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VITAMIN CATIONIC LIPIDS

Abstract

Disclosed are cationic lipids comprising a vitamin substructure and an ionizable nitrogen-containing group. Cationic lipids provided herein can be useful for delivery and expression of mRNA and encoded protein, e.g., as a component of liposomal delivery vehicle, and accordingly can be useful for treating various diseases, disorders and conditions, such as those associated with deficiency of one or more proteins.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] The present application is a divisional of U.S. patent application Ser. No. 17/058,574, filed Nov. 24, 2020, which is a 35 U.S.C. § 371 filing of International Patent Application No. PCT/US2019/034461, filed May 29, 2019, which claims the benefit of U.S Provisional Patent Application Nos. 62/677,818, filed May 30, 2018; 62/677,828, filed May 30, 2018; 62/677,851, filed May 30, 2018; 62/677,855, filed May 30, 2018;

62/807,671, filed Feb. 19, 2019; 62/807,672, filed Feb. 19, 2019; and 62/807,673, filed Feb. 19, 2019; each of which is incorporated by reference in its entirety.

BACKGROUND

[0002] Delivery of nucleic acids has been explored extensively as a potential therapeutic option for certain disease states. In particular, messenger RNA (mRNA) therapy has become an increasingly important option for treatment of various diseases, including for those associated with deficiency of one or more proteins.

SUMMARY

[0003] The present invention provides, among other things, cationic lipids useful for delivery of mRNA. Delivery of mRNA provided by cationic lipids described herein can result in targeted delivery, reduce administration frequency, improve patient tolerability, and provide more potent and less toxic mRNA therapy for the treatment of a variety of diseases, including but not limited to cancer, cardiovascular, cystic fibrosis, infectious, and neurological diseases.

[0004] In one aspect, the invention provides a liposome encapsulating an mRNA encoding a protein, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids, wherein the liposome comprises at least one cationic lipid that comprises the structure of Vitamin A, D, E, or K, and also comprises a moiety X.sup.1, wherein X.sup.1 is an ionizable nitrogen-containing group.

[0005] In another aspect, the invention provides a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that comprises the structure of Vitamin A, D, E, or K, and also comprises a moiety X.sup.1, wherein X.sup.1 is an ionizable nitrogen-containing group.

[0006] In embodiments, a cationic lipid is a cationic lipid having a structure according to any of the following structures:
##STR00001## [0007] wherein [0008] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0009] X.sup.1 is an ionizable nitrogen-containing group; [0010] X.sup.2 is S, C═O, or C═S; [0011] X.sup.3 is O, CR.sup.aR.sup.b, or NR.sup.c; [0012] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0013] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0014] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0015] In embodiments, the cationic lipid has a structure according to Formula (A-I):
##STR00002##

[0016] In embodiments, the cationic lipid has a structure according to Formula (A-Ia):
##STR00003##

[0017] In embodiments, the cationic lipid has a structure according to Formula (E-1),
##STR00004##

[0018] In embodiments, the cationic lipid has a structure according to formula (E-1a),
##STR00005##

[0019] In embodiments, the cationic lipid has a structure according to Formula (K-1),
##STR00006##

[0020] In embodiments, the cationic lipid has a structure according to Formula (K-1a),
##STR00007##

[0021] In embodiments, the cationic lipid has a structure according to Formula (K-1b),
##STR00008##

[0022] In embodiments, a cationic lipid has a structure according to Formula (D-A),
##STR00009## [0023] wherein [0024] custom-character represents a single or double bond; [0025] X.sup.1 is an ionizable nitrogen-containing group; [0026] X.sup.2 is O or S; [0027] Z is O or a covalent bond; [0028] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0029] R.sup.2 is H or C.sub.1-C.sub.4-alkyl.

[0030] In embodiments, a cationic lipid has a structure according to any one of the following formulas:
##STR00010##

[0031] In embodiments, a cationic lipid has a structure according to any one of the following formulas:
##STR00011## ##STR00012## ##STR00013##

[0032] In embodiments, X.sup.2 is O.

[0033] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkylene.

[0034] In embodiments, R.sup.1 is C.sub.1-C.sub.5-alkylene.

[0035] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene.

[0036] In embodiments, R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene.

[0037] In embodiments, R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —

C.sub.25H.sub.50—.
[0038] In embodiments, R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or —C.sub.4H.sub.8—.
[0039] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0040] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0041] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0042] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0043] In embodiments, R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0044] In embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.
[0045] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl.
[0046] In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.
[0047] In embodiments, X.sup.1 is dialkylamine.
[0048] In embodiments, X.sup.1 is N(Me).sub.2.
[0049] In embodiments, X.sup.1 is
##STR00014##
wherein [0050] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6.
[0051] In embodiments, X.sup.1 is
##STR00015##
[0052] In embodiments, X.sup.1 is
##STR00016##
[0053] In embodiments, X.sup.1 is
##STR00017##
[0054] In embodiments, the cationic lipid has the structure of:
##STR00018##
[0055] In embodiments, the cationic lipid has the structure of:
##STR00019##
[0056] In embodiments, the cationic lipid has the structure of:
##STR00020##
[0057] In embodiments, the cationic lipid has the structure of:
##STR00021##
[0058] In embodiments, the cationic lipid has the structure of:
##STR00022##
[0059] In embodiments, the cationic lipid has the structure of:
##STR00023##
[0060] In embodiments, the cationic lipid has the structure of:
##STR00024## ##STR00025##
[0061] In embodiments, the cationic lipid has the structure of:
##STR00026## ##STR00027## ##STR00028##
[0062] In embodiments, the cationic lipid has the structure of:
##STR00029##

[0063] In embodiments, the cationic lipid has the structure of:
##STR00030##
[0064] In another aspect, the invention features a composition comprising an mRNA encoding a peptide or a polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0065] In another aspect, the invention features a composition comprising an mRNA encoding a peptide or a polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0066] In embodiments, a composition comprises an mRNA encoding a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell.
[0067] In embodiments, a composition comprises an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein.
[0068] In embodiments, a composition comprises an mRNA encoding a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell.
[0069] In embodiments, a composition comprises an mRNA encoding for ornithine transcarbamylase (OTC) protein.
[0070] In embodiments, a composition comprises an mRNA encoding a peptide or polypeptide for use in a vaccine.
[0071] In embodiments, a composition comprises an mRNA encoding for an antigen (e.g., an antigen from an infectious agent).
[0072] In another aspect, the invention features a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0073] In another aspect, the invention features a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0074] In embodiments, a composition further comprises one or more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids.
[0075] In embodiments, a nucleic acid is an mRNA encoding a peptide or polypeptide.
[0076] In embodiments, an mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. In embodiments, an mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein.
[0077] In embodiments, an mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. In embodiments, an mRNA encodes ornithine transcarbamylase (OTC) protein.
[0078] In embodiments, an mRNA encodes a peptide or polypeptide for use in a vaccine. In embodiments, an mRNA encodes an antigen (e.g., an antigen from an infectious agent).
[0079] In some aspects, the present invention provides methods of treating a disease in a subject comprising administering to the subject a composition (e.g., a pharmaceutical composition) as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0080] In some other aspects, the present invention provides methods of treating a disease in a subject comprising administering to the subject a composition (e.g., a pharmaceutical composition) as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0081] In embodiments, a composition is formulated for intravenous (IV) administration.
[0082] In embodiments, a composition is formulated for intramuscular (IM) administration.
[0083] In embodiments, a composition is formulated for administration by inhalation (e.g., a composition is formulated for nebulization).

Description

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS
Definitions

[0084] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

[0085] Amino acid: As used herein, the term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure H.sub.2N—C(H)(R)—COOH. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an 1-amino acid. “Standard amino acid” refers to any of the twenty standard 1-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (e.g., methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, etc.). The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0086] Animal: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0087] Approximately or about: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0088] Biologically active: As used herein, the term “biologically active” refers to a characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active.

[0089] Delivery: As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA encompasses situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient's circulation system (e.g., serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery”).

[0090] Expression: As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a polypeptide, assemble multiple polypeptides into an intact protein (e.g., enzyme) and/or post-translational modification of a polypeptide or fully assembled protein (e.g., enzyme). In this application, the terms “expression” and “production,” and grammatical equivalent, are used inter-changeably.

[0091] Functional: As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0092] Half-life: As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0093] Improve, increase, or reduce: As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0094] In Vitro: As used herein, the term “in vitro” refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0095] In Vivo: As used herein, the term “in vivo” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, in vitro systems).

[0096] Isolated: As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about

99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (e.g., buffer, solvent, water, etc.).

[0097] Liposome: As used herein, the term "liposome" refers to any lamellar, multilamellar, or solid nanoparticle vesicle. Typically, a liposome as used herein can be formed by mixing one or more lipids or by mixing one or more lipids and polymer(s). In some embodiments, a liposome suitable for the present invention contains one or more cationic lipids and optionally non-cationic lipid(s), optionally cholesterol-based lipid(s), and/or optionally PEG-modified lipid(s).

[0098] messenger RNA (mRNA): As used herein, the term "messenger RNA (mRNA)" or "mRNA" refers to a polynucleotide that encodes at least one polypeptide. mRNA as used herein encompasses both modified and unmodified RNA. The term "modified mRNA" related to mRNA comprising at least one chemically modified nucleotide. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, etc. An mRNA sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (e.g., adenosine, guanosine, cytidine, uridine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C5 propynyl-cytidine, C5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 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); and/or modified phosphate groups (e.g., phosphorothioates and 5′-N-phosphoramidite linkages).

[0099] Nucleic acid: As used herein, the term "nucleic acid," in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a polynucleotide chain via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides). In some embodiments, "nucleic acid" refers to a polynucleotide chain comprising individual nucleic acid residues. In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. In some embodiments, "nucleic acid" encompasses ribonucleic acids (RNA), including but not limited to any one or more of interference RNAs (RNAi), small interfering RNA (siRNA), short hairpin RNA (shRNA), antisense RNA (aRNA), messenger RNA (mRNA), modified messenger RNA (mmRNA), long non-coding RNA (lncRNA), micro-RNA (miRNA) multimeric coding nucleic acid (MCNA), polymeric coding nucleic acid (PCNA), guide RNA (gRNA) and CRISPR RNA (crRNA). In some embodiments, "nucleic acid" encompasses deoxyribonucleic acid (DNA), including but not limited to any one or more of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) and complementary DNA (cDNA). In some embodiments, "nucleic acid" encompasses both RNA and DNA. In embodiments, DNA may be in the form of antisense DNA, plasmid DNA, parts of a plasmid DNA, pre-condensed DNA, a product of a polymerase chain reaction (PCR), vectors (e.g., P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, chromosomal DNA, or derivatives of these groups. In embodiments, RNA may be in the form of messenger RNA (mRNA), ribosomal RNA (rRNA), signal recognition particle RNA (7 SL RNA or SRP RNA), transfer RNA (tRNA), transfer-messenger RNA (tmRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), SmY RNA, small Cajal body-specific RNA (scaRNA), guide RNA (gRNA), ribonuclease P (RNase P), Y RNA, telomerase RNA component (TERC), spliced leader RNA (SL RNA), antisense RNA (aRNA or asRNA), cis-natural antisense transcript (cis-NAT), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), micro-RNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), transacting siRNA (tasiRNA), repeat associated siRNA (rasiRNA), 73K RNA, retrotransposons, a viral genome, a viroid, satellite RNA, or derivatives of these groups. In some embodiments, a nucleic acid is an mRNA encoding a protein such as an enzyme.

