(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 March 2006 (23.03.2006)

PCT

(10) International Publication Number
WO 2006/031291 A2

(51) International Patent Classification:
C12N 9/36 (2006.01) *C12N 1/21* (2006.01)
C07H 21/04 (2006.01) *C12N 15/75* (2006.01)

(21) International Application Number:
PCT/US2005/025831

(22) International Filing Date: 21 July 2005 (21.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/939,262 10 September 2004 (10.09.2004) US

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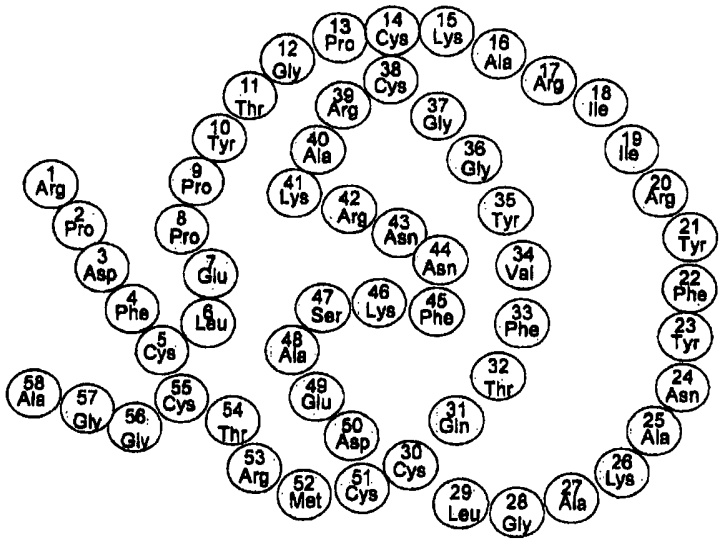
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** A NOVEL CLASS OF THERAPEUTIC PROTEIN BASED MOLECULES



(57) **Abstract:** The present invention provides new compositions and methods for preventing and treating pathogen infection. In particular, the present invention provides compounds having an anchoring domain that anchors the compound to the surface of a target cell, and a therapeutic domain that can act extracellularly to prevent infection of a target cell by a pathogen, such as a virus. The present invention also comprises therapeutic compositions having sialidase activity, including protein-based compounds having sialidase catalytic domains. Compounds of the invention can be used for treating or preventing pathogen infection, and for treating and reducing allergic and inflammatory responses. The invention also provides compositions and methods for enhancing transduction of target cells by recombinant viruses. Such compositions and methods can be used in gene therapy.

WO 2006/031291 A2

A NOVEL CLASS OF THERAPEUTIC PROTEIN BASED MOLECULES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to United States patent application serial number 10/939,262 filed 10 September 2004, entitled, "Novel Class of Therapeutic Protein Based Molecules," which is herein incorporated by reference in its entirety.

The present application also incorporates by reference in their entirety the following patent applications: United States application 10/718,986, filed 21 November 2003, entitled "Broad spectrum anti-viral therapeutics and prophylaxis", United States provisional patent application serial number 60/428,535, filed 22 November 2002, entitled "Broad spectrum anti-viral therapeutics and prophylaxis", United States provisional patent application serial number 60/464,217, filed 19 April 2003, entitled "Class of broad spectrum anti-viral protein", United States provisional patent application serial number 60/561,749, filed 13 April 13 2004, entitled "Anti-microbial therapeutics and prophylaxis", United States provisional patent application serial number 60/580,084, filed 16 June 16, 2004, entitled "Class of broad spectrum anti-microbial agents".

BACKGROUND OF THE INVENTION

The invention relates to therapeutic compositions that can be used to prevent and treat infection of human and animal subjects by a pathogen, and specifically to protein-based therapeutic compositions that can be used for the prevention and treatment of viral or bacterial infections. The invention also relates to therapeutic protein-based compositions that can be used to prevent or ameliorate allergic and inflammatory responses. The invention also relates to protein-based compositions for increasing transduction efficiency of a recombinant virus, such as a recombinant virus used for gene therapy.

Influenza is a highly infectious acute respiratory disease that has plagued the human race since ancient times. It is characterized by recurrent annual epidemics and periodic major worldwide pandemics. Because of the high disease-related morbidity and mortality, direct and indirect social economic impacts of influenza are enormous. Yearly epidemics cause approximately 300,000 hospitalizations and 25,000 deaths in the United States alone. Four pandemics occurred in the last century; together they caused tens of millions of deaths. Mathematical models based on earlier pandemic experiences have estimated that 89,000-207,000 deaths, 18-42 million outpatient visits and 20-47 million additional illnesses will occur during the next pandemic (Meltzer, MI, Cox, NJ and Fukuda, K. (1999) *Emerg Infect Dis* 5:659-671).

Influenza is typically caused by infection of two types of viruses, *Influenza virus A* and *Influenza virus B* (the third type *Influenza virus C* only causes minor common cold like symptoms). They belong to the *orthomyxoviridae* family of RNA viruses. Both type A and type B viruses have 8 segmented negative-strand RNA genomes enclosed in a lipid envelope derived from the host cell. The viral envelope is covered with spikes that are composed of three types of proteins: hemagglutinin (HA) which attaches virus to host cell receptors and mediates fusion of viral and cellular membranes; neuraminidase (NA) which facilitates the release of the new viruses from host cells; and a small number of M2 proteins which serve as ion channels.

Infections by influenza type A and B viruses are typically initiated at the mucosal surface of the upper respiratory tract. Viral replication is primarily limited to the upper respiratory tract but can extend to the lower respiratory tract and cause bronchopneumonia that can be fatal.

Influenza viral protein hemagglutinin (HA) is the major viral envelope protein. It plays an essential role in viral infection. The importance of HA is evidenced by the fact that it is the major target for protective neutralizing antibodies produced by the host immune response (Hayden, FG. (1996) In *Antiviral drug resistance* (ed. D. D. Richman), pp. 59-77. Chichester, UK: John Wiley & Sons Ltd.). It is now clear that HA has two different functions in viral infection. First, HA is responsible for the attachment of the virus to sialic acid cell receptors. Second, HA mediates viral entry into target cells by triggering fusion of the viral envelope with cellular membranes.

HA is synthesized as a precursor protein, HA0, which is transferred through the Golgi apparatus to the cell surface as a trimeric molecular complex. HA0 is further cleaved to generate the C terminus HA1 (residue 328 of HA0) and the N terminus of HA2. It is generally believed that the cleavage occurs at the cell surface or on released viruses. The cleavage of HAO into HA1/HA2 is not required for HA binding to sialic acid receptor; however, it is believed to be necessary for viral infectivity (Klenk, HD and Rott, R. (1988) *Adv Vir Res.* 34:247-281; Kido, H, Niwa, Y, Beppu, Y and Towatari, T. (1996) *Advan Enzyme Regul* 36:325-347; Skehel, JJ and Wiley, DC. (2000) *Annu Rev Biochem* 69:531-569; Zambon, M. (2001*) Rev Med Virol* 11:227-241.)

Currently, influenza is controlled by vaccination and anti-viral compounds. Inactivated influenza vaccines are now in worldwide use, especially in high-risk groups. The vaccine viruses are grown in fertile hen's eggs, inactivated by chemical means and purified. The vaccines are usually trivalent, containing representative influenza A viruses (H1N1 and H3N2) and influenza B strains. The vaccine strains need to be regularly updated in order to maintain efficacy; this effort is coordinated by the World Health Organization (WHO). During inter-pandemic periods, it usually takes 8 months before the updated influenza vaccines are ready for the market (Wood, J. (2001) *Phil Trans R Soc Lond B* 356:1953-1960). However, historically, pandemics spread to most continents within 6 months, and future pandemics are expected to spread even faster with increased international travel (Gust, ID, Hampson, AW., and Lavanchy, D. (2001) *Rev Med Virol* 11:59-70). Therefore it is inevitable that an effective vaccine will be unavailable or in very short supply during the first waves of future pandemics.

Anti-viral compounds have become the mainstay for treating inter-pandemic diseases. Currently, they are also the only potential alternative for controlling pandemics during the initial period when vaccines are not available. Two classes of antiviral compounds are currently on the market: the M2 inhibitors, such as amantadine and rimantadine; and the NA inhibitors, which include oseltamivir (Tamiflu) and zanamivir (Relenza). Both classes of molecules have proven efficacy in prevention and treatment of influenza. However, side effects and the risk of generating drug-resistant viruses remain the top two concerns for using them widely as chemoprophylaxis (Hayden, FG. (1996) In *Antiviral drug resistance* (ed. D. D. Richman), pp. 59-77. Chichester, UK: John Wiley

& Sons Ltd.). Most importantly, future pandemic strains, either evolved naturally or artificially created by genetic engineering in bio-warfare, may be resistant to all the available anti-viral compounds, and this will have devastating consequences globally.

In summary, currently available vaccination and anti-viral compounds are limited by some fundamental shortcomings. Novel therapeutic and prophylactic modalities are needed to address future influenza pandemics.

Respiratory tract infections (RTIs) are the most common, and potentially most severe, types of infectious diseases. Clinically, RTIs include sinusitis, otitis, laryngitis, bronchitis and pneumonia. Based on numerous etiology and epidemiology studies, it is clear that although many microorganisms have the potential to cause RTIs, only a handful of pathogens are responsible for vast majority of the cases. Such pathogens include *S. pneumoniae, M. pneumoniae, H. influenzae, M. catarrhalis,* influenza A & B, and parainfluenza virus. Besides causing CAP and AECB, several of the bacterial pathogens, such as *S. pneumoniae* and *H. influenzae,* are also the common cause of acute sinusitis, otitis media, as well as invasive infections leading to sepsis, meningitis, etc. Therefore these microorganisms are of the highest clinical importance.

One common feature of all respiratory pathogenic bacteria is that they establish commensal colonization on the mucosal surface of the upper airway; such colonization precedes an infection and is prerequisite for infections. The bacterial colonization in a neonate occurs shortly after birth. During lifetime, the upper airway, specifically the nasopharynx and oropharynx, remains a dynamic ecological reservoir of microbial species with bacteria being acquired, eliminated and re-acquired continually. In most cases the bacterial flora in the pharynx is harmless. However, when the condition of the host is altered, some microorganisms may invade adjacent tissues or bloodstream to cause diseases. In addition to serving as the port of entry for mucosal and invasive infections by both bacteria and viruses, the nasopharynx is also the major source of spreading the pathogenic microorganisms between individuals, as well as the reservoir where antibiotic-resistant bacteria are selected (Garcia-Rodriguez and Martinez, J Antimicrob Chemother, (2002) 50(Suppl S2), 59-73; Soriano and Rodriguez-Cerrato, J Antimicrob Chemother, (2002) 50 Suppl S2, 51-58). It is well established clinically

that individuals who are prone to RTIs tend to be persistent and recurrent carriers of the pathogenic bacteria (Garcia-Rodriguez and Martinez, J Antimicrob Chemother, (2002) 50(Suppl S2), 59-73; Mbaki et al., Tohoku J Exp. Med., (1987) 153(2), 111-121).

Helicobacter pylori is a human pathogen implicated in gastritis and peptic ulcer. The bacterium resides in the human stomach and binds to epithelial cells of the gastric antrum. It has been demonstrated that the bacterial adhesion is mediated by binding of *Helicobacter pylori* adhesin I and II to sialic acids on the epithelial surface.

Siglecs (sialic acid binding Ig-like lectins) are members of the immunoglobulin (Ig) superfamily that bind to sialic acid and are mainly expressed by cells of the hematopoietic system. At least 11 siglecs have been discovered and they seem to exclusively recognize cell surface sialic acid as the ligand. It is believed that the binding of siglecs to sialic acid mediates cell-cell adhesion and interactions (Crocker and Varki, Trends Immunol., (2001) 22(6), 337-342; Angata and Brinkman-Van der Linden, Biochim. Biophys. Acta, (2002) 1572(2-3), 294-316). Siglec-8 (SAF-2) is an adhesion molecule that is highly restricted to the surface of eosinophils, basophils, and mast cells, which are the central effector cells in allergic conditions including allergic rhinitis, asthma and eczema. Siglec-8 is considered to be responsible for mediating the recruitment of the three allergic cell types to the airway, the lungs and other sites of allergy. Siglec-1 (sialoadhesion) and siglec-2 (CD22) are the adhesion molecules on macrophages and B cells, both types of cells play central roles in immune reactions that lead to inflammation.

Recombinant viruses, in particular adeno-associated virus (AAV), can be used to transfer the wild type cystic fibrosis transmembrane conductance regulator (CFTR) gene into the epithelial cells to correct the genetic defect that causes cystic fibrosis (Flotte and Carter, Methods Enzymol., (1998) 292, 717-732). Clinical trials with AAV vectors have shown efficient and safe delivery of the CFTR gene into epithelial cells with low levels of gene transfer (Wagner et al., Lancet, (1998) 351(9117), 1702-1703). Compared to adenoviral vectors, AAV offers more stable gene expression and diminished cellular immunity. However, the transduction efficiency of AAV in vivo is rather low in the lung

(Wagner et al., Lancet, (1998) 351(9117), 1702-1703). A method that can improve transduction efficiency of AAV in vivo is needed to achieve full therapeutic potential of gene therapy for cystic fibrosis. It has been shown that negatively charged carbohydrates, such as sialic acid, inhibit the transduction efficiency of AAV vector to the well-differentiated airway epithelium, and treatment of the airway epithelium by glycosidases, including a neuraminidase, and endoglycosidase H, enhances transduction efficiency of the AAV vector (Bals et al., J Virol., (1999) 73(7), 6085-6088).

BRIEF SUMMARY OF THE INVENTON

The present invention recognizes that current therapeutics for preventing and treating infection by pathogens are often difficult to provide in a timely manner, can have undesirable side effects, and can lead to drug-resistant pathogen strains. The present invention also recognizes that the current approach to treat allergy and inflammation has limited efficacy and is associated with side effects. In addition, the present invention also recognizes that the current approach to administer recombinant viruses yield low transduction efficiency and unsatisfactory efficacy of the gene therapy.

The present invention provides new compositions and methods for preventing and treating pathogen infection. In particular, the present invention provides compounds that can act extracellularly to prevent infection of a cell by a pathogen. Some preferred embodiments of the present invention are therapeutic compounds having an anchoring domain that anchors the compound to the surface of a target cell, and a therapeutic domain that can act extracellularly to prevent infection of the target cell by a pathogen, such as a virus or bacterium.

In one aspect, the invention provides a protein-based composition for preventing or treating infection by a pathogen. The composition comprises a compound that comprises at least one therapeutic domain comprising a peptide or protein, where the therapeutic domain has at least one extracellular activity that can prevent the infection of a target cell by a pathogen, and at least one anchoring domain that can bind at or near the membrane of a target cell.

In some embodiments of this aspect of the present invention, the at least one therapeutic domain comprises an inhibitory activity that prevents or impedes the infection of a target cell by a pathogen. In a preferred embodiment, the inhibitory activity inhibits the activity of a protease that can process a viral protein necessary for infection of a target cell. In a particularly preferred embodiment, the compound comprises a therapeutic domain that can inhibit the processing of the HA protein of influenza virus, and the anchoring domain can bind the compound at the surface of a respiratory epithelial cell.

In some embodiments of the present invention, at least one therapeutic domain comprises a catalytic activity. In a preferred embodiment, the catalytic activity removes a moiety from the surface of a target cell that is necessary for infection of the target cell. In a particularly preferred embodiment, the therapeutic domain is a sialidase that can digest sialic acid moieties on the surface of epithelial target cells, and the anchoring domain is a GAG-binding domain of a human protein that can bind heparin or heparan sulfate moieties at the surface of an epithelial cell.

In another aspect, the present invention includes pharmaceutical compositions for treating or preventing pathogen infection in a subject. Pharmaceutical compositions comprise a compound of the present invention comprising at least one therapeutic domain and at least one anchoring domain. The pharmaceutical composition can also comprise solutions, stabilizers, fillers and the like. In some preferred embodiments, the pharmaceutical composition is formulated as an inhalant. In some preferred embodiments, the pharmaceutical composition is formulated as a nasal spray.

Another aspect of the present invention is a pharmaceutical composition comprising at least one sialidase. The sialidase can be isolated from any source, such as, for example, a bacterial or mammalian source, or can be a recombinant protein that is substantially homologous to a naturally occurring sialidase. A pharmaceutical composition comprising a sialidase can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops. A pharmaceutical composition comprising a sialidase can be used to treat or prevent pathogen infection, to treat or prevent allergy or inflammatory response, or to enhance the transduction efficiency of a recombinant virus for gene therapy.

Yet another aspect of the present invention is a sialidase catalytic domain protein. In this aspect, proteins that comprise the catalytic domain of a sialidase but comprise less than the entire sialidase the catalytic domain sequence is derived from are considered sialidase catalytic domain proteins. Sialidase catalytic domain proteins can comprise other protein sequences, such as but not limited to functional domains derived from other proteins. A pharmaceutical composition comprising a sialidase can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops. A pharmaceutical composition comprising a sialidase can be used to treat or prevent pathogen infection, to treat or prevent allergy or inflammatory response, or to enhance the transduction efficiency of a recombinant virus for gene therapy.

In yet another aspect, the present invention includes a method for treating or preventing infection by a pathogen. In preferred embodiments, the method comprises administering a siaidase activity, such as a sialidase or a sialidase catalytic domain protein, including a sialidase catalytic domain fusion protein, to a subject to prevent or treat an infection. A pathogen can be, for example, a viral or bacterial pathogen. The method includes applying a pharmaceutically effective amount of a compound of the present invention to at least one target cell of a subject. Preferably, the pharmaceutical composition can applied by the use of a spray, inhalant, or topical formulation.

The present invention also provides new compositions and methods for treating allergy and inflammation. In particular, the present invention provides compounds that can act extracellularly to prevent or inhibit adhesion and function of inflammatory cells. Some preferred embodiments of compounds for treating allergy or inflammation comprise at least one therapeutic domain that has the said extracellular activity and an at least one anchoring domain that anchors the compound to the surface of a target cell. In some preferred embodiments, the method comprises administering a siaidase activity, such as a sialidase or a sialidase catalytic domain protein, including a sialidase catalytic domain fusion protein to a subject to prevent or treat an allergic or inflammatory response. The allergic or inflammatory response can be asthma, allergic rhinitis, skin conditions such as eczema, or response to plant or animal toxins. The method includes applying a pharmaceutically effective amount of a compound of the present invention to

at least one target cell of a subject. Preferably, the pharmaceutical composition can applied by the use of a spray, inhalant, or topical formulation.

The present invention also provides new compositions and methods for improving efficiency of gene transfer by recombinant viral vectors during gene therapy. In particular, the present invention provides compounds that can act extracellularly to reduce the physical or chemical barrier that hinders transduction by gene therapy vectors, such as AAV vector. Some preferred compounds of the present invention for improving efficiency of gene transfer by recombinant viral vectors comprise at least one therapeutic domain that has an extracellular activity and an at least one anchoring domain that anchors the compound to the surface of a target cell. . In some preferred embodiments, the method comprises administering a siaidase activity, such as a sialidase or a sialidase catalytic domain protein, including a sialidase catalytic domain fusion protein to a subject to facilitate transduction of a target cell by a recombinant viral vector. The method includes applying an effective amount of a compound of the present invention along with a recombinant viral vector to at least one target cell. A pharmaceutical composition of the present invention can applied by the use of a spray, inhalant, or topical formulation.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

Figure 1 is a schematic depiction of the primary amino acid structure of aprotinin.

Figure 2 shows GAG-binding sequences of four human genes: PF4, human platelet factor 4; IL8, human interleukin 8; AT III, human antithrombin III; ApoE, human apolipoprotein E; AAMP, human angio-associated migratory cell protein.

Figure 3 is a sequence comparison between human sialidases NEU2 and NEU4.

Figure 4 is a table comparing substrate specificity of bacterial and fungal sialidases.

Figure 5 depicts the nucleotide and amino acid sequences of Construct #1 encoding His6-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

Figure 6 depicts the nucleotide and amino acid sequences of Construct #2 encoding AR-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

Figure 7 depicts the nucleotide and amino acid sequences of Construct #3 encoding AR-G_4S-AvCD. NcoI and HindIII sites used for cloning into pTrc99a are shown in bold.

Figure 8 is a graph of data from an experiment showing that the AR-tag enhances the removal of α(2,6)-linked sialic acid from MDCK cells. The Y axis shows the percentage of α(2,6)-linked sialic acid remaining on the surface of MDCK cells after treatment with various dilutions of recombinant AvCD (Construct #1) (diamonds) or recombinant AR-AvCD (Construct #2) (squares).

Figure 9 is a graph depicting the protection against influenza viruses conferred by treating MDCK cells with recombinant AR-AvCD protein made from Construct #2 or the isolated sialidase of *A. ureafaciens.* The challenge viral strains are: A/WS/33 (H1N1); A/PR/8 (H1N1); A/Japan/305/57 (H2N2); A/Victoria/504/2000 (H3N2); A/HongKong/8/68 (H3N2); B/Lee/40; 7. B/Maryland/1/59; and Turkey/Wis/66 (H9N2).

Figure 10 is a graph showing the level of inhibition of influenza virus amplification by the recombinant AR-AvCD sialidase and the recombinant AR-G_4S-AvCD sialidase. The challenge viral strains are: A/PR/8 (H1N1); A/WS/33 (H1N1); A/Japan/305/57 (H2N2); A/HongKong/8/68 (H3N2); B/Lee/40; 7. B/Maryland/1/59; and Turkey/Wis/66 (H9N2).

Figure 11 provides graphs showing that topical administration of recombinant AR-AvCD sialidase fusion protein reduces the inflammatory responses of ferrets infected with an influenza A (H1N1) virus. (**A**) The total number of inflammatory cells from nasal wash samples obtained from infected animals at the indicated times after infection. (**B**) The protein concentration was determined in cell-free nasal wash samples of infected ferrets. Infected ferrets were vehicle-treated (squares) or were treated with recombinant AR-AvCD sialidase fusion protein made from Construct #2 (triangles). Uninfected animals

were also treated with recombinant AR-AvCD sialidase fusion protein (diamonds). Statistically significant values are labeled with * ($p<0.05$) and ** ($p<0.01$).

Figure 12 is a table depicting inhibition of viral replication, cell protection EC50's, and selective indexes for two sialidase catalytic doman fusion proteins of the present invention. All EC50's are in mU/ml.

Figure 13 is a table depicting viral replication in the respiratory tract of ferrets treated with a sialidase catalytic doman fusion proteins of the present invention and ferrets treated with a control vehicle.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the manufacture or laboratory procedures described below are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references. Where a term is provided in the singular, the inventors also contemplate the plural of that term. Where there are discrepancies in terms and definitions used in references that are incorporated by reference, the terms used in this application shall have the definitions given herein. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

A "pathogen" can be any virus or microorganism that can infect a cell, a tissue or an organism. A pathogen can be a virus, bacterium, or protozoan.

A "target cell" is any cell that can be infected by a pathogen or any cell that can interact with inflammatory cells, or a host cell that is the intended destination for an exogenous gene transferred by a recombinant virus.

A "recombinant virus" or a "recombinant viral vector", a "gene therapy viral vector" or a "gene therapy vector" is defined as a genetically engineered virus that comprises one or more exogenous genes. When a target cell is transduced by a recombinant virus, the exogenous gene(s) is transferred to the target cell. Genes transferred to a target cell can be expressed in the cell to provide the intended therapeutic effects. Currently, most commonly used gene therapy viral vectors are based on four types of viruses: retrovirus (including lentivirus), adeno-virus, adeno-associated virus (AAV) and herpes simplex virus type 1.

"Inflammatory cells" are the cells that carry out or participate in inflammatory responses of the immune system. Inflammatory cells include include B lymphocytes, T lymphocytes, macrophages, basophils, eosinophils, mast cells, NK cells and monocytes.

An "extracellular activity that can prevent the infection of a target cell by a pathogen" is any activity that can block or impede infection of a target cell by a pathogen by acting at or near the exterior surface of a target cell. An extracellular activity that can prevent the infection of a target cell by a pathogen, can be an activity such as, but not limited to, a catalytic activity or an inhibitory activity. For example, a catalytic activity can be an enzymatic activity that degrades one or more entities (such as but not limited to ligands, receptors, or enzymes) on a pathogen, on a target cell, or in the vicinity of a target cell, in which the one or more entities contribute to the infection process. A catalytic activity can also modify one or more entities on a pathogen, on a target cell, or in the vicinity of a target cell, such that the infection-promoting property of the entity is reduced. An inhibitory activity can be an activity that, for example, binds to a receptor or ligand and prevents the receptor or ligand from binding a moiety, where the binding is necessary for or promotes the infection process. An inhibitory activity can also be an inhibitor of an enzyme or receptor that prevents the enzyme or receptor from performing a function that is necessary for or promotes the infection process. The exterior of a target cell includes the target cell membrane itself, as well as the extracellular milieu surrounding the target cell, including extracellular matrix, intracellular spaces, and

luminal spaces. For epithelial cells, the exterior of a target cell also includes the apical or luminal surface of the cell membrane that form luminal linings, and the extracellular milieu near the luminal surface. An "extracellular activity that can prevent the infection of a target cell by a pathogen" can be any type of chemical entity, including a protein, polypeptide, peptide, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, and the like, including combinations of any of these. Preferably, however, the activity comprises a peptide or protein or coupled to a peptide or protein.

An "extracellular activity that can improve transduction efficiency, or gene transfer efficiency, by a recombinant virus" is any activity that reduces or eliminates physical or chemical barriers that impedes host cell entry by a recombinant virus by acting at or near the exterior surface of a target cell. An extracellular activity that can improve transduction efficiency, or gene transfer efficiency, by a recombinant virus can be an activity such as, but not limited to, a catalytic activity or an inhibitory activity. For example, a catalytic activity can be an enzymatic activity that degrades one or more entities (such as but not limited to ligands, receptors, or enzymes) on a pathogen, on a target cell, or in the vicinity of a target cell, in which the one or more entities contribute to the infection process. A catalytic activity can also modify one or more entities on a pathogen, on a target cell, or in the vicinity of a target cell, such that the infection-promoting property of the entity is reduced. An inhibitory activity can be an activity that, for example, binds to a receptor or ligand and prevents the receptor or ligand from binding a moiety, where the binding is necessary for or promotes the infection process. An inhibitory activity can also be an inhibitor of an enzyme or receptor that prevents the enzyme or receptor from performing a function that is necessary for or promotes the infection process. The exterior of a target cell includes the target cell membrane itself, as well as the extracellular milieu surrounding the target cell, including extracellular matrix, intracellular spaces, and luminal spaces. For epithelial cells, the exterior of a target cell also includes the apical or luminal surface of the cell membrane that form luminal linings, and the extracellular milieu near the luminal surface. An "extracellular activity that can prevent the infection of a target cell by a pathogen" can be any type of chemical entity, including a protein, polypeptide, peptide, nucleic acid, peptide nucleic acid,

nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, and the like, including combinations of any of these. Preferably, however, the activity comprises a peptide or protein or coupled to a peptide or protein.

An "extracellular activity that can inhibit adhesion or function of inflammatory cells" is any activity that can prevent inflammatory cells from contacting the target cell and affecting the normal physiological status of the target cell.

A "domain that can anchor said at least one therapeutic domain to the membrane of a target cell", also called an "extracellular anchoring domain" or simply, "anchoring domain" refers to a chemical entity can that can stably bind a moiety that is at or on the exterior of a cell surface or is in close proximity to the surface of a cell. An extracellular anchoring domain can be reversibly or irreversibly linked to one or more moieties, such as, preferably, one or more therapeutic domains, and thereby cause the one or more attached therapeutic moieties to be retained at or in close proximity to the exterior surface of a eukaryotic cell. Preferably, an extracellular anchoring domain binds at least one molecule on the surface of a target cell or at least one molecule found in close association with the surface of a target cell. For example, an extracellular anchoring domain can bind a molecule covalently or noncovalently associated with the cell membrane of a target cell, or can bind a molecule present in the extracellular matrix surrounding a target cell. An extracellular anchoring domain preferably is a peptide, polypeptide, or protein, and can also comprise any additional type of chemical entity, including one or more additional proteins, polypeptides, or peptides, a nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or a combination of any of these.

As used herein, a protein or peptide sequences is "substantially homologous" to a reference sequence when it is either identical to a reference sequence, or comprises one or more amino acid deletions, one or more additional amino acids, or more one or more conservative amino acid substitutions, and retains the same or essentially the same activity as the reference sequence. Conservative substitutions may be defined as exchanges within one of the following five groups:

I. Small, aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly

II. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln

III. Polar, positively charged residues: His, Arg, Lys

IV. Large, aliphatic nonpolar residues: Met, Leu, Ile, Val, Cys

V. Large aromatic residues: Phe, Try, Trp

Within the foregoing groups, the following substitution are considered to be "highly conservative": Asp/Glu, His/Arg/Lys, Phe/Tyr/Trp, and Met/Leu/Ile/Val. Semi-conservative substitutions are defined to be exchanges between two of groups (I)-(IV) above which are limited to supergroup (A), comprising (I), (II), and (III) above, or to supergroup (B), comprising (IV) and (V) above. In addition, where hydrophobic amino acids are specified in the application, they refer to the amino acids Ala, Gly, Pro, Met, Leu, Ile, Val, Cys, Phe, and Trp, whereas hydrophilic amino acids refer to Ser, Thr, Asp, Asn, Glu, Gln, His, Arg, Lys, and Tyr.

A "sialidase" is an enzyme that can remove a sialic acid residue from a substrate molecule. The sialidases (N-acylneuraminosylglycohydrolases, EC 3.2.1.18) are a group of enzymes that hydrolytically remove sialic acid residues from sialo-glycoconjugates. Sialic acids are alpha-keto acids with 9-carbon backbones that are usually found at the outermost positions of the oligosaccharide chains that are attached to glycoproteins and glycolipids. One of the major types of sialic acids is N-acetylneuraminic acid (Neu5Ac), which is the biosynthetic precursor for most of the other types. The substrate molecule can be, as nonlimiting examples, an oligosaccharide, a polysaccharide, a glycoprotein, a ganglioside, or a synthetic molecule. For example, a sialidase can cleave bonds having alpha(2,3)-Gal, alpha(2,6)-Gal, or alpha(2,8)-Gal linkages between a sialic acid residue and the remainder of a substrate molecule. A sialidase can also cleave any or all of the linkages between the sialic acid residue and the remainder of the substrate molecule. Two major linkages between Neu5Ac and the penultimate galactose residues of carbohydrate side chains are found in nature, Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha (2,6)-Gal. Both Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha (2,6)-Gal molecules can be recognized by influenza viruses as the receptor, although human viruses seem to prefer Neu5Ac alpha (2,6)-Gal, avian and equine viruses predominantly recognize Neu5Ac alpha (2,3)-

Gal. A sialidase can be a naturally-occurring sialidase, an engineered sialidase (such as, but not limited to a sialidase whose amino acid sequence is based on the sequence of a naturally-occurring sialidase, including a sequence that is substantially homologous to the sequence of a naturally-occurring sialidase). As used herein, "sialidase" can also mean the active portion of a naturally-occurring sialidase, or a peptide or protein that comprises sequences based on the active portion of a naturally-occurring sialidase.

A "fusion protein" is a protein comprising amino acid sequences from at least two different sources. A fusion protein can comprise amino acid sequence that is derived from a naturally occurring protein or is substantially homologous to all or a portion of a naturally occurring protein, and in addition can comprise from one to a very large number of amino acids that are derived from or substantially homologous to all or a portion of a different naturally occurring protein. In the alternative, a fusion protein can comprise amino acid sequence that is derived from a naturally occurring protein or is substantially homologous to all or a portion of a naturally occurring protein, and in addition can comprise from one to a very large number of amino acids that are synthetic sequences.

A "sialidase catalytic domain protein" is a protein that comprises the catalytic domain of a sialidase, or an amino acid sequence that is substantially homologous to the catalytic domain of a sialidase, but does not comprises the entire amino acid sequence of the sialidase the catalytic domain is derived from, wherein the sialidase catalytic domain protein retains substantially the same activity as the intact sialidase the catalytic domain is derived from. A sialidase catalytic domain protein can comprise amino acid sequences that are not derived from a sialidase, but this is not required. A sialidase catalytic domain protein can comprise amino acid sequences that are derived from or substantially homologous to amino acid sequences of one or more other known proteins, or can comprise one or more amino acids that are not derived from or substantially homologous to amino acid sequences of other known proteins.

