cholix^{352} (SEQ ID NO: 37). In one embodiment, the truncated Cholix toxin is Cholix^{351} (SEQ ID NO: 38). In one embodiment, the truncated Cholix toxin is Cholix^{350} (SEQ ID NO: 39). In one embodiment, the truncated Cholix toxin is Cholix^{349} (SEQ ID NO: 40). In one embodiment, the truncated Cholix toxin is Cholix^{348} (SEQ ID NO: 41).

[0100] Cholix toxin Domain Ib (amino acids 387-425 of SEQ ID NO: 1) is not essential for any known activity of Cholix, including cell binding, translocation, ER retention or ADP ribosylation activity. In various embodiments, the non-naturally occurring fusion molecules comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain Ib, wherein the fusion molecule has the ability to activate the receptor for the biologically active cargo. In one embodiment, the truncated Cholix toxin is Cholix^{425} (SEQ ID NO: 42). In one embodiment, the truncated Cholix toxin is Cholix^{424} (SEQ ID NO: 43). In one embodiment, the truncated Cholix toxin is Cholix^{423} (SEQ ID NO: 44). In one embodiment, the truncated Cholix toxin is Cholix^{422} (SEQ ID NO: 45). In one embodiment, the truncated Cholix toxin is Cholix^{421} (SEQ ID NO: 46). In one embodiment, the truncated Cholix toxin is Cholix^{420} (SEQ ID NO: 47). In one embodiment, the truncated Cholix toxin is Cholix^{419} (SEQ ID NO: 48). In one embodiment, the truncated Cholix toxin is Cholix^{418} (SEQ ID NO: 49). In one embodiment, the truncated Cholix toxin is Cholix^{417} (SEQ ID NO: 50). In one embodiment, the truncated Cholix toxin is Cholix^{416} (SEQ ID NO: 51). In one embodiment, the truncated Cholix toxin is Cholix^{415} (SEQ ID NO: 52). In one embodiment, the truncated Cholix toxin is Cholix^{414} (SEQ ID NO: 53). In one embodiment, the truncated Cholix toxin is Cholix^{413} (SEQ ID NO: 54). In one embodiment, the truncated Cholix toxin is Cholix^{412} (SEQ ID NO: 55). In one embodiment, the truncated Cholix toxin is Cholix^{411} (SEQ ID NO: 56). In one embodiment, the truncated Cholix toxin is Cholix^{410} (SEQ ID NO: 57). In one embodiment, the truncated Cholix toxin is Cholix^{409} (SEQ ID NO: 58). In one embodiment, the truncated Cholix toxin is Cholix^{408} (SEQ ID NO: 59). In one embodiment, the truncated Cholix toxin is Cholix^{407} (SEQ ID NO: 60). In one embodiment, the truncated Cholix toxin is Cholix^{406} (SEQ ID NO: 61). In one embodiment, the truncated Cholix toxin is Cholix^{405} (SEQ ID NO: 62). In one embodiment, the truncated Cholix toxin is Cholix^{404} (SEQ ID NO: 63). In one embodiment, the truncated Cholix toxin is Cholix^{403} (SEQ ID NO: 64). In one embodiment, the truncated Cholix toxin is Cholix^{402} (SEQ ID NO: 65). In one embodiment, the truncated Cholix toxin is Cholix^{401} (SEQ ID NO: 66). In one embodiment, the truncated Cholix toxin is Cholix^{400} (SEQ ID NO: 67). In one embodiment, the truncated Cholix toxin is Cholix^{399} (SEQ ID NO: 68). In one embodiment, the truncated Cholix toxin is Cholix^{398} (SEQ ID NO: 69). In one embodiment, the truncated Cholix toxin is Cholix^{397} (SEQ ID NO: 70). In one embodiment, the truncated Cholix toxin is Cholix^{396} (SEQ ID NO: 71). In one embodiment, the truncated Cholix toxin is Cholix^{395} (SEQ ID NO: 72). In one embodiment, the truncated Cholix toxin is Cholix^{394} (SEQ ID NO: 73). In one embodiment, the truncated Cholix toxin is Cholix^{393} (SEQ ID NO: 74). In one embodiment, the truncated Cholix toxin is Cholix^{392} (SEQ ID NO: 75). In one embodiment, the truncated Cholix toxin is Cholix^{391} (SEQ ID NO: 76). In one embodiment, the truncated Cholix toxin is Cholix^{390} (SEQ ID NO: 77). In one embodiment, the truncated Cholix toxin is Cholix^{389} (SEQ ID NO: 78). In one embodiment, the truncated Cholix toxin is Cholix^{388} (SEQ ID NO: 79). In one embodiment, the truncated Cholix toxin is Cholix^{387} (SEQ ID NO: 80).

[0101] Cholix toxin Domain III (amino acids 426-634 of SEQ ID NO: 1) is responsible for cytotoxicity and includes an endoplasmic reticulum retention sequence. Domain III mediates ADP ribosylation of elongation factor 2 ("EF2"), which inactivates protein synthesis. A Cholix that “lacks endogenous ADP ribosylation activity” or a "detoxified Cholix" refers to any Cholix described herein (including modified variants) that does not comprise Cholix domain III or which has been modified within domain III in a manner which detoxifies the molecule. For example, deletion of the glutamic acid (Glu) residue at amino acid position 581 of SEQ ID NO: 1 detoxifies the molecule. This detoxified Cholix is referred to as "Cholix ΔE581". In various embodiments, the portion of Cholix domain III other than the ER retention signal can be replaced by another amino acid sequence. This amino acid sequence can itself be non-immunogenic, slightly immunogenic, or highly immunogenic. A highly immunogenic ER retention domain is preferable for use in eliciting a humoral immune response. For example, Cholix domain III is itself highly immunogenic and can be used in fusion molecules where a robust humoral immune response is desired.

[0102] As used herein, “a detoxified Cholix sequence” may be a full length sequence or portion(s) of the full length sequence. Generally, a detoxified Cholix sequence has one or more domains or portions of domains with certain biological activities of a detoxified Cholix, such as a cell recognition domain, a translocation domain, or an endoplasmic reticulum retention domain. For example, a detoxified Cholix sequence may include only domain II and detoxified domain III. In another example, a detoxified Cholix sequence may include only domain Ia, domain II, and detoxified domain III. In another example, a detoxified Cholix sequence may include all of domains Ia, Ib, II, and detoxified III. Therefore, a detoxified Cholix sequence may be a contiguous sequence of the native Cholix, or it can be a sequence comprised of non-contiguous subsequences of the native Cholix that lacks ADP ribosylation activity. In one embodiment of the present disclosure, the non-naturally occurring fusion molecule comprises a mutated modified Cholix toxin, designated herein as Cholix toxin ΔE581, having the amino acid sequence set forth in SEQ ID NO: 81.

Biologically Active Cargo

[0103] In addition to the modified Cholix toxin polypeptide, the fusion molecules of the present disclosure further comprise a biologically active cargo for delivery to a subject. A "biologically active cargo" as used herein includes, but is not limited to: a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material.

[0104] In various embodiments, the biologically active cargo is a macromolecule that can perform a desirable biological activity when introduced to the bloodstream of the subject. For example, the biologically active cargo can have receptor binding activity, enzymatic activity, messenger activity (i.e., act as a hormone, cytokine, neurotransmitter, or other signaling molecule), luminescent or other detectable activity, or regulatory activity, or any combination thereof. In certain diagnostic embodiments, the biologically active cargo can be conjugated to or can itself be a pharmaceutically acceptable gamma-emitting moiety, including but not limited to, indium and technetium, magnetic particles, radiopaque materials such as air or barium and fluorescent compounds.

[0105] In various embodiments, the biologically active cargo of the fusion molecule can exert its effects in biological compartments of the subject other than the subject's blood. For example, in various embodiments, the biologically active cargo can exert its effects in the lymphatic system. In other embodiments, the biologically active cargo can exert its effects in an organ or tissue, such as, for example, the subject's liver, heart, lungs, pancreas, kidney, brain, bone marrow, etc. In such embodiments, the biologically active cargo may or may not be present in the blood, lymph, or other biological fluid at detectable concentrations, yet may still accumulate at sufficient concentrations at its site of action to exert a biological effect.

[0106] In various embodiments, the biologically active cargo is a protein that comprises more than one polypeptide subunit. For example, the protein can be a dimer, trimer, or higher order multimer. In various embodiments, two or more subunits of the protein can be connected with a covalent bond, such as, for example, a disulfide bond. In other embodiments, the subunits of the protein can be held together with non-covalent interactions. One of skill in the art can routinely identify such proteins and determine whether the subunits are properly associated using, for example, an immunoassay.

[0107] In various embodiments, the biologically active cargo to be delivered is selected from, *e.g.,* cytokines and cytokine receptors such as Interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, lymphokine inhibitory factor, macrophage colony stimulating factor, platelet derived growth factor, stem cell factor, tumor growth factor-β, tumor necrosis factor, lymphotoxin, Fas, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, interferon-α, interferon-β, interferon-γ, growth factors and protein hormones such as erythropoietin, angiogenin, hepatocyte growth factor, fibroblast growth factor, keratinocyte growth factor, nerve growth factor, tumor growth factor-α, thrombopoietin, thyroid stimulating factor, thyroid releasing hormone, neurotrophin, epidermal growth factor, VEGF, ciliary neurotrophic factor, LDL, somatomedin, insulin growth factor, insulin-like growth factor I and II, chemokines such as ENA-78, ELC, GRO-α, GRO-β, GRO-γ, HRG, LEF, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1-α, MIP-1-β, MG, MDC, NT-3, NT-4, SCF, LIF, leptin, RANTES, lymphotactin, eotaxin-1, eotaxin-2, TARC, TECK, WAP-1, WAP-2, GCP-1, GCP-2; α-chemokine receptors, e.g., CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7; and β-chemokine receptors, e.g., CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7.

[0108] Other examples of biologically active cargo that can be delivered according to the present disclosure include, but are not limited to, antineoplastic compounds, such as nitrosoureas*, e.g.,* carmustine, lomustine, semustine, strepzotocin; methylhydrazines, e.g., procarbazine, dacarbazine; steroid hormones*, e.g.,* glucocorticoids, estrogens, progestins, androgens, tetrahydrodesoxycaricosterone; immunoactive compounds such as immunosuppressives, *e.g.,* pyrimethamine, trimethopterin, penicillamine, cyclosporine, azathioprine; and immunostimulants, *e.g.,* levamisole, diethyl dithiocarbamate, enkephalins, endorphins; antimicrobial compounds such as antibiotics, *e.g.,* β-lactam, penicillin, cephalosporins, carbapenims and monobactams, β-lactamase inhibitors, aminoglycosides, macrolides, tetracyclins, spectinomycin; antimalarials, amebicides; antiprotazoals; antifungals, *e.g.,* amphotericin β, antivirals, *e.g.,* acyclovir, idoxuridine, ribavirin, trifluridine, vidarbine, gancyclovir; parasiticides; antihalmintics; radiopharmaceutics; gastrointestinal drugs; hematologic compounds; immunoglobulins; blood clotting proteins, *e.g.*, antihemophilic factor, factor IX complex; anticoagulants, *e.g.,* dicumarol, heparin Na; fibrolysin inhibitors, *e.g.,* tranexamic acid; cardiovascular drugs; peripheral anti-adrenergic drugs; centrally acting antihypertensive drugs, *e.g.,* methyldopa, methyldopa HCl; antihypertensive direct vasodilators, *e.g.,* diazoxide, hydralazine HCl; drugs affecting renin-angiotensin system; peripheral vasodilators, *e.g.*, phentolamine; anti-anginal drugs; cardiac glycosides; inodilators, *e.g.,* amrinone, milrinone, enoximone, fenoximone, imazodan, sulmazole; antidysrhythmics; calcium entry blockers; drugs affecting blood lipids, *e.g.,* ranitidine, bosentan, rezulin; respiratory drugs; sypathomimetic drugs, *e.g.,* albuterol, bitolterol mesylate, dobutamine HCl, dopamine HCl, ephedrine So, epinephrine, fenfluramine HCl, isoproterenol HCl, methoxamine HCl, norepinephrine bitartrate, phenylephrine HCl, ritodrine HCl; cholinomimetic drugs, *e.g.,* acetylcholine Cl; anticholinesterases, *e.g.*, edrophonium Cl; cholinesterase reactivators; adrenergic blocking drugs, *e.g.*, acebutolol HCl, atenolol, esmolol HCl, labetalol HCl, metoprolol, nadolol, phentolamine mesylate, propanolol HCl; antimuscarinic drugs, *e.g.,* anisotropine methylbromide, atropine SO_4, clinidium Br, glycopyrrolate, ipratropium Br, scopolamine HBr;

neuromuscular blocking drugs; depolarizing drugs, *e.g.*, atracurium besylate, hexafluorenium Br, metocurine iodide, succinylcholine Cl, tubocurarine Cl, vecuronium Br; centrally acting muscle relaxants, *e.g.,* baclofen; neurotransmitters and neurotransmitter agents, *e.g.,* acetylcholine, adenosine, adenosine triphosphate; amino acid neurotransmitters, *e.g.*, excitatory amino acids, GABA, glycine; biogenic amine neurotransmitters, *e.g.,* dopamine, epinephrine, histamine, norepinephrine, octopamine, serotonin, tyramine; neuropeptides, nitric oxide, K^+ channel toxins; antiparkinson drugs, *e.g.*, amaltidine HCl, benztropine mesylate, carbidopa; diuretic drugs, *e.g.,* dichlorphenamide, methazolamide, bendroflumethiazide, polythiazide; antimigraine drugs, *e.g*, carboprost tromethamine mesylate, methysergide maleate.

[0109] Still other examples of biologically active cargo that can be delivered according to the present disclosure include, but are not limited to, hormones such as pituitary hormones, e.g., chorionic gonadotropin, cosyntropin, menotropins, somatotropin, iorticotropin, protirelin, thyrotropin, vasopressin, lypressin; adrenal hormones, e.g., beclomethasone dipropionate, betamethasone, dexarnethasone, triamcinolone; pancreatic hormones, e.g., glucagon, insulin; parathyroid hormone, e.g., dihydrochysterol; thyroid hormones, e.g., calcitonin etidronate disodium, levothyroxine Na, liothyronine Na, liotrix, thyroglobulin, teriparatide acetate; antithyroid drugs; estrogenic hormones; progestins and antagonists; hormonal contraceptives; testicular hormones; gastrointestinal hormones, e.g., cholecystokinin, enteroglycan, galanin, gastric inhibitory polypeptide, epidermal growth factor-urogastrone, gastric inhibitory polypeptide, gastrin-releasing peptide, gastrins, pentagastrin, tetragastrin, motilin, peptide YY, secretin, vasoactive intestinal peptide, or sincalide.

[0110] Still other examples of biologically active cargo that can be delivered according to the present disclosure include, but are not limited to, enzymes such as hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, PGE-adenosine deaminase; intravenous anesthetics such as droperidol, etomidate, fetanyl citrate/droperidol, hexobarbital, ketamine HCl, methohexital Na, thiamylal Na, thiopental Na; antiepileptics, e.g., carbamazepine, clonazepam, divalproex Na, ethosuximide, mephenyloin, paramethadione, phenyloin, primidone. In various embodiments, the biologically active cargo is an enzyme selected from hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, PGE-adenosine deaminase.

[0111] Yet other examples of biologically active cargo that can be delivered according to the present disclosure include, but are not limited to, chemotherapeutics, such as chemotherapy or anti-tumor agents which are effective against various types of human cancers, including leukemia, lymphomas, carcinomas, sarcomas, myelomas etc., such as, for example, doxorubicin, mitomycin, cisplatin, daunorubicin, bleomycin, actinomycin D, and neocarzinostatin.

Modulators of Inflammation (Interleukin-10 and related cytokines)

[0112] Interleukin-10 (IL-10) is an important immunoregulatory cytokine produced by many cell populations and whose main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. More recent data suggests that IL-10 also mediates immunostimulatory properties that help to eliminate infectious and noninfectious particles with limited inflammation; Asadullah et al., *Pharmacol Rev*, 55:241-269, 2003. Moreover, numerous investigations suggest a major impact of IL-10 in inflammatory, malignant, and autoimmune diseases, and IL-10 overexpression was found in certain tumors such as melanoma, basal cell and squamous cell carcinoma and several lymphomas; Id. Five new human molecules structurally related to IL-10 have been discovered, IL-19 (Gallagher et al., *Genes Immun.,* 1:442-450, 2000); IL-20 (Blumberg et al., *Cell,* 104:9-19, 2001), IL-22 (Dumoutier et al., *Genes Immun.,* 1:488-494, 2000), IL-24 (Jiang et al., *Oncogene,* 11:2477-2486, 1995) and IL-26 (Knappe et al., *J. Virol.,* 74:3881-3887, 2000) and data suggests that immune cells are a major source of the new IL-10 family members; Wolk et al., *J. Immunol.*, 168:5397-5402, 2002.

[0113] While there were some promising results from IL-10 delivery on the course of several inflammatory diseases in experimental models, several clinical studies evaluating IL-10 as a therapeutic agent for the treatment of inflammatory and/or immune disorders remain somewhat disappointing, with much of the data conflicting; Asadullah et al., *Pharmacol Rev*, 55:241-269, 2003. Overall, the data suggests that IL-10 is safe and generally well tolerated, however, the ultimate local IL-10 concentration in the intestine after systemic administration with standard doses is too low, resulting in only marginal efficacy. Id. Unfortunately, the ability to sufficiently increase the doses is limited due to side effects (e.g., anemia, headache), and there are concerns higher doses of systemically administered IL-10 may be detrimental rather than helpful in certain indications, e.g., Crohn’s; Herfarth et al, *Gut*, 50(2): 146-147, 2002.

[0114] In various embodiments, the biologically active cargo is a polypeptide that has been determined to be a modulator of inflammation in the GI tract selected from, *e.g.,* interleukin-10, interleukin-19, interleukin-20, interleukin-22, interleukin-24, or interleukin-26.

[0115] Interleukin-10 (IL-10) was first identified as a product of the type 2 helper T cell and later shown to be produced by other cell types including B cells and macrophages (Moore et al., Annu Rev Immunol, 19:683-765, 2001). It also inhibits the synthesis of several cytokines produced from type 1 helper T cells, such as γ-interferon, IL-2, and tumor necrosis factor-α (TNF-α) (Fiorentino et al., *J Immunol*, 146:3444-3451, 1991). The ability of IL-10 to inhibit cell-mediated immune response modulators and suppress antigen-presenting cell-dependent T cell responses demonstrates IL-10 has immunosuppressive properties. This cytokine also inhibits monocyte/macrophage production of other cytokines such as IL-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and TNF-α.

[0116] The IL-10 protein forms a functional dimer that becomes biologically inactive upon disruption of the non-covalent interactions connecting its two monomer subunits. The N-terminus does not appear to be directly involved with IL-10 receptor activation. Thus, in one aspect of the disclosure, a fusion molecule is constructed via conjugation through the N-terminus of the IL-10 protein to the C-terminus of a modified Cholix toxin using a cleavable linker. Such a construction may result in a solution dimer as a result of IL-10 interactions.

[0117] In various embodiments, the biologically active cargo is human interleukin-10 having the amino acid sequence set forth in SEQ ID NO: 82:

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQM
KDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENL
KTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKI
RN (SEQ ID NO: 82)

or a fragment or variant thereof.

[0118] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 82.

[0119] IL-19 a cytokine that belongs to the IL-10 cytokine subfamily. This cytokine is found to be preferentially expressed in monocytes. It can bind the IL-20 receptor complex and lead to the activation of the signal transducer and activator of transcription 3 (STAT3) (Yamamoto-Furusho JK, *et al.* Hum Immunol, 72(11):1029-32, 2011). In various embodiments, the biologically active cargo is human interleukin-19 having the amino acid sequence set forth in SEQ ID NO: 83:

MKLQCVSLWLLGTILILCSVDNHGLRRCLISTDMHHIEESFQEIKRAIQAKDTFPNVTILST
LETLQIIKPLDVCCVTKNLLAFYVDRVFKDHQEPNPKILRKISSIANSFLYMQKTLRQCQE
QRQCHCRQEATNATRVI HDNYDQLEVHAAAIKSLGELDVFLAWINKNHEVMSSA
(SEQ ID NO: 83)

or a fragment or variant thereof.

[0120] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 83.

[0121] IL-20 is a cytokine structurally related to interleukin 10 (IL-10). This cytokine has been shown to transduce its signal through signal transducer and activator of transcription 3 (STAT3) in keratinocytes. A specific receptor for this cytokine is found to be expressed in skin and upregulated dramatically in psoriatic skin, suggesting a role for this protein in epidermal function and psoriasis (Yamamoto-Furusho JK, *et al.* Immunol Lett, 149(1-2):50-3 2013). In various embodiments, the biologically active cargo is human interleukin-20 having the amino acid sequence set forth in SEQ ID NO: 84:

MKASSLAFSLLSAAFYLLWTPSTGLKTLNLGSCVIATNLQEIRNGFSEIRGSVQAKDGNI
DIRILRRTESLQDTKPANRCCLLRHLLRLYLDRVFKNYQTPDHYTLRKISSLANSFLTIKK
DLRLCHAHMTCHCGEEAMK KYSQILSHFEKLEPQAAVVKALGELDILLQWMEETE
(SEQ ID NO: 84)

or a fragment or variant thereof.

[0122] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 84.

[0123] IL-22 is a cytokine structurally related to interleukin 10 (IL-10). IL-22 secreting CD4(+) T (Th22) cells and IL-22 are involved in the pathogenesis of autoimmune disease, and may play an important role in the pathogenesis of NMO and MS (Xu et al., J Neuroimmunol., Aug 15;261(1-2):87-91, 2013). In various embodiments, the biologically active cargo is human interleukin-22 having the amino acid sequence set forth in SEQ ID NO: 85:

MAALQKSVSSFLMGTLATSCLLLLALLVQGGAAAPISSHCRLDKSNFQQPYITNRTFML
AKEASLADNNTDVRLIGEKLFHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQE
VVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNA
CI (SEQ ID NO: 85)

or a fragment or variant thereof.

[0124] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 85.

[0125] IL-24 is a cytokine structurally related to interleukin 10 (IL-10) which can induce apoptosis selectively in various cancer cells. Overexpression of this gene leads to elevated expression of several GADD family genes, which correlates with the induction of apoptosis. The phosphorylation of mitogen-activated protein kinase 14 (MAPK7/P38), and heat shock 27kDa protein 1 (HSPB2/HSP27) are found to be induced by this gene in melanoma cells, but not in normal immortal melanocytes (Lin BW, *et al.*, J Korean Med Sci, 28(6):833-9, 2013). In various embodiments, the biologically active cargo is human interleukin-24 having the amino acid sequence set forth in SEQ ID NO: 86:

MNFQQRLQSLWTLASRPFCPPLLATASQMQMVVLPCLGFTLLLWSQVSGAQGQEFHF
GPCQVKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLL
EFYLKTVFKNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLF
RRAFKQLDVEAALTKALGEVDILLTWMQKFYKL (SEQ ID NO: 86)

or a fragment or variant thereof.

[0126] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 86.

[0127] IL-26 was identified by its overexpression specifically in herpesvirus saimiri-transformed T cells. The encoded protein is a member of the IL-10 family of cytokines. It is a secreted protein and may function as a homodimer. This protein is thought to contribute to the transformed phenotype of T cells after infection by herpesvirus saimiri (Corvaisier M, *et al.* PLoS Biol, 10(9):e1001395, 2012). In various embodiments, the biologically active cargo is human interleukin-26 having the amino acid sequence set forth in SEQ ID NO: 87:

MLVNFILRCGLLLVTLSLAIAKHKQSSFTKSCYPRGTLSQAVDALYIKAAWLKATIPEDRI
KNIRLLKKKTKKQFMKNCQFQEQLLSFFMEDVFGQLQLQGCKKIRFVEDFHSLRQKLS
HCISCASSAREMKSITRMKRIFYRIGNKGIYKAISELDILLSWIKKLLESSQ
(SEQ ID NO: 87)

or a fragment or variant thereof.

[0128] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 87.

[0129] Importantly, the non-naturally occurring fusion molecules which lack a cleavable linker can be advantageous in that the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of, e.g., immune cells that also express the receptor for the IL-10 (but in considerably lower quantity) can allow for greater exposure of the IL-10 at the surface of the targeted cells, and provide a synergistic effect via the binding of the Cholix to its receptor and the binding of IL-10 to the IL-10R.

Tumor Necrosis Factor Super Family

[0130] Tumor necrosis factor is a rapidly growing superfamily of cytokines (hereinafter “TNFSF”) that interact with a corresponding superfamily of receptors (hereinafter “TNFSFR”). Since the discovery of tumor necrosis factor-alpha (“TNF-α”) about 25 years ago, the TNFSF has grown to a large family of related proteins consisting of over 20 members that signal through over 30 receptors (see, e.g., “Therapeutic Targets of the TNF Superfamily”, edited by Iqbal S. Grewal, Landes Bioscience/Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology, 2009). Members of TNFSF have wide tissue distribution and TNFSF ligand-receptor interactions are involved in numerous biological processes, ranging from hematopoiesis to pleiotropic cellular responses, including activation, proliferation, differentiation, and apoptosis. TNFSF ligand-receptor interactions have also been implicated in tumorigenesis, transplant rejection, septic shock, viral replication, bone resorption and autoimmunity. The particular response depends upon the receptor that is signaling, the cell type, and the concurrent signals received by the cell.

[0131] Because a number of TNFSF members are expressed on tumor cells, antibody based therapies are being developed to target these molecules and some are currently undergoing clinical trials (e.g., TNF-α for human use in the treatment of sarcomas and melanomas (Eggermont et al., Lancet Oncol, 4:429-437, 2003; Lans et al., Clin Cancer Res, 7:784-790, 2001). In addition, many of these molecules are also being exploited as targets for antibody-drug conjugates (e.g., CD30 and CD70), or exploited for radioimmunotherapy (e.g., the BLyS receptors TACI and BR3) (Buchsbaum et al., J Nucl Med, 44:434-436, 2003).

[0132] Similarly, because a number of TNFSF members have been implicated in both innate and adaptive immune responses such as defense against pathogens, inflammatory response and autoimmunity, approaches to target many of TNFSF receptors and ligands for treatment of autoimmunity and other inflammatory diseases are being exploited. Indeed, a number of biologic TNF blocking therapies (hereinafter “TNF inhibitors”) including humanized/human monoclonal antibodies (e.g., infliximab (REMICADE®) or adalimumab (HUMIRA®)) or recombinant fusion proteins of IgG and soluble TNFSF receptors (e.g., etanercept (ENBREL®)) have been developed and are now being used in humans to inhibit the inflammation associated with Crohn’s disease and rheumatoid arthritis (Mitoma et al., Arthritis Rheum, 58:1248-1257, 2008; Shealy et al., Handb Exp Pharmacol, 181:101-129, 2008). Thus, the potential to deliver such agents locally including, but not limited to, intestinal and pulmonary mucosa, would provide added benefits for efficacy and safety.

[0133] Although these various TNF inhibitors have been approved for human therapies and are being successfully used in human patients, there remains a number of toxicities associated with these TNF inhibitors, e.g., hepatotoxicity, thromboembolic complications, and increased risk of development of tuberculosis and lymphoma (Gardam et al., Lancet Infect Dis, 3:148-155, 2003). Moreover, while effective in halting progression of disease, these agents are very expensive, generally administered intravenously or subcutaneously, and do not cure the diseases. The continued examination of signal transduction of TNFSF members is needed to develop approaches for tissue specific interventions, which could allow targeted therapies to have fewer side effects.

[0134] In various embodiments, the biologically active cargo is a TNF inhibitor that is an isolated antibody or an antibody fragment. Isolated antibodies and antibody fragments useful in the constructs and methods of the present invention include, without limitation, monoclonal Abs (mAbs), polyclonal Abs, Ab fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric Abs, mini-Abs or domain Abs (dAbs), dual specific Abs, bispecific Abs, heteroconjugate Abs, single chain Abs (SCA), single chain variable region fragments (ScFv), fusion proteins comprising an Ab portion or multiple Ab portions, humanized Abs, fully human Abs, and any other modified configuration of the immunoglobulin (Ig) molecule that comprises an antigen recognition site of the required specificity.

