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(54) Title: PLANT VIRUS MOVEMENT PROTEINS AND METHODS OF USING THE SAME

(57) Abstract: Disclosed herein are transfection complexes comprising at least one cell surface ligand; at least one helper lipid component; and a transfection enhancer. Also disclosed are pharmaceutical compositions comprising the disclosed transfection complexes, and a pharmaceutically acceptable carrier. Further, disclosed are methods of transfecting a cell, the method comprising the steps of: obtaining a transfection complex as disclosed; and contacting a cell with the transfection complex.



PLANT VIRUS MOVEMENT PROTEINS AND METHODS OF USING THE SAME

RELATED APPLICATIONS

[001] The present application claims priority to the U.S. Provisional Application Serial No. 62/302,155, filed on March 1, 2016, by Jessee et al., and entitled “PLANT VIRUS MOVEMENT PROTEINS AND METHODS OF USING THE SAME,” and U.S. Provisional Application Serial No. 62/303,278, filed on March 3, 2016, by Jessee et al., and entitled “PLANT VIRUS MOVEMENT PROTEINS AND METHODS OF USING THE SAME.” The entire disclosure of both of these applications is hereby incorporated by reference herein in their entirety.

SEQUENCE STATEMENT

[002] The Sequence Listing associated with this application is filed in electronic format via EFS-Web and is hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is LT01229_SL.txt. The size of the text file is 195 kilobytes, and the text file was created on March 1, 2017.

FIELD OF THE INVENTION

[003] The present invention is in the field of transfection complexes suitable for the delivery of one or more biologically active agents to a cell and methods and kits for using the same.

BACKGROUND OF THE DISCLOSURE

[004] Lipid aggregates such as liposomes or cationic polymers can facilitate introduction of macromolecules, such as DNA, RNA, and proteins, into living cells. Aggregates comprising cationic lipid components can be used to effect delivery of large anionic molecules, such as nucleic acids, into certain types of cells.

[005] The use of cationic lipids has become increasingly popular since their introduction over 25 years ago. Several cationic lipids have been described in the literature and some of these are commercially available. DOTMA (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride; IUPAC: 1,2-di-O-octadecenyl-3-trimethylammonium propane (chloride salt); CAS Number: 104872-42-6) was the first cationic lipid

to be synthesized for the purpose of nucleic acid transfection. DOTMA can be formulated alone or can be combined with DOPE (dioleoylphosphatidylethanolamine) into a liposome, and such liposomes can be used to deliver plasmids into some cells. Other classes of lipids subsequently have been synthesized by various groups. For example, DOGS (5-carboxyspermylglycinedioctadecylamide) was the first polycationic lipid to be prepared and other polycationic lipids have since been prepared. The lipid DOSPA (2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium) has been described as an effective delivery agent.

[006] In other examples, cholesterol-based cationic lipids, such as DC-Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol) have been prepared and used for transfection. Also, 1,4-bis(3-N-oleylamino-propyl)piperazine was prepared and combined with histone H1 to generate a delivery reagent that was reported to be less toxic than other reagents. Some examples of commercially available lipids include Lipofectin® (DOTMA:DOPE) (Thermo-Fisher, Carlsbad, CA), LipofectAmine™ (DOSPA:DOPE) (Thermo-Fisher), LipofectAmine2000™ (Thermo-Fisher), Lipofectamine 3000 (Thermo-Fisher), Fugene®, Transfectam® (DOGS), ViaFect (Promega), DNA-In, GeneIn (MTI-GlobalStem) Effectene®, and DC-Chol. Further examples are PEI polymers and dendrimers such as jetPEI (PolyPlus), and Superfect (Qiagen). None of these reagents can be used universally for all cells. This is perhaps not surprising in light of the variation in composition of the membranes of different types of cells as well as the barriers that can restrict entry of extracellular material into cells. Moreover, the mechanism by which cationic lipids deliver nucleic acids into cells is not clearly understood. The reagents are less efficient than viral delivery methods and are toxic to cells, although the degree of toxicity varies from reagent to reagent.

[007] However, transfection agents, including cationic lipids, anionic lipids, cationic polymers, exosomes, and virasomes, are not universally effective in all cell types. Effectiveness of transfection of different cells depends on the particular transfection agent composition. In general, polycationic lipids are more efficient than monocationic lipids in transfecting eukaryotic cells. In many cases, cationic lipids alone are not effective or are only partially effective for transfection so helper lipids or transfection enhancers can be used in combination with cationic lipids.

[008] Many biological materials are taken up by cells via receptor-mediated endocytosis, in which a surface ligand binds to a cell-surface receptor, leading to clustering of ligand-bound receptors, and formation of coated pits followed by internalization of the

ligands into endosomes. Both enveloped viruses, like influenza virus and alphaviruses, and non-enveloped viruses, like Adenovirus, infect cells via endocytotic mechanisms. Enhancement of dendrimer-mediated transfection of some cells by chloroquine (a lysosomotropic agent) suggests that endocytosis is involved in at least some transfections.

[0009] Introduction of foreign DNA sequences into eukaryotic cells mediated by viral infection is generally orders of magnitude more efficient than transfection with anionic lipids, cationic lipid, PEI, peptides, or dendrimer transfection agents. Viral infection of all the cells in a culture requires fewer than 10 virus particles per cell. Although the detailed mechanism of fusion is not fully understood and varies among viruses, viral fusion typically involves specific fusogenic agents, such as viral proteins, viral spike glycoproteins and peptides of viral spike glycoproteins. Cell binding and internalization also can be enhanced, accelerated or made selective with peptides that bind cell receptors. For example, the penton-base protein of the Adenovirus coat contains the peptide motif RGD (Arg--Gly--Asp) which mediates virus binding to integrins and viral internalization via receptor-mediated endocytosis.

[0010] The efficiency of cationic lipid transfections has been shown to be enhanced by the addition of whole virus particles to the transfection mixture. Certain viral components may also enhance the efficiency of cationic lipid-mediated transfection. For example, it has been suggested that "Lipofectin™"-mediated transfections may be enhanced 3-4-fold by adding influenza virus hemagglutinin peptides to the transfection mixture. Antibodies have been shown to enhance cationic lipid transfections and transferrin-poly lysine or asialoglycoprotein polylysine have been shown to enhance cationic lipid transfection.

[0011] Nevertheless, these methods do not work for all cell types, require relatively complex protocols and are inconvenient. It is apparent, therefore, that new and improved methods for introducing macromolecules, and particularly nucleic acids, into cell, are greatly to be desired. In particular, improved methods for introducing nucleic acids into a wider variety of cells, and particularly into primary cells, are greatly to be desired.

SUMMARY OF THE INVENTION

[0012] Disclosed herein are transfection complexes comprising at least one cell surface ligand or a plant virus movement protein or peptide fragments; at least one helper lipid component; and a transfection enhancer. Also disclosed are pharmaceutical compositions comprising the disclosed transfection complexes, and a pharmaceutically

acceptable carrier. Further, disclosed are methods of transfecting a cell, the method comprising the steps of: obtaining a transfection complex as disclosed; and contacting a cell with the transfection complex.

DETAILED DESCRIPTION OF THE EMBODIMENTS

DEFINITIONS AND ABBREVIATIONS

[0013] It is to be understood that the present invention is not limited to particular devices or biological systems, which may, of course, vary. It is also to be understood that, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include singular and plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a lipid” includes one or more lipids. It is to be yet further understood that any terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0014] Unless stated otherwise, the following terms, definitions, and abbreviations as used herein are intended to have the following meanings:

[0015] As used herein, the term “labeled” is intended to mean that a compound has at least one element, isotope, or chemical compound attached to enable the detection of the compound by using a radioactive or heavy isotope label, or an immune label such as an antibody or antigen or a label derived from a colored, luminescent, phosphorescent, or fluorescent dye. Photoaffinity labeling employing, for example, o-, m- and p-azidobenzoyls, substituted with one or more halogen moieties, including, but not limited to 4-azido-2,3,5,6-tetrafluorobenzoic acid, is utilized for the direct elucidation of intermolecular interactions in biological systems.

[0016] The terms “subject” and “animal” are synonymous and, as used herein, refer to humans as well as non-human animals, including, for example, mammals (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a primate, or a pig), birds, reptiles, amphibians, and fish.

[0017] The term “cell” generally refers to eukaryotic cells of any type and from any source. Types of eukaryotic cells include epithelial, fibroblastic, neuronal, hematopoietic cells and the like from primary cells, tumor cells or immortalized cell lines. Sources of such cells include any animal such as human, canine, mouse, hamster, cat, bovine, porcine, monkey, ape, sheep, fish, insect, fungus, and any plant including crop plants, algae, ornamentals and trees.

[0018] “Delivery” is used to denote a process by which a desired compound is transferred to a target cell such that the desired compound is ultimately located inside the target cell or in, or on, the target cell membrane. In many uses of the compounds of the invention, the desired compound is not readily taken up by the target cell and delivery via lipid aggregates or transfection complexes a means for delivering the desired compound to the appropriate cellular compartment within a cell. In certain uses, especially under in vivo conditions, delivery to a specific target cell type is preferable and can be facilitated by transfection complexes comprising surface ligands of the invention.

[0019] Drug refers to any therapeutic or prophylactic agent other than food which is used in the prevention, diagnosis, alleviation, treatment, or cure of disease in man or animal.

[0020] “Kit” refers to transfection or protein expression kits which include one or more of the compounds of the present invention or mixtures thereof. Such kits may comprise a carrying means being compartmentalized to receive in close confinement one or more container means such as vials, test tubes and the like. Each of such container means comprises components or a mixture of components needed to perform transfection. Such kits may include one or more components selected from nucleic acids (preferably one or more vectors), cells, one or more compounds of the present invention, lipid-aggregate forming compounds, transfection enhancers, biologically active substances, etc.

[0021] The term “associated with”, when used in the context of molecular interactions, refers to two entities linked by a direct or indirect covalent or non-covalent interaction, such as hydrogen bonding, van der Waals interactions, hydrophobic interactions, magnetic interactions, electrostatic interactions, etc.

[0022] The term “biocompatible,” as used herein refers to compounds that are not toxic to cells. Compounds are biocompatible if their addition to cells in vitro results in less than or equal to 20% cell death, and their administration in vivo does not induce inflammation or other such adverse effects.

[0023] The term “biodegradable,” as used herein, refers to compounds that, when introduced into cells, are broken down into components that the cells can either reuse or dispose of without significant toxic effect on the cells (i.e., fewer than about 20% of the cells are killed when the components are added to cells in vitro). The components do not induce inflammation or other adverse effects in vivo. The chemical reactions relied upon to break down the biodegradable compounds are typically uncatalyzed. The term “effective amount,” as used herein with respect to an active agent, refers to the amount necessary to

elicit the desired biological response. The effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the composition of the encapsulating matrix, the target tissue, etc. Delivery of an “effective amount of a molecule” is the delivery of the molecule into a cell in sufficient amounts so that the molecule elicits a biological response, for example, modulating the expression of one or more genes in the cell. In specific embodiments, an effective amount of a molecule is delivered to a cell such that an amelioration or improvement in a disease, condition, or disorder related to the cell can be obtained. Delivery of an “effective amount of siRNA” or an “effective amount or RNAi” is the delivery of siRNA or other RNAi into a cell in sufficient amounts to cause a reduction in expression of the target gene in the cell.

[0024] The terms “biologically active agent”, “bioactive agents” or the like, generally refers to a composition, complex, compound or molecule which has a biological effect or that modifies, causes, promotes, enhances, blocks or reduces a biological effect, or that enhances or limits the production or activity of, reacts with and/or binds to a second molecules which has a biological effect. The second molecule can, but need not be, an endogenous molecule (e.g., a molecule, such as a protein or nucleic acid, normally present in the target cell). A biological effect may be, but is not limited to, one that stimulates or causes an immunoreactive response; one that impacts a biological process in a cell, tissue or organism (e.g., in an animal); one that imparts a biological process in a pathogen or parasite; one that generates or causes to generate a detectable signal; one that regulates the expression of a protein or polypeptide; one that stops or inhibits the expression of a protein or polypeptide; or one that causes or enhances the expression of a protein or polypeptide. Biologically active compositions, complexes, compounds or molecules may be used in investigative, therapeutic, prophylactic and diagnostic methods and compositions. Bioactive agents may include but are not limited to, pharmaceuticals, cell metabolites, proteins, nutrients (vitamins, amino acids, lipids, nucleotides, and carbohydrates), and exosomes.

[0025] The term “cationic lipid” refers to any cationic lipids which may be used for transfection and which under physiological conditions possess at least one positive charge. While it is to be understood that certain of the cell surface ligands that form the basis of the present disclosure can be formulated with cationic lipids the cationic lipids can be considered helper lipids.

[0026] The term “lysosomotropic agent” is any compound or molecule which inhibit lysosomal function that prevents or slows the acidification of the lysosomal compartment.

[0027] The term “nucleic acid binding moiety” as used herein refers to a compound or molecule capable binding to nucleic acid. In some embodiments, the binding molecule is capable of noncovalently binding to nucleic acid, while in other embodiments, the binding molecule links covalently to a cell binding adhesion sequence, a plant virus movement protein or peptide fragments, a nuclear localization sequence, transfection enhancer, and/or a fusion agent. The binding molecule can include but is not limited to spermine, spermine derivative, spermidine, histones or fragments thereof, protamines or fragments thereof, HMG proteins or fragments thereof, poly-lysine, poly-arginine, poly-histidine, polyamines and cationic peptides, nucleic acid intercalaters, protein nucleic acid sequences or aptamers. In addition, this includes but is not limited to analogs or derivatives of the above compounds. Non limiting examples are the cationic peptides that are repeats of lysine or arginine, for example a sequence having between 8-20 lysine residues (K8-K20) (SEQ ID NO:583) or between 8-20 arginine residues (R8-R20) (SEQ ID NO:584).

[0028] “Target cell” or “target tissue” refers to any cell or tissue to which a desired compound is delivered, using a lipid aggregate or transfection complex as carrier for the desired compound.

[0029] Transfection is used herein to mean the delivery of any nucleic acid, protein, peptide, lipid, cell nutrient, pharmaceutical agent, molecule or other macromolecule to a target cell or tissue in vitro or in vivo (i.e., in an animal, a plant or a human), such that the nucleic acid, protein or other macromolecule is expressed in, confers a phenotype to, causes enhanced growth, expression of a protein, or has a biological function in the cell.

[0030] The term “expressible nucleic acid” includes both DNA and RNA without regard to molecular weight, and the term “expression” means any manifestation of the functional presence of the nucleic acid within the cell including, without limitation, both transient expression and stable expression.

[0031] The term “fusion agent” as used herein refers to any chemical or molecules capable breaking down an endosomal membrane or cell membrane and freeing the transfection agent into the cytoplasm of the cell. This term includes but is not limited to viruses, synthetic compounds, proteins, fusion peptides, cell penetration peptides or

proteins, or derivatives thereof. As a result of the presence of the fusion agent the membrane can undergo lysis, fusion, or rearrangement or all three.

[0032] The term “fusion peptide” refers to any peptide grouping which penetrates a membrane such that the structural organization and integrity of the membrane is lost. Fusion peptides are fusion agents.

[0033] The term “transfection agent” as used herein generally refers to composition capable of delivering molecules to cells. Transfection agents can be organic such as lipid, carbohydrate, cationic polymers, dendrimers, peptide or protein based or combination of those depending cell type or tissue that one targets. Transfection agents can also be in-organic such as calcium salts. They included cationic lipids, anionic lipids, cationic peptides, cationic proteins, polycationic virus hybrids, cationic polymers, exosomes, and combinations of the above. Transfection agent as used herein may optionally include at least one or more of the transfection compounds optionally in combination with one or more helper lipids, one or more pegylated lipids, one or more lipids from exosomes, complete lipid mixtures form exosomes, optionally one or more targeting moieties, optionally one or more cell surface ligands, optionally one or more nuclear localization sequences, optionally one or more fusion agents, optionally one or more condensing agents, optionally one or more cell penetration agents, optionally one or more plant movement proteins or peptide fragments , optionally one or more exosomes and optionally one or more lysosomotropic agents.

[0034] The term “transfection enhancer” as used herein refers to a compound when added to a transfection agent increases the efficiency of transfection (i.e., increases the percent of cells transfected), increases the level of expression of a transfection agent, or reduces the requirement for the amount of nucleic acid or protein required to give a biological response, or any combination of the enhancements above. In some embodiments, the transfection enhancer also helps deliver molecules that help downregulate expression such as siRNA, LNA's and the like.

[0035] The term “surface ligand” or “cell surface ligand” refers to a chemical or structure which will bind to a surface receptor of a cell. The term “cell surface receptor” as used herein refers to any specific chemical grouping on the surface of a cell to which the surface ligand can attach, contact or associate with. A surface ligand is a targeting moiety. Furthermore, surface ligands include anything which is capable of binding to the cell and centering the cell through cytosin (e.g., endocytosis, potocytosis, and pinocytosis).

[0036] The term “transfection complex”, as used herein generally refers to a composition formulated for the delivery of a biologically active agent, such as a nucleic acid, a protein, a macromolecule, cell nutrient, bioactive molecule or the like, to a cell or to a tissue in vivo or in vitro. Transfection complexes as used herein may include at least one or more of the transfection compounds or agents in combination with the biologically active compound to be delivered, optionally in combination with; one or more helper lipids, one or more pegylated lipids, one or more targeting moieties, one or more nuclear localization sequences, one or more fusion agents, one or more condensing agents, one or more cell penetration agents, one or more plant movement proteins or peptide fragments, complete exosomes, total lipid extracts isolated from exosomes, one or more exosome lipids, one or more exosome protein components and one or more lysosomotropic agents in addition to the bioactive agent that is to be delivered. For the purposes described herein, the term “transfection complex” may be thought of as a lipoplex or a lipid aggregate contacted with a bioactive agent. Thus, in some instances in the following disclosure, terms such as lipoplex, lipid aggregate and the like may be used to make reference a transfection complex that lacks the one or more bioactive agents or “payloads”.

[0037] The term “helper lipid”, as used herein, generally refers to a lipid that is suitable for use in the preparation and formation of transfection complexes disclosed herein. Suitable helper lipids may include, though are not limited to DOPE, DPhPE, saturated and unsaturated DPPE, saturated and unsaturated DMPE, DOPC, Lyso-PE (1-acyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine), Lyso-PC (1-acyl-3-hydroxy-*sn*-glycero-3-phosphocholine), 3-alkyloxy-2-hydroxy-1-acetamidopropane, 4-alkyloxy-3-hydroxy-1-acetamidopropane, 5-alkyloxy-4-hydroxy-1-acetamidopropane, cholesterol, cholesterol derivatives, sterols, including phytosterols, zoosterols and hopanoids, or any of the neutral or cationic lipids that are known to allow or to facilitate the introduction of exogenous bioactive molecules to the interior of a cell or of a tissue. In some embodiments, more than one helper lipid may be used in the formulation of the transfection complexes described herein. Exemplary though non-limiting neutral or cationic lipids contemplated for use in the preparation of the presently disclosed transfection complexes may include one or more lipids selected from the following: N-(2-bromoethyl)-N,N-dimethyl-2,3-bis(9-octadecenyloxy)-propan-1-amine bromide (BMOP), dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DDPES), DSPC, dioleoylphosphatidylethanolamine (DOPE), formulation of cetyltrimethylammonium bromide (CTAB) and DOPE (CTAB:DOPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-

phosphocholine (POPC), DMG, 1,2-dimyristoylaminopropane (DMAP), dimyristoylphosphatidylethanolamine (DMPE), DOMG, DMA, Dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), Dipalmitoylethylphosphatidylcholine (DPEPC), dioleoydimethylammonium chloride (DODAC), 1,3-di-oleoyloxy-2-(6-carboxyspermyl)-propylamid (DOSPER), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniumchloride (DOTMA), N-[1-(2,3-dipalmitoleoyloxy)propyl]-N,N,N-trimethylammoniumchloride (DPTMA), didoceyl methylammonium bromide (DDAB), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP), DOTAP.Cl, 3,β-N,(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-chol), 2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA), O,O'-ditetradecanoyl-N-(alphatrimethylammonioacetyl) diethanolamine chloride (DC-6-14), dicaproylphosphatidylethanolamine (DCPE), dilauryl oxypropyl-3-dimethylhydroxyethylammonium bromide (DLRIE), 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), Ethyl-PC, 2,3-dioleoyloxy-N-[2-(sperminecarboxamidoethyl)-N,N-di-methyl-1-propanaminium trifluoroacetate (DOSPA), dioctadecylamidoglycyl carboxyspermine (DOGS), N-[1-(2,3 dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl) ammonium bromide (DMRIE), Dioleoyl-ethyl-phosphocholine (DOEPC), N-[1-(2,3-dioleoyloxy)propyl]-N-[1-(2-hydroxyethyl)]-N,Ndimethylammonium iodide (DOHME), N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propaniminium bromide/dioleoylphosphatidylethanolamine (GAP-DLRIE:DOPE), dipalmitoylphosphatidylcholine (DPPC), 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(3-lysyl(1-glycerol)).Cl (DOPG), N-lauroylsarcosine, (R)-(+)-limonene, lecithins (and derivatives thereof); phosphatidylethanolamine (and derivatives thereof); phosphatidylethanolamines, dioleoylphosphatidylethanolamine), diphytanoylphosphatidylethanolamine (DPhPE), dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylethanolamine, 3-β-[1-ornithinamidecarbonyl]-cholesterol (O-Chol), palmitoyl-oleoylphosphatidylethanolamine (POPE); distearoylphosphatidylethanolamine; phosphatidylcholine; phosphatidylcholines, dipalmitoylphosphatidylcholine (DPPC) palmitoyl-oleoylphosphatidylcholine (POPC); distearoylphosphatidylcholine; phosphatidylglycerol; piperazine-based cationic lipids, a phosphatidylglycerol, dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidyl-glycerol (DPPG), distearoylphosphatidylglycerol; phosphatidylserine (and derivatives thereof); phosphatidylserines, dioleoyl- or

dipalmitoylphosphatidylserine; a diquatery ammonium salt; N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,2-ethanediamine (TmedEce), N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,3-propanediamine (PropEce), N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,6-hexanediamine (HexEce), and their corresponding N,N'-dicetyl saturated analogues (TmedEce, PropEce and HexEce), a diphosphatidylglycerol; a fatty acid ester; a monocationic transfection lipid; 1-deoxy-1-[dihexadecyl(methyl)ammonio]-D-xylitol; 1-deoxy-1-[methyl(ditetradecyl)ammonio]-Darabinitol; 1-deoxy-1-[dihexadecyl(methyl)ammonio]-D-arabinitol; a 1-deoxy-1-[methyl(dioctadecyl)-ammonio]-darabinitol, glycerol ester; sphingolipids; cardolipin; a cerebroside; a ceramide, exosomes or lipids mixtures isolated from exosomes; and combinations thereof.