I. Composition for preventing or treating infection by a pathogen

The present invention includes peptide or protein-based compounds that comprise at least one domain that can anchor at least one therapeutic domain to the membrane of a eukaryotic cell and at least one therapeutic domain having an extracellular activity that can prevent the infection of a cell by a pathogen. By "peptide or protein-based" compounds, it is meant that the two major domains of the compound have an amino acid framework, in which the amino acids are joined by peptide bonds. A peptide or protein-based compound can also have other chemical compounds or groups attached to the amino acid framework or backbone, including moieties that contribute to the anchoring activity of the anchoring domain, or moieties that contribute to the infection-preventing activity or the therapeutic domain. For example, the protein-based therapeutics of the present invention can comprise compounds and molecules such as but not limited to: carbohydrates, fatty acids, lipids, steroids, nucleotides, nucleotide analogues, nucleic acid molecules, nucleic acid analogues, peptide nucleic acid molecules, small organic molecules, or even polymers. The protein-based therapeutics of the present invention can also comprise modified or non-naturally occurring amino acids. Non-amino acid portions of the compounds can serve any purpose, including but not limited to: facilitating the purification of the compound, improving the solubility or distribution or the compound (such as in a therapeutic formulation), linking domains of the compound or linking chemical moieties to the compound, contributing to the two-dimensional or three-dimensional structure of the compound, increasing the overall size of the compound, increasing the stability of the compound, and contributing to the anchoring activity or therapeutic activity of the compound.

The peptide or protein-based compounds of the present invention can also include protein or peptide sequences in addition to those that comprise anchoring domains or therapeutic domains. The additional protein sequences can serve any purpose, including but not limited to any of the purposes outlined above (facilitating the purification of the compound, improving the solubility or distribution or the compound, linking domains of the compound or linking chemical moieties to the compound, contributing to the two-dimensional or three-dimensional structure of the compound, increasing the overall size

of the compound, increasing the stability of the compound, or contributing to the anchoring activity or therapeutic activity of the compound). Preferably any additional protein or amino acid sequences are part of a single polypeptide or protein chain that includes the anchoring domain or domains and therapeutic domain or domains, but any feasible arrangement of protein sequences is within the scope of the present invention.

The anchoring domain and therapeutic domain can be arranged in any appropriate way that allows the compound to bind at or near a target cell membrane such that the therapeutic domain can exhibit an extracellular activity that prevents or impedes infection of the target cell by a pathogen. The compound will preferably have at least one protein or peptide-based anchoring domain and at least one peptide or protein-based therapeutic domain. In this case, the domains can be arranged linearly along the peptide backbone in any order. The anchoring domain can be N-terminal to the therapeutic domain, or can be C-terminal to the therapeutic domain. It is also possible to have one or more therapeutic domains flanked by at least one anchoring domain on each end. Alternatively, one or more anchoring domains can be flanked by at least one therapeutic domain on each end. Chemical, or preferably, peptide, linkers can optionally be used to join some or all of the domains of a compound.

It is also possible to have the domains in a nonlinear, branched arrangement. For example, the therapeutic domain can be attached to a derivatized side chain of an amino acid that is part of a polypeptide chain that also includes, or is linked to, the anchoring domain.

A compound of the present invention can have more than one anchoring domain. In cases in which a compound has more than one anchoring domain, the anchoring domains can be the same or different. A compound of the present invention can have more than one therapeutic domain. In cases in which a compound has more than one therapeutic domain, the therapeutic domains can be the same or different. Where a compound comprises multiple anchoring domains, the anchoring domains can be arranged in tandem (with or without linkers) or on alternate sides of other domains, such as therapeutic domains. Where a compound comprises multiple therapeutic domains, the therapeutic domains can be arranged in tandem (with or without linkers) or on alternate sides of other domains, such as, but not limited to, anchoring domains.

A peptide or protein-based compound of the present invention can be made by any appropriate way, including purifying naturally occurring proteins, optionally proteolytically cleaving the proteins to obtain the desired functional domains, and conjugating the functional domains to other functional domains. Peptides can also be chemically synthesized, and optionally chemically conjugated to other peptides or chemical moieties. Preferably, however, a peptide or protein-based compound of the present invention is made by engineering a nucleic acid construct to encode at least one anchoring domain and at least one therapeutic domain together (with or without nucleic acid linkers) in a continuous polypeptide. The nucleic acid constructs, preferably having appropriate expression sequences, can be transfected into prokaryotic or eukaryotic cells, and the therapeutic protein-based compound can be expressed by the cells and purified. Any desired chemical moieties can optionally be conjugated to the peptide or protein-based compound after purification. In some cases, cell lines can be chosen for expressing the protein-based therapeutic for their ability to perform desirable post-translational modifications (such as, but not limited to glycosylation).

A great variety of constructs can be designed and their protein products tested for desirable activities (such as, for example, binding activity of an anchoring domain, or a binding, catalytic, or inhibitory activity of a therapeutic domain). The protein products of nucleic acid constructs can also be tested for their efficacy in preventing or impeding infection of a target cell by a pathogen. *In vitro* and *in vivo* tests for the infectivity of pathogens are known in the art, such as those described in the Examples for the infectivity of influenza virus.

Anchoring Domain

As used herein, an "extracellular anchoring domain" or "anchoring domain" is any moiety that can stably bind an entity that is at or on the exterior surface of a target cell or is in close proximity to the exterior surface of a target cell. An anchoring domain serves to retain a compound of the present invention at or near the external surface of a target cell.

An extracellular anchoring domain preferably binds 1) a molecule expressed on the surface of a target cell, or a moiety, domain, or epitope of a molecule expressed on

the surface of a target cell, 2) a chemical entity attached to a molecule expressed on the surface of a target cell, or 3) a molecule of the extracellular matrix surrounding a target cell.

An anchoring domain is preferably a peptide or protein domain (including a modified or derivatized peptide or protein domain), or comprises a moiety coupled to a peptide or protein. A moiety coupled to a peptide or protein can be any type of molecule that can contribute to the binding of the anchoring domain to an entity at or near the target cell surface, and is preferably an organic molecule, such as, for example, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or any combination of any of these.

A molecule, complex, domain, or epitope that is bound by an anchoring domain may or may not be specific for the target cell. For example, an anchoring domain may bind an epitope present on molecules on or in close proximity to the target cell and that occur at sites other than the vicinity of the target cell as well. In many cases, however, localized delivery of a therapeutic compound of the present invention will restrict its occurrence primarily to the surface of target cells. In other cases, a molecule, complex, moiety, domain, or epitope bound by an anchoring domain may be specific to a target tissue or target cell type.

Target tissue or target cell type includes the sites in an animal or human body where a pathogen invades or amplifies. For example, a target cell can be an endothelial cell that can be infected by a pathogen. A composition of the present invention can comprise an anchoring domain that can bind a cell surface epitope, for example, that is specific for the endothelial cell type. In another example, a target cell can be an epithelial cell and a composition of the present invention can bind an epitope present on the cell surface of many epithelial cell types, or present in the extracellular matrix of different types of epithelial cells. In this case localized delivery of the composition can restrict its localization to the site of the epithelial cells that are targets of the pathogen.

A compound for preventing or treating infection by a pathogen can comprise an anchoring domain that can bind at or near the surface of epithelial cells. For example, heparan sulfate, closely related to heparin, is a type of glycosaminoglycan (GAG) that is

ubiquitously present on cell membranes, including the surface of respiratory epithelium. Many proteins specifically bind to heparin/heparan sulfate, and the GAG-binding sequences in these proteins have been identified (Meyer, FA, King, M and Gelman, RA. (1975) *Biochimica et Biophysica Acta* 392: 223-232; Schauer, S. ed., pp233. Sialic Acids Chemistry, Metabolism and Function. Springer-Verlag, 1982). For example, the GAG-binding sequences of human platelet factor 4 (PF4) (**SEQ ID NO:2**), human interleukin 8 (IL8) (**SEQ ID NO:3**), human antithrombin III (AT III) (**SEQ ID NO:4**), human apoprotein E (ApoE) (**SEQ ID NO:5**), human angio-associated migratory cell protein (AAMP) (**SEQ ID NO:6**), or human amphiregulin (**SEQ ID NO:7**) (**Figure 2**) have been shown to have very high affinity (in the nanomolar range) towards heparin (Lee, MK and Lander, AD. (1991) Pro Natl Acad Sci USA 88:2768-2772; Goger, B, Halden, Y, Rek, A, Mosl, R, Pye, D. Gallagher, J and Kungl, AJ. (2002) Biochem. 41:1640-1646; Witt, DP and Lander AD (1994) Curr Bio 4:394-400; Weisgraber, KH, Rall, SC, Mahley, RW, Milne, RW and Marcel, Y. (1986) J Bio Chem 261:2068-2076). The GAG-binding sequences of these proteins are distinct from their receptor-binding sequences, so they will not induce the biological activities associated with the full-length proteins or the receptor-binding domains. These sequences, or other sequences that have been identified or are identified in the future as heparin/heparan sulfate binding sequences, or sequences substantially homologous to identified heparin/heparan sulfate binding sequences that have heparin/heparan sulfate binding activity, can be used as epithelium-anchoring-domains in compounds of the present invention that can be used to prevent or treat, for example, respiratory epithelium-infecting viruses such as, but not limited to, influenza virus.

An anchoring domain can bind a moiety that is specific to the target cell type of a particular species or can bind a moiety that is found in the target cell type of more than one species. In cases where the anchoring domain can bind moieties that are present at the surface of target cells of more than one species, and a virus or pathogen can infect more than one species, a therapeutic compound can have utility for more than one species (providing that the therapeutic domain is also effective across the relevant species.) For example, in the case of therapeutic compounds that can be used against influenza virus, a therapeutic compound of the present invention that has an anchoring domain that binds

heparin/heparan sulfate, the compound can be used in mammals (including humans) as well as avians.

Therapeutic Domain

A compound of the present invention includes at least one therapeutic domain that has an extracellular activity that can prevent or impede the infection of a cell by a pathogen, can modulate the immune response of a subject, or can improve transduction efficiency of a recombinant virus. The therapeutic activity can be, as nonlimiting examples, a binding activity, a catalytic activity, or an inhibitory activity. In some embodiments of the present invention, the therapeutic activity acts to modify or inhibit a function of the pathogen that contributes to infectivity of the cell by the pathogen. In other embodiments, a therapeutic domain can modify or inhibit a function of the target cell or target organism.

For example, the therapeutic domain can bind a receptor on a target cell that is necessary for binding of the pathogen to a target cell. In this way the therapeutic moiety can block binding of the pathogen to a target cell and prevent infection. In an alternative, a therapeutic domain can bind a molecule or epitope on a pathogen to prevent an interaction of the molecule or epitope with a target cell that is necessary for infection. A therapeutic domain can also have a catalytic activity that can degrade a molecule or epitope of the pathogen or host that allows for or promotes infection of a target cell by a host. In yet other embodiments, a therapeutic domain can be an inhibitor of an activity that is necessary for target cell infection by a pathogen. The inhibited activity can be an activity of the host organism or of the pathogen.

The therapeutic domain preferably acts extracellularly, meaning that its infection-preventing, inflammatory response-modulating, or transduction-enhancing activity takes place at the target cell surface or in the immediate area surrounding the target cell, including sites within the extracellular matrix, intracellular spaces, or luminal spaces of tissues.

A therapeutic domain is preferably a peptide or protein domain (including a modified or derivatized peptide or protein domain), or comprises a moiety coupled to a peptide or protein. A moiety coupled to a peptide or protein can be any type of molecule

that can prevent or impede the infection of a target cell by a pathogen, and is preferably an organic molecule, such as, for example, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipids, steroid, fatty acid, carbohydrate, or any combination of any of these.

A therapeutic domain can be a synthetic peptide or polypeptide, or can comprise a synthetic molecule that can be conjugated to a peptide or polypeptide, can be a naturally-occurring peptide or protein, or a domain of naturally-occurring protein. A therapeutic domain can also be a peptide or protein that is substantially homologous to a naturally-occurring peptide or protein.

A therapeutic domain can have utility in a particular species, or can prevent or impede pathogen infection in more than one species. For example, therapeutic domains that inhibit pathogen functions can in general be used in a range of species that can be infected by the host, while therapeutic domains that interrupt host-pathogen interactions by interfering with a property of the host may or may not be species-specific. In many cases, anchoring domains and therapeutic domains can be effective in more than one species, so that compounds of the present invention can be used to advance human and animal health, while reducing propagation and spread of the virus through animal hosts. For example, when the therapeutic domain is a sialidase, a sialidase that can cleave more than one type of linkage between a sialic acid residue and the remainder of a substrate molecule, in particular, a sialidase that can cleave both alpha(2, 6)-Gal and alpha (2, 3)-Gal linkages, can protect humans from infections by a broad-spectrum of influenza viruses, including viruses that are naturally hosted in different species such as birds, pigs or horses.

Linkers

A compound of the present invention can optionally include one or more linkers that can join domains of the compound. Linkers can be used to provide optimal spacing or folding of the domains of a compound. The domains of a compound joined by linkers can be therapeutic domains, anchoring domains, or any other domains or moieties of the compound that provide additional functions such as enhancing compound stability, facilitating purification, etc. A linker used to join domains of compounds of the present

invention can be a chemical linker or an amino acid or peptide linker. Where a compound comprises more than one linker, the linkers can be the same or different. Where a compound comprises more than one linker, the linkers can be of the same or different lengths.

Many chemical linkers of various compositions, polarity, reactivity, length, flexibility, and cleavability are known in the art of organic chemistry. Preferred linkers of the present invention include amino acid or peptide linkers. Peptide linkers are well known in the art. Preferably linkers are between one and one hundred amino acids in length, and more preferably between one and thirty amino acids in length, although length is not a limitation in the linkers of the compounds of the present invention. Preferably linkers comprise amino acid sequences that do not interfere with the conformation and activity of peptides or proteins encoded by monomers of the present invention. Some preferred linkers of the present invention are those that include the amino acid glycine. For example, linkers having the sequence:

(GGGGS (**SEQ ID NO:10**))n, where n is a whole number between 1 and 20, or more preferably between 1 and 12, can be used to link domains of therapeutic compounds of the present invention.

The present invention also comprises nucleic acid molecules that encode protein-based compounds of the present invention that comprise at least one therapeutic domain and at least one anchoring domain. The nucleic acid molecules can have codons optimized for expression in particular cell types, such as, for example E. coli or human cells. The nucleic acid molecules or the present invention that encode protein-based compounds of the present invention that comprise at least one therapeutic domain and at least one anchoring domain can also comprise other nucleic acid sequences, including but not limited to sequences that enhance gene expression. The nucleic acid molecules can be in vectors, such as but not limited to expression vectors.

Composition comprising at least one anchoring domain and at least one protease inhibitor

In some aspects of the present invention, a therapeutic domain that has an extracellular activity that can prevent the infection of a cell by a pathogen is a protease

inhibitor. The protease inhibitor can be any type of chemical entity, such as, for example, a carbohydrate or polymer, but is preferably a protein or peptide that inhibits the activity of an enzyme. Preferably, the protease inhibitor inhibits the activity of an enzyme that at least partially processes at least one pathogen or host cell protein, where the processing of the pathogen or host cell protein is necessary for pathogen infectivity. The enzyme that can process a viral protein necessary for pathogen infectivity can be a pathogen enzyme, or an enzyme that originates from the host organism. Preferably, the processing enzyme acts at or near the target cell surface, so that a compound of the present invention that is anchored at or near the surface of a target cell can effectively inhibit the activity of the enzyme.

Compounds of the present invention that comprise protease inhibitory domains can be used to inhibit infection by any pathogen that requires a protease in its life cycle, in which the protease is active at or near the surface of the host cell. These protein-based compositions can have, for example, one of the following structures:

(Anchoring Domain)n-linker-(Protease Inhibitor)n (n=1,2, 3 or more)

or :

(Protease Inhibitor)n-linker-(Anchoring Domain)n (n=1,2,3 or more)

The protease inhibitor can be a monomeric form of a peptide or polypeptide or can be multiple copies of the same polypeptide that are either linked directly or with spacing sequence in between. Alternatively, different polypeptide-based protease inhibitors can be linked with each other, such as, for example, aprotinin linked with soybean protease inhibitor as protease inhibiting functional domains. The polypeptides or peptides can be linked directly or via a spacer composed of peptide linker sequence. The anchoring domain can be any peptide or polypeptide that can bind at or near the surface of target cells.

The protease inhibitor can be a naturally occurring protease inhibitor (or an active portion thereof) or can be an engineered protease inhibitor. A peptide protease inhibitor used in a compound of the present invention can have a sequence substantially homologous to a naturally occurring protease inhibitor, having one or more deletions,

additions, or substitutions while retaining the activity, or substantially retaining the same activity, of the naturally occurring protease inhibitor.

In one preferred embodiment of the present invention, a therapeutic compound of the present invention is for the prevention and treatment of influenza in humans, and the therapeutic domain is a protein or peptide protease inhibitor that can inhibit a serine protease that can cleave the influenza virus hemagglutinin precursor protein HA0 into HA1 and HA2.

A number of serine protease inhibitors have been shown to reduce HA cleavage and influenza virus activation in cultured cells, in chicken embryos and in lungs of infected mice. They include many of the commonly used trypsin inhibitors, such as: aprotinin (Zhirnov OP, Ikizler MR and Wright PF. (2002) *J Virol* 76:8682-8689), leupeptin (Zhirnov OP, Ikizler MR and Wright PF. (2002) *J Virol* 76:8682-8689; Tashiro M, Klenk HD and Rott R.(1987) *J Gen Virol* 68:2039-2043), soybean protease inhibitor (Barbey-Morel CL, Oeltmann TN, Edwards KM and Wright PF. (1987) *J Infect Dis* 155:667-672), e-aminocaproic acid (Zhirnov OP, Ovchartenko AV and Bukrinskaya AG. 1982. *Arch Virol* 73:263-272) and n-p-tosyl-L-lysine chloromethylketone (TLCK) (Barbey-Morel CL, Oeltmann TN, Edwards KM and Wright PF. (1987) *J Infect Dis* 155:667-672). Among these, aerosol inhalation of aprotinin has shown definitive therapeutic effects against influenza and parainfluenza bronchopneumonia in mice (Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1984) *J Gen Virol* 65:191-196; Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1985) *J Gen Virol* 66:1633-1638; Zhirnov OP. (1987) *J Med Virol* 21:161-167; Ovcharenko AV and Zhirnov OP. (1994) *Antiviral Res* 23:107-118) as well as in human (Zhirnov OP. (1983) *Problems Virol.* 4:9-12 (in Russian)).

Aprotinin (**SEQ ID NO: 1**; **Figure 1**) is a 58 amino acid polypeptide inhibitor (also called Trasylol or bovine pancreatic trypsin inhibitor (BPTI)). A compound of the present invention can have one or more aprotinin domains; for example, a therapeutic composition of the present invention can have from one to six aprotinin polypeptides, more preferably from one to three aprotinin polypeptides. A compound of the present invention can also have a therapeutic domain comprising a polypeptide or peptide having substantial homology to the amino acid sequence of aprotinin.

A compound for preventing or treating influenza that comprises a protease inhibitor preferably comprises an anchoring domain that can bind at or near the surface of epithelial cells. In some preferred embodiments, the epithelium anchoring domain is a GAG-binding sequence from a human protein, such as, for example, the GAG-binding sequence of human platelet factor 4 (PF4) (**SEQ ID NO:2**), human interleukin 8 (IL8) (**SEQ ID NO:3**), human antithrombin III (AT III) (**SEQ ID NO:4**), human apoprotein E (ApoE) (**SEQ ID NO:5**), human angio-associated migratory cell protein (AAMP) (**SEQ ID NO:6**), or human amphiregulin (**SEQ ID NO:7**) (**Figure 2**). A compound of the present invention can also have an anchoring domain comprising a polypeptide or peptide having substantial homology to the amino acid sequences of the GAG-binding domains listed in **SEQ ID NO:2**, **SEQ ID NO:3**, **SEQ ID NO:4**, **SEQ ID NO:5**, **SEQ ID NO:6**, and **SEQ ID NO:7**.

Clinically, a drug comprising aprotinin and an epithelial anchoring domain can be administered by aerosol inhalation to cover the entire respiratory tract to prevent and treat bronchopneumonia caused by influenza viruses, or any other virus, such as parainfluenza virus, that requires serine proteases in its life cycle. Alternatively, an aprotinin/epithtelial anchoring domain fusion protein can be administered as nasal spray to treat uncomplicated early stage influenza cases or other infections by respiratory viruses. In addition, an aprotinin/epithtelial anchoring domain fusion protein can be used as a prophylaxis for influenza or other viral infections before an infection occurs.

Composition comprising at least one anchoring domain and at least one catalytic activity

In some aspects of the present invention, a therapeutic domain that has an extracellular activity that can prevent the infection of a cell by a pathogen is a catalytic activity. The enzymatic activity can be a catalytic activity that removes, degrades or modifies a host molecule or complex or a pathogen molecule or complex that contributes to the infectivity of the pathogen. Preferably the host molecule or complex or pathogen molecule or complex that is removed, degraded, or modified by the enzymatic activity of a compound of the present invention is on, at, or near the surface of a target cell, so that a compound of the present invention that is anchored to the surface of a target cell can effectively inhibit the host or pathogen molecule or complex.

For example, a therapeutic domain can have a catalytic activity that can digest a molecule or epitope of the pathogen or target cell that is required for host-pathogen binding, and subsequent entry of the pathogen into the target cell. Receptors on target cells that allow for the entry of viruses into cells can be the target of an enzymatic activity of a compound of the present invention.

Compounds of the present invention that comprise catalytic domains can be used to inhibit infection by any pathogen that uses a receptor to gain entry to a target cell, as long as removal of the receptor does not impair the organism. These protein-based compositions can have, for example, one of the following structures:

(Anchoring Domain)n-[linker]-(Enzymatic Activity)n (n=1,2, 3 or more)

or :

(Enzymatic Activity)n (n=1,2, 3 or more)-[linker]-(Anchoring Domain)n,

where the linkers are optional.

The enzymatic activity can be a monomeric form of a peptide or polypeptide or can be multiple copies of the same polypeptide that are either linked directly or with spacing sequence in between. The polypeptides or peptides can be linked directly or via a spacer composed of peptide linker sequence. The anchoring domain can be any peptide or polypeptide that can bind to or near the surface of target cells.

In one preferred embodiment of the present invention, a therapeutic domain comprises a sialidase that can eliminate or greatly reduce the level of sialic acid on the surface of epithelial cells. Sialic acid is a receptor for influenza viruses. Thus, treating the surface of respiratory epithelial cells with a sialidase can prevent influenza infections or interrupt early infections. The therapeutic domain can comprise a complete sialidase protein, or an active portion thereof. . Sialic acid is a receptor for influenza viruses, and at least one of the receptors for parainfluenza virus, some coronavirus and rotavirus, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Helicobacter pylori*. Thus, treating the surface of respiratory epithelial cells with a sialidase can prevent influenza or other viral

infections or interrupt early infections, as well as prevent or reduce colonization of bacteria such as *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis,* and *Pseudomonas aeruginosa*. Treating the gastrointestinal epithelial cells with a sialidase can prevent or reduce colonization of *Helicobacter pylori* in the stomach.

Sialic acid also mediates cell adhesion and interactions between inflammatory cells and target cells. Therefore, treating the surface of respiratory epithelial cells with a sialidase can prevent the recruitment of inflammatory cells to the airway surface, and therefore can treat allergic reactions including asthma and allergic rhinitis.

Since sialic acid serves as a barrier that hinder cell entry by a gene therapy vector, treating the target cells with a sialidase can increase transduction efficiency, and therefore improve efficacy of the gene therapy.

Preferred sialidases are the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), A*ctinomyces viscosus* (Genbank Accession Number X62276), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) can be used. Therapeutic domains of compounds of the present invention can comprise all or a portion of the amino acid sequence of a large bacterial sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequence of a large bacterial sialidase. In one preferred embodiment, a therapeutic domain comprises a sialidase encoded by Actinomyces viscosus, such as that of **SEQ ID NO:12**, or such as sialidase sequence substantially homologous to **SEQ ID NO:12**. In yet another preferred embodiment, a therapeutic domain comprises the catalytic domain of the Actinomyces viscosus sialidase extending from amino acids 274-666 of **SEQ ID NO:12**, or a substantially homologous sequence.

Other preferred sialidases are the human sialidases such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9**; Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663) (**Figure 3**). Therapeutic domains of

compounds of the present invention can comprise all or a portion of the amino acid sequences of a human sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequences of a human sialidase. Preferably, where a therapeutic domain comprises a portion of the amino acid sequences of a naturally occurring sialidase, or sequences substantially homologous to a portion of the amino acid sequences of a naturally occurring sialidase, the portion comprises essentially the same activity as the human sialidase.

A compound for preventing or treating influenza that comprises an enzymatic domain preferably comprises an anchoring domain that can bind at or near the surface of epithelial cells. In some preferred embodiments, the epithelium-anchoring domain is a GAG-binding sequence from a human protein, such as, for example, the GAG-binding amino acid sequences of human platelet factor 4 (PF4) (**SEQ ID NO:2**), human interleukin 8 (IL8) (**SEQ ID NO:3**), human antithrombin III (AT III) (**SEQ ID NO:4**), human apoprotein E (ApoE) (**SEQ ID NO:5**), human angio-associated migratory cell protein (AAMP) (**SEQ ID NO:6**), and human amphiregulin (**SEQ ID NO:7**) (**Figure 2**). An epithelial anchoring domain can also be substantially homologous to a naturally occurring GAG-binding sequence, such as those listed in **Figure 2**.

It is also within the scope of the present invention to use compounds comprising a human sialidase, or comprising a sialidase with substantial homology to a sialidase, in the absence of an anchoring domain, in the treatment or prevention of pathogen infections, such as but not limited to influenza, paramyxovirus, coronavirus, rotavirus, and *Pseudomonas aeruginosa* infections or bacterial infections; in the treatment or prevention of allergic and inflammatory responses, and to improve the transduction efficiency of a recombinant virus.

The present invention recognizes that such infections may be prevented or abated by the use of sialidases, such as, but not limited to, the *A. viscosus* sialidase or human sialidases such as NEU2 and NEU4. The sialidases can optionally be adapted, by genetic or chemical engineering, or by pharmaceutical formulation, to improve their half life or retention at the respiratory epithelium.

Because influenza viruses primarily infect the upper respiratory tract, removing the receptor sialic acid locally in the nasal cavity and nasopharynx area can prevent infections or interrupt early infections. The sialidase can be delivered to the upper respiratory tract as a nasal spray, and it can be used either in therapeutic mode during early stage of influenza (or other infection) or in prophylactic mode before the infection occurs. Alternatively, it can be delivered to the lower respiratory tract as an inhalant to treat influenza and to prevent influenza complications, such as bronchopneumonia.

II. Therapeutic Composition Comprising at least one Sialidase Activity

The present invention includes a therapeutic composition that comprises at least one sialidase activity. The sialidase activity can be a sialidase isolated from any source, such as, for example, a bacterial or mammalian source, or can be a recombinant protein that is substantially homologous to at least a portion of a naturally occurring sialidase. Preferred sialidases are the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Actinomyces viscosus* (Genbank Accession Number L06898), *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) or substantially homologous proteins can be used.

For example, therapeutic compounds of the present invention can comprise a large bacterial sialidase or can comprise a protein with the amino acid sequence of a large bacterial sialidase or can comprise amino acid sequences that are substantially homologous to the amino acid sequence of a large bacterial sialidase. A preferred pharmaceutical composition of the present invention comprises the *A. viscosus* sialidase (**SEQ ID NO:12**), or comprises a protein substantially homologous to the *A. viscosus* sialidase.

Other preferred sialidases are the human sialidases such as those encoded by the genes NEU2 (**SEQ ID NO:8**; Genbank Accession Number Y16535; Monti, E, Preti, Rossi, E., Ballabio, A and Borsani G. (1999) *Genomics* 57:137-143) and NEU4 (**SEQ ID NO:9**; Genbank Accession Number NM080741; Monti, E, Preti, A, Venerando, B and

Borsani, G. (2002) *Neurochem Res* 27:646-663) (**Figure 3**). Therapeutic domains of compounds of the present invention can comprise a human sialidase protein that is substantially homologous to the amino acid sequences of a human sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequences of a human sialidase. Preferably, where a therapeutic domain comprises a portion of the amino acid sequences of a naturally occurring sialidase, or sequences substantially homologous to a portion of the amino acid sequences of a naturally occurring sialidase, the portion comprises essentially the same activity as the human sialidase.

A pharmaceutical composition comprising a sialidase can include other compounds, including but not limited to other proteins, that can also have therapeutic activity. A pharmaceutical composition comprising a sialidase can include other compounds that can enhance the stability, solubility, packaging, delivery, consistency, taste, or fragrance of the composition.

A pharmaceutical composition comprising a sialidase can be formulated for nasal, tracheal, bronchial, oral, or topical administration, or can be formulated as an injectable solution or as eyedrops. A pharmaceutical composition comprising a sialidase can be used to treat or prevent pathogen infection, to treat or prevent allergy or inflammatory response, or to enhance the transduction efficiency of a recombinant virus for gene therapy.

III. Sialidase Catalytic Domain Proteins

The present invention also includes sialidase catalytic domain proteins. As used herein a "sialidase catalytic domain protein" comprises a catalytic domain of a sialidase but does not comprise the entire amino acid sequence of the sialidase from which the catalytic domain is derived. A sialidase catalytic domain protein has sialidase activity. Preferably, a sialidase catalytic domain protein comprises at least 10%, at least 20%, at least 50%, at least 70% of the activity of the sialidase from which the catalytic domain sequence is derived. More preferably, a sialidase catalytic domain protein comprises at

least 90% of the activity of the sialidase from which the catalytic domain sequence is derived.

A sialidase catalytic domain protein can include other amino acid sequences, such as but not limited to additional sialidase sequences, sequences derived from other proteins, or sequences that are not derived from sequences of naturally-occurring proteins. Additional amino acid sequences can perform any of a number of functions, including contributing other activities to the catalytic domain protein, enhancing the expression, processing, folding, or stability of the sialidase catalytic domain protein, or even providing a desirable size or spacing of the protein.

A preferred sialidase catalytic domain protein is a protein that comprises the catalytic domain of the *A. viscosus* sialidase. Preferably, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 270-666 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**). Preferably, an *A. viscosus* sialidase catalytic domain protein comprises an amino acid sequence that begins at any of the amino acids from amino acid 270 to amino acid 290 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and ends at any of the amino acids from amino acid 665 to amino acid 901 of said *A. viscosus* sialidase sequence (**SEQ ID NO:12**), and lacks any *A. viscosus* sialidase protein sequence extending from amino acid 1 to amino acid 269. (As used herein "lacks any *A. viscosus* sialidase protein sequence extending from amino acid 1 to amino acid 269" means lacks any stretch of four or more consecutive amino acids as they appear in the designated protein or amino acid sequence.)

In some preferred embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 274-681 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks other *A. viscosus* sialidase sequence. In some preferred embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 274-666 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence. In some preferred embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 290-666 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence. In yet other preferred embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids

290-681 of the *A. viscosus* sialidase sequence (**SEQ ID NO:12**) and lacks any other *A. viscosus* sialidase sequence.

The present invention also comprises nucleic acid molecules that encode protein-based compounds of the present invention that comprise a catalytic domain of a sialidase. The nucleic acid molecules can have codons optimized for expression in particular cell types, such as, for example E. coli or human cells. The nucleic acid molecules or the present invention that encode protein-based compounds of the present invention that comprise at least one catalytic domain of a sialidase can also comprise other nucleic acid sequences, including but not limited to sequences that enhance gene expression. The nucleic acid molecules can be in vectors, such as but not limited to expression vectors.

Fusion Proteins

Sialidase catalytic domain proteins can be fusion proteins, in which the fusion protein comprises at least one sialidase catalytic domain and at least one other protein domain, including but not limited to: a purification domain, a protein tag, a protein stabiliy domain, a solubility domain, a protein size-increasing domain, a protein folding domain, a protein localization domain, an anchoring domain, an N-terminal domain, a C-terminl domain, a catalytic activity domain, a binding domain, or a catalytic activity-enhancing domain. Preferably, the at least one other protein domain is derived from another source, such as, but not limited to, sequences from another protein. The at least one other protein domain need not be based on any known protein sequence, but can be engineered and empirically tested to perform any function in the fusion protein.