[0135] Anti-TNF-α Antibodies. The FDA approved anti-TNF-α antibody, Adalimumab (Abbvie HUMIRA®; DrugBank DB 00051) has been used to treat humans. In various embodiments of the present invention, the biologically active cargo is a human antibody or antigen-binding fragment comprising the heavy chain variable region sequence set forth in SEQ ID NO: 88:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGK
GLEWVSAITWNSGHIDYADSVERGFTISRDNAKNSLYLQMNSLRAE
DTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO: 88)

and the light chain variable region sequence set forth in SEQ ID NO:89:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAA
STLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC (SEQ ID NO: 89)

or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

[0136] In various embodiments, the invention provides antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region, and wherein the heavy chain variable region comprises a sequence that has at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% identity to the amino acid sequence as set forth in SEQ ID NO:88; and wherein the light chain comprises a light chain variable region, and wherein the light chain variable region comprises a sequence that has at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% identity to the amino acid sequence as set forth in any of SEQ ID NO:89; wherein the antibody binds specifically to human TNF-α.

[0137] The FDA approved anti-TNF-α antibody, Infliximab (Centocor REMICADE®; DrugBank DB 00065) has been used to treat humans. In various embodiments of the present invention, the biologically active cargo is a human antibody or antigen-binding fragment comprising the heavy chain variable region sequence set forth in SEQ ID NO: 90:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQA
PGKGLEWVAIISFDGSNKSSADSVKGRFTUSRRNSKNALFLQM
NSLRAEDTAVFYCARDRGVSAGGNYYYYGMDVWGQGTTVTVSS
(SEQ ID NO: 90)

and the light chain variable region sequence set forth in SEQ ID NO:91:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQA
PRLLIYDASNRATGIPARFSGSGSGTRFTLTISSLEPEDFAVYYC
QQRSNWPPFTFGPGTKVDIL (SEQ ID NO: 91)

or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

[0138] In various embodiments, the invention provides antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region, and wherein the heavy chain variable region comprises a sequence that has at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% identity to the amino acid sequence as set forth in SEQ ID NO:90; and wherein the light chain comprises a light chain variable region, and wherein the light chain variable region comprises a sequence that has at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% identity to the amino acid sequence as set forth in any of SEQ ID NO:91; wherein the antibody binds specifically to human TNF-α.

[0139] Antibodies to several other TNFSF ligands or TNFSFRs have been described in the literature, and evaluated as therapeutic candidates in the treatment or prevention of a variety of inflammatory diseases, autoimmune diseases and cancer. Nucleotide and amino acid sequences of antibodies to the designated TNFSF polypeptides or TNFSFRs are readily available from publicly available databases. A comprehensive review of such antibodies as well as additional TNF inhibitors is provided in “Therapeutic Targets of the TNF Superfamily”, edited by Iqbal S. Grewal, Landes Bioscience/Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology, 2009, which is hereby incorporated by reference in its entirety for the purpose of teaching such TNF inhibitors.

[0140] In various embodiments, the biologically active cargo is a TNFSF inhibitor that comprises a soluble receptor or soluble co-ligand. The terms "soluble receptor", "soluble cytokine receptor" (SCR) and "immunoadhesin" are used interchangeably to refer to soluble chimeric molecules comprising the extracellular domain of a receptor, e.g., a receptor of a TNFSF member and an Ig sequence, which retains the binding specificity of the receptor and is capable of binding to the TNFSF member. In various embodiments, a TNFSFSCR comprises a fusion of a TNFSFR amino acid sequence (or a portion thereof) from a TNFSF member extracellular domain capable of binding the TNFSF member (in some embodiments, an amino acid sequence that substantially retains the binding specificity of the TNFSFR) and an Ig sequence. Two distinct types of TNFSFR are known to exist: Type I TNFSFR (TNFSFRI) and Type II TNFSFR (TNFSFRII). In various embodiments, the TNFSF receptor is a human TNFSF receptor sequence, and the fusion is with an Ig constant domain sequence. In other embodiments, the Ig constant domain sequence is an Ig heavy chain constant domain sequence. In other embodiments, the association of two TNF receptor-Ig heavy chain fusions (e.g., via covalent linkage by disulfide bond(s)) results in a homodimeric Ig-like structure.

[0141] An example of a commercially available soluble receptor useful in the present invention is ENBREL® (etanercept). ENBREL® consists of recombinant human TNFR-p75-Fc dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. Etanercept is produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian cell expression system. It consists of 934 amino acids. The product is made by encoding the DNA of the soluble portion of human TNFR-p75 with the Fc portion of IgG. In various embodiments of the present invention, the biologically active cargo is a TNF inhibitor that is dimeric fusion protein comprising the sequence set forth in SEQ ID NO: 92:

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTS
DTVCDSCEDSTYTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPG
WYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCKPCAPGTFSNTTSS
TDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQ
HTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK (SEQ ID NO: 92)

or a fragment or variant thereof.

[0142] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% identity to the sequence of SEQ ID NO: 92.

[0143] An illustrative, but not limiting list of suitable TNFSF ligands and TNFSFRs from which a TNF inhibitor will be derived and used as a biologically active cargo in the constructs and methods of the present invention is provided in Table 2.

Table 2

TNFSF Ligands	RefSeq (protein)
Tumor necrosis factor-α ("TNF-α")	NP_000585.2
lymphotoxin-α ("LT-α")	NP_ 000586.2
lymphotoxin-β ("LT-β")	NP_002332.1
CD30 ligand	NP_ 001235.1
CD40 ligand	NP_000065.1
CD70 ligand	NP_001243.1
OX40 ligand	NP_001284491.1
41BB ligand	NP_ 001552.2
Apo1 ligand (or FasL or CD95L)	NP_ 000630.1
Apo2 ligand (or TRAIL, AIM-1 or AGP-1)	NP_ 001177871.1
Apo3 ligand (or TWEAK)	NP_003800.1
APRIL	NP_ 001185551.1
LIGHT	NP_ 003798.2
OPG ligand (or RANK ligand)	NP_ 003692.1
BlyS (or THANK)	NP_ 001139117.1
BCMA	NP_ 001183.2
TACI	NP_ 036584.1
TNFSFRs	
TNFR1	NP_001056.1
TNFR2	NP_001057.1
lymphotoxin-βR	NP_001257916.1
CD40	NP_001241.1
CD95 (or FAS or APO-1)	NP_000034.1
OPG	NP_ 002537.3
RANK	NP_ 001257878.1
CD30	NP_001234.3
CD27	NP_001233.1
OX40 (or CD134)	NP_003318.1
41BB	NP_ 001552.2

NGFR	NP_ 002498.1
BCMA	NP_ 001183.2
TAC1	NP_ 036584.1
EDA2R	NP_001186616.1
TROY	NP_ 001191387.1
DR6	NP_ 055267.1
DR5 (or TRAILR2)	NP_ 003833.4
DR4	NP_ 003835.3
DR3	NP_ 001034753.1
HVEM	NP_ 001284534.1
LTβR	NP_ 001257916.1
GITR	NP_004186.1
DcR3	NP_003814
Fn14 (or TWEAKR)	NP_057723.1
BAFF	NP_443177.1

Glucose-lowering agents

[0144] In various embodiments, the biologically active cargo is a glucose-lowering agent. In various embodiments, the glucose-lowering agent is a peptide that comprises about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 400, about 500, about 600, about 700, about 800, about 900 or about 1000 amino acids.

[0145] An illustrative, but not limiting, list of suitable glucose metabolism-related proteins to be used as the glucose-lowering agent in the fusion molecules of the present disclosure, or from which the glucose-lowering agents contemplated for use as a glucose-lowering agent could be derived, is provided in Table 3.

Table 3

Glucose metabolism-related proteins	**RefSeq (NCBI/Uniprot)**
Glucagon proprotein	NP_002045.1
Glucagon peptide	NP_002045.1 (aa 53-81)

Glucagon-like peptide 1	NP_002045.1 (aa 98-128)
Glucagon-like peptide 2	NP_002045.1 (aa 146-178)
Glicentin	P01275 (aa 21-89)
Glicentin-related polypeptide	P01275 (aa 21-50)
Gastric inhibitory polypeptide preprotein	NP_004114.1
Gastric inhibitory polypeptide	NP_004114.1 (aa 52-93)
Dipeptidyl peptidase 4	P27487
Glucose transporter member 4	NP_001033.1
Preproglucagon	AAA52567.1
Insulin receptor substrate 1	NP_005535.1
Insulin	P01308
Apolipoprotein A-II	P02652
Solute carrier family 2, faciliated glucose transporter member 1	P11166
Glycogen synthase 1	P13807
Glycogen synthase 2	P54840
Tyrosin-protein phosphatase non-receptor type 1	P18031
RAC-alpha serinel threonine-protein kinase	P31749
Peroxisome proliferator-activated receptor gamma	P37231
Hexokinase 3	P52790
Phosphatidylinositol-3,4,5-triphosphate 3-phosphatase and dual-specificty protein	P60484
Pyruvate dehydrogenase kinase 1	Q15118
Calcium-binding and coiled-coil domain-containing protein 1	Q9P1Z2
Max-like protein X	Q9UH92
Fructose-bisphosphate aldolase A	P04075
Glucagon-like peptide 1 receptor	P43220
Glucagon-like peptide 2 receptor	O95838
Gastric inhibitory polypeptide receptor	P48546
Insulin-like growth factor 1 receptor	P08069.1
Insulin-like growth factor 2 receptor	P11717.3
Insulin Receptor	P06213
GLP-1 agonist-Exenatide	DB01276
GLP-1 agonist-Liraglutide	DB06655

[0146] Glucagon-like peptide-1 (GLP-1), a member of the pro-glucagon incretin family synthesized in intestinal L-cells by tissue-specific post-translational processing of the glucagon precursor preproglucagon, is a potent glucose-lowering agent implicated in the control of appetite and satiety. GLP-1 acts through GLP-1 receptor (GLP-1R), which is widely distributed in tissues, including brain, pancreas, intestine, lung, stomach, and kidney. The effects of GLP-1 appear to be both insulinotropic and insulinomimetic, depending on the ambient glucose concentration. Due to their ability to increase insulin secretion from the pancreas, increase insulin-sensitivity in both alpha cells and beta cells, and decrease glucagon secretion from the pancreas, GLP-1 and its analogs have attracted considerable attention as a therapeutic strategy for diabetes.

[0147] Several clinical trials have studied the addition of GLP-1 agonists in conjunction with ongoing insulin therapy and several GLP-1 agonists have been approved for treatment of T2D, including, e.g., exenatide (tradename Byetta®, Amylin/Astrazeneca); liraglutide (tradename Victoza®, Novo Nordisk A/S); lixisenatide (tradename Lyxumia®, Sanofi); albiglutide (tradename Tanzeum®, GlaxoSmithKline); dulaglutide (tradename Trulicity®, Eli Lilly). While proven efficacious, the major drawback associated with the clinical use of GLP-1 agonists is the short biological half-life, necessitating continuous administration intravenously or by frequent subcutaneous injections, and all GLP-1 drugs approved to date are subcutaneous administered on a twice daily or once weekly basis. Moreover, there are safety concerns associated with the use of these GLP-1 agonists, namely, pancreatitis and pancreatic neoplasia, hypoglycemia, and renal impairment. Other reported side effects include gastrointestinal disorders, such as dyspepsia, decreased appetite, nausea, vomiting, abdominal pain, diarrhea, dizziness, headache, and feeling jittery. As such, there continues to be extensive research directed to preparing analogs of the natural GLP-1 that are longer lasting, as well as development of sustained release and other related technologies in order to lower the frequency of injections for the T2D patients.

[0148] In various embodiments, the biologically active cargo is GLP-1 agonist having the amino acid sequence set forth in SEQ ID NO: 93:

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS (SEQ ID NO: 93)

or a fragment or variant thereof.

[0149] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 93.

[0150] In various embodiments, the biologically active cargo is GLP-1 agonist having the amino acid sequence set forth in SEQ ID NO: 94:

HAEGTFTSDVSSYLEGQAAKEEFIIAWLVKGRG (SEQ ID NO: 94)

or a fragment or variant thereof.

[0151] In various embodiments, the biologically active cargo contains an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 94.

Human Growth Hormone

[0152] Growth Hormone (GH) (also known as somatropin or somatotropin) is the master hormone in the human body, and is synthesized and secreted by the endocrinal system. This hormone controls essential functions like: growth and replication of cells in various organs of the body. Some of the essential functions of GH include: controlling muscle growth, improving bone mineralization and strength, reducing fat deposition, and sustaining good energy levels. The production and secretion of the growth hormone is controlled by Growth Hormone Releasing Hormone (GHRH), which is secreted by the hypothalamus. The GHRH stimulates the pituitary gland to produce GH, which is directly released into the blood stream. The GH in turn stimulates the liver to produce Insulin-like Growth Factor (IGF-1) which stimulates the proliferation of chondrocytes (cartilaginous cells), promotes differentiation of myoblasts and enhances protein synthesis, which in turn, helps in the growth of other muscles and tissue cells.

[0153] In the US, synthetically produced human growth hormone (HGH) has been used in the pediatric population to treat short stature due to growth hormone deficiency (GHD), Turner syndrome (TS), Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene (SHOX) deficiency, chronic renal insufficiency, idiopathic short stature and children small for gestational age. In adults, HGH has been used to treat short bowel syndrome, a condition in which nutrients are not properly absorbed due to sever intestinal disease or the surgical removal of a large portion of the small intestine, GH deficiency due to rare pituitary tumors or their treatment, and muscle-wasting disease associated with HIV/AIDS.

[0154] Growth hormone deficiency (GHD) is a rare disorder that includes a group of different pathologies characterized by the inadequate secretion of growth hormone (GH) from the anterior pituitary gland, a small gland located at the base of the brain that is responsible for the production of several hormones. GHD may occur by itself or in combination with other pituitary hormone deficiencies. GHD may be present from birth (congenital) or acquired as a result of trauma, infiltrations, tumor or radiation therapy. There is a third category that has no known cause (idiopathic). Childhood-onset GHD may be all three: congenital, acquired, or idiopathic. It results in growth retardation, short stature, and maturation delays reflected by the delay of lengthening of the bones of the extremities that is inappropriate to the chronological age of the child. Adult-onset GHD is most often acquired from a pituitary tumor or trauma to the brain but may also be idiopathic. It is characterized by a number of variable symptoms including reduced energy levels, altered body composition, osteoporosis (reduced bone mineral density), reduced muscle strength, lipid abnormalities such as increased LDL or cholesterol levels, insulin resistance, and impaired cardiac function. Adult GHD has been estimated to affect 1 in 100,000 people annually, while its incidence rate is approximately 2 cases per 100,000 population when childhood-onset GHD patients are considered. About 15-20 % of the cases represent the transition of childhood GHD into adulthood (Stochholm K et al., *Eur J Endocrinol.*, 155:61-71, 2006).

[0155] Turner (or Ullrich-Turner) syndrome (TS) is a chromosomal abnormality characterized by the absence of the entire chromosome X or a deletion within that chromosome and that affects development in females. The most common feature of Turner syndrome is short stature, which becomes evident by about age 5. This condition occurs in about 1 in 2,500 newborn girls worldwide, but it is much more common among pregnancies that do not survive to term (miscarriages and stillbirths). As a chromosomal condition, there is no cure for Turner syndrome.

[0156] Recombinant DNA-derived human growth hormone is the only drug approved specifically for treatment of GHD and TS. As of 2005, various recombinant human growth hormones (also referred to as somatropin [rDNA origin] for injection) available in the United States (and their manufacturers) included NUTROPIN® (Genentech), HUMATROPE® (Lilly), GENOTROPIN® (Pfizer), NORDITROPIN® (Novo), and SAIZEN® (Merck Serono). In 2006, the U.S. Food and Drug Administration (FDA) approved a version of rHGH called OMNITROPE® (Sandoz). A sustained-release form of human growth hormone, NUTROPIN DEPOT® (Genentech/Alkermes) was approved by the FDA in 1999, allowing for fewer injections (every 2 or 4 weeks instead of daily); however, the product was discontinued by Genentech/Alkermes in 2004 for financial reasons. Additional approved recombinant HGH products include SEROSTIM® (EMD Serono), TEV-TROPIN® (Teva) and ZORBITIVE® (Merck Serono) for short bowel syndrome.

[0157] While proven to be the most effective, spontaneous and trusted treatment option for the management of growth disorders such as GHD, these injectable rHGH's have some significant limitations including, e.g, 1) complications associated with prolonged use and high dosages which are severe and irreversible, and include, e.g, the probability of developing diabetes, cardiovascular disorders and colon cancer. Other common side effects include: joint pain, generalized edema, severe headache, hypoglycemia, wrist pain (carpel tunnel syndrome), increased level of LDL in the blood increasing the possibility of developing atherosclerosis, etc.; 2) HGH injections are not available over the counter, nevertheless, due to rigid FDA norms, black-marketing is rampant. The procurement of the HGH injections without medical prescription is considered illegal and is punishable by law, with imprisonment and fine; and 3) the cost of the treatment is exorbitant. Depending upon the pharmaceutical company the cost of HGH injections for a month of treatment, typically range from between $800 to $3000. Finally, conventional methods using rHGH typically involve multi-dose regimens in which the HGH is administered via subcutaneous injection. The inconvenience, pain and social stigma associated with such methods can be considerable. Management of the pediatric population to treat short stature due to growth hormone deficiency (GHD), Turner syndrome (TS) and related disorders, with these highly invasive and repetitive therapies can be especially difficult.

[0158] Full length human HGH consists of 191 amino acids. HGH produced using molecular biological techniques may have an amino acid sequence identical to naturally occurring HGH. Alternatively, the HGH used may be an HGH analog comprising one or more variations in amino acid sequence with respect to the native hormone. These amino acid variations may provide enhanced biological activity or some other biological or logistical advantages. In various embodiments, the recombinant HGH comprises the amino acid sequence set forth in Genbank Accession No. P01241. The HGH amino acid sequence (without the 26 aa signal sequence of P01241) is set forth in SEQ ID NO: 95:

FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFS
ESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNV
YDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLL
YCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO: 95)

[0159] HGH of the present disclosure refers to HGH from any source which has the sequence of SEQ ID NO: 95, including isolated, purified and/or recombinant HGH produced from any source or chemically synthesizes, for example using solid phase synthesis. Also included herein are conserved amino acid substitutions of native HGH. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In various embodiments, the HGH has an amino acid sequence that shares an observed homology of, *e.g.,* at least about 75%, at least about 80%, at least about 85%, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% with the sequence of SEQ ID NO: 95.

[0160] In various embodiments, the HGH contemplated for use in the fusion molecules of the present disclosure include human growth hormone variants and mutants which have been extensively described in the art (see, e.g. US Patent 8,637,646 (Wells et al) and references cited therein, and US 20110130331 (Guyon et al), each incorporated by reference in its entirety herein for the specific purpose of providing such growth hormone variants and mutants).

[0161] In various embodiments, the HGH contemplated for use in the fusion molecules of the present disclosure include, e.g., NUTROPIN® (Genentech), HUMATROPE® (Lilly), GENOTROPIN® (Pfizer), NORDITROPIN® (Novo), SAIZEN® (Merck Serono), OMNITROPE® (Sandoz), SEROSTIM® (EMD Serono), TEV-TROPIN® (Teva) and ZORBITIVE® (Merck Serono).

[0162] An illustrative, but not limiting, list of suitable growth hormone proteins to be used as the growth hormone in the fusion molecules of the present disclosure, or from which the growth hormones contemplated for use as a growth hormone could be derived, is provided in Table 4.

Table 4

Growth Hormone Related Proteins	RefSeq (NCBI/Uniprot)
Somatotropin	P01241
Synthetic Human Growth Hormone	AAA72260.1
Synthetic Human Growth Hormone Partial	CAA01435
Synthetic Human Growth Hormone Partial	CAA00380
Human Growth Hormone 2	P01242
Somatoliberin	P01286.1
Appetite-regulating Hormone	Q9UBU3
Leptin	P41159

Growth Hormone Receptor Proteins	
Growth Hormone Receptor	P10912
Growth Hormone-Releasing Hormone Receptor	Q02643
Growth Hormone Secretagogue Receptor	Q92847
Growth Hormone-Releasing Hormone Receptor form a	P78470
Growth Hormone Receptor	E9PCN7

Insertion site for attachment of the biologically active cargo

[0163] The biologically active cargo of the fusion molecule can be attached to the remainder of the fusion molecule by any method known by one of skill in the art without limitation. The biologically active cargo can be introduced into any portion of the fusion molecule that does not disrupt the cell-binding or transcytosis activity of the modified Cholix toxin. In various embodiments, the biologically active cargo is directly coupled to the N-terminus or C-terminus of the modified Cholix toxin. In various embodiments, the biologically active cargo can be connected with a side chain of an amino acid of the modified Cholix toxin. In various embodiments, the biologically active cargo is coupled to the modified Cholix with a non-cleavable peptide linker. In various embodiments, the biologically active cargo is coupled to the modified Cholix toxin with a cleavable linker such that cleavage at the cleavable linker(s) separates the biologically active cargo from the remainder of the fusion molecule. In various embodiments, the biologically active cargo is a polypeptide that may also comprise a short leader peptide that remains attached to the polypeptide following cleavage of the cleavable linker. For example, the biological active cargo can comprise a short leader peptide of greater than 1 amino acid, greater than 5 amino acids, greater than 10 amino acids, greater than 15 amino acids, greater than 20 amino acids, greater than 25 amino acids, greater than 30 amino acids, greater than 50 amino acids, or greater than 100 amino acids. In some cases, biological active cargo can comprise a short leader peptide of less than 100 amino acids, less than 50 amino acids, less than 30 amino acids, less than 25 amino acids, less than 20 amino acids, less than 15 amino acids, less than 10 amino acids, or less than 5 amino acids. In some cases, biological active cargo can comprise a short leader peptide of between 1- 100 amino acids, between 5-10 amino acids, between 10 to 50 amino acids, or between 20 to 80 amino acids. In native Cholix toxin, the domain Ib loop spans amino acids 387 to 425, and is structurally characterized by a disulfide bond between two cysteines at positions 395 and 402. This domain Ib portion of Cholix toxin is not essential for any known activity of Cholix toxin, including cell binding, translocation, ER retention or ADP ribosylation activity. Accordingly, domain Ib can be deleted entirely, or modified to contain a biologically active cargo. Thus, in various embodiments, the biologically active cargo can be inserted into Cholix toxin domain Ib. If desirable, the biologically active cargo can be inserted into Cholix toxin domain Ib between the cysteines at positions 395 and 402 that are not crosslinked. This can be accomplished by reducing the disulfide linkage between the cysteines, by deleting one or both of the cysteines entirely from the Ib domain, by mutating one or both of the cysteines to other residues, for example, serine, or by other similar techniques. Alternatively, the biologically active cargo can be inserted into the domain Ib loop between the cysteines at positions 395 and 402. In such embodiments, the disulfide linkage between the cysteines can be used to constrain the biologically active cargo domain.

[0164] In embodiments where the biologically active cargo is expressed together with another portion of the fusion molecule as a fusion protein, the biologically active cargo can be can be inserted into the fusion molecule by any method known to one of skill in the art without limitation. For example, amino acids corresponding to the biologically active cargo can be directly inserted into the fusion molecule, with or without deletion of native amino acid sequences. In various embodiments, all or part of the Ib domain of Cholix toxin can be deleted and replaced with the biologically active cargo. In various embodiments, the cysteine residues of the Ib loop are deleted so that the biologically active cargo remains unconstrained. In other embodiments, the cysteine residues of the Ib loop are linked with a disulfide bond and constrain the biologically active cargo.

[0165] In embodiments where the biologically active cargo is not expressed together with the remainder of the fusion molecule as a fusion protein, the biologically active cargo can be connected with the remainder of the fusion molecule by any suitable method known by one of skill in the art, without limitation. More specifically, the exemplary methods described above for connecting a receptor binding domain to the remainder of the molecule are equally applicable for connecting the biologically active cargo to the remainder of the molecule.

Production of Fusion Proteins

[0166] In various embodiments, the non-naturally occurring fusion molecule is synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the fusion molecule, placing the DNA in an expression cassette under the control of a particular promoter, expressing the molecule in a host, isolating the expressed molecule and, if required, renaturing the molecule.

[0167] DNA encoding the fusion molecules (e.g. Cholix^{415}-IL-10) described herein can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang et al. (1979) Meth. Enzymol. 68: 90-99; the phosphodiester method of Brown et al. (1979) Meth. Enzymol. 68: 109-151; the diethylphosphoramidite method of Beaucage et al. (1981) Tetra. Lett., 22: 1859-1862); the solid support method of U.S. Pat. No. 4,458,066, and the like.

[0168] Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

[0169] Alternatively subsequences can be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments can then be ligated to produce the desired DNA sequence.

[0170] In various embodiments, DNA encoding fusion molecules of the present disclosure can be cloned using DNA amplification methods such as polymerase chain reaction (PCR). Thus, for example, the gene for the IL-10 is PCR amplified, using a sense primer containing the restriction site for, e.g., NdeI and an antisense primer containing the restriction site for HindIII. This can produce a nucleic acid encoding the mature IL-10 sequence and having terminal restriction sites. A modified Cholix toxin having "complementary" restriction sites can similarly be cloned and then ligated to the IL-10 and/or to a linker attached to the IL-10. Ligation of the nucleic acid sequences and insertion into a vector produces a vector encoding the IL-10 joined to the modified Cholix toxin.

Non-cleavable Linkers

[0171] In various embodiments, the modified Cholix toxin and biologically active cargo can be separated by a peptide spacer consisting of one or more amino acids (e.g., up to 25 amino acids). Generally the spacer will have no specific biological activity other than to join the proteins or to preserve some minimum distance or other spatial relationship between them. In various embodiments, however, the constituent amino acids of the spacer can be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity.

[0172] In various embodiments, the linker is capable of forming covalent bonds to both the Cholix toxin and to the biologically active cargo. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. In various embodiments, the linker(s) can be joined to the constituent amino acids of the Cholix toxin and/or the biologically active cargo through their side groups (e.g., through a disulfide linkage to cysteine). In various embodiments, the linkers are joined to the alpha carbon amino and/or carboxyl groups of the terminal amino acids of the Cholix toxin and/or the biologically active cargo.

[0173] A bifunctional linker having one functional group reactive with a group on the Cholix toxin and another group reactive on the biologically active cargo, can be used to form the desired conjugate. Alternatively, derivatization can involve chemical treatment of the targeting moiety. Procedures for generation of, for example, free sulfhydryl groups on polypeptides, such as antibodies or antibody fragments, are known (See U.S. Pat. No. 4,659,839).

[0174] Many procedures and linker molecules for attachment of various compounds including radionuclide metal chelates, toxins and drugs to proteins such as antibodies are known. See, for example, European Patent Application No. 188,256; U.S. Pat. Nos. 4,671,958, 4,659,839, 4,414,148, 4,699,784; 4,680,338; 4,569,789; and 4,589,071; and Borlinghaus et al. (1987) Cancer Res. 47: 4071-4075.

[0175] In various embodiments, the biologically active cargo to be delivered to the subject is coupled to the modified Cholix toxin using one or more non-cleavable peptide linkers comprising, e.g., the amino acid sequence GGGGS (SEQ ID NO: 96), GGGGSGGGGS (SEQ ID NO: 97), GGGGSGGGGSGGGGS (SEQ ID NO: 98), or GGGGSGGG (SEQ ID NO: 99), wherein the modified Cholix toxin targets said biologically active cargo to specific cells, including but not limited to, cells of the immune system such as macrophages, antigen-presenting cells and dendritic cells.

Cleavable Linkers

[0176] In various embodiments, the biologically active cargo to be delivered to the subject is coupled to the modified Cholix toxin using one or more cleavable linkers. The number of cleavable linkers present in the fusion molecule depends, at least in part, on the location of the biologically active cargo in relation to the modified Cholix toxin and the nature of the biologically active cargo. When the biologically active cargo can be separated from the remainder of the fusion molecule with cleavage at a single linker, the fusion molecules can comprise a single cleavable linker. Further, where the biologically active cargo is, e.g., a dimer or other multimer, each subunit of the biologically active cargo can be separated from the remainder of the fusion molecule and/or the other subunits of the biologically active cargo by cleavage at the cleavable linker.

[0177] In various embodiments, the cleavable linkers are cleavable by a cleaving enzyme that is present at or near the basolateral membrane of an epithelial cell. By selecting the cleavable linker to be cleaved by such enzymes, the biologically active cargo can be liberated from the remainder of the fusion molecule following transcytosis across the mucous membrane and release from the epithelial cell into the cellular matrix on the basolateral side of the membrane. Further, cleaving enzymes could be used that are present inside the epithelial cell, such that the cleavable linker is cleaved prior to release of the fusion molecule from the basolateral membrane, so long as the cleaving enzyme does not cleave the fusion molecule before the fusion molecule enters the trafficking pathway in the polarized epithelial cell that results in release of the fusion molecule and biologically active cargo from the basolateral membrane of the cell.