[0100] Patient: As used herein, the term "patient" or "subject" refers to any organism to which a provided composition may be administered, e.g., for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre- and post-natal forms.

[0101] Pharmaceutically acceptable: The term "pharmaceutically acceptable," as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0102] Pharmaceutically acceptable salt: Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid, or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-

toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and N.sup.+(C.sub.1-4 alkyl).sub.4 salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium. quaternary ammonium, and amine cations formed using counterions, such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, sulfonate and aryl sulfonate. Further pharmaceutically acceptable salts include salts formed from the quarternization of an amine using an appropriate electrophile, e.g., an alkyl halide, to form a quarternized alkylated amino salt.

[0103] Systemic distribution or delivery: As used herein, the terms “systemic distribution,” “systemic delivery,” or the grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body's circulation system, e.g., blood stream. Compared to the definition of “local distribution or delivery.”

[0104] Subject: As used herein, the term “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0105] Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0106] Target tissues: As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

[0107] Therapeutically effective amount: As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0108] Treating: As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0109] Aliphatic: As used herein, the term aliphatic refers to C.sub.1-C.sub.40 hydrocarbons and includes both saturated and unsaturated hydrocarbons. An aliphatic may be linear, branched, or cyclic. For example, C.sub.1-C.sub.20 aliphatics can include C.sub.1-C.sub.20 alkyls (e.g., linear or branched C.sub.1-C.sub.20 saturated alkyls), C.sub.2-C.sub.20 alkenyls (e.g., linear or branched C.sub.4-C.sub.20 dienyls, linear or branched C.sub.6-C.sub.20 trienyls, and the like), and C.sub.2-C.sub.20 alkynyls (e.g., linear or branched C.sub.2-C.sub.20 alkynyls). C.sub.1-C.sub.20 aliphatics can include C.sub.3-C.sub.20 cyclic aliphatics (e.g., C.sub.3-C.sub.20 cycloalkyls, C.sub.4-C.sub.20 cycloalkenyls, or C.sub.8-C.sub.20 cycloalkynyls). In certain embodiments, the aliphatic may comprise one or more cyclic aliphatic and/or one or more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxyl, hydroxy, amino, aryl, ether, ester or amide. An aliphatic group is unsubstituted or substituted with one or more substituent groups as described herein. For example, an aliphatic may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In embodiments, the aliphatic is unsubstituted. In embodiments, the aliphatic does not include any heteroatoms.

[0110] Alkyl: As used herein, the term “alkyl” means acyclic linear and branched hydrocarbon groups, e.g. “C.sub.1-C.sub.20 alkyl” refers to alkyl groups having 1-20 carbons. An alkyl group may be linear or branched. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl tert-pentylhexyl, Isohexyletc. Other alkyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. An alkyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkyl group may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In embodiments, the alkyl is substituted (e.g., with 1, 2, 3, 4, 5, or 6 substituent groups as described herein). In embodiments, an alkyl group is substituted with a-OH group and may also be referred to herein as a “hydroxyalkyl” group, where the prefix denotes the —OH group and “alkyl” is as described herein.

[0111] Alkylene: The term “alkylene,” as used herein, represents a saturated divalent straight or branched chain hydrocarbon

group and is exemplified by methylene, ethylene, isopropylene and the like. Likewise, the term “alkenylene” as used herein represents an unsaturated divalent straight or branched chain hydrocarbon group having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, and the term “alkynylene” herein represents an unsaturated divalent straight or branched chain hydrocarbon group having one or more unsaturated carbon-carbon triple bonds that may occur in any stable point along the chain. In certain embodiments, an alkylene, alkenylene, or alkynylene group may comprise one or more cyclic aliphatic and/or one or more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxyl, hydroxy, amino, aryl, ether, ester or amide. For example, an alkylene, alkenylene, or alkynylene may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In certain embodiments, an alkylene, alkenylene, or alkynylene is unsubstituted. In certain embodiments, an alkylene, alkenylene, or alkynylene does not include any heteroatoms.

[0112] Alkenyl: As used herein, “alkenyl” means any linear or branched hydrocarbon chains having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, e.g., “C.sub.2-C.sub.20 alkenyl” refers to an alkenyl group having 2-20 carbons. For example, an alkenyl group includes prop-2-enyl, but-2-enyl, but-3-enyl, 2-methylprop-2-enyl, hex-2-enyl, hex-5-enyl, 2,3-dimethylbut-2-enyl, and the like. In embodiments, the alkenyl comprises 1, 2, or 3 carbon-carbon double bond. In embodiments, the alkenyl comprises a single carbon-carbon double bond. In embodiments, multiple double bonds (e.g., 2 or 3) are conjugated. An alkenyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkenyl group may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In embodiments, the alkenyl is unsubstituted. In embodiments, the alkenyl is substituted (e.g., with 1, 2, 3, 4, 5, or 6 substituent groups as described herein). In embodiments, an alkenyl group is substituted with a-OH group and may also be referred to herein as a “hydroxyalkenyl” group, where the prefix denotes the —OH group and “alkenyl” is as described herein.

[0113] Alkynyl: As used herein, “alkynyl” means any hydrocarbon chain of either linear or branched configuration, having one or more carbon-carbon triple bonds occurring in any stable point along the chain, e.g., “C.sub.2-C.sub.20 alkynyl” refers to an alkynyl group having 2-20 carbons. Examples of an alkynyl group include prop-2-ynyl, but-2-ynyl, but-3-ynyl, pent-2-ynyl, 3-methylpent-4-ynyl, hex-2-ynyl, hex-5-ynyl, etc. In embodiments, an alkynyl comprises one carbon-carbon triple bond. An alkynyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkynyl group may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In embodiments, the alkynyl is unsubstituted. In embodiments, the alkynyl is substituted (e.g., with 1, 2, 3, 4, 5, or 6 substituent groups as described herein).

[0114] Amine: The term “amine” or “amino,” used interchangeably throughout, is used herein to refer to the group NZ.sup.1Z.sup.2, where each of Z.sup.1 and Z.sup.2 are independently hydrogen or alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkoxy, aryloxy, amino, silyl and combinations thereof. Each of Z.sup.1 and Z.sup.2 can be independently unsubstituted or substituted with one or more substituent groups as described herein. For example, a dialkylamine group refers to the group N(alkyl).sub.2, wherein each of the alkyl groups may be unsubstituted or substituted with one or more substituents such as alkyl, halo, alkoxyl, hydroxy, amino, aryl, ether, ester or amide.

[0115] Aryl: The terms “aryl” and “ar-”, used alone or as part of a larger moiety, e.g., “aralkyl”, “aralkoxy”, or “aryloxyalkyl”, refer to an optionally substituted C.sub.6-14-aromatic hydrocarbon moiety comprising one to three aromatic rings. For example, the aryl group is a C.sub.6-10-aryl group (i.e., phenyl and naphthyl). Aryl groups include, without limitation, optionally substituted phenyl, naphthyl, or anthracenyl. The terms “aryl” and “ar-”, as used herein, also include groups in which an aryl ring is fused to one or more cycloaliphatic rings to form an optionally substituted cyclic structure such as a tetrahydronaphthyl, indenyl, or indanyl ring. The term “aryl” may be used interchangeably with the terms “aryl group”, “aryl ring”, and “aromatic ring”.

[0116] Cycloalkyl: As used herein, the term “cycloalkyl” means a nonaromatic, saturated, cyclic group, e.g., “C.sub.3-C.sub.10 cycloalkyl.” In embodiments, a cycloalkyl is monocyclic. In embodiments, a cycloalkyl is polycyclic (e.g., bicyclic or tricyclic). In polycyclic cycloalkyl groups, individual rings can be fused, bridged, or spirocyclic. Examples of a cycloalkyl group include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, norbornanyl, bicyclo[3.2.1]octanyl, octahydro-pentalenyl, and spiro[4.5]decanyl, and the like. The term “cycloalkyl” may be used interchangeably with the term “carbocycle”. A cycloalkyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, a cycloalkyl group may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, —COR′, —CO.sub.2H, —CO.sub.2R′, —CN, —OH, —OR′, —OCOR′, —OCO.sub.2R′, —NH.sub.2, —NHR′, —N(R′).sub.2, —SR′ or —SO.sub.2R′, wherein each instance of R′ independently is C.sub.1-C.sub.20 aliphatic (e.g., C.sub.1-C.sub.20 alkyl, C.sub.1-

C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is an unsubstituted alkyl (e.g., unsubstituted C.sub.1-C.sub.20 alkyl, C.sub.1-C.sub.15 alkyl, C.sub.1-C.sub.10 alkyl, or C.sub.1-C.sub.3 alkyl). In embodiments, R′ independently is unsubstituted C.sub.1-C.sub.3 alkyl. In embodiments, the cycloalkyl is unsubstituted. In embodiments, the cycloalkyl is substituted (e.g., with 1, 2, 3, 4, 5, or 6 substituent groups as described herein).

[0117] Halogen: As used herein, the term “halogen” means fluorine, chlorine, bromine, or iodine.

[0118] Heteroalkenyl. The term “heteroalkenyl” is meant a branched or unbranched alkenyl group having from 2 to 14 carbon atoms in addition to 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O, S, and P. A heteroalkenyl may optionally include monocyclic, bicyclic, or tricyclic rings, in which each ring desirably has three to six members. The heteroalkenyl group may be substituted or unsubstituted.

[0119] Heteroalkynyl. The term “heteroalkynyl” is meant a branched or unbranched alkynyl group having from 2 to 14 carbon atoms in addition to 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O, S, and P. A heteroalkynyl may optionally include monocyclic, bicyclic, or tricyclic rings, in which each ring desirably has three to six members. The heteroalkynyl group may be substituted or unsubstituted.