Purification domains can include, as nonlimiting examples, one or more of a his tag, a calmodulin binding domain, a maltose binding protein domain, a streptaidin domain, a streptavidin binding domain, an intein domain, or a chitin binding domain. Protein tags can comprise sequences that can be used for antibody detection of proteins, such as, for example, the myc tag, the hemaglutinin tag, or the FLAG tag. Protein domains that enhance protein expression, modification, folding, stability, size, or localization can be based on sequences of know proteins or engineered. Other protein domains can have binding or catalytic activity or enhance the catalytic activity of the sialidase catalytic domain.

Preferred fusion proteins of the present invention comprise at least one sialidase catalytic domain and at least one anchoring domain. Preferred anchoring domains include GAG-binding domains, such as the GAG-binding domain or human amphiregulin (**SEQ ID NO:7**).

Sialidase catalytic domains and other domains of a fusion protein of the present invention can optionally be joined by linkers, such as but not limited to peptide linkers. A variety of peptide linkers are known in the art. A preferred linker is a peptide linker comprising glycine, such as G-G-G-G-S (**SEQ ID NO:10**).

The present invention also comprises nucleic acid molecules that fusion proteins of the present invention that comprise a catalytic domain of a sialidase. The nucleic acid molecules can have codons optimized for expression in particular cell types, such as, for example E. coli or human cells. The nucleic acid molecules or the present invention that encode fusion proteins of the present invention can also comprise other nucleic acid sequences, including but not limited to sequences that enhance gene expression. The nucleic acid molecules can be in vectors, such as but not limited to expression vectors.

IV Pharmaceutical Compositions

The present invention includes compounds of the present invention formulated as pharmaceutical compositions. The pharmaceutical compositions comprise a pharmaceutically acceptable carrier prepared for storage and preferably subsequent administration, which have a pharmaceutically effective amount of the compound in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990)). Preservatives, stabilizers, dyes and even flavoring agents can be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives. In addition, antioxidants and suspending agents can be used.

Depending on the target cell, the compounds of the present invention can be formulated and used as tablets, capsules or elixirs for oral administration; salves or ointments for topical application; suppositories for rectal administration; sterile solutions, suspensions, and the like for use as inhalants or nasal sprays. Injectables can also be prepared in conventional forms either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride and the like. In addition, if desired, the injectable pharmaceutical compositions can contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents and the like.

The pharmaceutically effective amount of a test compound required as a dose will depend on the route of administration, the type of animal or patient being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. In practicing the methods of the present invention, the pharmaceutical compositions can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These products can be utilized *in vivo*, preferably in a mammalian patient, preferably in a human, or *in vitro*. In employing them *in vivo*, the pharmaceutical compositions can be administered to the patient in a variety of ways, including topically, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperiotoneally, employing a variety of dosage forms. Such methods can also be used in testing the activity of test compounds *in vivo*.

In preferred embodiments, these pharmaceutical compositions may be in the form of orally-administrable suspensions, solutions, tablets or lozenges; nasal sprays; inhalants; injectables, topical sprays, ointments, powders, or gels.

When administered orally as a suspension, compositions of the present invention are prepared according to techniques well-known in the art of

pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art. Components in the formulation of a mouthwash or rinse include antimicrobials, surfactants, cosurfactants, oils, water and other additives such as sweeteners/flavoring agents known in the art.

When administered by a drinking solution, the composition comprises one or more of the compounds of the present invention, dissolved in water, with appropriate pH adjustment, and with carrier. The compound may be dissolved in distilled water, tap water, spring water, and the like. The pH can preferably be adjusted to between about 3.5 and about 8.5. Sweeteners may be added, e.g., 1% (w/v) sucrose.

Lozenges can be prepared according to U.S. Patent No. 3,439,089, herein incorporated by reference for these purposes.

When administered by nasal aerosol or inhalation, the pharmaceutical compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, Ansel, H. C. et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, Sixth Ed. (1995). Preferably these compositions and formulations are prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these can be found in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact nature of the nasal dosage form desired, e.g., solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain

large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, jelling agents, or buffering and other stabilizing and solubilizing agents may also be present. Preferably, the nasal dosage form should be isotonic with nasal secretions.

Nasal formulations can be administers as drops, sprays, aerosols or by any other intranasal dosage form. Optionally, the delivery system can be a unit dose delivery system. The volume of solution or suspension delivered per dose can preferably be anywhere from about 5 to about 2000 microliters, more preferably from about 10 to about 1000 microliters, and yet more preferably from about 50 to about 500 microliters. Delivery systems for these various dosage forms can be dropper bottles, plastic squeeze units, atomizers, nebulizers or pharmaceutical aerosols in either unit dose or multiple dose packages.

The formulations of this invention may be varied to include; (1) other acids and bases to adjust the pH; (2) other tonicity imparting agents such as sorbitol, glycerin and dextrose; (3) other antimicrobial preservatives such as other parahydroxy benzoic acid esters, sorbate, benzoate, propionate, chlorbutanol, phenylethyl alcohol, benzalkonium chloride, and mercurials; (4) other viscosity imparting agents such as sodium carboxymethylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, polyvinyl alcohol and other gums; (5) suitable absorption enhancers; (6) stabilizing agents such as antioxidants, like bisulfite and ascorbate, metal chelating agents such as sodium edetate and drug solubility enhancers such as polyethylene glycols.

V. Method of preventing or treating infection by a pathogen

The present invention also includes methods of preventing or treating infection by a pathogen. In one aspect, the method includes: treating a subject that is infected with a pathogen or at risk of being infected with a pathogen with a pharmaceutical composition of the present invention that comprises a compound that comprises at least one anchoring

domain that can anchor the compound at or near the surface of a target cell and at least one therapeutic domain comprising a peptide or protein that has at least one extracellular activity that can prevent the infection of a target cell by a pathogen. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present invention to epithelial cells of a subject. The subject to be treated can be an animal or human subject.

In another aspect, the method includes: treating a subject that is infected with a pathogen or at risk of being infected with a pathogen with a pharmaceutical composition of the present invention that comprises a protein-based compound that comprises a sialidase activity. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present invention to epithelial cells of a subject. The sialidase activity can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**). The subject to be treated can be an animal or human subject.

In yet another aspect, the method includes: treating a subject that is infected with a pathogen or at risk of being infected with a pathogen with a pharmaceutical composition of the present invention that comprises a protein-based compound that comprises a sialidase catalytic domain. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present invention to epithelial cells of a subject. The sialidase catalytic domain is preferably can substantially homologous to the catalytic domain of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase catalytic domain with substantial homology to amino acids 274-666 the *A. viscosus* sialidase (**SEQ ID NO:12**). The subject to be treated can be an animal or human subject.

A pathogen can be a viral, bacterial, or protozoan pathogen. In some embodiments, the pathogen is one of the following: influenza viruses, parainfluenza virus, respiratory syncytial virus (RSV), coronavirus, rotavirus, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Helicobacter pylori*. In one preferred embodiment, the pathogen is influenza virus.

Compounds of the present invention can be designed for human use or animal use. In some aspects of the present invention, a compound of the present invention can be used to prevent pathogen infection in a class of animals, such as mammals. In some aspects of the present invention, a composition can be used for human and animal use (although the formulation may differ). In these aspects, the active domains of a compound can be effective against more than one pathogen species, type, subtype, or strain and can be active in more than one host species. For example, some preferred compounds of the present invention that comprise, for example, active domains such as protease inhibitors that prevent processing of the HA protein of influenza virus, or sialidases that remove sialic acid receptors from target cells, or anchoring domains such as domains that bind heparin or heparan sulfate, can be used in birds, mammals, or humans. Such compounds that can be effective against a range of pathogens with the capacity to infect different host species can also be used in humans to combat infection by pathogens that are naturally hosted in other species.

In some preferred embodiments of the present invention, the pharmaceutical composition prevents infection by influenza, and a therapeutically effective amount of the pharmaceutical composition is applied to the respiratory epithelial cells of a subject. This can be done by the use of an inhaler, or by the use of a nasal spray. Preferably, the inhaler or nasal spray is used from one to four times a day.

Because influenza viruses primarily infect the upper respiratory tract, removing the receptor sialic acid locally in the nasal cavity, pharynx, trachea and bronchi can prevent infections or interrupt early infections. The sialidase can be delivered to the upper respiratory tract as a nasal spray or as an inhalant, and it can be used either in therapeutic mode during early stage of influenza (or other infection) or in prophylactic mode before the infection occurs. Alternatively, it can be delivered to the lower respiratory tract as an inhalant to treat influenza and to prevent influenza complications, such as bronchopneumonia. Similarly, the sialidase can be delivered as nasal spray or inhalant to prevent or reduce infection by parainfluenza virus and coronavirus. It can also be delivered as an inhalant or nasal spray to prevent or reduce airway colonization by pathogenic bacteria, including *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*. The

therapeutic compounds can optionally be adapted, by genetic or chemical engineering, or by pharmaceutical formulation, to improve their half-life or retention at the respiratory epithelium. Additionally, it can be delivered topically to the eyes or to surgical wounds in the form of drops, sprays or ointments to prevent and treat bacterial infection including infection by *Pseudomonas aeruginosa*. It can also be administered orally to treat infection by *Helicobacter pylori*.

Dosage

As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above. In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced or disappear. The dosage for a compound of the present invention can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the compound. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, preferably between about 10 ng/kg and about 1 mg/kg, and more preferably between about 100 ng/kg and about 100 micrograms/kg.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, Fingle et al., in The Pharmacological Basis of Therapeutics (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust

administration due to toxicity, organ dysfunction or other adverse effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate. The magnitude of an administrated does in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight and response of the individual patient, including those for veterinary applications.

Thus, in accordance with the present invention, there is further provided a method of treating and a pharmaceutical composition for treating influenza virus infection and prevention of influenza virus infection. The treatment involves administering to a patient in need of such treatment a pharmaceutical carrier and a therapeutically effective amount of any composition of the present invention, or a pharmaceutically acceptable salt thereof.

In one preferred regimen, appropriate dosages are administered to each patient by either inhaler, nasal spray, or by oral lozenge. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific salt or other form employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

VI. Method of reducing, preventing, or treating allergic and inflammatory responses

The present invention also includes methods of reducing, preventing, or treating an allergic or inflammatory response of a subject.

In one aspect, the method includes: preventing or treating an allergic or inflammatory response of a subject with a pharmaceutical composition of the present

invention that comprises a protein-based compound that comprises a sialidase activity. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present invention to epithelial cells of a subject. The sialidase activity can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**). The subject to be treated can be an animal or human subject.

In yet another aspect, the method includes: preventing or treating an allergic or inflammatory response of a subject with a pharmaceutical composition of the present invention that comprises a protein-based compound that comprises a sialidase catalytic domain. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present invention to epithelial cells of a subject. The sialidase catalytic domain is preferably can substantially homologous to the catalytic domain of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase catalytic domain with substantial homology to amino acids 274-666 the *A. viscosus* sialidase (**SEQ ID NO:12**). The subject to be treated can be an animal or human subject.

The allergic or inflammatory response can be and acute or chronic condition, and can include, as nonlimiting examples, asthma, other allergic responses causing respiratory distress, allergic rhinitis, eczema, psoriasis, reactions to plant or animal toxins, or autoimmune conditions.

In some preferred embodiments, compounds of the present invention can be delivered as an inhalant or nasal spray to prevent or treat inflammation in the airway including, but not limited to, asthma and allergic rhinitis. Compounds of the present invention comprising sialidase activity (including sialidase catalytic domain proteins and sialidase fusion proteins) can also be administered as eye drops, ear drops, or sprays, ointments, lotions, or gels to be applied to the skin. In another aspect, the method includes treating a patient who has inflammatory diseases with the present invention that comprises a sialidase activity that is administered intravenously or as a local injection.

Dosage

As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above. In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced or disappear. The dosage for a compound of the present invention can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the compound. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, preferably between about 10 ng/kg and about 1 mg/kg, and more preferably between about 100 ng/kg and about 100 micrograms/kg.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, Fingle et al., in The Pharmacological Basis of Therapeutics (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust administration due to toxicity, organ dysfunction or other adverse effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate. The magnitude of an administrated does in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency,

will also vary according to the age, body weight and response of the individual patient, including those for veterinary applications.

In some preferred regimens, appropriate dosages are administered to each patient by either inhaler, nasal spray, or by topical application. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific salt or other form employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

VI. Method of enhancing gene delivery by a recombinant viral vector

The present invention also includes methods of gene delivery by a recombinant viral vector. In one aspect, the method includes: administering an effective amount of a compound of the present invention that comprises a protein having sialidase activity to at least one cell prior to or concomitant with the administration of at least one recombinant viral vector. A composition of the present invention can be provided in the same formulation as at least one recombinant viral vector, or in a separate formulation.

In some preferred embodiments, the method includes applying a therapeutically effective amount of a composition of the present invention and a recombinant viral vector to cells of a subject. The subject to be treated can be an animal or human subject. In a particularly preferred embodiment, a recombinant viral vector is used to transduce epithelial target cells of a subject for gene therapy. For example, a recombinant viral vector can be used to transduce airway epithelial cells of a subject with cystic fibrosis. In this case, a compound of the present invention can be administered by use of an inhaler. A recombinant virus comprising a therapeutic gene can be administered concurrently or separately.

In other embodiments, cells can be treated with a compound of the present invention and a recombinant viral vector in vitro or "ex vivo" (that is, cells removed from a subject to be transplanted into a subject after transduction).

The sialidase activity can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase, including a sialidase catalytic domain. A preferred pharmaceutical composition comprises a sialidase with substantial homology to the *A. viscosus* sialidase (**SEQ ID NO:12**).

A compound of the present invention can be administered to target cells from one day before to two hours subsequent to the administration of the recombinant virus. Preferably a compound of the present invention is administered to target cells from four hours to ten minutes before administration of the recombinant virus. Administration can be

A recombinant virus is preferably a recombinant virus that can be used to transfer genes to mammalian cells, such as, preferably human cells. For example, a recombinant virus can be a retrovirus (including lentivirus), adeno-virus, adeno-associated virus (AAV) or herpes simplex virus type 1. The recombinant virus comprises at least one exogenous gene that is to be transferred to a target cell. The gene is preferably a therapeutic gene, but this need not be the case. For example, the gene can be a gene used to mark cells or confer drug resistance.

In a preferred embodiment, the present invention includes methods of improving efficacy of a gene therapy vector. The method includes treating a patient with a compound of the present invention that comprises a sialidase activity and, in the same or a separate formation, with a recombinant virus. The compound of the present invention having sialidase activity can be administered to the patient prior to, concomitant to, or even subsequent to the administration of a recombinant virus. In one embodiment, the sialidase is substantially homologous to the *Actinomyces viscosus* sialidase (**SEQ ID NO:12**) or a portion thereof. In one preferred embodiment, the sialidase comprises the catalytic domain of the *Actinomyces viscosus* sialidase. In another embodiment, the recombinant virus is AAV. In yet another embodiment, the disease is cystic fibrosis. In yet another embodiment, the recombinant virus comprises the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

Dosage

As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above. In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced or disappear. The dosage for a compound of the present invention can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the compound. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, preferably between about 10 ng/kg and about 1 mg/kg, and more preferably between about 100 ng/kg and about 100 micrograms/kg.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, Fingle et al., in The Pharmacological Basis of Therapeutics (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust administration due to toxicity, organ dysfunction or other adverse effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate. The magnitude of an administrated does in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency,

will also vary according to the age, body weight and response of the individual patient, including those for veterinary applications.

In some preferred regimens, appropriate dosages are administered to each patient by either inhaler, nasal spray, or by topical application. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific salt or other form employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

Examples

Example 1: Synthesizing aprotinin genes, purifying and testing aprotinin fusion proteins.

Introduction

Influenza viral protein hemagglutinin (HA) is the major influenza envelope protein. It plays an essential role in viral infection. The importance of HA is evidenced by the fact that it is the major target for protective neutralizing antibodies produced by the host immune response (Hayden, FG. (1996) In *Antiviral drug resistance* (ed. D. D. Richman), pp. 59-77. Chichester, UK: John Wiley & Sons Ltd.). It is now clear that HA has two different functions in viral infection. First, HA is responsible for the attachment of the virus to sialic acid cell receptors. Second, HA mediates viral entry into target cells by triggering fusion of the viral envelope with cellular membranes.

HA is synthesized as a precursor protein, HA0, which is transferred through the Golgi apparatus to the cell surface as a trimeric molecular complex. HA0 is further cleaved to generate the C terminus HA1 (residue 328 of HA0) and the N terminus of HA2. It is generally believed that the cleavage occurs at the cell surface or on released viruses. The cleavage of HAO into HA1/HA2 is not required for HA binding to a sialic acid receptor; however, it is essential for viral infectivity (Klenk, HD and Rott, R. (1988)

Adv Vir Res. 34:247-281; Kido, H, Niwa, Y, Beppu, Y and Towatari, T. (1996) *Advan Enzyme Regul* 36:325-347; Skehel, JJ and Wiley, DC. (2000) *Annu Rev Biochem* 69:531-569).

Sensitivity of HA0 to host proteases is determined by the proteolytic site in the external loop of HA0 molecule. The proteolytic site may contain either a single Arg or Lys residue (monobasic cleavage site) or several Lys and/or Arg residues in R-X-K/R-R motif (multibasic cleavage site). Only the influenza A virus subtypes H5 and H7 have HA proteins carrying the multibasic cleavage site. All other influenza A, B and C viruses contain HA proteins having the monobasic cleavage site. Influenza A viruses having multibasic cleavage sites are more virulent and induce systemic infection in hosts whereas viruses with a monobasic HA site initiate infection only in the respiratory tract in mammals or in the respiratory and enteric tracts in avian species (Klenk, HD and Garten W. 1994. Trend Micro 2:39-43 for review). Fortunately, human infection by the highly virulent avian influenza A H5 and H7 subtypes, which carry the multibasic cleavage site, has so far only occurred in a handful of cases discovered mostly in Hong Kong. The vast majority of influenza infections are caused by viruses with HA proteins are cleaved at the monobasic cleavage site.

Influenza virus HA subtypes 5 and 7 that contain multibasic cleavage sites are activated by furin, a member of the subtilisin-like endoproteases, or the pre-protein convertase family. Furin cleaves the virus intracellularly and is ubiquitously present in many cell types, allowing the virulent, systemic infection seen with such viruses (Klenk, HD and Garten W. 1994. Trend Micro 2:39-43; Nakayama, K. 1997. *Biochem* 327:625-635). All other influenza viruses, which have HAs with monobasic cleavage sites, are activated by secreted, trypsin-like serine proteases. Enzymes that have been implicated in influenza virus activation include: plasmin (Lazarowitz SG, Goldberg AR and Choppin PW. 1973. *Virology* 56:172-180), mini-plasmin (Murakami M, Towatari T, Ohuchi M, Shiota M, Akao M, Okumura Y, Parry MA and Kido H. (2001) *Eur J Biochem* 268: 2847-2855), tryptase Clara (Kido H, Chen Y and Murakami M. (1999) In B.Dunn (ed.), Proteases of infectious agents. p.205-217, Academic Press, New York, N.Y), kallikrein, urokinase, thrombin (Scheiblauer H, Reinacher M, Tashiro M and Rott R. (1992) *J Infec Dis* 166:783-791), blood clotting factor Xa (Gotoh B, Ogasawara T, Toyoda T, Inocencio

N, Hamaguchi M and Nagai Y. (1990) *EMBO J* 9:4189-4195), acrosin (Garten W, Bosch FX, Linder D, Rott R and Klenk HD. (1981) *Virology* 115:361-374.), proteases from human respiratory lavage (Barbey-Morel CL, Oeltmann TN, Edwards KM and Wright PF. (1987) *J Infect Dis* 155:667-672) and bacterial proteases from *Staphylococcus aureus* (Tashiro M, Ciborowski P, Reinacher M, Pulverer G, Klenk HD and Rott R. (1987) *Virology* 157:421-430) and *Pseudomonas aeruginosa* (Callan RJ, Hartmann FA, West SE and Hinshaw VS. (1997) *J Virol* 71:7579-7585). Activation of influenza viruses by host serine proteases is generally considered to occur extracellularly either at the plasma membrane or after virus release from the cell.

Aprotinin, also called Trasylol, or bovine pancreatic trypsin inhibitor (BPTI) is a polypeptide having 58 amino acids. It belongs to the family of Kunitz-type inhibitors and competitively inhibits a wide spectrum of serine proteases, including trypsin, chymotrypsin, plasmin and plasma kallikrein. Aprotinin has long been used as a human therapeutics, such as treatment of pancreatitis, various states of shock syndrome, hyperfibrinolytic haemorrhage and myocardial infarction. It is also used in open-heart surgery, including cardiopulmonary bypass operations, to reduce blood loss (Fritz H and Wunderer G. (1983) *Arzneim-Forsch* 33:479-494).

The safety of aprotinin in human has been well documented through years of clinical applications. In addition, aprotinin is apparently a very weak immunogen as aprotinin-specific antibodies have not been observed in human sera so far (Fritz H and Wunderer G. (1983) *Arzneim-Forsch* 33:479-494). Another desired feature of aprotinin as a drug candidate is its superb stability. It can be kept at room temperature for at least 18 months without any loss of activity (Fritz H and Wunderer G. (1983) *Arzneim-Forsch* 33:479-494).

To achieve significant viral inhibition in animal studies that have been performed, aprotinin was administered at high doses. For example, 280 micrograms to 840 micrograms per day of aprotinin was injected intraperitoneally into each mouse for 6 days (Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1984) *J Gen Virol* 65:191-196); a lower dosage was required for aerosol inhalation, still, each mouse was given 63 - 126 micrograms per day for 6 days (Ovcharenko AV and Zhirnov OP. (1994) *Antiviral Res* 23:107-118). A very high dose of aprotinin would be required in human based on

extrapolation from the mouse data. Therefore to achieve better efficacy in human, the potency of aprotinin molecule needs to be significantly improved.

Aprotinin functions by competitively inhibiting serine proteases that are mostly on the surface of host respiratory epithelial cells. Local concentration of aprotinin in the vicinity of host proteases is therefore the key factor determining competitive advantage of aprotinin. We use two approaches that work synergistically to boost competitive advantage of aprotinin on the surface of respiratory epithelium.

First, the avidity (functional affinity) of aprotinin is increased by making multivalent aprotinin fusion proteins consisting of two, three, or more aprotinin proteins connected via linkers. Such a molecule is able to bind to membrane proteases in a multivalent fashion, which has significant kinetic advantage over the aprotinin monomer. Monomeric aprotinin binds to bovine trypsin very tightly with dissociation constant (Ki) being 6.0×10^{-14} mol/l. However, its affinity compared to other proteases, such as chymotrypsin, plasmin and Kallikrein, which have been implicated in activation of influenza viruses, is much lower with Ki being at the level of 10^{-8} to 10^{-9} mol/l (Fritz H and Wunderer G. (1983) *Arzneim-Forsch* 33:479-494). Multimerization can increase aprotinin's affinity to these proteases exponentially.

Second, we fuse aprotinin with a respiratory epithelium-anchoring domain. The anchoring domain localizes aprotinin to the proximity of host membrane-associated proteases and maintains a high local concentration of aprotinin on epithelial surface. The anchoring domain also increases retention time of the drug on the respiratory epithelium.

Cloning

Aprotinin is a single chain polypeptide having 58 amino acid residues and 3 intra-chain disulfide bonds (**SEQ ID NO:1**). The amino acid sequence of aprotinin is shown in **Figure 1**. Genes encoding aprotinin and aprotinin fusion proteins are synthesized by PCR using overlapping oligonucleotides with codons optimized for *E. Coli* expression as templates. The PCR products are cloned into pCR2.1-TOPO vector (Invitrogen). After sequencing, the genes are subcloned into an expression vector pQE (Qiagen). The vector carries a purification tag, Hisx6, to allow easy purification of the recombinant proteins. The constructs are used to transform *E. Coli*. The transformed cells grown in LB-

ampicillin medium to mid-log phase are induced by IPTG according to standard protocols. Cells are pelleted and lysed in phosphate-buffered-saline (PBS) by sonication. The enzymes, which have His_6 purification tag, are purified using a nickel column (Qiagen).

The following aprotinin fusion proteins are made:

1. Dimeric and trimeric aprotinin. Two or three aprotinin genes are linked via a flexible linker as the following constructs:

   Aprotinin—(GGGGS (**SEQ ID NO:10**))n (n=3, 4 or 5)—Aprotinin;

   and

   Aprotinin—(GGGGS(**SEQ ID NO:10**))n (n=3, 4 or 5)—Aprotinin—(GGGGS(**SEQ ID NO:10**))n (n=3, 4 or 5)—Aprotinin

The length of the linker sequence may determine three-dimensional flexibility of the multimeric aprotinin and thereby influence functional affinity of the molecule. Therefore constructs having linkers with various lengths are made.

Fully functional recombinant monomeric aprotinin has been produced in *E. Coli* (Auerswald EA, Horlein D, Reinhardt G, Schroder W and Schnabel E. (1988).*Biol Chem Hoppe-Seyler* Vol 369, Suppl., pp27-35). We therefore expect proper folding of multivalent aprotinin proteins in *E. coli* cells. Besides expressing protein in various common *E. Coli* cell strains, such as BL21, JM83, etc, the multivalent aprotinin proteins are also expressed in Origami™ cells (Novagen, Bad Soden, Germany). The Origami™ cell strain does not have thioredoxin and glutathione reductase and thus has an oxidizing cytoplasm. This cell strain has been used to successfully express a number of proteins that contain disulfide bonds (Bessette PH, Aslund F, Beckwith J and Georgiou G. (1999) *Pro Natl Acad Sci USA* 96:13703-13708; Venturi M, Seifert C and Hunte C. (2001) *J Mol Biol* 315:1-8.).

2. The epithelium cell-anchoring aprotinin. An epithelium cell-anchoring sequence is fused with aprotinin. The epithelium-anchoring sequence can be any peptide or polypeptide sequence that has affinity towards the surface of epithelial cells. We have

selected three human GAG-binding sequences: PF4 (aa 47-70; **SEQ ID NO: 2**), IL-8 (aa 46-72; **SEQ ID NO: 3**), and AT III (aa 118-151; **SEQ ID NO: 4**) (**Figure 2**). These sequences bind to heparin/heparan sulfate with nanomolar-level affinities (**Table 1**). Heparin/Heparan Sulfate are ubiquitously present on the respiratory epithelium. In separate constructs, the GAG-binding sequences are fused with the aprotinin gene on the N terminus and on the C terminus via a generic linker sequence GGGGS as the following constructs:

(GAG domain—GGGGS(**SEQ ID NO:10**)—Aprotinin); and

(Aprotinin—GGGGS(**SEQ ID NO:10**)—GAG domain)

Table 1. Affinities to Heparin

Protein	Kd nM	(ref)
PF4	27	(44)
IL-8	<5	(43)
ATIII	11	(42)
ApoE	620	(45)

Photometric trypsin inhibition assay

The trypsin inhibition activity of aprotinin and aprotinin fusion proteins is measured by a photometric assay described previously in detail (Fritz H and Wunderer G. (1983) Arzneim-Forsch 33:479-494). Briefly, in this assay aprotinin inhibits the trypsin-catalyzed hydrolysis of Na-benzoyl-L-arginine-p-nitroanilide (BzArgpNA or L-BAPA)

(Sigma), which is followed photometrically at 405 nm. One trypsin unit (U_{BAPA}) corresponds to the hydrolysis of 1 micromole substrate per min. One inhibitor unit (IU_{BAPA}) decreases the activity of two trypsin units by 50%, which corresponds arithmetically to the inhibition of 1 U_{BAPA} of trypsin. The specific activity of aprotinin is given in IU_{BAPA}/mg polypeptide.

Surface plasmon resonance assay

The affinities of dimeric and trimeric aprotinin with various linkers are compared against the monomeric aprotinin using surface plasmon resonance assay, or BIAcore analysis (BIAcore, Piscataway, NJ) with human plasmin as the target. Similarly, BIAcore assay with heparin as the target is used to analyze affinity between GAG binding aprotinin fusion proteins and heparin.

When plasmin is used as the target, purified human plasmin (Sigma) is immobilized on the CM5 chip according manufacturer's instructions (BIAcore, Piscataway, NJ). When heparin is the target, biotinylated albumin and albumin-heparin (Sigma) are captured on a streptavidin-coated BIAcore SA chip as described previously (Xiang Y and Moss B. (2003) *J Virol* 77:2623-2630).

Example 2: Establishing improved tissue culture models for studies on influenza virus infection.

Stocks of Influenza Viruses

Influenza viral strains are obtained from ATCC and the repository at St. Jude Children's Research Hospital. All experiments involving influenza viruses are conducted at Bio-safety level II.

Viruses are propagated by injection into the allantoic cavity of nine-day-old chicken embryos as described (Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1985) *J Gen Virol* 66:1633-1638). Alternatively, viral stocks are grown on Madin-Darby canine kidney (MDCK) cells in minimal essential medium (MEM) supplemented with 0.3% bovine serum albumin and 0.5 micrograms of trypsin per ml. After

incubating for 48 to 72 hours, the culture medium is clarified by low speed centrifugation. Viral particles are pelleted by ultracentrifugation through a 25% sucrose cushion. Purified viruses are suspended in 50% glycerol-0.1M Tris buffer (pH 7.3) and stored at -20^0C.

Plaque Assays

Infectivity and titer of the viral stocks are determined by two kinds of plaque assays, a conventional one and a modified one (Tobita, K, Sugiura, A, Enomoto, C and Furuyama, M. (1975) *Med Microbiol Immnuol* 162:9-14; Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1982) *Arch Virol* 71:177-183). The conventional plaque assay is routinely used as a virus titration method. It requires exogenous trypsin in agar overlay added immediately after virus infection to MDCK monolayers (Tobita, K, Sugiura, A, Enomoto, C and Furuyama, M. (1975) *Med Microbiol Immnuol* 162:9-14). This method artificially increases infectivity of the viral stocks being tested by activating all the viral particles that have uncleaved HA.

Zhirnov et. al. designed a modified plaque assay consisting of a double agar overlay, with trypsin being included in the second layer which is added 24 hours after infection (Zhirnov OP, Ovcharenko AV and Bukrinskaya AG. (1982) *Arch Virol* 71:177-183). Three days after infection, cells are fixed with a 10% formaldehyde solution, agarose layers are removed, fixed cells are stained with hematoxylin-eosin solution and plaques are counted. The modified plaque assay allows accurate determination of the real infectivity of viral stocks that contain both cleaved and uncleaved HA. Combining results from both conventional and modified plaque assays, one can distinguish viruses containing cleaved or uncleaved HA and correlate infectivity of viral stocks with the status of HA cleavage.

Human Cell Culture Models

1. Short-term culture of primary human epithelial cells. Conventional *in vitro* influenza virus infection is mostly carried out in MDCK cells with exogenous trypsin added to the culture medium. This is far from being physiological and is inappropriate for the work

proposed here because trypsin is not the protease that activate influenza viruses *in vivo*. Very limited numbers of *in vitro* tissue culture models that are able to support the growth of influenza virus without an exogenous protease have been reported so far, those being primary cultures with primate cells of renal origin, cells lining the allantoic and aminiotic cavities of embryonated eggs, fetal tracheal ring organ cultures and primary human adenoid epithelial cells (Endo Y, Carroll KN, Ikizler MR and Wright PF. (1996) *J Virol* 70:2055-2058). Among these, the latest work with primary human adenoid epithelial cells is the closest mimic of human conditions. In this case, Endo et. al. (Endo Y, Carroll KN, Ikizler MR and Wright PF. (1996) *J Virol* 70:2055-2058) isolated epithelial cells from surgical samples of human adenoids, and cultured the epithelial cells on a collagen matrix (Vitrogen 100, Celtrix Laboratories, Palo Alto, California) in Transwell inserts (Costar, Cambridge, Mass). Cells were maintained in 50% Ham's F12 and 50% Eagles minimal essential media with supplements of growth factors and trace elements. The cells reached confluency in 10 to 14 days, remaining largely as a monolayer but with discrete patches of ciliated cells, which maintained regular ciliary activity for 1 to 3 weeks after reaching confluency. In this system, influenza A virus grew to a titer of 10^6 PFU/ml with a multiplicity of infection of 0.001 (Endo Y, Carroll KN, Ikizler MR and Wright PF. (1996) *J Virol* 70:2055-2058). Progressive cytopathogenic effects were also present during infection. The biggest drawback of this system is that it requires fresh human adenoid tissue.

To solve this problem, primary human adenoid epithelial cells are replaced with primary human airway epithelial cells that are commercially available (Cambrex), and the cells are grown under the same conditions. Such short-term culture of primary human airway epithelial cells is relatively quick to establish and is useful as the first-line experimental model for most of the *in vitro* infection and antiviral experiments.