[0038] Helper lipids also include the neutral lipids cholesterol and other 3 β OH-sterols, as well as derivatives thereof, phosphatidyl choline, or commercially available cationic lipid mixtures such as, for example, LIPOFECTIN® CELLFECTIN® (1:1.5 (M/M) formulation of N,N,N',N'',N'''-tetramethyl-N,N,N',N'',N'''-tetrapalmitylspermine (TMTPS) and dioleoyl phosphatidylethanolamine (DOPE), LIPOFECTACE®, GS 2888 CYTOFECTIN®, FUGENE 6®, EFFECTENE®, and LIPOFECTAMINE®, LIPOFECTAMINE 2000®, LIPOFECTAMINE PLUS®, LIPOTAXI®, POLYECT®, SUPERFECT®, TFXNT™, TRANSFAST™, TRANSFECTAM®, TRANSMESSENGER®, vectamidine (3-tetradecylamino-N-tert-butyl-N'-tetradecylpropionamidine (a.k.a. diC14-amidine), OLIGOFECTAMINE MessengerMAX, GeneIn™, TransfeX™, LipofectAmine 3000, Lipofectin®, DMRIE-C, CellFectin®, LipofectAce®, Fugene®, Fugene® HD, Tfx-10®, Tfx-20®, Tfx-50®, DNA-In, Transfectin™, SilentFect™, Effectene®, ViaFect™, DC-chol, GenePorter®, DharmaFect 1®, DharmaFect 2®, DharmaFect 3®, DharmaFect 4®, Escort™ III, Escort™ IV, DOGS among others. Also contemplated are any mixtures of combination of the above listed helper lipids, exosomes, and lipids mixtures isolated from exosomes.

[0039] Examples of lipids examples isolated from exosomes are include, but are not limited to, Lyso-PC (non-limiting examples include C-18, C-16, C-14 and mixture), lyso-bisphosphahtidic acid (non-limiting example include C-18, C-16 and C-14), sphingomyelin, ceramides (non-limiting examples include C-8 and C-24), disaturated PC (non-limiting examples include DSPC, DPPC, DMPC, and compounds having a Cn length (where n= 8 – 25), diunsaturated PC-MIX (non-limiting examples include DOPC and DP(db)PC), phosphatidyl serine (PS), phosphatidyl inositol (PI), disaturated PE (non-limiting example include DSPE, DPPE, and DMPE), di-unsaturated PE-MIX (non-limiting

example include DOPE and DP(db)PE), phosphatidyl glycerol (PG), (non-limiting examples include C-18 – C-22), cholesterol, and diglycerides, such as cardiolipin.

[0040] Also contemplated are any mixtures of combination of the above listed helper lipids, exosomes, and lipids mixtures isolated from exosomes.

[0041] The following patent documents, patent applications, or references are incorporated by reference herein in their entirety and in particular for their disclosure of transfection agents containing cationic and neutral helper lipids, which may be used in the transfection complexes disclosed herein: U.S. Pat. Nos. 6,075,012; 6,020,202; 5,578,475; 5,736,392; 6,051,429; 6,376,248; 5,334,761; 5,316,948; 5,674,908; 5,834,439; 6,110,916; 6,399,663; 6,716,882; 5,627,159; 7,915,230; 7,531,693; 8,034,977; 7,166,745; 5,994,109; 6,033,884; 6,150,168; 6,177,554; 6,083,741 6,458,026; 7,598,421; 7,820,624; 7,256,043; 7,704,969; 8,026,341; 7,145,039; 7,531,693; and 8,785,200; and International Publications WO 2004/063342, WO 0027795, WO 2004/105697, WO 2007/130073, WO 2012/142622, and WO 2013/158127,

[0042] The term “pegylated lipid” as used herein generally refers to a lipid that is covalently conjugated to one or more polyethylene glycol moieties. Pegylated lipids for lipoplex embodiments herein include phosphatidylethanolamine (PE) based pegylated lipids such as, for example, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-MW] where MW refers to average MW of the polyethylene glycol moiety. Such dimyristoyl-PEG-PE lipids are commonly designated 14:0 PEG (MW) PE. The average MW of the polyethylene glycol moiety can be 25, 350, 550, 750, 1000, 2000, 3000, 5000, 6000, 8000 or 12000, for example. The fatty acid chains of the phosphatidylethanolamine based pegylated lipids may include, for example, a 1,2-dioleoyl group such as for 18:1 PEG (MW) PE, a 1,2-dipalmitoyl group such as for 16:0 PEG (MW) PE, or a 1,2-distearoyl-group such as for 18:0 PEG (MW) PE. Further phosphatidylethanolamine (PE) based pegylated lipids include, for example, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[MOD(polyethylene glycol)-MW], also referred to as DSPE-MOD PEG(MW) wherein MOD refers to a functional moiety such as an amine, biotin, carboxylic acid, folate, maleimide, PDP, or carboxyfluorescein moiety. The MW may be 2000 or 5000, for example. Pegylated lipids for the embodiments described herein also include ceramide based pegylated lipids such as, for example, N-octanoyl-sphingosine-1-{succinyl[methoxy(polyethylene glycol)MW]}, designated C8 PEG (MW) ceramide, where MW is 750, 2000, or 5000, for example. Alternatively, the

fatty acid moiety may have an N-palmitoyl (C16) group such as for C16 PEG (MW) ceramide.

[0043] A “liposomal composition” generally is a formulation that includes one or more liposomes. In some instances, the term “liposomal composition” may be used interchangeably with the term “transfection complex”. These formulations are typically colloids, but can be dried formulations as well. A liposome is a vesicular colloidal particle composed of self-assembled amphiphilic molecules. Surface ligands, plant virus movement proteins, or fragments thereof disclosed herein can be incorporated into liposomal compositions of one or more cationic lipids, or one or more anionic lipids either or one or more pH sensitive lipids alone or optionally in combination with one or more helper lipids (i.e., a neutral lipid, a cholesterol or cholesterol derivative, lysolipid, or cationic lipids) that are processed using standard methods to form a liposome-containing colloid suspension. Liposomal compositions disclosed herein are those containing one or more cationic lipids, one or more helper lipids, optionally, in combination with one or more neutral and/or helper lipids, exosomes, total lipid extract from exosomes, targeting moieties, fusion agents, cell penetration agents, lysomotropic agents which are treated by any of the standard methods known in the art without limitation to form liposomes. The liposomal compositions may optionally contain one or more fusion agents. The liposomal compositions may optionally contain one or more liposomal compositions can be distinguished one from another by particle size measurements. Different compositions will exhibit differences in particle size and uniformity of particle size, e.g., average particle size, and polydispersity. Different compositions will exhibit differences in the extent of the composition that is in the form of liposomes. In some non-limiting embodiments, liposomal compositions will exhibit particle size in the range 120 nm and 800 nm and will exhibit generally lower polydispersity. Lipoplex particle size (with siRNA or other cargo) may range from about 40 nm to 135 nm. In some embodiments, lipoplex particle size is 50 nm to 120 nm, 50 nm to 100 nm, 60 nm to 90 nm, 70 nm to 90 nm, or about 85 nm.

[0044] The term “Lipid aggregate” or “lipoplex” is a generic term that includes liposomes of all types, both unilamellar and multilamellar, as well as vesicles, micelles, exosomes, micro-vesicles and more amorphous aggregates. A cationic lipid aggregate is a lipid aggregate comprising a combination of one or more cationic compounds, optionally in combination with non-cationic lipids (including neutral lipids), exosomes, such that the lipid aggregate has a net positive charge. Surface ligands or plant virus movement proteins or fragments thereof disclosed herein can be incorporated into lipid aggregate, optionally

with a helper lipid and further optionally with one or more pegylated lipids and/or one or more targeting moieties, one or more fusion agents, one or more cell penetration agents and one or more lysosomotropic agents, one or more exosomes, which can then form a lipid-bioactive agent complex when contacted with a suitable bioactive agent. The terms “lipid aggregate” or “lipoplex” are generally used herein to refer to a “naked” transfection complex, i.e., a transfection complex that generally lacks a payload of bioactive agent to be delivered to a cell or to a tissue in vitro or in vivo.

[0045] The term “exosome” refers to the small membrane vesicles secreted by most cells that contain cell specific payloads of proteins, lipids and, genetic material and other biomolecules that are transported to other cells in different location of the tissue. Exosomes can be considered liposomal particles. Exosomes or lipid mixtures obtained therefrom, can be used in combination with other transfection agents or helper lipid mixtures. Exosomes are also referred to as microvesicles, epididimosomes, argosomes, exosome-like vesicles, microparticles, promininosomes, prostasomes, dexosomes, texosomes,, archeosomes and oncosomes In one example of lipid constituents of exosomes is Lyso-PC (non limiting examples of which C-18, C-16, C-14 and mixture) , Lyso-bisphosphahtidic acid (non limiting example of which is C-18, C-16 and C-14), Sphingomyelin, Ceramides (non limiting examples C-8- C-24), Disaturated PC (non limiting examples(DSPC, DPPC, DMPC and others where Cn (n= 8 – 25) Diunsaturated PC-MIX (non limiting examples of which are DOPC, DP(db)PC) phosphatidyl serine (PS), phosphatidyl inositol (PI), Disaturated PE (non limiting example, DSPE, DPPE, DMPE), Di-unsaturated PE-MIX (non limiting example DOPE DP(db)PE), posphatidyl glycerol (PG), (non limiting examples of which are C-18 – C-22, Cholesterol, Diglycerides such as cardiolipin

[0046] The term “lipid-bioactive agent” generally refers to the noncovalent association between a lipid or lipid aggregate and a bioactive agent, such as a nucleic acid, nucleotide, amino acid, peptide, a polypeptide, protein, protein nucleic complex, nutrient, exosome and the like.

[0047] As used herein “nucleic acid” and its grammatical equivalents will include the full range of polymers of single or double stranded nucleotides and includes nucleic acids (including DNA, RNA, and DNA-RNA hybrid molecules, Linked Nucleic acids (LNA), Bridged Nuclic acid (BNA)) that are isolated from a natural source; that are prepared in vitro, using techniques such as PCR amplification or chemical synthesis; that are prepared in vivo, e.g., via recombinant DNA technology; or that are prepared or

obtained by any known method. A nucleic acid typically refers to a polynucleotide molecule comprised of a linear strand of two or more nucleotides (deoxyribonucleotides and/or ribonucleotides) or variants, derivatives and/or analogs thereof. The exact size will depend on many factors, which in turn depends on the ultimate conditions of use, as is well known in the art. The nucleic acids of the present invention include without limitation primers, probes, oligonucleotides, vectors, constructs, plasmids, genes, transgenes, genomic DNA, cDNA, LNA, BNA, RNA, mRNA, tRNA, miRNA, RNAi, siRNA, shRNA, stRNA, guide-RNA, gBlock, PCR products, restriction fragments, oligonucleotides and the like.

[0048] As used herein, the term “nucleotide” includes any monomeric unit of DNA or RNA containing a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base and may also include mono-, di- and triphosphate forms of such nucleotides. The base is usually linked to the sugar moiety via the glycosidic carbon (at the 1' carbon of pentose) and that combination of base and sugar is called a “nucleoside.” The base characterizes the nucleotide with the four customary bases of DNA being adenine (A), guanine (G), cytosine (C) and thymine (T). Inosine (I) is an example of a synthetic base that can be used to substitute for any of the four, naturally occurring bases (A, C, G, or T). The four RNA bases are A, G, C, and uracil (U). Accordingly, a nucleic acid may be a nucleotide sequence comprising a linear array of nucleotides connected by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses. Other modified nucleotides are known and may be used in the practice of the invention. The term nucleotide includes ribonucleoside triphosphates ATP, UTP, ITP, CTG, GTP or derivatives such as but not limited to [α S] ATP, 7-deaza-GTP and 7-deaza-ATP, 5-methylCTP, pseudoUTP, 4-thioUTP and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [α S]dATP, 7-deaza-dGTP and 7-deaza-dATP, 5-methylCTP, pseudodUTP, 4-thiodUTP, LNA-Nucleosidetriphosphates and nucleotide derivatives that confer nuclease resistance on the nucleic acid molecule containing them. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. The term nucleotides as used here also refer to nucleotides that contain modifiable groups. Illustrated examples of nucleotides with modifiable group include, but are not limited to, allyamine-CTP, allyamine dCTP, allyamine UTP, allyamine dUTP. According to the present invention, a “nucleotide” may be unlabeled or detectably

labeled by well-known techniques. Detectable labels include, for example, biotin, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels. Various labeling methods known in the art can be employed in the practice of this invention. Transfection complexes of this invention can be used to deliver nucleotides to living cells to allow incorporation of modified nucleotides in nucleic acids.

[0049] “RNA” or “RNA molecule” refers to any RNA molecule or functional portion thereof, of any size, self-replicating, and having any sequence, from any source, including RNA viral, prokaryotic, and eukaryotic organisms. The RNA molecule may be chemically modified and in any form, including, but not limited to, linear or circular, and single or double stranded. Non-limiting examples of RNA molecules include mRNA, rRNA, tRNA, miRNA, mtRNA, tmRNA, RNAi, siRNA, shRNA, guideRNA, and stRNA. In some embodiments, siRNA molecules useful in the practice of the invention include, for example, those described in U.S. Patent Publication Nos. 2004/0014956, 2004/0054155, 2006/0009409, 2009/0023216, and 2010/0136695; and as described in International Publications WO 2003/064626, and WO 03/064625, all of which are incorporated by reference herein. Further siRNA molecules useful in the practice of the invention include, for example, those described in International Publication WO 2009/039173, which application is incorporated by reference herein.

[0050] The terms “peptide”, “polypeptide”, or “protein,” as used herein refer to a string of at least three amino acids linked together by peptide bonds. The terms “protein” and “peptide” may be used interchangeably, though it is generally understood that a “polypeptide” or “protein” is larger than a peptide. “Peptide” may refer to an individual peptide or a collection of peptides.

[0051] The terms “polynucleotide” or “oligonucleotide,” as used herein, refer to a polymer of nucleotides. Typically, a polynucleotide comprises at least three nucleotides. The polymer may include natural nucleosides (i.e., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine), nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, C5-propynylcytidine, C5-propynyluridine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-methylcytidine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine), chemically modified bases, biologically modified bases (e.g., methylated bases), intercalated bases, modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose), or modified phosphate groups (e.g., phosphorothioates and 5'-N-

phosphoramidite linkages). The term “lipid” refers to hydrophobic or amphiphilic organic compounds inclusive of fats, oils and triglycerides.

DISCLOSED ASPECTS AND EMBODIMENTS

[0052] Transfection complexes suitable for the delivery of one or more biologically active agents to a cell or a tissue in vitro or in vivo are provided for herein. The transfection complexes described herein include one or more cell surface ligands, plant virus movement proteins, or fragments thereof that enhance transfection in combination with a transfection agent as part of a transfection complex. In some embodiments, the transfection complexes disclosed herein optionally further comprise one or more components selected from the group consisting of one or more helper lipids, one or more pegylated lipids, one or more cationic lipids, one or more cationic polymers one or more targeting moieties, exosomes, total lipid isolated from exosomes, total lipid and protein isolated from exosomes, a combination thereof. In some embodiments, transfection complexes disclosed herein further comprise one or more components selected from the group consisting of peptide or non-peptide transfection enhancers, consisting of peptide or non-peptide surface ligand, cell penetration peptide or non-peptide cell penetration agent, fusogenic peptide or non-peptide fusion agents, peptide or non-peptide endosomal release agents, lysomolotropic agents, nuclear targeting agents (such as, e.g., a peptide containing one or more nuclear localization sequences), and a combination thereof.

[0053] Plant virus movement proteins are involved in moving the genome of plant viruses from cell to cell and from cell membrane to nucleus and back to the cell membrane and then into the neighboring cells using the plant host cell machinery in combination with the plant virus movement protein. All plant viruses have movement protein but not all have been studied. These virus movement proteins, or fragments of such have utility for moving DNA or RNA in eukaryotic cells from the cell cytoplasm to the nucleus or nucleus to cell membrane. The virus genera and virus families of Tobamovirus, Dianthovirus, Umbravirus, Bromovirus, Cucumovirus, Begomovirus, Potyvirus, Hordei-like virus, Potex-like virus, Begomoviruses, Geminiviridae have example of virus movement proteins that move nucleic acid to and from the cell membrane and to the nucleus if a DNA virus. Utilization of these plant proteins to enhance transfection of both DNA and RNA are disclosed.

[0054] In some embodiments, helper lipids suitable for use in the preparation and formation of transfection complexes disclosed herein include, though are not limited

to a cholesterol, a cholesterol derivative, one or more sterols, including phytosterols, zoosterols and hopanoids, or any of the neutral or cationic lipids that are known to allow or to facilitate the introduction of exogenous bioactive molecules to the interior of a cell or of a tissue. The helper lipid or helper lipids may be derived from exosomes or complete exosomes. In some embodiments, more than one helper lipid is used in the formulation of the transfection complexes described herein. In some embodiments, the transfection complexes disclosed herein comprise no helper lipid.

[0055] Illustrative though non-limiting neutral or cationic lipids suitable for use as helper lipids in accordance with some of the embodiments set forth herein include saturated and unsaturated alkyl and alicyclic ethers and esters of amines, amides or derivatives thereof. In some embodiments, straight-chain or branched alkyl and alkene groups of cationic lipids contain from 1 to about 25 carbon atoms. In certain embodiments, straight-chain or branched alkyl or alkene groups have six or more carbon atoms. In some embodiments, straight-chain or branched alkyl or alkene groups have eight to about twenty carbon atoms. In other embodiments, alicyclic groups contain from about 6 to 30 carbon atoms, or, alternatively, eight to twenty carbon atoms. In some embodiments, the alicyclic groups include cholesterol and other steroid groups. In certain embodiments, cationic lipids are prepared with a variety of counter ions (anions) including among others: a halide (i.e., Cl^- , Br^- , I^- , F^-), acetate, trifluoroacetate, sulfate, nitrite, triflate, and nitrate

[0056] Embodiments of pegylated lipids suitable for use in the preparation and formation of the transfection complexes disclosed herein are any lipid or mixture of lipids that are compatible with the formation of transfection complexes described herein, and with the administration thereof to an animal or to a human in vivo, or to tissues or cells in vitro. The pegylated lipids used with the presently described transfection complexes include, but are not limited to, a PEG polymer having a molecular weight between about 250 daltons and about 12,000, or in some embodiments, about 350 daltons and about 6,000 daltons, or, in some embodiments, between about 500 daltons and about 1,000 daltons, or, in some embodiments, between about 1,000 daltons and about 2,000 daltons, or, in some embodiments, between about 2,000 daltons and 5,000 daltons.

[0057] In some embodiments, the presently disclosed transfection complexes include one or more biologically active agents to be delivered to a cell or to a target tissue in vitro or in vivo. Suitable biologically active agents include, but are not limited to, any molecule that is capable of forming a transfection complex with the presently described transfection reagents and that elicits a biological response when delivered to the interior of

a cell or cells or to a tissue in vivo or in vitro. In some embodiments, biologically active agents contemplated for use in the presently described embodiments are cationic, neutral or anionic agents. In some embodiments, the biologically active agents suitable for formulation in the presently disclosed transfection complexes include a protein, DNA or RNA molecule, either alone or in combination with other protein, DNA or RNA molecules in various combinations, though are not limited to; nucleic acids (including but not limited to single or double stranded linear or circular DNA molecules including cDNA molecules, single or double stranded RNA molecules, mRNA, modified mRNA that has increase stability, small interfering RNA (siRNA) molecules, small hairpin RNA (shRNA) molecules, guideRNA (gRNA), Cas9 protein, Cas9 protein/guide RNA, Cas9DNA/guideRNA, Cas9mRNA/guideRNA, Cas9mRNA/gRNA/ssDNA/RecAprotein, Cas9mRNA/gRNA/ssDNA/recombination protein, Cas9protein/gRNA/ssDNA/RecAprotein, Cas9protein/gRNA/ssDNA/recombination protein, microRNA (miRNA) molecules, oligonucleotides, anti-sense oligonucleotides, sense oligonucleotides), polypeptides, antibodies, oligopeptides, therapeutic peptides or protein molecules, peptide nucleic acids (PNAs), cationic, anionic or neutral organic molecules or drugs, in addition to pharmaceutically acceptable salts thereof. In another embodiment, nutrients required for cell growth or nutrients that can be used to enhance protein expression can be delivered into cells by transfection complexes disclosed herein.

[0058] In certain non-limiting illustrative embodiments, the transfection complexes disclosed herein deliver nucleic acid molecules into cells or tissues in vitro or in vivo, including the delivery of RNA interference molecules (RNAi) or small interfering RNA molecules (siRNA, shRNA or miRNA) into cells for inhibition of gene expression.

[0059] In some embodiments, the cell surface ligands, the plant virus movement proteins, peptide fragments thereof, or the presently disclosed transfection complexes are used to deliver mRNA molecules or mixtures of mRNA and DNA molecules into a cell or a tissue in vivo or in vitro to promote the expression of a specific protein or proteins. mRNA reprogramming molecules or telomerase are non-limiting examples of mRNA molecules. Cas9 mRNA, and DNA molecules that code for gRNA, CRE mRNA and LoxP containing DNA molecules, SV40T antigen mRNA and DNA molecules with the SV40 origin of replication are non-limiting examples of mRNA and DNA pairs that have utility. Preformed transfection complexes that contain mRNA are non-limiting illustrative embodiments of transfection complexes disclosed herein. Telomerase mRNA transfection complexes or Telomerase mRNA and SV40 Large T-antigen complexes as a media

supplement are disclosed herein. In some embodiments, preformed transfection complexes containing mRNA are made and the DNA molecule is added to the complex at a later time.

[0060] In some embodiments, the cell surface ligands, plant virus movement proteins or peptide fragments thereof, or the presently disclosed transfection complexes are used to deliver DNA molecules (including cDNA molecules) into a cell or a tissue in vivo or in vitro to promote the expression of a specific protein or proteins or to synthesize specific RNA molecules, including but not limited to mRNA molecules or RNAi or miRNA or shRNA or sgRNA molecules are also provided.

[0061] In some embodiments, the cell surface ligands, plant virus movement proteins, or peptide fragments thereof presently disclosed, are used to deliver proteins or protein nucleic acid complexes into a cell or a tissue in vivo or in vitro to effect the function of the protein, as for example in gene editing. In some embodiments, the transfection complexes described herein contain, one or more surface ligands, one or more fusogenic peptides, one or more nuclear targeting peptide, one or more cationic lipid, or one or more neutral lipid. Non-limiting examples of proteins and protein nucleic acid complexes include, recombinases, CRISPR enzymes, Cre recombinase and Cre fusion proteins, transcription activator like effector nucleases (TALEN), genome editing proteins and CRISPR-Cas9 nuclease/guide RNA complex.

[0062] In some embodiments, proteins such as RNA polymerase, RNA binding proteins or peptides, and transcription factors are bound to nucleic acids and are delivered to cells with the transfection reagents disclosed herein. In certain embodiments, proteins are made anionic by the addition anionic peptides or anionic polymers designed to attach to the protein or an anionic amino acid is added to the C-terminus or N-terminus of the protein.

[0063] In some embodiments, the transfection complexes described herein may optionally include one or more fusogenic or cell-penetrating peptides. A fusogenic or cell-penetrating peptide is any peptide molecule that is capable of promoting the fusion of a lipid-containing complex to a cell membrane (either a plasma membrane or an endosomal membrane). A variety of fusogenic or cell-penetrating peptides are known in the art and it is well within the skill level of a practitioner to identify suitable fusogenic or cell-penetrating peptides and condition for the use thereof in the present invention without undue experimentation.

[0064] In some embodiments, the transfection complexes described herein optionally include one or more transfection helpers or targeting moieties in combination

with the cell surface ligands, plant virus movement proteins, or peptide fragment thereof described herein. In some embodiments, the targeting moiety is a peptide, a modified peptide, an antibody, a modified antibody, a receptor molecule, a modified receptor molecule, a single or a double stranded nucleic acid molecule, a modified single or double stranded nucleic acid molecule, a peptide or nucleic acid aptamer, a modified peptide or nucleic acid aptamer, an organic molecule, a polysaccharide, an exosome, or any other molecule that is capable of targeting a transfection complex to specific tissue or cell type for targeted delivery of a biologically agent thereto, such as will be readily apparent to those having ordinary skill level in the art. In some embodiments, modification of a peptide, an antibody, a nucleic acid, an aptamer, and the like includes conjugating the peptide, antibody, nucleic acid, aptamer, and the like to a PEG moiety. Alternatively, said modification includes conjugating the peptide, antibody, nucleic acid, aptamer, and the like to a PEG-lipid moiety. A variety of targeting moieties are widely known to those skilled in the art, and all are contemplated for use with the presently described embodiments, without limitation.