[0178] In various embodiments, the enzyme that is present at a basolateral membrane of a polarized epithelial cell is selected from, *e.g.,* Cathepsin GI, Chymotrypsin I, Elastase I, Subtilisin AI, Subtilisin AII, Thrombin I, or Urokinase I. Table 5 presents these enzymes together with an amino acid sequence that is recognized and cleaved by the particular peptidase.

Table 5

Peptidases Present Near Basolateral Mucous Membranes or in Latter Aspects of the Transcytosis Pathway

Peptidase	Amino Acid Sequence Cleaved
Cathepsin GI	AAPF (SEQ ID NO: 100)
Chymotrypsin I	GGF (SEQ ID NO: 101)
Elastase I	AAPV (SEQ ID NO: 102)
Subtilisin AI	GGL (SEQ ID NO: 103)
Subtilisin AII	AAL (SEQ ID NO: 104)
Thrombin I	FVR (SEQ ID NO: 105)
Urokinase I	VGR (SEQ ID NO: 106)
Furin	RKPR (SEQ ID NO: 107)

[0179] In various embodiments, the cleavable linker exhibits a greater propensity for cleavage than the remainder of the delivery construct. As one skilled in the art is aware, many peptide and polypeptide sequences can be cleaved by peptidases and proteases. In various embodiments, the cleavable linker is selected to be preferentially cleaved relative to other amino acid sequences present in the delivery construct during administration of the delivery construct. In various embodiments, the receptor binding domain is substantially (e.g., about 99%, about 95%, about 90%, about 85%, about 80, or about 75%) intact following delivery of the delivery construct to the bloodstream of the subject. In various embodiments, the translocation domain is substantially (e.g., about 99%, about 95%, about 90%, about 85%, about 80, or about 75%) intact following delivery of the delivery construct to the bloodstream of the subject. In various embodiments, the macromolecule is substantially (e.g., about 99%, about 95%, about 90%, about 85%, about 80, or about 75%) intact following delivery of the delivery construct to the bloodstream of the subject. In various embodiments, the cleavable linker is substantially (e.g., about 99%, about 95%, about 90%, about 85%, about 80, or about 75%) cleaved following delivery of the delivery construct to the bloodstream of the subject.

[0180] In other embodiments, the cleavable linker is cleaved by a cleaving enzyme found in the plasma of the subject. Any cleaving enzyme known by one of skill in the art to be present in the plasma of the subject can be used to cleave the cleavable linker. Uses of such enzymes to cleave the cleavable linkers is less preferred than use of cleaving enzymes found near the basolateral membrane of a polarized epithelial cell because it is believed that more efficient cleavage will occur in near the basolateral membrane. However, if the skilled artisan determines that cleavage mediated by a plasma enzyme is sufficiently efficient to allow cleavage of a sufficient fraction of the delivery constructs to avoid adverse effects, such plasma cleaving enzymes can be used to cleave the delivery constructs. Accordingly, in various embodiments, the cleavable linker can be cleaved with an enzyme that is selected from the group consisting of caspase-1, caspase-3, proprotein convertase 1, proprotein convertase 2, proprotein convertase 4, proprotein convertase 4 PACE 4, prolyl oligopeptidase, endothelin cleaving enzyme, dipeptidyl-peptidase IV, signal peptidase, neprilysin, renin, and esterase (see, e.g., U.S. Pat. No. 6,673,574, incorporated by reference in its entirety herein). Table 6 presents these enzymes together with an amino acid sequence(s) recognized by the particular peptidase. The peptidase cleaves a peptide comprising these sequences at the N-terminal side of the amino acid identified with an asterisk.

Table 6

Plasma Peptidases

Peptidase	Amino Acid Sequence Cleaved

Caspase-1	Tyr-Val-Ala-Asp-Xaa* (SEQ ID NO: 108)
Caspase-3	Asp-Xaa-Xaa-Asp-Xaa* (SEQ ID NO: 109)
Proprotein convertase 1	Arg-(Xaa)$_n$-Arg-Xaa*; n = 0, 2, 4 or 6 (SEQ ID NO: 110)
Proprotein convertase 2	Lys-(Xaa)$_n$-Arg-Xaa*; n = 0, 2, 4, or 6 (SEQ ID NO: 111)
Proprotein convertase 4	Glu-Arg-Thr-Lys-Arg-Xaa* (SEQ ID NO: 112)
Proprotein convertase 4 PACE 4	Arg-Val-Arg-Arg-Xaa* (SEQ ID NO: 113) Decanoyl-Arg-Val-Arg-Arg-Xaa* (SEQ ID NO: 114)
Prolyloligopeptidase Endothelin cleaving enzyme in combination with dipeptidyl-peptidase IV	Pro-Xaa*-Trp-Val-Pro-Xaa (SEQ ID NO: 115)
Signal peptidase	Trp-Val*-Ala-Xaa (SEQ ID NO: 116)
Neprilysin in combination with dipeptidyl-peptidase IV	Xaa-Phe*-Xaa-Xaa (SEQ ID NO: 117) Xaa-Tyr*-Xaa-Xaa (SEQ ID NO: 118) Xaa-Trp*-Xaa-Xaa (SEQ ID NO: 119)
Renin in combination with dipeptidyl-peptidase IV	Asp-Arg-Tyr-Ile-Pro-Phe-His-Leu*-Leu (Val, Ala or Pro)-Tyr-(Ser, Pro, or Ala) (SEQ ID NO: 120)

[0181] Thus, in various embodiments, the cleavable linker can be any cleavable linker known by one of skill in the art to be cleavable by an enzyme that is present at the basolateral membrane of an epithelial cell. In various embodiments, the cleavable linker comprises a peptide. In other embodiments, the cleavable linker comprises a nucleic acid, such as RNA or DNA. In still other embodiments, the cleavable linker comprises a carbohydrate, such as a disaccharide or a trisaccharide.

[0182] Alternatively, in various embodiments, the cleavable linker can be any cleavable linker known by one of skill in the art to be cleavable by an enzyme that is present in the plasma of the subject to whom the delivery construct is administered. In various embodiments, the cleavable linker comprises a peptide. In other embodiments, the cleavable linker comprises a nucleic acid, such as RNA or DNA. In still other embodiments, the cleavable linker comprises a carbohydrate, such as a disaccharide or a trisaccharide.

[0183] In various embodiments, the peptidases exhibit much higher (e.g., 100%, 200%, or more increase in activity relative to the apical side) on the baso-lateral side (also referred to as basolateral). Thus, in various embodiments, the cleavable linker is cleavable by an enzyme that exhibits 50% higher activity on the basolateral side of the membrane than on the apical side of the membrane. In various embodiments, the cleavable linker is cleavable by an enzyme that exhibits 100% higher activity on the basolateral side of the membrane than on the apical side of the membrane. In various embodiments, the cleavable linker is cleavable by an enzyme that exhibits 200% higher activity on the basolateral side of the membrane than on the apical side of the membrane. In various embodiments, the cleavable linker is cleavable by an enzyme that exhibits 500% higher activity on the basolateral side of the membrane than on the apical side of the membrane. In various embodiments, the cleavable linker is cleavable by an enzyme that exhibits 1,000% higher activity on the basolateral side of the membrane than on the apical side of the membrane.

[0184] In various embodiments, the fusion molecule comprises a cleavable linker having an amino acid sequence selected from, e.g., SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106 or SEQ ID NO: 107 and is cleavable by an enzyme that exhibits higher activity on the basolateral side of a polarized epithelial cell than it does on the apical side of the polarized epithelial cell, and/or is cleavable by an enzyme that exhibits higher activity in the plasma than it does on the apical side of a polarized epithelial cell.

[0185] In various embodiments, the cleavable linker can be a cleavable linker that is cleaved following a change in the environment of the fusion molecule. For example, the cleavable linker can be a cleavable linker that is pH sensitive and is cleaved by a change in pH that is experienced when the fusion molecule is released from the basolateral membrane of a polarized epithelial cell. For instance, the intestinal lumen is strongly alkaline, while plasma is essentially neutral. Thus, a cleavable linker can be a moiety that is cleaved upon a shift from alkaline to neutral pH. The change in the environment of the fusion molecule that cleaves the cleavable linker can be any environmental change that that is experienced when the fusion molecule is released from the basolateral membrane of a polarized epithelial cell known by one of skill in the art, without limitation.

[0186] In various embodiments, the cleavable linker is cleaved by a cleaving enzyme found in the plasma of the subject. Any cleaving enzyme known by one of skill in the art to be present in the plasma of the subject can be used to cleave the cleavable linker. Accordingly, in various embodiments, the cleavable linker can be cleaved with an enzyme that is selected from e.g., SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119 or SEQ ID NO: 120.

[0187] In various embodiment, the cleavable linker is a linker that contains an amino acid sequence that is a known substrate for the tobacco etch virus (TEV) protease. Accordingly, in various embodiments, the cleavable linker comprises the amino acid sequence set in forth in, e.g., GGGGSGGGENLYFQS (SEQ ID NO: 121).

Chemical Conjugation of the Cargo to the modified Cholix Toxin

[0188] In various embodiments, the biologically active cargo to be delivered to the subject is chemically conjugated to the modified Cholix toxin. Means of chemically conjugating molecules are well known to those of skill.

[0189] The procedure for conjugating two molecules varies according to the chemical structure of the agent. Polypeptides typically contain variety of functional groups; e.g., carboxylic acid (COOH) or free amine ($--NH_2$) groups, that are available for reaction with a suitable functional group on the other peptide, or on a linker to join the molecules thereto.

[0190] Alternatively, the antibody and/or the biologically active cargo can be derivatized to expose or attach additional reactive functional groups. The derivatization can involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford Ill.

[0191] In various embodiments, isolated modified Cholix toxins are prepared by bacterial fermentation and purified by established methods. The purified modified Cholix toxin is then modified at its C-terminus to allow direct chemical coupling through a free sulfhydryl residue located near the C-terminus of the protein. The C-terminal modification includes a cysteine-constrained loop harboring the consensus cleavage sequence for the highly selective protease from the tobacco etch virus (TEV), a second cysteine, and a hexa-histadine (His_6) tag. The second Cys is included to form a disulphide bridge with the Cys ultimately used for coupling. Adding the His_6 sequence to the protein simplifies the purification and the TEV cleavage sequence provides a mechanism to selectively remove the terminal Cys residue following mild reduction. TEV cleavage and mild reduction with 0.1 mM dithiotheitol following expression and isolation of the ntCholix constructs allows for the direct chemical coupling of a biologically active cargo via a maleimide-based reaction as a generic mechanism of cargo attachment. Following TEV protease cleavage, reduction, and cargo coupling through a maleimide reaction with the free sulfhydryl, removal of the freed C-terminal sequence was achieved by a second Ni^{2+} column chromatography step.

[0192] In various embodiments, the fusion molecule comprises particles which are decorated covalently with the modified Cholix toxin, and wherein the biologically active cargo is integrated into the particles. In various embodiments, the particles can be smaller than ~150 nm in diameter, smaller than ~100 nm, or smaller than ~50 nm.

[0193] In various embodiments, the fusion molecule comprises a biologically active cargo coupled non-covalently to the modified Cholix toxin. This fusion molecule could ferry, e.g., a non-covalently associated IL-10 across the epithelium such as a surface element of the IL-10 receptor (Josephson, K., Logsdon, N.J., Walter, M.R., Immunity 15: 35-46, 2001, incorporated by reference in its entirety herein).

Pharmaceutical Compositions and Delivery Methods

[0194] The pharmaceutical compositions of the present disclosure relate to compositions for administration to a human subject. The pharmaceutical compositions comprise the non-naturally occurring fusion molecules recited herein, alone or in combination. The pharmaceutical compositions may comprise additional molecules capable of altering the characteristics of the non-naturally occurring fusion molecules, for example, stabilizing, modulating and/or activating their function. The composition may, *e.g.,* be in solid or liquid form and may be, inter alia, in the form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). The pharmaceutical composition of the present disclosure may, optionally and additionally, comprise a pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material and any of the standard pharmaceutical carriers, vehicles, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants.

[0195] The pharmaceutical compositions are generally formulated appropriately for the immediate use intended for the fusion molecule. For example, if the fusion molecule is not to be administered immediately, the fusion molecule can be formulated in a composition suitable for storage. One such composition is a lyophilized preparation of the fusion molecule together with a suitable stabilizer. Alternatively, the fusion molecule composition can be formulated for storage in a solution with one or more suitable stabilizers. Any such stabilizer known to one of skill in the art without limitation can be used. For example, stabilizers suitable for lyophilized preparations include, but are not limited to, sugars, salts, surfactants, proteins, chaotropic agents, lipids, and amino acids. Stabilizers suitable for liquid preparations include, but are not limited to, sugars, salts, surfactants, proteins, chaotropic agents, lipids, and amino acids. Specific stabilizers than can be used in the compositions include, but are not limited to, trehalose, serum albumin, phosphatidylcholine, lecithin, and arginine. Other compounds, compositions, and methods for stabilizing a lyophilized or liquid preparation of the fusion molecules may be found, for example, in U.S. Pat. Nos. 6,573,237, 6,525,102, 6,391,296, 6,255,284, 6,133,229, 6,007,791, 5,997,856, and 5,917,021.

[0196] In various embodiments, the pharmaceutical compositions of the present disclosure are formulated for oral delivery. The pharmaceutical compositions formulated for oral administration take advantage of the modified Cholix toxin’s ability to mediate transcytosis across the gastrointestinal (GI) epithelium. It is anticipated that oral administration of these pharmaceutical compositions will result in absorption of the fusion molecule through polarized epithelial cells of the digestive mucosa, *e.g.,* the intestinal mucosa, followed by release of the biologically active cargo at the basolateral side of the mucous membrane. In various embodiments, the epithelial cell is selected from the group consisting of nasal epithelial cells, oral epithelial cells, intestinal epithelial cells, rectal epithelial cells, vaginal epithelial cells, and pulmonary epithelial cells. Pharmaceutical compositions of the disclosure may include the addition of a transcytosis enhancer to facilitate transfer of the fusion protein across the GI epithelium. Such enhancers are known in the art. See Xia et al., (2000) J. Pharmacol. Experiment. Therap., 295:594-600; and Xia et al. (2001) Pharmaceutical Res., 18(2):191-195, each incorporated by reference in its entirety herein.

[0197] It is anticipated that once transported across the GI epithelium, the fusion molecules of the disclosure will exhibit extended half-life in serum, that is, the biologically active cargo of the fusion molecules will exhibit an extended serum half-life compared to the biologically active cargo in its non-fused state. As such, the oral formulations of the pharmaceutical compositions of the present disclosure are prepared so that they are suitable for transport to the GI epithelium and protection of the fusion molecule in the stomach. Such formulations may include carrier and dispersant components and may be in any suitable form, including aerosols (for oral or pulmonary delivery), syrups, elixirs, tablets, including chewable tablets, hard or soft capsules, troches, lozenges, aqueous or oily suspensions, emulsions, cachets or pellets granulates, and dispersible powders. In various embodiments, the pharmaceutical compositions are employed in solid dosage forms, e.g., tablets, capsules, or the like, suitable for simple oral administration of precise dosages.

[0198] In various embodiments, the oral formulation comprises a fusion molecule and one or more compounds that can protect the fusion molecule while it is in the stomach. For example, the protective compound should be able to prevent acid and/or enzymatic hydrolysis of the fusion molecule. In various embodiments, the oral formulation comprises a fusion molecule and one or more compounds that can facilitate transit of the construct from the stomach to the small intestine. In various embodiments, the one or more compounds that can protect the fusion molecule from degradation in the stomach can also facilitate transit of the construct from the stomach to the small intestine. For example, inclusion of sodium bicarbonate can be useful for facilitating the rapid movement of intra-gastric delivered materials from the stomach to the duodenum as described in Mrsny et al., Vaccine 17:1425-1433, 1999. Other methods for formulating compositions so that the fusion molecules can pass through the stomach and contact polarized epithelial membranes in the small intestine include, but are not limited to, enteric-coating technologies as described in DeYoung, Int J Pancreatol, 5 Suppl:31-6, 1989 and the methods provided in U.S. Pat. Nos. 6,613,332, 6,174,529, 6,086,918, 5,922,680, and 5,807,832, each incorporated by reference in its entirety herein.

[0199] Pharmaceutical compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents in order to provide a pharmaceutically elegant and palatable preparation. For example, to prepare orally deliverable tablets, the fusion molecule is mixed with at least one pharmaceutical excipient, and the solid formulation is compressed to form a tablet according to known methods, for delivery to the gastrointestinal tract. The tablet composition is typically formulated with additives, e.g. a saccharide or cellulose carrier, a binder such as starch paste or methyl cellulose, a filler, a disintegrator, or other additives typically usually used in the manufacture of medical preparations. To prepare orally deliverable capsules, DHEA is mixed with at least one pharmaceutical excipient, and the solid formulation is placed in a capsular container suitable for delivery to the gastrointestinal tract. Compositions comprising fusion molecules may be prepared as described generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton Pa. 18042) at Chapter 89, which is herein incorporated by reference.

[0200] In various embodiments, the pharmaceutical compositions are formulated as orally deliverable tablets containing fusion molecules in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets. These excipients may be inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, maize starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid, or talc. The tablets may be uncoated or they may be coated with known techniques to delay disintegration and absorption in the gastrointestinal track and thereby provide a sustained action over a longer period of time. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

[0201] In various embodiments, the pharmaceutical compositions are formulated as hard gelatin capsules wherein the fusion molecule is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, or kaolin or as soft gelatin capsules wherein the fusion molecule is mixed with an aqueous or an oil medium, for example, arachis oil, peanut oil, liquid paraffin or olive oil.

[0202] In various embodiments, aqueous suspensions may contain a fusion molecule in the admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecylethyloxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives for example, ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents such as sucrose or saccharin.

[0203] In various embodiments, oily suspensions may be formulated by suspending the fusion molecule in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oil suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

[0204] In various embodiments, the pharmaceutical compositions may be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil for example, gum acacia or gum tragacanth, naturally-occurring phosphotides, for example soybean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, and condensation products of the same partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0205] In various embodiments wherein the pharmaceutical composition is in the form of a tablet or capsule, the tablet or capsule is coated or encapsulated to protect the biologically active cargo from enzymatic action in the stomach and to ensure that there is sufficient biologically active cargo to be absorbed by the subject to produce an effective response. Such coating or encapsulation methods include, e.g., encapsulation in nanoparticles composed of polymers with a hydrophobic backbone and hydrophilic branches as drug carriers, encapsulation in microparticles, insertion into liposomes in emulsions, and conjugation to other molecules. Examples of nanoparticles include mucoadhesive nanoparticles coated with chitosan and Carbopol (Takeuchi et al., Adv. Drug Deliv. Rev. 47(1):39-54, 2001) and nanoparticles containing charged combination polyesters, poly(2-sulfobutyl-vinyl alcohol) and poly(D,L-lactic-co-glycolic acid) (Jung et al., Eur. J. Pharm. Biopharm. 50(1):147-160, 2000).

[0206] Encapsulated or coated tablets can be used that release the biologically active cargo in a layer-by-layer manner, thereby releasing biologically active cargo over a pre-determined time frame while moving along the gastrointestinal tract. In addition, tablets comprising the biologically active cargo can be placed within a larger tablet, thereby protecting the inner tablet from environmental and processing conditions, such as temperature, chemical agents (e.g., solvents), pH, and moisture. The outer tablet and coatings further serve to protect the biologically active cargo in the gastric environment.

[0207] In various embodiments, pharmaceutical compositions may be formulated for oral delivery using polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, Oral Delivery of Microencapsulated Proteins, in Protein Delivery: Physical Systems, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)).

[0208] Surface active agents or surfactants promote absorption of polypeptides through mucosal membrane or lining. Useful surface active agents or surfactants include fatty acids and salts thereof, bile salts, phospholipid, or an alkyl saccharide. Examples of fatty acids and salts thereof include sodium, potassium and lysine salts of caprylate (C_8), caprate (C_{10}), laurate (C_{12}) and myristate (C_{14}). Examples of bile salts include cholic acid, chenodeoxycholic acid, glycocholic acid, taurocholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, lithocholic acid, and ursodeoxycholic acid. Examples of phospholipids include single-chain phospholipids, such as lysophosphatidylcholine, lysophosphatidylglycerol, lysophosphatidylethanolamine, lysophosphatidylinositol and lysophosphatidylserine; or double-chain phospholipids, such as diacylphosphatidylcholines, diacylphosphatidylglycerols, diacylphosphatidylethanolamines, diacylphosphatidylinositols and diacylphosphatidylserines. Examples of alkyl saccharides include alkyl glucosides or alkyl maltosides, such as decyl glucoside and dodecyl maltoside.

[0209] In another aspect, the present disclosure relates to methods of orally administering the pharmaceutical compositions of the disclosure. Without intending to be bound to any particular theory or mechanism of action, it is believed that oral administration of the fusion molecules results in absorption of the fusion molecule through polarized epithelial cells of the digestive mucosa, e.g., the intestinal mucosa, followed by cleavage of the fusion molecule and release of the biologically active cargo at the basolateral side of the mucous membrane. Thus, when the biologically active cargo exerts a biological activity in the liver, such as, for example, activities mediated by IL-10 binding to its cognate receptor, the biologically active cargo is believed to exert an effect in excess of what would be expected based on the plasma concentrations observed in the subject, i.e., oral administration of the fusion molecule can deliver a higher effective concentration of the delivered biologically active cargo to the liver of the subject than is observed in the subject's plasma.

[0210] In another aspect, the present disclosure relates to methods of orally administering the pharmaceutical compositions of the disclosure. Such methods may include, but are not limited to, steps of orally administering the compositions by the patient or a caregiver. Such administration steps may include administration on intervals such as once or twice per day depending on the fusion molecule, disease or patient condition or individual patient. Such methods also include the administration of various dosages of the individual fusion molecule. For instance, the initial dosage of a pharmaceutical composition may be at a higher level to induce a desired effect, such as reduction in blood glucose levels. Subsequent dosages may then be decreased once a desired effect is achieved. These changes or modifications to administration protocols may be done by the attending physician or health care worker.

[0211] These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. The therapeutically effective amount for a given situation will readily be determined by routine experimentation and is within the skills and judgment of the ordinary clinician or physician. The skilled person knows that the effective amount of a pharmaceutical composition administered to an individual will, inter alia, depend on the nature of the biologically active cargo. The length of treatment needed to observe changes and the interval following treatment for responses to occur vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art.

[0212] The amount of biologically active cargo is an amount effective to accomplish the purpose of the particular active agent. The amount in the composition typically is a pharmacologically, biologically, therapeutically, or chemically effective amount. However, the amount can be less than a pharmacologically, biologically, therapeutically, or chemically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of carrier/biologically or chemically active agent compositions or may contain a divided pharmacologically, biologically, therapeutically, or chemically effective amount. The total effective amounts can then be administered in cumulative units containing, in total, pharmacologically, biologically, therapeutically or chemically active amounts of biologically active cargo.

[0213] In various embodiments, an amount of fusion molecule administered to the subject is at most 0.001 pg, at most 1 pg, at most 2 pg, at most 3 pg, at most 4 pg, at most 5 pg, at most 10 pg, at most 50 pg, at most 100 pg, at most 1 µg, at most 2 µg, at most 3 µg, at most 4 µg, at most 5 µg, at most 10 µg, at most 50 µg, at most 100 µg, at most 1 mg, at most 2 mg, at most 3 mg, at most 4 mg, at most 5 mg, at most 10 mg, at most 50 mg, at most 100 mg, or at most 1g.

[0214] In various embodiments, an amount of fusion molecule administered to the subject is at least 0.001 pg, at least 1 pg, at least 2 pg, at least 3 pg, at least 4 pg, at least 5 pg, at least 10 pg, at least 50 pg, at least 100 pg, at least 1 µg, at least 2 µg, at least 3 µg, at least 4 µg, at least 5 µg, at least 10 µg, at least 50 µg, at least 100 µg, at least 1 mg, at least 2 mg, at least 3 mg, at least 4 mg, at least 5 mg, at least 10 mg, at least 50 mg, at least 100 mg, or at least 1g.

[0215] In various embodiments, an amount of fusion molecule administered to the subject is from 0.001 pg and about 1 g , from1 pg to 10 pg, from 50 pg to 100 pg, from1 µg to 5 µg , from 10 µg to 20 µg, from10 µg to 500 mg, from10 µg to 100 mg, from 10 µg to 1000 µg, from 10 µg to 250 µg, from 10 µg to 100 µg, from 10 µg to 50 µg, from 1 mg to 5 mg, or from 10 mg to 100mg.

[0216] The volume of a composition comprising the fusion molecule that is administered will generally depend on the concentration of fusion molecule and the formulation of the composition. In various embodiments, a unit dose of the fusion molecule composition is from 0.001 µl to 1 ml, from 1 µl to 100 µl, from 50 µl to 500 µl, from 0.01 ml to 1 ml, from 1 ml to 100 ml, from 0.05 ml to 1 ml. For example, the unit dose of the fusion molecule composition can be about 0.5 ml.

[0217] In some embodiments, a unit dose of the fusion molecule composition is at most about 0.001 µl, at most 1 µl, at most 10 µl, at most 50 µl, at most 200 µl, at most 0.01 ml, at most 0.05 ml, at most 0.1 ml, at most 0.2 ml, at most 0.5 ml, or at most 1 ml.

[0218] In some a unit dose of the fusion molecule composition is at least 0.001 µl, at least 1 µl, at least 10 µl, at least 50 µl, at least 200 µl, at least 0.01 ml, at least 0.05 ml, at least 0.1 ml, at least 0.2 ml, at least 0.5 ml, or at least 1 ml.

[0219] The fusion molecule compositions can be prepared in dosage forms containing between 1 and 50 doses (e.g., 0.5 ml to 25 ml), more usually between 1 and 10 doses (e.g., 0.5 ml to 5 ml).

[0220] The fusion molecule compositions of the disclosure can be administered in one dose or in multiple doses. A dose can be followed by one or more doses spaced by about 1 to about 6 hours, by about 6 to about 12 hours, by about 12 to about 24 hours, by about 1 day to about 3 days, by about 1 day to about 1 week, by about 1 week to about 2 weeks, by about 2 weeks to about 1 month, by about 4 to about 8 weeks, by about 1 to about 3 months, or by about 1 to about 6 months.

[0221] In various embodiments, the pharmaceutical compositions comprising the fusion molecules may be, though not necessarily, administered daily, in an effective amount to ameliorate a symptom. Generally, the total daily dosage can be administered at an amount of at least about 0.001 pg, at least about 0.1 mg, at least about 1 mg, at least about 10 mg, at least about 50 mg, at least about 100 mg, at least about 150 mg, at least about 200 mg, at least about 250 mg, at least about 300 mg, at least about 350 mg, at least about 400 mg, at least about 450 mg, at least about 500 mg per day, or at least about 1000 mg per day. For example, the dosage can be formulated for oral administration in capsules or tablets, such that 4 capsules or tablets, each containing 50 mg fusion molecule. Capsules or tablets for oral delivery can conveniently contain up to a full daily oral dose, e.g., 200 mg or more per day.

[0222] In various embodiments, the pharmaceutical compositions comprising the fusion molecules may be, though not necessarily, administered daily, in an effective amount to ameliorate a symptom. Generally, the total daily dosage can be administered at an amount of at most 50 mg per day, at most 100 mg per day, at most 150 mg per day, at most 200 mg per day, at most 250 mg per day, at most 300 mg per day, at most 350 mg per day, at most 400 mg per day, at most 450 mg per day, at most 500 mg per day, or at most 1000 mg per day.

[0223] As used herein, the terms "co-administration", "co-administered" and "in combination with", referring to the fusion molecules of the disclosure and one or more other therapeutic agents, is intended to mean, and does refer to and include the following: simultaneous administration of such combination of fusion molecules of the disclosure and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said patient; substantially simultaneous administration of such combination of fusion molecules of the disclosure and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said patient, whereupon said components are released at substantially the same time to said patient; sequential administration of such combination of fusion molecules of the disclosure and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said patient with a significant time interval between each administration, whereupon said components are released at substantially different times to said patient; and sequential administration of such combination of fusion molecules of the disclosure and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are released in a concurrent, consecutive, and/or overlapping manner at the same and/or different times to said patient, where each part may be administered by either the same or a different route.