2. Well-differentiated human airway epithelium (WD-HAE). In order to best mimic the *in vivo* condition of human airway, the model of well-differentiated human airway epithelium (WD-HAE) is used. WD-HAE is stratified epithelium that has all the differentiated cells of the normal human airway epithelium, including functional ciliated cells and mucus secreting cells. Therefore, in this model system influenza viruses are

most likely to be activated by host proteases that are physiologically relevant. Although WD-HAE has been widely used to study respiratory viral infections, such as respiratory syncytial virus (RSV) Zhang L, Peeples ME, Boucher RC, Collins PL and Pickles RJ. (2002) *J Virol* 76:5654-5666) measles virus (Sinn PL, Williams G, Vongpunsawad S, Cattaneo R and McCray PB. (2002) *J Virol* 76:2403-2409, or human rhinovirus, it has not previously been used to study influenza viruses.

A detailed protocol of WD-HAE has been described previously (Krunkosky TM, Fischer BM, Martin LD, Jones N, Akley NJ and Adler KB. (2000) *Am J Respir Cell Mol Biol* 22:685-692). Briefly, commercial primary human bronchial epithelial cells (Cambrex) are cultured on Transwell-clear culture inserts (Costar) that are thin-coated with rat-tail collagen I. Cells are cultured submerged for the first 5 to 7 days in medium containing a 1:1 mixture of bronchial epithelial cell growth medium (BEGM) (Cambrex) and DMEM with high glucose with supplement of growth factors (Krunkosky TM, Fischer BM, Martin LD, Jones N, Akley NJ and Adler KB. (2000) *Am J Respir Cell Mol Biol* 22:685-692). When cultures are 70% confluent (days 5 to 7), the air-liquid interface is created by removing the apical medium and exposing cells only to medium on their basal surface. Cells are cultured for additional 14 days in air-liquid interphase, for a total of 21 days in culture, and are then ready for experiments. The differentiated epithelium can be maintained *in vitro* for weeks.

Epithelial morphology and degree of differentiation is documented by routine histology (Endo Y, Carroll KN, Ikizler MR and Wright PF. (1996) *J Virol* 70:2055-2058). Briefly, following fixation with 10% buffered formalin, the epithelial cells are embedded in paraffin, sectioned and stained with hematoxylin and eosin, and with periodic acid-Schiff stain for mucus secreting cells.

Influenza infection is carried out in the above two model systems by adding 0.001 to 1 MOI of viruses to the differentiated cells. The titer and infectivity of viruses in the supernatant are followed over a period of 3 to 7 days. The level of influenza viral amplification and the infectivity of influenza viruses are evaluated using conventional and modified plaque assays.

Example 3: Comparing functions of the aprotinin fusion proteins in vitro

Anti-Viral Effects of Aprotinin Fusion Proteins

1. Pre-infection treatment. Aprotinin fusion proteins are added to primary human cell cultures at various concentrations and allowed to incubate with the cells for 1 hour. The cells are washed with fresh medium and immediately inoculated with influenza viruses at MOI 0.01 to 1. Cells are washed again after 1 hour and cultured for 3 to 5 days. Titer and infectivity of viruses in the supernatant are measured at various time points by two plaque assays. The cytopathic effect caused by viral infection is evaluated by staining viable cells with crystal violet and quantifying by measuring absorption at 570 nm at the end of the experiment. The percentage of cell protection by aprotinin fusion proteins is calculated by 100x{(aprotinin treated sample-untreated infected sample)/(uninfected control-untreated infected sample)}. The drug efficacy for cell protection is described by its Effective Concentration that achieves 50% of the cell protection (EC_{50}). Since HA activation only occurs to newly released viral particles, the first round of viral infection occurs normally and viral titer rises in the first 24 hours after infection. However, starting from the second round, infectivity of viruses drops and viral titer gradually decreases as result of aprotinin treatment. Results from this experiment differentiate various types of different aprotinin fusion proteins by their efficacies in a single prophylactic treatment.

Alternatively, timing of initial viral inoculation is altered from immediately after aprotinin treatment to 2-24 hours post treatment. Viral titer, infectivity and cytopathic effect are measured for 3 to 5 day after infection as described above. Results from these experiments distinguish various aprotinin fusion proteins by the lengths of the effective window after a single prophylactic treatment.

2. Post-infection Treatment. For multi-dose treatment, cells are first infected by viral inoculations at 0.001 to 0.1 MOI for 1 hour. Various concentrations of aprotinin fusion proteins are added immediately afterwards, additional treatments are applied at 8-hour intervals during the first 48 hours post infection. Cells are cultured until day 7 post

infection. Viral titer and infectivity in the media are followed during the whole process. Cytopathic effect is evaluated at the end of the experiment.

For single dose treatment, cells are first infected by viral inoculations at 0.001 to 0.1 MOI for 1 hour. Treatments of aprotinin fusion proteins at various concentrations are applied at different time points during the first 48 hours after infection, but each cell sample only receives one treatment during the whole experiment. Cells are cultured until day 7 post infection. Viral titer and infectivity in the media are followed during the whole process. Cytopathic effect is evaluated at the end of the experiment. Results from these experiments distinguish different types of aprotinin fusion proteins for their therapeutic potency.

Inhibition of HA Cleavage by Aprotinin Fusion Proteins

To demonstrate that aprotinin fusion proteins inhibit influenza viral infection by inhibiting cleavage of influenza HA protein, a human primary epithelial cell culture is infected with influenza virus at MOI of 1. Aprotinin fusion proteins are added to the culture either right before viral inoculation or immediately after the viral infection. At 6.5 hour post infection, the culture is incubated for 1 hour in MEM lacking cold methionine and containing ^{35}S-labeled methionine (Amersham) at a concentration of 100 microCi/ml (pulse). Thereafter, the cells are washed twice with MEM containing a 10-fold concentration of cold methionine and incubated in MEM for additional 3 hours (chase). After labeling, cells are dissolved in radioimmunoprecipitation assay (RIPA) buffer, HA is precipitated by anti-serum against the particular strain of virus used for infection (anti-influenza sera can be obtained from ATCC and Center of Disease Control and Prevention), and immunocomplex is then purified by protein G-Sepharose (Amersham). Samples are fractionated by SDS-PAGE followed by autoradiography. In samples untreated by aprotinin fusion proteins, HA1 and HA2 are expected to be the predominant HA species; while in aprotinin treated samples, HA0 is expected to be the major type of HA present.

Example 4: Synthesizing genes of five sialidases, expressing and purifying the sialidase proteins.

Introduction

Influenza viruses belong to the *orthomyxoviridae* family of RNA viruses. Both type A and type B viruses have 8 segmented negative-strand RNA genomes enclosed in a lipid envelope derived from the host cell. The viral envelope is covered with spikes that are composed of three proteins: hemagglutinin (HA), that attaches virus to host cell receptors and mediates fusion of viral and cellular membranes; neuraminidase (NA), which facilitates the release of the new viruses from the host cell; and a small number of M2 proteins that serve as ion channels. For *Influenza A virus*, HA and NA both undergo antigenic drift and antigenic shift, the viral subtypes are distinguished by serologic differences between their HA and NA proteins. There are total 15 types of HA (H1-H15) and 9 types of NA (N1-N9), but only three HA (H1-H3) and two NA (N1 and N2) have been found in human *Influenza A virus* so far (Granoff, A. & Webster, R. G., ed. *Encyclopedia of Virology*, 2nd Edition, Vol 2). In contrast to *Influenza A virus*, no distinct antigenic subtypes are recognized for *Influenza virus B*.

While *Influenza B virus* circulates only in humans, *Influenza A virus* can be isolated from a whole host of animals, such as pigs, horses, chickens, ducks and other kinds of birds, which accounts for genetic reassortment of *Influenza A virus* that results in antigenic shift. Wild aquatic birds are considered to be the primordial reservoir of all influenza viruses for avian and mammalian species. There is extensive evidence for transmission of the virus between aquatic birds and other species including pigs and horses and indirect transmission to humans through pigs. Direct transmission from pigs or chickens to humans has also been documented (Ito, T. (2000) *Microbiol Immunol* 44(6):423-430).

The host cell receptor for influenza viruses is the cell surface sialic acid. Sialic acids are α-keto acids with 9-carbon backbones that are usually found at the outermost positions of the oligosaccharide chains that are attached to glycoproteins and glycolipids. One of the major types of sialic acids is N-acetylneuraminic acid (Neu5Ac), which is the biosynthetic precursor for most of the other types. Two major linkages between Neu5Ac

and the penultimate galactose residues of carbohydrate side chains are found in nature, Neu5Ac α(2,3)-Gal and Neu5Ac α(2,6)-Gal. Both Neu5Ac α(2,3)-Gal and Neu5Ac α(2,6)-Gal molecules can be recognized by *Influenza A virus* as the receptor (Schauer, R. (1982) Adv. *Carbohydrate Chem & Biochem* 40:131-235), while human viruses seem to prefer Neu5Ac α(2,6)-Gal, avian and equine viruses predominantly recognize Neu5Ac α(2,3)-Gal (Ito, T. (2000) *Microbiol Immunol* 44(6):423-430).

Infections by influenza type A and B viruses are typically initiated at the mucosal surface of the upper respiratory tract. Viral replication is primarily limited to the upper respiratory tract but can extend to the lower respiratory tract and causes bronchopneumonia that can be fatal. The risk of death is one per 10,000 infections, but is significantly greater for high-risk groups with pre-existing cardiopulmonary conditions and for immunologically naïve individuals during a pandemic.

A therapeutic compound comprising a sialidase that can effectively degrade both receptor sialic acids, Neu5Ac α(2,6)-Gal and Neu5Ac α(2,3)-Gal, can confer protection against the broadest range of influenza viruses, including animal viruses. It can also remain effective as the viral strains change yearly. Because sialidase targets the host cell rather than virus and acts at the "choking point" in a viral life cycle, generation of resistant virus is improbable. Protein-bound sialic acid turns over homogeneously on cell surface with half-life of 33 hours (Kreisel, W, Volk, BA, Buchsel, R. and Reutter, W. (1980) *Proc Natl Acad Sci USA* 77:1828-1831). Therefore we estimate that once-a-day or twice-a-day administration of a sialidase would confer sufficient protection against influenza.

Sialidases are found in higher eukaryotes, as well as in some mostly pathogenic microbes, including viruses, bacteria and protozoans. Viral and bacterial sialidases have been well characterized, and the three-dimensional structures of some of them have been determined (Crennell, SJ, Garman, E, Laver, G, Vimr, E and Taylor, G. (1994) *Structure* 2:535-544; Janakiraman, MN, White, CL, Laver, WG, Air, GM and Luo, M. (1994) *Biochemistry* 33:8172-8179; Pshezhetsky, A, Richard, C, Michaud, L, Igdoura, S, Wang, S, Elsliger, M, Qu, J, Leclerc, D, Gravel, R, Dallaire, L and Potier, M. (1997) *Nature Genet* 15: 316-320). Several human sialidases have also been cloned in the recent years (Milner, CM, Smith, SV, Carrillo MB, Taylor, GL, Hollinshead, M and Campbell, RD.

(1997) *J Bio Chem* 272:4549-4558; Monti, E, Preti, A, Nesti, C, Ballabio, A and Borsani G. 1999. *Glycobiol* 9:1313-1321; Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351). All the sialidases characterized share a four amino acid motif in the amino terminal portion followed by the Asp box motif which is repeated three to five times depending on the protein. (Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292). While the overall amino acid identity of the sialidase superfamily is relatively low at about 20-30%, the overall fold of the molecules, especially the catalytic amino acids, are remarkably similar (Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292).

The sialidases are generally divided into two families: "small" sialidases have molecular weight of about 42 kDa and do not require divalent metal ion for maximal activity; "large" sialidases have molecular weight above 65 kDa and may require divalent metal ion for activity (Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27; Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351; Copley, RR, Russell, RB and Ponting, CP. (2001) *Protein Sci* 10:285-292).

Over fifteen sialidase proteins have been purified and they vary greatly from one another in substrate specificities and enzymatic kinetics. To confer a broad-spectrum protection against influenza viruses, a sialidase needs to effectively degrade sialic acid in both α(2,6)-Gal and α(2,3)-Gal linkages and in the context of glycoproteins and some glycolipids. Viral sialidases, such as those from *influenza A virus*, *fowl plague virus* and *Newcastle disease virus*, are generally specific for Neu5Ac α(2,3)-Gal and only degrade

Neu5Ac α(2,6)-Gal very inefficiently. Small bacterial sialidases generally react poorly to sialic acid in the context of glycoproteins and glycolipids. By contrast, large bacterial sialidases can effectively cleave sialic acid in both (α,2-6) linkage and (α,2-3) linkage in the context of most natural substrates (**Figure 4**; Vimr, DR. (1994) *Trends Microbiol* 2: 271-277; Drzeniek, R. (1973) *Histochem J* 5:271-290; Roggentin, P, Kleineidam, RG and Schauer, R. (1995) *Biol Chem Hoppe-Seyler* 376:569-575; Roggentin, P, Schauer, R, Hoyer, LL and Vimr, ER. (1993) Mol Microb 9:915-921). Because of their broad substrate specificities, large bacterial sialidases are better candidates.

Among the large bacterial sialidases with known substrate specificity shown in **Figure 4**, *Vibrio cholerae* sialidase requires Ca2+ for activity making it less preferred. More preferred sialidases include the 71 kDa enzyme from *Clostridium perfringens*, the 113 kDa enzyme from A*ctinomyces viscosus* and sialidase of *Arthrobacter ureafaciens*. A third sialidase, the 68 kDa enzyme from *Micromonospora viridifaciens*, has been known to destroy influenza viral receptor (Air, GM and Laver, WG. (1995) *Virology* 211:278-284), and is also a candidate.

These enzymes have high specific activity (600 U/mg protein for *C. perfringens* (Corfield, AP, Veh, RW, Wember, M, Michalski, JC and Schauer, R. (1981) *Bichem J* 197:293-299) and 680 U/mg protein for *A. viscosus* (Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443)), are fully active without divalent metal iron, and have been cloned and purified as recombinant proteins from *E. coli* (Roggentin, P, Kleineidam, RG and Schauer, R. (1995) *Biol Chem Hoppe-Seyler* 376:569-575, Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443 , Sakurada, K, Ohta, T and Hasegawa, M. (1992) *J Bacteriol* 174: 6896-6903). In addition, *C. perfringens* is stable in solution at 2-8^{0}C for several weeks, and at 4^{0}C in the presence of albumin for more than two years (Wang, FZ, Akula, SM, Pramod, NP, Zeng, L and Chandran, B. (2001) *J Virol* 75:7517-27). *A. viscosus* is labile towards freezing and thawing, but is stable at 4^{0}C in 0.1 M acetate buffer, pH 5 (Teufel, M, Roggentin, P. and Schauer, R. (1989) *Biol Chem Hoppe Seyler* 370:435-443).

Although the chances of inducing immune reactions using bacterial sialidases is very low because the proteins will be used topically in the upper respiratory tract and will

not be absorbed systemically a human enzyme would be more desirable for long-term use in human subjects.

Four sialidase genes have been cloned from human so far: NEU1/G9/lysosomal sialidase (Pshezhetsky, A, Richard, C, Michaud, L, Igdoura, S, Wang, S, Elsliger, M, Qu, J, Leclerc, D, Gravel, R, Dallaire, L and Potier, M. (1997) *Nature Genet* 15: 316-320. , Milner, CM, Smith, SV, Carrillo MB, Taylor, GL, Hollinshead, M and Campbell, RD. (1997). *J Bio Chem* 272:4549-4558); NEU3, a membrane-associated sialidase isolated from human brain (Wada, T, Yoshikawa, Y, Tokuyama, S, Kuwabara, M, Akita, H and Miyagi, T. (1999) *Biochem Biophy Res Communi* 261:21-27, Monti, E, Bassi, MT, Papini, N, Riboni, M, Manzoni, M, Veneranodo, B, Croci, G, Preti, A, Ballabio, A, Tettamanti, G and Borsani, G. (2000) *Bichem J* 349:343-351), NEU2 a 42 kDa sialidase expressed in human skeletal muscle at a very low level (Monti, E, Preti, A, Nesti, C, Ballabio, A and Borsani G. (1999) *Glycobiol* 9:1313-1321), and NEU4 a 497 amino acid protein (Genbank NM080741) expressed in all human tissues examined (Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663).

Amino acid sequence comparison reveals NEU2 (**SEQ ID NO:8**) and NEU4 (**SEQ ID NO:9**) are both cytosolic sialidases. 9 out of 12 of the amino acid residues which form the catalytic site of *S. typhimurium* sialidase are conserved in both NEU2 and NEU4 (Monti, E, Preti, A, Nesti, C, Ballabio, A and Borsani G. (1999) *Glycobiol* 9:1313-1321, **Figure 3**). In addition, NEU4 also shows a stretch of about 80 amino acid residues (aa 294-373) that appears unique among known mammalian sialidases (Monti, E, Preti, A, Venerando, B and Borsani, G. (2002) *Neurochem Res* 27:646-663). Unlike the selected large bacterial sialidases, the substrate specificity of NEU2 and NEU4 is unknown. It will need to be tested if NEU2 and NEU4 can effectively degrade the influenza virus receptors.

Sialidase assay

NEU2, NEU4 and M. viridifaciens enzymes will be stored in PBS and 50% glycerol at -20^0C. C. perfringens and A. viscosus enzymes are stored in 10mM acetate buffer (pH5) at 4^0C. Protein preps are characterized by HPLC and SDS-PAGE

electrophoresis. Specific activities and stability of the enzymes will be monitored by sialidase assay.

The enzymatic activity of sialidases are determined by fluorimetric 2'-(4-methylumbelliferyl)-alpha-D-N-acetylneuraminic acid) (4Mu-NANA) (Sigma) as the substrate. Specifically, reactions are set up in duplicate in 0.1M Na citrate/phosphate buffer pH5.6, in the presence of 400 micrograms bovine serum albumin, with 0.2 mM 4MU-NANA in a final volume of 100 microliters, and incubated at 37^0C for 5-10min. Reactions are stopped by addition of 1 ml of 0.2 M glycines/NaOH pH10.2. Fluorescence emission is measured on a fluorometer with excitation at 365 nm and emission at 445 nm, using 4-methylumbelliferone (4-MU) to obtain a calibration curve.

Example 5: Comparing functions of the sialidases in vitro and selecting one sialidase for further studies.

1. Stocks of Influenza Viruses

Influenza viral strains are obtained from the ATCC and the repository at St. Jude Children's Research Hospital.Viral stocks are grown on Madin-Darby canine kidney (MDCK) cells in minimal essential medium (MEM) supplemented with 0.3% bovine serum albumin and 0.5 micrograms of trypsin per ml. After incubating for 48 to 72 hours, the culture medium is clearified by low speed centrifugation. Viral particles are pelleted by ultracentrifugation through a 25% sucrose cushion. Purified viruses are suspended in 50% glycerol-0.1M Tris buffer (pH 7.3) and stored at -20^0C. Viral titer is determined by plaque assay (Tobita, K, Sugiura, A, Enomoto, C and Furuyama, M. (1975) *Med Microbiol Immnuol* 162: 9-14), or $TCID_{50}$, which is the dose of virus required to infect 50% of the MDCK cells.

Selected human and animal influenza A strains with specificity towards Neu5Ac alpha(2,6)-Gal or Neu5Aç alpha(2,3)-Gal and have high affinity to the receptors (measured by high hemagglutination activity) are chosen for *in vitro* tests:

1. Strains that recognize receptor Neu5Ac alpha(2,6)-Gal include human isolates A/aichi/2/68, A/Udorn/307/72, A/Prot Chaimers/1/73 and A/Victoria/3/75, etc.

(Connor, RJ, Kawaoka, Y, Webster, RG and Paulson JC. (1994) *Virology* 205:17-23).

2. Strains that have Neu5Ac alpha(2,3)-Gal specificity include animal isolates A/duckUkraine/1/63, A/duckMemphis/928/74, A/duckhokk/5/77, A/Eq/Miami/1/63, A/Eq/Ur/1/63, A/Eq/Tokyo/71, A/Eq/Prague/71, etc (Connor, RJ, Kawaoka, Y, Webster, RG and Paulson JC. (1994) *Virology* 205:17-23).

2. Hemagglutination Assay

This assay is used to rapidly determine the efficiency of each enzyme to destroy receptors Neu5Ac alpha(2,6)-Gal and Neu5Ac alpha(2,3)-Gal.

Specifically, 6 ml of Chicken red blood cells (SPAFAS Inc., Norwich, CT) are diluted in two times the volume of PBS, centrifuge for 5 min at 500 x g and re-suspended in PBS of original volume. Sialidases are added to the chicken erythrocytes at various concentrations and allowed to incubate at room temperature for 30 min. The cells are then washed three times to remove sialidase proteins, and then are resuspended in PBS to 6 ml. Control cells are incubated with BSA and washed. Various strains of influenza virus, which recognize either Neu5Ac alpha(2,6)-Gal or Neu5Ac salpha(2,3)-Gal as the receptor as listed above, are prepared in microtiter plates as serial dilutions in PBS (100 microliters) of the original viral stocks. Sialidase-treated or control chicken red blood cell suspensions (100 microliters of the 0.5% solution prepared above) are added to each well at 4^0C. The plates are read after 2 h. The lowest concentration of virus that causes the blood cell to agglutinate is defined as one hemagglutination unit. We will be looking for enzymes that effectively abolish hemagglutination by all viral strains.

3. Viral Inhibition Assay

Confluent monolayers of MDCK cells are treated with various concentrations of sialidases for 1 h, washed twice with buffer, then infected with various strains of influenza virus. After incubation for 1 hr, the cells are washed again to remove unbound virus. To estimate the decrease in viral binding sites on cell surface, the cells are overlaid

with agar and incubated at 37^0C. The number of plaques in the sialidase treated cells will be compared against those in control cells. Alternatively, the cells will be cultured in regular medium at 37^0C, and viral titers in the culture media are measured at various time during culture as $TCID_{50}$.

To demonstrate that sialidase treatment can inhibit a pre-existing infection, MDCK monolayers are first infected with a low titer of virus. After washing off the unbound virus, the cells are then cultured in the presence of a sialidase. Fresh sialidase is added to cell culture very 24 h. Viral titer in the cultured medium is measured over a 72-hour period.

4. Cytotoxicity assay

Primary human bronchial epithelial cells are purchased (Clonetics) and cultured in supplemented minimal medium following manufacture's instruction. Sialidases are added to the culture medium at various concentrations. Cell growth over a period of 7-10 days will be measured. Cells will also be observed regularly for microscopic cytopathic effects.

Example 6: Constructing and testing sialidase fusion proteins.

1. Choosing a GAG-binding sequence as the anchoring domain.

One sialidase is selected for its best overall properties, including anti-viral activity, toxicity, stability, ease of production, etc. We will then genetically link it to a GAG-binding sequence, sub-clone the fusion genes into pQE vector, express and purify the fusion proteins from *E. coli*.

We have selected six possible human GAG-binding sequences: PF4 (aa 47-70) (**SEQ ID NO:2**), IL-8 (aa 46-72) (**SEQ ID NO:3**), AT III (aa 118-151) (**SEQ ID NO:4**), ApoE (aa 132-165) (**SEQ ID NO:5**), amphiregulin (aa 25-45) (**SEQ ID NO:6**), and human angio-associated migratory cell protein (AAMP) (aa 14-25) (**SEQ ID NO:7**) (**Figure 2**). These sequences generally bind to heparin with nanomolar-level affinities; however, their affinities may vary from one another by an order of magnitude (**Table 1**). Since it is not clear which anchoring domain will enable the most effective functioning of

the sialidase, all four GAG-binding sequences are fused with the sialidase gene either on the N terminus or the C terminus via a generic linker sequence GGGGS as the following constructs:

(GAG binding domain— GGGGS(**SEQ ID NO:10**) —Sialidase); or

(Sialidase—GGGGS(**SEQ ID NO:10**)—GAG binding domain)

Different fusion proteins are compared by a modified viral inhibition assay. Specifically, confluent monolayers of MDCK cells are treated with same amount of each fusion protein for a limited duration, such as 30 min. The cells are then washed twice with buffer to remove unbound sialidase fusion proteins, and incubated in culture medium for an additional 1 hour. Afterwards, strains of influenza virus are added to the cells for 1 hr and then cells are washed again to remove unbound virus. Viral titers in the culture media are measured during 72-h cultures as $TCID_{50}$. The un-fused sialidase protein will be used to compare against the fusion proteins in this assay. If the results are too close to rank all fusion proteins, we will make the assay more stringent by shortening treatment window for the fusion proteins, lowering protein concentrations and increasing the level of viral challenge.

2. Optimizing the fusion protein construct

After selecting the best fusion protein from the earlier experiments, the construct is further optimized by testing different linker length. In this regard, the following constructs are made:

(Sialidase— (GGGGS(**SEQ ID NO:10**))n (n=0, 1, 2, 3, or 4) —GAG binding domain)

The proteins are expressed and purified and compared in the modified viral protection assay as described above.

In addition, if earlier data indicate that higher affinity of the fusion protein towards heparan sulfate brings better potency, we also plan to test if the potency can be further improved by increasing the GAG-binding affinity. This can be achieved by creating a multivalent GAG binding mechanism in the fusion protein in constructs like these:

(Sialidase—(GGGGS(**SEQ ID NO:10**))n—HS binding domain—GAG binding domain);

or:

(GAG binding domain—(GGGGS(**SEQ ID NO:10**))n—Sialidase—

(GGGGS(**SEQ ID NO:10**))n—GAG binding domain)

The purified fusion proteins are ranked based on their activities in the modified viral protection assay as described above.

3. Cytotoxicity assay

The effects of the fusion proteins on normal cell growth and morphology are monitored by culturing primary human bronchial epithelial cells with various concentrations of the fusion proteins and following growth curve of the cells and observing any microscopic cytopathic effects.

Example 7: Fusion Proteins against Other Infectious Microbes

Fusion proteins composed of a functional domain and an anchorage domain are designed for many more different applications. For example, a sialidase fusion protein as proposed here can also be used as a therapeutic/prophylatictic agent against infections by other viruses and bacteria besides influenza viruses, because many other infectious microbes, such as paramyxoviruses (Wassilewa, L. (1977) *Arch Virol* 54:299-305), coronaviruses (Vlasak, R., Luytjes, W., Spaan, W. and Palese, P. (1988) *Proc Natl Acad Sci USA* 85:4526-4529), rotaviruses (Fukudome, K., Yoshie, O. and Konno, T. (1989) *Virology* 172:196-205) and Pseudomonas aeruginosa (Ramphal, R. and Pyle, M. (1983) *Infect Immun* 41:339-44) etc, are also known to use sialic acid as cellular receptors. For example, aprotinin fused with a heparin-binding domain can make a fusion protein that be used to prevent/treat infection of other viruses besides influenza that require host serine proteases for activation, such as parainfluenza virus.

Example 8: Cloning Sialidase Catalytic Domain Fusion Proteins

According to the published literature on the large bacterial sialidases, the 51 kDa *Arthrobacter ureafaciens* sialidase, the 71 kDa sialidase from *Clostridium perfringens* and the 113 kDa sialidase from *Actinomyces viscosus* seem to have similar specific activities and broad substrate specificity toward various sialic acid conjugates (Biology of the Sialic Acids (1995) , 270-273; Corfield et al., Biochem. J., (1981) 197(2), 293-299; Roggentin et al., Biol. Chem. Hoppe Seyler, (1995) 376(9), 569-575; Teufel et al., Biol. Chem. Hoppe Seyler, (1989) 370(5), 435-443). A third sialidase, the 68 kDa enzyme from *Micromonospora viridifaciens*, was also known to destroy the influenza viral receptor (Air and Laver, Virology, (1995) 211(1), 278-284; (1995) , 270-273).

A. viscosus is part of the normal flora of human oral cavity and gastrointestinal tract (Sutter, Rev. Infect. Dis., (1984) 6 Suppl 1, S62-S66). Since the sialidase from *A. viscosus* is normally secreted by the bacterium hosted on human mucosal surface, it should be tolerated by the human mucosal immune system. Therefore, it is unlikely that *A. viscosus* sialidase will be immunogenic when delivered topically to the human airway surface. We think that this feature makes *A. viscosus* sialidase a good candidate for a therapeutic agent.

We determined that a fragment of the *A. viscosus* sialidase, extending from amino acid 274 to amino acid 667, should contain the catalytic domain (referred to as AvCD) of the sialidase and should be fully active on it own. We later cloned the AvCD fragment and demonstrated that this AvCD fragment and other *A. viscosus* sialidase fragments comprising at least amino acids 290-666 of the A. viscosus sialidase protein sequence (**SEQ ID NO:12**), such as the fragment extending from amino acid 274 to amino acid 681, the fragment extending from amino acid 274 to amino acid 666, the fragment extending from amino acid 290 to amino acid 666, and the fragment extending from amino acid 290 to amino acid 681, have sialidase activity.

The complete sequence of *A. viscosus* sialidase protein and gene were obtained from GenBank (A49227 and L06898). Based on homology with sialidases with known 3D structures (*M. viridifaciens* and *S. typhimurium*), we assigned the catalytic domain (CD) sequence to be located between amino acids 274-667 (**SEQ ID NO:16**). To clone the catalytic domain of *A. viscosus* sialidase (AvCD), this region of the *A. viscosus*

sialidase gene was engineered with codons optimized for expression in E.coli (**SEQ ID NO:15**). The codon-optimized AvCD nucleotide sequence encoding amino acids 274-667 of the *A. viscosus* sialidase (**SEQ ID NO:15**) was produced by chemical synthesis of overlapping oligonucleotides which were annealed, amplified by PCR and cloned into the expression vector pTrc99a (Amersham, New Jersey, USA).

Sialidase fusion constructs were made using standard molecular cloning methods. The His_6-AR construct was made by fusing six histidines (His_6) to the N-terminal residue of the AvCD sequence. The His_6-AvCD construct has the nucleotide sequence of **SEQ ID NO:17** and translated amino acid sequence of **SEQ ID NO:18**. These sequences are depicted in **Figure 7**.

To make the AR-AvCD construct, an anchoring domain was directly fused with the N-terminal residue of the AvCD sequence. The anchoring domain, referred to as AR, was derived from the GAG binding sequence of human amphiregulin precursor (GenBank # AAH09799). Nucleotide sequences encoding amino acids 125 to 145 (**Figure 2, SEQ ID NO:7**) of the human amphiregulin precursor were synthesized chemically as two overlapping oligonucleotides. The AR-AvCD construct has the nucleotide sequence of **SEQ ID NO:19** and translated amino acid sequence of **SEQ ID NO:20.**

Another construct, AR-G4S-AvCD, was made by fusing the same AR-encoding sequence used in the AR-AvCD construct with a sequence encoding a five-amino-acid linker (GGGGS; **SEQ ID NO:10**) which then was fused with the AvCD sequence such that in a translation product, the linker was fused to N-terminus of the catalytic domain of the A. viscosus sialidase. The nucleotide sequence (**SEQ ID NO:34**) and translated amino acid sequence (**SEQ ID NO:35**) of this construct are depicted in **Figure 9**. All constructs were cloned into the pTrc99a expression vector.

In addition, four constructs were made in which the catalytic domain of the *A. viscosus* sialidase was fused to the N-terminus of the AR (GAG-binding domain of human amphiregulin; **SEQ ID NO:7**). In Construct #4 (**SEQ ID NO:27**), the catalytic domain of the *A. viscosus* sialidase consisted of amino acids 274-666 of **SEQ ID NO:12** fused to the GAG-binding domain of amphiregulin (**SEQ ID NO:7**). In Construct #5 (**SEQ ID NO:29**), the catalytic domain of the *A. viscosus* sialidase consisted of amino acids 274-

681 of **SEQ ID NO:12** fused to the GAG-binding domain of amphiregulin (**SEQ ID NO:7**). In Construct #6 (**SEQ ID NO:31**), the catalytic domain of the A. *viscosus* sialidase consisted of amino acids 290-666 of **SEQ ID NO:12** fused to the GAG-binding domain of amphiregulin (**SEQ ID NO:7**). In Construct #7 (**SEQ ID NO:33**), the catalytic domain of the A. *viscosus* sialidase consisted of amino acids 290-681 of **SEQ ID NO:12** fused to the GAG-binding domain of amphiregulin (**SEQ ID NO:7**). All of these constructs displayed comparable sialidase activity in assays.