[0065] In some embodiments, the transfection complexes disclosed herein are stable for up to one year and are either contacted with the cells or tissues to be transfected, or are administered to a subject immediately or shortly after being formed. In some embodiments, the transfection complexes disclosed herein are optionally stored for a period of time prior to being contacted with the cells or tissues, or being administered to a subject. The transfection complexes are stable and may be stored for a time period of at least 30 minutes, at least 45 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 10 hours, at least 15 hours, at least 20 hours, at least 24 hours, at least 48 hours, at least 72 hours, at least 5 days, at least 7 days, at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months or at least 1 year at room temperature, or at a temperature greater than freezing, up to about room temperature. In some embodiments, the formulations described herein include one or more stabilizing agents, preservatives, buffers, and the like, that aid in the long-term stabilization and storage of bioactive formulation, such as will be readily understood by the skilled practitioner of the biological and pharmaceutical arts, and without requiring undue experimentation to achieve. It is also understood, that the storage period can be between any of the aforesaid time periods, for example between 31 minutes and 1 hour or between 1 hour and 24 hours.

[0066] In another aspect, disclosed herein are methods for the preparation of functional transfection complexes containing cell surface ligands, plant virus movement proteins, or peptides derived therefrom, the complexes being as described herein. In some embodiment the functional transfection complex may be used for the preparation of synthetic liposomes or exosomes that contain biologically active macromolecules. In some embodiments, the methods include the step of forming a liposomes or exosome composition containing one or more plant virus movement proteins or peptide fragments or from surface ligands described herein, optionally in combination with one or more helper lipids, stabilizing lipids, transfection helpers, exosomal lipids or lipid extracts, pegylated lipids, targeting moieties, fusion agents, cell penetration agent, lysosomotropic agent. In some embodiments, the methods include the step of forming a lipid-aggregate by encapsulating a biologically active agent in a composition containing one or more surface ligands, plant virus proteins, or peptide fragments thereof described herein, optionally in combination with one or more helper lipids, stabilizing lipids, transfection helpers, pegylated lipids, targeting moieties, fusion agents, lysosomotropic agent and/or exosomes. In some embodiments, the methods alternatively include: 1) mixing one or more surface ligands, plant virus movement proteins, or peptide fragments thereof, with one or more transfection compounds, which optionally include one or more helper lipids, exosomes, surface ligands, stabilizing lipids, transfection helpers, targeting moieties, fusion agents, lysosomotropic agent, optionally with one or more pegylated lipids, or a salt thereof, in an, an aqueous, alcohol/aqueous, or alcohol solution wherein the alcohol concentration is <10%, <25%, <50%, or <99%; 2) mixing one or more surface ligands, plant virus movement proteins, or peptide fragments thereof, with one or more transfection compounds, which optionally include one or more helper lipids, exosomes, stabilizing lipids, transfection helpers, targeting moieties, surface ligands, fusion agents, cell penetration agents, lysosomotropic agent, and one or more pegylated lipids, or a salt thereof, in a molar percentage such that the one or more transfection compounds are present at 1%-90%; 3) mixing one or more surface ligands, plant virus movement protein, or peptide fragment thereof, with one or more transfection compounds, which optionally include one or more helper lipid, exosomes, stabilizing lipids, transfection helpers, targeting moieties, surface ligands, fusion agents, lysosomotropic agent, one or more pegylated lipids, or a salt thereof, in a molar percentage such that the Pegylated lipids are present at <50%; and 4) mixing one or more surface ligands, plant virus movement proteins, or peptides derived therefrom, with one or more transfection compounds, which optionally

include one or more helper lipid, exosomes, stabilizing lipids, transfection helpers, targeting moieties, surface ligands fusion agents, cell penetration agents, lysosomotropic agent, one or more pegylated lipids, or a salt thereof, wherein the pegylated lipid has a polyethylene glycol molecular weight of about 2000-12000 and a fatty acid chain length of C₆-C₂₀ alkyl, or C₁₀-C₂₀ alkenyl; and complexing the lipid aggregate in an aqueous, alcohol/aqueous, or alcohol solution with the bioactive agent to form a transfection complex, wherein the alcohol concentration is <50%, preferably less than 40% if pegylated lipids are present. In some embodiments, the alcohol is ethanol. In some embodiments, the alcohol is a pharmaceutically acceptable alcohol such as an alcohol that is liquid at about room temperature, for example, ethanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol (Transcutol™), benzyl alcohol, glycerol, polyethylene glycol 200, polyethylene glycol 300, polyethylene glycol 400 or a mixture thereof. In some embodiments, the alcohol for mixing is different than the alcohol for complexing. Formulations of lipid aggregates as provided above can be provided in 0% to 100% ethanol. In some embodiments, the helper lipid is a neutral lipid. The ratio of cationic to neutral lipid can vary from 100% to 0.1% cationic lipid.

[0067] In another embodiment preformed lipid aggregates, exosomes, cationic exosomes or liposomes containing cationic, anionic, and or neutral lipids are mixed with cell surface ligands, plant virus movement proteins, or peptides derived therefrom describe herein. In certain embodiments, the lipid aggregates, exosomes or liposomes are optionally formulated with one or more transfection enhancers such as helper lipids, stabilizing lipids, transfection helpers, pegylated lipids, other targeting moieties, cell surface ligands, fusion agents, cell penetration agents, and or lysosomotropic agents. In some embodiments, the cell surface ligands, plant virus movement proteins, or peptides derived therefrom as described herein are added to these formulations at any point prior to or after the bioactive agent is loaded into the transfection complex.

[0068] In another embodiment a mixture of cationic lipid, neutral lipids and or total lipid extract of exosomes are dissolved in organic solvent such as chloroform and mixed with aqueous solutions optionally containing buffers and one or more cell surface ligands, plant virus movement proteins, or peptides derived therefrom, and optionally one or more transfection enhancers, one or more fusion agents, one or more cell penetration agents, one or more nuclear localization agents and subjected to reverses evaporation to remove the organic solvent leaving behind a lipid aggregate or liposome in solution.

[0069] In another embodiment a mixture of cationic lipid, neutral lipids and or total lipid extract of exosomes are dissolved in organic solvent such as chloroform or ethanol and mixed with aqueous solutions optionally containing buffers and one or more plant virus movement proteins or peptides derived from plant virus movement proteins, and optionally one or more transfection enhancers, one or more fusion agents, one or more nuclear localization agents, one or more cell surface ligand and subjected to reverses evaporation to remove the organic solvent leaving behind a lipid aggregate or liposome in solution.

[0070] In another embodiment, a mixture of cationic lipids and, lipids (total lipid extract of exosomes, lipids from exosomes, neutral lipids) in a water, alcohol or in a alcohol water mixture is added to an alcohol solution or alcohol water solution containing buffers and plant virus movement proteins or peptides derived from plant virus movement proteins, and optionally one or more transfection enhancers, one or more exosomes, one or more fusion agents, one or more nuclear localization agents, one or more surface ligands and this solution is optionally micro fluidization, extruded or sonicated to form lipid aggregates or liposomes.

[0071] In another embodiment, a mixture of cationic lipids and neutral lipid in a water, alcohol or in an alcohol/water mixture is added to an aqueous solution containing buffers and one or more cell surface ligands, plant virus movement proteins, or peptides derived therefrom, and optionally one or more transfection enhancers, one or more exosomes, one or more fusion agents, one or more cell penetration agents, one or more nuclear localization agents one or more surface ligands and this solution is optionally micro fluidization, extruded or sonicated to form lipid aggregates or liposomes.

[0072] In another aspect, disclosed herein are methods for screening for a tissue-based delivery of a transfection complex or cell type. In some embodiments, the method comprises the step of preparing a plurality of transfection complexes, each transfection complex having at least one cell surface ligand, plant virus movement protein, or peptides derived therefrom, in combination with at least one nucleic acid that facilitates detection of delivery to a tissue or cell type. In some embodiments, the nucleic acid is an RNA molecule or a DNA molecule that encodes a protein that can be directly detected (such as, e.g., Green Fluorescent Protein (GFP), Red Fluorescent Protein, Luciferase, or the like), or encode a protein that effects expression of a protein that can be directly detected.

[0073] In an embodiment, a method for screening for a cell based or tissue-based delivery of at least one cell surface ligand, plant virus movement protein, or peptide derived therefrom, comprises the step of preparing a plurality of unique transfection complexes, each transfection complex having at least cell surface ligand, plant virus movement protein, or peptide derived therefrom in combination with an mRNA or a cDNA that encodes the detectable protein or a specific transcription factor. Each unique transfection complex is delivered either to cells in culture, intravenously, subcutaneously, or to a tissue to a subject. After a predetermined amount of time, cells in culture or tissues from the subject are harvested and the expression of the detectable protein in various tissues is detected by gross examination, histological examination or by molecular detection (PCR, Western blotting, or the like), or imaged in vivo using the IVIS® Imaging System (Caliper), to determine to which tissue or tissues the transfection complexes containing specific transfection compounds are delivered.

[0074] In an embodiment, a method for screening cells in culture or tissue-based delivery of a transfection complex comprises the step of preparing a plurality of unique transfection complexes, each transfection complex having at least one test cell surface ligand, plant virus movement protein, or peptide derived therefrom in combination with an mRNA or a cDNA that encodes a specific transcription factor. Each unique transfection complex may be delivered to cells in culture, intravenously, subcutaneously, or to a tissue to a transgenic animal that expresses a reporter gene (such as, e.g., luciferase) under the control of the specific transcription factor. After a predetermined amount of time, tissues from the transgenic animal may be harvested and the expression of reporter gene in various tissues may be detected by gross examination, histological examination or by molecular detection (PCR, Western blotting, or the like). If the reporter gene is luciferase, detection may be accomplished in-vivo using the IVIS® Imaging System (Caliper).

[0075] In some embodiments, cell surface ligand, plant virus movement protein, or peptide derived therefrom of the presently disclosed transfection complexes are used to deliver exosomes into a cell or a tissue in vivo or in vitro to effect the function of the biological cargo in the exosomes. In some embodiments, the transfection complex described herein comprise one or more fusogenic peptides, one or more cell penetration agents, one or more nuclear targeting peptide, one or more cationic lipid, one or more plant virus movement proteins or peptides derived from plant virus movement proteins, one or more surface ligands or one or more neutral lipids. In certain embodiments, the transfection

complexes described herein comprise one or more of the cationic lipids described in Formula I.

[0076] In another aspect, disclosed herein are compositions and methods that provide improved efficiency for introducing molecules and macromolecules, such as nucleic acids, proteins, peptides, nutrients and pharmaceuticals into cells. Accordingly, provided herein are compositions comprising a nucleic acid molecule, a transfection agent and a transfection enhancer.

[0077] In some embodiments, the transfection enhancer is a surface ligand that comprises amino acid sequences derived from a cell binding adhesion proteins. Collagen, fibronectin, lamin, veronectin, cadherin, nidogen, fibrinogen, elastin, bone asialoprotein, osteopontin and tenascin-C are non-limiting examples of cell binding adhesion proteins. In some embodiments, the surface ligand is the above-listed full-length protein. In other embodiments, the surface ligand is a fragment of the above-listed protein having greater than 5 amino acids in length. In other embodiments, the length of the fragment of the above-listed protein is greater than 5 amino acids is greater than 10 amino acids, greater than 15 amino acids, greater than 20 amino acids, greater than 25 amino acids, greater than 30 amino acids, greater than 35 amino acids, or greater than 40 amino acids.

[0078] In some embodiments, cell surface ligand, plant virus movement protein, or peptide derived therefrom proteins and describe herein comprise a nucleic acid binding moiety functionally linked to the amino acid sequence of the cell surface ligand, plant virus movement protein, or peptide derived therefrom proteins. Suitable nucleic acid binding moieties include, but are not limited to, a polycationic peptide sequence, a polyamine, a peptide nucleic acid, spermine, spermidine, carboxyspermidine, carboxy spermine, spermine and spermidine analogs, nucleic acid intercalaters, and the like. In certain embodiments, the nucleic acid binding moiety is covalently linked to the transfection promoting cell surface ligand comprising adhesion protein amino acid sequences. In further embodiments, the transfection agent is a cationic lipid, such as those described below, a polyamine, a polycationic peptide sequence, a cationic dendrimer, or the like. In some embodiments, the cell surface ligand adhesion sequence or peptide derived from a plant movement protein is a multimer of itself or other adhesion sequences. In certain embodiments, the cell binding adhesion or peptide derived from a plant movement protein amino sequence is cyclized. In other embodiments, the surface ligands or peptide derived from a plant movement protein also contain other peptide sequence that enhance transfection efficiency, such as linkers, spacers, or nuclear targeting sequences.

[0079] In some embodiments, cell surface ligand, plant virus movement protein, or peptide derived therefrom described herein are attached directly to the binding molecule by covalent bonding, or are connected to the binding molecule via a spacer. The term “spacer,” or “linker,” which are used interchangeably herein, as used herein refers to a chemical structure that links two molecules to each other. In some embodiments, the spacer binds each molecule on a different part of the spacer molecule. In other embodiments, the spacer is a hydrophilic moiety and comprises about 6 to 30 carbon atoms. In other embodiments, the spacer comprises a polyether, for example $-\text{CH}_2-\text{O}-(\text{CH}_2-\text{CH}_2-\text{O})_i-\text{CH}_2-$. In other embodiments, the spacer comprises a hydrophilic polymer, for example $[(\text{gly})_i(\text{ser})_j]_k$ (SEQ ID NO: 585). In these formulae i ranges from 1 to 6, j ranges from 1 to 6, and k ranges from 3 to 20. In some embodiments, the spacer is a peptide of sequence APYKAWK (SEQ ID NO:505). In other embodiments, the spacer is a sequence that is degraded in vivo by a peptidase.

[0080] In some embodiments, the cell surface ligand, plant virus movement protein, or peptide derived therefrom described herein are functionally linked to a lipid, such as a cationic or neutral lipid. In some of these embodiments, the linked moiety is used for delivery of macromolecules into cells. For example, a cell surface ligand, plant virus movement protein, or peptide derived therefrom, or a cell binding adhesion peptide sequences amino acid sequence is covalently linked to a lipid, such as a cationic lipid, a lysolipid, using methods known in the art.

[0081] In certain embodiments, the cell surface ligand, plant virus movement protein, or peptide derived therefrom sequences described herein also are functionally linked to an amino acid sequence that inserts itself into lipid membranes, such as membrane anchor peptides or proteins. In other embodiments, the cell surface ligand peptide sequences are linked to chemical compositions that associate with lipids.

[0082] In other embodiments, the transfection complexes or liposomal compositions with the cell surface ligand, plant virus movement protein, or peptide derived therefrom described herein also comprise other transfection enhancing agents, such as a nuclear localization protein or peptide, a fusogenic peptide or protein, a transport peptide or protein, a viral peptide or protein, or a lysomotropic agent. In certain embodiments, the viral peptide is derived from a virus enveloped or non-enveloped virus, for example an influenza virus, a vesicular stomatitis virus, an adenovirus, an alphavirus, a Semliki Forest Virus, a hepatitis virus, a herpes virus, an HIV virus, or a simian virus. In some embodiments, the transfection enhancing agent is, for example, insulin, a transferrin, a

epidermal growth factor, a fibroblast growth factor, a cell targeting antibody or fragment from an antibody, a lactoferrin, a fibronectin, an adenovirus penton base, Knob, a hexon protein, a vesicular stomatitis virus glycoprotein, a Semliki Forest Virus core protein, an influenza hemagglutinin, a hepatitis B core protein, an HIV Tat protein, a herpes simplex virus VP22 protein, a histone protein, an arginine rich cell permeability protein, a high mobility group protein, and invasin protein, an internalin protein, an endotoxin, a diphtheria toxin, a shigella toxin, a melittin, a magainin, a gramicidin, a cecrophin, a defensin, a protegrin, a tachyplesin, a thionin, a indolicidin, a bactenecin, a drosomycin, an apidaecin, a cathelicidin, a bacteriacidal-permeability-increasing protein, a nisin, a buforin, or fragments thereof. In other embodiments, the transfection enhancing agent is chloroquine, a lysosomotropic compound or combinations thereof. In other embodiments exosomes or exosomal derived lipids or proteins are the transfection enhanceagent. In other embodiments, the transfection enhancer agent comprises multimers of the same or different peptides or proteins.

[0083] Suitable nuclear localization peptides or proteins included in transfection complexes or liposomal compositions include, but are not limited to, a sequence selected from the group consisting of SEQ ID NOs:1-41, as set forth in Table 1, below, or in the sequence listings.

[0084] Proteins such as histones, protamines, HMG proteins, and viral core proteins or coat proteins comprise nuclear localization proteins. In some embodiments, these proteins or fragments thereof are used to enhance transfection. In some embodiments, the nuclear localization peptide is optionally linked to a nucleic acid binding moiety, for example via a covalent linkage. Spacer sequences are optionally used between the DNA binding sequence and the nuclear localization sequence. In some embodiments, the nuclear localization sequences are linked to helper lipids or other peptides proteins or compounds that associate with lipid bilayers.

[0085] In some embodiments, the compositions described herein also comprise a fusion agent or combinations of fusion agents, which in some embodiments also function as an amphipathic peptide. Suitable fusion peptides include, but are not limited to, a sequence selected from the group consisting of SEQ ID NOs:42-92, as set forth in Table 1, below, or in the sequence listings.

[0086] In some embodiments, the fusion agent is optionally linked to a nucleic acid binding moiety, for example via a covalent linkage. The peptides KK, KKK, KKKK (SEQ ID NO:97), RR, RRR, RRRR (SEQ ID NO:105) can be linked to fusion agents of

SEQ ID NOs:42-92. In certain embodiments, fusion peptides are linked to helper lipids, cationic lipids, or other peptides or proteins that associate with lipid bilayers. Spacer sequences are optionally used between the DNA binding sequence and the fusion agent sequence

[0087] In certain embodiments, the compositions disclosed herein comprise a cell penetration agent or combinations of cell penetration agents. Suitable cell penetration agents include, but are not limited to, a sequence selected from the group consisting of SEQ ID NOs:93-96 as set forth in Table 1, below, or in the sequence listings.

[0088] In some embodiments, the cell penetration agents are optionally linked to a nucleic acid binding moiety, for example via a covalent linkage. In other embodiments, the cell penetration agents are linked to helper lipids or other peptides or proteins that associate with lipid bilayers.

[0089] In some embodiments, the nuclear localization sequences, the fusion agents, cell surface ligand or the cell penetration agents are linked to the GPI anchor peptides, the sequence FTLTGLLGTLVTMGLLT (SEQ ID NO:504) being a non limiting example.

[0090] In some embodiments, the nucleic acid binding moieties that are linked to different transfection enhancer and are part of transfection complexes have different binding affinity for nucleic acids depending on the needed functionality for attachment, condensation of nucleic acid, and the rate of release of nucleic acid from the nucleic acid binding moiety. Suitable nucleic acid binding moieties include, but are not limited to a polycationic peptide sequence, a polyamine, a peptide nucleic acid, spermine, spermidine, carboxyspermidine, carboxy spermine, spermine and spermidine analogs, nucleic acid intercalators, and the like.

[0091] In some embodiments, the compositions described herein comprise combinations of different transfection enhancers with different nucleic acid binding moieties. Suitable nucleic acid binding peptides include, but are not limited to a sequence of SEQ ID NOs:97-149, as set forth in Table 1, below, or in the sequence listings.

[0092] In some embodiments, the nucleic acid binding moieties also serve as transfection enhancers when bound to nucleic acids, or alternatively serve as condensing agents. Suitable nucleic acid condensing peptides include, but are not limited to, a sequence selected from the group consisting of the peptides of SEQ ID NOs:97-149 as set forth in Table 1, below, or in the sequence listings. In some embodiments, multimers of these peptides are also synthesized and used as condensing agents. In some embodiments,

nuclear localization sequences are also used as condensing agents if they have enough cationic charge.

[0093] Suitable nucleic acid binding moieties include, but are not limited to a polycationic peptide sequence, a polyamine, a peptide nucleic acid, spermine, spermidine, carboxyspermidine, carboxy spermine, spermine and spermidine analogs, nucleic acid intercalaters, and the like

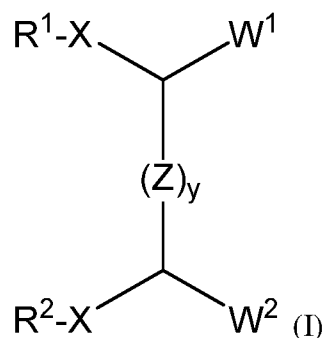
[0094] One skilled in the art will readily recognize that the surface ligand chosen depends on which receptor is being bound. Since different types of cells have different receptors, this provides a method of targeting nucleic acid, peptides, protein, and compounds to specific cell types, depending on which cell surface ligand is used. Thus, the preferred cell surface ligand or ligands may depend on the targeted cell type.

[0095] In some embodiments, the transfection enhancers that are used in combination with the cell surface ligand, plant virus movement protein, or peptide derived therefrom disclose herein include, but are not limited to, the peptides or proteins selected from the group consisting of a collagen, a fibronectin, a lamin, a veronectin, a cadherin, a nidogen, a fibrinogen, a elastin, a bone asialoprotein, a osteopontin, a tenascin-C, Avadin, insulin, a transferrin, a epidermal growth factor, a fibroblast growth factor, a cell targeting antibody, a lactoferrin, an enveloped virus, a non-enveloped virus, an adenovirus penton base, a knob protein, a hexon protein, a vesicular stomatitis virus glycoprotein, a Semliki Forest Virus core protein, an influenza hemagglutinin, a hepatitis B core protein, an HIV Tat protein, a herpes simplex virus VP22 protein, a histone protein, an arginine rich cell permeability protein, a high mobility group protein, invasin protein, internalin protein, an endotoxin, a non-toxic diptheria toxin, a non-toxic shigella toxin, a melittin, a magainin, a gramicidin, a cecrophin, a defensin, a protegrin, a tachyplesin, a thionin, a indolicidin, a bactenecin, a drosomycin, an apidaecin, a cathelicidin, a bacteriacidal-permability-increasing protein, a nisin, a buforin, a fragment thereof, and a sequence selected from the group consisting of SEQ ID NOs:150-503, as set forth in Table 1, below, or in the sequence listings.

[0096] In some embodiments, the transfection enhancing agent is chloroquine, a lysosomotropic compound or combinations thereof. In certain embodiments, the transfection enhancer agent comprises multimers of the same or different peptide enhancers, protein or protein fragments of transfection enhancers.

[0097] In some embodiments, the aforementioned peptides are optionally linked to a moiety selected from the group consisting of a nucleic acid binding moiety, a helper lipid, a cationic lipid, a cationic polymer, and a GPI anchor peptide.