[0224] In various embodiments, the pharmaceutical compositions comprising the fusion molecules may be co-administered with a second component, wherein the second component is a hormone, toxin, or bioactive agent which is capable of binding to the GM-1 (monosialotetrahexosylganglioside) receptor (Hakomori, Advances in Exp. Medicine and Biology, 174:333-339, 1984). In various embodiments, the second component is SV40 virus, polyoma virus, or a toxin such as cholera toxin, or exotoxin A from *Pseudomonas aeruginosa* (PE).

[0225] As used herein, the terms “cholera toxin” or “CT” refer to the eponymous virulence agent of *Vibrio cholerae* bacterium, which can cause acute, life-threatening massive watery diarrhea. CT is a protein complex composed of a single A subunit organized with a pentamer of B subunits that binds to cell surface G_{M1} ganglioside structures at the apical surface of epithelia. CT is secreted by *V. cholera* following horizontal gene transfer with virulent strains of *V. cholerae* carrying a variant of lysogenic bacteriophage called CTXf or CTXφ. Recent cholera outbreaks, however, have suggested that strains of some serogroups (non-O1, non-O139) do not express CT but rather use other virulence factors. Detailed analyses of non-O1, non-O139 environmental and clinical data suggested the presence of a novel putative secreted exotoxin with some similarity to PE. The sequence of CT is known and has been described (Mekalanos J. J. et al Nature 306, page 551 (1983)).

[0226] As used herein the terms "exotoxin A from *Pseudomonas aeruginosa*”, "*Pseudomonas* exotoxin A" or “PE” refer to an extremely active monomeric protein (molecular weight 66 kD), secreted by *Pseudomonas aeruginosa*, which inhibits protein synthesis in eukaryotic cells. The 613-residue sequence of PE is well known in the art and is set forth, for example, in U.S. Pat. No. 5,602,095. Domain Ia (amino acids 1-252) mediates cell binding. Domain II (amino acids 253-364) is responsible for translocation into the cytosol and domain III (amino acids 400-613) mediates ADP ribosylation of elongation factor 2. The function of domain Ib (amino acids 365-399) remains undefined, although it has been known a large part of it, amino acids 365-380, can be deleted without loss of cytotoxicity. See Siegall et al., J Biol Chem, 264:14256-61 (1989).

[0227] Certain cytotoxic fragments of PE are known in the art and are often referenced by the molecular weight of the fragment, which designates for the person of skill in the art the particular composition of the PE fragment. For example, PE40 was one of the first fragments that was studied and used as the toxic portion of immunotoxins. The term designates a truncated form of PE in which domain Ia, the domain responsible for non-specific binding. See, e.g., Pai et al., Proc. Nat'l Acad. Sci. USA, 88:3358-3362 (1991); and Kondo et al., J. Biol. Chem., 263:9470-9475 (1988). Elimination of non-specific binding, however, can also be achieved by mutating certain residues of domain Ia. U.S. Pat. No. 5,512,658, for instance, discloses that a mutated PE in which domain Ia is present but in which the basic residues of domain Ia at positions 57, 246, 247, and 249 are replaced with acidic residues (glutamic acid, or "E")) exhibits greatly diminished non-specific cytotoxicity. This mutant form of PE is sometimes referred to as "PE4E."

[0228] In various embodiments, the combination therapy comprises administering the isolated fusion molecule composition and the second agent composition simultaneously, either in the same pharmaceutical composition or in separate pharmaceutical compositions. In various embodiments, isolated fusion molecule composition and the second agent composition are administered sequentially, i.e., the isolated fusion molecule composition is administered either prior to or after the administration of the second agent composition.

[0229] In various embodiments, the administrations of the isolated fusion molecule composition and the second agent composition are concurrent, i.e., the administration period of the isolated fusion molecule composition and the second agent composition overlap with each other.

[0230] In various embodiments, the administrations of the isolated fusion molecule composition and the second agent composition are non-concurrent. For example, in various embodiments, the administration of the isolated fusion molecule composition is terminated before the second agent composition is administered. In various embodiments, the administration second agent composition is terminated before the isolated fusion molecule composition is administered. In various embodiments, the administrations of the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be archived with a meal, e.g. prior to the meal, during the meal or after the meal.

[0231] In some embodiments, the administration of the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be archived prior to a meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered more than 12 hours, more than 11 hours, more than 10 hours, more than 9 hours, more than 8 hours, more than 7 hours, more than 6 hours, more than 5 hours, more than 4 hours, more than 3 hours, more than 2 hours, more than 1 hour, more than 50 minutes, more than 40 minutes, more than 30 minutes, more than 20 minutes, more than 10 minutes, more than 5 minutes, or more than 1 minute prior to the meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered less than 12 hours, less than 11 hours, less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, less than 50 minutes, less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute prior to the meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered between about 1 minute to about 10 minutes, between about 5 minutes to about 30 minutes, between about 20 minutes to about 60 minutes, between about 1 hour to about 3 hours, between about 2 hours to about 10 hours, or between about 5 hours to about 12 hour prior to the meal.

[0232] In some embodiments, the administration of the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be archived after a meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered more than 12 hours, more than 11 hours, more than 10 hours, more than 9 hours, more than 8 hours, more than 7 hours, more than 6 hours, more than 5 hours, more than 4 hours, more than 3 hours, more than 2 hours, more than 1 hour, more than 50 minutes, more than 40 minutes, more than 30 minutes, more than 20 minutes, more than 10 minutes, more than 5 minutes, or more than 1 minute after the meal. In some embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered less than 12 hours, less than 11 hours, less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, less than 50 minutes, less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute after the meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered less than 12 hours, less than 11 hours, less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, less than 50 minutes, less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute prior to the meal. In various embodiments, the fusion molecule of the invention, whether alone or in combination with a therapeutic agent, can be administered between about 1 minute to about 10 minutes, between about 5 minutes to about 30 minutes, between about 20 minutes to about 60 minutes, between about 1 hour to about 3 hours, between about 2 hours to about 10 hours, or between about 5 hours to about 12 hour after the meal.

Methods of Use

[0233] In another aspect, the pharmaceutical compositions formulated for oral delivery are used to treat certain classes of diseases or medical conditions that are particularly amenable for oral formulation and delivery. Such classes of diseases or conditions include, e.g., viral disease or infections, cancer, a metabolic diseases, obesity, autoimmune diseases, inflammatory diseases, allergy, graft-vs-host disease, systemic microbial infection, anemia, cardiovascular disease, psychosis, genetic diseases, neurodegenerative diseases, disorders of hematopoietic cells, diseases of the endocrine system or reproductive systems, gastrointestinal diseases. In many chronic diseases, oral formulations of the fusion molecules of the disclosure are particularly useful because they allow long-term patient care and therapy via home oral administration without reliance on injectable treatment or drug protocols.

[0234] In various embodiments of the present disclosure, pharmaceutical compositions comprising the fusion molecules of the disclosure are provided for use in treating and/or preventing inflammatory diseases. "Inflammatory diseases" include all diseases associated with acute or chronic inflammation. Acute inflammation is the initial response of the body to harmful stimuli and results from an increased movement of plasma and leukocytes (such as e.g. granulocytes) from the blood into the injured tissues. A number of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation is referred to as chronic inflammation, which leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Inflammatory diseases can be caused by e.g. burns, chemical irritants, frostbite, toxins, infection by pathogens, physical injury, immune reactions due to hypersensitivity, ionizing radiation, or foreign bodies, such as e.g. splinters, dirt and debris. Examples of inflammatory diseases are well known in the art.

[0235] In various embodiments, the inflammatory disease is selected from the group consisting of inflammatory bowel disease, psoriasis and bacterial sepsis. The term "inflammatory bowel disease", as used herein, refers to a group of inflammatory conditions of the colon and small intestine including, for example, Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome and indeterminate colitis.

[0236] “Crohn’s disease”, in accordance with the present disclosure, is a T-helper Type 1 (Th 1) inflammatory bowel disease, which has an immune response pattern that includes an increased production of interleukin-12, tumour necrosis factor (TNF), and interferon-γ (Romagnani. Inflamm Bowel Dis 1999; 5:285-94), and which can have a devastating impact on the lifestyle of a patient afflicted therewith. Common symptoms of Crohn's disease include diarrhea, cramping, abdominal pain, fever, and even rectal bleeding. Crohn's disease and complications associated with it often results in the patient requiring surgery, often more than once. There is no known cure for Crohn's disease, and long-term, effective treatment options are limited. The goals of treatment are to control inflammation, correct nutritional deficiencies, and relieve symptoms like abdominal pain, diarrhea, and rectal to bleeding. While treatment can help control the disease by lowering the number of times a person experiences a recurrence, there is no cure. Treatment may include drugs, nutrition supplements, surgery, or a combination of these options. Common treatments which may be administered for treatment include anti-inflammation drugs, including sulfasalazine, cortisone or steroids, including prednisone, immune system suppressors, such as 6-mercaptopurine or azathioprine, and antibiotics.

[0237] "Psoriasis", in accordance with the present disclosure, is a disease which affects the skin and joints. It commonly causes red scaly patches to appear on the skin. The scaly patches caused by psoriasis, called psoriatic plaques, are areas of inflammation and excessive skin production. Skin rapidly accumulates at these sites and takes a silvery-white appearance. Plaques frequently occur on the skin of the elbows and knees, but can affect any area including the scalp and genitals. Psoriasis is hypothesized to be immune-mediated and is not contagious. The disorder is a chronic recurring condition which varies in severity from minor localised patches to complete body coverage. Fingernails and toenails are frequently affected (psoriatic nail dystrophy)--and can be seen as an isolated finding. Psoriasis can also cause inflammation of the joints, which is known as psoriatic arthritis. Ten to fifteen percent of people with psoriasis have psoriatic arthritis.

[0238] The term "bacterial sepsis", as used herein, refers to life-threatening conditions resulting from the circulation of bacteria in the blood stream. Sepsis results in generalized systemic production of proinflammatory cytokines that results in tissue damage and ultimately septic shock due to failure of the microcirculation.

[0239] Another aspect of the present disclosure relates to methods for treatment, prophylaxis and/or prevention of an autoimmune disease, comprising administering to said patient a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a fusion molecule described herein, in pharmaceutically acceptable carrier.

[0240] An autoimmune disease, as pertains to the present disclosure, is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. In various embodiments the autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), pemphigus vulgaris, myasthenia gravis, hemolytic anemia, thrombocytopenia purpura, Grave's disease, Sjogren's disease, dermatomyositis, Hashimoto's disease, polymyositis, inflammatory bowel disease, multiple sclerosis (MS), diabetes mellitus, rheumatoid arthritis, and scleroderma.

[0241] "Rheumatoid arthritis", in accordance with the present disclosure, is an autoimmune disorder that causes the body's immune system to attack the bone joints (Muller B et al., Springer Semin Immunopathol., 20:181-96, 1998). Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue under the skin.

[0242] In various embodiments of the present disclosure, pharmaceutical compositions comprising the fusion molecules of the disclosure are provided for use in the treatment, prophylaxis and/or prevention of a cancer, comprising administering to said patient a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a fusion molecule described herein, in pharmaceutically acceptable carrier. Cancers to be treated include, but are not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, chronic lymphocytic leukemia, hairy cell leukemia, acute lymphoblastic leukemia, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney ovary, cervix, breast, lung, nasopharynx, malignant melanoma and rituximab resistant NHL and leukemia.

[0243] In various embodiments, the therapeutically effective amount of a fusion molecule described herein will be administered in combination with one or more other therapeutic agents. Such therapeutic agents may be accepted in the art as a standard treatment for a particular disease state as described herein, such as inflammatory disease, autoimmune disease, or cancer. Exemplary therapeutic agents contemplated include, but are not limited to, cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, or other active and ancillary agents.

[0244] In various embodiments, the present disclosure provides a method of treating a subject having a metabolic disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said metabolic disorder is diabetes, obesity, diabetes as a consequence of obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, impaired glucose tolerance (IGT), diabetic dyslipidemia, or hyperlipidemia.

[0245] In another aspect, the present disclosure provides a method of treating a subject having a fatty liver disease (e.g., nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH)), a gastrointestinal disease, or a neurodegenerative disease, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disease.

[0246] In another aspect, the present disclosure relates to the use of a non-naturally occurring fusion molecule of the present disclosure for the preparation of a medicament for treatment, prophylaxis and/or prevention of GH deficient growth disorders in a subject in need thereof.

[0247] In another aspect, the present disclosure provides a method of treating a subject having a GH deficient growth disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said disorder is growth hormone deficiency (GHD), Turner syndrome (TS), Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene (SHOX) deficiency, chronic renal insufficiency, and idiopathic short stature short bowel syndrome, GH deficiency due to rare pituitary tumors or their treatment, and muscle-wasting disease associated with HIV/AIDS.

Polynucleotides Encoding Fusion molecules

[0248] In another aspect, the disclosure provides polynucleotides comprising a nucleotide sequence encoding the non-naturally occurring fusion molecules. These polynucleotides are useful, for example, for making the fusion molecules. In yet another aspect, the disclosure provides an expression system that comprises a recombinant polynucleotide sequence encoding a modified Cholix toxin, and a polylinker insertion site for a polynucleotide sequence encoding a biologically active cargo. The polylinker insertion site can be anywhere in the polynucleotide sequence so long as the polylinker insertion does not disrupt the receptor binding domain or the transcytosis domain of the modified Cholix toxin. In various embodiments, the expression system may comprise a polynucleotide sequence that encodes a cleavable linker so that cleavage at the cleavable linker separates a biologically active cargo encoded by a nucleic acid inserted into the polylinker insertion site from the remainder of the encoded fusion molecule. Thus, in embodiments where the polylinker insertion site is at an end of the encoded construct, the polynucleotide comprises one nucleotide sequence encoding a cleavable linker between the polylinker insertion site and the remainder of the polynucleotide. In embodiments where the polylinker insertion site is not at the end of the encoded construct, the polylinker insertion site can be flanked by nucleotide sequences that each encode a cleavable linker.

[0249] Various *in vitro* methods that can be used to prepare a polynucleotide encoding a modified Cholix toxin useful in the fusion molecules of the disclosure include, but are not limited to, reverse transcription, the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR) and the QP replicase amplification system (QB). Any such technique known by one of skill in the art to be useful in construction of recombinant nucleic acids can be used. For example, a polynucleotide encoding the protein or a portion thereof can be isolated by polymerase chain reaction of cDNA using primers based on the DNA sequence of a modified Cholix toxin or a nucleotide encoding, e.g., a receptor binding domain.

[0250] Guidance for using these cloning and *in vitro* amplification methodologies are described in, for example, U.S. Pat. No. 4,683,195; Mullis et al., 1987, Cold Spring Harbor Symp. Quant. Biol. 51:263; and Erlich, ed., 1989, PCR Technology, Stockton Press, NY. Polynucleotides encoding a fusion molecule or a portion thereof also can be isolated by screening genomic or cDNA libraries with probes selected from the sequences of the desired polynucleotide under stringent, moderately stringent, or highly stringent hybridization conditions.

[0251] Construction of nucleic acids encoding the fusion molecules of the disclosure can be facilitated by introducing an insertion site for a nucleic acid encoding the biologically active cargo into the construct. In various embodiments, an insertion site for the biologically active cargo can be introduced between the nucleotides encoding the cysteine residues of domain Ib of the modified Cholix toxin. In other embodiments, the insertion site can be introduced anywhere in the nucleic acid encoding the construct so long as the insertion does not disrupt the functional domains encoded thereby. In various embodiments, the insertion site can be in the ER retention domain.

[0252] Further, the polynucleotides can also encode a secretory sequence at the amino terminus of the encoded fusion molecule. Such constructs are useful for producing the fusion molecules in mammalian cells as they simplify isolation of the immunogen.

[0253] Furthermore, the polynucleotides of the disclosure also encompass derivative versions of polynucleotides encoding a fusion molecule. Such derivatives can be made by any method known by one of skill in the art without limitation. For example, derivatives can be made by site-specific mutagenesis, including substitution, insertion, or deletion of one, two, three, five, ten or more nucleotides, of polynucleotides encoding the fusion molecule. Alternatively, derivatives can be made by random mutagenesis. One method for randomly mutagenizing a nucleic acid comprises amplifying the nucleic acid in a PCR reaction in the presence of 0.1 mM $MnCl_2$ and unbalanced nucleotide concentrations. These conditions increase the inaccuracy incorporation rate of the polymerase used in the PCR reaction and result in random mutagenesis of the amplified nucleic acid.

[0254] Accordingly, in various embodiments, the disclosure provides a polynucleotide that encodes a fusion molecule. The fusion molecule comprises a modified Cholix toxin and a biologically active cargo to be delivered to a subject; and, optionally, a non-cleavable or cleavable linker. Cleavage at the cleavable linker can separate the biologically active cargo from the remainder of the fusion molecule. The cleavable linker can be cleaved by an enzyme that is present at a basolateral membrane of a polarized epithelial cell of the subject or in the plasma of the subject.

[0255] In various embodiments, the polynucleotide hybridizes under stringent hybridization conditions to any polynucleotide of this disclosure. In further embodiments, the polynucleotide hybridizes under stringent conditions to a nucleic acid that encodes any fusion molecule of the disclosure.

[0256] In still another aspect, the disclosure provides expression vectors for expressing the fusion molecules. Generally, expression vectors are recombinant polynucleotide molecules comprising expression control sequences operatively linked to a nucleotide sequence encoding a polypeptide. Expression vectors can readily be adapted for function in prokaryotes or eukaryotes by inclusion of appropriate promoters, replication sequences, selectable markers, etc. to result in stable transcription and translation or mRNA. Techniques for construction of expression vectors and expression of genes in cells comprising the expression vectors are well known in the art. See, e.g., Sambrook et al., 2001, Molecular Cloning--A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., and Ausubel et al., eds., Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY.

[0257] Useful promoters for use in expression vectors include, but are not limited to, a metallothionein promoter, a constitutive adenovirus major late promoter, a dexamethasone-inducible MMTV promoter, a SV40 promoter, a MRP pol III promoter, a constitutive MPSV promoter, a tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), and a constitutive CMV promoter.

[0258] The expression vectors should contain expression and replication signals compatible with the cell in which the fusion molecules are expressed. Expression vectors useful for expressing fusion molecules include viral vectors such as retroviruses, adenoviruses and adeno-associated viruses, plasmid vectors, cosmids, and the like. Viral and plasmid vectors are preferred for transfecting the expression vectors into mammalian cells. For example, the expression vector pcDNA1 (Invitrogen, San Diego, Calif.), in which the expression control sequence comprises the CMV promoter, provides good rates of transfection and expression into such cells.

[0259] The expression vectors can be introduced into the cell for expression of the fusion molecules by any method known to one of skill in the art without limitation. Such methods include, but are not limited to, e.g., direct uptake of the molecule by a cell from solution; facilitated uptake through lipofection using, e.g., liposomes or immunoliposomes; particle-mediated transfection; etc. See, e.g., U.S. Pat. No. 5,272,065; Goeddel et al., eds, 1990, Methods in Enzymology, vol. 185, Academic Press, Inc., CA; Krieger, 1990, Gene Transfer and Expression--A Laboratory Manual, Stockton Press, NY; Sambrook et al., 1989, Molecular Cloning--A Laboratory Manual, Cold Spring Harbor Laboratory, NY; and Ausubel et al., eds., Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY.

[0260] The expression vectors can also contain a purification moiety that simplifies isolation of the fusion molecule. For example, a polyhistidine moiety of, e.g., six histidine residues, can be incorporated at the amino terminal end of the protein. The polyhistidine moiety allows convenient isolation of the protein in a single step by nickel-chelate chromatography. In various embodiments, the purification moiety can be cleaved from the remainder of the fusion molecule following purification. In other embodiments, the moiety does not interfere with the function of the functional domains of the fusion molecule and thus need not be cleaved.

[0261] In yet another aspect, the disclosure provides a cell comprising an expression vector for expression of the fusion molecules, or portions thereof. The cell is selected for its ability to express high concentrations of the fusion molecule to facilitate purification of the protein. In various embodiments, the cell is a prokaryotic cell, for example, *E. coli*. As described in the examples, the fusion molecules are properly folded and comprise the appropriate disulfide linkages when expressed in *E. coli*.

[0262] In other embodiments, the cell is a eukaryotic cell. Useful eukaryotic cells include yeast and mammalian cells. Any mammalian cell known by one of skill in the art to be useful for expressing a recombinant polypeptide, without limitation, can be used to express the fusion molecules. For example, Chinese hamster ovary (CHO) cells can be used to express the fusion molecules.

[0263] The fusion molecules of the disclosure can be produced by recombination, as described below. However, the fusion molecules may also be produced by chemical synthesis using methods known to those of skill in the art.

[0264] Methods for expressing and purifying the fusion molecules of the disclosure are described extensively in the examples below. Generally, the methods rely on introduction of an expression vector encoding the fusion molecule to a cell that can express the fusion molecule from the vector. The fusion molecule can then be purified for administration to a subject.

Transcytosis Testing

[0265] The function of the transcytosis domain can be tested as a function of the fusion molecule's ability to pass through an epithelial membrane. Because transcytosis first requires binding to the cell, these assays can also be used to assess the function of the cell recognition domain.

[0266] The fusion molecule's transcytosis activity can be tested by any method known by one of skill in the art, without limitation. In various embodiments, transcytosis activity can be tested by assessing the ability of a fusion molecule to enter a non-polarized cell to which it binds. Without intending to be bound to any particular theory or mechanism of action, it is believed that the same property that allows a transcytosis domain to pass through a polarized epithelial cell also allows molecules bearing the transcytosis domain to enter non-polarized cells. Thus, the fusion molecule's ability to enter the cell can be assessed, for example, by detecting the physical presence of the construct in the interior of the cell. For example, the fusion molecule can be labeled with, for example, a fluorescent marker, and the fusion molecule exposed to the cell. Then, the cells can be washed, removing any fusion molecule that has not entered the cell, and the amount of label remaining determined. Detecting the label in this traction indicates that the fusion molecule has entered the cell.

[0267] In other embodiments, the fusion molecule's transcytosis ability can be tested by assessing the fusion molecule's ability to pass through a polarized epithelial cell. For example, the fusion molecule can be labeled with, for example, a fluorescent marker and contacted to the apical membranes of a layer of epithelial cells. Fluorescence detected on the basolateral side of the membrane formed by the epithelial cells indicates that the transcytosis domain is functioning properly.

Cleavable Linker Cleavage Testing

[0268] The function of the cleavable linker can generally be tested in a cleavage assay. Any suitable cleavage assay known by one of skill in the art, without limitation, can be used to test the cleavable linkers. Both cell-based and cell-free assays can be used to test the ability of an enzyme to cleave the cleavable linkers.

[0269] An exemplary cell-free assay for testing cleavage of cleavable linkers comprises preparing extracts of polarized epithelial cells and exposing a labeled fusion molecule bearing a cleavable linker to the fraction of the extract that corresponds to membrane-associated enzymes. In such assays, the label can be attached to either the biologically active cargo to be delivered or to the remainder of the fusion molecule. Among these enzymes are cleavage enzymes found near the basolateral membrane of a polarized epithelial cell, as described above. Cleavage can be detected, for example, by binding the fusion molecule with, for example, an antibody and washing off unbound molecules. If label is attached to the biologically active cargo to be delivered, then little or no label should be observed on the molecule bound to the antibodies. Alternatively, the binding agent used in the assay can be specific for the biologically active cargo, and the remainder of the construct can be labeled. In either case, cleavage can be assessed.

[0270] Cleavage can also be tested using cell-based assays that test cleavage by polarized epithelial cells assembled into membranes. For example, a labeled fusion molecule, or portion of a fusion molecule comprising the cleavable linker, can be contacted to either the apical or basolateral side of a monolayer of suitable epithelial cells, such as, for example, Coco-2 cells, under conditions that permit cleavage of the linker. Cleavage can be detected by detecting the presence or absence of the label using a reagent that specifically binds the fusion molecule, or portion thereof. For example, an antibody specific for the fusion molecule can be used to bind a fusion molecule comprising a label distal to the cleavable linker in relation to the portion of the fusion molecule bound by the antibody. Cleavage can then be assessed by detecting the presence of the label on molecules bound to the antibody. If cleavage has occurred, little or no label should be observed on the molecules bound to the antibody. By performing such experiments, enzymes that preferentially cleave at the basolateral membrane rather than the apical membrane can be identified, and, further, the ability of such enzymes to cleave the cleavable linker in a fusion molecule can be confirmed.

[0271] Further, cleavage can also be tested using a fluorescence reporter assay as described in U.S. Pat. No. 6,759,207. Briefly, in such assays, the fluorescence reporter is contacted to the basolateral side of a monolayer of suitable epithelial cells under conditions that allow the cleaving enzyme to cleave the reporter. Cleavage of the reporter changes the structure of the fluorescence reporter, changing it from a non-fluorescent configuration to a fluorescent configuration. The amount of fluorescence observed indicates the activity of the cleaving enzyme present at the basolateral membrane.

[0272] Further, cleavage can also be tested using an intra-molecularly quenched molecular probe, such as those described in U.S. Pat. No. 6,592,847. Such probes generally comprise a fluorescent moiety that emits photons when excited with light of appropriate wavelength and a quencher moiety that absorbs such photons when in close proximity to the fluorescent moiety. Cleavage of the probe separates the quenching moiety from the fluorescent moiety, such that fluorescence can be detected, thereby indicating that cleavage has occurred. Thus, such probes can be used to identify and assess cleavage by particular cleaving enzymes by contacting the basolateral side of a monolayer of suitable epithelial cells with the probe under conditions that allow the cleaving enzyme to cleave the probe. The amount of fluorescence observed indicates the activity of the cleaving enzyme being tested.

Exemplary Cholix Toxin-Biologically Active Cargo Fusion Molecules

[0273] Embodiments of the present disclosure include, but are not limited to, the fusion molecules described in Table 7.

Table 7

Modified Cholix Toxin (SEQ ID NO)	**Cleavable Linker (SEQ ID NO)**	**Biologically Active Cargo (SEQ ID NO)**
SEQ ID NO: 3	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 4	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 5	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 6	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 7	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 8	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95

SEQ ID NO: 9	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 10	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 11	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 12	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 13	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 14	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 15	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 16	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 17	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 18	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 19	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 20	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 21	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 22	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 23	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 24	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 25	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 26	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 27	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 28	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 29	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 30	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 31	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 32	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 33	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 34	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 35	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 36	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 37	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 38	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95

SEQ ID NO: 39	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 40	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 41	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 42	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 43	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 44	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 45	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 46	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 47	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 48	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 49	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 50	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 51	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 52	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 53	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 54	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 55	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 56	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 57	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 58	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 59	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 60	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 61	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 62	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 63	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 64	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 65	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 66	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 67	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 68	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95

SEQ ID NO: 69	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 70	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 71	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 72	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 73	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 74	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 75	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 76	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 77	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 78	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 79	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 80	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95
SEQ ID NO: 81	SEQ ID NOs: 96 – 121 No Linker	SEQ ID NOs: 82 - 95

[0274] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 82.

[0275] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 82.

[0276] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 82.

[0277] In various embodiments, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 114.

[0278] In various embodiments, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0279] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 88 and a light chain variable having the amino acid sequence of SEQ ID NO: 89.

[0280] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 90 and a light chain variable having the amino acid sequence of SEQ ID NO: 91

[0281] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 92.

[0282] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 93.

[0283] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 94.

[0284] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 52 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 95.

[0285] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 88 and a light chain variable having the amino acid sequence of SEQ ID NO: 89.

[0286] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 90 and a light chain variable having the amino acid sequence of SEQ ID NO: 91

[0287] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 92.

[0288] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 93.

[0289] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 94. In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 80 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 95.

[0290] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 88 and a light chain variable having the amino acid sequence of SEQ ID NO: 89.

[0291] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 90 and a light chain variable having the amino acid sequence of SEQ ID NO: 91

[0292] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 92.

[0293] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 93.

[0294] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 94.

[0295] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 70 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 95.

[0296] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 88 and a light chain variable having the amino acid sequence of SEQ ID NO: 89.

[0297] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo that is an antibody comprising a heavy chain variable having the amino acid sequence of SEQ ID NO: 90 and a light chain variable having the amino acid sequence of SEQ ID NO: 91.

[0298] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 92.

[0299] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 93.

[0300] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 94.

[0301] In various embodiments, the fusion molecule comprises a modified Cholix toxin having the amino acid sequence of SEQ ID NO: 42 and a biologically active cargo having the amino acid sequence of SEQ ID NO: 95.

[0302] The following examples merely illustrate the disclosure, and are not intended to limit the disclosure in any way.