[0120] Heteroalkyl. The term “heteroalkyl” is meant a branched or unbranched alkyl group having from 1 to 14 carbon atoms in addition to 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O, S, and P. Heteroalkyls include, without limitation, tertiary amines, secondary amines, ethers, thioethers, amides, thioamides, carbamates, thiocarbamates, hydrazones, imines, phosphodiesters, phosphoramidates, sulfonamides, and disulfides. A heteroalkyl may optionally include monocyclic, bicyclic, or tricyclic rings, in which each ring desirably has three to six members. The heteroalkyl group may be substituted or unsubstituted. Examples of heteroalkyls include, without limitation, polyethers, such as methoxymethyl and ethoxyethyl.

[0121] Heteroaryl: The terms “heteroaryl” and “heteroar-”, used alone or as part of a larger moiety, e.g., “heteroaralkyl”, or “heteroaralkoxy”, refer to groups having 5 to 14 ring atoms, preferably 5, 6, 9, or 10 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. A heteroaryl group may be mono-, bi-, tri-, or polycyclic, for example, mono-, bi-, or tricyclic (e.g., mono- or bicyclic). The term “heteroatom” refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. For example, a nitrogen atom of a heteroaryl may be a basic nitrogen atom and may also be optionally oxidized to the corresponding N-oxide. When a heteroaryl is substituted by a hydroxy group, it also includes its corresponding tautomer. The terms “heteroaryl” and “heteroar-,” as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocycloaliphatic rings. Nonlimiting examples of heteroaryl groups include thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, pteridinyl, indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H-quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3-b]-1,4-oxazin-3(4H)-one. The term “heteroaryl” may be used interchangeably with the terms “heteroaryl ring,” “heteroaryl group,” or “heteroaromatic,” any of which terms include rings that are optionally substituted. The term “heteroaralkyl” refers to an alkyl group substituted by a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0122] Heterocyclyl. As used herein, the terms “heterocycle,” “heterocyclyl,” “heterocyclic radical,” and “heterocyclic ring” are used interchangeably and refer to a stable 3- to 8-membered monocyclic or 7-10-membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, such as one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term “nitrogen” includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl), or NR.sup.+ (as in N-substituted pyrrolidinyl).

[0123] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothienyl, piperidinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and thiamorpholinyl. A heterocyclyl group may be mono-, bi-, tri-, or polycyclic, preferably mono-, bi-, or tricyclic, more preferably mono- or bicyclic. The term “heterocyclylalkyl” refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted. Additionally, a heterocyclic ring also includes groups in which the heterocyclic ring is fused to one or more aryl rings.

Cationic Lipids

[0124] Liposomal-based vehicles are considered an attractive carrier for therapeutic agents and remain subject to continued development efforts. While liposomal-based vehicles that comprise a cationic lipid component have shown promising results with regards to encapsulation, stability and site localization, there remains a great need for improvement of liposomal-based delivery systems. For example, a significant drawback of liposomal delivery systems relates to the construction of liposomes that have sufficient cell culture or in vivo stability to reach desired target cells and/or intracellular compartments, and the ability of such liposomal delivery systems to efficiently release their encapsulated materials to such target cells.

[0125] In particular, there remains a need for improved cationic lipids that demonstrate improved pharmacokinetic properties and which are capable of delivering macromolecules, such as nucleic acids to a wide variety cell types and tissues with enhanced efficiency. Importantly, there also remains a particular need for novel cationic lipids that are characterized as having reduced toxicity and are capable of efficiently delivering encapsulated nucleic acids and polynucleotides to targeted cells, tissues and organs.

[0126] Described herein are novel cationic lipids, compositions comprising such lipids, and related methods of their use. In embodiments, the compounds described herein are useful as liposomal compositions or as components of liposomal compositions to facilitate the delivery to, and subsequent transfection of one or more target cells.
[0127] Cationic lipids disclosed herein comprise a basic, ionizable functional group (e.g., an amine or a nitrogen-containing heteroaryl as described herein), which is present in neutral or charged form.
[0128] In embodiments, cationic lipids described herein can provide one or more desired characteristics or properties. That is, in certain embodiments, cationic lipids described herein can be characterized as having one or more properties that afford such compounds advantages relative to other similarly classified lipids. For example, cationic lipids disclosed herein can allow for the control and tailoring of the properties of liposomal compositions (e.g., lipid nanoparticles) of which they are a component. In particular, cationic lipids disclosed herein can be characterized by enhanced transfection efficiencies and their ability to provoke specific biological outcomes. Such outcomes can include, for example enhanced cellular uptake, endosomal/lysosomal disruption capabilities and/or promoting the release of encapsulated materials (e.g., polynucleotides) intracellularly.
[0129] Exemplary vitamin-based cationic lipids are described herein. Such exemplary cationic lipids can be used in any of the compositions and methods described herein. For example, any of the vitamin-based cationic lipids can be used in any of the liposomes described herein and any of the nucleic acids encapsulated within a liposome described herein, as well as compositions and methods of use thereof.
Cationic Lipids
Vitamin A Cationic Lipids
[0130] In one aspect, the present invention provides a cationic lipid derived from Vitamin A.
Cationic Lipids of Formula (A-I)
[0131] In one aspect, the present invention provides a cationic lipid of Formula (A-I):
##STR00031##
wherein [0132] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0133] X.sup.1 is an ionizable nitrogen-containing group; [0134] X.sup.2 is S, C═O, or C═S; [0135] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0136] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0137] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0138] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0139] In embodiments, X.sup.2 is S.
[0140] In embodiments, X.sup.2 is C═O.
[0141] In embodiments, X.sup.2 is C═S.
[0142] In embodiments, X.sup.3 is S.
[0143] In embodiments, X.sup.3 is O.
[0144] In embodiments, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. Alternatively, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring.
[0145] In embodiments, X.sup.3 is NR.sup.c, wherein R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0146] In embodiments, the cationic lipid has a structure according to Formula (A-Ia):
##STR00032##
[0147] In embodiments, R.sup.1 is C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl.
[0148] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkyl.
[0149] In embodiments, R.sup.1 is C.sub.1-C.sub.5-alkyl.
[0150] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkyl.
[0151] In embodiments, R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkyl.
[0152] In embodiments, R.sup.1 is —C.sub.1H.sub.2—, —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, C.sub.4H.sub.8—, —C.sub.5H.sub.10—, C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0153] In embodiments, R.sup.1 is —C.sub.6H.sub.13—, —C.sub.7H.sub.25—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0154] In embodiments, R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or —C.sub.4H.sub.8—.
[0155] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen,

hydroxyl, amino, thiol, ester, and thioester.
[0156] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0157] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0158] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0159] In embodiments, R.sup.1 is —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2-3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0160] An ionizable nitrogen-containing group can refer to a nitrogen functional group (e.g., NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl) that can be converted to a charged group by protonation with an acid or deprotonation with a base. Accordingly, in embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.
[0161] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. Suitable 5- to 6-membered heterocycloalkyl groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolyl, pyrrolinyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, and oxazinyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.
[0162] In embodiments, X.sup.1 is dialkylamine. In embodiments, X.sup.1 is unsubstituted dialkylamine. In embodiments, X.sup.1 is substituted dialkylamine.
[0163] In embodiments, X.sup.1 is N(Me).sub.2.
[0164] In embodiments, X.sup.1 is
##STR00033##
wherein [0165] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6.
[0166] In embodiments, R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl.
[0167] In embodiments, R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl.
[0168] In embodiments, R.sup.3a and R.sup.3b are each independently —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51.
[0169] In embodiments, R.sup.3a and R.sup.3b are each independently substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0170] In embodiments, R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenyl or C.sub.5-C.sub.20-alkenyl.
[0171] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenyl, C.sub.9-alkenylene, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl.
[0172] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from unsubstituted C.sub.8-alkenyl, unsubstituted C.sub.9-alkenyl, unsubstituted C.sub.10-alkenyl, unsubstituted C.sub.11-alkenyl, unsubstituted C.sub.12-alkenyl, unsubstituted C.sub.13-alkenyl, unsubstituted C.sub.14-alkenyl, unsubstituted C.sub.15-alkenyl, unsubstituted C.sub.16-alkenyl, unsubstituted C.sub.17-alkenyl, unsubstituted C.sub.18-alkenyl, unsubstituted C.sub.19-alkenyl, and unsubstituted C.sub.20-alkenyl.
[0173] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH.sub.2, —

(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —
(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —
(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —
(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —
(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3
—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —
(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3,
—(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —
(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —
(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —
(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —
(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —
(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3

[0174] In embodiments, X.sup.1 is

##STR00034##

[0175] In embodiments, an ionizable nitrogen-containing group is

##STR00035##

[0176] In embodiments, X.sup.1 is

##STR00036##

Cationic Lipids of Formula (A-II)

[0177] In one aspect, the present invention provides a cationic lipid of Formula (A-II):

##STR00037##

wherein [0178] X.sup.4 is an ionizable nitrogen-containing group; and [0179] each X.sup.5 is independently S, C═O, or C═S; [0180] each X.sup.6 is independently S, O, CR.sup.aR.sup.b, or NR.sup.c; [0181] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0182] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0183] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0184] In embodiments, each X.sup.5 is S.

[0185] In embodiments, each X.sup.5 is C═O.

[0186] In embodiments, each X.sup.5 is C═S.

[0187] In embodiments, each X.sup.6 is S.

[0188] In embodiments, each X.sup.6 is O.

[0189] In embodiments, each X.sup.6 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. Alternatively, each X.sup.6 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring.

[0190] In embodiments, each X.sup.6 is NR.sup.c, wherein R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0191] In embodiments, the cationic lipid of Formula (A-II) has a structure according to Formula (A-IIa):

##STR00038##

[0192] In embodiments, X.sup.4 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. For example, in embodiments, X.sup.4 is

##STR00039##

[0193] In embodiments, X.sup.4 is any ionizable nitrogen-containing group described herein (e.g., X.sup.4 can be any group recited for X.sup.1 of Formula (I) or (Ia)).

Vitamin D Cationic Lipids

[0194] In one aspect, the present invention provides a cationic lipid derived from Vitamin D.

Cationic Lipids of Formula (D-A)

[0195] In one aspect, the invention provides a cationic lipid having a structure according to Formula (D-A):

##STR00040## [0196] wherein [0197] custom-character represents a single or double bond; [0198] X.sup.1 is an ionizable nitrogen-containing group; [0199] X.sup.2 is O or S; [0200] Z is O or a covalent bond; [0201] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0202] R.sup.2 is H or C.sub.1-C.sub.4-alkyl.