[0098] In certain embodiments, the aforementioned peptides are optionally linked to a chemical moiety of Formula I,



or a pharmaceutically acceptable salt thereof, where

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-OH}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(OH)-CH}_2\text{-(CH}_2\text{)}_n\text{-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH-iso-butyl)-CH}_2\text{-(CH}_2\text{)}_n\text{-O-iso-butyl}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-C(=NH)-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-His}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-spermine}$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{H}$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2\text{(CH}_2\text{)}_m\text{-NH}_2$; $m = 1\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2\text{(CH}_2\text{)}_m\text{-NH-C(=O)-spermine}$; $m = 1\text{--}6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-(C=O)$ -amino acid side chain; $m = 1-6$, $y = 0$; or
 $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $y = 0$; or
 $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $y = 0$; or
 $W^1 = H$, $W^2 = CH_2-N(R^3R^4)CH_2-R^5$; $R^3 = R^4 = CH_3$; $R^5 = H$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -amino acid side chain, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-N^+(CH_3)_3Cl$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(O^-)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-}iso\text{-}butyl)-CH_2-(CH_2)_n-O\text{-}iso\text{-}butyl$, $n = 0-6$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-His$, $n = 0-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)$ -spermine, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m = 1-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-(C=O)$ -amino acid side chain; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$;
 $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$;
 $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_p-N(R^3)-(CH_2)_p$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$;
 $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n=0-6$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)\text{-spermine}$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-(C=O)\text{-amino acid side chain}$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=(CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=0$ or 1 ; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $y=0$ where B is selected from the group –
 $O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n=0-6$, $y=0$ where B is selected from the group –
 $O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $y = 0$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-(C=O)\text{-amino acid side chain}$; $m = 1-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = H$ $W^2 = -CH_2-B-CH_2-N(R^3R^4)CH_2-R^5$; $R^3 = R^4 = CH_3$; $R^5 = H$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$; or
 $W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -amino acid side chain,
 $y = 0$; or
 $W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-N^+(CH_3)_3Cl$, $y = 0$; or
 $W^1=H$; $W^2 = CH_2-O-P(=O)(O^-)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6, y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)$ -spermine, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or
 $Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)$ -amino acid side chain; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_p-N(R^3)-(CH_2)_p$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$

where B is selected from the group $-\text{O}(\text{CH}_2)_i$, $-\text{S}(\text{CH}_2)_i$, $-\text{S}-\text{S}(\text{CH}_2)_i$, $-\text{SO}_2-(\text{CH}_2)_i$, $i=1-5$;
or

Z=(CH₂)_q-N(R³)-(CH₂)_q, W¹ = W² = -CH₂-B-CH₂-N(R³R⁴), R¹ = R² = C₈-C₂₂ alkyl, X = O; R³ = CH₃, R⁴ = -C(=O)-spermine, q=1-3, y=1 where B is selected from the group -O(CH₂)_i, -S(CH₂)_i, -S-S(CH₂)_i, -SO₂-(CH₂)_i, i=1-5; or

$Z = (CH_2)_q - N(R^3) - (CH_2)_q$, $W^1 = W^2 = -CH_2 - B - CH_2 - N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q = 1-3$, $y = 1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i = 1-5$; or

$$\text{Z}=(\text{CH}_2)_q\text{-N}(\text{R}^3)\text{-(CH}_2)_q, \text{W}^1=\text{W}^2=\text{-CH}_2\text{-B-CH}_2\text{-N}(\text{R}^3\text{R}^4), \text{R}^1=\text{R}^2=\text{C}_8\text{-C}_{22}$$

alkyl, X = O; R³ = CH₃; R⁴ = CH₂(CH₂)_m-NH₂; m = 1-6, q=1-3, y=1 where B is selected from the group -O(CH₂)_i, -S(CH₂)_i, -S-S(CH₂)_i, -SO₂-(CH₂)_i, i=1-5; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$;
or

$$Z=(CH_2)_q-N(R^3)-(CH_2)_q, W^1=W^2=-CH_2-B-CH_2-N(R^3R^4), R^1=R^2=C_8-C_{22}$$

alkyl, X = O; R³ = CH₃; R⁴ = CH₂(CH₂)_m-NH-(C=O)-amino acid side chain; m = 1-6,
q=1-3, y=1 where B is selected from the group -O(CH₂)_i, -S(CH₂)_i, -S-S(CH₂)_i, -SO₂-
(CH₂)_i, i=1-5; or

$Z = (CH_2)_q - N(R^3) - (CH_2)_q$, $W^1 = W^2 = -CH_2 - B - CH_2 - N(R^3 R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$;

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (\text{CH}_2)_q\text{-S-S-}(\text{CH}_2)_q$, $W^1 = W^2 = \text{-CH}_2\text{-B-CH}_2\text{-N(R}^3\text{R}^4\text{)}$, $R^1 = R^2 = \text{C}_8\text{-C}_{22}$ alkyl, $X = \text{O}$; $R^3 = \text{CH}_3$, $R^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-OH}$, $n = 0\text{-}6$, $q = 1\text{-}3$, $y = 1$ where B is selected from the group $\text{-O(CH}_2\text{)}_i$, $\text{-S(CH}_2\text{)}_i$, $\text{-S-S(CH}_2\text{)}_i$, $\text{-SO}_2\text{-(CH}_2\text{)}_i$, $i = 1\text{-}5$; or

$$\text{Z}=(\text{CH}_2)_q\text{-S-S-}(\text{CH}_2)_q, \text{W1}=\text{W}^2=\text{-CH}_2\text{-B-CH}_2\text{-N(R}^3\text{R}^4\text{)}, \text{R}^1=\text{R}^2=\text{C}_8\text{-C}_{22}$$

alkyl, X = O; R³ = CH₃, R⁴ = -C(=O)-C(OH)-CH₂-(CH₂)_n-NH₂, n = 0-6, q=1-3, y=1

where B is selected from the group $-\text{O}(\text{CH}_2)_i$, $-\text{S}(\text{CH}_2)_i$, $-\text{S}-\text{S}(\text{CH}_2)_i$, $-\text{SO}_2-(\text{CH}_2)_i$, $i=1-5$;
or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH\text{-}iso\text{-}butyl)-CH_2-(CH_2)_n-O\text{-}iso\text{-}butyl$, $n=0-6, y=1$ where B is selected from the group $-O(CH_2)_i, -S(CH_2)_i, -S-S(CH_2)_i, -SO_2-(CH_2)_i$, $i=1-5$; or

$$\begin{aligned} &Z=(CH_2)_q-S-S-(CH_2)_q, W^1=W^2=-CH_2-B-CH_2-N(R^3R^4), R^1=R^2=C_8-C_{22} \\ &\text{alkyl, } X=O; R^3=CH_3, R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2, n=0-6, q=1-3, \\ &y=1 \text{ where } B \text{ is selected from the group } -O(CH_2)_i, -S(CH_2)_i, -S-S(CH_2)_i, -SO_2-(CH_2)_i, \\ &i=1-5; \text{ or} \end{aligned}$$

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-His$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)$ -spermine, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q = 1-3$, $y = 1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i = 1-5$; or

$Z = (CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m = 1-6$, $q = 1-3$, $y = 1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i = 1-5$; or

$Z = (\text{CH}_2)_q - \text{S} - \text{S} - (\text{CH}_2)_q$, $W^1 = W^2 = -\text{CH}_2 - \text{B} - \text{CH}_2 - \text{N}(\text{R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8 - \text{C}_{22}$ alkyl,
 $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2(\text{CH}_2)_m - \text{NH} - (\text{C}=\text{O}) - \text{amino acid side chain}$; $m = 1-6$, $q = 1-3$,
 $y = 1$ where B is selected from the group $-\text{O}(\text{CH}_2)_i$, $-\text{S}(\text{CH}_2)_i$, $-\text{S}-\text{S}(\text{CH}_2)_i$, $-\text{SO}_2-(\text{CH}_2)_i$,
 $i = 1-5$; or

$Z = (CH_2)_q - S - S - (CH_2)_q$, $W^1 = W^2 = -CH_2 - B - CH_2 - N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8-C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-(C=O)$ -amino acid side chain; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$.

[0099] In some embodiments, the compound of Formula I is a compound where:

$R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$, $W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$;

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$;

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)$ -spermine, $y = 0$;

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $y = 0$.

[00100] In other embodiments, the compound of Formula I is a compound where:

$R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$, $W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$.

[00101] In other embodiments, the compound of Formula I is a compound where:

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 -C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $y = 0$.

[00102] In other embodiments, the compound of Formula I is a compound where:

$R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$, $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)$ -spermine, $y = 0$;

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)$ -spermine, $y = 0$.

[00103] In some embodiments, the transfection agent comprises at least one or more cationic lipid from Formula I and may optionally also contain one or more neutral lipids; DOPE, DPhPE, saturated and unsaturated DPPE, saturated and unsaturated DMPE, cholesterol, DOPC, Lyso-PE (1-acyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine), Lyso-PC (1-acyl-3-hydroxy-*sn*-glycero-3-phosphocholine), 3-alkyloxy-2-hydroxy-1-acetamidopropane, 4-alkyloxy-3-hydroxy-1-acetamidopropane, 5-alkyloxy-4-hydroxy-1-acetamidopropane or 6-alkyloxy-5-hydroxy-1-acetamidopropane. In some embodiments, the alkyloxy in the above list is selected from the group consisting of myristyloxy, myristeyleyloxy lauryloxy, palmityloxy, palmitoleyloxy, oleyloxy and streayloxy. In some embodiments transfection agents contains Lyso-phosphatidylcholine, Sphingomyelin, Disaturated phosphatidylcholine, saturated and unsaturated Phosphatidylcholine, Disaturated phosphatidylethanolamine, Phosphatidylethanolamine saturated and unsaturated, Phosphatidylserine, phosphatidylinositol, Lyso-bis phosphatidic acid, Cholesterol, and Diglyceride. Helper Lipids may include, complete exosomes solutions, total extract of lipids isolated from exosomes.

[00104] In other embodiment, the transfection agent comprises at least one or more cationic lipid of Formula I. In certain embodiments, the transfection agent optionally comprises one or more of cationic lipid, while in other embodiments, the agent optionally comprises one or more neutral lipids or one or more exosomes, or lipid extract for exosomes. In certain embodiments, the transfection agent comprises both one or more cationic lipid and one or more neutral lipid.

[00105] In some embodiments, the cationic lipid is selected from the group consisting of GeneInTM (MTI-GlobalStem), TransfeXTM (ATCC), LipofectAmineTM 2000, LipofectAmine 3000, LipofectAmineTM, Lipofectin®, DMRIE-C, CellFectin® (Invitrogen), Oligofectamine® (Invitrogen), LipofectAce® (Invitrogen), Fugene® (Promega), Fugene® HD (Promega), Transfectam® (Promega), Tfx-10® (Promega), Tfx-20® (Promega), Tfx-50® (Promega), DNA-In (MTI-GlobalStem), TransfectinTM (BioRad, Hercules, CA), SilentFectTM (Bio-Rad), Effectene® (Qiagen, Valencia, CA), DC-chol

(Avanti Polar Lipids), GenePorter® (Gene Therapy Systems, San Diego, CA), DharmaFect 1® (Dharmacon, Lafayette, CO), DharmaFect 2® (Dharmacon), DharmaFect 3® (Dharmacon), DharmaFect 4® (Dharmacon), Escort™ III (Sigma, St. Louis, MO), Escort™ IV (Sigma), ViaFect™ (Promega), DOTMA, DOTAP, DMRIE, DC-Chol, DDAB, DOSPA, DOSPER, DOGS, TMTPS, TMTOS, TMTLS, TMTMS, TMDOS, N-1-dimethyl-N-1-(2,3-diaoleoyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diaoleoyloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, L-spermine-5-carboxyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine), 3,5-(N,N-di-lysyl)-diaminobenzoyl-glycyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine), L-Lysine-bis(O,O'-oleoyl-β-hydroxyethyl)-amide dihydrochloride, L-Lysine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-alkylamino)-2-hydroxypropyl]piperazine, L-Lysine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxypropyl]piperazine, L-Ornithine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-butane-2,3-diol, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-butane-2,3-diol, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-butane-2,3-diol, 1,4-bis[(3-oleylamino)propyl]piperazine, L-Arginine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxypropyl]piperazine, L-Arginine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Serine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]piperazine, Glycine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, Sarcosine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Histidine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, cholesteryl-3β-carboxyl-amidoethylenetriethylammonium iodide, 1,4-bis[(3-myristylamino)propyl]piperazine, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl carboxylate iodide, cholesteryl-3β-carboxyamidoethyleneamine, cholesteryl-3β-oxysuccinamidoethylenetriethylammonium iodide, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-

cholesteryl-3 β -oxysuccinate iodide, 2-[(2-trimethylammonio)-ethylmethylamino] ethyl-cholesteryl-3 β -oxysuccinate iodide, 3 β [N-(N',N'-dimethylaminoethane)carbamoyl]-cholesterol, and 3 β -[N-(polyethyleneimine)-carbamoyl] cholesterol, 1,4-bis[(3-palmitylamino)propyl]piperazine, L-Ornithylglycyl-N-(1-heptadecyloctadecyl)glycinamide, N²,N⁵-Bis(3-aminopropyl)-L-ornithylglycyl-N-(1-heptadecyloctadecyl)glycinamide, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-alkylamino)-2-hydroxypropyl]piperazine N²-[N²,N⁵-Bis(3-aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L-glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L- α -glutamine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleylamino)-2-hydroxypropyl]piperazine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L- α -asparagine, N-[N²-[N²,N⁵-Bis[(1,1-dimethylethoxy)-carbonyl]-N²,N⁵-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl]-N,N-dioctadecyl-L-glutaminyl]-L-glutamic acid, N²-[N²,N⁵-Bis(3-aminopropyl)-L-ornithyl]-N,N-diethyl-L-glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dioleil-L- α -glutamine, 4-bis[(3-(3-amino-2-hydroxypropyl)-myristylamino)-2-hydroxypropyl]piperazine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dioleil-L- α -asparagine, N-[N²-[N²,N⁵-Bis[(1,1-dimethylethoxy)carbonyl]-N²,N⁵-bis[3-[(1,1-dimethylethoxy)carbonyl]-aminopropyl]-L-ornithyl]-N,N-dioleil-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-oleylamino)propyl]piperazine, N²-[N²,N⁵-Bis(3-aminopropyl)-L-ornithyl]-N,N-dipalmityl-L-glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dipalmityl-L- α -glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dipalmityl-L- α -asparagine, N-[N²-[N²,N⁵-Bis[(1,1-dimethylethoxy)carbonyl]-N²,N⁵-bis[3-[(1,1-dimethylethoxy)-carbonyl]aminopropyl]-L-ornithyl]-N,N-dipalmityl-L-glutaminyl]-L-glutamic acid, N²-[N²,N⁵-Bis(3-aminopropyl)-L-ornithyl]-N,N-dimyristyl-L-glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dimyristyl-L- α -glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dimyristyl-L- α -asparagine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmitylamino)-2-hydroxypropyl]piperazine, N-[N²-[N²,N⁵-Bis[(1,1-dimethylethoxy)-carbonyl]-N²,N⁵-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl]-N,N-dimyristyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-propyl]piperazine, N²-[N²,N⁵-Bis(3-aminopropyl)-L-ornithyl]-N,N-dilaureyl-L-glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dilaureyl-L- α -glutamine, N²-[N²,N⁵-Bis(aminopropyl)-L-ornithyl]-N,N-dilaureyl-L- α -asparagine, N-[N²-[N²,N⁵-Bis[(1,1-dimethylethoxy)carbonyl]-N²,N⁵-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl]-N,N-dilaureyl-L-glutaminyl]-L-glutamic acid, 3-[N',N''-bis(2-tert-butylloxycarbonylaminoethyl)guanidino]-N,N-dioctadec-9-enylpropionamide, 3-[N',N''-

bis(2-tertbutyloxycarbonylaminoethyl)guanidino]-N,N-dipalmitylpropionamide, 3-
 [N',N''-bis(2-tertbutyloxycarbonylaminoethyl)guanidino]-N,N-dimyristylpropionamide,
 1,4-bis[(3-(3-aminopropyl)-palmitylamino)propyl]piperazine, 1,4-bis[(3-(3-amino-2-
 hydroxypropyl)-oleylamino)propyl]piperazine, N,N-(2-hydroxy-3-aminopropyl)-N-2-
 hydroxypropyl-3-N,N-diolyaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-
 hydroxypropyl-3-N,N-dipalmitylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-
 hydroxypropyl-3-N,N-dimyristylaminopropane, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-
 myristylamino)propyl]piperazine, [(3-aminopropyl)-bis-(2-tetradecyloxyethyl)]methyl
 ammonium bromide, [(3-aminopropyl)-bis-(2-oleyloxyethyl)]methyl ammonium
 bromide, [(3-aminopropyl)-bis-(2-palmitoxyethyl)]methyl ammonium bromide,
 Oleoyl-2-hydroxy-3-N,N-dimethylamino propane, 2-didecanoyl-1-N,N-dimethylamino-
 propane, palmitoyl-2-hydroxy-3-N,N-dimethylamino propane, 1,2-dipalmitoyl-1-N,N-
 dimethylaminopropane, myristoyl-2-hydroxy-3-N,N-dimethylamino propane, 1,2-
 dimyristoyl-1-N,N-dimethylaminopropane, (3-Amino-propyl)->4-(3-amino-propyl-
 amino)-4-tetradecylcarbamoyle-butylcarbamic acid cholesteryl ester, (3-Amino-propyl)->4-
 (3-amino-propylamino)-4-carbamoylbutylcarbamic acid cholesteryl ester, (3-Amino-
 propyl)->4-(3-amino-propylamino)-4-(2-dimethylamino-ethylcarbamoyle)-butylcarbamic
 acid cholesteryl ester, Spermine-5-carboxyglycine (N'-stearyl-N'-oleyl) amide
 tetratrifluoroacetic acid salt, Spermine-5-carboxyglycine (N'-stearyl-N'-elaidyl) amide
 tetratrifluoroacetic acid salt, Agmatinyl carboxycholesterol acetic acid salt, Spermine-5-
 carboxy-β-alanine cholesteryl ester tetratrifluoroacetic acid salt, 2,6-Diaminohexanoyle β-
 alanine cholesteryl ester bistrifluoroacetic acid salt, 2,4-Diaminobutyroyle β-alanine
 cholesteryl ester bistrifluoroacetic acid salt, N,N-Bis (3-aminopropyl)-3-aminopropionyle
 β-alanine cholesteryl ester tristrifluoroacetic acid salt., [N,N-Bis(2-hydroxyethyl)-2-
 aminoethyl]aminocarboxy cholesteryl ester, Stearyl carnitine ester, Palmityl carnitine
 ester, Myristyl carnitine ester, Stearyl stearoyl carnitine ester chloride salt, L-Stearyl
 Stearoyl Carnitine Ester, Stearyl oleoyl carnitine ester chloride, Palmityl palmitoyl
 carnitine ester chloride, Myristyl myristoyl carnitine ester chloride, L-Myristyl myristoyl
 carnitine ester chloride, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmitylamino)-
 propyl]piperazine, N-(3-aminopropyl)-N,N'-bis-(dodecyloxyethyl)-piperazinium
 bromide, N-(3-aminopropyl)-N,N'-bis-(oleyloxyethyl)-piperazinium bromide, N-(3-
 aminopropyl)-N,N'-bis-(palmitoxyethyl)-piperazinium bromide, N-(3-aminopropyl)-
 N,N'-bis-(myristyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-
 (bis-2-dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-

2-oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-palmitoyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-myristoyloxyethyl)-piperazinium bromide, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxy-propyl]piperazine, or 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxy-propyl]piperazine, 3-alkyloxy-2-hydroxy-1-histidylamidopropane, 3-alkyloxy-2-hydroxy-1-aminopropane, 4-alkyloxy-3-hydroxy-1-histidylamidopropane, 4-alkyloxy-3-hydroxy-1-aminopropane, 5-alkyloxy-4-hydroxy-1-histidylamidopropane, 5-alkyloxy-4-hydroxy-1-aminopropane, 6-alkyloxy-5-hydroxy-1-histidylamidopropane, 6-alkyloxy-4-hydroxy-1-aminopropane; 2,3-dialkoxy-1,4-bis(N-methyl-N-carboxyspermineamido)-aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-histidinylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-arginylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-lysinylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N'-ornithinylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-serinylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-homoerinylamido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-(diaminobutanyl)amido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-(diaminopropyl)amido)aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-(2-hydroxypropylamine))aminobutane, 2,3-dialkoxy-1,4-bis(N-methyl-N-(2-diaminopropyl))aminobutane, 2,3-dialkoxy-1,4- and bis(N-methyl-N-propylamine)aminobutane. The alkoxy in the above list may be myristyloxy, myristeylexy lauryloxy, palmityloxy, palmitoleyloxy, oleyloxy and streaylox.

[00106] In some embodiments transfection agents contains lyso-phosphatidylcholine, sphingomyelin, disaturated phosphatidylcholine, saturated and unsaturated phosphatidylcholine, disaturated phosphatidylethanolamine, phosphatidylethanolamine saturated and unsaturated, phosphatidylserine, phosphatidylinositol, lyso-bis phosphatidic acid, cholesterol, and diglyceride. Helper Lipids may include, complete exosomes solutions, total extract of lipids isolated from exosomes. The Helper lipids can be formulated into exosome like lipid particles so that lyso- phosphatidylcholine is from 3.75 to 6.21% of total lipid content , a sphingomyelin, a sphingosin, or ceramide is from 8.26 to 12, 41% of total, phosphatidylcholine-disaturated is from 3.00 to 4.81 % of total, Phosphatidylethanolamine-mix is from 12.00 to 19.22 %, phosphatidylserine is from 5.17 to 6.89%, phosphatidylinositol is from 5.17 to 6.89%, Phosphatidylethanolamine disaturated is from 2.61 to 2.85%, Phosphatidylethanolamine mix is from 13.42 to 14.65 %, cholesterol is from 13.01 to 16.61%, diglycerides are from

4.76 to 7.05% and cationic lipids can be the remaining % in such formulations. Cationic lipids can be substituted or used in combination with a sphingomyelin, a sphingosin, or ceramides. Depending on cell type the % composition of helper lipids and can be adjusted to optimize formulations of synthetic exosomes with various transfection enhancers.

[00107] When the composition contains a neutral lipid, that lipid a saturated or unsaturated, or mixed acyl phosphatidyl ethanol mine (PE) or phospahtidyl choline (PC), for example, DOPE, DPhPE, saturated and unsaturated DPPE, saturated and unsaturated DMPE, cholesterol, DOPC, Lyso-PE (1-acyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine), Lyso-PC (1-acyl-3-hydroxy-*sn*-glycero-3-phosphocholine), 3-alkyloxy-2-hydroxy-1-acetamidopropane, 4-alkyloxy-3-hydroxy-1-acetamidopropane, 5-alkyloxy-4-hydroxy-1-acetamidopropane or 6-alkyloxy-5-hydroxy-1-acetamidopropane. The alkyloxy in the above list may be myristyloxy, myristeyleyloxy lauryloxy, palmityloxy, palmitoleyloxy, oleyloxy or streayloxy. In some embodiments, the tranfection agent. may contain more than one of these neutral lipids or exosomes, lipids from exosomes or total lipid extracts from exosomes compositions.

[00108] In other embodiments the transfection agent comprises at least one polyamine moiety. Suitable polyamines include dense star dendrimers, PAMAM dendrimers, NH₃ core dendrimers, ethylenediamine core dendrimers, dendrimers of generation 5 or higher, dendrimers with substituted groups, dendrimers having one or more amino acids, grafted dendrimers, activated dendrimers, polyethylenimine, and polyethylenimine conjugates, polycationic peptides such as polylysine, polyorinthine, polyhistidine, polyarginine

[00109] In other embodiments, cell surface ligand containing adhesion peptide sequences is conjugated to a nucleic acid binding group. In some of these embodiments, the nucleic acid binding group is linked to a polyamine or peptide nucleic acid. The polyamine optionally comprises at least one spermine moiety.

[00110] Suitable cell surface ligands containing adhesion peptide sequences that are derived from cell adhesion proteins include, but are not limited to, a sequence selected from the group consisting of SEQ ID NOs:202-503, as set forth in Table 1, below, or in the sequence listings.