Example 9: Production of Sialidase Catalytic Domain Fusion Proteins

To produce the sialidase fusion proteins, the expression constructs were transformed into E.coli BL21. A single colony was inoculated into 2.5 ml of LB broth and grown overnight at 37°C with shaking. In the morning 2 ml of overnight culture was inoculated into 500 ml of TB medium in a 2 liter shake flask and the culture was allowed to grow to OD_{600}=4.0 (2-4 hours) at 37°C with shaking. Protein expression was induced by addition of IPTG to a final concentration of 1 mM and continued for 3 hr with shaking. Cells were harvested by centrifugation at 5,000xg for 10 min. Cell were washed once (resuspended in PBS and recentrifuged) and resuspended in 15 ml of Lysis buffer.

Compositions of media and buffers used in protein expression and purification.

TB medium for protein expression

Solution 1

Bacto-tryptone - 12 g

Yeast extract - 24 g

H_2O to 800 ml

Solution 2

KH_2PO_4 (anhydrous) - 2.3 g

K_2HPO_4 (anhydrous) – 12.5 g

H_2O to 100 ml

Autoclave solutions 1 and 2 separately, cool, mix and add the following:

60 ml of 20% glycerol (filter sterilized)

20 ml of 20% glucose (filter sterilized)

Lysis buffer

50 mM phosphate, pH 8.0

10% glycerol

300 mM NaCl

Bacterial cells suspended in lysis buffer were lysed by sonication and cell debris was removed by centrifugation. Clarified lysate was passed through an SP-Sepharose column (bed volume 15 ml, flow rate 120 cm/hour). The column was reconditioned to lower pH and salt with one volume of PBS to ensure good retention of Fludase during endotoxin removal. Endotoxin was removed by washing the column with 5 volumes of PBS containing 1% Triton X-100, 0.5% Sodium Deoxycholate and 0.1% SDS. The detergents were washed away with 3 volumes of PBS and 3 volumes of lysis buffer. Proteins were eluted from the column with lysis buffer that contained 0.8 M NaCl. The fraction eluted from SP-Sepharose was adjusted to 1.9 M $(NH_4)_2SO_4$ (most contaminating proteins are salted out at this step) and clarified by centrifugation. The supernatant was loaded onto Butyl-Sepharose column (flow rate 120 cm/hour). The column was washed with 2 volumes of 1.3 M $(NH_4)_2SO_4$ and the fusion was eluted with 0.65 M $(NH_4)_2SO_4$. For the final step, size exclusion chromatography was performed on Sephacryl S-200 equilibrated with PBS buffer at a flow rate of 25 cm/hour. Sialidase activity was determined against 4-MU-NANA as described in the following paragraph. Protein concentration was determined using Bio-Rad's Bradford kit. Protein purity was assessed by SDS-PAGE and estimated to be >98%. Specific activity of the enzyme was about 937 U/mg. Endotoxin in final preparations was measured using LAL test (Cambrex) and estimated to be <0.5 EU/ml.

For purification of His6 containing fusion protein, cation exchange on SP-Sepharose was replaced with Metal Chelate Affinity Chromatography on Ni-NTA. All buffers remained the same with the exception that elution from Ni-NTA was performed by 0.25 M imidazole in lysis buffer.

Example 10: Sialidase Assay to Measure Activity of Sialidase Catalytic Domain Fusion Proteins

The sialidase activity of the AR-AvCD protein encoded by Construct #2 was assayed and compared with that of native sialidases purfied from *C. perfringens* (Sigma, St. Louis, MO) and *A. ureafaciens* (Prozyme, San Leandro, CA). In addition, a fusion protein produced from a construct in which the amphiregulin GAG sequence (**SEQ ID NO: 7**) was fused to the Neu 2 human sialidase (**SEQ ID NO:8**) was also assayed for sialidase activity.

The sialidase activity expressed as units per mg sialidase was measured by the sialidase assay using the artificial fluorogenic substrate 4-MU-NANA (Sigma). One unit of sialidase is defined as the amount of enzyme that releases 10 nmol of MU from 4-MU-NANA in 10 min at 37°C (50 mM CH_3COOH - NaOH buffer, pH 5.5) in reaction that contains 20 nmol of 4-MU-NANA in a 0.2 ml volume. Reactions are stopped by addition of 1 ml of 0.2 M glycine/NaOH pH 10.2. Fluorescence emission is measured on a fluorometer with excitation at 365 nm and emission at 445 nm, using 4-methylumbelliferone (4-MU) to obtain a calibration curve (Potier et al., Anal. Biochem., (1979) 94(2), 287-296).

Table 2. Specific activity of sialidases (units per mg).

Sialidase	Specific activity
AR-NEU2	8
AR-AvCD	937
C. perfringens	333
A. ureafaciens	82

Our results show that the AvCD fusion protein (AR-AvCD) has the highest specific activity among all the tested sialidases (**Table 2**). The specific activity of AR-AvCD is over 100 times higher than that of a human sialidase fusion (AR-NEU2), and over two times higher than that of *C. perfringens* sialidase. Experimental results comparing the stability of the sialidases indicate very high stability of AR-AvCD: No

loss of activity for AR-AvCD was detected after 20 weeks at 25°C or 4°C in solution. By comparison, AR-NEU2 solution exhibited a half-life of 5 and 2 weeks when stored at 25°C and 37°C, respectively.

Example 11: Optimization of the N-terminus of Sialidase Catalytic Domain Fusion Proteins

The N-terminus of the AR-AvCD fusion protein was partially cleaved under certain conditions that resulted in small degrees of protein heterogeneity in the purified AR-AvCD prep. To solve this problem, we designed an approach to optimize the N-terminus of the sialidase fusion construct. A library containing AR-AvCD with random amino acids at the N-terminus was constructed as follows. AR-AvCD was amplified by PCR using a primer pair in which the primer annealing on 5'-end of the gene contained a randomized sequence in positions corresponding to amino acids 2 and 3. The nucleotide sequence of the primer and the encoded amino acid sequence are shown below.

ttttcgtctcccatgvnnvnnaagcgcaaaaaaaaaggcggca (**SEQ ID NO:21**)

MetXxxXxxLysArgLysLysLysGlyGly (**SEQ ID NO:22**)

In **SEQ ID NO:21**, "n" stands for any nucleotide (a, c, g, or t) and "v" stands for nucleotides a, g or c. By designing the sequence in such a way (disallowing the nucleotide t in the first position of codons) we avoided introduction of stop codons as well as aromatic amino acids (Phe, Tyr, Trp) and Cys. The *Esp*3I restriction endonuclease site (shown in bold) was introduced to allow generation of *Nco*I compatible overhang. The primer annealing to 3'-end of the gene carried *Hind*III site following the stop codon. The PCR product was digested with *Esp*3I - *Hind*III was ligated into pTrc99a expression vector digested with *Nco*I - *Hind*III. The ligation mix was transformed into *E.coli* and the cells were grown overnight in liquid culture containing Ampicillin.

The next day the culture was diluted with fresh medium, grown to OD_{600}=0.8 and induced with IPTG for 2 hours. Cells were harvested, homogenized and the fusions were subjected to two-step purification by liquid chromatography. Clarified lysate was loaded onto SP-Sepharose equilibrated with lysis buffer (50 mM HEPES, pH 8.0, 0.3 M NaCl,

10% glycerol). The column was washed with 0.45 M NaCl and the fusions were eluted with 0.9 M NaCl. The eluate was diluted with 10% glycerol to bring the concentration of NaCl to 0.2 M and loaded onto Heparin-Sepharose column. The column was developed with a linear gradient of NaCl. The fractions that contained sialidase activity were resolved on SDS-PAGE, electroblotted onto PVDF membrane and the 43 kDa band was subjected to amino-terminal sequencing.

The predominant N-terminal residues of the isolated sialidase fusion protein were either Val or Gly followed by the N-terminal residues of the AR tag. We then synthesized new sialidase fusion constructs, Constructs #2 and #3, by introducing a Val in front of the AR sequence such that the first six amino acids encoded by Constructs #2 and #3 were (Met-Val-Lys-Arg-Lys-Lys (**SEQ ID NO:23**)). N-terminal sequencing of proteins made from these new fusion constructs showed 100% homogeneity with the initiation Met being completely removed (which is desirable for therapeutic proteins) and Val being the first N-terminal residue followed by the AR tag sequence. These data are consistent with earlier publications that reported the common rules of N-terminal processing and protein stability as function of protein's N-terminal amino acid residue (Hirel et al., Proc. Natl. Acad. Sci. U. S. A, (1989) 86(21), 8247-8251; Varshavsky, Proc. Natl. Acad. Sci. U. S. A, (1996) 93(22), 12142-12149).

The nucleotide sequences of new fusion Construct #2 (AR-AvCD with optimized N-terminus) (**SEQ ID NO:24**) and its amino acid sequence translation (**SEQ ID NO:25**) is depicted in **Figure 10**. The nucleotide sequences of new fusion Construct #3 (AR-G4S-AvCD with optimized N-terminus) (**SEQ ID NO:36**) and its amino acid sequence translation (**SEQ ID NO:37**) is depicted in **Figure 11**. The amino acid sequence of processed proteins isolated from E. coli infected with Construct #2 is provided herein as **SEQ ID NO:38** and the amino acid sequence of processed proteins isolated from E. coli infected with Construct #3 is provided herein as **SEQ ID NO:39**.

Example 12: Comparing Activities of Sialidase Constructs with or without an Anchoring Domain

To evaluate if the AR sequence indeed improves the cell-surface activity of a sialidase fusion protein, we incubated proteins purified from E. coli that were

transformed with Construct #2; **SEQ ID NO:24**, depicted in **Figure 7**) or Construct #1 (His_6-AvCD; **SEQ ID NO:17**, depicted in **Figure 5**) with primary human bronchial epithelial cells and measured cell-bound sialidase activity after extensive washing. For cells incubated with Construct #2 protein (**SEQ ID NO:25**), up to 10% of the sialidase was found to be cell-bound, and the cell-bound sialidase activity increased in a dose-dependent manner with the input concentration of Construct #2 protein. However, Construct #1 protein (**SEQ ID NO:18**) incubated cells only exhibited background level of sialidase activity. Furthermore, we treated MDCK cells with either Construct #2 protein or Construct #1 protein and measured the level of residual α(2,6)-linked sialic acid on the surface of the cells (**Figure 8**). At equal levels of enzymatic activity below 100 mU per well, Construct #2 protein demonstrated significantly higher potency than Construct #1 protein. These results indicate that the AR domain indeed enhances the function of sialidase.

Example 13: In vitro Activities of Sialidase Fusion Proteins

Stocks of Influenza Viruses

Influenza viral strains are obtained from ATCC and the repository at St. Jude Children's Research Hospital. All experiments involving influenza viruses are conducted at Bio-safety level II.

Viruses are propagated on Madin-Darby canine kidney (MDCK) cells in minimal essential medium (MEM) supplemented with 0.3% bovine serum albumin and 0.5 micrograms of trypsin per ml. After incubating for 48 to 72 hours, the culture medium is clarified by low speed centrifugation. Viral particles are pelleted by ultracentrifugation through a 25% sucrose cushion. Purified viruses are suspended in 50% glycerol-0.1M Tris buffer (pH 7.3) and stored at -20^0C.

Cell protection assay

To evaluate the ability of the Construct #2 AR-AvCD protein to protect cells against influenza viruses, we first treated MDCK cells with AR-AvCD made from Construct #2 or a broad-spectrum bacterial sialidase isolated from *A. ureafaciens*, and challenged the cells with a broad selection of human influenza viruses (IFV), including

human IFV A of H1, H2 and H3 subtypes, human IFV B as well as an avian IFV strain. As shown in **Figure 9**, the fusion protein made from Construct #2 demonstrated 80 to 100% of cell protection that was comparable to the effect of *A. ureafaciens* sialidase.

To perform the assay, MDCK cells were treated with 10 mU of AR-AvCD protein (made using Construct #2) or the isolated sialidase of *A. ureafaciens* at 37^0C for 2 hrs. The cells were subsequently challenged with influenza viruses at MOI 0.1 for 1 hr. The cells were washed and incubated in fresh DMDM:F12 supplemented with 0.2% ITS (GIBCO) and 0.6 μg/ml acetylated trypsin (Sigma). The cells were stained with 0.5% crystal violet and 20% methanol for 5 min and rinsed with tap water. The level of viable cells in each well was quantitated by extracting crystal violet by 70% ethanol and reading at 570 nM. Cell protection was calculated by 100 x {(sialidase treated sample - virus only)/(uninfected sample-virus only)}.

IFV inhibition assay

We evaluated inhibition of IFV amplification by AR-AvCD protein (made using Construct #2) and AR-G_4S-AvCD protein (made using Construct #3) using a cell-based ELISA method (Belshe et al., J Virol., (1988) 62(5), 1508-1512).

To perform the assay, MDCK monolayers in 96 well plates were treated with 16 mU of the sialidases AR-AvCD made from Construct #2 or AR-G_4S-AvCD made from Construct #3 in EDB/BSA buffer (10 mM Sodium Acetate, 150 mM NaCl, 10 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 0.5% BSA) for 2 hrs at 37 °C. Both the sialidase treated and the untreated control cells (treated with only EDB/BSA buffer) were infected with 0.1 MOI of virus. After 1 hour, the cells were washed two times with PBS and incubated in DMEM:F12 supplemented with 0.2% ITS (Gibco) and 0.6 ug/ml acetylated trypsin (Sigma). Forty to 48 hours post-infection, the levels of cell-bound virus were determined by using a cell-based ELISA assay. Specifically, cells were fixed in 0.05% glutaraldehyde in PBS and were incubated with 50 μl of 10^3 dilution of either anti-influenza A NP antiserum or anti-influenza B (Fitzgerald Inc.) in 0.5% BSA and PBS at 37^0C for 1 hr. After washing, each well was incubated with HRP-protein G in 0.5% BSA and PBS for 1 hr. After final washes, 50μl of 25 mM sodium citrate (pH 4.5) containing 0.02% 3,3',5,5'-tetramethylbenzidine dihydrochloride (Sigma) and 0.01% hydrogen

peroxide was allowed to react with the cells at room temperature for 5 min. The reactions were stopped by adding 50 µl of 1M H_2SO_4, and quantitated by measuring optical densities at 450 nM. Percentage viral replication inhibition is calculated by 100% x {(virus only samples – sialidase treated samples)/(virus only samples – uninfected samples)}.

Data on inhibition of viral replication and cell protection EC50's and selective indexes for recombinant sialidase fusion proteins AR-AvCD made from Construct #2 and AR-G_4S-AvCD made from Construct #3 for a variety of human influenza A and influenza B viruses, as well as equine viruses are shown in **Figure 12**.

As shown in **Figure 10**, sialidase fusion proteins strongly inhibited amplification of a broad selection of influenza viruses. Notably, 80-100% viral inhibition (**Figure 10**) as well as cell protection (**Figure 9**) was achieved although a maximum of 70-80% of cell surface sialic acid was removed by the sialidase treatment (**Figure 8**). This finding demonstrates that it is unnecessary to completely eliminate cell surface sialic acid in order to achieve the desired therapeutic effect of treating with the sialidase fusion proteins of the present invention. The residual 20-30% of the surface sialic acid, while being inaccessible for the sialidase fusion proteins, is probably inaccessible for influenza viruses as well.

Cytotoxicity of sialidase fusion proteins

To evaluate the cytotoxicity of AR-AvCD or AR-G_4S-AvCD proteins (made from Constructs #2 and #3), MDCK cells were seeded at low density in 96-well plates and cultured for 5 days in DMEM containing 10% FBS and up to 20 U of AR-AvCD protein or AR-G_4S-AvCD protein per well (both sialidases remained fully active during the entire experiment). Cell density in AR-AvCD or AR-G_4S-AvCD treated or control wells were determined every day by staining the cells with crystal violet and measuring absorption at 570 nM. No inhibition of cell growth was observed even at the highest concentration of AR-AvCD or AR-G_4S-AvCD (100 U/ ml) in the culture. Therefore IC_{50}, which is the drug concentration that inhibits cell growth by 50%, for AR-AvCD or AR-G_4S-AvCD is above 100 U/ml.

Example 14: In vivo Activities of Sialidase Catalytic Domain Fusion Protein

Ferrets can be infected with human unadapted influenza viruses and produce signs of disease comparable to those of humans, which can be treated by antiviral compounds, such as zanamivir (Relenza). (Mendel et al., Antimicrob Agents Chemother, (1998) 42(3), 640-646; Smith and Sweet, Rev. Infect. Dis., (1988) 10(1), 56-75; Reuman et al., J. Virol. Methods, (1989) 24(1-2), 27-34). To evaluate in vivo efficacy of our compounds, we tested AR-AvCD protein (made from Construct #2) in the ferret model. Specifically, 24 young female ferrets (0.5-0.8 kg) (Marshall Farms, North Rose, NY) that tested negative for the presence of anti-hemagglutinin antibodies in sera were included in the study. Two animals were placed in each cage and allowed to acclimate for 3 days before the experiment. The animals were randomly divided into three groups: 8 animals were treated with drug dilution buffer and viral challenge, 12 animals were treated with AR-AvCD and viral challenge, and 4 animals were treated with AR-AvCD only. A preparation of AR-AvCD dissolved in phosphate buffered saline (PBS) that contains 500 U/ml in sialidase activity and 0.7 mg/ml in protein concentration was used in the study. Animals in the drug treatment groups received 1 ml of AR-AvCD at each dose, which amounts to about 1 mg/kg in dosage level.

Ferrets were anesthetized and inoculated intranasally (0.5 ml into each nostril) with AR-AvCD or PBS twice (8 am and 6pm) and daily for a total of 7 days (2 days prior to the viral challenge and 5 days post virus inoculation). The ferrets were observed following the drug application for signs of intolerance. Viral inoculation was carried out on day 3 between 10-11 am. The viral challenge was done with human A/Bayern/7/95 (H1N1)-like virus at dose 10^5 $TCID_{50}$ ($\geq 10^4$ ferret ID_{50}). The nasal washes were collected from all animals starting day 2 post AR-AvCD treatment and continued until day 7. To collect nasal washes, 1 ml of sterile PBS was administered intranasally, the sneezed liquid was harvested and its volume was recorded. The nasal washes were centrifuged. The pelleted cells were re-suspended and counted in a hemacytometer under a microscope. The supernatants were collected, aliquoted and stored at –80°C. The protein concentration in cell-free nasal washes was determined by using the Bio-Rad protein reagent according to manufacturer's protocol (Bio-Rad, Hercules, CA). For virus titration of the nasal washes, inoculated MDCK cells were incubated for 3 days at 36°C

in a CO_2 incubator. The monolayers were inspected visually for cytopathic effect (CPE) and aliquots of the cell culture supernatants from each well were tested for the virus presence by a standard hemagglutination assay with guinea pig red blood cells. Viral titer was determined by the Spearman Karber method ((1996)).

In uninfected animals given intranasal AR-AvCD (n=4), no apparent effect on the inflammatory cell counts and protein concentrations in the nasal washes was observed (Figure 15 A and B). Nasal washes from these animals were followed for 7 days and were all negative for viral shedding. No signs of drug-related toxicity were detected in these animals at the drug dose used in this study. In the vehicle-treated group, virus replicated in the nasal epithelium of all 8 ferrets. Viral shedding reached peak values of 4.4 to 5.9 $\log_{10}TCID_{50}$ (mean peak titer of 4.9) on day 1 or 2 post challenge, diminished over time and became negative by day 5 (**Figure 13**). By contrast, only 3 of 12 AR-AvCD-treated ferrets were positive for viral shedding on day 1 post challenge (**Figure 13**), and their nasal viral titers were about 100 times lower than those in the vehicle-treated animals (mean 2.4±0.3 vs. 4.4±0.4 $\log_{10}TCID_{50}$) (**Figure 13**). After day 1, the response to the AR-AvCD treatment varied substantially. Three animals were completely protected against infection, signs of illness, and inflammatory response (**Figure 13**), ferret tag # 803, 805, 806). The protection was also confirmed by a lack of seroconversion on day-14 post challenge. One ferret (tag #780) did not shed virus during the first three days post challenge, but it died on day 4 post infection from an unrelated injury. The shedding in the remaining 8 ferrets varied during the course of infection, ranging from ferret #812 that shed virus for a day only, to the ferret #791 that shed virus for 5 days.

Infection in the ferrets that shed virus for at least one day was confirmed by more than a 16-fold rise in the post-challenge anti-HA antibody titer (seroconversion). There was no apparent effect of AR-AvCD treatment on the anti-HA titers in post-challenge sera (320-1280, vs. 160-1280, vehicle- and drug-treated group, respectively).

In ferrets that shed the virus despite the AR-AvCD treatment (n=8), the inflammatory response was reduced and animals appeared to be more alert and active compared to the untreated ferrets that were invariably lethargic and feverish. For this group of 8 infected, AR-AvCD-treated animals, the mean AUC (area under the curve)

value calculated for the nasal protein concentrations was reduced by approximately 40% (2.68 vs. 4.48, arbitrary units) compared to the vehicle-treated infected animals (**Figure 11B**). In vehicle-treated infected animals, the number of inflammatory cells in nasal washes was increased to approximately 100-fold above those in uninfected animals on day 2 post challenge. These levels were sustained for 4 additional days. The AR-AvCD-treated animals exhibited a significant reduction in the number of inflammatory cells in the nasal washes. Specifically, the AUC value for cell counts was reduced by approximately 3-fold in the AR-AvCD-treated animals compared to the vehicle-treated infected animals (1965 vs. 674, arbitrary units, **Figure 11A**). The observed reduction in the inflammatory response indicates the importance of inhibiting viral replication at the early stage of infection.

Example 15. Inhibition of Bacterial Cell Adhesion by Sialidase Fusion Proteins

Bacteria

S. pneumoniae: 10 encapsulated strains of different serotypes are selected from the clinical isolates deposited at ATCC. Bacteria are maintained as frozen stocks and passaged on tryptic soy agar plates containing 3% sheep blood (Difco & Micropure Medical Inc.) for 18 hr at 37^0C in 5% CO_2. To label pneumococci with radioisotope, an inoculum is taken from a 1- to 2-day plate culture, added to lysine-deficient tryptic soy broth containing 70 μCi of [^{3}H] lysine per ml and incubated at 37^0C in 5% CO_2. The growth of each culture is monitored by light absorbance at 595 nm. At late logarithmic phase, the bacteria are harvested, washed twice by centrifugation (13,000rpm x 3min), and resuspended in L-15 medium (without phenol red) plus 0.1% BSA (L-15-BSA) (Cundell and Tuomanen, Microb. Pathog., (1994) 17(6), 361-374) (Barthelson et al., Infect. Immun., (1998) 66(4), 1439-1444).

H. influenzae: 5 strains of type b (Hib) and 10 nontypable strains (NTHi) are obtained from the clinical isolates deposited at ATCC. All strains are stocked in brain heart infusion (BHI, Difco) containing hemin (ICN) and NAD (Sigma) and kept frozen until use; then they are cultured on BHI agar supplemented with hemin and NAD and grown

for 14 hr at 37^{0}C with 5% CO_2. (Kawakami et al., Microbiol. Immunol., (1998) 42(10), 697-702). To label the bacteria with [^{3}H], *H. influenzae* cells are inoculated in BHI broth containing hemin, NAD and [^{3}H]leucine at 250 μCi/ml and allowed to grow until late logarithmic phase and then harvested, washed and resuspended in L-15-BSA (Barthelson et al., Infect. Immun., (1998) 66(4), 1439-1444).

Cell Adhesion Assay

All [^{3}H]-labeled bacteria are suspended in L-15-BSA after washing, the bacterial concentration is determined by visual counting with a Petroff-Hausser chamber, radioactivity is determined by scintillation counting, and the specific activity of the [^{3}H]-labeled cells is calculated. Preparations of bacteria with 7cpm/1000 cells or greater are used. The bacteria are diluted to 5x 10^8 cells/ml. BEAS-2B cell monolayers are incubated with [^{3}H]-labeled bacterial suspension containing 5 x 10^7 bacteria at 37^{0}C in 5% CO_2. After 30 min, unbound bacteria are removed by washing with L-15-BSA for 5 times. Bacteria attached to the WD-HAE tissue samples are quantitated by scintillation counting.

Desialylation of BEAS-2B cells by sialidase fusion proteins and effects on cell adhesion by *H. influenzae* and *S. pneumoniae.*

BEAS-2B cells are incubated with 1-50 mU of AR-AvCD for 2 hours. Cell adhesion assay will be carried out using *H. influenzae* and *S. pneumoniae* strains as described above. Mock treated cells are used as positive control. Efficacy of AR-AvCD is quantitated as the EC_{50}, which is the amount of enzyme to achieve 50% inhibition on bacterial adhesion.

Example 16. Improving Transduction Efficiency of AAV Vector using Sialidase Fusion Proteins

In vitro Experiments

An experiment demonstrating effect of AR-AvCD is performed in a way similar to the procedure published (Bals et al., J Virol., (1999) 73(7), 6085-6088). A monolayer

of Well-Differentiated Airway Epithelium (WDAE) cells is maintained in transwells (Karp et al., Methods Mol. Biol., (2002) 188, 115-137; Wang et al., J Virol., (1998) 72(12), 9818-9826). In order to eliminate sialic acid from the cell surface the culture medium is replaced with serum free medium in which 0.5-10 units of AR-AvCD are dissolved. The cells are treated for 30 min to 6 hours. The cell monolayers are washed, transduced with AAV, and transduction efficiency is estimated using standard procedures. Several transwells are treated with medium only (without AR-AvCD) to serve the purpose of control (basal transduction efficiency). Additional controls may include the transwells treated with AR-AvCD only to assess cytotoxic effect of desialylation. A reporter virus is used for facile detection of transduced cells. Examples of reporter AAV and their use have been described in literature and include AAV-CMV-eGFP, AAV2LacZ (Bals et al., J Virol., (1999) 73(7), 6085-6088; Wang et al., Hum. Gene Ther., (2004) 15(4), 405-413) and alkaline phosphatase (Halbert et al., Nat. Biotechnol., (2002) 20(7), 697-701). The efficiency is estimated by light microscopy of the cells that were fixed and treated with appropriate substrate (if lacZ or AP containing virus is used) or fluorescent microscopy of live cells (if GFP is used). According to the experiments conducted at NexBio with NHBE primary epithelial cells (Cambrex, Walkersville, MD) the maximum amount of removal of sialic acid is achieved in less than one hour when 10 units of AR-AvCD per transwell are used. Other cell lines used (e.g. MDCK) become desialylated with much less AR-AvCD administered (0.1 U for 1 hour). It is therefore our estimate that a treatment of WDAE with 10 U of AR-AvCD for 2 hours will be sufficient to remove accessible sialic acid and provide significant enhancement of transduction of WDAE cells with AAV.

Testing Effect of AR-AvCD Treatment on AAV Transduction in an Animal Model.

To demonstrate effect of AR-AvCD treatment in animal model an experiment similar to previously described is conducted (Flotte et al., Proc. Natl. Acad. Sci. U. S. A, (1993) 90(22), 10613-10617; Halbert et al., Nat. Biotechnol., (2002) 20(7), 697-701). Several hours (1-6) prior to administration of AAV AR-AvCD is delivered to mice lungs by nasal aspiration of aerosole or lyophilized AR-AvCD powder according to previously published protocol (Flotte et al., Proc. Natl. Acad. Sci. U. S. A, (1993) 90(22), 10613-

10617). AAV carrying reporter gene (alkaline phosphatase) is delivered by nasal aspiration, mice are euthanized 4 weeks later and transduced cells are detected in fixed lungs as previously described (Halbert et al., J Virol., (1998) 72(12), 9795-9805).

Example 17. Sialidase Treatment Inhibits Mast Cell Functions and Smooth Muscle Contraction in the Trachea.

Using experimental methods described previously (Cocchiara et al., J Neuroimmunol., (1997) 75(1-2), 9-18), it will be demonstrated that treatment by compounds of the present invention prevents substance P (SP) induced histamine release by mast cells. Using another set of experiments (Stenton et al., J Pharmacol. Exp. Ther., (2002) 302(2), 466-474), treatment by compounds of the present invention will inhibit β-hexosaminidase release by mast cells stimulated by two PAR-activating peptides (PAR stands for proteinase-activated receptors).

Compounds of the present invention will be administered intratracheally in guinea pigs and the airway reactivity will be assessed in the animals as described previously (Jarreau et al., Am. Rev. Respir. Dis., (1992) 145(4 Pt 1), 906-910; Stenton et al., J Pharmacol. Exp. Ther., (2002) 302(2), 466-474). Sialidase treatment should not induce nonspecific airway hyperreactivity judged by the reaction to multiple inducers. In addition, sialidase treatment should reduce substance P-induced bronchoconstriction. Similarly, compounds of the present invention will be used to treated the isolated guinea pig and rat trachea and lung (Kai et al., Eur. J. Pharmacol., (1992) 220(2-3), 181-185; Stenton et al., J Pharmacol. Exp. Ther., (2002) 302(2), 466-474). Again recombinant sialidase treatment will have no effect on smooth muscle contractions induced by acetylcholine, histamine and 5-hydroxytryptamine. In addition, it will inhibit tracheal contraction induced by antigen (ovalbumin) or compound 48/80.

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All publications, including patent documents, Genbank sequence database entries including nucleotide and amino acid sequences and accompanying information, and scientific articles, referred to in this application and the bibliography and attachments are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

CLAIMS

We Claim:

1. A sialidase catalytic domain protein, wherein said sialidase catalytic domain protein comprises an amino acid sequence that begins at any of the amino acids from amino acid 270 to amino acid 290 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**) and ends at any of the amino acids from amino acid 665 to amino acid 901 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**); wherein said sialidase catalytic domain protein lacks the *Actinomyces viscosus* sialidase protein sequence that consists of the sequence extending from amino acid 1 to amino acid 269, further wherein said sialidase catalytic domain protein has sialidase activity.

2. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 1.

3. The nucleic acid molecule of claim 2 in an expression vector.

4. The sialidase catalytic domain protein of claim 1 comprising **SEQ ID NO:14**.

5. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 4.

6. The nucleic acid molecule of claim 5 in an expression vector.

7. The sialidase catalytic domain protein of claim 1, wherein said sialidase catalytic domain protein comprises an amino acid sequence that begins at any of the amino acids from amino acid amino acids 270 to 290 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**) and ends at any of amino acid residues 665 to 681 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**).

8. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 7.

9. The nucleic acid molecule of claim 8 in an expression vector.

10. The sialidase catalytic domain protein of claim 7, wherein said sialidase catalytic domain protein comprises **SEQ ID NO:16**.

11. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 10.

12. The nucleic acid molecule of claim 11 in an expression vector.

13. The sialidase catalytic domain protein of claim 7, wherein said sialidase catalytic domain protein comprises an amino acid sequence that begins amino acid 274 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**) and ends at amino acid residues 681 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**).

14. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 13.

15. The nucleic acid molecule of claim 14 in an expression vector.

16. The sialidase catalytic domain protein of claim 7, wherein said sialidase catalytic domain protein comprises an amino acid sequence that begins amino acid 290 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**) and ends at amino acid residues 666 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**).

17. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 16.

18. The nucleic acid molecule of claim 17 in an expression vector.

19. The sialidase catalytic domain protein of claim 7, wherein said sialidase catalytic domain protein comprises an amino acid sequence that begins amino acid 290 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**) and ends at amino acid residues 681 of said *Actinomyces viscosus* sialidase protein sequence (**SEQ ID NO:12**).

20. A nucleic acid molecule comprising a nucleotide sequence encoding the sialidase catalytic domain protein of claim 19.

21. The nucleic acid molecule of claim 20 in an expression vector.

22. A fusion protein comprising:

at least one catalytic domain of a sialidase; and

a purification domain, a protein tag, a protein stabiliy domain, a solubility domain, a protein size-increasing domain, a protein folding domain, a protein localization domain, an anchoring domain, an N-terminal domain, a C-terminal domain, a catalytic activity domain, a binding domain, or a catalytic activity-enhancing domain

23. The fusion protein of claim 22, further comprising at least one peptide linker.

24. The fusion protein of claim 22, wherein said catalytic domain is substantially homologous to the catalytic domain of the *Clostridium perfringens* sialidase, substantially homologous to the *Actinomyces viscosus* sialidase, substantially homologous to the *Arthrobacter ureafaciens* sialidase, substantially homologous to the *Micromonospora viridifaciens* sialidase, substantially homologous to the human Neu2 sialidase, or substantially homologous to the human Neu4 sialidase.

25. The fusion protein of claim 24, wherein said catalytic domain is substantially homologous to the catalytic domain of the *Actinomyces viscosus* sialidase.