[0203] In embodiments, custom-character represents a single bond.

[0204] In embodiments, custom-character represents a double bond.

[0205] In embodiments, Z is a covalent bond (e.g., compounds of Formula (D-I)).

[0206] In embodiments, Z is a O (e.g., compounds of Formula (D-III)).

[0207] In embodiments, X.sup.2 is O.

[0208] In embodiments, X.sup.2 is S.

[0209] In embodiments, R.sup.1 is C.sub.1-C.sub.5-alkylene.

[0210] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkylene.
[0211] In embodiments, R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene.
[0212] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene.
[0213] In embodiments, R.sup.1 is —C.sub.1H.sub.2—, —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, C.sub.4H.sub.8—, —C.sub.5H.sub.10—, C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0214] In embodiments, R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—.
[0215] In embodiments, R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0216] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0217] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0218] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0219] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0220] In embodiments, R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0221] In embodiments, R.sup.2 is H.
[0222] In embodiments, R.sup.2 is C.sub.1-C.sub.4-alkyl. In embodiments, R.sup.2 is unsubstituted C.sub.1-C.sub.4-alkyl. In embodiments, R.sup.2 is substituted C.sub.1-C.sub.4-alkyl. In embodiments, R.sup.2 is CH.sub.3. In embodiments, R.sup.2 is CH.sub.2CH.sub.3.
[0223] In embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.
[0224] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. Suitable 5- to 6-membered heterocycloalkyl groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolyl, pyrrolinyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, and oxazinyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.
[0225] In embodiments, X.sup.1 is dialkylamine. In embodiments, X.sup.1 is unsubstituted dialkylamine. In embodiments, X.sup.1 is substituted dialkylamine.
[0226] In embodiments, X.sup.1 is N(Me).sub.2.
[0227] In embodiments, X.sup.1 is
##STR00041##
wherein [0228] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0229] each n is independently an integer having a value between about 1 and about 6.
[0230] In embodiments, R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkylene.
[0231] In embodiments, R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkylene.

[0232] In embodiments, R.sup.3a and R.sup.3b are each independently —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —Cn H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0233] In embodiments, R.sup.3a and R.sup.3b are each independently substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0234] In embodiments, R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenylene or C.sub.5-C.sub.20-alkenylene.
[0235] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0236] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0237] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0238] In embodiments, X.sup.1 is
##STR00042##
[0239] In embodiments, X.sup.1 is
##STR00043##
[0240] In embodiments, X.sup.1 is
##STR00044##
Suitable 5- to 6-membered heterocycloalkyl groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolyl, pyrrolinyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, and oxazinyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl, or
##STR00045##
Cationic Lipids of Formula (D-I)
[0241] In one aspect, the present invention provides a cationic lipid of Formula (D-A) having a structure according to Formula (D-I):
##STR00046## [0242] wherein [0243] custom-character represents a single or double bond; [0244] X.sup.1 is an ionizable nitrogen-containing group; [0245] X.sup.2 is O or S; [0246] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0247] R.sup.2 is H or C.sub.1-C.sub.4-alkyl. In embodiments, R.sup.2 is H. Alternatively, in embodiments, R.sup.2 is C.sub.1-C.sub.4-alkyl, such as, for example, methyl, ethyl, propyl, isopropyl, or butyl. In preferred embodiments, R.sup.2 is H, methyl, or ethyl.
[0248] In embodiments, the cationic lipid has a structure according to Formula (D-Ia):
##STR00047##
[0249] In embodiments, the cationic lipid has a structure according to Formula (D-Ib):
##STR00048##
[0250] In embodiments, the cationic lipid has a structure according to Formula (D-Ic):
##STR00049##
[0251] In embodiments, the cationic lipid has a structure according to Formula (D-Id):
##STR00050##
[0252] In embodiments, X.sup.2 is O.
[0253] In embodiments, X.sup.2 is S.

[0254] In embodiments, R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene.
[0255] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkylene.
[0256] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene.
[0257] In embodiments, R.sup.1 is —C.sub.6H.sub.12, —C.sub.7H.sub.14, —C.sub.8H.sub.16, —C.sub.9H.sub.18, —C.sub.10H.sub.20, —C.sub.11H.sub.22, —C.sub.12H.sub.24, —C.sub.13H.sub.26, —C.sub.14H.sub.28, —C.sub.15H.sub.30, —C.sub.16H.sub.32, —C.sub.17H.sub.34, —C.sub.18H.sub.36, —C.sub.19H.sub.38, —C.sub.20H.sub.40, —C.sub.21H.sub.42, —C.sub.22H.sub.44, —C.sub.23H.sub.46, —C.sub.24H.sub.48, or —C.sub.25H.sub.50.
[0258] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0259] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0260] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0261] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0262] In embodiments, R.sup.1 is —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.1CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0263] As used herein, an ionizable nitrogen-containing group can refer to a nitrogen functional group (e.g., NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl) that can be converted to a charged group by protonation with an acid or deprotonation with a base.
[0264] Accordingly, in embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. For example, in embodiments, X.sup.1 is
##STR00051##
[0265] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. Suitable 5- to 6-membered heterocycloalkyl groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolyl, pyrrolinyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, and oxazinyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.
[0266] In embodiments, X.sup.1 is dialkylamine. In embodiments, X.sup.1 is unsubstituted dialkylamine. In embodiments, X.sup.1 is substituted dialkylamine.
[0267] In embodiments, X.sup.1 is N(Me).sub.2.
[0268] In embodiments, X.sup.1 is
##STR00052##
wherein [0269] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0270] each n is independently an integer having a value between about 1 and about 6.
[0271] In embodiments, X.sup.1 is
##STR00053##
[0272] In embodiments, X.sup.1 is
##STR00054##
Cationic Lipids of Formula (D-II)
[0273] In one aspect, the present invention provides a cationic lipid of Formula (D-II):
##STR00055## [0274] wherein [0275] X.sup.3 is an ionizable nitrogen-containing group; and [0276] each X.sup.4 is

independently S or O.
[0277] In embodiments, each X.sup.4 is S.
[0278] In embodiments, each X.sup.4 is O.
[0279] In embodiments, the cationic lipid of Formula (D-II) has a structure according to Formula (D-IIa):
##STR00056##
[0280] In embodiments, X.sup.3 is any ionizable nitrogen-containing group described herein (e.g., X.sup.3 can be any group recited for X.sup.1 of Formula (D-A), (D-I), (D-III), (D-Ia), (D-Ib), (D-Ic), (D-Id), (D-IIIa), (D-IIIb), (D-IIIc), or (D-IIId)).
[0281] In embodiments, X.sup.3 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. For example, in embodiments, X.sup.3 is
##STR00057##
Cationic Lipids of Formula (D-III)
[0282] In one aspect, the present invention provides a cationic lipid of Formula (D-III):
##STR00058## [0283] wherein [0284] custom-character represents a single or double bond; [0285] X.sup.1 is an ionizable nitrogen-containing group; [0286] X.sup.2 is O or S; [0287] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0288] R.sup.2 is H or C.sub.1-C.sub.4-alkyl.
[0289] In embodiments, R.sup.2 is H. Alternatively, in embodiments, R.sup.2 is C.sub.1-C.sub.4-alkyl, such as, for example, methyl, ethyl, propyl, isopropyl, or butyl. In preferred embodiments, R.sup.2 is H, methyl, or ethyl.
[0290] In embodiments, the cationic lipid has a structure according to Formula (D-IIIa):
##STR00059##
[0291] In embodiments, the cationic lipid has a structure according to Formula (D-IIIb):
##STR00060##
[0292] In embodiments, the cationic lipid has a structure according to Formula (D-IIIc):
##STR00061##
[0293] In embodiments, the cationic lipid has a structure according to Formula (D-IIId):
##STR00062##
(D-IIId).
[0294] In embodiments, X.sup.2 is O.
[0295] In embodiments, X.sup.2 is S.
[0296] In embodiments, R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene.
[0297] In embodiments, R.sup.1 is C.sub.1-C.sub.5-alkylene.
[0298] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkylene.
[0299] In embodiments, R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene.
[0300] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene.
[0301] In embodiments, R.sup.1 is —C.sub.1H.sub.2—, —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, C.sub.4H.sub.8—, —C.sub.5H.sub.10—, C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0302] In embodiments, R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—.
[0303] In embodiments, R.sup.1 is C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0304] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0305] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0306] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0307] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0308] In embodiments, R.sup.1 is —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —

(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —
(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —
(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —
(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —
(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —
(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —
(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —
(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.

[0309] As used herein, an ionizable nitrogen-containing group can refer to a nitrogen functional group (e.g., NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl) that can be converted to a charged group by protonation with an acid or deprotonation with a base. Accordingly, in embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.

[0310] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl, such as, for example, In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.

[0311] In embodiments, X.sup.1 is substituted dialkylamine.

[0312] In embodiments, X.sup.1 is

##STR00063##

wherein [0313] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0314] each n is independently an integer having a value between about 1 and about 6.

[0315] In embodiments, X.sup.1 is

##STR00064##

In embodiments X.sup.1 is

##STR00065##

In embodiments, X.sup.1 is

##STR00066##

Vitamin E Cationic Lipids

[0316] In one aspect, the present invention provides a cationic lipid derived from Vitamin E.

Cationic Lipids of Formula (E-I)

[0317] In one aspect, the present invention provides a cationic lipid of Formula (E-I):

##STR00067##

wherein [0318] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0319] X.sup.1 is an ionizable nitrogen-containing group; [0320] X.sup.2 is S, C═O, or C═S; [0321] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0322] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0323] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0324] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0325] In embodiments, X.sup.2 is S.

[0326] In embodiments, X.sup.2 is C═O.

[0327] In embodiments, X.sup.2 is C═S.

[0328] In embodiments, X.sup.3 is S.

[0329] In embodiments, X.sup.3 is O.

[0330] In embodiments, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. Alternatively, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring.