[00111] In some embodiments, the peptides of SEQ ID NOs:202-503 are optionally linked to a nucleic acid binding moiety, a helper lipid, a cationic lipid, a cationic polymer, a GPI anchor peptide or other chemical moieties that associate with transfection complexes.

[00112] In some embodiments, the peptides of SEQ ID NOs:202-500 are used with other surface ligands, such as antibodies, antibody fragments, single chain antibodies, aptamers, or peptides from phage display. In certain embodiments, these surface ligands are optionally attached to nucleic acid binding moieties, to lipids or lipid associating moieties.

[00113] In particular embodiments, the transfection agent comprises at least one cationic lipid, and optionally also contains in various combinations with one or more neutral and/or helper lipids, targeting moieties, cell penetration agent, fusion agents, and lysomotropic agents.

[00114] In some embodiments, the presently disclosed complexes comprise one more agents selected from the group consisting of fusogenic agents, nuclear localization sequences, cell penetration agent, transport peptides, receptor-ligand or cell adhesion peptides.

[00115] It is to be understood that while some peptides are disclosed herein in the context of one particular use, all of the peptides presently disclosed can be used in other uses as well. Thus, by way of example only, the peptides of SEQ ID NOs:1-41 are disclosed to be nuclear localization peptides. However, these peptides can be used as a nucleic acid binding peptide, a nucleic acid condensing peptide, a transfection enhancer.

[00116] In specific embodiments, the cell surface ligand containing adhesion peptide sequences is covalently linked to the transfection agents, the cationic lipid, the neutral lipid, helper lipid, a chemical group that associates with lipids or liposomes, and/or the polyamine.

[00117] In specific embodiments, the plant virus movement protein or peptide derived from plant virus movement proteins is covalently linked to the transfection agents, the cationic lipid, the neutral lipid, helper lipid, a chemical group that associates with lipids or liposomes, and/or the polyamine.

[00118] In other embodiments, the plant virus movement protein or peptide derived from plant virus movement proteins is conjugated to a nucleic acid binding group. In some of these embodiments, the nucleic acid binding group is linked to a polyamine or peptide nucleic acid. The polyamine optionally comprises at least one spermine moiety.

[00119] Suitable virus movement proteins and peptides derived from plant virus movement proteins sequences include, but are not limited to, a sequence selected from the group consisting of SEQ ID NOs:506-580, as set forth in Table 1, below, or in the sequence listings.

[00120] In some embodiments, the peptides of SEQ ID NOs:506-580 are optionally linked to a nucleic acid binding moiety, a helper lipid, a cationic lipid, a cationic polymer, a GPI anchor peptide or other chemical moieties that associate with transfection complexes.

[00121] In some embodiments, the peptides of SEQ ID NOs:506-580 are used with other surface ligands, such as antibodies, antibody fragments, single chain antibodies, aptamers, or peptides from phage display. In certain embodiments, these surface ligands are optionally attached to nucleic acid binding moieties, to lipids or lipid associating moieties.

[00122] In particular embodiments, the transfection agent comprises at least one cationic lipid, and optionally also contains in various combinations with one or more neutral and/or helper lipids, targeting moieties, surface ligand, fusion agents, and lysomotropic agents.

[00123] In some embodiments, the presently disclosed complexes comprise one more agents selected from the group consisting of fusogenic agents, cell penetration agents, nuclear localization sequences, transport peptides, plant movement protein or peptide derived from, receptor-ligand, surface ligand or cell adhesion peptides.

[00124] It is to be understood that while some peptides are disclosed herein in the context of one particular use, all of the peptides presently disclosed can be used in other uses as well. Thus, by way of example only, the peptides of SEQ ID NOs:1-41 are disclosed to be nuclear localization peptides. However, these peptides can be used as a nucleic acid binding peptide, a nucleic acid condensing peptide, a transfection enhancer.

[00125] In another aspect, disclosed herein are pharmaceutical compositions containing a complex as described herein, and a pharmaceutically acceptable carrier.

[00126] In another aspect, disclosed herein are methods of transfecting a cell, by contacting a cell with a complex as described herein. In some embodiments, the cell is selected from the group consisting of a primary cell culture, a passaged cell culture, suspension cell line and an attached cell line. Suitable cells include all human cell lines and all animal cell lines. In some embodiments, the cell is a blood derived cell or the cell is a stem cell, while in other embodiments, the cell is a neuron.

[00127] In one method, a nucleic acid, protein or, peptide, or pharmaceutical is contacted with a cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, capable associating with nucleic acid, protein,

peptide or pharmaceutical and the resulting mixture is added to a transfection agent then contacted to cells.

[00128] In one embodiment of the transfection methods, cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, are contacted with a transfection agent capable of associating with a nucleic acid, a protein, a peptide or a pharmaceutical composition, followed by addition of a nucleic acid, a protein, peptide or pharmaceutical then contacted to cells.

[00129] In another method, cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, linked to a nucleic acid binding moiety is contacted with transfection agent capable of associating with nucleic acid, protein, peptide or pharmaceutical followed by addition of a nucleic acid, protein, peptide or pharmaceutical then contacted to cells

[00130] In another method, cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, contacted with transfection agent followed by addition of a fusion agent and then contacted with a nucleic acid, protein, peptide or pharmaceutical then contacted to cells

[00131] In another method, cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, linked to a nucleic acid binding moiety is contacted with transfection agent followed by addition of a fusion agent and then contacted with a nucleic acid, protein, peptide or pharmaceutical then contacted to cells

[00132] In another method cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, is contacted with a fusion agent followed by addition of a transfection agent and then contacted with a nucleic acid, protein, peptide or pharmaceutical then contacted to cells.

[00133] In another method cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, linked to a nucleic acid binding moiety is contacted with a fusion agent followed by addition of a transfection agent and then contacted with a nucleic acid, protein, peptide or pharmaceutical then contacted to cells.

[00134] In another method cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, linked to a nucleic acid binding moiety is contacted with a transfection agent , then contacted with a nucleic acid, protein, peptide or pharmaceutical, then contacted with a fusion agent, and then contacted to cells.

[00135] In another method cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, linked to a nucleic acid binding moiety is contacted with a transfection agent containing exosomes, or lipid extracts from exosomes, then contacted with a nucleic acid, protein, peptide or pharmaceutical, then contacted with a fusion agent, and then contacted to cells

[00136] In another method an aqueous solution of transfection enhancers, that may optionally contain one or more a plant virus movement proteins or peptides derived from plant virus movement proteins linked to a nucleic acid binding moiety, that may optionally contain buffers, that may optionally contain one or more cell surface ligands, that may optionally contain one or more nuclear localization agents, that may optionally contain one or more cell penetration peptides, that may optionally containing one or more fusion peptides or proteins, all or some of these transfection enhancers may optionally be linked to nucleic acid binding domain is mixed with an ethanol or aqueous solution of lipids that may contain cationic lipids, helper lipids, exosomes or total lipid extracts of exosomes or various mixtures of such lipids or lipid particles The mixture is then contacted with nucleic acids, protein, peptides, exosomes, growth enhancers, and then cells.

[00137] In another method an ethanol solution of transfection enhancers, that may optionally contain one or more a plant virus movement proteins or peptides derived from plant virus movement proteins linked to a nucleic acid binding moiety, that may optionally contain buffers, that may optionally contain one or more cell surface ligands, that may optionally contain one or more nuclear localization agents, that may optionally contain one or more cell penetration peptides, that may optionally containing one or more fusion peptides or proteins, all or some of these transfection enhancers may optionally be linked to nucleic acid binding domain is mixed with an ethanol or aqueous solution of lipids that may contain cationic lipids, helper lipids, exosomes or total lipid extracts of exosomes or various mixtures of such lipids or lipid particles The mixture is then contacted with nucleic acids, protein, peptides, exosomes, growth enhancers, and then cells.

[00138] In another method an aqueous solution of transfection enhancers, that may optionally contain one or more a plant virus movement proteins or peptides derived from plant virus movement proteins linked to a nucleic acid binding moiety, that may optionally contain buffers, that may optionally containing one or more cell surface ligands, that may optionally one or more nuclear localization agents, that may optionally contain one or more cell penetration peptides, all or some of these transfection enhancers may optionally be linked to nucleic acid binding domain is mixed with an ethanol or aqueous

solution of lipids that may contain cationic lipids, helper lipids, exosomes or total lipid extracts of exosomes or various mixtures of such lipids or lipid particles. This mixture is then contacted with one or more fusion peptides or proteins which may optionally be in a buffer. The mixture is then contacted with nucleic acids, protein, peptides, exosomes, growth enhancers, and then cells.

[00139] In another method, a transfection complex is contacted with a nucleic acid, protein or, peptide then contacted with cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, and then contacted to cells.

[00140] Those skilled in the art understand that concentrations of the various components, such as cationic lipid, helper lipid, the plant virus protein or peptide derived therefrom, cell surface ligand, fusogenic reagent, nuclear ligand, cationic polymer, condensing agent, cell penetration, lysomotrophic agent, exosomes or exosome lipids or proteins from exosomes, peptides derived for exosomal proteins and transfection enhancers are optimized according to the cell to be transfected or the in vivo application. In some embodiments, the concentration of each component ranges from 0.001 to 20 mg/mL, depending on solubility and formulation solvent.

[00141] In some embodiments, the plant virus movement protein derived peptides disclosed herein are suitable for use in the disclosed transfection enhancers to enhance transfection into HeLa cells, HuVec cells, iPS cells, NL-1 cells, C2C12 cells, human fibroblast cells, stem cells, Jurkat cells, rat cortical neurons, THP-1 cells, and human skeletal muscle cells, among others.

[00142] In some embodiments, the peptide useful to enhance the transfection of HeLa cells is selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503 can be used in combination with the plant virus movement protein or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of HeLa cells is selected from the group consisting of SEQ ID NOs:205, 237, 268, 326, 328, 335, 336, 342, 347, 348, 352, 353, 355, 357, 358, 365, 367, 377, 379, 381, 450, 454, and 503 can be used in combination with the plant virus movement protein or peptide derived therefrom. In some embodiments, the peptide useful to enhance the transfection of HuVec cells is selected from the group consisting of SEQ ID NOs:205, 216,

218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of HuVec cells is selected from the group consisting of SEQ ID NOs:236, 358, 373 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00143] In some embodiments, the peptide useful to enhance the transfection of NL-1 iPS cells is selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of NL-1 iPS cells is selected from the group consisting of SEQ ID NOs:216, 224, 226, 236, 236, 323, 327, 341, 343, 347, 348, 349, 350, 351, 354, 358, 360, 373, 383, 450, 454, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00144] In some embodiments, the peptide useful to enhance the transfection of C2C12 cells is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, 502, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of C2C12 cells is selected from the group consisting of SEQ ID NOs:218, 230, 237, 239, 256, 323, 326, 328, 335, 336, 342, 343, 345, 347, 348, 352, 357, 359, 367, 375, 379, 381, 450, 452, 454, 501, 502, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom,

[00145] In some embodiments, the peptide useful to enhance the transfection of human fibroblast cells is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, 503 can be used in combination with cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of human fibroblast cells is selected from the group consisting of SEQ ID NOs:205, 218, 219, 229, 230, 335, 336, 342, 344, 348, 349, 350, 351, 353, 355, 357, 361, 367, 369, 375, 379, 381, 450, 454, 501, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00146] In some embodiments, the peptide useful to enhance the transfection of Jurkat cells is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, 503 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom. In some of these embodiments, the peptide useful to enhance the transfection of Jurkat cells is selected from the group consisting of SEQ ID NOs:218, 349, 358 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00147] In some embodiments, the peptide useful to enhance the transfection of rat cortical neuron cells is selected from the group consisting of SEQ ID NOs: 220, 236, 238, 323, 327, 336, 338, 341, 343, 347, 348, 350, 351, 352, 354, 367, 369, 373, 375, 377, 454 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00148] In some embodiments, the peptide useful to enhance the transfection of THP-1 cells is selected from the group consisting of SEQ ID NOs:219, 229, 230, 239, 323, 328, 332, 341, 343, 350, 351, 357, 358, 375, 450, 454 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00149] In some embodiments, the peptide useful to enhance the transfection of human skeletal muscle cells is selected from the group consisting of SEQ ID NOs: 218, 219, 230, 328, 336, 344, 350, 351, 353, 355, 365, 375 can be used in combination with the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom.

[00150] In some embodiments combination of different surface ligands that have preference for a given cell types are mixed together to target multiple cell types in a single formulation.

[00151] In another aspect, disclosed herein are kits containing a transfection agent and a peptide or protein or a modified peptide or modified protein with cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, as described herein. In some embodiments, the kit further comprises instructions for the preparation and the use of the transfection complexes. In certain embodiments, the kit further comprises separate compartments for the various components. In certain embodiments transfection agents may comprise various peptides selected from SEQ ID NOs 1-505 in combination with SEQ ID NOs 506 -580 that are suitable for transfecting cells in vivo or in vitro.

EXAMPLES

Example 1: Surface Ligands

[00152] Cells were plated to so that on the day of transfection the cells were 70% confluent in 96 well tissue culture plates. A DOTMA/DOPE Lipid solution (1:1 molar ratio) at 2 mg/mL in water were mixed with an equal volume cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, that had the nucleic acid binding moiety RRRRRRRRRRRR (SEQ ID NO:109) or KKKKKKKKKKKKKKKKKK (SEQ ID NO: 102) covalently linked to the N-terminus, or C-terminus of each peptide during peptide synthesis. Peptides were at 2 mg/mL in water. The DOTMA/DOPE and the solution containing the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, surface ligand peptide the was diluted 1 to 1 (v/v) in water. DOTMA/DOPE/PEPTIDE solutions were added to 0.1 mL of a plasmid DNA solution (EF1Alpha eGFP plasmid) at 10 µg/mL in OptiMEM.

[00153] All solutions were at room temperature. A volume of 0.2 mL of a 10 µg/mL solution of DNA was aliquoted into each well of a non-tissue cultured treated plate. 1-12 µL of transfection reagents were added to DNA solutions respectively. The transfection reagent and DNA solution was mixed by pipetting up and down twice. Transfection complexes were formed for 10 minutes. After 10 minutes, 0.01 or 0.02 mL of the transfection complex was added to cells. HuVEC, HeLa, human adult keratinocytes, human primary adult fibroblast, and A549 cells all respectively received 0.01 mL of the transfection complex. Human skeletal muscle cells, mouse C2C12 cells, and Jurkat, cells received 0.02 mL of the transfection complex. Cells were incubated for 42 hours at 37 °C at 5% CO₂. Plates were read on a fluorescent plate reader. Cells were also examined visually under a microscope to assess the extent of transfection (in terms of the percent of cells transfected) with a fluorescent microscope. Other modes of analysis, for example quantification with B-galactocidase or luciferase reports plasmids can also be used. If the plate reader did not show a sufficient signal to noise ratio, then plates were scored for cells transfected and those peptides that show increase over DOTMA alone were noted.

[00154] Not all surface ligands increased transfection efficiency even though they were suggested to be used to attach cell to tissue culture plates. Surface ligand that caused increase expression of GFP over the lipid DOTMA with no surface ligand were considered to enhance transfection or had higher % cells transfected were considered to enhance transfection. Peptides that gave greater than 2-fold enhancement are noted in bold and could be further optimized by adding different transfection enhancers or could be used with different cationic lipids or polymers.

[00155] Tables 2-7, below, provide the results of the transfection enhancements with the various cell types, while Table 8 lists the peptides that were determined by visual inspection to enhance the transfection of the denoted cell lines over the lipid DOTMA.

[00156] The following sequences increased transfection expression and the % cells transfected over the no peptide control on some examined cell types: SEQ ID NOs: 205, 216, 218, 219, 220, 226, 229, 230, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 365, 367, 369, 373, 375, 377, 379, 381, 393, 450, 452, 454, 501, and 503.

[00157] Various DOTMA/peptide transfection reagents that showed enhanced transfection efficiency over DOTMA alone were further formulated with the fusogenic

peptide (SEQ ID NO:72). The fusogenic peptide was added to the DOMTA/peptide formulation to achieve a final concentration of 0.1 mg/mL to see if transfection reagents were further enhanced by the addition of a fusogenic peptide and could provide higher transfection efficiency into HeLa cells and expression than the commercially available Lipofectamine 2000. The results are shown in Table 9.

[00158] The following Table 1 lists the peptide sequences that are referenced herein.

TABLE 1

SEQ ID NO	Sequence
1	GYSTPPKKRKVEDP
2	GYSTPPKTRRRP
3	GYSTPGRKKR
4	GYSTPRRNRRRRW
5	PDEVKRKKKPPTSYP
6	PRRRTKPPTSYP
7	RKKGPTSYP
8	WRRRRNRRPTSYP
9	GYGPPKKRKVEAPYKA
10	PAAKRVKLD
11	RQRRNELKRSP
12	KRPAATKKAGQAKKKK
13	VRKKRKTEESPLKDKDAKSKQE
14	RLRRDAGGRGGVYHLGGAPRRRK
15	KRKGDEVGDGVDECAKSKK
16	NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY
17	GGKRTADGSEFESPKKARKVEAYPKAW
18	GGKRTADGSEFESPKKKRAVEAYPKAW
19	GGKRTADGSEFESPKKKAKVEAYPKAW
20	GGKRTADGSEFESPKKKRKVEAPYKAWK
21	GGKRTADGSEFESPKKKRKVEYKAWK
22	GYGPAAKRVKLDEAYPKAWK
23	GGKRTADGSEFEPAAKRVKLDEAYPKAWK
24	GTGPKKKRKVGGGGYGPKKKRLVG
25	KRPAATKKAGQAKKKKLEAYPKAWK
26	ATKGTKRSYEQMETGE
27	GKWERKPIRCAS

SEQ ID NO	Sequence
28	GYGKRTADSQHSTPPKKKRKVEAPYKAWK
29	KRTADSQHSTPPKKKRKVEAPYKAWK
30	GYGPPKKKRKVEAPYKAWKWAKYPAMRRAHRRRRRASHRRRTTT GT
31	GYGPPKKKRKVEAPYKAWKRGARRYSKMKRRRRRVARRHRRRP
32	FWGYGYGPPKKKRKVEAPYKAWK
33	GKPSSDDEATADSQHSTPPKKKERKVED
34	GKPTADDQHSTPPKKKRKVED
35	GGKRTADGSEFESPPKARKVEAYPKAK
36	EKIRLRPGRKKRYRLKHL
37	PEGTRQARRNRRRRWRKR
38	PEGTRQPRRNRRRRWRKR
39	GVKRSYGAARGDDRRRPNVVAPYKAW
40	KSVPNRTRTYIKLRRLRFKGAPYKAW
41	EMRRRREEEGLQLRKQKREEQLFKRRN
42	FEAALAEALAEALA
43	Ac-LARLLPRLARL-NHCH ₃
44	GLLEELLEELLEELWHEELLEG
45	GWEGLIEGIEGGWEGLIEG
46	GLFEALAEFIEGGWEGLIEG
47	GLFEALLELLESLWELLLEA
48	GGYCLEKWMIVASELKCFGNTA
49	GGYCLTRWMLIEAELKCFGNTAV
50	WEAALAEALAEALAEHLAEALAEALEALAA
51	GLFGAIAGFIENGWEGMIDGWYG
52	GIGAVLKVLTTGLPALISWIKRKRQQ
53	GRKKRRQRRRPPQ
54	RQIKIWFQNRRMKWKK
55	GWTLSAGYLLGKINLKALAALAKKIL
56	WEAKLAKALAKALAKHLAKALAKALKACEA
57	GLFKALLKLLKSLWKLLLKA
58	GLFRALLRLLRSLWRLLLRA
59	GLFEALLELLESLYELLLEA
60	GLFEALEELWEA
61	GLFLLEEWLE
62	GLFLLEEWLEK
63	GLFEALLELLESLWELLLEAK

SEQ ID NO	Sequence
64	Suc-GLFKLLEEWLE
65	Suc-GLFKLLEEWLEK
66	GLFEAIAEFIEGGWEGLIEG
67	GLFKAIAKFIKGGWKGLIKG
68	IRFKKTKLIASIAMALC
69	ALAGTIIAGASLTFQVLDKVEELGKVSRL
70	GLFEAIEGFIENGWEGMIDGWYG
71	GYICRRARGDNPDDRCT
72	GLFEAIAEFIEGGWEGLIEGCA
73	GLFHAIAHFIHGGWHGLIHGWYWG
74	RRRQRRKKRGGDIMGEWGNEIFGAIAGFLG
75	GLFEAIAEFDIENGWEGMIDGGG
76	ALAGTIIAGASLTFQVLDKVEELGKVSRLK
77	IRFKKTKLIASIAMAA
78	GLWHLLLHLWRLLRLLR
79	KKIMLLMTLLLVSLPLAQEQ
80	GLFEALLELESWELLLEAWYG
81	RLLRLLRLWRLLRLLR
82	LLELELELELELELELELELELE
83	GLFEALLELESWELLLEARRRRRRR
84	GLFEALLELESWELLLEARRRRRR
85	GLFEALLELESWELLLEAKKKKKKK
86	GLFEALLELESWELLLEAKKKKKK
87	GLFEALLELESWELLLEAKK
88	GLFEALLELESWELLLEAKKKK
89	GLFEALLELESWELLLEAEE
90	GLFEALLELESWELLLEAEEEE
91	GLFEALLELESWELLLEAEEEEEE
92	GLFEALLELESWELL
93	PLSSIFSRIGDPRGARRYAKMKRRRRRVARRHRRRP
94	GPFHYFQFLFPPV
95	GSSSWWQRWWPPW
96	RRRQRRKKR
97	KKKK
98	KKKKKK
99	KKKKKKKK
100	KKKKKKKKKK

SEQ ID NO	Sequence
101	KKKKKKKKKKKK
102	KKKKKKKKKKKKKKKKKK
103	KKKKKKKKKKKKKKKKKKKK
104	KKKKKKKKKKKKKKKKKKKKKKKK
105	RRRR
106	RRRRRR
107	RRRRRRRR
108	RRRRRRRRRR
109	RRRRRRRRRRRR
110	RRRRRRRRRRRRRRRR
111	RRRRRRRRRRRRRRRRRRRR
112	RRRRRRRRRRRRRRRRRRRRRR
113	YKA
114	KKKKKKKKWKGGGGACYGLPHLFCG
115	YKAKKKKKKKKKWK
116	KTPKKAKKPKTPKKAKKP
117	KKAKKPAATRKSNNPKPKTVKPKKVAK
118	RGARRYSKMKRRRRRVARRHRRRP
119	TRQARRNRRRRWRERQRGSGSG
120	KRPRGRPKGSKKNWRRRKRRASRRSPRR
121	KRGRGRPRKQPPKEPSEVPTPKRPRGRPKGSKNK
122	KEYEKDIAAYRAKGKPAAKKGVVKAESKSKK
123	YKAKKKKKKKKKKKWK
124	KKKKKKKGGC
125	YRARRRRRRRRWR
126	YRARRRRRRRRRRWR
127	KGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSK
128	KKQLKKQLKKQLKQWK
129	KKSPKKSPKKSPKSK
130	KLSKLEKSKLEK
131	KLSKLEKKLSKLEKSKLEK
132	KSLKSLKKSLKSK
133	KIRRRGKNKVAARTCRQRRTDR
134	KIRRRGKNKVAAQNCRKRKLET
135	KRRIRREKNKMAAAKCRNRRRELT
136	KDRSNLLERHTR
137	KRPAATKKAGQAKKKL