Example 1

[0303] In this Example, the preparation of a non-naturally occurring fusion molecule as a single amino acid sequence and comprising a modified Cholix toxin sequence, a cleavable linker sequence, and a biologically active cargo, is generally described.

[0304] Seven exemplary fusion molecule expression vectors for delivering the polypeptides interleukin-10 (SEQ ID NO: 82), interleukin-19 (SEQ ID NO: 83), interleukin-20 (SEQ ID NO: 84), interleukin-22 (SEQ ID NO: 85), interleukin-24 (SEQ ID NO: 86), or interleukin-26 (SEQ ID NO: 87) are constructed as generally described below. First, the polypeptide genes are amplified by PCR, incorporating restriction enzymes pairs of NdeI and EcoRI, PstI and PstI, AgeI and EcoRI, or PstI and EcoRI sites at two ends of the PCR products. After restriction enzyme digestion, the PCR products are cloned into an appropriate plasmid for cellular expression, which is digested with the corresponding restriction enzyme pairs. The resulting constructs comprise a modified Cholix toxin comprising an amino acid sequence encoding amino acids 1-386 of SEQ ID NO: 1 (Cholix^{386}) and the respective polypeptides, and are also tagged with a 6-His motif at the N-terminus of the polypeptide to facilitate purification. The final plasmids are verified by restriction enzyme digestions and DNA sequencing.

[0305] Also prepared was a non-naturally occurring fusion molecule comprising a Cholix^{415} (SEQ ID NO: 52), a cleavable linker sequence having the amino acid sequence set forth in SEQ ID NO: 121, and a biologically active cargo that is a IL-10 polypeptide consisting of amino acid residues 20-178 of SEQ ID NO: 82 (this fusion molecule is designated “Cholix^{415}-

TEV-IL-10”, see FIG. 1 (SEQ ID NO: 122)), and a non-naturally occurring fusion molecule comprising a Cholix^{415} (SEQ ID NO: 52), a non-cleavable linker sequence having the amino acid sequence set forth in SEQ ID NO: 98, and a biologically active cargo that is a IL-10 polypeptide consisting of amino acid residues 20-178 of SEQ ID NO: 82 (this fusion molecule is designated “Cholix^{415}-$(G_4S)_3$-IL-10”, see FIG. 1 (SEQ ID NO: 123)).

[0306] Expression vectors comprising non-cleavable or cleavable linkers are constructed by introducing sequences encoding the appropriate amino acid sequence. To do so, oligonucleotides that encode sequences complementary to appropriate restriction sites and the amino acid sequence of the desired linker are synthesized, then ligated into an expression vector prepared as described above between the modified Cholix sequence and the polypeptide sequence.

[0307] In various embodiments, the fusion molecules are expressed as follows: *E. coli* BL21(DE3) pLysS competent cells (Novagen, Madison, Wis.) are transformed using a standard heat-shock method in the presence of the appropriate plasmid to generate fusion molecule expression cells, selected on ampicillin-containing media, and isolated and grown in Luria-Bertani broth (Difco; Becton Dickinson, Franklin Lakes, N.J.) with antibiotic, then induced for protein expression by the addition of 1 mM isopropyl-D-thiogalactopyranoside (IPTG) at OD 0.6. Two hours following IPTG induction, cells are harvested by centrifugation at 5,000 rpm for 10 min. Inclusion bodies are isolated following cell lysis and proteins are solubilized in the buffer containing 100 mM Tris-HCl (pH 8.0), 2 mM EDTA, 6 M guanidine HCl, and 65 mM dithiothreitol. Solubilized fusion molecule is refolded in the presence of 0.1 M Tris, pH=7.4, 500 mM L-arginine, 0.9 mM GSSG, 2 mM EDTA. The refolded proteins are purified by Q sepharose Ion Exchange and Superdex 200 Gel Filtration chromatography (Amersham Biosciences, Inc., Sweden). The purity of proteins is assessed by SDS-PAGE and analytic HPLC (Agilent, Inc. Palo Alto, Calif.).

[0308] FIG. 2 is a ribbon diagram representation of an exemplary fusion molecule, e.g., Cholix^{415}-TEV-IL-10 after refolding that would be driven by IL-10 dimerization. IL-10 dimerization is envisaged to result in purple Cholix^{415} /blue hIL-10 and orange Cholix^{415} /green organization shown.

[0309] Cholix^{415}-TEV-IL-10 and Cholix^{415}-$(G_4S)_3$-IL-10 were evaluated to verify the proper folding with regard to their anticipated molecular size. Following induction, expressed protein was collected from inclusion bodies. The extent of Cholix^{415}-TEV-IL-10 (depicted as “C” on the gel) expression and Cholix^{415}-$(G_4S)_3$-IL-10 (depicted as “N” on the gel) expression in inclusion bodies showed an apparent molecular weight of ~ 66 kDa that was comparable to the calculated mass of 66380.78 and 65958.25 Daltons, respectively. See FIG. 3. The lack of these proteins in supernatant media following inclusion body removal for the TEV linker (Cs) and non-TEV linker (Ns) are shown to demonstrate the extent and specificity of chimera induction. SeeBlue® Plus2 Prestained MW standards are shown.

Example 2

[0310] This example describes *in vitro* methods to verify the proper folding of the fusion molecules with regard to their ability to carry a biologically active cargo across an intact epithelium.

[0311] The J774 mouse macrophage cell line can be used as an IL-10 responsive cell line (O'Farrell AM, et al., EMBO J, 17(4):1006-18, 1998). IL-10 naturally forms a dimer that is required for its optimal activity. Cholix^{415}-$(G_4S)_3$-IL-10 expressed by *E coli* was collected from inclusion bodies and folded using a disulphide shuffle exchange buffer system. The resulting material was purified by ion exchange and size exclusion chromatography that resulted in the isolation of a protein of ~130 kDa, the anticipated size of an IL-10 dimer conjoined to two Cholix^{415} molecules (hereinafter “dimer Cholix^{415}-IL-10” fusion molecule). The preparation had a protein purity of ~ 85-90% based upon SDS PAGE. Cultures of the J774.2 cell line were treated for 48 h with dimer Cholix^{415}-IL-10 fusion molecule at concentrations of 25nM and 250 nM. Compared to untreated matched cells, dimer Cholix^{415}-IL-10 fusion molecule produced a dose-dependent decrease in cell number as assessed by flow cytometry of live/dead cells (see FIG. 4). Values represent n=4 ± standard deviation.

[0312] Alternatively, one could co-culture the IL-10 responsive cells in the basal compartment of the cell monolayers used for apical to basolateral transcytosis (Rubas W, et al., Pharm Res. 13(1):23-6, 1996).

Example 3

[0313] In this example, dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule was evaluated for its effect on the barrier properties of Caco-2 cell monolayers *in vitro*. Caco-2 cells (a human colon cancer derived cell line) with media from the basolateral compartment being sampled periodically for several hours (Rubas W, et al., J Pharm Sci., 85(2):165-9, 1996). Caco-2 (ATCC HTB-37™) cells are maintained in 5% CO_2 at 37℃ in complete media: Dulbecco’s modified Eagle’s medium F12 (DMEM F12) supplemented with 10% fetal bovine serum, 2.5 mM glutamine, 100 U of penicillin/ml, and 100 µg of streptomycin/ml (Gibco BRL, Grand Island, N.Y.). Cells are fed every 2 to 3 days with this media (designated complete medium) and passaged every 5 to 7 days. For assays, cells are seeded into 24- or 96-well plates and grown to confluence.

[0314] Established Caco-2 monolayers used for these studies had transepithelial electrical resistance (TER) values of between ~450-600 Ω·cm^2 (579 Ω·cm^2 average) as measured using a chopstick Millicell-ERS® voltmeter (Millipore). Fluorescein-labeled 70 kDa dextran and varying concentrations (4.7 nM, 23.6 nM and 236 nM) of dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule were added to the apical surface of these monolayers and the cumulative amount of florescence detected in the basal compartment monitored over time by collecting 150 µL volumes with replacement. As depicted in FIG. 5 and FIG. 6, in the absence of Caco-2 cells on the filter support, the dextran rapidly moved from the apical to basal compartment. By comparison, the extent of 70 kDa dextran transport was much less across Caco-2 monolayers and the various dimer Cholix^{415}-IL-10 fusion molecules failed to have any dose-dependent effect on the extent of 70 kDa dextran transport across these Caco-2 monolayers and were not strikingly different from results obtained with Caco-2 monolayers not exposed to dimer Cholix^{415}-IL-10 fusion molecules. The dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule does not overtly affect the barrier properties of Caco-2 cell monolayers *in vitro*.

Example 4

[0315] In this example, an ELISA assay is performed to evaluate the ability of the dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule to move across Caco-2 cell monolayers. A549 (ATCC CCL-185™), L929 (ATCC CRL-2148™), and Caco-2 (ATCC HTB-37™) cells are maintained in 5% CO_2 at 37°C in complete media: Dulbecco's modified Eagle's medium F12 (DMEM F12) supplemented with 10% fetal bovine serum, 2.5 mM glutamine, 100 U of penicillin/ml, and 100 µg of streptomycin/ml (Gibco BRL, Grand Island, N.Y.). Cells are fed every 2 to 3 days with this media (designated complete medium) and passaged every 5 to 7 days. For assays, cells are seeded into 24- or 96-well plates and grown to confluence.

[0316] Caco-2 cells are grown as confluent monolayers on collagen-coated 0.4-µm pore size polycarbonate membrane transwell supports (Corning-Costar, Cambridge, MA) and used 18-25 days after attaining a trans-epithelial electrical resistance (TER) of >250 Ω·cm2 as measured using a chopstick Millicell-ERS® voltmeter (Millipore). Apical to basolateral (A→B) transport of dimer Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule across these monolayer is determined by measuring the amount of transported protein 4 hr after a 4.7 nM, 23.6 nM and 236 nM application at 37°C. TER measurements and the extent of 10 kDa fluorescent dextran (measured using an HPLC size exclusion protocol) are used to verify monolayer barrier properties during the course of the study. The extent of Cholix transport is determined by titration of collected media in the cell-based cytotoxicity assay. Transported dimer $Cholix^{415}$-$(G_4S)_3$-IL-10 fusion molecule is measured by enzyme linked immunosorbant assay (ELISA) using anti-IL-10 antibody for capture and the polyclonal sera to Cholix for detection. As depicted in FIG. 7 (A and B), dimer $Cholix^{415}$-$(G_4S)_3$-IL-10 fusion molecule moves across Caco-2 cell monolayers.

Example 5

[0317] In this Example, the preparation of a non-naturally occurring fusion molecule that lacks a cleavable sequence is described. These fusions molecules are designed to specifically target the submucosal/GI space and limit the actions of the biologically active cargo to that space.

[0318] A plasmid construct is prepared encoding the non-toxic mutant form of the Cholix toxin, Cholix toxin ΔE581 (SEQ ID NO: 81). Protein expression is achieved using *E. coli* DH5α cells (Invitrogen, Carlsbad, CA) following transformation by heat-shock (1 min at 42 °C) with the appropriate plasmid. Transformed cells, selected on antibiotic-containing media, are isolated and grown in Luria-Bertani broth (Difco). Protein expression is induced by addition of 1 mM isopropyl-D-thiogalactopyranoside (IPTG). Two hours following IPTG induction, cells are harvested by centrifugation at 5,000 x*g* for 10 min at 4°C. Inclusion bodies are isolated following cell lysis and proteins are solubilized in 6 M guanidine HCl and 2 mM EDTA (pH 8.0) plus 65 mM dithiothreitol. Following refolding and purification, proteins are stored at ~5 ml/ml in PBS (pH 7.4) lacking Ca^{2+} and Mg^{2+} at -80°C. All proteins used in these studies are confirmed to be at >90% purity based upon size exclusion chromatography.

[0319] The Cholix toxin ΔE581 protein is then modified at its C-terminus to allow direct chemical coupling through a free sulfhydryl residue located near the C-terminus of the protein. The C-terminal modification includes a cysteine-constrained loop harboring the consensus cleavage sequence for the highly selective protease from the tobacco etch virus (TEV), a second cysteine, and a hexa-histadine (His_6) tag. The second Cys is included to form a disulphide bridge with the Cys ultimately used for coupling. Adding the His_6 sequence to the protein simplifies the purification and the TEV cleavage sequence provides a mechanism to selectively remove the terminal Cys residue following mild reduction. TEV cleavage and mild reduction with 0.1 mM dithiotheitol following expression and isolation of the ntCholix constructs allows for the direct chemical coupling of a biologically active cargo via a maleimide-based reaction as a generic mechanism of cargo attachment. Following TEV protease cleavage, reduction, and cargo coupling through a maleimide reaction with the free sulfhydryl, removal of the freed C-terminal sequence was achieved by a second Ni^{2+} column chromatography step.

Example 6

[0320] Trans-epithelial transport of Cholix toxin ΔE581-cargo is assessed using Caco-2 monolayers *in vitro*. Caco-2 cells (passage number 25-35) are grown to confluent monolayers as previously described; Rubas, W. et al., *Pharm Res,* 10:113-118 (1993). Briefly, cells are maintained at 37℃ in DMEM/high growth media enriched with 2 mM L-glutamine, 10% fetal bovine serum, and 100 Units of penicillin/streptomycin in an atmosphere of 5% CO_2 and 90% humidity. Cells are passaged every week at a split ratio of 1:3 in $75cm^2$ flasks and seeded onto prewetted and collagen-coated permeable (0.4 µm pore size) polycarbonate (Transwell^{TM}) filter supports from Corning Costar (Cambridge, MA) at a density of 63,000 cells/cm^2. Growth media is replaced every other day. Confluent monolayers, determined by the acquisition of significant trans-epithelial resistance (TEER) determine using an volt-ohm-meter (World Precision Instruments, Sarasota, FL), are used 20-26 days post seeding.

[0321] Trans-epithelial transport flux rates are measured *in vitro* in the apical (Ap) to basolateral (Bl) and the Bl to Ap directions using polarized monolayers of Caco-2 cells to describe mucosal to serosal and serosal to mucosal flux events, respectively. Just prior to initiation of a transport study, the transepithelial resistance (TEER) of each filter is measured; monolayers TEER reading of <200 Ω·cm2 are excluded from the study. Ap and Bl media is removed from included monolayers and these surfaces are washed once with phosphate buffered saline (PBS). One set of monolayers then receives an Ap (donor) application of 100 µL PBS containing 10 µg Cholix toxin ΔE581-cargo and 10 µg TRITC-Dextran or 10 µg BSA-cargo and 10 µg TRITC-Dextran. Receiver (Bl) compartments then receive 500 µL PBS to set the T_0 for the transport study. Both donor and receiver compartments are sampled after 4 hr of incubation at 37℃ to determine the amount of material transported across the monolayer and the amount retained at the apical surface, respectively.

Example 7

[0322] This example describes the preparation and expression in *E. coli.* of a fusion molecule comprising a modified Cholix toxin comprising a sequence encoding amino acids 1-415 of SEQ ID NO: 1 directly fused at its C-terminus to an IL-10 polypeptide (referred to as a "Cholix^{415}-IL-10 fusion molecule"). Protein expression is achieved using *E. coli* DH5α cells (Invitrogen, Carlsbad, CA) following transformation by heat-shock (1 min at 42 °C) with the appropriate plasmid. Transformed cells, selected on antibiotic-containing media, are isolated and grown in Luria-Bertani broth (Difco). Protein expression is induced by addition of 1 mM isopropyl-D-thiogalactopyranoside (IPTG). Two hours following IPTG induction, cells are harvested by centrifugation at 5,000 x*g* for 10 min at 4°C. Inclusion bodies are isolated following cell lysis and proteins are solubilized in 6 M guanidine HCl and 2 mM EDTA (pH 8.0) plus 65 mM dithiothreitol. Following refolding and purification, proteins are stored at ~5 ml/ml in PBS (pH 7.4) lacking Ca^{2+} and Mg^{2+} at -80°C. All proteins used in these studies were confirmed to be at >90% purity based upon size exclusion chromatography.

[0323] Polystyrene beads (10 nm diameter) containing a covalently integrated red fluorescent dye with excitation/emission properties of 468/508 nm and having aldehyde surface functional groups (XPR-582) are obtained from Duke Scientific (Palo Alto, CA). One hundred μl of XPR-582 beads (at 2% solids) are mixed with approximately 2.5 nmoles IL-10 or Cholix^{415}-IL-10 fusion molecule in a final volume of 200 μl neutral (pH 7.0) phosphate buffered saline (PBS). After 2 hr of gentle rocking at room temperature, 20 μl of a 2 mg/ml solution of bovine serum albumin (BSA; Sigma, St. Louis, MO) in PBS is added. Preparations are then dialyzed by three cycles of dilution with PBS and concentration using a 100,000 molecular weight cutoff Microcon filter device from Millipore (Bedford, MA). Final preparations of coated beads were at 1% solids.

Example 8

[0324] In this Example, non-naturally occurring isolated fusion molecules comprising the modified Cholix toxin sequence of SEQ ID NO: 52 (Cholix^{415}), a cleavable linker sequence (SEQ ID NO: 121) or a non-cleavable linker (SEQ ID NO: 98), and a biologically active cargo that is a TNFSF inhibitor, are prepared as described in Example 1, and evaluated as described in the Examples above to confirm proper folding, proper size,

[0325] Six exemplary fusion molecule expression vectors (3 for each linker) were prepared to test for the ability of the fusion molecules to transport apical to basal across epithelial cells a TNFSF inhibitor selected from: 1) a TNF inhibitor that is an antibody comprising the heavy chain variable region and light chain variable region sequences of SEQ ID NO: 88 and 89; 2) a TNF inhibitor that is an antibody comprising the heavy chain variable region and light chain variable region sequences of SEQ ID NO: 90 and 91; and 3) a TNFSF inhibitor that is a dimer of a soluble human TNFR-p75 with the Fc portion of IgG comprising the sequence of SEQ ID NO: 92.

Example 9

[0326] In this Example, non-naturally occurring isolated fusion molecules comprising the modified Cholix toxin sequence of SEQ ID NO: 52 (Cholix^{415}), a cleavable linker sequence (SEQ ID NO: 121) or a non-cleavable linker (SEQ ID NO: 98), and a biologically active cargo that is a glucose-lowering agent, are prepared as described in Example 1, and evaluated as described in the Examples above to confirm proper folding, proper size,

[0327] Four exemplary fusion molecule expression vectors (2 for each linker) were prepared to test for the ability of the fusion molecules to transport apical to basal across epithelial cells a glucose-lowering agent selected from: 1) a GLP-1 agonist comprising the sequence of SEQ ID NO: 93; and 2) a GLP-1 agonist comprising the sequence of SEQ ID NO: 94.

Example 10

[0328] In this Example, non-naturally occurring isolated fusion molecules comprising the modified Cholix toxin sequence of SEQ ID NO: 52 (Cholix^{415}), a cleavable linker sequence (SEQ ID NO: 121) or a non-cleavable linker (SEQ ID NO: 98), and a biologically active cargo that is a human growth hormone, are prepared as described in Example 1, and evaluated as described in the Examples above to confirm proper folding, proper size,

[0329] Two exemplary fusion molecule expression vectors (one for each linker) were prepared to test for the ability of the fusion molecules to transport apical to basal across epithelial cells a human growth hormone comprising the sequence of SEQ ID NO: 95.

Example 11

[0330] This example describes histological detection in tissues of a representative biologically active cargo of the fusion molecules prepared in Example 1. Following administration of a fusion molecule, animals are euthanized by CO_2 asphyxiation and exsanguinated by cardiac puncture. Specific tissues (lymph nodes, trachea, brain, spleen liver, GI tract) are removed, briefly rinsed in PBS to remove any residual blood and frozen in OCT. Sections (5 microns thick) are placed onto slides. Slides are fixed in acetone for 10 min and rinsed with PBS. Slides are incubated with 3% peroxidase for 5 min. Slides are then blocked with protein for an additional 5 min. Primary antibody to the respective biologically active cargo is incubated onto slides for 30 min at a 1:100 dilution followed by PBS washes. Biotin-labeled secondary antibody is then incubated for approximately 15 minutes followed by PBS washes. Streptavidin HRP label is incubated onto slides for 15 min followed by PBS washes. HRP Chromagen is applied for 5 min followed by several rinses in distilled H_20. Finally, the slides are counterstained with hematoxylin for 1 min, coverslipped, and examined for the presence of the biologically active cargo.

[0331] The fusion molecules of the disclosure offer several advantages over conventional techniques for local or systemic delivery of macromolecules to a subject. Foremost among such advantages is the ability to deliver the biologically active cargo to a subject without using a needle to puncture the skin of the subject. Many subjects require repeated, regular doses of macromolecules. For example, diabetics must inject insulin several times per day to control blood sugar concentrations. Such subjects' quality of life would be greatly improved if the delivery of a macromolecule could be accomplished without injection, by avoiding pain or potential complications associated therewith.

[0332] In addition, coupling of the biologically active cargo to the remainder of the fusion molecule with a linker that is cleaved by an enzyme present at a basolateral membrane of an epithelial cell allows the biologically active cargo to be liberated from the fusion molecule and released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane. Such liberation reduces the probability of induction of an immune response against the biologically active cargo. It also allows the biologically active cargo to interact with its target free from the remainder of the fusion molecule.

[0333] In addition, the non-naturally occurring fusion molecules which lack a cleavable linker can be advantageous in that the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of, e.g., immune cells that also express the receptor for the biologically active cargo (but in considerably lower quantity) can allow for greater exposure of the biologically active cargo at the surface of the targeted cells, and provide a synergistic effect via the binding of the Cholix to its receptor and, e.g., binding of IL-10 to the IL-10R.

[0334] Moreover, once transported across the GI epithelium, the fusion molecules of the disclosure will exhibit extended half-life in serum, that is, the biologically active cargo of the fusion molecules will exhibit an extended serum half-life compared to the biologically active cargo in its non-fused state, and oral administration of the fusion molecule can deliver a higher effective concentration of the delivered biologically active cargo to the liver of the subject than is observed in the subject's plasma.

[0335] Furthermore, the embodiments of the fusion molecules can be constructed and expressed in recombinant systems. Recombinant technology allows one to make a fusion molecule having an insertion site designed for introduction of any suitable biologically active cargo. Such insertion sites allow the skilled artisan to quickly and easily produce fusion molecules for delivery of new biologically active cargo, should the need to do so arise.

[0336] All of the articles and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the articles and methods of this disclosure have been described in terms of embodiments, it will be apparent to those of skill in the art that variations may be applied to the articles and methods without departing from the spirit and scope of the disclosure. All such variations and equivalents apparent to those skilled in the art, whether now existing or later developed, are deemed to be within the spirit and scope of the disclosure as defined by the appended claims. All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the disclosure pertains. All patents, patent applications, and publications are herein incorporated by reference in their entirety for all purposes and to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety for any and all purposes. The disclosure illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising”, “consisting essentially of”, and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by the appended claims.

Sequence Listings

[0337] The amino acid sequences listed in the accompanying sequence listing are shown using standard three letter code for amino acids, as defined in 37 C.F.R. 1.822.

[0338] SEQ ID NO: 1 is the 634 amino acid sequence of mature *Vibrio cholera* Cholix toxin.

[0339] SEQ ID NO: 2 is a nucleic acid sequence encoding the 634 amino acid sequence mature *V. cholera* Cholix toxin.

[0340] SEQ ID NOs: 3-80 are the amino acid sequences of various truncated Cholix toxins derived from the mature Cholix toxin sequence set forth in SEQ ID NO: 1.

[0341] SEQ ID NO: 81 is the amino acid sequence of a mutated Cholix toxin wherein the amino acid residue E581 of SEQ ID NO: 1 has been deleted.

[0342] SEQ ID NO: 82 is the amino acid sequence of human interleukin-10 (IL-10).

[0343] SEQ ID NO: 83 is the amino acid sequence of human interleukin-19 (IL-19).

[0344] SEQ ID NO: 84 is the amino acid sequence of human interleukin-20 (IL-20).

[0345] SEQ ID NO: 85 is the amino acid sequence of human interleukin-22 (IL-22).

[0346] SEQ ID NO: 86 is the amino acid sequence of human interleukin-24 (IL-24).

[0347] SEQ ID NO: 87 is the amino acid sequence of human interleukin-26 (IL-26).

[0348] SEQ ID NO: 88 - heavy chain variable region sequence for an anti-TNF-alpha antibody.

[0349] SEQ ID NO: 89 - light chain variable region sequence for an anti-TNF-alpha antibody.

[0350] SEQ ID NO: 90 - heavy chain variable region sequence for an anti-TNF-alpha antibody.

[0351] SEQ ID NO: 91 - light chain variable region sequence for an anti-TNF-alpha antibody.

[0352] SEQ ID NO: 92 - amino acid sequence of human TNFR-p75-Fc dimeric fusion protein.

[0353] SEQ ID NO: 93 – GLP-1 agonist peptide amino acid sequence (exenatide)

[0354] SEQ ID NO: 94 - GLP-1 agonist peptide amino acid sequence (Liraglutide)

[0355] SEQ ID NO: 95 - amino acid sequence of human growth hormone (somatotropin)

[0356] SEQ ID NOs: 96-121 are the amino acid sequences of various peptide linkers

[0357] SEQ ID NO: 122 is the amino acid sequence of a Cholix^{415}-TEV-IL-10 fusion molecule.

[0358] SEQ ID NO: 123 is the amino acid sequence of a Cholix^{415}-$(G_4S)_3$-IL-10 fusion molecule.