[0331] In embodiments, X.sup.3 is NR.sup.c, wherein R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0332] In embodiments, the cationic lipid has a structure according to Formula (E-Ia):

##STR00068##

[0333] In embodiments, R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered

heteroarylene.
[0334] In embodiments, R.sup.1 is C.sub.1-C.sub.5-alkylene.
[0335] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkylene.
[0336] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene.
[0337] In embodiments, R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene.
[0338] In embodiments, R.sup.1 is —C.sub.1H.sub.2—, —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, C.sub.4H.sub.8—, —C.sub.5H.sub.10—, C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0339] In embodiments, R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
[0340] In embodiments, R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—.
[0341] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0342] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
[0343] In embodiments, R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, Cis-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene.
[0344] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene.
[0345] In embodiments, R.sup.1 is —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—.
[0346] An ionizable nitrogen-containing group can refer to a nitrogen functional group (e.g., NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl) that can be converted to a charged group by protonation with an acid or deprotonation with a base. Accordingly, in embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.
[0347] In embodiments, X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. Suitable 5- to 6-membered heterocycloalkyl groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, pyrrolyl, pyrrolinyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, and oxazinyl. In embodiments, X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl.
[0348] In embodiments, X.sup.1 is dialkylamine. In some embodiments, X.sup.1 is unsubstituted dialkylamine. In some embodiments, X.sup.1 is substituted dialkylamine.
[0349] In embodiments, X.sup.1 is
##STR00069##
wherein [0350] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6.
[0351] In embodiments, R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl.
[0352] In embodiments, R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl.
[0353] In embodiments, R.sup.3a and R.sup.3b are each independently —C.sub.6H.sub.13, —C.sub.7H.sub.15, —

C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25Hsi.

[0354] In embodiments, R.sup.3a and R.sup.3b are each independently substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.

[0355] In embodiments, R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenyl or C.sub.5-C.sub.20-alkenyl.

[0356] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenyl, C.sub.9-alkenylene, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl.

[0357] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from unsubstituted C.sub.8-alkenyl, unsubstituted C.sub.9-alkenyl, unsubstituted C.sub.10-alkenyl, unsubstituted C.sub.11-alkenyl, unsubstituted C.sub.12-alkenyl, unsubstituted C.sub.13-alkenyl, unsubstituted C.sub.14-alkenyl, unsubstituted C.sub.15-alkenyl, unsubstituted C.sub.16-alkenyl, unsubstituted C.sub.17-alkenyl, unsubstituted C.sub.18-alkenyl, unsubstituted C.sub.19-alkenyl, and unsubstituted C.sub.20-alkenyl.

[0358] In embodiments, R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3

[0359] In embodiments, X.sup.1 is

##STR00070##

[0360] In embodiments, an ionizable nitrogen-containing group is

##STR00071##

[0361] In embodiments, X.sup.1 is

##STR00072##

Cationic Lipids of Formula (E-II)

[0362] In one aspect, the present invention provides a cationic lipid of Formula (E-II):

##STR00073## [0363] wherein [0364] X.sup.4 is an ionizable nitrogen-containing group; and [0365] each X.sup.5 is independently S, C═O, or C═S; [0366] each X.sup.6 is independently S, O, CR.sup.aR.sup.b, or NR.sup.c; [0367] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0368] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0369] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1—C-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0370] In embodiments, each X.sup.5 is S.

[0371] In embodiments, each X.sup.5 is C═O.

[0372] In embodiments, each X.sup.5 is C═S.

[0373] In embodiments, each X.sup.6 is S.

[0374] In embodiments, each X.sup.6 is O.

[0375] In embodiments, each X.sup.6 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. Alternatively, each X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring.

[0376] In embodiments, each X.sup.6 is NR.sup.c, wherein R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.

[0377] In embodiments, the cationic lipid of Formula (E-II) has a structure according to Formula (E-IIa):

##STR00074##

[0378] In embodiments, X.sup.4 is any ionizable nitrogen-containing group described herein (e.g., X.sup.4 can be any group recited for X.sup.1 of Formula (E-I) or (E-Ia)).

[0379] In embodiments, X.sup.4 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. For example, in embodiments, X.sup.4 is

##STR00075##

Vitamin K Cationic Lipids

[0380] In one aspect, the present invention provides a cationic lipid derived from Vitamin K.

Cationic Lipids of Formula (K-I)
[0381] In one aspect, the present invention provides a cationic lipid of Formula (K-I):
##STR00076##
wherein [0382] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0383] X.sup.1 is an ionizable nitrogen-containing group; [0384] X.sup.2 is S, C═O, or C═S; [0385] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0386] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0387] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0388] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0389] In embodiments, X.sup.2 is S.
[0390] In embodiments, X.sup.2 is C═O.
[0391] In embodiments, X.sup.2 is C═S.
[0392] In embodiments, X.sup.3 is S.
[0393] In embodiments, X.sup.3 is O.
[0394] In embodiments, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0395] In embodiments, X.sup.3 is CR.sup.aR.sup.b, wherein R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring.
[0396] In embodiments, X.sup.3 is NR′, wherein R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0397] In embodiments, the cationic lipid of Formula (K-I) has a structure according to Formula (K-Ia):
##STR00077##
[0398] In embodiments, the cationic lipid of Formula (K-I) has a structure according to Formula (K-Ib):
##STR00078##
[0399] In embodiments, R.sup.1 is C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl.
[0400] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkyl.
[0401] In embodiments, R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkyl.
[0402] In embodiments, R.sup.1 is —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51
[0403] In embodiments, R.sup.1 is substituted C.sub.6-C.sub.30-alkyl with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester.
[0404] In embodiments, R.sup.1 is C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl.
[0405] In embodiments, R.sup.1 is selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl.
[0406] In embodiments, R.sup.1 is selected from unsubstituted C.sub.8-alkenyl, unsubstituted C.sub.9-alkenyl, unsubstituted C.sub.10-alkenyl, unsubstituted C.sub.11-alkenyl, unsubstituted C.sub.12-alkenyl, unsubstituted C.sub.13-alkenyl, unsubstituted C.sub.14-alkenyl, unsubstituted C.sub.15-alkenyl, unsubstituted C.sub.16-alkenyl, unsubstituted C.sub.17-alkenyl, unsubstituted C.sub.18-alkenyl, unsubstituted C.sub.19-alkenyl, and unsubstituted C.sub.20-alkenyl.
[0407] In embodiments, R.sup.1 is —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3.
[0408] An ionizable nitrogen-containing group can refer to a nitrogen functional group (e.g., NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl) that can be converted to a charged group by protonation with an acid or deprotonation with a base. Accordingly, in embodiments, X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-

containing heteroaryl. For example, in embodiments, an ionizable nitrogen-containing group is
##STR00079##
Cationic Lipids of Formula (K-II)
[0409] In one aspect, the present invention provides a cationic lipid of Formula (K-II):
##STR00080##
wherein [0410] X.sup.4 is an ionizable nitrogen-containing group; [0411] each X.sup.5 is independently S, C═O, or C═S; [0412] each X.sup.6 is independently S, O, CR.sup.aR.sup.b, or NR′; [0413] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0414] each combination of R.sup.a and R.sup.b, together with the carbon atom through which they are connected, may form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0415] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
[0416] In embodiments, each X.sup.5 is S.
[0417] In embodiments, each X.sup.5 is C═O.
[0418] In embodiments, each X.sup.5 is C═S.
[0419] In embodiments, each X.sup.6 is S.
[0420] In embodiments, each X.sup.6 is O.
[0421] In embodiments, the cationic lipid of Formula (K-II) has a structure according to Formula (K-IIa):
##STR00081##
[0422] In embodiments, X.sup.4 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. For example, in embodiments, X.sup.4 is
##STR00082##
Exemplary Cationic Lipids
[0423] One exemplary cationic lipid of the present invention is Cationic Lipid (A1),
##STR00083##
[0424] Another exemplary cationic lipid of the present invention is Cationic Lipid (A2),
##STR00084##
[0425] One exemplary cationic lipid of the present invention is Cationic Lipid (3),
##STR00085##
[0426] One exemplary cationic lipid of the present invention is Cationic Lipid (A4),
##STR00086##
[0427] One exemplary cationic lipids of the present invention is Cationic Lipid (D1),
##STR00087##
[0428] Another exemplary cationic lipids of the present invention is Cationic Lipid (D2),
##STR00088##
[0429] Yet another exemplary cationic lipids of the present invention is Cationic Lipid (D3),
##STR00089##
[0430] Yet another exemplary cationic lipids of the present invention is Cationic Lipid (D4),
##STR00090##
[0431] Another exemplary cationic lipids of the present invention is Cationic Lipid (D5),
##STR00091##
[0432] An exemplary cationic lipids of the present invention is Cationic Lipid (D6),
##STR00092##
[0433] An exemplary cationic lipids of the present invention is Cationic Lipid (D7),
##STR00093##
[0434] One exemplary cationic lipid of the present invention is Cationic Lipid (E1),
##STR00094##
[0435] Another exemplary cationic lipid of the present invention is Cationic Lipid (E2),
##STR00095##
[0436] Yet another exemplary cationic lipid of the present invention is Cationic Lipid (E3),
##STR00096##
[0437] Still another exemplary cationic lipid of the present invention is Cationic Lipid (E4),
##STR00097##
[0438] Another exemplary cationic lipid of the present invention is Cationic Lipid (E5),
##STR00098##
[0439] One exemplary cationic lipids of the present invention is Cationic Lipid (K1),
##STR00099##
[0440] Another exemplary cationic lipids of the present invention is Cationic Lipid (K2),
##STR00100##
[0441] Yet another exemplary cationic lipids of the present invention is Cationic Lipid (K3),
##STR00101##
[0442] Still another exemplary cationic lipid of the present invention is Cationic Lipid (K4),
##STR00102##
Synthesis of Cationic Lipids

[0443] Cationic lipids described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) can be prepared according to methods known in the art.

[0444] Exemplary synthetic methods are shown in the Examples.

Nucleic Acids

[0445] Cationic lipids described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) can be used to prepare compositions useful for the delivery of nucleic acids.

Synthesis of Nucleic Acids

[0446] Nucleic acids according to the present invention may be synthesized according to any known methods. For example, mRNAs according to the present invention may be synthesized via in vitro transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7, mutated T7 or SP6 RNA polymerase), DNAse I, pyrophosphatase, and/or RNAse inhibitor. The exact conditions will vary according to the specific application.

[0447] In some embodiments, for the preparation of mRNA according to the invention, a DNA template is transcribed in vitro. A suitable DNA template typically has a promoter, for example a T3, T7, mutated T7 or SP6 promoter, for in vitro transcription, followed by desired nucleotide sequence for desired mRNA and a termination signal.