SEQ ID NO	Sequence
138	RRRRRREEEE
139	RRRRRREEEEE
140	RRRRRREEEEEEE
141	RRRRRRRREEEE
142	RRRRRRRREEEEE
143	RRRRRRRREEEEEEE
144	RRRRRRRRRRRREEEE
145	RRRRRRRRRRRREEEEE
146	RRRRRRRRRRRREEEEE
147	KLKLEKK
148	SKLEK
149	KLKLEKKLSEKK
150	PKKKRKVGGRGDSP
151	LPHKSMPCG
152	GACLQHKSMPCG
153	YGLPHLFCG
154	SERSMNFCE
155	DHYSLYEDLERGTDK
156	ISLPRTSGAQRATR
157	EKLQTKYGLPHKVEFCG
158	TRISEQAKPGD
159	LVFFDY
160	WGGNGPTTFDCSGYTKYVFAK
161	INIGTTGWGDHYSLY
162	YDNIHG
163	AGWGKFLVGFRV
164	SIGYPLP
165	TTHWGFTL
166	HLQIQPYPQISG
167	KLNIVSVNG
168	RGH
169	DNRIRLQAKAA
170	KIKMVISWKG
171	LPWYSYLYAVSA
172	WNLPWYYSVSPT
173	WNL
174	PWYYSVSPT

SEQ ID NO	Sequence
175	SSWESYKSGGGTRL
176	RDWSSQHPGRCNGETHLK
177	SLPTLTL
178	VICTGGDYSFALPVGQWPVMT
179	DKPSYQFGGHNSVDFEEDTLPKV
180	RARRRKRASATQLYQTCKASGTCPPD
181	SGDYSFALPVGQWPWMTG
182	CTGGDYSFALPVGQWPW
183	FYYDYDFFFDYWGG
184	HLRRLRRRLLEAEG
185	DYYCAAWDDSLNGYSVF
186	YYCLQSMEDPYTFGG
187	YYCARSDGNYGYYYALDYDY
188	AARSPSYRYDY
189	GPYYAMDYD
190	YYCQQRSSYPYTEGGAYPKAWK
191	YYCQRYDSDFSFGQGTKL
192	YYCARSGYYAMDYWGQGT
193	RVRRGACRGDCLG
194	RVRRGACRYDCLG
195	YYCAKGTHWGFWSGYFDYWGGGT
196	GRENYHGCTTHWGFTLC
197	VQATQSNQHTPRGGGSK
198	DPRAPGS
199	YYCQQRSSYPYTEFGG
200	AARSPSYRYDYGPYYAMDYD
201	GPKLTGILISILSFVES
202	KYILRWRPKNS
203	IKVAV
204	WTPPRAQITGYRLTVGLTRR
205	AASIKVAVSADR
206	KLDAPT
207	NRWHSIYITRFG
208	PHSRN
209	SSFHFDGSGYAM
210	RGDS
211	IAFQRN

SEQ ID NO	Sequence
212	GRGDSP
213	TWYKIAFQRRK
214	EDGIHEL
215	SLVRNRRVITIQ
216	YRVRVTPKEKTGPMKE
217	LQVQLSR
218	SPPRRARVT
219	RKRLQVQLSIRT
220	ATETTITIS
221	NAPFPKLSWTIQ
222	VSPRRARVTDATETTITISWRTKTETITGG
223	WTIQTTVDRGLL
224	KPDVRSYTITG
225	DTINNGRDHMILI
226	ANGQTPIQRYIK
227	MILISIGKSQKRM
228	PRARITGYIIKYEKPGSPPREVVPRPRPGV
229	PPFLMLLKGSTR
230	WQPPRARI
231	NQRLASFSNAQQS
232	WQPPRARITGYIIKYEKPG
233	ISNVFVQRMSQSPEVLD
234	YEKPGSPPREVVPRPRPGV
235	KARSFNVNQLLQD
236	KNNQKSEPLIGRKKT
237	KNSFMALYLSKG
238	EILDVPST
239	KNSFMALYLSKGRLVFALG
240	IDAPS
241	RDSFVALYLSEGHVIFAGLG
242	VVIDASTAIDAPSNL
243	KPRLQFSLDIQT
244	LDVPS
245	DGQWHSVTVSIK
246	REDV
247	FVLYLGSKNAKK
248	PHSRNRGDSP

SEQ ID NO	Sequence
249	LAIKNDNLVYVY
250	LWVTVRSQQRGLF
251	AYFSIVKIERVG
252	GTNNWWQSPSIQN
253	DVISLYNFKHIY
254	WVTVTLDLRQVFQ
255	FFDGSSYAVVRD
256	RQVFQVAYIIKA
257	LHVFYDFGFGFSNG
258	LTRYKITPRRGPT
259	LKKAQINDAKYREISIIYHN
260	LLEFTSARYIRL
261	RAYFNGQSFIAS
262	YIRLRLQRIRTL
263	SRLRGKNPTKGK
264	RRYYYSIKDISV
265	LHKKGKNSSKPK
266	SINNTAVNQRLT
267	RLKTRSSHGMIF
268	GGFLKYTVSYDI
269	GEKSQFSIRLKT
270	RDQLMTVLANVT
271	TLFLAHGRLVFM
272	ANVTHLLIRANY
273	LVFMFNVGHKKL
274	AGTFALRGDNPQG
275	TLFLAHGRLVFMFNVGHKKL
276	VLIKGGARKHV
277	DFMTLFLAHGRLVFMGNVG
278	LSNIDYLIKAS
279	HKCLKIRSQEKY
280	LQQSRIANISME
281	GAAWKIKGPIYL
282	NLLLLLVKANLK
283	VIRDSNVVQLDV
284	HRDELLWARKI
285	GLIYYVAHQNM

SEQ ID NO	Sequence
286	KRRARDLVHRAE
287	DYATLQLQEGR LHFMFDLG
288	SQFQESVDNITK
289	KKGSYNNIVVHV
290	PGGMREKGRKAR
291	ADNLLFYLGSAK
292	MEMQANLLLDRL
293	GSAKFIDFLAIE
294	LSEIKLLISAR
295	KVSFLWWVGSGV
296	RDFTKATNIRLRFLR
297	SYWYRIEASRTG
298	ISTVMFKFRTFS
299	YFDGTGFAKAVG
300	KQANISIVDIDSN
301	NGQWHKVTAKKI
302	FSTRNESGIILL
303	AKKIKNRLELVV
304	RRQTTQAYYAIF
305	GFPGGLNQFGLTTN
306	YAIFLNKGRLEV
307	NQFGLTTNIRFRG
308	KNRLTIELEVRT
309	IRSLKLTGKGTP
310	GLLFYMARINHA
311	AKALELRGVQPVS
312	VQLRNGFPYFSY
313	GQLFHVAYILIKF
314	HKIKIVRVKQEG
315	NVLSLYNFKTTF
316	DFGTVQLRNGFPFFSYDLG
317	SQRIYQFAKLNYT
318	NIRLRFLRTNTL
319	EVNVTLDLGQVFH
320	GKNTGDHFVLYM
321	GQVFHVAYVLIKF
322	VVSLYNFEQTFML

SEQ ID NO	Sequence
323	HQQDLGTAGSCLRKFSTMFLF
324	RFDQELRLVSYN
325	HQQDLGTAGSCLRKFSTMFLFCNI
326	RLVSYSGVLFFLK
327	VAEIDGIEL
328	NWRHISYITRFG
329	GIIFFL
330	KRLQVQLRSIRT
331	ASKAIQVFLGG
332	TWYKIAFQRNRK
333	VLVRVERATVFS
334	QVFQVAYIIKA
335	TVFSVDQDNMLE
336	GEFYFDLRLKGDK
337	RLRGPQRVFDLH
338	GTPGPQGIA
339	FDLHQNMGSVN
340	GQRDVV
341	LRAHAVDVNG
342	TAGSCLRKFSTM
343	LFSHAVSSNG
344	KGHRGF
345	TAGSCLRKFSTMFLF
346	TAGSCLRKFSTMFLFCNI
347	DLGTAGSCLRKFSTM
348	HQQDLGTAGSCLRKFSTM
349	RNIAEIIKDI
350	SIGFRGDGQTC
351	LNRQELFPFG
352	RIQNLLKITNLRIKFVK
353	KKQRFRRNRKGYRSQ
354	SINNTAVMQRLT
355	FRHRNRKGY
356	RYRVRVTPKEKTGPMKE
357	SETTVKYIFRLHE
358	GHRGPTGRPGKRGKQGQKGDS
359	KAFDITYVRLKF

SEQ ID NO	Sequence
360	GDLGRPGRKGRPGPP
361	YIGSR
362	RGEFYFDLRLKGDK
363	LAGSCLARFSTM
364	LALFLSNGHFVA
365	ISRCQVCMKKRH
366	PGRWHKVSVRWE
367	TDIPPCPHGWISLWK
368	VRWGMQQIQLVV
369	TAIPSCPEGTVP LYS
370	KMPYVSLELEMR
371	GPAGKDGEAGAQG
372	VLLQANDGAGEF
373	GLPGER
374	DGRWHRVAVIMG
375	LAGSCLPVFSTL
376	APVNV T ASVQIQ
377	TAGSCLRRFSTM
378	KQGKALTQRHAK
379	TAGSCLRKF
380	RYVVLPR
381	TAGSCL
382	SPYTFIDSLV LMPY
383	TAG
384	PDSGR
385	QQNLGSVNVSTG
386	SRATAQKVSRRS
387	DPGYIGSR
388	GSLSSHLEFVGI
389	VILQQSAADIAR
390	RNRLHLSMLVRP
391	KDISEKVAVYST
392	APMSGRSPSLVLK
393	LG TIPG
394	AFGV LALWGTRV
395	TDIRVTLNRLNTF
396	IENVVTTFAPNR

SEQ ID NO	Sequence
397	AFSTLEGRPSAY
398	LEAEFHFTHLIM
399	TSAEAYNLLRT
400	HLIMTFKTRPA
401	LNRRYEQARNIS
402	KTWGVYRYFAYD
403	SLLSQLNNLLDQ
404	TNLRIKFVKLHT
405	RDIAEIIKDI
406	KRLVTGQR
407	SHAVSS
408	GPGVVVVERQYI
409	ADTPPV
410	NEPKVLKSYYYAI
411	LRAHAVDING
412	YYAISDFAVGGR
413	DSITKYFQMSLE
414	LPFFNDRPWRRAT
415	YTALIIATDN
416	FDPELYRSTGHGGH
417	VITVKDINDN
418	TNAVGYSVYDIS
419	GLDRESYPYY
420	APVKFLGNQVLSY
421	MKVSATDADD
422	SFSFRVDRRDTR
423	PQVTRGDVFTMP
424	TWSKVGGHLRPGIVQSG
425	KEAEREVTDLLR
426	RGDV
427	AAEPLKNIGILF
428	FALWDAIIGEL
429	VGVAPG
430	LWPLLAVLAAVA
431	PGVGV
432	VFDNFVLK
433	TSIKIRGTYSER

SEQ ID NO	Sequence
434	TTWSQCSKS
435	DPETGV
436	KRSR
437	QGADTPPVGV
438	SVVYGLR
439	PLDREAIKY
440	DGRGDSVAYG
441	HAVDI
442	LALERKDHSG
443	DQNDN
444	YSMKKTTMKIIPFNRLTIG
445	QDPELPDKNM
446	RGDF
447	LVVQAADLQG
448	GVYYQGGTYSKAS
449	NDDGGQFVVT
450	TAGSCLRKFSC
451	YILHVAVTN
452	CNYYSNSYSFWLASLNPER
453	TYRIWRDTAN
454	TGLSCLQRFTTM
455	GFTCECSIGFRGDGQTCYGIVFWSEV
456	HHLGGAKQAGDV
457	SCLPGFSGDGRACRDVDECGH
458	MAPRPSLAKKQRFHRNRKGYRSQRGHSRG
459	KKQKFRHRNRKGYRSQ
460	KKQKFKHRNRKGYRS
461	KKQKFRRRNRKGYRSH
462	TAIPPCPHGWISLWK
463	KKQKSRHRSRKRYRS
464	KKQKSRRRSRKGYRS
465	ISRCTVC
466	ISRCQVCMKRRH
467	VSRCTVC
468	TDIPPCPQGWISLWK
469	TVKAGELEKIISRCQVMKRRH
470	TDIPSCPHGWISLWK

SEQ ID NO	Sequence
471	TDIPPCPAGWISLWK
472	TEIPPCPQGWISLWK
473	TDVPPCPQGWISLWK
474	RLVSYNGILFFLK
475	RLVSYSGVIFFLK
476	RLVSYNGILFFL
477	RLVSYSGIIFFLK
478	RFEQELRLVSYSGVLFFLKQ
479	RLVSYNGIIFFLK
480	DPAFKIEDPYSPRIQNLLKITNLRIKFVKL
481	TKRFEQELRLVSYSGVLFFL
482	GGRLKYSVAF
483	GGFLRYTVSYDI
484	GGFLKYTVSYDV
485	LGNKLTAFGGFLKYTVSYDIPV
486	GGYLKYTVSYDI
487	GEIFFDMRLKGDK
488	GEIYFDLRLKGDK
489	GEIYLDMRLKGDK
490	IGQPGAKGEPGEFYFDLRLKGDKGDPGFPG
491	GEVFFDMRLKGDK
492	LAGSCLPIFSTL
493	AHNQDLGLAGSCLARFSTMPFLYCNP GDIC
494	QEKAHNQDLGLAGSCLPVFSTL PFAYCNIH
495	LAGSCLPVFSTM
496	GNKRAHGQDLGTAGSCLRRFSTMPFMFCNI
497	RAHGQDLGTAGSCLRRFSTMP
498	RKRLQVQLNIRT
499	HLVLPLQQSDVRKRLQVQLSIRTFASSGLI
500	RKRLSVQLRIRT
501	DLGTAGSCLRRFSTM
502	RNIAEIIKDI
503	TAGSCLRKFSTMRRRRRRRRRRRRR
504	FTLTGLLGTLVTMGLLT
505	APYKAWK
506	STSKTNRGDDSNWSKRVTNNKPS
507	STSKRKRGGDDSNWSKRVTKKKPS

SEQ ID NO	Sequence
508	STSKRKRGGDSNWSKRVSKKKPS
509	STSKRKRGGDANWSKRVTKKKPS
510	PLAGSKRKRADÉVAWSKRGTKKKPER
511	PLAGSKRKRADÉVAWSKRGTKKKPERTSAARAGPSRRIR
512	STSKRKRGGDANWSKRRTTKKKPSS
513	STSKRKRGGDANWSKRRTTKKKPSSAGLKRAAGSKADRPSL
514	PTTAGKRKRSDDAAWSKRARPKAGRT
515	PTTAGKRKRSDDAAWSKRARPKAGRTSAARPGTSVRRIR
516	SSSLGKRKRSDÉGAWSKGKSKKKAMR
517	SSSLGKRKRSDÉGAWSKGKSKKKAMRGSSSRPGPVRGP
518	PTTAGKRKRRTDDAAWSKRARPKAGR
519	PTTAGKRKRRTDDAAWSKRARPKAGRTSAARPGTAVRRVR
520	PATAGKRKRSDDAAWSKRARPKAGRTSAAR
521	PATAGKRKRSDDAAWSKRARPKAGRTSAARPGTSVRRIR
522	SSSLGKRKRSDGGDWSKRSAKKKPA
523	SSSLGKRKRSDGGDWSKRSAKKKPAAGTPSRRAGPGRGPR
524	SSSLGKRKRSDÉGAWSKGKSKKKAMR
525	SSSLGKRKRSDÉGAWSKGKSKKKAMRGSSSRPGPVRGP
526	STSKRKRGGDANWNKRPTKKKPSS
527	STSKRKRGGDANWNKRPTKKKPSSAGLKKAGSKAERPSL
528	SGALKRKRSDÉVAWSRRRPVKKPV
529	SGALKRKRSDÉVAWSRRRPVKKPVRRAPPPRAGPSVRRG
530	SGALKRKRSDÉVAWSRRKPAKKPAR
531	SGALKRKRSDÉVAWSRRKPAKKPARQPPPPRAGPSVRRG
532	AGALKRKRSDÉVAWSRRKPAKKPAR
533	AGALKRKRSDÉVAWSRRKPAKKPARAPPPRAGPSVRRGL
534	STSKRKRGGDSNWSKRVTKKKPSSAGLKRAAGSKADRPSLQIQTQH AGTTMITVPSGGVCDLINTYARGSDÉGNRHTSETLTYKIAIDYHFVA DAAACRYSTGTGVMWLVDYDTPGGQAPTPQTIFSYPDTLKA WPA TWKVSRELCHRFVVKRRWLFNMETDGRIGSDIPPSNASWKPKRNI YFHKFTSGLGVRTQWKNVTDGGVGAIQRGALYMVIAPGNGLTFTA HGQTRL YFKSVGNQ
535	DPQNALYYQPRVPTAAPTSGGVPWSRVGEVAILS FVALICFYLLYL WVLRDLILVLKARQGRSTEELIFGGQAVDRSNPIPNIPAPPSQGNPGP FVPGTG
536	GSQLVPPPSAFNYIESQRDEFQLSHDLTEIVLQFPSTASQITARLSRSC MKIDHCVIEYRQQVPINASGTVIVEIHDKRMTDNESLQASWTFPIRC NIDLHYFSSSFFSLKDPIPWKLYYRVSDSNVHQMTHFAKFKGKLKLS SAKHSVDIPFRAPT VKILAKQFSEKDIDFWHVG YGKWERRLVKSASS SRFGLRGPIEINPGESWATKSAIVTPNRNADLDIEEELLPYRELNRLG

SEQ ID NO	Sequence
	TNILDPGESASIVGIQRSQSNITMSMSQLNELVRSTVHECIKTSCIPSTP KSLS
537	RTGVKRSYGAARGDDRRRPNVV
538	SYVKTVPNRTRTYIKLRVR
539	MYSTSNRRGRSQTQRGSHVRRRTGVKRSYGAARGDDRRRPNVVSKT QVEPRMTIQRVQENQFGPEFVLSQNSALSTFVTYPSYVKTVPNRTRT YIKLRVRFKGTCLKIERGQGDITMDGPSSNIEGVFSMVIVVDRKPHV SQSGRLHTFDELFGARIHCHGNLSVVPALKDRYYIRHVTKRVVSLEK DTLLIDLHGTTQLSNKRYNCWASFSDLERDCNGVYGNITKNALLVY YCWLSDAQSKASTYVSFELDYL
540	R ₁₂ -VDYGKWERKPIRCASMSR
541	R ₁₂ -GKWERKPIRCAS
542	K ₁₆ -GKWERKPIRCAS
543	R ₁₂ -VDFSHVDYGKWERKPIRCASMSRLGLRG
544	GVKRSYGAARGDDRRRPNVVAPYKAW-R ₁₂
545	KSVPNRTRTYIKLRRLRFKGAPYKAW-R ₁₂
546	RTGVKRSYGAARGDDRRRPNVV-R ₁₂
547	SYVKTVPNRTRTYIKGGGGG-R ₁₂
548	VDIPFRAPTIKILSKQFTEDDIDFWHVGYGKWERKLVRPASLSGRRG LRR
549	IDFWHVGYGKWERKLVRPASLSGRRGLRR
550	IDFWSVEKGETRRRLNPTPHAHSPRPIAHR
551	IDFSHVGYGKWERKMIRSASISRLGLHN
552	VDFSHVGYGKWERKLIRSASTVKYGLPS
553	IDFSHVDYGKVERKLKCESSRLGLHS
554	IDFWSVGRKAQQRKLVQGPSLIGSRSMRY
555	IDFWSVGSKPQTRRLVDGSRLIGHSSRSLRV
556	IDFWSVERGETRRRLNPTPSAGSNRALSQR
557	VDFWSVGKPKPIRRLIQNDPGTDYDTGPKYR
558	VDFWSVEKPKPIRRLNPGPNQGPYPNTGHR
559	VDFSHVDYGKWERKLIRSASTSRYGLRS
560	VDFSHVDYGKWERKTLRSRSLRIGLTG
561	IDFWHVGYGKWERRLVKSASSSRFGIRG
562	VDFHVDYGRWERKHIRCASMSRVGLRG
563	GTFQHVDYGKWERKPIRCQSMSRVGYRR
564	VGYGKWERKLVRPASLS
565	VEKGETRRRLNPTPHA
566	VGYGKWERKLIRSASTV
567	VEKPKPIRRLNPGPNQ

SEQ ID NO	Sequence
568	VDYGKWERKLIRSASTS
569	VDYGKWERKTLRSRSL
570	VGYGKWERRLVKSASSS
571	VDYGRWERKHRCASMS
572	VERPKPIRLLTPTPGC
573	PFRAPTILSKQFTEDDIDFWHVGYGKWERKLVRPASLSGRRGLRR
574	PFRAPTVKILSKQFTDKDIDFSHVGYGKWERKMIRSASISRLGL
575	DIAFRAPTVKILSKQFTDRDVFDFSHVGYGKWERKLIRSASTVKYGL
576	DIRFKPPTINILSKDYTADCVDVFSVVEKPKPIRLLNPGPNQGPYPNT G
577	DIPFRAPTVKIHSKQFSHRDVFDFSHVDYGKWERKTLRSRSLSRIGL
578	DIPFRAPTVKILAKQFSEKDIDFWHVGYGKWERRLVKSASSSRFGI
579	DIPFRAPTVKILSKQFTDKDVFDFHVDYGRWERKHRCASMSRVGL
580	DIKYKPPTIKILSKDYTADCVDVFSVERPKPIRLLTPTPGCG
581	ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVK KPHRYR PGTVA
582	SGRGKGGKG LGKGGAKRHRKVLRDNIQGITKPAI

TABLE 2: Relative Fluorescence of Transfection of HeLa Cells

SEQ ID NO	RF*	SEQ ID NO	RF	SEQ ID NO	RF
DOTMA	7551	327	5345	357	22679
107	75	328	32528	358	15132
205	26368	332	9198	359	3136
216	6181	335	26954	360	8516
218	11179	336	25262	361	3698
219	13721	338	6241	363	2590
220	9398	341	4108	365	36093
224	5559	342	33666	367	39008
226	7340	343	5910	369	14444
229	8816	344	14415	371	5180
230	8944	345	8493	373	8992
234	3867	347	22527	375	41076
236	7202	348	22289	377	15091
236	5042	349	4433	379	27424
237	21288	350	13313	381	22652
238	6067	351	12279	383	8012
239	2717	352	32126	450	21045
256	2243	353	29119	452	13888
268	25885	354	4403	454	34550
323	14067	355	32628	503	25368
326	18946				

*: RF = Relative Fluorescence

TABLE 3: Relative Fluorescence of Transfection of HuVec Cells

SEQ ID NO	RF*	SEQ ID NO	RF	SEQ ID NO	RF
DOTMA	12230	328	3614	359	3024
205	8309	332	4221	360	24042
216	14693	335	6350	361	6017
218	14827	336	5366	363	1383
219	6237	338	12000	365	14764
220	8737	341	15906	367	13354
224	15945	342	12804	369	6536
226	20864	343	16239	371	10669
229	5452	344	11867	373	28811
230	9067	345	2939	375	5542
234	13956	347	20728	377	9326
235	26940	348	21214	379	14573
236	14418	349	22907	381	17542
237	2302	350	13163	383	20267
238	11083	351	15379	450	20911
239	1404	352	3130	452	1841
256	3030	353	8772	454	13336
268	5403	354	19284	503	12123
323	12815	355	14810		
326	4932	357	6009		
327	21018	358	25398		