SEQUENCE LISTINGS

SEQ ID NO: 1 - mature *Vibrio cholera* Cholix toxin amino acid sequence

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYLPENRAVITPQGVTNWTYQELEATHQAL
TREGYVFVGYHGTNHVAAQTIVNRIAPVPRGNNTENEEKWGGLYVATHAEVAHGYARIKEGTG
EYGLPTRAERDARGVMLRVYIPRASLERFYRTNTPLENAEEHITQVIGHSLPLRNEAFTGPESA
GGEDETVIGWDMAIHAVAIPSTIPGNAYEELAIDEEAVAKEQSISTKPPYKERKDELK

SEQ ID NO: 2 - nucleic acid sequence encoding the mature *V. cholera* Cholix toxin

ATGGTCGAAGAAGCTTTAAACATCTTTGATGAATGCCGTTCGCCATGTTCGTTGACCCCGGAACCGG
GTAAGCCGATTCAATCAAAACTGTCTATCCCTAGTGATGTTGTTCTGGATGAAGGTGTTCTGTATTAC
TCGATGACGATTAATGATGAGCAGAATGATATTAAGGATGAGGACAAAGGCGAGTCCATTATCACTAT
TGGTGAATTTGCCACAGTACGCGCGACTAGACATTATGTTAATCAAGATGCGCCTTTTGGTGTCATCC
ATTTAGATATTACGACAGAAAATGGTACAAAAACGTACTCTTATAACCGCAAAGAGGGTGAATTTGCA
ATCAATTGGTTAGTGCCTATTGGTGAAGATTCTCCTGCAAGCATCAAAATCTCCGTTGATGAGCTCGA
TCAGCAACGCAATATCATCGAGGTGCCTAAACTGTATAGTATTGATCTCGATAACCAAACGTTAGAGC
AGTGGAAAACCCAAGGTAATGTTTCTTTTTCGGTAACGCGTCCTGAACATAATATCGCTATCTCTTGG
CCAAGCGTGAGTTACAAAGCAGCGCAGAAAGAGGGTTCACGCCATAAGCGTTGGGCTCATTGGCAT
ACAGGCTTAGCACTGTGTTGGCTTGTGCCAATGGATGCTATCTATAACTATATCACCCAGCAAAATTG
TACTTTAGGGGATAATTGGTTTGGTGGCTCTTATGAGACTGTTGCAGGCACTCCGAAGGTGATTACG
GTTAAGCAAGGGATTGAACAAAAGCCAGTTGAGCAGCGCATCCATTTCTCCAAGGGGAATGCGATGA
GCGCACTTGCTGCTCATCGCGTCTGTGGTGTGCCATTAGAAACTTTGGCGCGCAGTCGCAAACCTC
GTGATCTGACGGATGATTTATCATGTGCCTATCAAGCGCAGAATATCGTGAGTTTATTTGTCGCGACG
CGTATCCTGTTCTCTCATCTGGATAGCGTATTTACTCTGAATCTTGACGAACAAGAACCAGAGGTGGC
TGAACGTCTAAGTGATCTTCGCCGTATCAATGAAAATAACCCGGGCATGGTTACACAGGTTTTAACC
GTTGCTCGTCAGATCTATAACGATTATGTCACTCACCATCCGGGCTTAACTCCTGAGCAAACCAGTG
CGGGTGCACAAGCTGCCGATATCCTCTCTTTATTTTGCCCAGATGCTGATAAGTCTTGTGTGGCTTCA
AACAACGATCAAGCCAATATCAACATCGAGTCTCGTTCTGGCCGTTCATATTTGCCTGAAAACCGTGC
GGTAATCACCCCTCAAGGCGTCACAAATTGGACTTACCAGGAACTCGAAGCAACACATCAAGCTCTG
ACTCGTGAGGGTTATGTGTTCGTGGGTTACCATGGTACGAATCATGTCGCTGCGCAAACCATCGTGA
ATCGCATTGCCCCTGTTCCGCGCGGCAACAACACTGAAAACGAGGAAAAGTGGGGCGGGTTATATG
TTGCAACTCACGCTGAAGTTGCCCATGGTTATGCTCGCATCAAAGAAGGGACAGGGGAGTATGGCC

TTCCGACCCGTGCTGAGCGCGACGCTCGTGGGGTAATGCTGCGCGTGTATATCCCTCGTGCTTCAT
TAGAACGTTTTTATCGCACGAATACACCTTTGGAAAATGCTGAGGAGCATATCACGCAAGTGATTGGT
CATTCTTTGCCATTACGCAATGAAGCATTTACTGGTCCAGAAAGTGCGGGCGGGGAAGACGAAACTG
TCATTGGCTGGGATATGGCGATTCATGCAGTTGCGATCCCTTCGACTATCCCAGGGAACGCTTACGA
AGAATTGGCGATTGATGAGGAGGCTGTTGCAAAAGAGCAATCGATTAGCACAAAACCACCTTATAAA
GAGCGCAAAGATGAACTTAAG

SEQ ID NO: 3 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{386}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
A

SEQ ID NO: 4 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{385}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ

SEQ ID NO: 5 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{384}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGA

SEQ ID NO: 6 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{383}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAG

SEQ ID NO: 7 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{382}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSA

SEQ ID NO: 8 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{381}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTS

SEQ ID NO: 9 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{380}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQT

SEQ ID NO: 10 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{379}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQ

SEQ ID NO: 11 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{378}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPE

SEQ ID NO: 12 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{377}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTP

SEQ ID NO: 13 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{376}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLT

SEQ ID NO: 14 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{375}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGL

SEQ ID NO: 15 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{374}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPG

SEQ ID NO: 16 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{373}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHP

SEQ ID NO: 17 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{372}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHH

SEQ ID NO: 18 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{371}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTH

SEQ ID NO: 19 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{370}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVT

SEQ ID NO: 20 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{369}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYV

SEQ ID NO: 21 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{368}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDY

SEQ ID NO: 22 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{367}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYND

SEQ ID NO: 23 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{366}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYN

SEQ ID NO: 24 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{365}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIY

SEQ ID NO: 25 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{364}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQI

SEQ ID NO: 26 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{363}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQ

SEQ ID NO: 27 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{362}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVAR

SEQ ID NO: 28 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{361}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVA

SEQ ID NO: 29 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{360}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTV

SEQ ID NO: 30 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{359}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLT

SEQ ID NO: 31 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{358}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVL

SEQ ID NO: 32 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{357}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQV

SEQ ID NO: 33 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{356}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQ

SEQ ID NO: 34 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{355}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVT

SEQ ID NO: 35 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{354}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMV

SEQ ID NO: 36 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{353}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGM

SEQ ID NO: 37 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{352}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPG

SEQ ID NO: 38 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{351}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNP

SEQ ID NO: 39 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{350}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENN

SEQ ID NO: 40 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{349}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINEN

SEQ ID NO: 41 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{348}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINE

SEQ ID NO: 42 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{425}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYLPEN

SEQ ID NO: 43 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{424}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYLPE

SEQ ID NO: 44 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{423}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYLP

SEQ ID NO: 45 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{422}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYL

SEQ ID NO: 46 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{421}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSY

SEQ ID NO: 47 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{420}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRS

SEQ ID NO: 48 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{419}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGR

SEQ ID NO: 49 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{418}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSG

SEQ ID NO: 50 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{417}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRS

SEQ ID NO: 51 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{416}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESR

SEQ ID NO: 52 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{415}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIES

SEQ ID NO: 53 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{414}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIE

SEQ ID NO: 54 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{413}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK

RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINI

SEQ ID NO: 55 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{412}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANIN

SEQ ID NO: 56 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{411}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANI

SEQ ID NO: 57 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{410}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQAN

SEQ ID NO: 58 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{409}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQA

SEQ ID NO: 59 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{408}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD

SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQ

SEQ ID NO: 60 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{407}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNND

SEQ ID NO: 61 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{406}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNN

SEQ ID NO: 62 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{405}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASN

SEQ ID NO: 63 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{404}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVAS

SEQ ID NO: 64 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{403}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVA

SEQ ID NO: 65 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{402}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCV

SEQ ID NO: 66 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{401}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSC

SEQ ID NO: 67 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{400}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKS

SEQ ID NO: 68 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{399}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADK

SEQ ID NO: 69 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{398}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDAD

SEQ ID NO: 70 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{397}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD

ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDA

SEQ ID NO: 71 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{396}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPD

SEQ ID NO: 72 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{395}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCP

SEQ ID NO: 73 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{394}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFC

SEQ ID NO: 74 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{393}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLF

SEQ ID NO: 75 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{392}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD

SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSL

SEQ ID NO: 76 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{391}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILS

SEQ ID NO: 77 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{390}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADIL

SEQ ID NO: 78 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{389}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADI

SEQ ID NO: 79 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{388}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AAD

SEQ ID NO: 80 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix^{387}

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AA

SEQ ID NO: 81 - modified *Vibrio cholera* Cholix toxin amino acid sequence Cholix Δ581

VEDELNIFDECRSPCSLTPEPGKPIQSKLSIPSDVVLDEGVLYYSMTINDEQNDIKDEDKGESIITI
GEFATVRATRHYVNQDAPFGVIHLDITTENGTKTYSYNRKEGEFAINWLVPIGEDSPASIKISVD
ELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKEGSRHK
RWAHWHTGLALCWLVPMDAIYNYITQQNCTLGDNWFGGSYETVAGTPKVITVKQGIEQKPVE
QRIHFSKGNAMSALAAHRVCGVPLETLARSRKPRDLTDDLSCAYQAQNIVSLFVATRILFSHLD
SVFTLNLDEQEPEVAERLSDLRRINENNPGMVTQVLTVARQIYNDYVTHHPGLTPEQTSAGAQ
AADILSLFCPDADKSCVASNNDQANINIESRSGRSYLPENRAVITPQGVTNWTYQELEATHQAL
TREGYVFVGYHGTNHVAAQTIVNRIAPVPRGNNTENEEKWGGLYVATHAEVAHGYARIKEGTG
EYGLPTRAERDARGVMLRVYIPRASLERFYRTNTPLENAEEHITQVIGHSLPLRNEAFTGPESA
GGEDTVIGWDMAIHAVAIPSTIPGNAYEELAIDEEAVAKEQSISTKPPYKERKDELK

SEQ ID NO: 82 - human interleukin-10 amino acid sequence

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLD
NLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRC
HRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN

SEQ ID NO: 83 - human interleukin-19 amino acid sequence

MKLQCVSLWLLGTILILCSVDNHGLRRCLISTDMHHIEESFQEIKRAIQAKDTFPNVTILSTLETLQ
IIKPLDVCCVTKNLLAFYVDRVFKDHQEPNPKILRKISSIANSFLYMQKTLRQCQEQRQCHCRQE
ATNATRVI HDNYDQLEVHAAAIKSLGELDVFLAWINKNHEVMSSA

SEQ ID NO: 84 - human interleukin-20 amino acid sequence

MKASSLAFSLLSAAFYLLWTPSTGLKTLNLGSCVIATNLQEIRNGFSEIRGSVQAKDGNIDIRILR
RTESLQDTKPANRCCLLRHLLRLYLDRVFKNYQTPDHYTLRKISSLANSFLTIKKDLRLCHAHMT
CHCGEEAMK KYSQILSHFEKLEPQAAVVKALGELDILLQWMEETE

SEQ ID NO: 85 - human interleukin-22 amino acid sequence

MAALQKSVSSFLMGTLATSCLLLLALLVQGGAAAPISSHCRLDKSNFQQPYITNRTFMLAKEAS
LADNNTDVRLIGEKLFHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQEVVPFLARLSN
RLSTCHIEGDDLHIQRNVQKLKDTVKKLGESGEIKAIGELDLLFMSLRNACI

SEQ ID NO: 86 - human interleukin-24 amino acid sequence

MNFQQRLQSLWTLASRPFCPPLLATASQMQMVVLPCLGFTLLLWSQVSGAQGQEFHFGPCQ
VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTVFKN
YHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQLDVEAALT
KALGEVDILLTWMQKFYKL

SEQ ID NO: 87 - human interleukin-26 amino acid sequence

MLVNFILRCGLLLVTLSLAIAKHKQSSFTKSCYPRGTLSQAVDALYIKAAWLKATIPEDRIKNIRLL
KKKTKKQFMKNCQFQEQLLSFFMEDVFGQLQLQGCKKIRFVEDFHSLRQKLSHCISCASSARE
MKSITRMKRIFYRIGNKGIYKAISELDILLSWIKKLLESSQ

SEQ ID NO: 88 - heavy chain variable region sequence for an anti-TNF-alpha antibody

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYA
DSVERGFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST

KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

SEQ ID NO: 89 - light chain variable region sequence for an anti-TNF-alpha antibody

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSG
SGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 90 - heavy chain variable region sequence for an anti-TNF-alpha antibody

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAIISFDGSNKSSAD
SVKGRFTYSRRNSKNALFLQMNSLRAEDTAVFYCARDRGVSAGGNYYYYGMDVWGQGTTVT
VSS

SEQ ID NO: 91 - light chain variable region sequence for an anti-TNF-alpha antibody

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSG
SGSGTRFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIL

SEQ ID NO: 92 - amino acid sequence of human TNFR-p75-Fc dimeric fusion protein

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRP
GFGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 93 – GLP-1 agonist peptide amino acid sequence (exenatide)

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS

SEQ ID NO: 94 - GLP-1 agonist peptide amino acid sequence (Liraglutide)

HAEGTFTSDVSSYLEGQAAKEEFIIAWLVKGRG

SEQ ID NO: 95 - amino acid sequence of human growth hormone (somatotropin)

FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNRE
ETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLED
GSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF

SEQ ID NO: 96 - amino acid sequence of a peptide linker

GGGGS

SEQ ID NO: 97 - amino acid sequence of a peptide linker

GGGGSGGGGS

SEQ ID NO: 98 - amino acid sequence of a peptide linker
GGGGSGGGGSGGGGS

SEQ ID NO: 99 - amino acid sequence of a peptide linker
GGGGSGGG

SEQ ID NO: 100 - amino acid sequence of a peptide linker
AAPF

SEQ ID NO: 101 - amino acid sequence of a peptide linker
GGF

SEQ ID NO: 102 - amino acid sequence of a peptide linker
AAPV

SEQ ID NO: 103 - amino acid sequence of a peptide linker
GGL

SEQ ID NO: 104 - amino acid sequence of a peptide linker
AAL

SEQ ID NO: 105 - amino acid sequence of a peptide linker
FVR

SEQ ID NO: 106 - amino acid sequence of a peptide linker
VGR

SEQ ID NO: 107 - amino acid sequence of a peptide linker
RKPR

SEQ ID NO: 108 - amino acid sequence of a peptide linker
Y V A D Xaa Xaa = any amino acid

SEQ ID NO: 109 - amino acid sequence of a peptide linker
D Xaa Xaa D Xaa Xaa = any amino acid

SEQ ID NO: 110 - amino acid sequence of a peptide linker

R $(Xaa)_n$ R Xaa Xaa = any amino acid n = 0, 2, 4 or 6

SEQ ID NO: 111 - amino acid sequence of a peptide linker
K $(Xaa)_n$ R Xaa Xaa = any amino acid n = 0, 2, 4 or 6

SEQ ID NO: 112 - amino acid sequence of a peptide linker
E R T K R Xaa Xaa = any amino acid

SEQ ID NO: 113 - amino acid sequence of a peptide linker
R V R R Xaa Xaa = any amino acid

SEQ ID NO: 114 - amino acid sequence of a peptide linker
Decanoyl-R V R R Xaa Xaa = any amino acid

SEQ ID NO: 115 - amino acid sequence of a peptide linker
P Xaa W V P Xaa Xaa = any amino acid

SEQ ID NO: 116 - amino acid sequence of a peptide linker
W V A Xaa Xaa = any amino acid

SEQ ID NO: 117 - amino acid sequence of a peptide linker
Xaa F Xaa Xaa Xaa = any amino acid

SEQ ID NO: 118 - amino acid sequence of a peptide linker
Xaa Y Xaa Xaa Xaa = any amino acid n = 0, 2, 4 or 6

SEQ ID NO: 119 - amino acid sequence of a peptide linker
Xaa W Xaa Xaa Xaa = any amino acid n = 0, 2, 4 or 6

SEQ ID NO: 120 - amino acid sequence of a peptide linker
D R W I P F H L L in combination with (V, A or P)-Y-(S, P or A)

SEQ ID NO: 121 - amino acid sequence of a peptide linker
GGGGSGGGENLYFQS

SEQ ID NO: 122 - amino acid sequence of a Cholix^{415}-TEV-IL-10 fusion molecule

MVEEALNIFDECRSPCSLTPEPGKPIQSKLSIPGDVVLDEGVLYYSMTINDEQNDIKDED
KGESIITIGEFATVRATRHYVSQDAPFGVINLDITTENGTKTYSFNRKESEFAINWLVPIGEDSPA
SIKISIDELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKE

GSRHKRWAHWHTGLALCWLVPIDAIYNYITQQNCTLGDNWFGGSYETVAGTPKAITVKQGIEQ
KPVEQRIHFSKKNAMEALAAHRVCGVPLETLARSRKPRDLPDDLSCAYNAQQIVSLFLATRILFT
HIDSIFTLNLDGQEPEVAERLDDLRRINENNPGMVIQVLTVARQIYNDYVTHHPGLTPEQTSAGA
QAADILSLFCPDADKSCVASNSDQANINIES**GGGGSGGGENLYFQS**PGQGTQSENSCTHFPG
NLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQA
ENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEF
DIFINYIEAYMTMKIRN

SEQ ID NO: 123 - amino acid sequence of a Cholix^{415}-(G$_4$S)$_3$-IL-10 fusion molecule

MVEEALNIFDECRSPCSLTPEPGKPIQSKLSIPGDVVLDEGVLYYSMTINDEQNDIKDED
KGESIITIGEFATVRATRHYVSQDAPFGVINLDITTENGTKTYSFNRKESEFAINWLVPIGEDSPA
SIKISIDELDQQRNIIEVPKLYSIDLDNQTLEQWKTQGNVSFSVTRPEHNIAISWPSVSYKAAQKE
GSRHKRWAHWHTGLALCWLVPIDAIYNYITQQNCTLGDNWFGGSYETVAGTPKAITVKQGIEQ
KPVEQRIHFSKKNAMEALAAHRVCGVPLETLARSRKPRDLPDDLSCAYNAQQIVSLFLATRILFT
HIDSIFTLNLDGQEPEVAERLDDLRRINENNPGMVIQVLTVARQIYNDYVTHHPGLTPEQTSAGA
QAADILSLFCPDADKSCVASNSDQANINIES**GGGGSGGGGSGGGGS**PGQGTQSENSCTHFPG
NLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQA
ENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEF
DIFINYIEAYMTMKIRN

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a non-naturally occurring, dimeric fusion molecule and one or more pharmaceutically acceptable carriers, wherein each monomer of the dimeric fusion molecule comprises

(i) a polypeptide consisting of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or a fragment thereof, wherein the fragment consists of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS: 3, 42, 52, 70, or 80, coupled to

(ii) a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

2. The pharmaceutical composition of claim 1, wherein the interleukin-10 has at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 82.

3. The pharmaceutical composition of claim 1 or claim 2, wherein the interleukin-10 has at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 82.

4. The pharmaceutical composition according to any one of claims 1-3, wherein the polypeptide consists of an amino acid sequence having at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 3.

5. The pharmaceutical composition according to any one of claims 1-4, wherein the polypeptide consists of an amino acid sequence having at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 3.

6. The pharmaceutical composition according to any one of claims 1-5, wherein the polypeptide is directly coupled to the biologically active cargo.

7. The pharmaceutical composition according to any one of claims 1-5, wherein polypeptide is coupled to the biologically active cargo by a cleavable linker.

8. The pharmaceutical composition according to claim 7, wherein the cleavable linker comprises the amino acid sequence of any one of SEQ ID NOS: 100-121.

9. A method for delivering a biologically active cargo to a subject, the method comprising orally delivering to the subject a pharmaceutical composition comprising a non-naturally occurring dimeric fusion molecule and one or more pharmaceutically acceptable carriers, wherein each monomer of the dimeric fusion molecule comprises

(i) a polypeptide consisting of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or a fragment thereof, wherein the fragment consists of an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS: 3, 42, 52, 70, or 80, coupled to

(ii) a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

10. The method according to claim 9, wherein the interleukin-10 has at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 82.

11. The method according to claim 9 or claim 10, wherein the interleukin-10 has at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 82.

12. The method according to any one of claims 9-11, wherein the polypeptide consists of an amino acid sequence having at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 3.

13. The method according to any one of claims 9-12, wherein the polypeptide consists of an amino acid sequence having at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 3.

14. The method according to any one of claims 9-13, wherein the polypeptide is directly coupled to the biologically active cargo.

15. The method according to any one of claims 9-13, wherein the polypeptide is coupled to the biologically active cargo by a cleavable linker.

16. The method according to claim 15, wherein the cleavable linker comprises the amino acid sequence of any one of SEQ ID NOS: 100-121.

17. The method according to any one of claims 9-16, wherein the subject has or is suspected of having an inflammatory disease, an autoimmune disease, a cancer, or a metabolic disorder.

18. The method according to any one of claims 9-17, wherein the subject has or is suspected of having an inflammatory disease.

19. The method according to claim 17 or 18, wherein the inflammatory disease is selected from the group consisting of inflammatory bowel disease, psoriasis or bacterial sepsis.

20. The method according to any one of claims 17-19, wherein the inflammatory disease is an inflammatory bowel disease.

21. The method according to claim 17 or claim 18, wherein the inflammatory disease is ulcerative colitis.

22. The method according to any one of claims 9-21, further comprising administering the pharmaceutical composition to the subject once daily.

23. The method according to any one of claims 9-22, further comprising administering to the subject a total daily dosage of at least 0.1 mg and at most 350 mg of the dimeric fusion molecule.

24. The method according to any one of claims 9-23, further comprising formulating the pharmaceutical composition in a tablet or capsule.

25. The method according to claim 24, wherein the capsule or tablet is enterically coated.

26. The method according to any one of claims 9-13, wherein the polypeptide is coupled to the biologically active cargo by a non-cleavable linker.

27. The method according to claim 26, wherein the non-cleavable linker comprises the amino acid sequence set forth in any one of SEQ ID NOS: 96-99.

28. The method according to claim 26 or claim 27, wherein the non-cleavable linker comprises the amino acid sequence set forth in SEQ ID NO: 97.

29. The method according to claim 26 or claim 27, wherein the non-cleavable linker comprises the amino acid sequence set forth in SEQ ID NO: 98.

30. The method according to any one of claims 9-29, wherein the biologically active cargo consists of the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

31. The method according to claim 9, wherein the biologically active cargo consists of amino acid residues 20-178 of SEQ ID NO: 82.

32. The method according to any one of claims 9-31, wherein dimerization of the fusion molecule is driven by IL-10 dimerization.

33. The method according to any one of claims 9-32, wherein each monomer of the dimeric fusion molecule comprises an N-terminal methionine residue.

34. The method according to claim 9, wherein the dimeric fusion molecule further comprises an N-terminal methionine residue, wherein the polypeptide consists of an amino acid sequence having at least 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 3, wherein the polypeptide is covalently coupled to the interleukin-10 by a linker, wherein the linker consists of the amino acid sequence set forth in SEQ ID NO: 98, and wherein the interleukin-10 consists of amino acids 20-178 of SEQ ID NO: 82.

35. The pharmaceutical composition according to any one of claims 1 to 5, wherein the polypeptide is coupled to the biologically active cargo by a non-cleavable linker.

36. The pharmaceutical composition according to claim 35, wherein the non-cleavable linker comprises the amino acid sequence set forth in any one of SEQ ID NOS: 96-99.

37. The pharmaceutical composition according to claim 35 or claim 36, wherein the non-cleavable linker comprises the amino acid sequence set forth in SEQ ID NO: 97.

38. The pharmaceutical composition according to 35 or claim 36, wherein the non-cleavable linker comprises the amino acid sequence set forth in SEQ ID NO: 98.

39. The pharmaceutical composition according to any one of claims 1-8 or any one of claims 35-38, formulated into a solid dosage form for oral delivery.

40. The pharmaceutical composition according to claim 39, wherein the solid dosage form is a capsule.

41. The pharmaceutical composition according to claim 39, wherein the solid dosage form is a tablet.

42. The pharmaceutical composition according to any one of claims 39-41, wherein the pharmaceutical composition is enterically coated.

43. The pharmaceutical composition according to any one of claims 1-8 or any one of claims 35-42, wherein the pharmaceutical composition comprises up to 200 mg of the dimeric fusion molecule.

44. The pharmaceutical composition according to any one of claims 1-8 or any one of claims 35-43, wherein the biologically active cargo consists of the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

45. The pharmaceutical composition according to claim 1 or any one of claims 35-44, wherein the biologically active cargo consists of amino acid residues 20-178 of SEQ ID NO: 82.

46. The pharmaceutical composition according to any one of claims 1-8 or any one of claims 35-45, wherein dimerization of the fusion molecule is driven by IL-10 dimerization.

47. The pharmaceutical composition according to any one of claims 1-8 or any one of claims 35-46, wherein each monomer of the dimeric fusion molecule comprises an N-terminal methionine residue.

48. The pharmaceutical composition according to claim 1, wherein each monomer of the dimeric fusion molecule further comprises an N-terminal methionine residue, wherein the polypeptide consists of an amino acid sequence having at least 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 3, wherein the polypeptide is covalently coupled to the interleukin-10 by a linker, wherein the linker consists of the amino acid sequence set forth in SEQ ID NO: 98, and wherein the interleukin-10 consists of amino acids 20-178 of SEQ ID NO: 82.

Cholix^{415}-TEV-IL-10 Fusion Molecule

MVEEALNIFD ECRSPCSLTP EPGKPIQSKL SIPGDVVLDE GVLYYSMTIN DEQNDIKDED KGESIITIGE
FATVRATRHY VSQDAPFGVI NLDITTENGT KTYSFNRKES EFAINWLVPI GEDSPASIKI SIDELDQQRN
IIEVPKLYSI DLDNQTLEQW KTQGNVSFSV TRPEHNIAIS WPSVSYKAAQ KEGSRHKRWA HWHTGLALCW
LVPIDAIYNY ITQQNCTLGD NWFGGSYETV AGTPKAITVK QGIEQKPVEQ RIHFSKKNAM EALAAHRVCG
VPLETLARSR KPRDLPDDLS CAYNAQQIVS LFLATRILFT HIDSIFTLNL DGQEPEVAER LDDLRRINEN
NPGMVIQVLT VARQIYNDYV THHPGLTPEQ TSAGAQAADI LSLFCPDADK SCVASNSDQA NINIES**GGGG**
SGGGENLYFQ **S**PGQGTQSEN SCTHFPGNLP NMLRDLRDAF SRVKTFFQMK DQLDNLLLKE SLLEDFKGYL
GCQALSEMIQ FYLEEVMPQA ENQDPDIKAH VNSLGENLKT LRLRLRRCHR FLPCENKSKA VEQVKNAFNK
LQEKGIYKAM SEFDIFINYI EAYMTMKIRN (SEQ ID NO: 122)

Cholix^{415}-(G$_4$S)$_3$-IL-10 Fusion Molecule

MVEEALNIFD ECRSPCSLTP EPGKPIQSKL SIPGDVVLDE GVLYYSMTIN DEQNDIKDED KGESIITIGE
FATVRATRHY VSQDAPFGVI NLDITTENGT KTYSFNRKES EFAINWLVPI GEDSPASIKI SIDELDQQRN
IIEVPKLYSI DLDNQTLEQW KTQGNVSFSV TRPEHNIAIS WPSVSYKAAQ KEGSRHKRWA HWHTGLALCW
LVPIDAIYNY ITQQNCTLGD NWFGGSYETV AGTPKAITVK QGIEQKPVEQ RIHFSKKNAM EALAAHRVCG
VPLETLARSR KPRDLPDDLS CAYNAQQIVS LFLATRILFT HIDSIFTLNL DGQEPEVAER LDDLRRINEN
NPGMVIQVLT VARQIYNDYV THHPGLTPEQ TSAGAQAADI LSLFCPDADK SCVASNSDQA NINIES**GGGG**
SGGGGSGGGG **S**PGQGTQSEN SCTHFPGNLP NMLRDLRDAF SRVKTFFQMK DQLDNLLLKE SLLEDFKGYL
GCQALSEMIQ FYLEEVMPQA ENQDPDIKAH VNSLGENLKT LRLRLRRCHR FLPCENKSKA VEQVKNAFNK
LQEKGIYKAM SEFDIFINYI EAYMTMKIRN (SEQ ID NO: 123)

FIG. 1

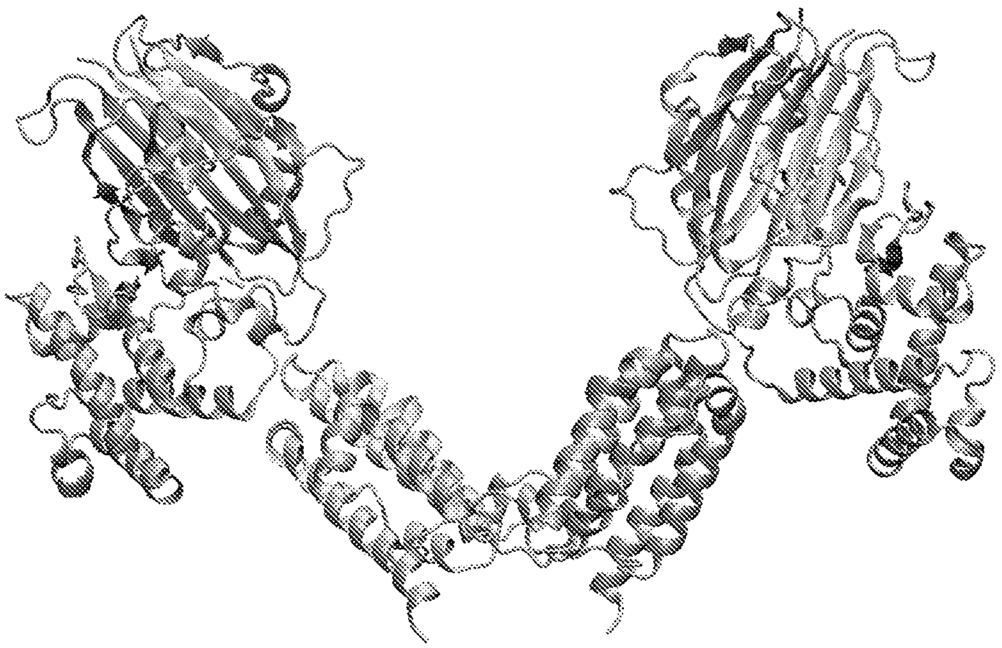


FIG. 2

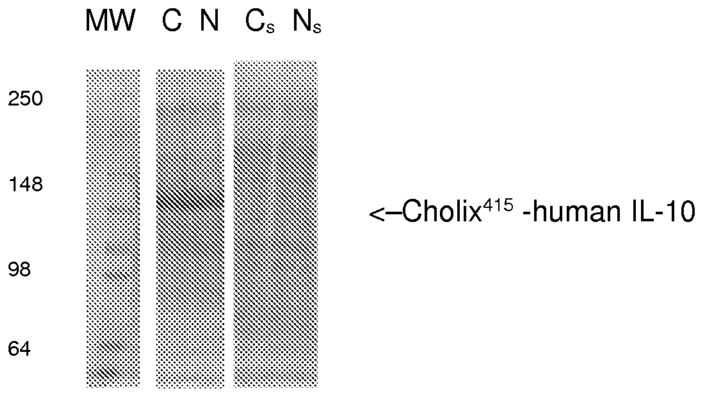


FIG. 3

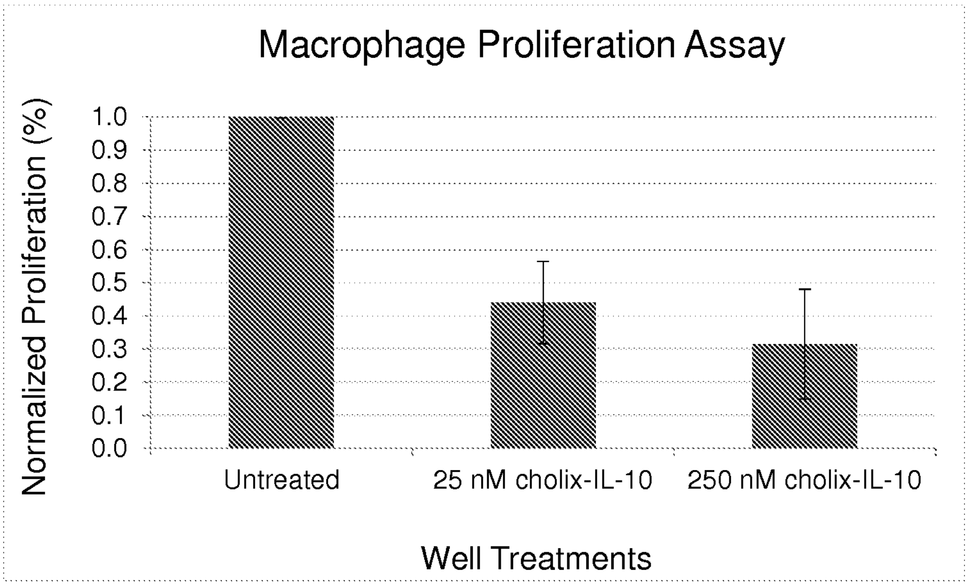

Macrophage Proliferation Assay
Normalized Proliferation (%)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Untreated
25 nM cholix-IL-10
250 nM cholix-IL-10
Well Treatments