[0448] Desired mRNA sequence(s) according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence (e.g., an enzyme sequence), a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

[0449] As described above, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. DNA may be in the form of antisense DNA, plasmid DNA, parts of a plasmid DNA, pre-condensed DNA, a product of a polymerase chain reaction (PCR), vectors (e.g., P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, chromosomal DNA, or derivatives of these groups. RNA may be in the form of messenger RNA (mRNA), ribosomal RNA (rRNA), signal recognition particle RNA (7 SL RNA or SRP RNA), transfer RNA (tRNA), transfer-messenger RNA (tmRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), SmY RNA, small Cajal body-specific RNA (scaRNA), guide RNA (gRNA), ribonuclease P (RNase P), Y RNA, telomerase RNA component (TERC), spliced leader RNA (SL RNA), antisense RNA (aRNA or asRNA), cis-natural antisense transcript (cis-NAT), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), microRNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), transacting siRNA (tasiRNA), repeat associated siRNA (rasiRNA), 73K RNA, retrotransposons, a viral genome, a viroid, satellite RNA, or derivatives of these groups. In some embodiments, a nucleic acid is a mRNA encoding a protein.

Synthesis of mRNA

[0450] mRNAs according to the present invention may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via in vitro transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNAse I, pyrophosphatase, and/or RNAse inhibitor. The exact conditions will vary according to the specific application. The exact conditions will vary according to the specific application. The presence of these reagents is undesirable in the final product according to several embodiments and may thus be referred to as impurities and a preparation containing one or more of these impurities may be referred to as an impure preparation. In some embodiments, the in vitro transcribing occurs in a single batch.

[0451] In some embodiments, for the preparation of mRNA according to the invention, a DNA template is transcribed in vitro. A suitable DNA template typically has a promoter, for example a T3, T7 or SP6 promoter, for in vitro transcription, followed by desired nucleotide sequence for desired mRNA and a termination signal.

[0452] Desired mRNA sequence(s) according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence (e.g., an enzyme sequence), a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

Modified mRNA

[0453] In some embodiments, mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Modified mRNA comprise nucleotide modifications in the RNA. A modified mRNA according to the invention can

thus include nucleotide modification that are, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5′-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, .beta.-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g., from the U.S. Pat. Nos. 4,373,071, 4,401,796, 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, 5,047,524, 5,132,418, 5,153,319, 5,262,530 and 5,700,642, the disclosures of which are incorporated by reference in their entirety.

[0454] In some embodiments, mRNAs may contain RNA backbone modifications. Typically, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides contained in the RNA are modified chemically. Exemplary backbone modifications typically include, but are not limited to, modifications from the group consisting of methylphosphonates, methylphosphoramidates, phosphoramidates, phosphorothioates (e.g. cytidine 5′-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc., which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0455] In some embodiments, mRNAs may contain sugar modifications. A typical sugar modification is a chemical modification of the sugar of the nucleotides it contains including, but not limited to, sugar modifications chosen from the group consisting of 4′-thio-ribonucleotide (see, e.g., US Patent Application Publication No. US 2016/0031928, incorporated by reference herein), 2′-deoxy-2′-fluoro-oligoribonucleotide (2′-fluoro-2′-deoxycytidine 5′-triphosphate, 2′-fluoro-2′-deoxyuridine 5′-triphosphate), 2′-deoxy-2′-deamine-oligoribonucleotide (2′-amino-2′-deoxycytidine 5′-triphosphate, 2′-amino-2′-deoxyuridine 5′-triphosphate), 2′-O-alkyloligoribonucleotide, 2′-deoxy-2′-C-alkyloligoribonucleotide (2′-O-methylcytidine 5′-triphosphate, 2′-methyluridine 5′-triphosphate), 2′-C-alkyloligoribonucleotide, and isomers thereof (2′-aracytidine 5′-triphosphate, 2′-arauridine 5′-triphosphate), or azidotriphosphates (2′-azido-2′-deoxycytidine 5′-triphosphate, 2′-azido-2′-deoxyuridine 5′-triphosphate).

[0456] In some embodiments, mRNAs may contain modifications of the bases of the nucleotides (base modifications). A modified nucleotide which contains a base modification is also called a base-modified nucleotide. Examples of such base-modified nucleotides include, but are not limited to, 2-amino-6-chloropurine riboside 5′-triphosphate, 2-aminoadenosine 5′-triphosphate, 2-thiocytidine 5′-triphosphate, 2-thiouridine 5′-triphosphate, 4-thiouridine 5′-triphosphate, 5-aminoallylcytidine 5′-triphosphate, 5-aminoallyluridine 5′-triphosphate, 5-bromocytidine 5′-triphosphate, 5-bromouridine 5′-triphosphate, 5-iodocytidine 5′-triphosphate, 5-iodouridine 5′-triphosphate, 5-methylcytidine 5′-triphosphate, 5-methyluridine 5′-triphosphate, 6-azacytidine 5′-triphosphate, 6-azauridine 5′-triphosphate, 6-chloropurine riboside 5′-triphosphate, 7-deazaadenosine 5′-triphosphate, 7-deazaguanosine 5′-triphosphate, 8-azaadenosine 5′-triphosphate, 8-azidoadenosine 5′-triphosphate, benzimidazole riboside 5′-triphosphate, N1-methyladenosine 5′-triphosphate, N1-methylguanosine 5′-triphosphate, N6-methyladenosine 5′-triphosphate, 06-methylguanosine 5′-triphosphate, pseudouridine 5′-triphosphate, puromycin 5′-triphosphate or xanthosine 5′-triphosphate.

[0457] Typically, mRNA synthesis includes the addition of a “cap” on the N-terminal (5′) end, and a “tail” on the C-terminal (3′) end. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The presence of a “tail” serves to protect the mRNA from exonuclease degradation.

[0458] Thus, in some embodiments, mRNAs include a 5′ cap structure. A 5′ cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5′ nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5′5′5 triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5′)ppp (5′(A,G(5′)ppp(5′)A and G(5′)ppp(5′)G.

[0459] In some embodiments, mRNAs include a 3′ poly(A) tail structure. A poly-A tail on the 3′ terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about 10 to 100 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, mRNAs include a 3′ poly(C) tail structure. A suitable poly-C tail on the 3′ terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0460] In some embodiments, mRNAs include a 5′ and/or 3′ untranslated region. In some embodiments, a 5′ untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5′ untranslated region may be between about 50 and 500 nucleotides in length.

[0461] In some embodiments, a 3′ untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3′ untranslated region may be between 50 and 500 nucleotides in length or longer.

Cap Structure

[0462] In some embodiments, mRNAs include a 5′ cap structure. A 5′ cap is typically added as follows: first, an RNA terminal

phosphatase removes one of the terminal phosphate groups from the 5′ nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5′5′5 triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5′)ppp (5′(A,G(5′)ppp(5′)A and G(5′)ppp(5′)G.

[0463] Naturally occurring cap structures comprise a 7-methyl guanosine that is linked via a triphosphate bridge to the 5′-end of the first transcribed nucleotide, resulting in a dinucleotide cap of m.sup.7G(5′)ppp(5′)N, where N is any nucleoside. In vivo, the cap is added enzymatically. The cap is added in the nucleus and is catalyzed by the enzyme guanylyl transferase. The addition of the cap to the 5′ terminal end of RNA occurs immediately after initiation of transcription. The terminal nucleoside is typically a guanosine, and is in the reverse orientation to all the other nucleotides, i.e., G(5′)ppp(5′)GpNpNp.

[0464] A common cap for mRNA produced by in vitro transcription is m.sup.7G(5′)ppp(5′)G, which has been used as the dinucleotide cap in transcription with T7 or SP6 RNA polymerase in vitro to obtain RNAs having a cap structure in their 5′-termini. The prevailing method for the in vitro synthesis of caPPEd mRNA employs a pre-formed dinucleotide of the form m.sup.7G(5′)ppp(5′)G (“m.sup.7GpppG”) as an initiator of transcription.

[0465] To date, a usual form of a synthetic dinucleotide cap used in in vitro translation experiments is the Anti-Reverse Cap Analog (“ARCA”) or modified ARCA, which is generally a modified cap analog in which the 2′ or 3′ OH group is replaced with —OCH.sub.3.

[0466] Additional cap analogs include, but are not limited to, a chemical structures selected from the group consisting of m.sup.7GpppG, m.sup.7GpppA, m.sup.7GpppC; unmethylated cap analogs (e.g., GpppG); dimethylated cap analog (e.g., m.sup.2° 7GpppG), trimethylated cap analog (e.g., m.sup.2,2,7GpppG), dimethylated symmetrical cap analogs (e.g., m.sup.7Gpppm.sup.7G), or anti reverse cap analogs (e.g., ARCA; m.sup.7,2′OmeGpppG, m.sup.72′dGpppG, m.sup.7,3′OmeGpppG, m.sup.7,3′dGpppG and their tetraphosphate derivatives) (see, e.g., Jemielity, J. et al., “*Novel ‘anti-reverse’ cap analogs with superior translational properties*”, RNA, 9: 1108-22 (2003)).

[0467] In some embodiments, a suitable cap is a 7-methyl guanylate (“m.sup.7G”) linked via a triphosphate bridge to the 5′-end of the first transcribed nucleotide, resulting in m.sup.7G(5′)ppp(5′)N, where N is any nucleoside. A preferred embodiment of a m.sup.7G cap utilized in embodiments of the invention is m.sup.7G(5′)ppp(5′)G.

[0468] In some embodiments, the cap is a Cap0 structure. Cap0 structures lack a 2′-O-methyl residue of the ribose attached to bases 1 and 2. In some embodiments, the cap is a Cap1 structure. Cap1 structures have a 2′-O-methyl residue at base 2. In some embodiments, the cap is a Cap2 structure. Cap2 structures have a 2′-O-methyl residue attached to both bases 2 and 3.

[0469] A variety of m.sup.7G cap analogs are known in the art, many of which are commercially available. These include the m.sup.7GpppG described above, as well as the ARCA 3′-OCH.sub.3 and 2′-OCH.sub.3 cap analogs (Jemielity, J. et al., RNA, 9: 1108-22 (2003)). Additional cap analogs for use in embodiments of the invention include N7-benzylated dinucleoside tetraphosphate analogs (described in Grudzien, E. et al., RNA, 10: 1479-87 (2004)), phosphorothioate cap analogs (described in Grudzien-Nogalska, E., et al., RNA, 13: 1745-55 (2007)), and cap analogs (including biotinylated cap analogs) described in U.S. Pat. Nos. 8,093,367 and 8,304,529, incorporated by reference herein.