*: RF = Relative Fluorescence

TABLE 4: Relative Fluorescence of Transfection of NL-1 iPS Cells

SEQ ID NO	RF*	SEQ ID NO	RF	SEQ ID NO	RF
DOTMA	8965	327	31383	357	15624
107	1441	328	8611	358	21956
205	13140	332	8725	359	5548
216	19626	335	14424	360	27572
218	17049	336	14875	361	9363
219	5770	338	17288	363	4189
220	15892	341	20446	365	6364
224	25209	342	11493	367	17545
226	24920	343	21726	369	13124
229	13491	344	10895	371	17587
230	14524	345	7836	373	36290
234	14410	347	21865	375	5196
236	43542	348	21645	377	5474
236	22256	349	33103	379	6894
237	8632	350	19132	381	9644
238	15856	351	24646	383	21983
239	4129	352	11628	450	29856

SEQ ID NO	RF*		SEQ ID NO	RF		SEQ ID NO	RF
256	1825		353	9248		452	10349
268	15697		354	24043		454	18100
323	21694		355	7910		503	19014
326	9239						

*: RF = Relative Fluorescence

TABLE 5: Relative Fluorescence of Transfection of C2C12 Cells

SEQ ID NO	RF*		SEQ ID NO	RF		SEQ ID NO	RF
DOTMA	1289		328	5507		359	5693
205	2102		332	537		360	2090
216	1001		335	4689		361	585
218	3706		336	9647		363	1080
219	1346		338	2290		365	5390
220	1470		341	2141		367	16207
224	1469		342	9496		369	3322
226	1664		343	3218		371	983
229	1164		344	1678		373	3037
230	2606		345	6569		375	5633
234	681		347	4457		377	1710
236	958		348	8625		379	3189
236	2142		350	2631		381	8834
237	6491		351	3530		383	1876
238	1550		352	3324		450	3795
239	10620		353	4291		452	21798
256	3207		354	1912		454	9280
268	2568		355	1900		501	5913
323	7698		357	4506		502	2589
326	8553		358	2271		503	3973
327	2043						

*: RF = Relative Fluorescence

TABLE 6: Relative Fluorescence of Transfection of Human Fibroblast Cells

SEQ ID NO	RF*		SEQ ID NO	RF		SEQ ID NO	RF
DOTMA	3397		328	5641		358	5665
205	8550		332	5178		359	3206
216	3117		335	8534		360	5347
218	11230		336	10449		361	7014
219	7389		338	5625		363	3008
220	4516		341	2284		365	5219
224	5995		342	9525		367	10036
226	3431		343	4590		369	7590
229	6811		344	12704		371	4584
230	11771		345	2816		373	4872
234	4145		347	9255		375	7305

SEQ ID NO	RF*	SEQ ID NO	RF	SEQ ID NO	RF
236	3630	348	11802	377	6638
236	3000	349	8810	379	8520
237	2184	350	7105	381	9953
238	3348	351	8061	383	5896
239	4084	352	3059	450	6996
256	2656	353	8653	452	2779
268	4568	354	4998	454	7568
323	4826	355	6821	501	9473
326	3070	357	7167	503	13085
327	4662				

*: RF = Relative Fluorescence

TABLE 7: Relative Fluorescence of Transfection of Jurkat Cells

SEQ ID NO	RF*	SEQ ID NO	RF	SEQ ID NO	RF
DOTMA	9084	328	4770	358	18820
205	2298	332	3052	359	1880
216	3570	335	4567	360	16607
218	21451	336	4813	361	7006
219	10762	338	4475	363	2432
220	6428	341	18042	365	5298
224	13170	342	12825	367	4365
226	12976	343	23499	369	11085
229	8079	344	10160	371	6093
230	10229	345	2462	373	12628
234	5441	347	17720	375	4272
236	11006	348	15585	377	6785
236	4593	349	23167	379	7711
237	1501	350	9184	381	10806
238	6278	351	9730	383	14910
239	2343	352	3150	450	8779
256	3322	353	11107	452	2554
268	3236	354	16705	454	8224
323	2900	355	8132	501	12350
326	3018	357	2256	503	9411
327	14546				

*: RF = Relative Fluorescence

TABLE 8: Visual Confirmation of Transfection of Cells

Cell Line	SEQ ID NOs
Rat Cortical Neurons	220, 236, 238, 323, 327, 336, 338, 341, 343, 347, 348, 350, 351, 352, 354, 367, 369, 373, 375, 377, 454
THP-1	219, 229, 230, 239, 323, 328, 332, 341, 343, 350, 351, 357, 358, 375, 450, 454

Human Skeletal Muscle	218, 219, 230, 328, 336, 344, 350, 351, 353, 355, 365, 375
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TABLE 9: Relative Fluorescence DOTMA/SEQ ID NO:72/Peptide as Compared with Lipofectamine 2000 for the Transfection of HeLa Cells

Transfection Agent	RF*			
	0.1 [†]	0.2 [†]	0.3 [†]	0.4 [†]
Lipo [#]	12694	22154	21542	19286
DOTMA/72 [§]	10178	11133	8021	7032
SEQ ID NO [‡]				
218	12686	13103	10151	10081
219	12135	13841	13531	11712
236	5993	8846	8492	7316
237	8986	9753	8015	8288
268	10162	9934	10792	9519
323	15537	16620	10839	9244
326	6887	9487	10330	9227
328	11037	12229	10801	10951
335	12866	12458	11547	9753
336	14442	16702	13434	11334
341	6406	7469	8020	7546
342	41299	36040	28841	18351
343	6961	8626	8577	8160
344	13315	16387	12564	12511
347	23188	21493	16814	14140
348	17413	18615	16164	15054
350	24092	23238	19802	22255
351	7678	11499	10575	8791
353	36315	44772	36882	25802
355	18362	27990	22367	18289
357	7718	11798	9229	9107
358	12331	12683	13686	11097
360	9946	11168	10381	8995
365	26620	43775	36054	21540
367	10427	21411	23001	17529
369	8737	16706	14880	12645
375	22457	30891	34887	29024
377	39115	40072	32914	22457
379	50018	47049	39036	26448
381	34187	37463	27007	20088
450	11962	17346	12553	10241
452	6276	12671	12047	10918
454	34033	37099	27412	16023
501	23571	23993	20410	14749
503	12827	20006	17101	16206

*: RF = Relative Fluorescence

†: Volume of DOTMA/SEQ ID NO:72/Peptide in μL in well of a 96 well plate

#: Lipofectamine 2000

§: DOTMA and SEQ ID NO:72 with no additional peptide

‡: Peptide mixed with DOTMA/SEQ ID NO:72

Example 2: Plant Movement Proteins

[00159] Cells were plated so that on the day of transfection the cells were 70% confluent in 96 well tissue culture plates. A DOTMA/DOPE Lipid solution (1:1 molar ratio) at 2 mg/mL in water were mixed with an equal volume of plant virus movement protein derived peptides that had the nucleic acid binding moiety RRRRRRRRRRRR (SEQ ID NO:109) or KKKKKKKKKKKKKKKKKKK (SEQ ID NO: 102) covalently linked to the N-terminus, or C-terminus of each peptide during peptide synthesis. Peptides were at 2 mg/mL in water. The DOTMA/DOPE and the plant virus movement peptide solution was diluted 1 to 1 (v/v) in water. DOTMA/DOPE/PEPTIDE solutions were added to 0.1 mL of a plasmid DNA solution (EF1Alpha eGFP plasmid) at 10 $\mu\text{g/mL}$ in OptiMEM.

[00160] All solutions were at room temperature. A volume of 0.2 mL of a 10 $\mu\text{g/mL}$ solution of DNA was aliquoted into each well of a non-tissue cultured treated plate. 1-6 μL of transfection reagents were added to DNA solutions respectively. The transfection reagent and DNA solution was mixed by pipetting up and down twice. Transfection complexes were formed for 10 minutes. After 10 minutes, 0.01 or 0.02 mL of the transfection complex was added to cells. HuVEC, HeLa, human adult keratinocytes, human primary adult fibroblast, and A549 cells all respectively received 0.01 mL of the transfection complex. Human skeletal muscle cells, mouse C2C12 cells, and Jurkat, cells received 0.02 mL of the transfection complex. Cells were incubated for 42 hours at 37 °C at 5% CO_2 . Plates were read on a fluorescent plate reader. Cells were also examined visually under a microscope to assess the extent of transfection (in terms of the percent of cells transfected) with a fluorescent microscope. Other modes of analysis, for example quantification with B-galactosidase or luciferase reports plasmids can also be used. If the plate reader did not show a sufficient signal to noise ratio, then plates were scored for cells transfected and those peptides that show increase over DOTMA alone were noted.

Table 10: Relative Fluorescence of Transfection of Hela Cells

Transfection Agent						
SEQ ID NO						

543	12174	42502	38779	28451	13793	7705
545	11056	28529	28943	14693	13009	10902
544	10683	19151	19877	16908	12759	8938
540	6422	35653	27970	18620	13917	10336
541	17145	36476	37105	23644	20473	15523
542	9010	32901	47293	42152	35631	18856
DOTMA 1µg/ml	4424	4782	5377	6992	9694	11720
109	4157	5578	6606	8520	8007	7803
547	5899	9491	10275	11241	8829	7989
546	15190	15022	12772	11535	8117	7181
Blank	3912	3941	3860	3805	3990	3871

Table 11: Relative Fluorescence of Transfection of Human Primary Fibroblast Cells

Transfection Agent	Rf					
SEQ ID NO	1 µl	1.5 µl	2 µl	3 µl	4 µl	6 µl
Blank	26467	26415	26510	26317	26738	26620
543	30416	45401	39786	33091	28648	28698
545	34309	48184	37497	29067	28928	29256
544	31368	39311	37634	32892	31428	28367
540	29368	40610	35505	33853	33063	30219
541	35030	44894	42097	31837	29537	28921
542	30818	43609	46966	36343	33706	31787
DOTMA 1 µg/ml	28527	28021	28035	28646	29613	34911
109	28368	30373	29858	29895	30941	29386
547	29141	33704	35175	35563	33951	30539
546	31980	39259	41747	35020	32220	29476
Blank	26822	26658	26624	25988	27112	26703

Table 12: Relative Fluorescence of Transfection of HuVEC Cells

Transfection Agent	Rf					
SEQ ID NO	1 µl	1.5 µl	2 µl	3 µl	4 µl	6 µl
Blank	23420	23592	23275	23437	23969	23973
543	26622	36742	42802	40584	32754	25492
545	26545	36649	46890	34047	30893	27133
544	25788	28622	31245	34718	31159	28639
540	24771	34062	44744	44087	45137	30023
541	27405	46446	OVER	40401	31568	28125
542	24827	33896	42546	43231	35294	29326
DOTMA 1 µg/ml	24590	24571	24811	24463	26972	29850
109	24603	25735	25421	26641	26529	25907
547	24554	26172	28255	32494	31180	26775
546	25990	32762	37899	35528	31614	27690

Blank	24151	23427	23055	23320	23689	24147
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Example 3: Formulation 1

[00161] Formulation 1 comprised a combination of cationic lipids, neutral lipids, and peptides, as described above. Specifically, the cationic lipids used in Formulation 1 included DPePE-Sp-OMe and DPB-Arg. These cationic lipids are compounds of Formula 1, where:

DPePE-Sp-OMe = $R^1 = R^2 = C_{16}H_{31}$, $X = O$, $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)-spermine$, $y=0$;

DPB-Arg = $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_{16}H_{31}$, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$.

[00162] The neutral lipids included dioleoylphosphatidylethanolamine (DOPE), diphytanoylphosphatidylethanolamine (DPhPE), and 3-oleyloxy-2-hydroxy-1-acetamidopropane (HOAP).

[00163] The cationic lipid and the neutral lipid are combined in ethanol in the ratio of 1:8 (cationic lipid:neutral lipid). Formulation 1 was obtained by mixing the lipid with water and the peptide in the ratio of 0.2:1:1 (lipid mix:water:peptide mix). Specifically, the following peptides were used to prepare Formulation 1:

Peptide A: SEQ ID NO:507 covalently linked to SEQ ID NO:109 (4.1 mg/mL in HEPES);

Peptide B: SEQ ID NO:540 (4.1 mg/mL in HEPES);

Peptide C: SEQ ID NO:350 covalently linked to SEQ ID NO:109 (4.1 mg/mL in HEPES);

Peptide D: SEQ ID NO:47 (1 mg/mL in TRIS).

[00164] For Formulation 1, the lipid component comprised lipids in the molar ratio of 0.50:0.50:2.666:2.666:2.666 (DPePe-Sp-OMe:DPB-Arg: DOPE:DPhPE:HOAP). This translates to a cationic lipid:neutral lipid ratio of 1:8. The mixture of peptides in the formulation were present in the ratio of 1:2:2:0.5 by volume (Peptide A:Peptide B:Peptide C:Peptide D).

Example 4: Transfection

[00165] Human Adult Dermal Fibroblast was obtained from ScienCell Research Laboratories (Carlsbad, CA) and grown according to manufacturer's instructions. Human Adult Keratinocytes were obtained from ThermoFisher Scientific and grown according to manufactures instructions in Keratinocyte-SFM. Cells were plated to obtain approximately 70% confluency the day of transfection in 96-well tissue culture treated plates. Green Fluorescent Protein expression plasmid was used in these experiments. Transfection complexes for Lipofectamine 3000 were prepared according to manufacturer's instructions. All solutions used to form transfection complexes were at room temperature for transfection. Into each well of a non-tissue culture treated plate containing 0.05 mL of OptiMEM were aliquoted 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 μ L of the Formulation 1 transfection reagent. A volume of 0.05 mL of a 20 μ g/mL solution of DNA in OptiMEM was aliquoted into each well of a non-tissue culture treated plate that contained transfection reagent aliquots. The transfection reagent and DNA solution were mixed by pipetting up and down twice. Transfection complexes were formed for 10 minutes at room temperature. After 10 minutes, 0.01 mL of the transfection complex was added to cells. Cells were incubated for 42 hours at 37 °C at 5% CO₂. Plates were read on a fluorescent plate reader. The results are shown in Tables 13 and 14. Cells were also examined visually under a microscope to assess the extent of transfection (regarding the percent of cells transfected) with a fluorescent microscope. Other modes of analysis, for example, quantification with B-galactosidase or luciferase reports plasmids, can also be used.

Table 13: Relative Fluorescence of Transfection of Human Primary Adult Fibroblasts

Volume of Transfection Reagent /well	Formulation 1	Lipofectamine 3000	Blank
0.1 μ L	21851	9648	9373
0.15 μ L	21359	10041	9017
0.2 μ L	26793	12509	9135
0.3 μ L	35137	15193	9088
0.4 μ L	42224	16971	9200
0.6 μ L	42154	18095	9345

Table 14: Relative Fluorescence of Transfection of Human Primary Adult Keratinocytes

Volume of Transfection Reagent /well	Formulation 1	Lipofectamine 3000	Blank
0.1 μ L	41003	15028	13843
0.15 μ L	36375	15981	13820
0.2 μ L	33300	15538	13674
0.3 μ L	23132	14780	13412
0.4 μ L	19578	15176	13987
0.6 μ L	17126	14934	13704

WHAT IS CLAIMED IS:

1. A transfection complex comprising a transfection enhancer and a cell surface ligand containing adhesion peptide, a plant virus movement protein, or peptide derived therefrom.
2. The transfection complex of claim 1, wherein the transfection enhancer is a peptide.
3. The transfection complex of claim 2, wherein the peptide is an amphipatic peptide.
4. The transfection complex of claim 2, wherein the peptide is a fusion peptide.
5. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:1-580.
6. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.
7. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 237, 268, 326, 328, 335, 336, 342, 347, 348, 352, 353, 355, 357, 358, 365, 367, 377, 379, 381, 450, 454, and 503.
8. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.
9. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:236, 358, and 373.
10. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.
11. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:216, 224, 226, 236, 236, 323, 327, 341, 343, 347, 348, 349, 350, 351, 354, 358, 360, 373, 383, 450, 454, and 503.

12. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, 502, and 503.

13. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:218, 230, 237, 239, 256, 323, 326, 328, 335, 336, 342, 343, 345, 347, 348, 352, 357, 359, 367, 375, 379, 381, 450, 452, 454, 501, 502, and 503.

14. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, and 503.

15. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 218, 219, 229, 230, 335, 336, 342, 344, 348, 349, 350, 351, 353, 355, 357, 361, 367, 369, 375, 379, 381, 450, 454, 501, and 503.

16. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, and 503.

17. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:218, 349, and 358.

18. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs: 220, 236, 238, 323, 327, 336, 338, 341, 343, 347, 348, 350, 351, 352, 354, 367, 369, 373, 375, 377, and 454.

19. The transfection complex of claim 1, wherein the peptide is selected from the group consisting of SEQ ID NOs:219, 229, 230, 239, 323, 328, 332, 341, 343, 350, 351, 357, 358, 375, 450, and 454.

20. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs: 218, 219, 230, 328, 336, 344, 350, 351, 353, 355, 365, and 375.

21. The transfection complex of claim 2, wherein the peptide is selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 226, 229, 230, 236, 237, 238,

239, 256, 268, 323, 326, 327, 328, 332, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 365, 367, 369, 373, 375, 377, 379, 381, 393, 450, 452, 454, 501, and 503.

22. The transfection complex of any one of claims 1-21, further comprising at least one helper lipid component.

23. The transfection complex of claim 22, wherein the helper lipid component is selected from the group consisting of a pegylated lipid, a cationic lipid, a pegylated cationic lipid, a neutral lipid, a pegylated neutral lipid, an exosome mixture, total lipid from exosomes, total lipid and protein derived from exosomes, a cationic polymer, a pegylated cationic polymer, a targeting moiety, and a combination thereof.

24. The transfection complex of claim 22, wherein the helper lipid is selected from the group consisting of cholesterol, cholesterol derivatives, sterols, phytosterols, zoosterols, and hopanoids.

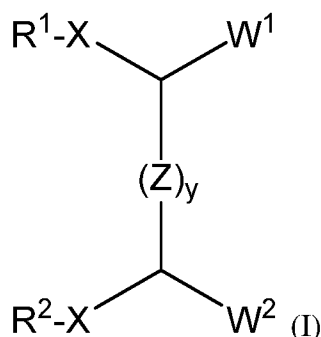
25. The transfection complex of claim 22, wherein the cationic lipid is selected from the group consisting of N-(2-bromoethyl)-N,N-dimethyl-2,3-bis(9-octadecenyloxy)-propana minimum bromide (BMOP), dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DDPES), DSPC, dioleoylphosphatidylethanolamine (DOPE), formulation of cetyltrimethylammonium bromide (CATB) and DOPE (CTAB:DOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), DMG, 4-dimethylaminopyridine (DMAP), dimyristoylphosphatidylethanolamine (DMPE), DOMG, DMA, Dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), Dipalmitoylethylphosphatidylcholine (DPEPC), dioleoyldimethylammonium chloride (DODAC), 1,3-di-oleoyloxy-2-(6-carboxyspermyl)-propylamid (DOSPER), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniumchloride (DOTMA), didoceyl methylammonium bromide (DDAB), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP), DOTAP.Cl, 3,β-N,(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-chol), 2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA), O,O'-ditetradecanoyl-N-(alphatrimethylammonioacetyl) diethanolamine chloride (DC-6-14), dicaproylphosphatidylethanolamine (DCPE), dilauryl oxypropyl-3-dimethylhydroxy ethylammonium bromide (DLRIE), 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), Ethyl-PC, 2,3-dioleoyloxy-N-[2-(sperminecarboxamidoethyl)-N,N-di-met-hyl-1-propanaminium trifluoroacetate (DOSPA), dioctadecylamidoglycyl carboxyspermine (DOGS), N-[1-(2,3 dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl) ammonium

bromide (DMRIE), Dioleoyl-ethyl-phosphocholine (DOEPC), N-[1-(2,3-dioleoyloxy)propyl]-N-[1-(2-hydroxyethyl)]-N,N-dimethylammonium iodide (DOHME), N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propaniminium bromide/dioleoylphosphatidylethanolamine (GAP-DLRIE:DOPE), dipalmitoylphosphatidylcholine (DPPC), 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(3-lysyl(1-glycerol))].Cl (DOPG), N-lauroylsarcosine, (R)-(+)-limonene, lecithins (and derivatives thereof); phosphatidylethanolamine (and derivatives thereof); phosphatidylethanolamines, dioleoylphosphatidylethanolamine, diphytanoylphosphatidylethanolamine (DPhPE), dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylethanolamine, 3-β-[1-ornithinamidocarbonyl]-cholesterol (O-Chol), palmitoyl-oleoylphosphatidylethanolamine (POPE); distearoylphosphatidylethanolamine; phosphatidylcholine; phosphatidylcholines; dipalmitoylphosphatidylcholine (DPPC) palmitoyl-oleoylphosphatidylcholine (POPC); distearoylphosphatidylcholine; phosphatidylglycerol; piperazine-based cationic lipids; a phosphatidylglycerol; dioleoylphosphatidylglycerol (DOPG); dipalmitoylphosphatidylglycerol (DPPG); distearoylphosphatidylglycerol; phosphatidylserine (and derivatives thereof); phosphatidylserines; dioleoyl- or dipalmitoylphosphatidylserine; a diquaternary ammonium salt; 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmitylamino)propyl]piperazine; N-(3-aminopropyl)-N,N'-bis-(dodecyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N,N'-bis-(oleoyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N,N'-bis-(palmitoyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N,N'-bis-(myristyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-dodecyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-oleoyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-palmitoyloxyethyl)-piperazinium bromide; N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-myristyloxyethyl)-piperazinium bromide; 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine; 1,4-bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxy-propyl]piperazine; or 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxy-propyl]piperazine; 3-alkyloxy-2-hydroxy-1-histidylamidopropane, 3-alkyloxy-2-hydroxy-1-aminopropane; 4-alkyloxy-3-hydroxy-1-histidylamidopropane, 4-alkyloxy-3-hydroxy-1-aminopropane; 5-alkyloxy-4-hydroxy-1-histidylamidopropane, 5-alkyloxy-4-hydroxy-1-aminopropane; 6-alkyloxy-5-hydroxy-1-histidylamidopropane; 6-alkyloxy-4-hydroxy-1-aminopropane; 2,3-dialkoxy-1,4-bis(N-methyl-N-carboxyspermineamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-

methyl-N-histidinylamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-arginyl-amido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-lysinylamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N'-ornithinylamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-serinylamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-homoerinylamido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-(diaminobutanyl)amido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-(diaminopropyl)amido)aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-(2-hydroxypropylamine))aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-(2-diaminopropyl))aminobutane; 2,3-dialkoxy-1,4-bis(N-methyl-N-propylamine)aminobutane; N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,2-ethanediamine (TmedEce); N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,3-propanediamine (PropEce); N,N'-dioleoyl-N,N,N',N'-tetramethyl-1,6-hexanediamine (HexEce); and their corresponding N,N'-dicetyl saturated analogues (TmedEce, PropEce and HexEce); a diphosphatidylglycerol; a fatty acid ester; a monocationic transfection lipid; 1-deoxy-1-[dihexadecyl(methyl)ammonio]-D-xylitol; 1-deoxy-1-[methyl(ditetradecyl)ammonio]-Darabinitol; 1-deoxy-1-[dihexadecyl(methyl)ammonio]-D-arabinitol; a 1-deoxy-1-[methyl(dioctadecyl)-ammonio]-darabinitol; glycerol ester; sphingolipids; cardolipin; a cerebroside; a ceramide, a lipid serine ; and combinations thereof.