FIG. 4

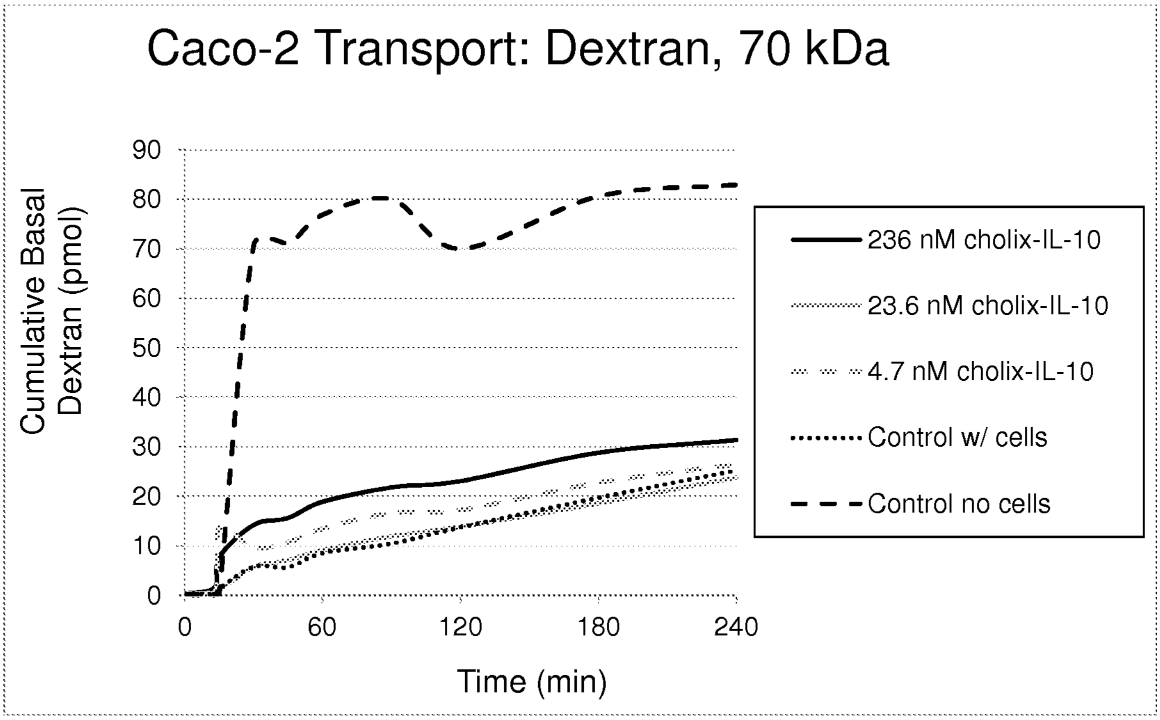

Caco-2 Transport: Dextran, 70 kDa
Cumulative Basal Dextran (pmol)
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
Time (min)
236 nM cholix-IL-10
23.6 nM cholix-IL-10
4.7 nM cholix-IL-10
Control w/ cells
Control no cells

FIG. 5

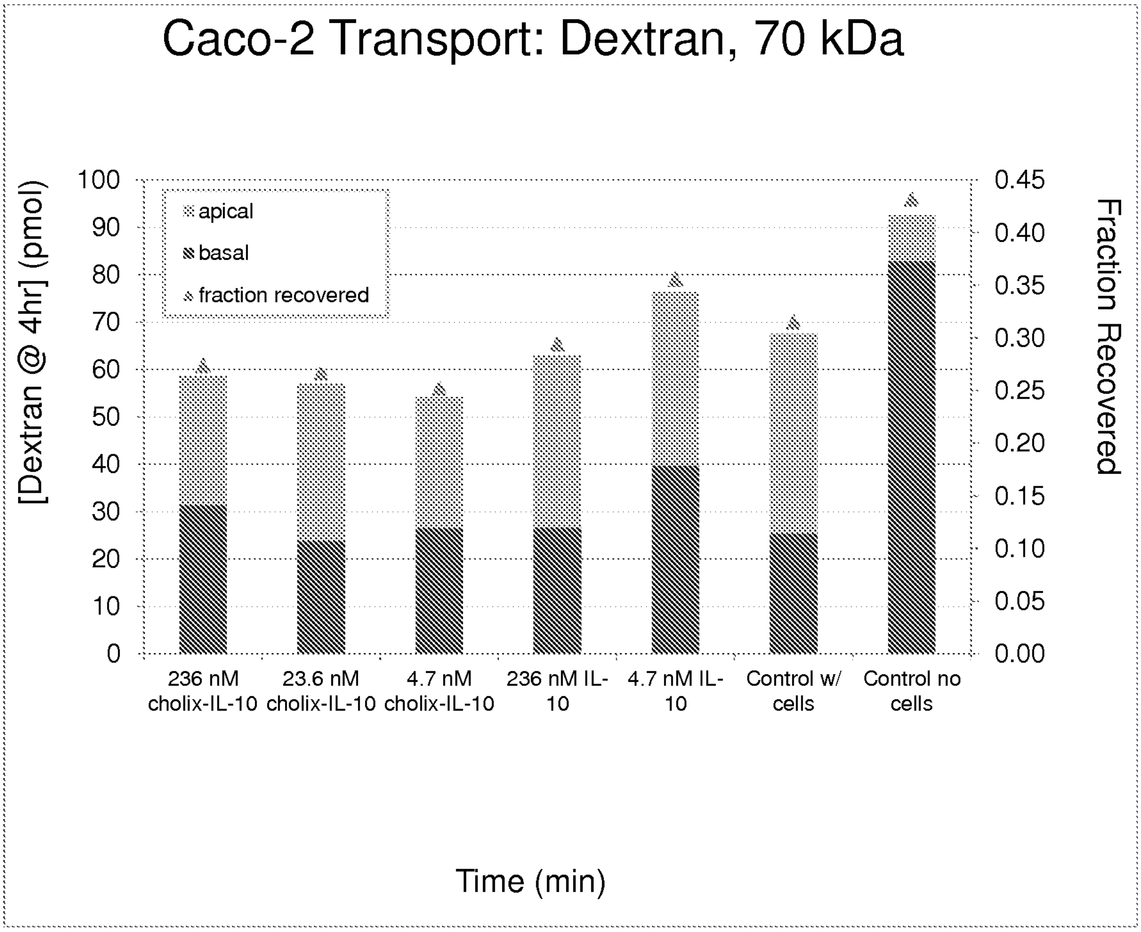
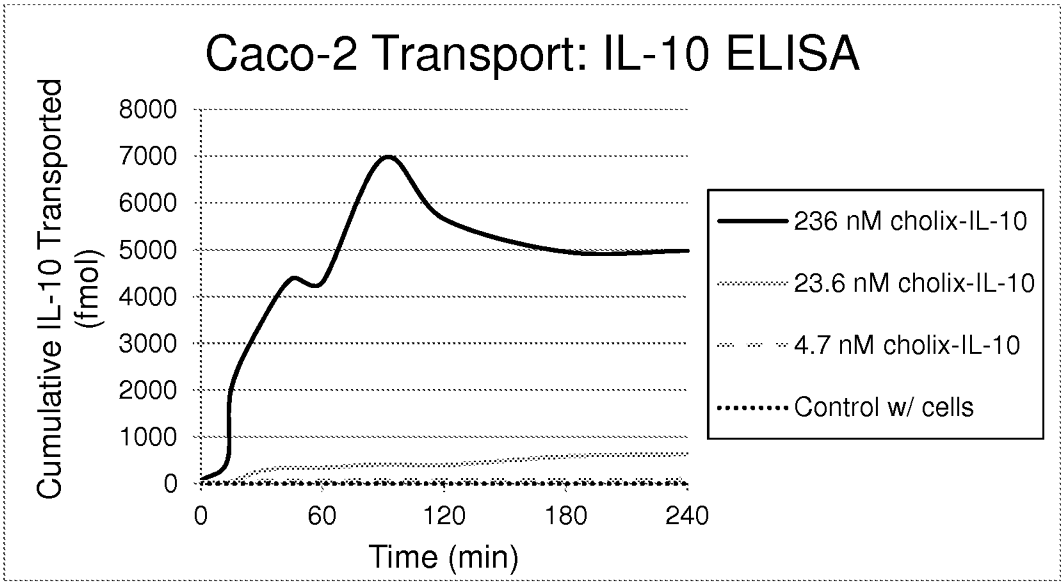

Caco-2 Transport: Dextran, 70 kDa
[Dextran @ 4hr] (pmol)
100
90
80
70
60
50
40
30
20
10
0
apical
basal
fraction recovered
Fraction Recovered
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
236 nM cholix-IL-10
23.6 nM cholix-IL-10
4.7 nM cholix-IL-10
236 nM IL-10
4.7 nM IL-10
Control w/ cells
Control no cells
Time (min)

FIG. 6

A


Caco-2 Transport: IL-10 ELISA
Cumulative IL-10 Transported (fmol)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
60
120
180
240
Time (min)
236 nM cholix-IL-10
23.6 nM cholix-IL-10
4.7 nM cholix-IL-10
Control w/ cells

B

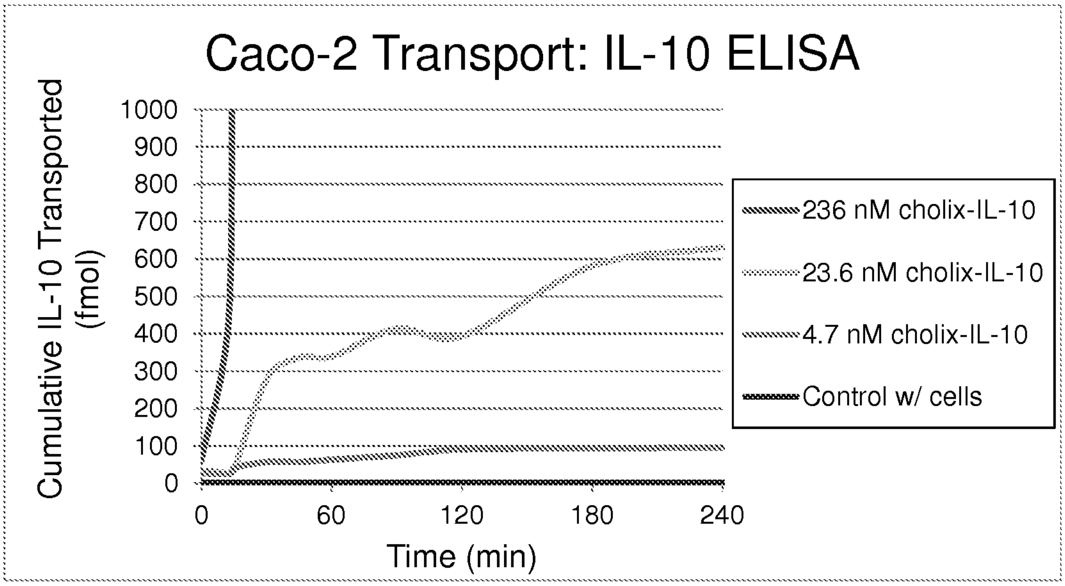

Caco-2 Transport: IL-10 ELISA
Cumulative IL-10 Transported (fmol)
1000
900
800
700
600
500
400
300
200
100
0
0
60
120
180
240
Time (min)
236 nM cholix-IL-10
23.6 nM cholix-IL-10
4.7 nM cholix-IL-10
Control w/ cells

FIG. 7

SEQUENCE LISTING

```
<110>  APPLIED MOLECULAR TRANSPORT LLC

<120>  CHOLIX TOXIN-DERIVED FUSION MOLECULES FOR ORAL DELIVERY OF
       BIOLOGICALLY ACTIVE CARGO

<130>  CACAM1.0002WO

<160>  123

<170>  PatentIn version 3.5

<210>  1
<211>  634
<212>  PRT
<213>  Vibreo cholerae - mature Cholix Toxin amino acid sequence

<400>  1

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190
```

```
Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415

Ser Gly Arg Ser Tyr Leu Pro Glu Asn Arg Ala Val Ile Thr Pro Gln
            420                 425                 430

Gly Val Thr Asn Trp Thr Tyr Gln Glu Leu Glu Ala Thr His Gln Ala
        435                 440                 445

Leu Thr Arg Glu Gly Tyr Val Phe Val Gly Tyr His Gly Thr Asn His
    450                 455                 460
```

```
Val Ala Ala Gln Thr Ile Val Asn Arg Ile Ala Pro Val Pro Arg Gly
465                 470                 475                 480

Asn Asn Thr Glu Asn Glu Glu Lys Trp Gly Gly Leu Tyr Val Ala Thr
                485                 490                 495

His Ala Glu Val Ala His Gly Tyr Ala Arg Ile Lys Glu Gly Thr Gly
            500                 505                 510

Glu Tyr Gly Leu Pro Thr Arg Ala Glu Arg Asp Ala Arg Gly Val Met
        515                 520                 525

Leu Arg Val Tyr Ile Pro Arg Ala Ser Leu Glu Arg Phe Tyr Arg Thr
    530                 535                 540

Asn Thr Pro Leu Glu Asn Ala Glu Glu His Ile Thr Gln Val Ile Gly
545                 550                 555                 560

His Ser Leu Pro Leu Arg Asn Glu Ala Phe Thr Gly Pro Glu Ser Ala
                565                 570                 575

Gly Gly Glu Asp Glu Thr Val Ile Gly Trp Asp Met Ala Ile His Ala
            580                 585                 590

Val Ala Ile Pro Ser Thr Ile Pro Gly Asn Ala Tyr Glu Glu Leu Ala
        595                 600                 605

Ile Asp Glu Glu Ala Val Ala Lys Glu Gln Ser Ile Ser Thr Lys Pro
    610                 615                 620

Pro Tyr Lys Glu Arg Lys Asp Glu Leu Lys
625                 630
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<210> 2
<211> 1905
<212> DNA
<213> Vibreo cholerae - nucleic acid sequence encoding mature Cholix Toxin

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<400>  2
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gaaccgggta agccgattca atcaaaactg tctatcccta gtgatgttgt tctggatgaa      120

ggtgttctgt attactcgat gacgattaat gatgagcaga atgatattaa ggatgaggac      180

aaaggcgagt ccattatcac tattggtgaa tttgccacag tacgcgcgac tagacattat      240

gttaatcaag atgcgccttt tggtgtcatc catttagata ttacgacaga aaatggtaca      300

aaaacgtact cttataaccg caaagagggt gaatttgcaa tcaattggtt agtgcctatt      360

ggtgaagatt ctcctgcaag catcaaaatc tccgttgatg agctcgatca gcaacgcaat      420

atcatcgagg tgcctaaact gtatagtatt gatctcgata accaaacgtt agagcagtgg      480

aaaacccaag gtaatgtttc tttttcggta acgcgtcctg aacataatat cgctatctct      540
```

```
tggccaagcg tgagttacaa agcagcgcag aaagagggtt cacgccataa gcgttgggct      600

cattggcata caggcttagc actgtgttgg cttgtgccaa tggatgctat ctataactat      660

atcacccagc aaaattgtac tttaggggat aattggtttg gtggctctta tgagactgtt      720

gcaggcactc cgaaggtgat tacggttaag caagggattg aacaaaagcc agttgagcag      780

cgcatccatt tctccaaggg gaatgcgatg agcgcacttg ctgctcatcg cgtctgtggt      840

gtgccattag aaactttggc gcgcagtcgc aaacctcgtg atctgacgga tgatttatca      900

tgtgcctatc aagcgcagaa tatcgtgagt ttatttgtcg cgacgcgtat cctgttctct      960

catctggata gcgtatttac tctgaatctt gacgaacaag aaccagaggt ggctgaacgt     1020

ctaagtgatc ttcgccgtat caatgaaaat aacccgggca tggttacaca ggttttaacc     1080

gttgctcgtc agatctataa cgattatgtc actcaccatc cgggcttaac tcctgagcaa     1140

accagtgcgg gtgcacaagc tgccgatatc ctctctttat tttgcccaga tgctgataag     1200

tcttgtgtgg cttcaaacaa cgatcaagcc aatatcaaca tcgagtctcg ttctggccgt     1260

tcatatttgc ctgaaaaccg tgcggtaatc acccctcaag gcgtcacaaa ttggacttac     1320

caggaactcg aagcaacaca tcaagctctg actcgtgagg gttatgtgtt cgtgggttac     1380

catggtacga atcatgtcgc tgcgcaaacc atcgtgaatc gcattgcccc tgttccgcgc     1440

ggcaacaaca ctgaaaacga ggaaaagtgg ggcgggttat atgttgcaac tcacgctgaa     1500

gttgcccatg gttatgctcg catcaaagaa gggacagggg agtatggcct tccgacccgt     1560

gctgagcgcg acgctcgtgg ggtaatgctg cgcgtgtata tccctcgtgc ttcattagaa     1620

cgttttatc gcacgaatac acctttggaa aatgctgagg agcatatcac gcaagtgatt      1680

ggtcattctt tgccattacg caatgaagca tttactggtc cagaaagtgc gggcggggaa     1740

gacgaaactg tcattggctg ggatatggcg attcatgcag ttgcgatccc ttcgactatc     1800

ccagggaacg cttacgaaga attggcgatt gatgaggagg ctgttgcaaa agagcaatcg     1860

attagcacaa aaccacctta taaagagcgc aaagatgaac ttaag                     1905


<210>  3
<211>  386
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-386

<400>  3

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60
```

```
Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335
```

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340   345   350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355   360   365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370   375   380

Gln Ala
385

<210> 4
<211> 385
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-385

<400> 4

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1   5   10   15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20   25   30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35   40   45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50   55   60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65   70   75   80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85   90   95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100   105   110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115   120   125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130   135   140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145   150   155   160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165   170   175

```
Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln
385


<210>  5
<211>  384
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-384

<400>  5

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15
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```
Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285
```

```
Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


<210>  6
<211>  383
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-383

<400>  6

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
```

```
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly
    370                 375                 380


<210>  7
<211>  382
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-382

<400>  7

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15
```

```
Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285
```

```
Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala
    370                 375                 380


<210>  8
<211>  381
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-381

<400>  8

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140
```

```
Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser
    370                 375                 380
```

<210> 9
<211> 380
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-380

<400> 9

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Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270
```

```
Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr
    370                 375                 380


<210>  10
<211>  379
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-379

<400>  10

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
```

```
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln
    370                 375


<210>  11
<211>  378
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-378
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<400>  11

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270
```

```
Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu
    370                 375


<210>  12
<211>  377
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-377

<400>  12

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro
    370                 375


<210>  13
<211>  376
<212>  PRT
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<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-376

<400>  13

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
                20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
            35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
        50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255
```

```
Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr
    370                 375


<210>  14
<211>  375
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-375

<400>  14

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
```

```
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu
    370                 375


<210>  15
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<211>  374
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-374

<400>  15

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255
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```
Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly
    370


<210>  16
<211>  373
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-373

<400>  16

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110
```

```
Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro
    370
```

```
<210>  17
<211>  372
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-372

<400>  17

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240
```

```
Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His
    370


<210>  18
<211>  371
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-371

<400>  18

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
```

```
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His
```

```
    370


<210>  19
<211>  370
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-370

<400>  19

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240
```

```
Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr
    370


<210>  20
<211>  369
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-369

<400>  20

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95
```

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365
```

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Val



<210>  21
<211>  368
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-368

<400>  21

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220
```

```
Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


<210>  22
<211>  367
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-367

<400>  22

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
```

```
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp
        355                 360                 365

<210>  23
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<211>  366
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-366

<400>  23

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255
```

```
Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn
        355                 360                 365


<210>  24
<211>  365
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-365

<400>  24

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr
        355                 360                 365


<210>  25
<211>  364
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-364

<400>  25
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Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270
```

```
Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile
        355                 360


<210>  26
<211>  363
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-363

<400>  26

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
```

```
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln
        355                 360


<210>  27
<211>  362
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-362

<400>  27

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30
```

```
Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300
```

```
Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg
        355                 360


<210>  28
<211>  361
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-361

<400>  28

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175
```

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
180 185 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
195 200 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
210 215 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225 230 235 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
245 250 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
260 265 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
275 280 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
290 295 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305 310 315 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
325 330 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
340 345 350

Met Val Thr Gln Val Leu Thr Val Ala
355 360

<210> 29
<211> 360
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-360

<400> 29

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1 5 10 15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
20 25 30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
35 40 45

```
Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320
```

```
Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val
        355                 360


<210>  30
<211>  359
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-359

<400>  30

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
```

```
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr
        355


<210>  31
<211>  358
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-358

<400>  31

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80
```

```
Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350
```

```
Met Val Thr Gln Val Leu
        355


<210>  32
<211>  357
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-357

<400>  32

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220
```

```
Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val
        355


<210>  33
<211>  356
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-356

<400>  33

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95
```

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln
        355
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<210>  34
<211>  355
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-355

<400>  34

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
```

```
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr
        355


<210>  35
<211>  354
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-354

<400>  35

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val
```

<210> 36
<211> 353
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-353

<400> 36

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Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270
```

```
Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met



<210>  37
<211>  352
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-352

<400>  37

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140
```

```
Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


<210>  38
<211>  351
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-351

<400>  38

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
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        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
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```
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro
            340                 345                 350


<210>  39
<211>  350
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-350

<400>  39

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205
```

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
210 215 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225 230 235 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
245 250 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
260 265 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
275 280 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
290 295 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305 310 315 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
325 330 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn
340 345 350

<210> 40
<211> 349
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-349

<400> 40

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1 5 10 15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
20 25 30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
35 40 45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
50 55 60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65 70 75 80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
85 90 95

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn
            340                 345


<210>  41
<211>  348
<212>  PRT
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<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-348

<400>  41

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255
```

```
Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu
            340                 345


<210>  42
<211>  425
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-425

<400>  42

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
```

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145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415

Ser Gly Arg Ser Tyr Leu Pro Glu Asn
```

```
            420                 425


<210>  43
<211>  424
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-424

<400>  43

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240
```

```
Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly Arg Ser Tyr Leu Pro Glu
            420


<210>  44
<211>  423
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-423

<400>  44

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45
```

```
Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320
```

```
Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly Arg Ser Tyr Leu Pro
            420


<210>  45
<211>  422
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-422

<400>  45

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400
```

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405   410   415

Ser Gly Arg Ser Tyr Leu
            420

<210> 46
<211> 421
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-421

<400> 46

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1   5   10   15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20   25   30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35   40   45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50   55   60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65   70   75   80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85   90   95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100   105   110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115   120   125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130   135   140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145   150   155   160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165   170   175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180   185   190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195   200   205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn

```
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly Arg Ser Tyr
            420


<210>  47
<211>  420
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-420

<400>  47

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30
```

```
Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300
```

```
Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly Arg Ser
            420


<210>  48
<211>  419
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-419

<400>  48

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110
```

```
Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380
```

```
Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly Arg



<210>  49
<211>  418
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-418

<400>  49

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190
```

```
Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


Ser Gly



<210>  50
<211>  417
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-417

<400>  50

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
```

```
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
```

275 280 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
290 295 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305 310 315 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
325 330 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
340 345 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
355 360 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
370 375 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385 390 395 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
405 410 415

Ser

<210> 51
<211> 416
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-416

<400> 51

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1 5 10 15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
20 25 30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
35 40 45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
50 55 60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65 70 75 80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
85 90 95

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365
```

```
Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415


<210>  52
<211>  415
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-415

<400>  52

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190
```

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195   200   205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210   215   220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225   230   235   240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245   250   255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260   265   270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275   280   285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290   295   300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305   310   315   320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325   330   335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340   345   350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355   360   365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370   375   380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385   390   395   400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser
                405   410   415

<210> 53
<211> 414
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-414

<400> 53

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1   5   10   15

```
Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285
```

```
Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu
                405                 410


<210>  54
<211>  413
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-413

<400>  54

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
```

```
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
```

385   390   395   400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile
                405   410

<210> 55
<211> 412
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-412

<400> 55

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1   5   10   15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20   25   30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35   40   45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50   55   60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65   70   75   80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85   90   95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100   105   110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115   120   125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130   135   140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145   150   155   160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165   170   175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180   185   190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195   200   205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210   215   220

```
Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn
                405                 410


<210>  56
<211>  411
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-411

<400>  56

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45
```

```
Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320
```

```
Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile
                405                 410


<210>  57
<211>  410
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-410

<400>  57

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140
```

```
Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn
                405                 410
```

```
<210>  58
<211>  409
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-409

<400>  58

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
```

```
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln Ala
                405


<210>  59
<211>  408
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-408

<400>  59

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80
```

```
Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350
```

```
Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp Gln
                405


<210>  60
<211>  407
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-407

<400>  60

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175
```

```
Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn Asp
                405


<210>  61
<211>  406
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-406

<400>  61
```

```
Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270
```

```
Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn Asn
                405


<210>  62
<211>  405
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-405

<400>  62

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
```

```
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
```

```
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser Asn
                405


<210>  63
<211>  404
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-404

<400>  63

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205
```

```
Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala Ser



<210>  64
<211>  403
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-403

<400>  64

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30
```

```
Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300
```

```
Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys Val Ala



<210>  65
<211>  402
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-402

<400>  65

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400
```

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Cys Val



<210>  66
<211>  401
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-401

<400>  66

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
```

```
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400


Cys



<210>  67
<211>  400
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-400

<400>  67

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60
```

```
Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335
```

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
340 345 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
355 360 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
370 375 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385 390 395 400

<210> 68
<211> 399
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-399

<400> 68

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1 5 10 15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
20 25 30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
35 40 45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
50 55 60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65 70 75 80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
85 90 95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
100 105 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
115 120 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
130 135 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145 150 155 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
165 170 175

```
Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys
385                 390                 395


<210>  69
<211>  398
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-398

<400>  69

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15
```

```
Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285
```

```
Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp
385                 390                 395


<210>  70
<211>  397
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-397

<400>  70

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
```

130 135 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145 150 155 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
165 170 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
180 185 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
195 200 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
210 215 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225 230 235 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
245 250 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
260 265 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
275 280 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
290 295 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305 310 315 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
325 330 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
340 345 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
355 360 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
370 375 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala
385 390 395

<210> 71

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<211>  396
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-396

<400>  71

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255
```

```
Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp
385                 390                 395


<210>  72
<211>  395
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-395

<400>  72

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95
```

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365
```

```
Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro
385                 390                 395


<210>  73
<211>  394
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-394

<400>  73

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205
```

```
Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys
385                 390


<210>  74
<211>  393
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-393

<400>  74

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
```

```
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
```

```
            325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser Leu Phe
385                 390


<210>  75
<211>  392
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-392

<400>  75

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175
```

```
Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu
385                 390