Tail Structure

[0470] Typically, the presence of a “tail” serves to protect the mRNA from exonuclease degradation. The poly A tail is thought to stabilize natural messengers and synthetic sense RNA. Therefore, in certain embodiments a long poly A tail can be added to an mRNA molecule thus rendering the RNA more stable. Poly A tails can be added using a variety of art-recognized techniques. For example, long poly A tails can be added to synthetic or in vitro transcribed RNA using poly A polymerase (Yokoe, et al. Nature Biotechnology. 1996; 14: 1252-56). A transcription vector can also encode long poly A tails. In addition, poly A tails can be added by transcription directly from PCR products. Poly A may also be ligated to the 3′ end of a sense RNA with RNA ligase (see, e.g., Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1991 edition)).

[0471] In some embodiments, mRNAs include a 3′ poly(A) tail structure. Typically, the length of the poly A tail can be at least about 10, 50, 100, 200, 300, 400 at least 500 nucleotides. In some embodiments, a poly-A tail on the 3′ terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about 10 to 100 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, mRNAs include a 3′ poly(C) tail structure. A suitable poly-C tail on the 3′ terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0472] In some embodiments, the length of the poly A or poly C tail is adjusted to control the stability of a modified sense mRNA molecule of the invention and, thus, the transcription of protein. For example, since the length of the poly A tail can influence the half-life of a sense mRNA molecule, the length of the poly A tail can be adjusted to modify the level of resistance of the mRNA to nucleases and thereby control the time course of polynucleotide expression and/or polypeptide production in a target cell.

5′ and 3′ Untranslated Region

[0473] In some embodiments, mRNAs include a 5′ and/or 3′ untranslated region. In some embodiments, a 5′ untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5′ untranslated region may be between about 50 and 500 nucleotides in length.

[0474] In some embodiments, a 3′ untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3′ untranslated region may be between 50 and 500 nucleotides in length or longer.

[0475] Exemplary 3′ and/or 5′ UTR sequences can be derived from mRNA molecules which are stable (e.g., globin, actin,

GAPDH, tubulin, histone, or citric acid cycle enzymes) to increase the stability of the sense mRNA molecule. For example, a 5′ UTR sequence may include a partial sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof to improve the nuclease resistance and/or improve the half-life of the polynucleotide. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof to the 3′ end or untranslated region of the polynucleotide (e.g., mRNA) to further stabilize the polynucleotide. Generally, these modifications improve the stability and/or pharmacokinetic properties (e.g., half-life) of the polynucleotide relative to their unmodified counterparts, and include, for example modifications made to improve such polynucleotides' resistance to in vivo nuclease digestion.

Pharmaceutical Formulations of Cationic Lipids and Nucleic Acids

[0476] In certain embodiments cationic lipids described herein described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)), as well as pharmaceutical and liposomal compositions comprising such lipids, can be used in formulations to facilitate the delivery of encapsulated materials (e.g., one or more polynucleotides such as mRNA) to, and subsequent transfection of one or more target cells. For example, in certain embodiments cationic lipids described herein (and compositions such as liposomal compositions comprising such lipids) are characterized as resulting in one or more of receptor-mediated endocytosis, clathrin-mediated and caveolae-mediated endocytosis, phagocytosis and macropinocytosis, fusogenicity, endosomal or lysosomal disruption and/or releasable properties that afford such compounds advantages relative other similarly classified lipids.

[0477] According to the present invention, a nucleic acid, e.g., mRNA encoding a protein (e.g., a full length, fragment or portion of a protein) as described herein may be delivered via a delivery vehicle comprising a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).

[0478] As used herein, the terms “delivery vehicle,” “transfer vehicle,” “nanoparticle” or grammatical equivalent, are used interchangeably.

[0479] For example, the present invention provides a composition (e.g., a pharmaceutical composition) comprising a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) and one or more polynucleotides. A composition (e.g., a pharmaceutical composition) may further comprise one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and/or one or more PEG-modified lipids.

[0480] In certain embodiments a composition exhibits an enhanced (e.g., increased) ability to transfect one or more target cells. Accordingly, also provided herein are methods of transfecting one or more target cells. Such methods generally comprise the step of contacting the one or more target cells with the cationic lipids and/or pharmaceutical compositions disclosed herein (e.g., a liposomal formulation comprising a cationic lipid described (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) encapsulating one or more polynucleotides) such that the one or more target cells are transfected with the materials encapsulated therein (e.g., one or more polynucleotides). As used herein, the terms “transfect” or “transfection” refer to the intracellular introduction of one or more encapsulated materials (e.g., nucleic acids and/or polynucleotides) into a cell, or preferably into a target cell. The introduced polynucleotide may be stably or transiently maintained in the target cell. The term “transfection efficiency” refers to the relative amount of such encapsulated material (e.g., polynucleotides) up-taken by, introduced into, and/or expressed by the target cell which is subject to transfection. In practice, transfection efficiency may be estimated by the amount of a reporter polynucleotide product produced by the target cells following transfection. In certain embodiments, the compounds and pharmaceutical compositions described herein demonstrate high transfection efficiencies thereby improving the likelihood that appropriate dosages of the encapsulated materials (e.g., one or more polynucleotides) will be delivered to the site of pathology and subsequently expressed, while at the same time minimizing potential systemic adverse effects or toxicity associated with the compound or their encapsulated contents.

[0481] Following transfection of one or more target cells by, for example, the polynucleotides encapsulated in the one or more lipid nanoparticles comprising the pharmaceutical or liposomal compositions disclosed herein, the production of the product (e.g., a polypeptide or protein) encoded by such polynucleotide may be preferably stimulated and the capability of such target cells to express the polynucleotide and produce, for example, a polypeptide or protein of interest is enhanced. For example, transfection of a target cell by one or more compounds or pharmaceutical compositions encapsulating mRNA will enhance (i.e., increase) the production of the protein or enzyme encoded by such mRNA.

[0482] Further, delivery vehicles described herein (e.g., liposomal delivery vehicles) may be prepared to preferentially distribute to other target tissues, cells or organs, such as the heart, lungs, kidneys, spleen. In embodiments, the lipid nanoparticles of the present invention may be prepared to achieve enhanced delivery to the target cells and tissues. For example, polynucleotides (e.g., mRNA) encapsulated in one or more of the compounds or pharmaceutical and liposomal compositions described herein can be delivered to and/or transfect targeted cells or tissues. In some embodiments, the encapsulated polynucleotides (e.g., mRNA) are capable of being expressed and functional polypeptide products produced (and in some instances excreted) by the target cell, thereby conferring a beneficial property to, for example the target cells or tissues. Such encapsulated polynucleotides (e.g., mRNA) may encode, for example, a hormone, enzyme, receptor, polypeptide, peptide or other protein of interest.

Liposomal Delivery Vehicles

[0483] In some embodiments, a composition is a suitable delivery vehicle. In embodiments, a composition is a liposomal delivery vehicle, e.g., a lipid nanoparticle.
[0484] The terms “liposomal delivery vehicle” and “liposomal composition” are used interchangeably.
[0485] Enriching liposomal compositions with one or more of the cationic lipids disclosed herein may be used as a means of improving (e.g., reducing) the toxicity or otherwise conferring one or more desired properties to such enriched liposomal composition (e.g., improved delivery of the encapsulated polynucleotides to one or more target cells and/or reduced in vivo toxicity of a liposomal composition). Accordingly, also contemplated are pharmaceutical compositions, and in particular liposomal compositions, that comprise one or more of the cationic lipids disclosed herein.
[0486] Thus, in certain embodiments, the compounds described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) are cationic lipids that may be used as a component of a liposomal composition to facilitate or enhance the delivery and release of encapsulated materials (e.g., one or more therapeutic agents) to one or more target cells (e.g., by permeating or fusing with the lipid membranes of such target cells).
[0487] As used herein, liposomal delivery vehicles, e.g., lipid nanoparticles, are usually characterized as microscopic vesicles having an interior aqua space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphophilic polymers and surfactants (e.g., polymerosomes, niosomes, etc.). In the context of the present invention, a liposomal delivery vehicle typically serves to transport a desired mRNA to a target cell or tissue.
[0488] In certain embodiments, such compositions (e.g., liposomal compositions) are loaded with or otherwise encapsulate materials, such as for example, one or more biologically-active polynucleotides (e.g., mRNA).
[0489] In embodiments, a composition (e.g., a pharmaceutical composition) comprises an mRNA encoding a protein, encapsulated within a liposome. In embodiments, a liposome comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids, and at least one cationic lipid is a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)). In embodiments, a composition comprises an mRNA encoding for a protein (e.g., any protein described herein). In embodiments, a composition comprises an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. In embodiments, a composition comprises an mRNA encoding for ornithine transcarbamylase (OTC) protein.
[0490] In embodiments, a composition (e.g., a pharmaceutical composition) comprises a nucleic acid encapsulated within a liposome, wherein the liposome comprises any cationic lipid (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) as described herein.
[0491] In embodiments, a nucleic acid is an mRNA encoding a peptide or polypeptide. In embodiments, an mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell (e.g., an mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein). In embodiments, an mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell (e.g., an mRNA encodes ornithine transcarbamylase (OTC) protein). Still other exemplary mRNAs are described herein.
[0492] In embodiments, a liposomal delivery vehicle (e.g., a lipid nanoparticle) can have a net positive charge.
[0493] In embodiments, a liposomal delivery vehicle (e.g., a lipid nanoparticle) can have a net negative charge.
[0494] In embodiments, a liposomal delivery vehicle (e.g., a lipid nanoparticle) can have a net neutral charge.
[0495] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (e.g., mRNA encoding a peptide or polypeptide) comprises one or more cationic lipids described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)).
[0496] For example, the amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) in a composition can be described as a percentage (“wt %”) of the combined dry weight of all lipids of a composition (e.g., the combined dry weight of all lipids present in a liposomal composition).
[0497] In embodiments of the pharmaceutical compositions described herein, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 0.5 wt % to about 30 wt % (e.g., about 0.5 wt % to about 20 wt %) of the combined dry weight of all lipids present in a composition (e.g., a liposomal composition).
[0498] In embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 1 wt % to about 30 wt %, about 1 wt % to about 20 wt %, about 1 wt % to about 15 wt %, about 1 wt % to about 10 wt %, or about 5 wt % to about 25 wt % of the combined dry weight of all lipids present in a

composition (e.g., a liposomal composition). In embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 0.5 wt % to about 5 wt %, about 1 wt % to about 10 wt %, about 5 wt % to about 20 wt %, or about 10 wt % to about 20 wt % of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle.