26. The fusion protein of claim 25 herein said catalytic domain comprises **SEQ ID NO: 16**.

27. The fusion protein of claim 26, comprising at least one protein purification domain.

28. The fusion protein of claim 27, wherein said at least one protein purification domain is a His tag, a calmodulin binding domain, a maltose binding protein domain, a streptavidin domain, a streptavidin binding domain; an intein domain, or a chitin binding domain.

29. The fusion protein of claim 28, wherein said protein purification domain is a His tag.

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30. The fusion protein of claim 29, wherein said fusion protein comprises **SEQ ID NO:18**.

31. A nucleic acid molecule encoding the fusion protein of claim 30.

32. The nucleic acid molecule of claim 31 in an expression vector.

33. The nucleic acid molecule of claim 31, comprising **SEQ ID NO:17**.

34. The nucleic acid molecule of claim 33 in an expression vector.

35. The fusion protein of claim 25, comprising at least one anchoring domain.

36. The fusion protein of claim 35, wherein said anchoring domain is a GAG-binding domain.

37. The fusion protein of claim 36, wherein said anchoring domain is substantially homologous to the GAG-binding domain of human platelet factor 4 (**SEQ ID NO:2**), substantially homologous to the GAG-binding domain of human interleukin 8 (**SEQ ID NO:3**), substantially homologous to the GAG-binding domain of human antithrombin III (**SEQ ID NO:4**), substantially homologous to the GAG-binding domain of human apoprotein E (**SEQ ID NO:5**), substantially homologous to the GAG-binding domain of human angio-associated migratory protein (**SEQ ID NO:6**), or substantially homologous to the GAG-binding domain of human amphiregulin (**SEQ ID NO:7**).

38. The fusion protein of claim 37, wherein said anchoring domain is substantially homologous to the human amphiregulin GAG-binding domain (**SEQ ID NO:7**).

39. The fusion protein of claim 38, wherein said anchoring domain comprises the human amphiregulin GAG-binding domain (**SEQ ID NO:7**).

40. The fusion protein of claim 39, wherein said catalytic domain of a sialidase comprises **SEQ ID NO:16**.

41. The fusion protein of claim 40, comprising **SEQ ID NO:20**.

42. A nucleic acid molecule encoding the fusion protein of claim 41.

43. The nucleic acid molecule of claim 42 in an expression vector.

44. The nucleic acid molecule of claim 42, comprising **SEQ ID NO:19**.

45. The nucleic acid molecule of claim 44 in an expression vector.

46. The fusion protein of claim 40, comprising **SEQ ID NO:25**.

47. A nucleic acid molecule encoding the fusion protein of claim 46.

48. The nucleic acid molecule of claim 47 in an expression vector.

49. The nucleic acid molecule of claim 47, comprising **SEQ ID NO:24**.

50. The nucleic acid molecule of claim 49 in an expression vector.

51. The fusion protein of claim 40, comprising **SEQ ID NO:38**.

52. The fusion protein of claim 40, further comprising a peptide linker connecting said human amphiregulin GAG-binding domain to said catalytic domain of a sialidase.

53. The fusion protein of claim 52, comprising **SEQ ID NO:35**.

54. A nucleic acid molecule molecule encoding the fusion protein of claim 53.

55. The nucleic acid molecule of claim 54, comprising **SEQ ID NO:34**.

56. An expression vector comprising the nucleic acid molecule of claim 55.

57. The fusion protein of claim 52, comprising **SEQ ID NO:37**.

58. A nucleic acid molecule encoding the fusion protein of claim 57.

59. The nucleic acid molecule of claim 58, comprising **SEQ ID NO:36**.

60. An expression vector comprising the nucleic acid molecule of claim 59.

61. The fusion protein of claim 52, comprising **SEQ ID NO:39**.

62. The fusion protein of claim 39, comprising **SEQ ID NO:27**.

63. A nucleic acid molecule encoding the fusion protein of claim 62.

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64. An expression vector comprising the nucleic acid molecule of claim 63.

65. The nucleic acid molecule of claim 63, comprising **SEQ ID NO:26**.

66. An expression vector comprising the nucleic acid molecule of claim 65.

67. The fusion protein of claim 39, comprising **SEQ ID NO:29**.

68. A nucleic acid molecule encoding the fusion protein of claim 67.

69. An expression vector comprising the nucleic acid molecule of claim 68.

70. The nucleic acid molecule of claim 68, comprising **SEQ ID NO:28**.

71. An expression vector comprising the nucleic acid molecule of claim 70.

72. The fusion protein of claim 39, comprising **SEQ ID NO:31**.

73. A nucleic acid molecule encoding the fusion protein of claim 72.

74. An expression vector comprising the nucleic acid molecule of claim 73.

75. The nucleic acid molecule of claim 73, comprising **SEQ ID NO:30**.

77. An expression vector comprising the nucleic acid molecule of claim 75.

78. The fusion protein of claim 39, comprising **SEQ ID NO:33**.

79. A nucleic acid molecule encoding the fusion protein of claim 78.

80. An expression vector comprising the nucleic acid molecule of claim 79.

81. The nucleic acid molecule of claim 79, comprising **SEQ ID NO:32**.

82. An expression vector comprising the nucleic acid molecule of claim 81.

83. A pharmaceutical formulation comprising a sialidase.

84. The pharmaceutical formulation of claim 83, wherein said sialidase is substantially homologous to at least a portion of *Clostridium perfringens* sialidase, substantially homologous to at least a portion of the *Actinomyces viscosus* sialidase, substantially homologous to at least a portion of the *Arthrobacter ureafaciens* sialidase, substantially homologous to at least a portion of the *Micromonospora viridifaciens* sialidase, substantially homologous to at least a portion of the human Neu2 sialidase, or substantially homologous to at least a portion of the human Neu4 sialidase.

85. The pharmaceutical formulation of claim 84, wherein said sialidase is substantially homologous to at least a portion of the *Actinomyces viscosus* sialidase.

86. The pharmaceutical formulation of claim 85, wherein said sialidase comprises **SEQ ID NO:12**.

87. A pharmaceutical formulation comprising the composition of claim 1.

88. A pharmaceutical formulation comprising the composition of claim 7

89. A pharmaceutical formulation comprising the composition of claim 22.

90. A pharmaceutical formulation comprising the composition of claim 26.

91. A pharmaceutical formulation comprising the composition of claim 46.

92. A pharmaceutical formulation comprising the composition of claim 62.

93. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92 formulated as a spray.

94. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92, formulated as an inhalant.

95. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92, formulated as a solution for injection.

96. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92, formulated as a solution for eye drops.

97. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92 formulated as a cream, salve, gel, or ointment.

98. The pharmaceutical formulation of claim 83, 85, 87, 88, 89, 90, 91, or 92, formulated as a pill, tablet, lozenge, suspension, or solution that can be administered orally.

99. A method of treating or preventing viral infection by influenza, parainfluenza, or respiratory syncytial virus, comprising:
applying a therapeutically effective amount of the composition of any of claims 83, 85, 87, 88, 89, 90, 91, or 92 to epithelial cells of a subject.

100. The method of claim 99, wherein said applying is by use of a nasal spray.

101. The method of claim 99, wherein said applying is by use of an inhaler.

102. The method of claim 99, wherein said applying is performed from once to four times a day.

103. A method of treating or preventing infection by a bacterial pathogen, comprising: administering a therapeutically effective amount of the composition of claim 83, 85, 87, 88, 89, 90, 91, or 92 to a subject.

104. The method of claim 103, wherein said pathogen is *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* , *Pseudomonas aeruginosa*, or *Heliobacter pylori*.

105. The method of claim 103, wherein said administering is by use of a nasal spray.

106. The method of claim 103, wherein said administering is by use of an inhaler.

107. The method of claim 103, wherein said administering is by topical application.

108. The method of claim 103, wherein said administering is by oral administration.

109. The method of claim 103, wherein said administering is performed from once to four times a day.

110. A method of treating or preventing allergy or inflammation, comprising: administering a therapeutically effective amount of the composition of claim 83, 85, 87, 88, 89, 90, 91, or 92 to a subject.

111. The method of claim 110, wherein said inflammation is associated with asthma, allergic rhinitis, eczema, psoriasis, exposure to plant or animal toxins, or autoimmune conditions.

112. The method of claim 110, wherein said administering is by use of a nasal spray.

113. The method of claim 110, wherein said administering is by use of an inhaler.

114. The method of claim 110, wherein said administering is by use of eye drops.

115. The method of claim 110, wherein said administering is by topical application.

116. The method of claim 110, wherein said administering is by local or intravenous injection.

117. The method of claim 110, wherein said administering is performed from once to four times a day.

118. A method of enhancing gene delivery by a recombinant viral vector, comprising: administering an effective amount of the composition of claim 83, 85, 87, 88, 89, 90, 91, or 92 to epithelial cells of a subject prior to or concomitant with the administration of said recombinant viral vector.

119. The method of claim 118, wherein said recombinant viral vector is a retroviral vector, a Herpes viral vector, an adenoviral vector, or an adeno-associated viral vector.

120. The method of claim 119, wherein said recombinant viral vector is a recombinant adeno-associated viral vector.

121. The method of claim 120, wherein said recombinant adeno-associated viral vector comprises a gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR).

122. The method of claim 119, wherein said administering is by use of an inhaler.

123. The method of claim 119, wherein said administering is performed from once to four times a day.

124. A pharmaceutical formulation comprising the composition of claim 62.

125. The pharmaceutical formulation of claim 124, formulated as a spray.

126. The pharmaceutical formulation of claim 124, formulated as an inhalant.

127. A method of treating or preventing influenza infection, comprising:
applying a therapeutically effective amount of the composition of claim 124 to epithelial cells of a subject.

128. The method of claim 127, wherein said applying is by use of a nasal spray.

129. The method of claim 127, wherein said applying is by use of an inhaler.

130. The method of claim 127, wherein said applying is performed from once to four times a day.

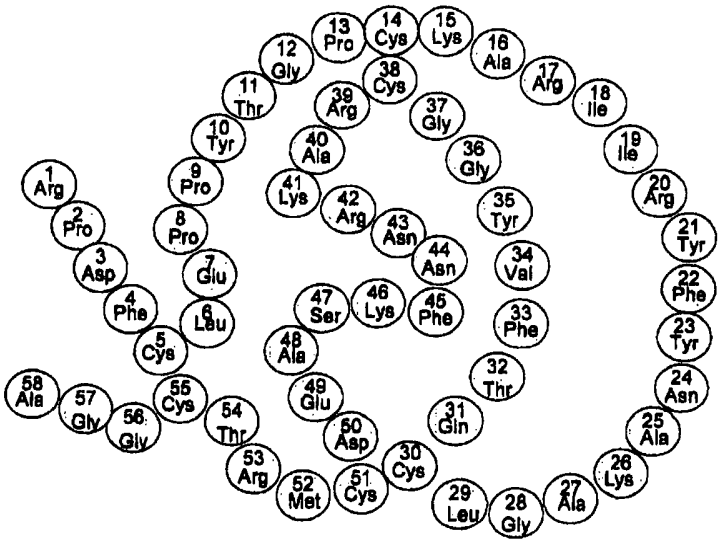
1 Arg
2 Pro
3 Asp
4 Phe
5 Cys
6 Leu
7 Glu
8 Pro
9 Pro
10 Tyr
11 Thr
12 Gly
13 Pro
14 Cys
15 Lys
16 Ala
17 Arg
18 Ile
19 Ile
20 Arg
21 Tyr
22 Phe
23 Tyr
24 Asn
25 Ala
26 Lys
27 Ala
28 Gly
29 Leu
30 Cys
31 Gln
32 Thr
33 Phe
34 Val
35 Tyr
36 Gly
37 Gly
38 Cys
39 Arg
40 Ala
41 Lys
42 Arg
43 Asn
44 Asn
45 Phe
46 Lys
47 Ser
48 Ala
49 Glu
50 Asp
51 Cys
52 Met
53 Arg
54 Thr
55 Cys
56 Gly
57 Gly
58 Ala

FIG. 1

PF4(**SEQ ID NO:2**): ^{47}NGRRICLDLQAPLYKKIIKKLLES^{70}

IL-8(**SEQ ID NO:3**): ^{46}GRELCLDPKENWVQRVVEKFLKRAENS^{72}

ATIII(**SEQ ID NO:4**): ^{118}QIHFFFAKLNCRLYRKANKSSKLVSANRLFGDKS^{151}

ApoE(**SEQ ID NO:5**): ^{132} ELRVRLASHLRKLRKRLLRDADDLQKRLAVYQAG^{165}

AAMP(**SEQ ID NO:6**): ^{14}RRLRRMESESES^{25}

Ampiregulin(**SEQ ID NO:7**): ^{25}KRKKKGGKNGKNTTNTKKKNP^{45}

FIG. 2

```
NEU2(SEQ ID NO:8): 1 MASLPVLQKE SVFQSGAHA- -YRIPALLYL PGQQSLLAFA EQRASKKDEH
                                              YR+P+LL + P    +LLAF EQR S  D H
NEU4(SEQ ID NO:9): 1 MGVPRTPSRT VLFERERTGL TYRVPSLLPV PPGPTLLAFV EQRLSPDDSH

NEU2: 49  AELIVLRRGD YDAPTHQVQW QAQEVVAQAR LDGHRSMNPC PLYDAQTGTL FLFFIAIPGQ
          A  +VLRRG          +W  A  ++  A     HRSMNPC P++DA TGT+ FLFFIA+ G
NEU4: 51  AHRLVLRRGT LAGGSV--RW GALHVLGTAA LAEHRSMNPC PVHDAGTGTV FLFFIAVLGH

NEU2: 110 VTEQQQLQTR ANVTRLCQVT STDHGRTWSS PRDLTDAAIG PAYREWSTFA VGPGHCLQLN
            E  Q+ T   N  RLC V  S D G +W S  RDLT+ AIG  A ++W+TFA VGPGH +QL
NEU4: 109 TPEAVQIATG RNAARLCCVA SRDAGLSWGS ARDLTEEAIG GAVQDWATFA VGPGHGVQLP

NEU2: 170 DRARSLVVPA YAYRKLHP-- ---IQRPIPS AFCFLSHDHG RTWARGHFVA QD-TLECQVA
            R L+VPA Y YR           I R  P  +F F S DHG RTW  G  V     + ECQ+A
NEU4: 169 S-GR-LLVPA YTYRVDRLEC FGKICRTSPH SFAFYSDDHG RTWRCGGLVP NLRSGECQLA

NEU2: 224  EVETGEQRVV TL-NARSHLR ARVQAQSTND GLDFQESQLV KKLVEPPPQG CQGSVISFPS
             V+ G+       NARS L  +RVQA  ST++ G F  ++ V   L E    G CQGS++ FP
NEU4: 227  AVDGGQAGSF LYCNARSPLG SRVQALSTDE GTSFLPAERV ASLPETAW-G CQGSIVGFPA

NEU2: 283  P--------- ---------- ---------- ---------- ---------  ---------

NEU4: 286  PAPNRPRDDS WSVGPRSPLQ PPLLGPGVHE PPEEAAVDPR GGQVPGGPFS RLQPRGDGP

NEU2: 284 ---------- ---------- ---RSGPGSP QWLLYTHPTH SWQRADLGAY LNPRPPAPEA
                                            WLLY+HP      R  +G   L+  P  P +
NEU4: 346 RQPGPRPGVSG DVGSWTLALP MPFAAPPQSP TWLLYSHPVG RRARLHMGIR LSQSPLDPRS

NEU2: 321  WSEPVLLAKG SCAYSDLQSM GTGPDGSPLF GCLYEANDY- --EEIVFLMF TLKQAFPAEY
             W+EP ++ +    YSDL S+ G  P+G  +F +CLYE                +L++
NEU4: 406  WTEPWVIYEG PSGYSDLASI GPAPEGGLVF ACLYESGART SYDEISFCTF SLREVLENVP

NEU2: 378 LPQ

NEU4: 466 ASPKPPNLGD KPRGCCWPS
```

FIG. 3

Substrate Specificity of Bacteria and Fungal Sialidases

Substrates	Sialidase activity*					
	Vibrio Cholerae	Clostridium perfringens (71Kd)	Clostridium perfringens (43Kd)	Arthrobacter ureafaciens	Salmonella typhimurium	Actinomyces viscosus
Oligo- and polysaccharides						
II^3Neu5AcLac	100	100	100	100	100	100
II^6Neu5AcLac	53	44	19	157	0.4	462
Colominic acid (α2-8)	30	33	4.0	63	0.1	300
Glycoproteins						
Fetuin (α2-3>α2-6)	340	272	6.6	59	17	—
α1-Acid glycoprotein (α2-6>α2-3)	1000	555	—	—	—	761
Submandibular gland mucin (α2-6)	400	139	5.1	—	—	123
Submaxillary gland mucin (α2-6)	—	—	—	56	—	—
Gangliosides						
Gangliosides mixtures	(360)	(350)	1.6	78	34	285
Synthetic						
4MU-Neu5Ac	1580	605	58	—	1050	—

* Each value represents a relative sialidase activity when the activity directed toward II^3Neu5AcLac is regard as 100.

FIG. 4

ccatggggcatcaccatcaccatcatctagagggagatcatccacaagctacaccagcacct
M G H H H H H H L E G D H P Q A T P A P
gcaccagatgctagcactgagctgccagcaagcatgtctcaggctcagcatcttgcagca
A P D A S T E L P A S M S Q A Q H L A A
aatacggctactgataattatcgcattccagcgattacaaccgctccgaatggtgattta
N T A T D N Y R I P A I T T A P N G D L
ctgattagctatgatgaacggccgaaggacaatggaaatggtggttccgatgcccctaac
L I S Y D E R P K D N G N G G S D A P N
ccgaatcatattgttcagcgtcgctccacagatggcggtaaaacttggagcgcgccaacc
P N H I V Q R R S T D G G K T W S A P T
tatattcatcagggtacggagactggcaagaaagtgggatattccgacccctcttatgtg
Y I H Q G T E T G K K V G Y S D P S Y V
gtggatcatcaaaccggtacaatcttcaattttcatgtgaaatcatacgatcagggctgg
V D H Q T G T I F N F H V K S Y D Q G W
ggaggtagccgtgggggaacagacccggaaaaccgcgggattattcaggcagaggtgtct
G G S R G G T D P E N R G I I Q A E V S
acgagcacggataatggatggacgtggacacatcgcaccatcaccgcggatattacgaaa
T S T D N G W T W T H R T I T A D I T K
gataaaccgtggaccgcgcgttttgcggcgtccggccaaggcattcagatccagcatggg
D K P W T A R F A A S G Q G I Q I Q H G
ccgcatgccggccgtctggtgcaacagtataccattcgtacggccggtggagcggtgcag
P H A G R L V Q Q Y T I R T A G G A V Q
gctgtatcggtttattccgatgatcatgggaaaacgtggcaggctggcaccccgattggg
A V S V Y S D D H G K T W Q A G T P I G
acgggtatggatgaaaacaaagttgtagagctgtctgacggctctctgatgctgaacagt
T G M D E N K V V E L S D G S L M L N S
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R A S D G S G F R K V A H S T D G G Q T
tggtccgaaccggtttcggacaaaaatttgccggattcggttgataatgcccagataatt
W S E P V S D K N L P D S V D N A Q I I
cgtgcgtttcctaatgctgcccccgatgacccgcgcgcgaaagtacttcttctgagtcat
R A F P N A A P D D P R A K V L L L S H
tccccaaatccacgtccgtggtcccgggatcgtggtacgataagcatgtcatgtgatgac
S P N P R P W S R D R G T I S M S C D D
ggggcctcatggaccacttccaaagtttttcacgaaccgtttgtgggctacacgactatt
G A S W T T S K V F H E P F V G Y T T I
gcagttcagagtgatggaagcatcggtctgctgtcggaggacgcgcacaatggcgctgat
A V Q S D G S I G L L S E D A H N G A D
tatggtggcatctggtatcgtaattttacgatgaactggctgggagaacaatgtggacaa
Y G G I W Y R N F T M N W L G E Q C G Q
aaacccgcggaat**aagctt**
K P A E

FIG. 5

ccatggttaagcgcaaaaaaaaaggcggcaaaaacggtaaaaatcgtcgtaaccgtaagaaa
M V K R K K K G G K N G K N R R N R K K
aaaaatcctggagatcatccacaagctacaccagcacctgcaccagatgctagcactgag
K N P G D H P Q A T P A P A P D A S T E
ctgccagcaagcatgtctcaggctcagcatcttgcagcaaatacggctactgataattat
L P A S M S Q A Q H L A A N T A T D N Y
cgcattccagcgattacaaccgctccgaatggtgatttactgattagctatgatgaacgg
R I P A I T T A P N G D L L I S Y D E R
ccgaaggacaatggaaatggtggttccgatgcccctaacccgaatcatattgttcagcgt
P K D N G N G G S D A P N P N H I V Q R
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R S T D G G K T W S A P T Y I H Q G T E
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T G K K V G Y S D P S Y V V D H Q T G T
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I F N F H V K S Y D Q G W G G S R G G T
gacccggaaaaccgcgggattattcaggcagaggtgtctacgagcacggataatggatgg
D P E N R G I I Q A E V S T S T D N G W
acgtggacacatcgcaccatcaccgcggatattacgaaagataaaccgtggaccgcgcgt
T W T H R T I T A D I T K D K P W T A R
tttgcggcgtccggccaaggcattcagatccagcatgggccgcatgccggccgtctggtg
F A A S G Q G I Q I Q H G P H A G R L V
caacagtataccattcgtacggccggtggagcggtgcaggctgtatcggtttattccgat
Q Q Y T I R T A G G A V Q A V S V Y S D
gatcatgggaaaacgtggcaggctggcaccccgattgggacgggtatggatgaaaacaaa
D H G K T W Q A G T P I G T G M D E N K
gttgtagagctgtctgacggctctctgatgctgaacagtcgtgcgtcggacgggagcggc
V V E L S D G S L M L N S R A S D G S G
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F R K V A H S T D G G Q T W S E P V S D
aaaaatttgccggattcggttgataatgcccagataattcgtgcgtttcctaatgctgcc
K N L P D S V D N A Q I I R A F P N A A
cccgatgacccgcgcgcgaaagtacttcttctgagtcattccccaaatccacgtccgtgg
P D D P R A K V L L L S H S P N P R P W
tcccgggatcgtggtacgataagcatgtcatgtgatgacggggcctcatggaccacttcc
S R D R G T I S M S C D D G A S W T T S
aaagtttttcacgaaccgtttgtgggctacacgactattgcagttcagagtgatggaagc
K V F H E P F V G Y T T I A V Q S D G S
atcggtctgctgtcggaggacgcgcacaatggcgctgattatggtggcatctggtatcgt
I G L L S E D A H N G A D Y G G I W Y R
aattttacgatgaactggctgggagaacaatgtggacaaaaacccgcggaat**aagctt**
N F T M N W L G E Q C G Q K P A E - A

FIG. 6

ccatggttaagcgcaaaaaaaaaggcggcaaaaacggtaaaaatcgtcgtaaccgtaagaaa
M V K R K K K G G K N G K N R R N R K K
aaaaatcctggtggtggtggttctggagatcatccacaagctacaccagcacctgcacca
K N P G G G G S G D H P Q A T P A P A P
gatgctagcactgagctgccagcaagcatgtctcaggctcagcatcttgcagcaaatacg
D A S T E L P A S M S Q A Q H L A A N T
gctactgataattatcgcattccagcgattacaaccgctccgaatggtgatttactgatt
A T D N Y R I P A I T T A P N G D L L I
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S Y D E R P K D N G N G G S D A P N P N
catattgttcagcgtcgctccacagatggcggtaaaacttggagcgcgccaacctatatt
H I V Q R R S T D G G K T W S A P T Y I
catcagggtacggagactggcaagaaagtgggatattccgacccctcttatgtggtggat
H Q G T E T G K K V G Y S D P S Y V V D
catcaaaccggtacaatcttcaattttcatgtgaaatcatacgatcagggctggggaggt
H Q T G T I F N F H V K S Y D Q G W G G
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P W T A R F A A S G Q G I Q I Q H G P H
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A G R L V Q Q Y T I R T A G G A V Q A V
tcggtttattccgatgatcatgggaaaacgtggcaggctggcaccccgattgggacgggt
S V Y S D D H G K T W Q A G T P I G T G
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M D E N K V V E L S D G S L M L N S R A
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S D G S G F R K V A H S T D G G Q T W S
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E P V S D K N L P D S V D N A Q I I R A
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F P N A A P D D P R A K V L L L S H S P
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N P R P W S R D R G T I S M S C D D G A
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S W T T S K V F H E P F V G Y T T I A V
cagagtgatggaagcatcggtctgctgtcggaggacgcgcacaatggcgctgattatggt
Q S D G S I G L L S E D A H N G A D Y G
ggcatctggtatcgtaattttacgatgaactggctgggagaacaatgtggacaaaaaccc
G I W Y R N F T M N W L G E Q C G Q K P
gcggaat**aagctt**
A E - A

FIG. 7

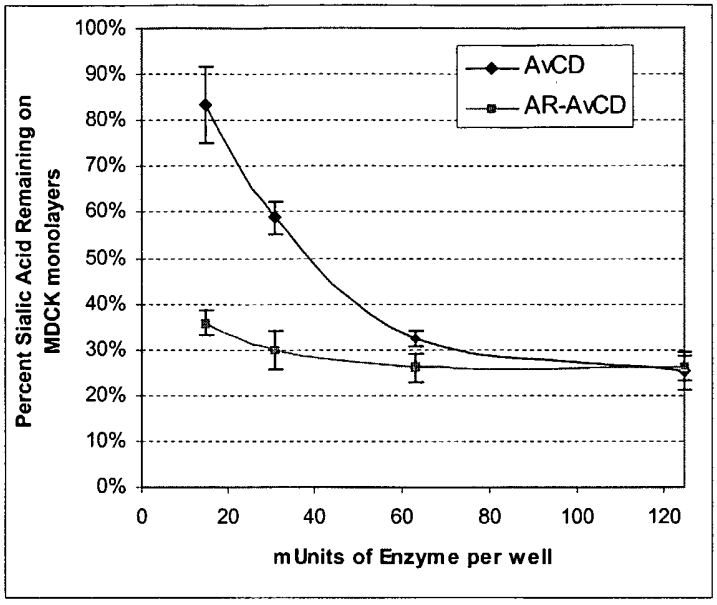
Percent Sialic Acid Remaining on MDCK monolayers
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
20
40
60
80
100
120
mUnits of Enzyme per well
AvCD
AR-AvCD

FIG. 8

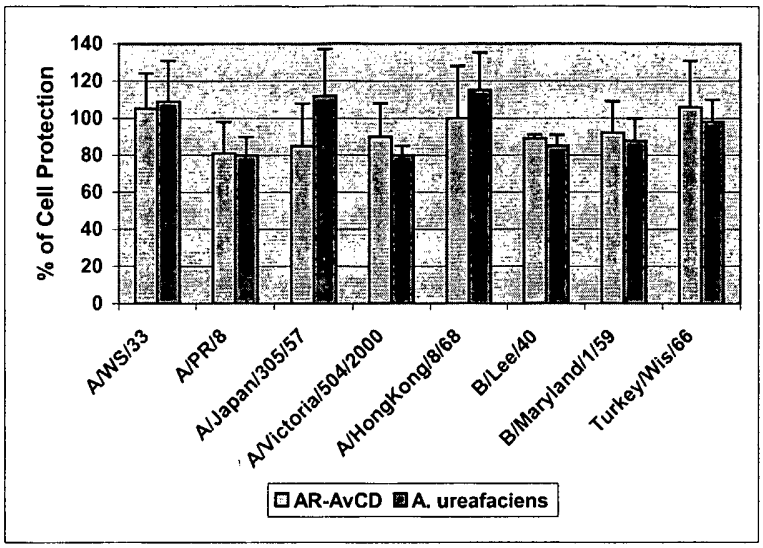
% of Cell Protection
140
120
100
80
60
40
20
0
A/WS/33
A/PR/8
A/Japan/305/57
A/Victoria/504/2000
A/HongKong/8/68
B/Lee/40
B/Maryland/1/59
Turkey/Wis/66
AR-AvCD
A. ureafaciens

FIG. 9

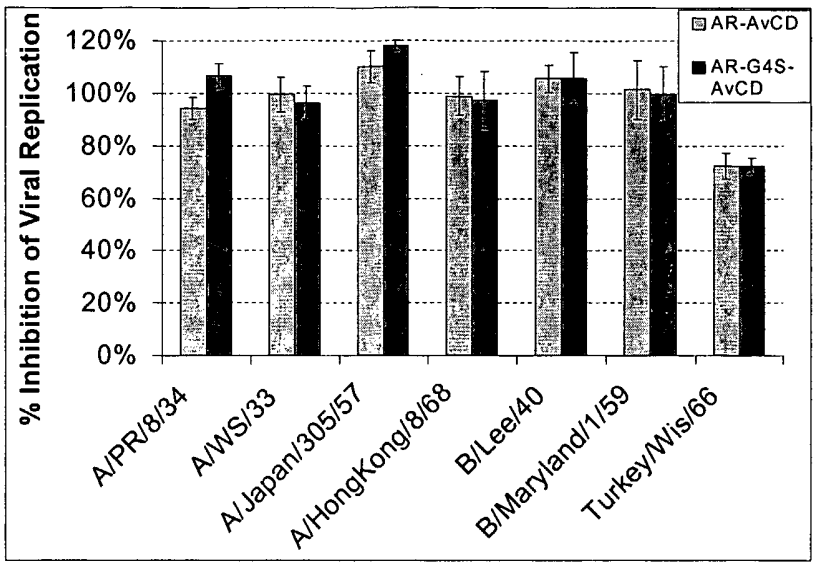
% Inhibition of Viral Replication
120%
100%
80%
60%
40%
20%
0%
AR-AvCD
AR-G4S-AvCD
A/PR/8/34
A/WS/33
A/Japan/305/57
A/HongKong/8/68
B/Lee/40
B/Maryland/1/59
Turkey/Wis/66

FIG. 10

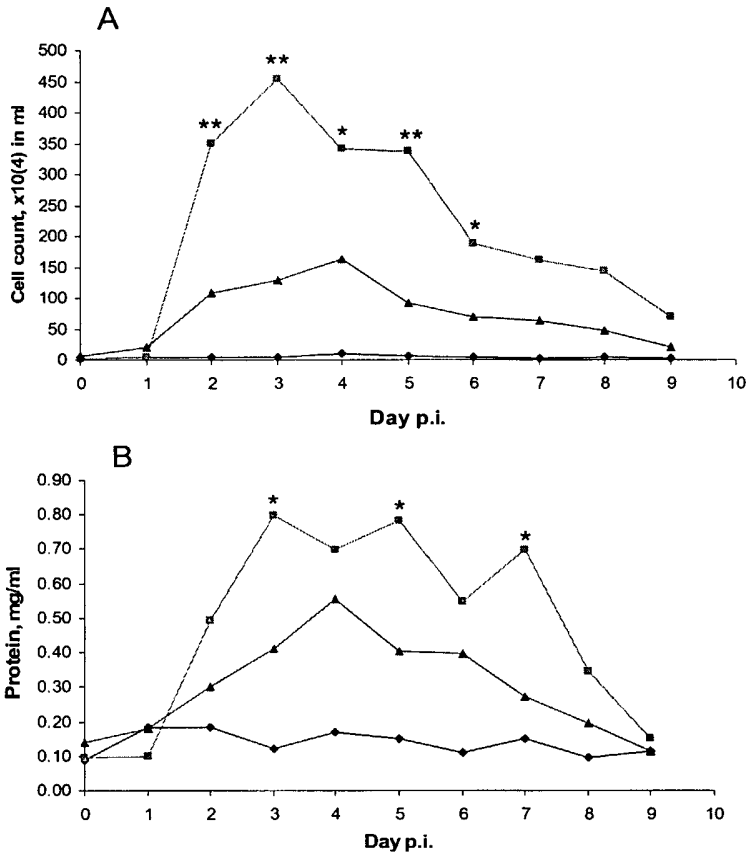
A
Cell count, x10(4) in ml
500
450
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Day p.i.
B
Protein, mg/ml
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0 1 2 3 4 5 6 7 8 9 10
Day p.i.