<210>  76
<211>  391
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-391

<400>  76

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15
```

```
Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285
```

```
Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp Ile Leu Ser
385                 390


<210>  77
<211>  390
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-390

<400>  77

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125
```

```
Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu
385                 390
```

```
<210>  78
<211>  389
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-389

<400>  78

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205


Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
```

245 250 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
260 265 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
275 280 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
290 295 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305 310 315 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
325 330 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
340 345 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
355 360 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
370 375 380

Gln Ala Ala Asp Ile
385

<210> 79
<211> 388
<212> PRT
<213> Vibreo cholerae Cholix Toxin amino acid residues 1-388

<400> 79

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1 5 10 15

Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
20 25 30

Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
35 40 45

Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
50 55 60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65 70 75 80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
85 90 95

```
Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320

Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365
```

```
Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala Asp
385


<210>  80
<211>  387
<212>  PRT
<213>  Vibreo cholerae Cholix Toxin amino acid residues 1-387

<400>  80

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45


Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60


Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80


Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95


Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110


Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125


Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140


Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160


Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175


Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190


Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205
```

```
Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220


Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240


Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255


Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270


Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285


Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300


Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320


Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335


Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350


Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365


Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380


Gln Ala Ala
385
```

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<210>  81
<211>  633
<212>  PRT
<213>  Vibreo cholerae mature Cholix Toxin deleted at amio acid residue 581

<400>  81

Val Glu Asp Glu Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys Ser
1               5                   10                  15


Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile Pro
            20                  25                  30


Ser Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr Ile
        35                  40                  45
```

```
Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser Ile
    50                  55                  60

Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr Val
65                  70                  75                  80

Asn Gln Asp Ala Pro Phe Gly Val Ile His Leu Asp Ile Thr Thr Glu
                85                  90                  95

Asn Gly Thr Lys Thr Tyr Ser Tyr Asn Arg Lys Glu Gly Glu Phe Ala
            100                 105                 110

Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile Lys
        115                 120                 125

Ile Ser Val Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val Pro
    130                 135                 140

Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp Lys
145                 150                 155                 160

Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn Ile
                165                 170                 175

Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu Gly
            180                 185                 190

Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu Cys
        195                 200                 205

Trp Leu Val Pro Met Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln Asn
    210                 215                 220

Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val Ala
225                 230                 235                 240

Gly Thr Pro Lys Val Ile Thr Val Lys Gln Gly Ile Glu Gln Lys Pro
                245                 250                 255

Val Glu Gln Arg Ile His Phe Ser Lys Gly Asn Ala Met Ser Ala Leu
            260                 265                 270

Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg Ser
        275                 280                 285

Arg Lys Pro Arg Asp Leu Thr Asp Asp Leu Ser Cys Ala Tyr Gln Ala
    290                 295                 300

Gln Asn Ile Val Ser Leu Phe Val Ala Thr Arg Ile Leu Phe Ser His
305                 310                 315                 320
```

```
Leu Asp Ser Val Phe Thr Leu Asn Leu Asp Glu Gln Glu Pro Glu Val
                325                 330                 335

Ala Glu Arg Leu Ser Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro Gly
            340                 345                 350

Met Val Thr Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp Tyr
        355                 360                 365

Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly Ala
    370                 375                 380

Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys Ser
385                 390                 395                 400

Cys Val Ala Ser Asn Asn Asp Gln Ala Asn Ile Asn Ile Glu Ser Arg
                405                 410                 415

Ser Gly Arg Ser Tyr Leu Pro Glu Asn Arg Ala Val Ile Thr Pro Gln
            420                 425                 430

Gly Val Thr Asn Trp Thr Tyr Gln Glu Leu Glu Ala Thr His Gln Ala
        435                 440                 445

Leu Thr Arg Glu Gly Tyr Val Phe Val Gly Tyr His Gly Thr Asn His
    450                 455                 460

Val Ala Ala Gln Thr Ile Val Asn Arg Ile Ala Pro Val Pro Arg Gly
465                 470                 475                 480

Asn Asn Thr Glu Asn Glu Glu Lys Trp Gly Gly Leu Tyr Val Ala Thr
                485                 490                 495

His Ala Glu Val Ala His Gly Tyr Ala Arg Ile Lys Glu Gly Thr Gly
            500                 505                 510

Glu Tyr Gly Leu Pro Thr Arg Ala Glu Arg Asp Ala Arg Gly Val Met
        515                 520                 525

Leu Arg Val Tyr Ile Pro Arg Ala Ser Leu Glu Arg Phe Tyr Arg Thr
    530                 535                 540

Asn Thr Pro Leu Glu Asn Ala Glu Glu His Ile Thr Gln Val Ile Gly
545                 550                 555                 560

His Ser Leu Pro Leu Arg Asn Glu Ala Phe Thr Gly Pro Glu Ser Ala
                565                 570                 575

Gly Gly Glu Asp Thr Val Ile Gly Trp Asp Met Ala Ile His Ala Val
            580                 585                 590
```

```
Ala Ile Pro Ser Thr Ile Pro Gly Asn Ala Tyr Glu Glu Leu Ala Ile
        595                 600                 605


Asp Glu Glu Ala Val Ala Lys Glu Gln Ser Ile Ser Thr Lys Pro Pro
    610                 615                 620


Tyr Lys Glu Arg Lys Asp Glu Leu Lys
625                 630


<210>  82
<211>  178
<212>  PRT
<213>  Homo sapiens - Interleukin-10

<400>  82

Met His Ser Ser Ala Leu Leu Cys Cys Leu Val Leu Leu Thr Gly Val
1               5                   10                  15


Arg Ala Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His
            20                  25                  30


Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe
        35                  40                  45


Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu
    50                  55                  60


Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys
65                  70                  75                  80


Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro
                85                  90                  95


Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu
            100                 105                 110


Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg
        115                 120                 125


Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn
    130                 135                 140


Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu
145                 150                 155                 160


Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile
                165                 170                 175


Arg Asn



<210>  83
```

```
<211>  177
<212>  PRT
<213>  Homo sapiens - Interleukin-19

<400>  83

Met Lys Leu Gln Cys Val Ser Leu Trp Leu Leu Gly Thr Ile Leu Ile
1               5                   10                  15


Leu Cys Ser Val Asp Asn His Gly Leu Arg Arg Cys Leu Ile Ser Thr
            20                  25                  30


Asp Met His His Ile Glu Glu Ser Phe Gln Glu Ile Lys Arg Ala Ile
        35                  40                  45


Gln Ala Lys Asp Thr Phe Pro Asn Val Thr Ile Leu Ser Thr Leu Glu
    50                  55                  60


Thr Leu Gln Ile Ile Lys Pro Leu Asp Val Cys Cys Val Thr Lys Asn
65                  70                  75                  80


Leu Leu Ala Phe Tyr Val Asp Arg Val Phe Lys Asp His Gln Glu Pro
                85                  90                  95


Asn Pro Lys Ile Leu Arg Lys Ile Ser Ser Ile Ala Asn Ser Phe Leu
            100                 105                 110


Tyr Met Gln Lys Thr Leu Arg Gln Cys Gln Glu Gln Arg Gln Cys His
        115                 120                 125


Cys Arg Gln Glu Ala Thr Asn Ala Thr Arg Val Ile His Asp Asn Tyr
    130                 135                 140


Asp Gln Leu Glu Val His Ala Ala Ala Ile Lys Ser Leu Gly Glu Leu
145                 150                 155                 160


Asp Val Phe Leu Ala Trp Ile Asn Lys Asn His Glu Val Met Ser Ser
                165                 170                 175


Ala



<210>  84
<211>  176
<212>  PRT
<213>  Homo sapiens - Interleukin-20

<400>  84

Met Lys Ala Ser Ser Leu Ala Phe Ser Leu Leu Ser Ala Ala Phe Tyr
1               5                   10                  15


Leu Leu Trp Thr Pro Ser Thr Gly Leu Lys Thr Leu Asn Leu Gly Ser
            20                  25                  30
```

```
Cys Val Ile Ala Thr Asn Leu Gln Glu Ile Arg Asn Gly Phe Ser Glu
        35                  40                  45


Ile Arg Gly Ser Val Gln Ala Lys Asp Gly Asn Ile Asp Ile Arg Ile
    50                  55                  60


Leu Arg Arg Thr Glu Ser Leu Gln Asp Thr Lys Pro Ala Asn Arg Cys
65                  70                  75                  80


Cys Leu Leu Arg His Leu Leu Arg Leu Tyr Leu Asp Arg Val Phe Lys
                85                  90                  95


Asn Tyr Gln Thr Pro Asp His Tyr Thr Leu Arg Lys Ile Ser Ser Leu
            100                 105                 110


Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp Leu Arg Leu Cys His Ala
        115                 120                 125


His Met Thr Cys His Cys Gly Glu Glu Ala Met Lys Lys Tyr Ser Gln
    130                 135                 140


Ile Leu Ser His Phe Glu Lys Leu Glu Pro Gln Ala Ala Val Val Lys
145                 150                 155                 160


Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln Trp Met Glu Glu Thr Glu
                165                 170                 175


<210>  85
<211>  179
<212>  PRT
<213>  Homo sapiens - Interleukin-22

<400>  85

Met Ala Ala Leu Gln Lys Ser Val Ser Ser Phe Leu Met Gly Thr Leu
1               5                   10                  15


Ala Thr Ser Cys Leu Leu Leu Leu Ala Leu Leu Val Gln Gly Gly Ala
            20                  25                  30


Ala Ala Pro Ile Ser Ser His Cys Arg Leu Asp Lys Ser Asn Phe Gln
        35                  40                  45


Gln Pro Tyr Ile Thr Asn Arg Thr Phe Met Leu Ala Lys Glu Ala Ser
    50                  55                  60


Leu Ala Asp Asn Asn Thr Asp Val Arg Leu Ile Gly Glu Lys Leu Phe
65                  70                  75                  80


His Gly Val Ser Met Ser Glu Arg Cys Tyr Leu Met Lys Gln Val Leu
                85                  90                  95
```

```
Asn Phe Thr Leu Glu Glu Val Leu Phe Pro Gln Ser Asp Arg Phe Gln
            100                 105                 110

Pro Tyr Met Gln Glu Val Val Pro Phe Leu Ala Arg Leu Ser Asn Arg
        115                 120                 125

Leu Ser Thr Cys His Ile Glu Gly Asp Asp Leu His Ile Gln Arg Asn
    130                 135                 140

Val Gln Lys Leu Lys Asp Thr Val Lys Lys Leu Gly Glu Ser Gly Glu
145                 150                 155                 160

Ile Lys Ala Ile Gly Glu Leu Asp Leu Leu Phe Met Ser Leu Arg Asn
                165                 170                 175

Ala Cys Ile



<210>  86
<211>  207
<212>  PRT
<213>  Homo sapiens - Interleukin-24

<400>  86

Met Asn Phe Gln Gln Arg Leu Gln Ser Leu Trp Thr Leu Ala Ser Arg
1               5                   10                  15

Pro Phe Cys Pro Pro Leu Leu Ala Thr Ala Ser Gln Met Gln Met Val
            20                  25                  30

Val Leu Pro Cys Leu Gly Phe Thr Leu Leu Leu Trp Ser Gln Val Ser
        35                  40                  45

Gly Ala Gln Gly Gln Glu Phe His Phe Gly Pro Cys Gln Val Lys Gly
    50                  55                  60

Val Val Pro Gln Lys Leu Trp Glu Ala Phe Trp Ala Val Lys Asp Thr
65                  70                  75                  80

Met Gln Ala Gln Asp Asn Ile Thr Ser Ala Arg Leu Leu Gln Gln Glu
                85                  90                  95

Val Leu Gln Asn Val Ser Asp Ala Glu Ser Cys Tyr Leu Val His Thr
            100                 105                 110

Leu Leu Glu Phe Tyr Leu Lys Thr Val Phe Lys Asn Tyr His Asn Arg
        115                 120                 125

Thr Val Glu Val Arg Thr Leu Lys Ser Phe Ser Thr Leu Ala Asn Asn
    130                 135                 140

Phe Val Leu Ile Val Ser Gln Leu Gln Pro Ser Gln Glu Asn Glu Met
```

```
145                 150                 155                 160


Phe Ser Ile Arg Asp Ser Ala His Arg Arg Phe Leu Leu Phe Arg Arg
                165                 170                 175


Ala Phe Lys Gln Leu Asp Val Glu Ala Ala Leu Thr Lys Ala Leu Gly
            180                 185                 190


Glu Val Asp Ile Leu Leu Thr Trp Met Gln Lys Phe Tyr Lys Leu
        195                 200                 205


<210>  87
<211>  171
<212>  PRT
<213>  Homo sapiens - Interleukin-26

<400>  87

Met Leu Val Asn Phe Ile Leu Arg Cys Gly Leu Leu Leu Val Thr Leu
1               5                   10                  15


Ser Leu Ala Ile Ala Lys His Lys Gln Ser Ser Phe Thr Lys Ser Cys
            20                  25                  30


Tyr Pro Arg Gly Thr Leu Ser Gln Ala Val Asp Ala Leu Tyr Ile Lys
        35                  40                  45


Ala Ala Trp Leu Lys Ala Thr Ile Pro Glu Asp Arg Ile Lys Asn Ile
    50                  55                  60


Arg Leu Leu Lys Lys Lys Thr Lys Lys Gln Phe Met Lys Asn Cys Gln
65                  70                  75                  80


Phe Gln Glu Gln Leu Leu Ser Phe Phe Met Glu Asp Val Phe Gly Gln
                85                  90                  95


Leu Gln Leu Gln Gly Cys Lys Lys Ile Arg Phe Val Glu Asp Phe His
            100                 105                 110


Ser Leu Arg Gln Lys Leu Ser His Cys Ile Ser Cys Ala Ser Ser Ala
        115                 120                 125


Arg Glu Met Lys Ser Ile Thr Arg Met Lys Arg Ile Phe Tyr Arg Ile
    130                 135                 140


Gly Asn Lys Gly Ile Tyr Lys Ala Ile Ser Glu Leu Asp Ile Leu Leu
145                 150                 155                 160


Ser Trp Ile Lys Lys Leu Leu Glu Ser Ser Gln
                165                 170


<210>  88
<211>  224
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<212>  PRT
<213>  Artificial

<220>
<223>  heavy chain variable region sequence for an anti-TNF-alpha
       antibody

<400>  88

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
            20                  25                  30


Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
    50                  55                  60


Glu Arg Gly Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
            100                 105                 110


Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125


Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
    130                 135                 140


Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160


Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175


Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190


Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205


Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
    210                 215                 220


<210>  89
<211>  214
<212>  PRT
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```
<213>  Artificial

<220>
<223>  light chain variable region sequence for an anti-TNF-alpha
       antibody

<400>  89

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110


Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125


Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140


Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160


Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175


Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190


Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205


Phe Asn Arg Gly Glu Cys
    210


<210>  90
<211>  126
<212>  PRT
<213>  Artificial
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<220>
<223>  heavy chain variable region sequence for an anti-TNF-alpha
      antibody

<400>  90

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30


Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Ile Ile Ser Phe Asp Gly Ser Asn Lys Ser Ser Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Tyr Ser Arg Arg Asn Ser Lys Asn Ala Leu Phe
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Phe Tyr Cys
                85                  90                  95


Ala Arg Asp Arg Gly Val Ser Ala Gly Gly Asn Tyr Tyr Tyr Tyr Gly
            100                 105                 110


Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125


<210>  91
<211>  108
<212>  PRT
<213>  Artificial

<220>
<223>  light chain variable region sequence for an anti-TNF-alpha
      antibody

<400>  91

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15


Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45


Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
```

```
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
                85                  90                  95


Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Leu
            100                 105


<210>  92
<211>  467
<212>  PRT
<213>  Artificial

<220>
<223>  amino acid sequence of human TNFR-p75-Fc dimeric fusion protein

<400>  92

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
1               5                   10                  15


Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys
            20                  25                  30


Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr
        35                  40                  45


Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
    50                  55                  60


Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
65                  70                  75                  80


Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
                85                  90                  95


Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
            100                 105                 110


Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala
        115                 120                 125


Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
    130                 135                 140


Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
145                 150                 155                 160


Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala
                165                 170                 175


Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
            180                 185                 190
```

```
His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr
        195                 200                 205

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
    210                 215                 220

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460
```

```
Pro Gly Lys
465


<210>  93
<211>  39
<212>  PRT
<213>  Artificial

<220>
<223>  GLP-1 agonist peptide amino acid sequence (exenatide)

<400>  93

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
1               5                   10                  15


Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
            20                  25                  30


Ser Gly Ala Pro Pro Pro Ser
        35


<210>  94
<211>  33
<212>  PRT
<213>  Artificial

<220>
<223>  GLP-1 agonist peptide amino acid sequence (Liraglutide)

<400>  94

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                   10                  15


Gln Ala Ala Lys Glu Glu Phe Ile Ile Ala Trp Leu Val Lys Gly Arg
            20                  25                  30


Gly



<210>  95
<211>  191
<212>  PRT
<213>  Artificial

<220>
<223>  human growth hormone (somatotropin)

<400>  95

Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1               5                   10                  15


Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
            20                  25                  30


Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
        35                  40                  45
```

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50 55 60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65 70 75 80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
85 90 95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

<210> 96
<211> 5
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 96

Gly Gly Gly Gly Ser
1 5

<210> 97
<211> 10
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 97

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> 98
<211> 15

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<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  98

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1               5                   10                  15


<210>  99
<211>  8
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  99

Gly Gly Gly Gly Ser Gly Gly Gly
1               5


<210>  100
<211>  4
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  100

Ala Ala Pro Phe
1


<210>  101
<211>  3
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  101

Gly Gly Phe
1


<210>  102
<211>  4
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  102

Ala Ala Pro Val
1
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<210> 103
<211> 3
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 103

Gly Gly Leu
1

<210> 104
<211> 3
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 104

Ala Ala Leu
1

<210> 105
<211> 3
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 105

Phe Val Arg
1

<210> 106
<211> 3
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 106

Val Gly Arg
1

<210> 107
<211> 4
<212> PRT
<213> Artificial

<220>
<223> peptide

<400> 107

Arg Lys Pro Arg
1

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<210>  108
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  peptide     X = any amino acid


<220>
<221>  misc_feature
<222>  (5)..(5)
<223>  Xaa can be any naturally occurring amino acid

<400>  108

Tyr Val Ala Asp Xaa
1               5


<210>  109
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  peptide     X = any amino acid


<220>
<221>  misc_feature
<222>  (2)..(3)
<223>  Xaa can be any naturally occurring amino acid

<220>
<221>  misc_feature
<222>  (5)..(5)
<223>  Xaa can be any naturally occurring amino acid

<400>  109

Asp Xaa Xaa Asp Xaa
1               5


<210>  110
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  peptide      X = any amino acid   N= 0, 2, 4 or 6


<220>
<221>  misc_feature
<222>  (2)..(2)
<223>  Xaa can be any naturally occurring amino acid

<220>
<221>  misc_feature
<222>  (5)..(5)
<223>  Xaa can be any naturally occurring amino acid

<400>  110
```

```
Arg Xaa Asn Arg Xaa
1               5


<210>  111
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  peptide     X = any amino acid   N = 0, 2, 4 or 6


<220>
<221>  misc_feature
<222>  (2)..(2)
<223>  Xaa can be any naturally occurring amino acid

<220>
<221>  misc_feature
<222>  (5)..(5)
<223>  Xaa can be any naturally occurring amino acid

<400>  111

Lys Xaa Asn Arg Xaa
1               5


<210>  112
<211>  6
<212>  PRT
<213>  Artificial

<220>
<223>  peptide    X = any amino acid


<220>
<221>  misc_feature
<222>  (6)..(6)
<223>  Xaa can be any naturally occurring amino acid

<400>  112

Glu Arg Thr Lys Arg Xaa
1               5


<210>  113
<211>  5
<212>  PRT
<213>  Artificial

<220>
<223>  peptide   X = any amino acid


<220>
<221>  misc_feature
<222>  (5)..(5)
<223>  Xaa can be any naturally occurring amino acid

<400>  113

Arg Val Arg Arg Xaa
1               5
```

```
<210>  114
<211>  6
<212>  PRT
<213>  Artificial

<220>
<223>  peptide       D = decanoyl   X = any amino acid


<220>
<221>  misc_feature
<222>  (6)..(6)
<223>  Xaa can be any naturally occurring amino acid

<400>  114

Asp Arg Val Arg Arg Xaa
1               5


<210>  115
<211>  6
<212>  PRT
<213>  Artificial

<220>
<223>  peptide      X = any amino acid


<220>
<221>  misc_feature
<222>  (2)..(2)
<223>  Xaa can be any naturally occurring amino acid

<220>
<221>  misc_feature
<222>  (6)..(6)
<223>  Xaa can be any naturally occurring amino acid

<400>  115

Pro Xaa Trp Val Pro Xaa
1               5


<210>  116
<211>  4
<212>  PRT
<213>  Artificial

<220>
<223>  peptide        X = any amino acid


<220>
<221>  misc_feature
<222>  (4)..(4)
<223>  Xaa can be any naturally occurring amino acid

<400>  116

Trp Val Ala Xaa
1


<210>  117
<211>  4
```

<212> PRT
<213> Artificial

<220>
<223> peptide   X = any amino acid

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (3)..(4)
<223> Xaa can be any naturally occurring amino acid

<400> 117

Xaa Phe Xaa Xaa
1

<210> 118
<211> 4
<212> PRT
<213> Artificial

<220>
<223> peptide   X = any amino acid

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (3)..(4)
<223> Xaa can be any naturally occurring amino acid

<400> 118

Xaa Tyr Xaa Xaa
1

<210> 119
<211> 4
<212> PRT
<213> Artificial

<220>
<223> peptide   X = any amino acid

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (3)..(4)
<223> Xaa can be any naturally occurring amino acid

<400> 119

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Xaa Trp Xaa Xaa
1


<210>  120
<211>  16
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  120

Asp Arg Tyr Ile Pro Phe His Leu Leu Val Ala Pro Tyr Ser Pro Ala
1               5                   10                  15


<210>  121
<211>  15
<212>  PRT
<213>  Artificial

<220>
<223>  peptide

<400>  121

Gly Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu Tyr Phe Gln Ser
1               5                   10                  15


<210>  122
<211>  590
<212>  PRT
<213>  Artificial

<220>
<223>  amino acid sequence of a Cholix415-TEV-IL-10 fusion molecule

<400>  122

Met Val Glu Glu Ala Leu Asn Ile Phe Asp Glu Cys Arg Ser Pro Cys
1               5                   10                  15


Ser Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile
            20                  25                  30


Pro Gly Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr
        35                  40                  45


Ile Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser
    50                  55                  60


Ile Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr
65                  70                  75                  80


Val Ser Gln Asp Ala Pro Phe Gly Val Ile Asn Leu Asp Ile Thr Thr
                85                  90                  95


Glu Asn Gly Thr Lys Thr Tyr Ser Phe Asn Arg Lys Glu Ser Glu Phe
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            100                 105                 110

Ala Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile
        115                 120                 125

Lys Ile Ser Ile Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val
    130                 135                 140

Pro Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp
145                 150                 155                 160

Lys Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn
                165                 170                 175

Ile Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu
            180                 185                 190

Gly Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu
        195                 200                 205

Cys Trp Leu Val Pro Ile Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln
    210                 215                 220

Asn Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val
225                 230                 235                 240

Ala Gly Thr Pro Lys Ala Ile Thr Val Lys Gln Gly Ile Glu Gln Lys
                245                 250                 255

Pro Val Glu Gln Arg Ile His Phe Ser Lys Lys Asn Ala Met Glu Ala
            260                 265                 270

Leu Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg
        275                 280                 285

Ser Arg Lys Pro Arg Asp Leu Pro Asp Asp Leu Ser Cys Ala Tyr Asn
    290                 295                 300

Ala Gln Gln Ile Val Ser Leu Phe Leu Ala Thr Arg Ile Leu Phe Thr
305                 310                 315                 320

His Ile Asp Ser Ile Phe Thr Leu Asn Leu Asp Gly Gln Glu Pro Glu
                325                 330                 335

Val Ala Glu Arg Leu Asp Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro
            340                 345                 350

Gly Met Val Ile Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp
        355                 360                 365

Tyr Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly
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370 375 380

Ala Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys
385 390 395 400

Ser Cys Val Ala Ser Asn Ser Asp Gln Ala Asn Ile Asn Ile Glu Ser
405 410 415

Gly Gly Gly Gly Ser Gly Gly Gly Glu Asn Leu Tyr Phe Gln Ser Pro
420 425 430

Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro Gly Asn
435 440 445

Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg Val Lys
450 455 460

Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu Lys Glu
465 470 475 480

Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala Leu Ser
485 490 495

Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala Glu Asn
500 505 510

Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu Asn Leu
515 520 525

Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys
530 535 540

Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe Asn Lys
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Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp Ile Phe
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Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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Ser Leu Thr Pro Glu Pro Gly Lys Pro Ile Gln Ser Lys Leu Ser Ile
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Pro Gly Asp Val Val Leu Asp Glu Gly Val Leu Tyr Tyr Ser Met Thr
        35                  40                  45

Ile Asn Asp Glu Gln Asn Asp Ile Lys Asp Glu Asp Lys Gly Glu Ser
    50                  55                  60

Ile Ile Thr Ile Gly Glu Phe Ala Thr Val Arg Ala Thr Arg His Tyr
65                  70                  75                  80

Val Ser Gln Asp Ala Pro Phe Gly Val Ile Asn Leu Asp Ile Thr Thr
                85                  90                  95

Glu Asn Gly Thr Lys Thr Tyr Ser Phe Asn Arg Lys Glu Ser Glu Phe
            100                 105                 110

Ala Ile Asn Trp Leu Val Pro Ile Gly Glu Asp Ser Pro Ala Ser Ile
        115                 120                 125

Lys Ile Ser Ile Asp Glu Leu Asp Gln Gln Arg Asn Ile Ile Glu Val
    130                 135                 140

Pro Lys Leu Tyr Ser Ile Asp Leu Asp Asn Gln Thr Leu Glu Gln Trp
145                 150                 155                 160

Lys Thr Gln Gly Asn Val Ser Phe Ser Val Thr Arg Pro Glu His Asn
                165                 170                 175

Ile Ala Ile Ser Trp Pro Ser Val Ser Tyr Lys Ala Ala Gln Lys Glu
            180                 185                 190

Gly Ser Arg His Lys Arg Trp Ala His Trp His Thr Gly Leu Ala Leu
        195                 200                 205

Cys Trp Leu Val Pro Ile Asp Ala Ile Tyr Asn Tyr Ile Thr Gln Gln
    210                 215                 220

Asn Cys Thr Leu Gly Asp Asn Trp Phe Gly Gly Ser Tyr Glu Thr Val
225                 230                 235                 240

Ala Gly Thr Pro Lys Ala Ile Thr Val Lys Gln Gly Ile Glu Gln Lys
                245                 250                 255

Pro Val Glu Gln Arg Ile His Phe Ser Lys Lys Asn Ala Met Glu Ala
            260                 265                 270

Leu Ala Ala His Arg Val Cys Gly Val Pro Leu Glu Thr Leu Ala Arg
        275                 280                 285
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Ser Arg Lys Pro Arg Asp Leu Pro Asp Asp Leu Ser Cys Ala Tyr Asn
    290                 295                 300

Ala Gln Gln Ile Val Ser Leu Phe Leu Ala Thr Arg Ile Leu Phe Thr
305                 310                 315                 320

His Ile Asp Ser Ile Phe Thr Leu Asn Leu Asp Gly Gln Glu Pro Glu
                325                 330                 335

Val Ala Glu Arg Leu Asp Asp Leu Arg Arg Ile Asn Glu Asn Asn Pro
            340                 345                 350

Gly Met Val Ile Gln Val Leu Thr Val Ala Arg Gln Ile Tyr Asn Asp
        355                 360                 365

Tyr Val Thr His His Pro Gly Leu Thr Pro Glu Gln Thr Ser Ala Gly
    370                 375                 380

Ala Gln Ala Ala Asp Ile Leu Ser Leu Phe Cys Pro Asp Ala Asp Lys
385                 390                 395                 400

Ser Cys Val Ala Ser Asn Ser Asp Gln Ala Asn Ile Asn Ile Glu Ser
                405                 410                 415

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Pro
            420                 425                 430

Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro Gly Asn
        435                 440                 445

Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg Val Lys
    450                 455                 460

Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu Lys Glu
465                 470                 475                 480

Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala Leu Ser
                485                 490                 495

Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala Glu Asn
            500                 505                 510

Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu Asn Leu
        515                 520                 525

Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys
    530                 535                 540

Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe Asn Lys
545                 550                 555                 560
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Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp Ile Phe
                565                 570                 575

Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
            580                 585                 590
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