26. The transfection complex of claim 22, wherein the neutral lipid is selected from the group consisting of cholesterol and derivatives thereof, a 3β OH-sterol and derivatives thereof, phosphatidyl choline, LIPOFECTIN® CELLFECTIN® (1:1.5 (M/M) formulation of N,N',N'',N'''-tetramethyl-N,N,N',N'',N'''-tetrapalmitylspermine (TMTPS), dioleoyl phosphatidylethanolamine (DOPE), LIPOFECTACE®, GS 2888 CYTOFECTIN®, FUGENE 6®, EFFECTENE®, and LIPOFECTAMINE®, LIPOFECTAMINE 2000®, LIPOFECTAMINE PLUS®, LIPOTAXI®, POLYECT®, SUPERFECT®, TFXNT™, TRANSFAST™, TRANSFECTAM®, TRANSMESSENGER®, vectamidine (3-tetradecylamino-N-tert-butyl-N'-tetradecylpropionamidine (a.k.a. diC14-amidine), OLIGOFECTAMINE MessengerMAX, GeneIn™, TransfeX™, LipofectAmine 3000, Lipofectin®, DMRIE-C, CellFectin®, LipofectAce®, Fugene®, Fugene® HD, Tfx-10®, Tfx-20®, Tfx-50®, DNA-In, Transfectin™, SilentFect™, Effectene®, ViaFect™, DC-chol, GenePorter®, DharmaFect 1®, DharmaFect 2®, DharmaFect 3®, DharmaFect 4®, Escort™ III, Escort™ IV, and DOGS.

27. The transfection complex of claim 22, where in the helper lipid is the cationic lipid of Formula I:



or a pharmaceutically acceptable salt thereof, where

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-OH}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(OH)-CH}_2\text{-(CH}_2\text{)}_n\text{-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH-iso-butyl)-CH}_2\text{-(CH}_2\text{)}_n\text{-O-iso-butyl}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-C(=NH)-NH}_2$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-C(NH}_2\text{)-CH}_2\text{-(CH}_2\text{)}_n\text{-His}$, $n = 0\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$, $\text{R}^4 = \text{-C(=O)-spermine}$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{H}$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2\text{(CH}_2\text{)}_m\text{-NH}_2$; $m = 1\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2\text{(CH}_2\text{)}_m\text{-NH-C(=O)-spermine}$; $m = 1\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{CH}_2\text{(CH}_2\text{)}_m\text{-NH-(C=O)-amino acid side chain}$; $m = 1\text{--}6$, $y = 0$; or

$\text{W}^1 = \text{W}^2 = \text{CH}_2\text{-N(R}^3\text{R}^4)$, $\text{R}^1 = \text{R}^2 = \text{C}_8\text{--C}_{22}$ alkyl, $\text{X} = \text{O}$; $\text{R}^3 = \text{CH}_3$; $\text{R}^4 = \text{(CH}_2\text{)}_m\text{(CH-OH)(CH}_2\text{)}_m\text{-NH}_2$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $y = 0$; or
 $W^1 = H$, $W^2 = CH_2-N(R^3R^4)CH_2-R^5$; $R^3 = R^4 = CH_3$; $R^5 = H$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)-spermine$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)-amino\ acid\ side\ chain$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-N^+(CH_3)_3Cl$, $y = 0$; or
 $W^1 = H$; $W^2 = CH_2-O-P(=O)(O^-)-O-CH_2CH_2-NH-C(=O)-spermine$, $y = 0$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6, q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6, q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6, q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-}iso\text{-}butyl)-CH_2-(CH_2)_n-O\text{-}iso\text{-}butyl$, $n = 0-6, y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6, q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-His$, $n = 0-6, q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-spermine$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)-spermine$; $m = 1-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)-amino\ acid\ side\ chain$; $m = 1-6$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH_2-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or
 $Z = (CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_p-N(R^3)-(CH_2)_p$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n=0-6$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)\text{-spermine}$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=(CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n=0-6$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-His$, $n=0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$, $R^4=-C(=O)-spermine$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)-spermine$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-(C=O)-amino\ acid\ side\ chain$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=(CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=0$ or 1 ; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$;
 $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$; or

$W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $y=0$ where B is selected from the group –
 $O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n=0-6$, $y=0$ where B is selected from the group –
 $O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $y=0$ where B is selected from the group –
 $O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $y = 0$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m = 1-6$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1 = H$ $W^2 = -CH_2-B-CH_2-N(R^3R^4)CH_2-R^5$; $R^3 = R^4 = CH_3$; $R^5 = H$, $y = 0$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)\text{-spermine}$, $y = 0$; or

$W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)\text{-amino acid side chain}$, $y = 0$; or

$W^1=H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-N^+(CH_3)_3Cl$, $y = 0$; or

$W^1=H$; $W^2 = CH_2-O-P(=O)(O^-)-O-CH_2CH_2-NH-C(=O)-spermine$, $y = 0$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6, q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6, y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-(C=O)$ -amino acid side chain; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_p-N(R^3)-(CH_2)_p$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)$ -spermine, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)$ -spermine; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)$ -amino acid side chain; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=(CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n=0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n=0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n=0-6, y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n=0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n=0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$, $R^4=-C(=O)\text{-spermine}$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=(CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1=W^2=-CH_2-B-CH_2-N(R^3R^4)$, $R^1=R^2=C_8-C_{22}$ alkyl, $X=O$; $R^3=CH_3$; $R^4=CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-S-S-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m=1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-N(R^3)-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = COCH_2(OCH_2CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-amino acid side chain}$; $m = 1-6$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH-OH)(CH_2)_m-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_n-NH_2$, $q=1-3$, $y=1$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-OH$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(OH)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH\text{-iso-butyl})-CH_2-(CH_2)_n-O\text{-iso-butyl}$, $n = 0-6$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n\text{-His}$, $n = 0-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)\text{-spermine}$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

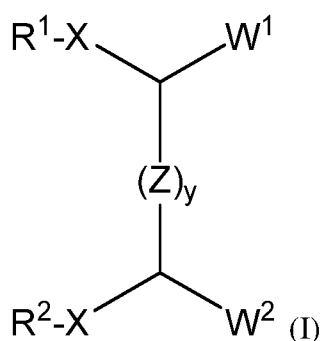
$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = H$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH_2$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z=(CH_2)_q-O-(CH_2)_q$, $W^1 = W^2 = -CH_2-B-CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m-NH-C(=O)\text{-spermine}$; $m = 1-6$, $q=1-3$, $y=1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i=1-5$; or

$Z = (CH_2)_q - O - (CH_2)_q$, $W^1 = W^2 = -CH_2 - B - CH_2 - N(R^3 R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = CH_2(CH_2)_m - NH - (C=O) - \text{amino acid side chain}$; $m = 1-6$, $q = 1-3$, $y = 1$ where B is selected from the group $-O(CH_2)_i$, $-S(CH_2)_i$, $-S-S(CH_2)_i$, $-SO_2-(CH_2)_i$, $i = 1-5$.

28. The transfection complex of claim 22, where in the helper lipid is the cationic lipid of Formula I:



or a pharmaceutically acceptable salt thereof, where

$R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$, $W^1 = H$; $W^2 = CH_2 - O - P(=O)(OMe) - O - CH_2CH_2 - NH - C(=O) - \text{spermine}$, $y = 0$;

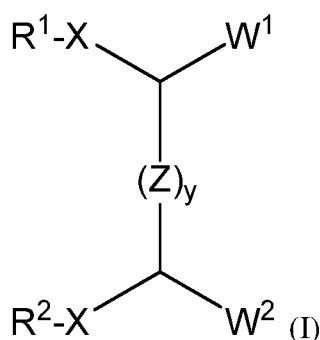
$W^1 = W^2 = CH_2 - N(R^3 R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O) - C(NH_2) - CH_2 - (CH_2)_n - C(=NH) - NH_2$, $n = 0-6$, $y = 0$;

$W^1 = W^2 = CH_2 - N(R^3 R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O) - \text{spermine}$, $y = 0$;

$W^1 = W^2 = CH_2 - N(R^3 R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O) - C(NH_2) - CH_2 - (CH_2)_n - NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2 - N(R^3 R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH_2)_m - OH)(CH_2)_m - NH_2$, $y = 0$.

29. The transfection complex of claim 22, where in the helper lipid is the cationic lipid of Formula I:

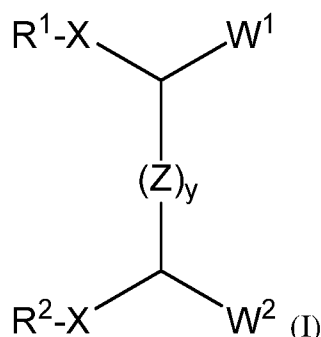


or a pharmaceutically acceptable salt thereof, where

$R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$, $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)-spermine$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$.

30. The transfection complex of claim 22, where in the helper lipid is the cationic lipid of Formula I:

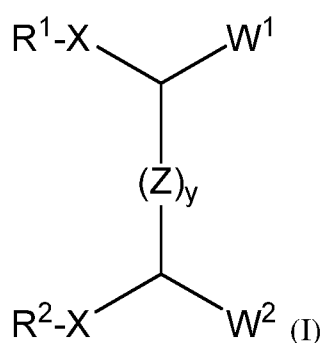


or a pharmaceutically acceptable salt thereof, where

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$; $R^4 = (CH_2)_m(CH_2OH)(CH_2)_m-NH_2$, $y = 0$.

31. The transfection complex of claim 22, where in the helper lipid is the cationic lipid of Formula I:



or a pharmaceutically acceptable salt thereof, where

$R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$, $W^1 = H$; $W^2 = CH_2-O-P(=O)(OMe)-O-CH_2CH_2-NH-C(=O)-spermine$, $y = 0$;

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^1 = R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-C(NH_2)-CH_2-(CH_2)_n-C(=NH)-NH_2$, $n = 0-6$, $y = 0$; or

$W^1 = W^2 = CH_2-N(R^3R^4)$, $R^2 = C_8 - C_{22}$ alkyl, $X = O$; $R^3 = CH_3$, $R^4 = -C(=O)-$ spermine, $y = 0$.

32. The transfection complex of any one of claims 1-31, wherein the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, comprise a nucleic acid binding moiety.

33. The transfection complex of claim 32, wherein the nucleic acid binding moiety is a DNA binding domain.

34. The transfection complex of claim 32, wherein the nucleic acid binding moiety comprises a group selected from the group consisting of a polycationic peptide sequence, a polyamine, a peptide nucleic acid, spermine, spermidine, carboxyspermidine, carboxy spermine, spermine and spermidine analogs, and nucleic acid intercalaters.

35. The transfection complex of claim 32, wherein the nucleic acid binding moiety comprises a peptide.

36. The transfection complex of claim 35, wherein the peptide comprises an amino acid sequence derived from a cell binding adhesion proteins or a plant virus movement protein.

37. The transfection complex of claim 34, wherein the plant virus movement is obtained from Gemini Viruses.

38. The transfection complex of claim 35, wherein the peptide comprises at least 5 amino acid residues of the cell binding adhesion proteins or a plant virus movement protein.

39. The transfection complex of any one of claims 1-38, wherein the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, facilitates the delivery of a nucleic acid molecule into a cell or a tissue.

40. The transfection complex of any one of claims 1-39, wherein the delivery into the cell or the tissue is either in vivo or in vitro.

41. The transfection complex of claim 32, wherein the nucleic acid binding moiety binds to nucleic acid molecule selected from the group consisting of primers, probes, oligonucleotides, vectors, constructs, plasmids, genes, transgenes, genomic DNA, cDNA, RNA, mRNA, tRNA, miRNA, RNAi, siRNA, shRNA, stRNA, guide-RNA, PCR products, restriction fragments, mRNA reprogramming molecules, telomerase mRNA molecules, Cas9 mRNA or DNA molecules that code for gRNA, CRE mRNA molecules, LoxP containing DNA molecules, and SV40T antigen mRNA and DNA molecules.

42. The transfection complex of claim 32, wherein the nucleic acid binding moiety comprises a sequence selected from the group consisting of SEQ ID NOs:93 and 97-137.

43. The transfection complex of claim 32, wherein the nucleic acid binding moiety is a nucleic acid condensing peptide.

44. The transfection complex of claim 43, wherein the nucleic acid condensing peptide comprises a sequence selected from the group consisting of SEQ ID NOs:33, 93, 97-127, 129, 132-137, and 147-149.

45. The transfection complex of claim 32, wherein the nucleic acid binding moiety is functionally linked, by a linkage comprising a covalent link or a spacer, to an amino acid sequence of the cell surface ligand containing adhesion peptide, the plant virus movement protein, or peptide derived therefrom, or a lipid

46. The transfection complex of claim 45, wherein the spacer comprises:

- i) a hydrophilic moiety and comprises about 6 to 30 carbon atoms;
- ii) a polyether;
- iii) a hydrophilic polymer;
- iv) a peptide of sequence APYKAWK (SEQ ID NO:505); or
- v) the GPI anchor peptide FTLTGLLGTLVTMGLLT (SEQ ID NO:504).

47. The transfection complex of claim 32, wherein the nucleic acid binding moiety comprises spermine, spermine derivative, spermidine, histones or fragments thereof, protamines or fragments thereof, HMG proteins or fragments thereof, poly-lysine, poly-arginine, poly-histidine, polyamines, cationic peptides, nucleic acid intercalators, protein nucleic acid sequences, and aptamers.

48. The transfection complex of claim 47, wherein the cationic peptide comprises repeats of lysine or arginine.

49. The transfection complex of claim 48, wherein the cationic peptide comprises between 8-20 lysine residues (K8-K20) (SEQ ID NO: 583) or between 8-20 arginine residues (R8-R20) (SEQ ID NO: 584).

50. The transfection complex of any one of claims 1-49, the plant virus movement protein, or peptide derived therefrom, is used in combination with the cell surface ligand selected from the group consisting of SEQ ID NOs:202-500.

51. The transfection complex of any one of claims 1-50, wherein the transfection enhancer is selected from the group consisting of a peptide or non-peptide

transfection enhancer, an exosome, an exosome mixture, total lipid from exosomes, total lipid and protein derived from exosomes, a nuclear localization protein or peptide, a fusogenic peptide or non-peptide agent, a peptide or non-peptide endosomal release agent, a viral peptide or protein, a lysomolotropic agent, a cell penetration agent, a nuclear targeting agent, a cell surface ligand and a combination thereof.

52. The transfection complex of claim 51, wherein the viral peptide or protein is derived from a virus selected from the group consisting of an enveloped or non enveloped virus, influenza virus, a vesicular stomatitis virus, an adenovirus, an alphavirus, a Semliki Forest Virus, a hepatitis virus, a herpes virus, an HIV virus, and a simian virus.

53. The transfection complex of claim 51, wherein the transfection enhancer is selected from the group consisting of insulin, a transferrin, a epidermal growth factor, a fibroblast growth factor, a cell targeting antibody or fragment from an antibody, a lactoferrin, a fibronectin, an adenovirus penton base, Knob, a hexon protein, a vesicular stomatitis virus glycoprotein, a Semliki Forest Virus core protein, an influenza hemagglutinin, a hepatitis B core protein, an HIV Tat protein, a herpes simplex virus VP22 protein, a histone protein, an arginine rich cell permeability protein, a high mobility group protein, and invasins protein, an internalin protein, an endotoxin, a diphtheria toxin, a shigella toxin, a melittin, a magainin, a gramicidin, a cecrophin, a defensin, a protegrin, a tachyplesin, a thionin, a indolicidin, a bactenecin, a drosomycin, an apidaecin, a cathelicidin, a bacteriacidal-permeability-increasing protein, a nisin, a buforin, and fragments thereof.

54. The transfection complex of claim 51, wherein the nuclear localization protein or peptide is a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:40.

55. The transfection complex of claim 1, wherein the transfection enhancer comprises chloroquine, a lysosomotropic compound or combinations thereof.

56. The transfection complex of claim 1, further comprising a biologically active agent.

57. The transfection complex of claim 1, further comprising one or more transfection helpers or targeting moieties in combination with the cell surface ligand.

58. The transfection complex of claim 57, wherein the targeting moiety is a molecule that targets a transfection complex to a specific tissue or cell type for targeted delivery of a biologically agent thereto.

59. The transfection complex of claim 58, wherein the targeting moiety is a peptide, a modified peptide, an antibody, a modified antibody, a receptor molecule, a modified receptor molecule, a single or a double stranded nucleic acid molecule, a modified single or double stranded nucleic acid molecule, a peptide or nucleic acid aptamer, a modified peptide or nucleic acid aptamer, an organic molecule, and a polysaccharide.

60. The transfection complex of any one of claims 1-59, further comprising a fusion agent.

61. The transfection complex of claim 60, wherein the fusion agent is a sequence selected from the group consisting of SEQ ID NOs:42-92.

62. The transfection complex of any one of claims 1-61, further comprising a cell penetration agent.

63. The transfection complex of claim 62, wherein the cell penetration agent is a sequence selected from the group consisting of SEQ ID NOs:74 and 93-96.

64. The transfection complex of any one of claims 1-63, further comprising a peptide or protein selected from the group consisting of a collagen, a fibronectin, a lamin, a veronectin, a cadherin, a nidogen, a fibrinogen, a elastin, a bone asialoprotein, a osteopontin, a tenascin-C, Avadin, insulin, a transferrin, a epidermal growth factor, a fibroblast growth factor, a cell targeting antibody, a lactoferrin, an adenovirus penton base, a knob protein, a hexon protein, a vesicular stomatitis virus glycoprotein, a Semliki Forest Virus core protein, an influenza hemagglutinin, a hepatitis B core protein, an HIV Tat protein, a herpes simplex virus VP22 protein, a histone protein, an arginine rich cell permeability protein, a high mobility group protein, invasin protein, internalin protein, an endotoxin, a non-toxic diphtheria toxin, a non-toxic shigella toxin, a melittin, a magainin, a gramicidin, a cecrophin, a defensin, a protegrin, a tachyplesin, a thionin, an indolicidin, a battenecin, a drosomycin, an apidaecin, a cathelicidin, a bacteriacidal-permeability-increasing protein, a nisin, a buforin, a fragment thereof, and a sequence selected from the group consisting of SEQ ID NOs:150-197.

65. The transfection complex of any one of claims 1-64, further comprising at least one polyamine moiety selected from the group consisting of dense star dendrimers, PAMAM dendrimers, NH₃ core dendrimers, ethylenediamine core dendrimers, dendrimers of generation 5 or higher, dendrimers with substituted groups, dendrimers having one or more amino acids, grafted dendrimers, activated dendrimers, polyethylenimine, and polyethylenimine conjugates, polycationic peptides such as polylysine, polyornithine, polyhistidine, polyarginine,

66. A pharmaceutical composition comprising the transfection complex of any one of claims 1-65, and a pharmaceutically acceptable carrier.

67. A method of transfecting a cell, the method comprising the steps of:
obtaining a transfection complex of any one of claims 1-62; and
contacting a cell with the transfection complex.

68. The method of claim 67, wherein the cell is selected from the group consisting of a primary cell culture, a passaged cell culture, suspension cell line and an attached cell line.

69. The method of claim 67, wherein the cell is a HeLa cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.

70. The method of claim 67, wherein the cell is a HuVec cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.

71. The method of claim 67, wherein the cell is an NL-1 iPS cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:107, 205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, and 503.

72. The method of claim 67, wherein the cell is a C2C12 cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, 502, and 503.

73. The method of claim 67, wherein the cell is a human fibroblast cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID

NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, and 503.

74. The method of claim 67, wherein the cell is a Jurkat cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:205, 216, 218, 219, 220, 224, 226, 229, 230, 234, 236, 236, 237, 238, 239, 256, 268, 323, 326, 327, 328, 332, 335, 336, 338, 341, 342, 343, 344, 345, 347, 348, 349, 350, 351, 352, 353, 354, 355, 357, 358, 359, 360, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 450, 452, 454, 501, and 503.

75. The method of claim 67, wherein the cell is a rat cortical neuron cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs: 220, 236, 238, 323, 327, 336, 338, 341, 343, 347, 348, 350, 351, 352, 354, 367, 369, 373, 375, 377, and 454.

76. The method of claim 67, wherein the cell is a THP-1 cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs:219, 229, 230, 239, 323, 328, 332, 341, 343, 350, 351, 357, 358, 375, 450, and 454.

77. The method of claim 67, wherein the cell is a human skeletal muscle cell and the transfection complex comprises a peptide selected from the group consisting of SEQ ID NOs: 218, 219, 230, 328, 336, 344, 350, 351, 353, 355, 365, and 375.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/20145

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 32-54, 60-77
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please see supplemental page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 22-31, 55-59; SEQ ID NO: 1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/20145

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07D 233/64; C12N 15/88; C07C 217/88, 237/10; A61K 47/48 (2017.01)

CPC - A61K 31/195, 47/48046; C07D 233/64; C12N 15/88, 15/1137; C07C 217/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0213637 A1 (MOLECULAR TRANSFER, INC.) July 31, 2014; abstract; paragraphs [0003], [0010], [0015], [0016], [0025], [0043], [0044], [0089], [0090], [0105], [0112], [0115], [0122]; SEQ ID NO: 1	1-5, 22/1-5, 23/22/1-5, 24/22/1-5, 25/22/1-5, 26/22/1-5, 27/22/1-5, 28/22/1-5, 29/22/1-5, 30/22/1-5, 31/22/1-5, 56-59 ----- 55
Y	WO 2007/130073 A2 (MOLECULAR TRANSFER, INC.) November 15, 2007; page 4, line 21; page 31, line 34	55
A	US 2002/0138497 A1 (LEWANDOWSKI, DJ et al.) September 26, 2002; entire document	1-5, 22/1-5, 23/22/1-5, 24/22/1-5, 25/22/1-5, 26/22/1-5, 27/22/1-5, 28/22/1-5, 29/22/1-5, 30/22/1-5, 31/22/1-5, 55-59
A	US 2005/0177900 A1 (NISHIGUCHI, M et al.) August 11, 2005; entire document	1-5, 22/1-5, 23/22/1-5, 24/22/1-5, 25/22/1-5, 26/22/1-5, 27/22/1-5, 28/22/1-5, 29/22/1-5, 30/22/1-5, 31/22/1-5, 55-59

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 June 2017 (29.06.2017)

Date of mailing of the international search report

19 JUL 2017

Name and mailing address of the ISA/

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/20145

-Continued from Box No.III: Lack of Unity of Invention-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-31, 55-59 and SEQ ID NO: 1 are directed toward a transfection complex comprising a transfection enhancer and a cell surface ligand containing adhesion peptide, a plant virus movement protein, or peptide derived therefrom.

The transfection complex will be searched to the extent it encompasses a transfection enhancer peptide encompassing SEQ ID NO: 1 (first exemplary transfection enhancer peptide). Applicant is invited to elect additional transfection enhancer peptide(s), with specified SEQ ID NO: for each, to be searched. Additional transfection enhancer peptide sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-4, 5 (in-part), 22 (in-part), 23 (in-part), 24 (in-part), 25 (in-part), 26 (in-part), 27 (in-part), 28 (in-part), 29 (in-part), 30 (in-part), 31 (in-part) and 55-59 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (transfection enhancer peptide). Applicants must specify the claims that encompass any additionally elected transfection enhancer peptide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a transfection enhancer peptide sequence encompassing SEQ ID NO: 2 (first exemplary elected transfection enhancer peptide sequence).

No technical features are shared between the transfection enhancer peptide sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a transfection complex comprising a transfection enhancer and a cell surface ligand containing adhesion peptide, a plant virus movement protein, or peptide derived therefrom.

However, these shared technical features are previously disclosed by US 2014/0213637 A1 to Molecular Transfer, Inc. (hereinafter 'Molecular').

Molecular discloses a transfection complex (a transfection enhancing complex; abstract) comprising a transfection enhancer (comprising fusion enhancer (a transfection enhancer); paragraph [0003]) and a cell surface ligand containing adhesion peptide (a cell surface ligand containing adhesion peptide; paragraphs [0003], [0088]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Molecular reference, unity of invention is lacking.