FIG. 11

Virus	AR-AvCD				AR-G_4S-AvCD			
	Inhibition of Viral Replication EC50	Selective Index	Cell Protection EC50	Selective Index	Inhibition of Viral Replication EC50	Selective Index	Cell Protection EC50	Selective Index
A/PR/8/34 (H1N1)	12.3 ± 7.4	>8163	50.3 ± 13.1	>1990	13.5 ± 1.4	>7407	43.8 ± 22.3	>2286
A/WS/33 (H1N1)	6.5 ± 3.9	>15444	17.7 ± 13.9	>5666	12.2 ± 11.0	>8197	19.1 ± 17.6	>5249
A/NWS/33 (H1N1)	2.5	>20000	10.8 ± 12.4	>9302	2.5	>40000	15.9 ± 19.3	>6249
A/Japan/305/57 (H2N2)	5.1 ± 3.4	>19512	11.3 ± 0.4	>8889	3.8 ± 1.8	>26667	14.9 ± 0.2	>6273
A/Victoria/504/2000 (H3N2)	2.5 ± 0.0	>40000	18.3 ± 1.0	>5457	2.6 ± 0.2	>38095	55.3 ± 0.4	>1810
A/HongKong/8/68 (H3N2)	2.5 ± 0.0	>40000	31.5 ± 38.2	>3175	2.5 ± 0.0	>40000	30.6 ± 30.2	>3265
B/Lee/40	4.1 ± 1.3	>24540	11.4 ± 3.7	>8791	3.5 ± 1.3	>28986	8.4 ± 2.7	>11940
B/Maryland/1/59	4.3 ± 2.5	>23392	2.5 ± 0.0	>40000	5.5 ± 4.2	>18265	5.6 ± 3.0	>17778
Turkey/Wis/66 (H9N2)	3.9 ± 0.5	>25478	16.6 ± 16.2	>6033	7.1 ± 0.9	>14035	20.4 ± 18.6	>4914
Equine/Prague/2/62 (H7N7)	n/a	n/a	15.8 ± 5.3	>6349	n/a	n/a	25.5 ± 5.7	>3922

FIG. 12

No.	Ferret tag no.	Virus titer log_{10} $TCID_{50}$/ml on day p.i. [a]					Post-challenge HI titers
		1	2	3	4	5	
Vehicle-treated group							
1	228	5.7	4.2	4.2	1.7	-	640
2	784	3.9	4.9	1.9	1.9	-	640
3	793	4.4	4.2	2.4	3.9	-	640
4	794	4.9	5.9	1.4	-	-	160
5	789	4.4	4.2	3.4	3.4	-	640
6	799	3.7	4.4	3.4	-	-	320
7	811	4.4	4.4	-	-	-	1280
8	841	4.2	4.7	2.7	1.9	-	320
	mean [b]	*4.4*	*4.7*	*2.7*	*3.7*	-	
	SD	*0.4*	*0.7*	*1.0*	*0.4*	-	
	Shed/total	*8/8*	*8/8*	*7/8*	*5/8*	*0/8*	
Fludase-treated group							
1	780	-	-	-	NA	NA	NA
2	791	2.2	5.2	4.9	4.2	1.7	640
3	804	-	4.7	3.7	1.7	-	1280
4	803	-	-	-	-	-	≤10
5	805	-	-	-	-	-	≤10
6	806	-	-	-	-	-	≤10
7	810	2.2	4.7	3.2	2.9	-	160
8	812	-	-	4.4	-	-	640
9	813	-	3.2	4.4	4.7	-	160
10	819	2.7	5.2	-	-	-	320
11	828	-	4.9	1.9	1.7	-	320
12	843	-	4.4	4.9	4.9	3.4	320
	mean [b]	*2.4*	*4.6*	*3.9*	*3.4*	*2.6*	
	SD	*0.3*	*0.7*	*1.1*	*1.5*	*1.2*	
	Shed/total	*3/12*	*7/12*	*7/12*	*6/11*	*2/11*	

[a] – all nasal washes collected after day 5 post challenge were negative for virus presence.
[b] - mean value was calculated for the ferrets that shed virus.
Nasal washes recovered from the uninfected treated ferrets were negative for virus (not shown).
NA – not applicable, the ferret died on day 4 post infection due to an accident.

FIG. 13

SEQUENCE LISTING

<110> FANG, Fang
MALAKHOV, Michael

<120> A NOVEL CLASS OF THERAPEUTIC PROTEIN BASED MOLECULES

<130> NB-101.P11PC

<150> US 60/428,535
<151> 2002-11-22

<150> US 10/939,262
<151> 2004-09-10

<150> US 10/718,986
<151> 2003-11-21

<150> US 60/580,084
<151> 2004-06-16

<150> US 60/561,749
<151> 2004-04-13

<150> US 60/464,217
<151> 2003-04-19

<160> 39

<170> PatentIn version 3.3

<210> 1
<211> 58
<212> PRT
<213> Bos taurus

<400> 1

Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly Pro Cys Lys Ala
1 5 10 15

Arg Ile Ile Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gln Thr
20 25 30

Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg Asn Asn Phe Lys Ser Ala
35 40 45

Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
50 55

<210> 2
<211> 24
<212> PRT

<213> Homo sapiens

<400> 2

Asn Gly Arg Arg Ile Cys Leu Asp Leu Gln Ala Pro Leu Tyr Lys Lys
1 5 10 15

Ile Ile Lys Lys Leu Leu Glu Ser
20

<210> 3
<211> 27
<212> PRT
<213> Homo sapiens

<400> 3

Gly Arg Glu Leu Cys Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val
1 5 10 15

Val Glu Lys Phe Leu Lys Arg Ala Glu Asn Ser
20 25

<210> 4
<211> 34
<212> PRT
<213> Homo sapiens

<400> 4

Gln Ile His Phe Phe Phe Ala Lys Leu Asn Cys Arg Leu Tyr Arg Lys
1 5 10 15

Ala Asn Lys Ser Ser Lys Leu Val Ser Ala Asn Arg Leu Phe Gly Asp
20 25 30

Lys Ser

<210> 5
<211> 34
<212> PRT
<213> Homo sapiens

<400> 5

Glu Leu Arg Val Arg Leu Ala Ser His Leu Arg Lys Leu Arg Lys Arg
1 5 10 15

Leu Leu Arg Asp Ala Asp Asp Leu Gln Lys Arg Leu Ala Val Tyr Gln
20 25 30

Ala Gly

<210> 6
<211> 12
<212> PRT
<213> Homo sapiens

<400> 6

Arg Arg Leu Arg Arg Met Glu Ser Glu Ser Glu Ser
1 5 10

<210> 7
<211> 21
<212> PRT
<213> Homo sapiens

<400> 7

Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Thr Thr Asn Thr
1 5 10 15

Lys Lys Lys Asn Pro
20

<210> 8
<211> 379
<212> PRT
<213> Homo sapiens

<400> 8

Met Ala Ser Leu Pro Val Leu Gln Lys Glu Ser Val Phe Gln Ser Gly
1 5 10 15

Ala His Ala Tyr Arg Ile Pro Ala Leu Leu Tyr Leu Pro Gly Gln Gln
20 25 30

Ser Leu Leu Ala Phe Ala Glu Gln Arg Ala Ser Lys Lys Asp Glu His
35 40 45

Ala Glu Leu Ile Val Leu Arg Arg Gly Asp Tyr Asp Ala Pro Thr His
50 55 60

```
Gln Val Gln Trp Gln Ala Gln Glu Val Val Ala Gln Ala Arg Leu Asp
65                  70                  75                  80

Gly His Arg Ser Met Asn Pro Cys Pro Leu Tyr Asp Ala Gln Thr Gly
                85                  90                  95

Thr Leu Phe Leu Phe Phe Ile Ala Ile Pro Gly Gln Val Thr Glu Gln
            100                 105                 110

Gln Gln Leu Gln Thr Arg Ala Asn Val Thr Arg Leu Cys Gln Val Thr
        115                 120                 125

Ser Thr Asp His Gly Arg Thr Trp Ser Ser Pro Arg Asp Leu Thr Asp
    130                 135                 140

Ala Ala Ile Gly Pro Ala Tyr Arg Glu Trp Ser Thr Phe Ala Val Gly
145                 150                 155                 160

Pro Gly His Cys Leu Gln Leu Asn Asp Arg Ala Arg Ser Leu Val Val
                165                 170                 175

Pro Ala Tyr Ala Tyr Arg Lys Leu His Pro Ile Gln Arg Pro Ile Pro
            180                 185                 190

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

Gly His Phe Val Ala Gln Asp Thr Leu Glu Cys Gln Val Ala Glu Val
    210                 215                 220

Glu Thr Gly Glu Gln Arg Val Val Thr Leu Asn Ala Arg Ser His Leu
225                 230                 235                 240

Arg Ala Arg Val Gln Ala Gln Ser Thr Asn Asp Gly Leu Asp Phe Gln
                245                 250                 255

Glu Ser Gln Leu Val Lys Lys Leu Val Glu Pro Pro Pro Gln Gly Cys
            260                 265                 270

Gln Gly Ser Val Ile Ser Phe Pro Ser Pro Arg Ser Gly Pro Gly Ser
        275                 280                 285
```

```
Pro Gln Trp Leu Leu Tyr Thr His Pro Thr His Ser Trp Gln Arg Ala
    290                 295                 300

Asp Leu Gly Ala Tyr Leu Asn Pro Arg Pro Pro Ala Pro Glu Ala Trp
305                 310                 315                 320

Ser Glu Pro Val Leu Leu Ala Lys Gly Ser Cys Ala Tyr Ser Asp Leu
                325                 330                 335

Gln Ser Met Gly Thr Gly Pro Asp Gly Ser Pro Leu Phe Gly Cys Leu
            340                 345                 350

Tyr Glu Ala Asn Asp Tyr Glu Glu Ile Val Phe Leu Met Phe Thr Leu
        355                 360                 365

Lys Gln Ala Phe Pro Ala Glu Tyr Leu Pro Gln
    370                 375
```

<210> 9
<211> 424
<212> PRT
<213> Homo sapiens

<400> 9

```
Leu Ala Gly Gly Ser Val Arg Trp Gly Ala Leu His Val Leu Gly Thr
1               5                   10                  15

Ala Ala Leu Ala Glu His Arg Ser Met Asn Pro Cys Pro Val His Asp
            20                  25                  30

Ala Gly Thr Gly Thr Val Phe Leu Phe Phe Ile Ala Val Leu Gly His
        35                  40                  45

Thr Pro Glu Ala Val Gln Ile Ala Thr Gly Arg Asn Ala Ala Arg Leu
    50                  55                  60

Cys Cys Val Ala Ser Arg Asp Ala Gly Leu Ser Trp Gly Ser Ala Arg
65                  70                  75                  80

Asp Leu Thr Glu Glu Ala Ile Gly Gly Ala Val Gln Asp Trp Ala Thr
                85                  90                  95

Phe Ala Val Gly Pro Gly His Gly Val Gln Leu Pro Ser Gly Arg Leu
            100                 105                 110
```

```
Leu Val Pro Ala Tyr Thr Tyr Arg Val Asp Arg Leu Glu Cys Phe Gly
        115                 120                 125

Lys Ile Cys Arg Thr Ser Pro His Ser Phe Ala Phe Tyr Ser Asp Asp
    130                 135                 140

His Gly Arg Thr Trp Arg Cys Gly Gly Leu Val Pro Asn Leu Arg Ser
145                 150                 155                 160

Gly Glu Cys Gln Leu Ala Ala Val Asp Gly Gly Gln Ala Gly Ser Phe
                165                 170                 175

Leu Tyr Cys Asn Ala Arg Ser Pro Leu Gly Ser Arg Val Gln Ala Leu
            180                 185                 190

Ser Thr Asp Glu Gly Thr Ser Phe Leu Pro Ala Glu Arg Val Ala Ser
        195                 200                 205

Leu Pro Glu Thr Ala Trp Gly Cys Gln Gly Ser Ile Val Gly Phe Pro
    210                 215                 220

Ala Pro Ala Pro Asn Arg Pro Arg Asp Asp Ser Trp Ser Val Gly Pro
225                 230                 235                 240

Arg Ser Pro Leu Gln Pro Pro Leu Leu Gly Pro Gly Val His Glu Pro
                245                 250                 255

Pro Glu Glu Ala Ala Val Asp Pro Arg Gly Gly Gln Val Pro Gly Gly
            260                 265                 270

Pro Phe Ser Arg Leu Gln Pro Arg Gly Asp Gly Pro Arg Gln Pro Gly
        275                 280                 285

Pro Arg Pro Gly Val Ser Gly Asp Val Gly Ser Trp Thr Leu Ala Leu
    290                 295                 300

Pro Met Pro Phe Ala Ala Pro Pro Gln Ser Pro Thr Trp Leu Leu Tyr
305                 310                 315                 320

Ser His Pro Val Gly Arg Arg Ala Arg Leu His Met Gly Ile Arg Leu
                325                 330                 335
```

```
Ser Gln Ser Pro Leu Asp Pro Arg Ser Trp Thr Glu Pro Trp Val Ile
            340                 345                 350

Tyr Glu Gly Pro Ser Gly Tyr Ser Asp Leu Ala Ser Ile Gly Pro Ala
        355                 360                 365

Pro Glu Gly Gly Leu Val Phe Ala Cys Leu Tyr Glu Ser Gly Ala Arg
    370                 375                 380

Thr Ser Tyr Asp Glu Ile Ser Phe Cys Thr Phe Ser Leu Arg Glu Val
385                 390                 395                 400

Leu Glu Asn Val Pro Ala Ser Pro Lys Pro Pro Asn Leu Gly Asp Lys
                405                 410                 415

Pro Arg Gly Cys Cys Trp Pro Ser
            420
```

<210> 10
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 10

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Gly Gly Gly Gly Ser
1               5
```

<210> 11
<211> 2706
<212> DNA
<213> Actinomyces viscosus

<400> 11

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ctggccgcca ccggcctgat cgccgccgca cccccggcgc acgccgtccc cacgtctgac    120

ggcctggccg acgtcaccat cacgcaggtg aacgcgcccg cggacggcct ctactccgtc    180

ggcgatgtca tgaccttcaa catcaccctg accaacacca gcggcgaggc ccactcctac    240

gccccggcct cgacgaacct gtccgggaac gtctccaagt gccggtggcg caacgtcccg    300

gccgggacga ccaagaccga ctgcaccggc ctggccacgc acacggtgac cgccgaggac    360
```

ctcaaggccg gtggcttcac cccgcagatc gcctacgagg tcaaggccgt ggagtacgcc 420

gggaaggccc tgagcacccc ggagacgatc aagggcgcga cgagcccagt caaggccaac 480

tcgctgcggg tcgagtcgat cacgccgtcg tcgagccagg agaactacaa gctgggcgac 540

accgtcagct acacggtgcg cgtgcgctcg gtgtcggaca agacgatcaa cgtcgccgcc 600

accgaatcct ccttcgacga cctgggccgc cagtgccact ggggcggcct caagccgggc 660

aagggcgccg tctacaactg caagccgctc acccacacga tcacgcaagc cgacgtcgac 720

gccggccgct ggacgccatc gatcaccctg acggccaccg gaaccgacgg cgccaccctc 780

cagacgctca ccgccaccgg caacccgatc aacgtcgtcg gcgaccaccc gcaggccacg 840

cccgcaccgg cgcccgacgc gagcacggag ctgccggcct caatgagcca ggcccagcac 900

ctggccgcca acacggccac cgacaactac cgcatcccgg cgatcaccac cgcccccaat 960

ggggacctgc tcatctccta cgacgagcgc ccgaaggaca acggcaacgg cggcagcgac 1020

gcccccaacc cgaaccacat cgtccagcgc cgctccaccg acggcggcaa gacctggtcg 1080

gcgcccacct acatccacca gggcacggag accggcaaga aggtcggcta ctccgacccg 1140

agctacgtcg tcgatcacca gacgggcacg atcttcaact tccacgtcaa gtcctacgac 1200

cagggctggg gcggctcgcg cggcggcacc gacccggaga accggggcat catccaggcc 1260

gaggtgtcga cctccacgga caacggctgg acctggacgc accgcacgat caccgcggac 1320

atcacgaagg acaagccgtg gaccgcgcgt ttcgcggcct cgggccaggg catccagatt 1380

cagcacgggc cccacgccgg gcgcctggtg cagcagtaca cgatcaggac cgccggcggc 1440

gcggtgcagg ccgtctcggt ctactccgac gaccacggga agacgtggca ggccggcacg 1500

ccgatcggga ccggcatgga tgagaacaag gtcgttgagc tctccgacgg ctccctcatg 1560

ctcaactcgc gcgcctcgga tggctccggc ttccgcaagg tggcccactc caccgacggt 1620

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tgcgacgacg gcgcctcctg gacgaccagc aaggtcttcc acgagccctt cgtcggatac 1860

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tgcggccaga agccggcgga gccgagcccg gcgccgtcgc cgacggcggc accctcagcg 2040

gcaccgacgg agaagccggc cccgtcggcc gcgccgagcg ctgagcccac gcaggcaccg 2100

gcaccatcct ccgcgcccga gccgagcgct gcgcccgagc cgagcagcgc cccggcgccg 2160

gagcccacga ccgctccgag cacggagccc acaccggctc ctgcgcccag ctccgcacct 2220

gagcagaccg atgggccgac cgctgcgccc gcaccggaga cgtcctctgc accggccgcc 2280

gaaccgacgc aggccccgac ggtggcgcct tctgttgagc ccacgcaggc tccgggtgcg 2340

cagccgagct cagcacccaa gccgggggcg acgggtcggg ccccgtcggt ggtgaacccg 2400

aaggcgaccg gggcggcgac ggagcctggg acgccgtcat cgagcgcgag cccggcaccg 2460

agccggaacg cggcgccgac gccgaagccg ggcatggagc ccgatgagat tgatcggccg 2520

tctgacggca ccatggcgca gccgaccggt ggcgccagcg cgccgagtgc cgcgccgacg 2580

caggcggcga aggccggcag caggctgtct cgcacgggga ccaacgcgct gctgatcctg 2640

ggccttgcgg gtgtcgcggt tgtcggcggg tacctgctgc tgcgggctcg ccgttcgaag 2700

aactga 2706

<210> 12
<211> 901
<212> PRT
<213> Actinomyces viscosus

<400> 12

Met Thr Ser His Ser Pro Phe Ser Arg Arg Arg Leu Pro Ala Leu Leu
1 5 10 15

Gly Ser Leu Pro Leu Ala Ala Thr Gly Leu Ile Ala Ala Ala Pro Pro
20 25 30

Ala His Ala Val Pro Thr Ser Asp Gly Leu Ala Asp Val Thr Ile Thr
35 40 45

Gln Val Asn Ala Pro Ala Asp Gly Leu Tyr Ser Val Gly Asp Val Met
50 55 60

Thr Phe Asn Ile Thr Leu Thr Asn Thr Ser Gly Glu Ala His Ser Tyr
65 70 75 80

Ala Pro Ala Ser Thr Asn Leu Ser Gly Asn Val Ser Lys Cys Arg Trp
85 90 95

Arg Asn Val Pro Ala Gly Thr Thr Lys Thr Asp Cys Thr Gly Leu Ala
100 105 110

```
Thr His Thr Val Thr Ala Glu Asp Leu Lys Ala Gly Gly Phe Thr Pro
        115                 120                 125

Gln Ile Ala Tyr Glu Val Lys Ala Val Glu Tyr Ala Gly Lys Ala Leu
    130                 135                 140

Ser Thr Pro Glu Thr Ile Lys Gly Ala Thr Ser Pro Val Lys Ala Asn
145                 150                 155                 160

Ser Leu Arg Val Glu Ser Ile Thr Pro Ser Ser Ser Gln Glu Asn Tyr
                165                 170                 175

Lys Leu Gly Asp Thr Val Ser Tyr Thr Val Arg Val Arg Ser Val Ser
            180                 185                 190

Asp Lys Thr Ile Asn Val Ala Ala Thr Glu Ser Ser Phe Asp Asp Leu
        195                 200                 205

Gly Arg Gln Cys His Trp Gly Gly Leu Lys Pro Gly Lys Gly Ala Val
    210                 215                 220

Tyr Asn Cys Lys Pro Leu Thr His Thr Ile Thr Gln Ala Asp Val Asp
225                 230                 235                 240

Ala Gly Arg Trp Thr Pro Ser Ile Thr Leu Thr Ala Thr Gly Thr Asp
                245                 250                 255

Gly Ala Thr Leu Gln Thr Leu Thr Ala Thr Gly Asn Pro Ile Asn Val
            260                 265                 270

Val Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
        275                 280                 285

Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
    290                 295                 300

Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
305                 310                 315                 320

Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
                325                 330                 335
```

```
Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
            340                 345                 350

Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
        355                 360                 365

Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
    370                 375                 380

Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
385                 390                 395                 400

Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
                405                 410                 415

Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
            420                 425                 430

Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
        435                 440                 445

Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro
    450                 455                 460

His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
465                 470                 475                 480

Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
                485                 490                 495

Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
            500                 505                 510

Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
        515                 520                 525

Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
    530                 535                 540

Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
545                 550                 555                 560
```

```
Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
                565                 570                 575

Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
            580                 585                 590

Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
        595                 600                 605

Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
    610                 615                 620

Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
625                 630                 635                 640

Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
                645                 650                 655

Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro
            660                 665                 670

Ser Pro Thr Ala Ala Pro Ser Ala Ala Pro Thr Glu Lys Pro Ala Pro
        675                 680                 685

Ser Ala Ala Pro Ser Ala Glu Pro Thr Gln Ala Pro Ala Pro Ser Ser
    690                 695                 700

Ala Pro Glu Pro Ser Ala Ala Pro Glu Pro Ser Ser Ala Pro Ala Pro
705                 710                 715                 720

Glu Pro Thr Thr Ala Pro Ser Thr Glu Pro Thr Pro Ala Pro Ala Pro
                725                 730                 735

Ser Ser Ala Pro Glu Gln Thr Asp Gly Pro Thr Ala Ala Pro Ala Pro
            740                 745                 750

Glu Thr Ser Ser Ala Pro Ala Ala Glu Pro Thr Gln Ala Pro Thr Val
        755                 760                 765

Ala Pro Ser Val Glu Pro Thr Gln Ala Pro Gly Ala Gln Pro Ser Ser
    770                 775                 780
```

```
Ala Pro Lys Pro Gly Ala Thr Gly Arg Ala Pro Ser Val Val Asn Pro
785                 790                 795                 800

Lys Ala Thr Gly Ala Ala Thr Glu Pro Gly Thr Pro Ser Ser Ser Ala
                805                 810                 815

Ser Pro Ala Pro Ser Arg Asn Ala Ala Pro Thr Pro Lys Pro Gly Met
            820                 825                 830

Glu Pro Asp Glu Ile Asp Arg Pro Ser Asp Gly Thr Met Ala Gln Pro
        835                 840                 845

Thr Gly Gly Ala Ser Ala Pro Ser Ala Ala Pro Thr Gln Ala Ala Lys
    850                 855                 860

Ala Gly Ser Arg Leu Ser Arg Thr Gly Thr Asn Ala Leu Leu Ile Leu
865                 870                 875                 880

Gly Leu Ala Gly Val Ala Val Val Gly Gly Tyr Leu Leu Leu Arg Ala
                885                 890                 895

Arg Arg Ser Lys Asn
            900
```

<210> 13
<211> 1887
<212> DNA
<213> Actinomyces viscosus

<400> 13

```
ggcgaccacc cgcaggccac gcccgcaccg gcgcccgacg cgagcacgga gctgccggcc     60

tcaatgagcc aggcccagca cctggccgcc aacacggcca ccgacaacta ccgcatcccg    120

gcgatcacca ccgcccccaa tggggacctg ctcatctcct acgacgagcg cccgaaggac    180

aacggcaacg gcggcagcga cgcccccaac ccgaaccaca tcgtccagcg ccgctccacc    240

gacggcggca agacctggtc ggcgcccacc tacatccacc agggcacgga gaccggcaag    300

aaggtcggct actccgaccc gagctacgtc gtcgatcacc agacgggcac gatcttcaac    360

ttccacgtca agtcctacga ccagggctgg ggcggctcgc gcggcggcac cgacccggag    420

aaccggggca tcatccaggc cgaggtgtcg acctccacgg acaacggctg gacctggacg    480

caccgcacga tcaccgcgga catcacgaag gacaagccgt ggaccgcgcg tttcgcggcc    540

tcgggccagg gcatccagat tcagcacggg ccccacgccg ggcgcctggt gcagcagtac    600
```

acgatcagga ccgccggcgg cgcggtgcag gccgtctcgg tctactccga cgaccacggg 660

aagacgtggc aggccggcac gccgatcggg accggcatgg atgagaacaa ggtcgttgag 720

ctctccgacg gctccctcat gctcaactcg cgcgcctcgg atggctccgg cttccgcaag 780

gtggcccact ccaccgacgg tgggcagacc tggagcgagc cggtgtccga caagaacctg 840

cccgactcgg tggacaacgc ccagatcatc cgagccttcc cgaacgccgc gccggacgac 900

ccgcgcgcca aggtgctgct gctgagccac tcaccgaacc cgcggccgtg gtcgcgtgac 960

cgcggcacca tctcgatgtc ctgcgacgac ggcgcctcct ggacgaccag caaggtcttc 1020

cacgagccct tcgtcggata cacgacgatc gcggtgcagt ccgacggcag catcgggctg 1080

ctcagcgagg acgcccacaa cggcgccgac tacggcggca tctggtaccg caacttcacg 1140

atgaactggc tcggcgagca gtgcggccag aagccggcgg agccgagccc ggcgccgtcg 1200

ccgacggcgg caccctcagc ggcaccgacg gagaagccgg ccccgtcggc cgcgccgagc 1260

gctgagccca cgcaggcacc ggcaccatcc tccgcgcccg agccgagcgc tgcgcccgag 1320

ccgagcagcg ccccggcgcc ggagcccacg accgctccga gcacggagcc cacaccggct 1380

cctgcgccca gctccgcacc tgagcagacc gatgggccga ccgctgcgcc cgcaccggag 1440

acgtcctctg caccggccgc cgaaccgacg caggccccga cggtggcgcc ttctgttgag 1500

cccacgcagg ctccgggtgc gcagccgagc tcagcaccca agccgggggc gacgggtcgg 1560

gccccgtcgg tggtgaaccc gaaggcgacc ggggcggcga cggagcctgg gacgccgtca 1620

tcgagcgcga gcccggcacc gagccggaac gcggcgccga cgccgaagcc gggcatggag 1680

cccgatgaga ttgatcggcc gtctgacggc accatggcgc agccgaccgg tggcgccagc 1740

gcgccgagtg ccgcgccgac gcaggcggcg aaggccggca gcaggctgtc tcgcacgggg 1800

accaacgcgc tgctgatcct gggccttgcg ggtgtcgcgg ttgtcggcgg gtacctgctg 1860

ctgcgggctc gccgttcgaa gaactga 1887

<210> 14
<211> 628
<212> PRT
<213> Actinomyces viscosus

<400> 14

Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr
1 5 10 15

```
Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr
            20                  25                  30

Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly
        35                  40                  45

Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly
    50                  55                  60

Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr
65                  70                  75                  80

Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr
                85                  90                  95

Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
            100                 105                 110

His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln
        115                 120                 125

Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile
    130                 135                 140

Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr
145                 150                 155                 160

His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala
                165                 170                 175

Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His
            180                 185                 190

Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala
        195                 200                 205

Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln
    210                 215                 220

Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu
225                 230                 235                 240
```

```
Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser
                245                 250                 255

Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser
            260                 265                 270

Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln
        275                 280                 285

Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys
    290                 295                 300

Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp
305                 310                 315                 320

Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr
                325                 330                 335

Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val
            340                 345                 350

Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly
        355                 360                 365

Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu
    370                 375                 380

Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro Ser
385                 390                 395                 400

Pro Thr Ala Ala Pro Ser Ala Ala Pro Thr Glu Lys Pro Ala Pro Ser
                405                 410                 415

Ala Ala Pro Ser Ala Glu Pro Thr Gln Ala Pro Ala Pro Ser Ser Ala
            420                 425                 430

Pro Glu Pro Ser Ala Ala Pro Glu Pro Ser Ser Ala Pro Ala Pro Glu
        435                 440                 445

Pro Thr Thr Ala Pro Ser Thr Glu Pro Thr Pro Ala Pro Ala Pro Ser
    450                 455                 460
```

```
Ser Ala Pro Glu Gln Thr Asp Gly Pro Thr Ala Ala Pro Ala Pro Glu
465                 470                 475                 480

Thr Ser Ser Ala Pro Ala Ala Glu Pro Thr Gln Ala Pro Thr Val Ala
                485                 490                 495

Pro Ser Val Glu Pro Thr Gln Ala Pro Gly Ala Gln Pro Ser Ser Ala
            500                 505                 510

Pro Lys Pro Gly Ala Thr Gly Arg Ala Pro Ser Val Val Asn Pro Lys
        515                 520                 525

Ala Thr Gly Ala Ala Thr Glu Pro Gly Thr Pro Ser Ser Ser Ala Ser
    530                 535                 540

Pro Ala Pro Ser Arg Asn Ala Ala Pro Thr Pro Lys Pro Gly Met Glu
545                 550                 555                 560

Pro Asp Glu Ile Asp Arg Pro Ser Asp Gly Thr Met Ala Gln Pro Thr
                565                 570                 575

Gly Gly Ala Ser Ala Pro Ser Ala Ala Pro Thr Gln Ala Ala Lys Ala
            580                 585                 590

Gly Ser Arg Leu Ser Arg Thr Gly Thr Asn Ala Leu Leu Ile Leu Gly
        595                 600                 605

Leu Ala Gly Val Ala Val Val Gly Gly Tyr Leu Leu Leu Arg Ala Arg
    610                 615                 620

Arg Ser Lys Asn
625
```

<210> 15
<211> 1182
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 15
ggagatcatc cacaagctac accagcacct gcaccagatg ctagcactga gctgccagca 60

agcatgtctc aggctcagca tcttgcagca aatacggcta ctgataatta tcgcattcca 120

gcgattacaa ccgctccgaa tggtgattta ctgattagct atgatgaacg gccgaaggac 180

aatggaaatg gtggttccga tgcccctaac ccgaatcata ttgttcagcg tcgctccaca 240

gatggcggta aaacttggag cgcgccaacc tatattcatc agggtacgga gactggcaag 300

aaagtgggat attccgaccc ctcttatgtg gtggatcatc aaaccggtac aatcttcaat 360

tttcatgtga aatcatacga tcagggctgg ggaggtagcc gtgggggaac agacccggaa 420

aaccgcggga ttattcaggc agaggtgtct acgagcacgg ataatggatg gacgtggaca 480

catcgcacca tcaccgcgga tattacgaaa gataaaccgt ggaccgcgcg ttttgcggcg 540

tccggccaag gcattcagat ccagcatggg ccgcatgccg gccgtctggt gcaacagtat 600

accattcgta cggccggtgg agcggtgcag gctgtatcgg tttattccga tgatcatggg 660

aaaacgtggc aggctggcac cccgattggg acgggtatgg atgaaaacaa agttgtagag 720

ctgtctgacg gctctctgat gctgaacagt cgtgcgtcgg acgggagcgg ctttcgtaag 780

gttgcgcata gcactgatgg tgggcagacc tggtccgaac cggtttcgga caaaaatttg 840

ccggattcgg ttgataatgc ccagataatt cgtgcgtttc ctaatgctgc ccccgatgac 900

ccgcgcgcga aagtacttct tctgagtcat tccccaaatc cacgtccgtg gtcccgggat 960

cgtggtacga taagcatgtc atgtgatgac ggggcctcat ggaccacttc caaagttttt 1020

cacgaaccgt ttgtgggcta cacgactatt gcagttcaga gtgatggaag catcggtctg 1080

ctgtcggagg acgcgcacaa tggcgctgat tatggtggca tctggtatcg taattttacg 1140

atgaactggc tgggagaaca atgtggacaa aaacccgcgg aa 1182

<210> 16
<211> 394
<212> PRT
<213> Actinomyces viscosus

<400> 16

Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr
1 5 10 15

Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr
20 25 30

Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly
35 40 45

```
Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly
    50                  55                  60

Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr
65                  70                  75                  80

Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr
                85                  90                  95

Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp
            100                 105                 110

His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln
        115                 120                 125

Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile
    130                 135                 140

Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr
145                 150                 155                 160

His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala
                165                 170                 175

Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His
            180                 185                 190

Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala
        195                 200                 205

Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln
    210                 215                 220

Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu
225                 230                 235                 240

Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser
                245                 250                 255

Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser
            260                 265                 270
```

Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln
275 280 285

Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys
290 295 300

Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp
305 310 315 320

Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr
325 330 335

Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val
340 345 350

Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly
355 360 365

Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu
370 375 380

Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
385 390

<210> 17
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 17

Met Val Lys Arg Lys Lys
1 5

<210> 18
<211> 1281
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 18
ccatggttaa gcgcaaaaaa aaaggcggca aaaacggtaa aaatcgtcgt aaccgtaaga 60

aaaaaaatcc tggagatcat ccacaagcta caccagcacc tgcaccagat gctagcactg 120

agctgccagc aagcatgtct caggctcagc atcttgcagc aaatacggct actgataatt 180

atcgcattcc agcgattaca accgctccga atggtgattt actgattagc tatgatgaac 240

ggccgaagga caatggaaat ggtggttccg atgcccctaa cccgaatcat attgttcagc 300

gtcgctccac agatggcggt aaaacttgga gcgcgccaac ctatattcat cagggtacgg 360

agactggcaa gaaagtggga tattccgacc cctcttatgt ggtggatcat caaaccggta 420

caatcttcaa ttttcatgtg aaatcatacg atcagggctg gggaggtagc cgtgggggaa 480

cagacccgga aaaccgcggg attattcagg cagaggtgtc tacgagcacg gataatggat 540

ggacgtggac acatcgcacc atcaccgcgg atattacgaa agataaaccg tggaccgcgc 600

gttttgcggc gtccggccaa ggcattcaga tccagcatgg gccgcatgcc ggccgtctgg 660

tgcaacagta taccattcgt acggccggtg gagcggtgca ggctgtatcg gtttattccg 720

atgatcatgg gaaaacgtgg caggctggca ccccgattgg gacgggtatg gatgaaaaca 780

aagttgtaga gctgtctgac ggctctctga tgctgaacag tcgtgcgtcg gacgggagcg 840

gctttcgtaa ggttgcgcat agcactgatg gtgggcagac ctggtccgaa ccggtttcgg 900

acaaaaattt gccggattcg gttgataatg cccagataat tcgtgcgttt cctaatgctg 960

cccccgatga cccgcgcgcg aaagtacttc ttctgagtca ttccccaaat ccacgtccgt 1020

ggtcccggga tcgtggtacg ataagcatgt catgtgatga cggggcctca tggaccactt 1080

ccaaagtttt tcacgaaccg tttgtgggct acacgactat tgcagttcag agtgatggaa 1140

gcatcggtct gctgtcggag gacgcgcaca atggcgctga ttatggtggc atctggtatc 1200

gtaattttac gatgaactgg ctgggagaac aatgtggaca aaaacccgcg gaataagctt 1260

aaaaacccgc ggaataagct t 1281

<210> 19
<211> 444
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 19

Met Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg
1 5 10 15

```
Asn Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala
            20                  25                  30

Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala
        35                  40                  45

Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala
    50                  55                  60

Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg
65                  70                  75                  80

Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His
                85                  90                  95

Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro
            100                 105                 110

Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser
        115                 120                 125

Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe
    130                 135                 140

His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr
145                 150                 155                 160

Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr
                165                 170                 175

Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr
            180                 185                 190

Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile
        195                 200                 205

Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr
    210                 215                 220

Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp
225                 230                 235                 240
```

```
Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met
                245                 250                 255

Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn
            260                 265                 270

Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr
        275                 280                 285

Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro
    290                 295                 300

Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala
305                 310                 315                 320

Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn
                325                 330                 335

Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp
            340                 345                 350

Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val
        355                 360                 365

Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu
    370                 375                 380

Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg
385                 390                 395                 400

Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala
                405                 410                 415

Glu Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn
            420                 425                 430

Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
        435                 440
```

<210> 20
<211> 1248
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 20
atgggagatc atccacaagc tacaccagca cctgcaccag atgctagcac tgagctgcca 60

gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg ctactgataa ttatcgcatt 120

ccagcgatta caaccgctcc gaatggtgat ttactgatta gctatgatga acggccgaag 180

gacaatggaa atggtggttc cgatgcccct aacccgaatc atattgttca gcgtcgctcc 240

acagatggcg gtaaaacttg gagcgcgcca acctatattc atcagggtac ggagactggc 300

aagaaagtgg gatattccga cccctcttat gtggtggatc atcaaaccgg tacaatcttc 360

aattttcatg tgaaatcata cgatcagggc tggggaggta gccgtggggg aacagacccg 420

gaaaaccgcg ggattattca ggcagaggtg tctacgagca cggataatgg atggacgtgg 480

acacatcgca ccatcaccgc ggatattacg aaagataaac cgtggaccgc gcgttttgcg 540

gcgtccggcc aaggcattca gatccagcat gggccgcatg ccggccgtct ggtgcaacag 600

tataccattc gtacggccgg tggagcggtg caggctgtat cggtttattc cgatgatcat 660

gggaaaacgt ggcaggctgg caccccgatt gggacgggta tggatgaaaa caaagttgta 720

gagctgtctg acggctctct gatgctgaac agtcgtgcgt cggacgggag cggctttcgt 780

aaggttgcgc atagcactga tggtgggcag acctggtccg aaccggtttc ggacaaaaat 840

ttgccggatt cggttgataa tgcccagata attcgtgcgt ttcctaatgc tgcccccgat 900

gacccgcgcg cgaaagtact tcttctgagt cattccccaa atccacgtcc gtggtcccgg 960

gatcgtggta cgataagcat gtcatgtgat gacggggcct catggaccac ttccaaagtt 1020

tttcacgaac cgtttgtggg ctacacgact attgcagttc agagtgatgg aagcatcggt 1080

ctgctgtcgg aggacgcgca caatggcgct gattatggtg gcatctggta tcgtaatttt 1140

acgatgaact ggctgggaga acaatgtgga caaaaacccg cgaagcgcaa aaaaaaaggc 1200

ggcaaaaacg gtaaaaatcg tcgtaaccgt aagaaaaaaa atccttga 1248

<210> 21
<211> 415
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 21

```
Met Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
1               5                  10                  15

Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
            20                  25                  30

Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
        35                  40                  45

Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
    50                  55                  60

Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
65                  70                  75                  80

Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
                85                  90                  95

Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
            100                 105                 110

Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
        115                 120                 125

Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
    130                 135                 140

Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
145                 150                 155                 160

Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
                165                 170                 175

Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro
            180                 185                 190

His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
        195                 200                 205

Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
    210                 215                 220
```

```
Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
225                 230                 235                 240

Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
                245                 250                 255

Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
            260                 265                 270

Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
        275                 280                 285

Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
    290                 295                 300

Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
305                 310                 315                 320

Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
                325                 330                 335

Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
            340                 345                 350

Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
        355                 360                 365

Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
    370                 375                 380

Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Lys Arg Lys Lys Lys Gly
385                 390                 395                 400

Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
                405                 410                 415
```

<210> 22
<211> 1293
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 22

atgggagatc atccacaagc tacaccagca cctgcaccag atgctagcac tgagctgcca 60

gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg ctactgataa ttatcgcatt 120

ccagcgatta caaccgctcc gaatggtgat ttactgatta gctatgatga acggccgaag 180

gacaatggaa atggtggttc cgatgcccct aacccgaatc atattgttca gcgtcgctcc 240

acagatggcg gtaaaacttg gagcgcgcca acctatattc atcagggtac ggagactggc 300

aagaaagtgg gatattccga cccctcttat gtggtggatc atcaaaccgg tacaatcttc 360

aattttcatg tgaaatcata cgatcagggc tggggaggta gccgtggggg aacagacccg 420

gaaaaccgcg ggattattca ggcagaggtg tctacgagca cggataatgg atggacgtgg 480

acacatcgca ccatcaccgc ggatattacg aaagataaac cgtggaccgc gcgttttgcg 540

gcgtccggcc aaggcattca gatccagcat gggccgcatg ccggccgtct ggtgcaacag 600

tataccattc gtacggccgg tggagcggtg caggctgtat cggtttattc cgatgatcat 660

gggaaaacgt ggcaggctgg caccccgatt gggacgggta tggatgaaaa caaagttgta 720

gagctgtctg acggctctct gatgctgaac agtcgtgcgt cggacgggag cggctttcgt 780

aaggttgcgc atagcactga tggtgggcag acctggtccg aaccggtttc ggacaaaaat 840

ttgccggatt cggttgataa tgcccagata attcgtgcgt ttcctaatgc tgccccgat 900

gacccgcgcg cgaaagtact tcttctgagt cattccccaa atccacgtcc gtggtcccgg 960

gatcgtggta cgataagcat gtcatgtgat gacggggcct catggaccac ttccaaagtt 1020

tttcacgaac cgtttgtggg ctacacgact attgcagttc agagtgatgg aagcatcggt 1080

ctgctgtcgg aggacgcgca caatggcgct gattatggtg gcatctggta tcgtaatttt 1140

acgatgaact ggctgggaga acaatgtgga caaaaacccg cggaaccgag cccagcccct 1200

agccctactg cagcaccgtc cgctgcaaag cgcaaaaaaa aaggcggcaa aaacggtaaa 1260

aatcgtcgta accgtaagaa aaaaaatcct tga 1293

<210> 23
<211> 430
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 23

Met Gly Asp His Pro Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser
1 5 10 15

```
Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn
            20                  25                  30

Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn
        35                  40                  45

Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn
    50                  55                  60

Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser
65                  70                  75                  80

Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly
                85                  90                  95

Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val
            100                 105                 110

Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp
        115                 120                 125

Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly
    130                 135                 140

Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp
145                 150                 155                 160

Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr
                165                 170                 175

Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro
            180                 185                 190

His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly
        195                 200                 205

Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp
    210                 215                 220

Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val
225                 230                 235                 240
```

```
Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly
                245                 250                 255

Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp
            260                 265                 270

Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala
        275                 280                 285

Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala
    290                 295                 300

Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg
305                 310                 315                 320

Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr
                325                 330                 335

Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala
            340                 345                 350

Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn
        355                 360                 365

Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp
    370                 375                 380

Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala Pro
385                 390                 395                 400

Ser Pro Thr Ala Ala Pro Ser Ala Ala Lys Arg Lys Lys Lys Gly Gly
                405                 410                 415

Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
            420                 425                 430
```

<210> 24
<211> 1203
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 24
atgggagagc tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact 60

gataattatc gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat 120

gatgaacggc cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt 180

gttcagcgtc gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag 240

ggtacggaga ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa 300

accggtacaa tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt 360

gggggaacag acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat 420

aatggatgga cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg 480

accgcgcgtt ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc 540

cgtctggtgc aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt 600

tattccgatg atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat 660

gaaaacaaag ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac 720

gggagcggct ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg 780

gtttcggaca aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct 840

aatgctgccc ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc cccaaatcca 900

cgtccgtggt cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg 960

accacttcca aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt 1020

gatggaagca tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc 1080

tggtatcgta attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcgaag 1140

cgcaaaaaaa aaggcggcaa aaacggtaaa aatcgtcgta accgtaagaa aaaaaatcct 1200

tga 1203

<210> 25
<211> 400
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 25

Met Gly Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala
1 5 10 15

```
Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro
            20                  25                  30

Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly
        35                  40                  45

Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg
    50                  55                  60

Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln
65                  70                  75                  80

Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val
                85                  90                  95

Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr
            100                 105                 110

Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg
        115                 120                 125

Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr
    130                 135                 140

Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp
145                 150                 155                 160

Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly
                165                 170                 175

Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly
            180                 185                 190

Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr
        195                 200                 205

Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val
    210                 215                 220

Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp
225                 230                 235                 240
```

```
Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr
                245                 250                 255

Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn
            260                 265                 270

Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg
        275                 280                 285

Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser
    290                 295                 300

Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp
305                 310                 315                 320

Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile
        .       325                 330                 335

Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His
            340                 345                 350

Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn
        355                 360                 365

Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Lys Arg Lys Lys Lys
    370                 375                 380

Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
385                 390                 395                 400
```

<210> 26
<211> 1248
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 26

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atgggagagc tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact     60

gataattatc gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat    120

gatgaacggc cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt    180

gttcagcgtc gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag    240
```

ggtacggaga ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa 300

accggtacaa tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt 360

gggggaacag acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat 420

aatggatgga cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg 480

accgcgcgtt ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc 540

cgtctggtgc aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt 600

tattccgatg atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat 660

gaaaacaaag ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac 720

gggagcggct ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg 780

gtttcggaca aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct 840

aatgctgccc ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc cccaaatcca 900

cgtccgtggt cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg 960

accacttcca aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt 1020

gatggaagca tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc 1080

tggtatcgta attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcggaa 1140

ccgagcccag cccctagccc tactgcagca ccgtccgctg caaagcgcaa aaaaaaaggc 1200

ggcaaaaacg gtaaaaatcg tcgtaaccgt aagaaaaaaa atccttga 1248

<210> 27
<211> 415
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 27

Met Gly Glu Leu Pro Ala Ser Met Ser Gln Ala Gln His Leu Ala Ala
1 5 10 15

Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro
20 25 30

Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly
35 40 45

```
Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile Val Gln Arg Arg
    50                  55                  60

Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr Tyr Ile His Gln
65                  70                  75                  80

Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val
                85                  90                  95

Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His Val Lys Ser Tyr
            100                 105                 110

Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg
        115                 120                 125

Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp Asn Gly Trp Thr
    130                 135                 140

Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp
145                 150                 155                 160

Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln Ile Gln His Gly
                165                 170                 175

Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly
            180                 185                 190

Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp His Gly Lys Thr
        195                 200                 205

Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp Glu Asn Lys Val
    210                 215                 220

Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser Arg Ala Ser Asp
225                 230                 235                 240

Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp Gly Gly Gln Thr
                245                 250                 255

Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp Ser Val Asp Asn
            260                 265                 270
```

Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg
275 280 285

Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro Arg Pro Trp Ser
290 295 300

Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp Gly Ala Ser Trp
305 310 315 320

Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly Tyr Thr Thr Ile
325 330 335

Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser Glu Asp Ala His
340 345 350

Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn
355 360 365

Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu Pro Ser Pro Ala
370 375 380

Pro Ser Pro Thr Ala Ala Pro Ser Ala Ala Lys Arg Lys Lys Lys Gly
385 390 395 400

Gly Lys Asn Gly Lys Asn Arg Arg Asn Arg Lys Lys Lys Asn Pro
405 410 415

<210> 28
<211> 1221
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 28
ccatggggca tcaccatcac catcatctag agggagatca tccacaagct acaccagcac 60

ctgcaccaga tgctagcact gagctgccag caagcatgtc tcaggctcag catcttgcag 120

caaatacggc tactgataat tatcgcattc cagcgattac aaccgctccg aatggtgatt 180

tactgattag ctatgatgaa cggccgaagg acaatggaaa tggtggttcc gatgccccta 240

acccgaatca tattgttcag cgtcgctcca cagatggcgg taaaacttgg agcgcgccaa 300

cctatattca tcagggtacg gagactggca agaaagtggg atattccgac ccctcttatg 360

tggtggatca tcaaaccggt acaatcttca attttcatgt gaaatcatac gatcagggct 420

ggggaggtag ccgtggggga acagacccgg aaaaccgcgg gattattcag gcagaggtgt 480

ctacgagcac ggataatgga tggacgtgga cacatcgcac catcaccgcg gatattacga 540

aagataaacc gtggaccgcg cgttttgcgg cgtccggcca aggcattcag atccagcatg 600

ggccgcatgc cggccgtctg gtgcaacagt ataccattcg tacggccggt ggagcggtgc 660

aggctgtatc ggtttattcc gatgatcatg ggaaaacgtg gcaggctggc accccgattg 720

ggacgggtat ggatgaaaac aaagttgtag agctgtctga cggctctctg atgctgaaca 780

gtcgtgcgtc ggacgggagc ggctttcgta aggttgcgca tagcactgat ggtgggcaga 840

cctggtccga accggtttcg gacaaaaatt tgccggattc ggttgataat gcccagataa 900

ttcgtgcgtt tcctaatgct gcccccgatg acccgcgcgc gaaagtactt cttctgagtc 960

attccccaaa tccacgtccg tggtcccggg atcgtggtac gataagcatg tcatgtgatg 1020

acggggcctc atggaccact tccaaagttt ttcacgaacc gtttgtgggc tacacgacta 1080

ttgcagttca gagtgatgga agcatcggtc tgctgtcgga ggacgcgcac aatggcgctg 1140

attatggtgg catctggtat cgtaatttta cgatgaactg gctgggagaa caatgtggac 1200

aaaaacccgc ggaataagct t 1221

<210> 29
<211> 404
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 29

Met Gly His His His His His His Leu Glu Gly Asp His Pro Gln Ala
1 5 10 15

Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met
20 25 30

Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg
35 40 45

Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr
50 55 60

```
Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn
65                  70                  75                  80

Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp
                85                  90                  95

Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val
            100                 105                 110

Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile
        115                 120                 125

Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg
    130                 135                 140

Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser
145                 150                 155                 160

Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala
                165                 170                 175

Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly
            180                 185                 190

Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln
        195                 200                 205

Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val
    210                 215                 220

Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly
225                 230                 235                 240

Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu
                245                 250                 255

Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala
            260                 265                 270

His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys
        275                 280                 285
```

```
Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro
    290                 295                 300

Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His
305                 310                 315                 320

Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met
                325                 330                 335

Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu
            340                 345                 350

Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile
        355                 360                 365

Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile
    370                 375                 380

Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln
385                 390                 395                 400

Lys Pro Ala Glu
```

<210> 30
<211> 1257
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 30

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ccatgaagcg caaaaaaaaa ggcggcaaaa acggtaaaaa tcgtcgtaac cgtaagaaaa     60

aaaatcctgg agatcatcca caagctacac cagcacctgc accagatgct agcactgagc    120

tgccagcaag catgtctcag gctcagcatc ttgcagcaaa tacggctact gataattatc    180

gcattccagc gattacaacc gctccgaatg gtgatttact gattagctat gatgaacggc    240

cgaaggacaa tggaaatggt ggttccgatg cccctaaccc gaatcatatt gttcagcgtc    300

gctccacaga tggcggtaaa acttggagcg cgccaaccta tattcatcag ggtacggaga    360

ctggcaagaa agtgggatat tccgacccct cttatgtggt ggatcatcaa accggtacaa    420

tcttcaattt tcatgtgaaa tcatacgatc agggctgggg aggtagccgt gggggaacag    480
```

acccggaaaa ccgcgggatt attcaggcag aggtgtctac gagcacggat aatggatgga 540

cgtggacaca tcgcaccatc accgcggata ttacgaaaga taaaccgtgg accgcgcgtt 600

ttgcggcgtc cggccaaggc attcagatcc agcatgggcc gcatgccggc cgtctggtgc 660

aacagtatac cattcgtacg gccggtggag cggtgcaggc tgtatcggtt tattccgatg 720

atcatgggaa aacgtggcag gctggcaccc cgattgggac gggtatggat gaaaacaaag 780

ttgtagagct gtctgacggc tctctgatgc tgaacagtcg tgcgtcggac gggagcggct 840

ttcgtaaggt tgcgcatagc actgatggtg ggcagacctg gtccgaaccg gtttcggaca 900

aaaatttgcc ggattcggtt gataatgccc agataattcg tgcgtttcct aatgctgccc 960

ccgatgaccc gcgcgcgaaa gtacttcttc tgagtcattc cccaaatcca cgtccgtggt 1020

cccgggatcg tggtacgata agcatgtcat gtgatgacgg ggcctcatgg accacttcca 1080

aagtttttca cgaaccgttt gtgggctaca cgactattgc agttcagagt gatggaagca 1140

tcggtctgct gtcggaggac gcgcacaatg gcgctgatta tggtggcatc tggtatcgta 1200

attttacgat gaactggctg ggagaacaat gtggacaaaa acccgcggaa taagctt 1257

<210> 31
<211> 416
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 31

Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1 5 10 15

Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala Pro
20 25 30

Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln
35 40 45

His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile
50 55 60

Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro
65 70 75 80

```
Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile
                85                  90                  95

Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr
            100                 105                 110

Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp
        115                 120                 125

Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His
    130                 135                 140

Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp
145                 150                 155                 160

Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp
                165                 170                 175

Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys
            180                 185                 190

Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln
        195                 200                 205

Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile
    210                 215                 220

Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp
225                 230                 235                 240

His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp
                245                 250                 255

Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser
            260                 265                 270

Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp
        275                 280                 285

Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp
    290                 295                 300
```

```
Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro
305                 310                 315                 320

Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro
                325                 330                 335

Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp
            340                 345                 350

Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly
        355                 360                 365

Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser
    370                 375                 380

Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn
385                 390                 395                 400

Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
                405                 410                 415
```

<210> 32
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<220>
<221> misc_feature
<222> (17)..(18)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (20)..(21)
<223> n is a, c, g, or t

<400> 32
ttttcgtctc ccatgvnnvn naagcgcaaa aaaaaaggcg gca 43

<210> 33
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<220>
<221> misc_feature
<222> (2)..(3)
<223> Xaa can be any naturally occurring amino acid

<400> 33

Met Xaa Xaa Lys Arg Lys Lys Lys Gly Gly
1 5 10

<210> 34
<211> 1272
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 34
ccatgaagcg caaaaaaaaa ggcggcaaaa acggtaaaaa tcgtcgtaac cgtaagaaaa 60

aaaatcctgg tggtggtggt tctggagatc atccacaagc tacaccagca cctgcaccag 120

atgctagcac tgagctgcca gcaagcatgt ctcaggctca gcatcttgca gcaaatacgg 180

ctactgataa ttatcgcatt ccagcgatta caaccgctcc gaatggtgat ttactgatta 240

gctatgatga acggccgaag gacaatggaa atggtggttc cgatgcccct aacccgaatc 300

atattgttca gcgtcgctcc acagatggcg gtaaaacttg gagcgcgcca acctatattc 360

atcagggtac ggagactggc aagaaagtgg gatattccga cccctcttat gtggtggatc 420

atcaaaccgg tacaatcttc aattttcatg tgaaatcata cgatcagggc tggggaggta 480

gccgtggggg aacagacccg gaaaaccgcg ggattattca ggcagaggtg tctacgagca 540

cggataatgg atggacgtgg acacatcgca ccatcaccgc ggatattacg aaagataaac 600

cgtggaccgc gcgttttgcg gcgtccggcc aaggcattca gatccagcat gggccgcatg 660

ccggccgtct ggtgcaacag tataccattc gtacggccgg tggagcggtg caggctgtat 720

cggtttattc cgatgatcat gggaaaacgt ggcaggctgg caccccgatt gggacgggta 780

tggatgaaaa caaagttgta gagctgtctg acggctctct gatgctgaac agtcgtgcgt 840

cggacgggag cggctttcgt aaggttgcgc atagcactga tggtgggcag acctggtccg 900

aaccggtttc ggacaaaaat ttgccggatt cggttgataa tgcccagata attcgtgcgt 960

ttcctaatgc tgcccccgat gacccgcgcg cgaaagtact tcttctgagt cattccccaa 1020

atccacgtcc gtggtcccgg gatcgtggta cgataagcat gtcatgtgat gacggggcct 1080

catggaccac ttccaaagtt tttcacgaac cgtttgtggg ctacacgact attgcagttc 1140

agagtgatgg aagcatcggt ctgctgtcgg aggacgcgca caatggcgct gattatggtg 1200

gcatctggta tcgtaatttt acgatgaact ggctgggaga acaatgtgga caaaaacccg 1260

cggaataagc tt 1272

<210> 35
<211> 421
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 35

Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1 5 10 15

Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro Gln
20 25 30

Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser
35 40 45

Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr
50 55 60

Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser
65 70 75 80

Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro
85 90 95

Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr
100 105 110

Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys
115 120 125

Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr
130 135 140

```
Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser
145                 150                 155                 160

Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val
                165                 170                 175

Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr
            180                 185                 190

Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser
        195                 200                 205

Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val
    210                 215                 220

Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser
225                 230                 235                 240

Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile
                245                 250                 255

Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser
            260                 265                 270

Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val
        275                 280                 285

Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp
    290                 295                 300

Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe
305                 310                 315                 320

Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser
                325                 330                 335

His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser
            340                 345                 350

Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His
        355                 360                 365
```

Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser
370 375 380

Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly
385 390 395 400

Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly
405 410 415

Gln Lys Pro Ala Glu
420

<210> 36
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 36
ccatggttaa gcgcaaaaaa aaaggcggca aaaacggtaa aaatcgtcgt aaccgtaaga 60

aaaaaaatcc tggtggtggt ggttctggag atcatccaca agctacacca gcacctgcac 120

cagatgctag cactgagctg ccagcaagca tgtctcaggc tcagcatctt gcagcaaata 180

cggctactga taattatcgc attccagcga ttacaaccgc tccgaatggt gatttactga 240

ttagctatga tgaacggccg aaggacaatg gaaatggtgg ttccgatgcc cctaacccga 300

atcatattgt tcagcgtcgc tccacagatg gcggtaaaac ttggagcgcg ccaacctata 360

ttcatcaggg tacggagact ggcaagaaag tgggatattc cgacccctct tatgtggtgg 420

atcatcaaac cggtacaatc ttcaattttc atgtgaaatc atacgatcag ggctggggag 480

gtagccgtgg gggaacagac ccggaaaacc gcgggattat tcaggcagag gtgtctacga 540

gcacggataa tggatggacg tggacacatc gcaccatcac cgcggatatt acgaaagata 600

aaccgtggac cgcgcgtttt gcggcgtccg gccaaggcat tcagatccag catgggccgc 660

atgccggccg tctggtgcaa cagtatacca ttcgtacggc cggtggagcg gtgcaggctg 720

tatcggttta ttccgatgat catgggaaaa cgtggcaggc tggcaccccg attgggacgg 780

gtatggatga aaacaaagtt gtagagctgt ctgacggctc tctgatgctg aacagtcgtg 840

cgtcggacgg gagcggcttt cgtaaggttg cgcatagcac tgatggtggg cagacctggt 900

ccgaaccggt ttcggacaaa aatttgccgg attcggttga taatgcccag ataattcgtg 960

cgtttcctaa tgctgccccc gatgacccgc gcgcgaaagt acttcttctg agtcattccc 1020

caaatccacg tccgtggtcc cgggatcgtg gtacgataag catgtcatgt gatgacgggg 1080

cctcatggac cacttccaaa gtttttcacg aaccgtttgt gggctacacg actattgcag 1140

ttcagagtga tggaagcatc ggtctgctgt cggaggacgc gcacaatggc gctgattatg 1200

gtggcatctg gtatcgtaat tttacgatga actggctggg agaacaatgt ggacaaaaac 1260

ccgcggaata agctt 1275

<210> 37
<211> 422
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 37

Met Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg
1 5 10 15

Asn Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro
20 25 30

Gln Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala
35 40 45

Ser Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn
50 55 60

Tyr Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile
65 70 75 80

Ser Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala
85 90 95

Pro Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys
100 105 110

Thr Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys
115 120 125

Lys Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly
130 135 140

```
Thr Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly
145                 150                 155                 160

Ser Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu
                165                 170                 175

Val Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile
            180                 185                 190

Thr Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala
        195                 200                 205

Ser Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu
    210                 215                 220

Val Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val
225                 230                 235                 240

Ser Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro
                245                 250                 255

Ile Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly
            260                 265                 270

Ser Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys
        275                 280                 285

Val Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser
    290                 295                 300

Asp Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala
305                 310                 315                 320

Phe Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu
                325                 330                 335

Ser His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile
            340                 345                 350

Ser Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe
        355                 360                 365
```

```
His Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly
    370                 375                 380

Ser Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly
385                 390                 395                 400

Gly Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys
                405                 410                 415

Gly Gln Lys Pro Ala Glu
            420
```

<210> 38
<211> 416
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 38

```
Met Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1               5                   10                  15

Arg Lys Lys Lys Asn Pro Gly Asp His Pro Gln Ala Thr Pro Ala Pro
            20                  25                  30

Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser Met Ser Gln Ala Gln
        35                  40                  45

His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr Arg Ile Pro Ala Ile
    50                  55                  60

Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser Tyr Asp Glu Arg Pro
65                  70                  75                  80

Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro Asn Pro Asn His Ile
                85                  90                  95

Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr Trp Ser Ala Pro Thr
            100                 105                 110

Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys Val Gly Tyr Ser Asp
        115                 120                 125
```

```
Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr Ile Phe Asn Phe His
    130                 135                 140

Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser Arg Gly Gly Thr Asp
145                 150                 155                 160

Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val Ser Thr Ser Thr Asp
                165                 170                 175

Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr Ala Asp Ile Thr Lys
            180                 185                 190

Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser Gly Gln Gly Ile Gln
        195                 200                 205

Ile Gln His Gly Pro His Ala Gly Arg Leu Val Gln Gln Tyr Thr Ile
    210                 215                 220

Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser Val Tyr Ser Asp Asp
225                 230                 235                 240

His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile Gly Thr Gly Met Asp
                245                 250                 255

Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser Leu Met Leu Asn Ser
            260                 265                 270

Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val Ala His Ser Thr Asp
        275                 280                 285

Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp Lys Asn Leu Pro Asp
    290                 295                 300

Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe Pro Asn Ala Ala Pro
305                 310                 315                 320

Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser His Ser Pro Asn Pro
                325                 330                 335

Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser Met Ser Cys Asp Asp
            340                 345                 350
```

```
Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His Glu Pro Phe Val Gly
        355                 360                 365

Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser Ile Gly Leu Leu Ser
    370                 375                 380

Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly Ile Trp Tyr Arg Asn
385                 390                 395                 400

Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly Gln Lys Pro Ala Glu
                405                 410                 415
```

<210> 39
<211> 421
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 39

```
Val Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg Arg Asn
1               5                   10                  15

Arg Lys Lys Lys Asn Pro Gly Gly Gly Gly Ser Gly Asp His Pro Gln
            20                  25                  30

Ala Thr Pro Ala Pro Ala Pro Asp Ala Ser Thr Glu Leu Pro Ala Ser
        35                  40                  45

Met Ser Gln Ala Gln His Leu Ala Ala Asn Thr Ala Thr Asp Asn Tyr
    50                  55                  60

Arg Ile Pro Ala Ile Thr Thr Ala Pro Asn Gly Asp Leu Leu Ile Ser
65                  70                  75                  80

Tyr Asp Glu Arg Pro Lys Asp Asn Gly Asn Gly Gly Ser Asp Ala Pro
                85                  90                  95

Asn Pro Asn His Ile Val Gln Arg Arg Ser Thr Asp Gly Gly Lys Thr
            100                 105                 110

Trp Ser Ala Pro Thr Tyr Ile His Gln Gly Thr Glu Thr Gly Lys Lys
        115                 120                 125
```

```
Val Gly Tyr Ser Asp Pro Ser Tyr Val Val Asp His Gln Thr Gly Thr
    130                 135                 140

Ile Phe Asn Phe His Val Lys Ser Tyr Asp Gln Gly Trp Gly Gly Ser
145                 150                 155                 160

Arg Gly Gly Thr Asp Pro Glu Asn Arg Gly Ile Ile Gln Ala Glu Val
                165                 170                 175

Ser Thr Ser Thr Asp Asn Gly Trp Thr Trp Thr His Arg Thr Ile Thr
            180                 185                 190

Ala Asp Ile Thr Lys Asp Lys Pro Trp Thr Ala Arg Phe Ala Ala Ser
        195                 200                 205

Gly Gln Gly Ile Gln Ile Gln His Gly Pro His Ala Gly Arg Leu Val
    210                 215                 220

Gln Gln Tyr Thr Ile Arg Thr Ala Gly Gly Ala Val Gln Ala Val Ser
225                 230                 235                 240

Val Tyr Ser Asp Asp His Gly Lys Thr Trp Gln Ala Gly Thr Pro Ile
                245                 250                 255

Gly Thr Gly Met Asp Glu Asn Lys Val Val Glu Leu Ser Asp Gly Ser
            260                 265                 270

Leu Met Leu Asn Ser Arg Ala Ser Asp Gly Ser Gly Phe Arg Lys Val
        275                 280                 285

Ala His Ser Thr Asp Gly Gly Gln Thr Trp Ser Glu Pro Val Ser Asp
    290                 295                 300

Lys Asn Leu Pro Asp Ser Val Asp Asn Ala Gln Ile Ile Arg Ala Phe
305                 310                 315                 320

Pro Asn Ala Ala Pro Asp Asp Pro Arg Ala Lys Val Leu Leu Leu Ser
                325                 330                 335

His Ser Pro Asn Pro Arg Pro Trp Ser Arg Asp Arg Gly Thr Ile Ser
            340                 345                 350
```

```
Met Ser Cys Asp Asp Gly Ala Ser Trp Thr Thr Ser Lys Val Phe His
        355                 360                 365

Glu Pro Phe Val Gly Tyr Thr Thr Ile Ala Val Gln Ser Asp Gly Ser
    370                 375                 380

Ile Gly Leu Leu Ser Glu Asp Ala His Asn Gly Ala Asp Tyr Gly Gly
385                 390                 395                 400

Ile Trp Tyr Arg Asn Phe Thr Met Asn Trp Leu Gly Glu Gln Cys Gly
                405                 410                 415

Gln Lys Pro Ala Glu
            420
```