[0499] In embodiments, the amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is at least about 5 wt %, about 10 wt %, about 15 wt %, about 20 wt %, about 25 wt %, about 30 wt %, about 35 wt %, about 40 wt %, about 45 wt %, about 50 wt %, about 55 wt %, about 60 wt %, about 65 wt %, about 70 wt %, about 75 wt %, about 80 wt %, about 85 wt %, about 90 wt %, about 95 wt %, about 96 wt %, about 97 wt %, about 98 wt %, or about 99 wt % of the combined dry weight of total lipids in a composition (e.g., a liposomal composition).

[0500] In embodiments, the amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is no more than about 5 wt %, about 10 wt %, about 15 wt %, about 20 wt %, about 25 wt %, about 30 wt %, about 35 wt %, about 40 wt %, about 45 wt %, about 50 wt %, about 55 wt %, about 60 wt %, about 65 wt %, about 70 wt %, about 75 wt %, about 80 wt %, about 85 wt %, about 90 wt %, about 95 wt %, about 96 wt %, about 97 wt %, about 98 wt %, or about 99 wt % of the combined dry weight of total lipids in a composition (e.g., a liposomal composition).

[0501] In embodiments, a composition (e.g., a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.1 wt % to about 20 wt % (e.g., about 0.1 wt % to about 15 wt %) of a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)). In embodiments, a delivery vehicle (e.g., a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.5 wt %, about 1 wt %, about 3 wt %, about 5 wt %, or about 10 wt % a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)). In embodiments, a delivery vehicle (e.g., a liposomal delivery vehicle such as a lipid nanoparticle) comprises up to about 0.5 wt %, about 1 wt %, about 3 wt %, about 5 wt %, about 10 wt %, about 15 wt %, or about 20 wt % of a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)). In embodiments, the percentage results in an improved beneficial effect (e.g., improved delivery to targeted tissues such as the liver or the lung).

[0502] The amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) in a composition also can be described as a percentage (“mol %”) of the combined molar amounts of total lipids of a composition (e.g., the combined molar amounts of all lipids present in a liposomal delivery vehicle).

[0503] In embodiments of pharmaceutical compositions described herein, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 0.5 mol % to about 30 mol % (e.g., about 0.5 mol % to about 20 mol %) of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle.

[0504] In embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 0.5 mol % to about 5 mol %, about 1 mol % to about 10 mol %, about 5 mol % to about 20 mol %, or about 10 mol % to about 20 mol % of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle. In embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is about 1 mol % to about 30 mol %, about 1 mol % to about 20 mol %, about 1 mol % to about 15 mol %, about 1 mol % to about 10 mol %, or about 5 mol % to about 25 mol % of the combined dry weight of all lipids present in a composition such as a liposomal delivery vehicle

[0505] In certain embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) can comprise from about 0.1 mol % to about 50 mol %, or from 0.5 mol % to about 50 mol %, or from about 1 mol % to about 25 mol %, or from about 1 mol % to about 10 mol % of the total amount of lipids in a composition (e.g., a

liposomal delivery vehicle).
[0506] In certain embodiments, a cationic lipid as described herein (e.g., a cationic lipid of Formula (I), such as the cationic lipid of Formula (Ia), compound (1), compound (2), compound (3), and/or compound (4)) can comprise greater than about 0.1 mol %, or greater than about 0.5 mol %, or greater (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) than about 1 mol %, or greater than about 5 mol % of the total amount of lipids in the lipid nanoparticle.
[0507] In certain embodiments, a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) can comprise less than about 25 mol %, or less than about 10 mol %, or less than about 5 mol %, or less than about 1 mol % of the total amount of lipids in a composition (e.g., a liposomal delivery vehicle).
[0508] In embodiments, the amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is at least about 5 mol %, about 10 mol %, about 15 mol %, about 20 mol %, about 25 mol %, about 30 mol %, about 35 mol %, about 40 mol %, about 45 mol %, about 50 mol %, about 55 mol %, about 60 mol %, about 65 mol %, about 70 mol %, about 75 mol %, about 80 mol %, about 85 mol %, about 90 mol %, about 95 mol %, about 96 mol %, about 97 mol %, about 98 mol %, or about 99 mol % of the combined dry weight of total lipids in a composition (e.g., a liposomal composition).
[0509] In embodiments, the amount of a cationic lipid as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) is present in an amount that is no more than about 5 mol %, about 10 mol %, about 15 mol %, about 20 mol %, about 25 mol %, about 30 mol %, about 35 mol %, about 40 mol %, about 45 mol %, about 50 mol %, about 55 mol %, about 60 mol %, about 65 mol %, about 70 mol %, about 75 mol %, about 80 mol %, about 85 mol %, about 90 mol %, about 95 mol %, about 96 mol %, about 97 mol %, about 98 mol %, or about 99 mol % of the combined dry weight of total lipids in a composition (e.g., a liposomal composition).
[0510] In embodiments, the percentage results in an improved beneficial effect (e.g., improved delivery to targeted tissues such as the liver or the lung).
[0511] In embodiments, a composition further comprises one more lipids (e.g., one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids).
[0512] In certain embodiments, such pharmaceutical (e.g., liposomal) compositions comprise one or more of a PEG-modified lipid, a non-cationic lipid and a cholesterol lipid. In embodiments, such pharmaceutical (e.g., liposomal) compositions comprise: one or more PEG-modified lipids; one or more non-cationic lipids; and one or more cholesterol lipids. In embodiments, such pharmaceutical (e.g., liposomal) compositions comprise: one or more PEG-modified lipids and one or more cholesterol lipids.
[0513] In embodiments, a composition (e.g., lipid nanoparticle) that encapsulates a nucleic acid (e.g., mRNA encoding a peptide or polypeptide) comprises one or more cationic lipids as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) and one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid.
[0514] In embodiments, a composition (e.g., lipid nanoparticle) that encapsulates a nucleic acid (e.g., mRNA encoding a peptide or polypeptide) comprises one or more cationic lipids as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)); one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid; and further comprises a cholesterol-based lipid.
[0515] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (e.g., mRNA encoding a peptide or polypeptide) comprises one or more cationic lipids as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)), as well as one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, a PEGylated lipid, and a cholesterol-based lipid.
[0516] According to various embodiments, the selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells, the characteristics of the mRNA to be delivered. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus, the molar ratios may be adjusted accordingly. Further Cationic Lipids
[0517] In addition to any of the cationic lipids as described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)), a composition may comprise one or more further cationic lipids.

[0518] In some embodiments, liposomes may comprise one or more further cationic lipids. As used herein, the phrase “cationic lipid” refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have been described in the literature, many of which are commercially available.
[0519] Suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2010/144740, which is incorporated herein by reference. In certain embodiments, the compositions include a cationic lipid, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate, having a compound structure of:
##STR00103##
and pharmaceutically acceptable salts thereof.
[0520] Other suitable additional cationic lipids for use in the compositions include ionizable cationic lipids as described in International Patent Publication WO 2013/149140, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid of one of the following formulas:
##STR00104##
or a pharmaceutically acceptable salt thereof, wherein R.sub.1 and R.sub.2 are each independently selected from the group consisting of hydrogen, an optionally substituted, variably saturated or unsaturated C.sub.1-C.sub.20 alkyl and an optionally substituted, variably saturated or unsaturated C.sub.6-C.sub.20 acyl; wherein L.sub.1 and L.sub.2 are each independently selected from the group consisting of hydrogen, an optionally substituted C.sub.1-C.sub.30 alkyl, an optionally substituted variably unsaturated C.sub.1-C.sub.30 alkenyl, and an optionally substituted C.sub.1-C.sub.30 alkynyl; wherein m and o are each independently selected from the group consisting of zero and any positive integer (e.g., where m is three); and wherein n is zero or any positive integer (e.g., where n is one). In certain embodiments, the compositions include the cationic lipid (15Z, 18Z)—N,N-dimethyl-6-(9Z,12Z)-octadeca-9,12-dien-1-yl) tetracosa-15,18-dien-1-amine (“HGT5000”), having a compound structure of:
##STR00105##
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions include the cationic lipid (15Z, 18Z)—N,N-dimethyl-6-((9Z,12Z)-octadeca-9,12-dien-1-yl) tetracosa-4,15,18-trien-1-amine (“HGT5001”), having a compound structure of:
##STR00106##
and pharmaceutically acceptable salts thereof. In certain embodiments, the include the cationic lipid and (15Z,18Z)—N,N-dimethyl-6-((9Z,12Z)-octadeca-9,12-dien-1-yl) tetracosa-5,15,18-trien-1-amine (“HGT5002”), having a compound structure of:
##STR00107##
and pharmaceutically acceptable salts thereof.
[0521] Other suitable additional cationic lipids for use in the compositions include cationic lipids described as aminoalcohol lipidoids in International Patent Publication WO 2010/053572, which is incorporated herein by reference. In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00108##
and pharmaceutically acceptable salts thereof.
[0522] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2016/118725, which is incorporated herein by reference. In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00109##
and pharmaceutically acceptable salts thereof.
[0523] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2016/118724, which is incorporated herein by reference. In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00110##
and pharmaceutically acceptable salts thereof.
[0524] Other suitable cationic lipids for use in the compositions include a cationic lipid having the formula of 14,25-ditridecyl 15,18,21,24-tetraaza-octatriacontane, and pharmaceutically acceptable salts thereof.
[0525] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publications WO 2013/063468 and WO 2016/205691, each of which are incorporated herein by reference. In some embodiments, the compositions include a cationic lipid of the following formula:
##STR00111##
or pharmaceutically acceptable salts thereof, wherein each instance of R.sup.L is independently optionally substituted C.sub.6-C.sub.40 alkenyl. In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00112##
and pharmaceutically acceptable salts thereof.
[0526] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00113##
and pharmaceutically acceptable salts thereof.
[0527] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00114##
and pharmaceutically acceptable salts thereof.
[0528] In certain embodiments, the compositions include a cationic lipid having a compound structure of:

##STR00115##
and pharmaceutically acceptable salts thereof.
[0529] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2015/184256, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid of the following formula:
##STR00116##
or a pharmaceutically acceptable salt thereof, wherein each X independently is O or S; each Y independently is O or S; each m independently is 0 to 20; each n independently is 1 to 6; each R.sub.A is independently hydrogen, optionally substituted C1-50 alkyl, optionally substituted C2-50 alkenyl, optionally substituted C2-50 alkynyl, optionally substituted C3-10 carbocyclyl, optionally substituted 3-14 membered heterocyclyl, optionally substituted C6-14 aryl, optionally substituted 5-14 membered heteroaryl or halogen; and each R.sub.B is independently hydrogen, optionally substituted C1-50 alkyl, optionally substituted C2-50 alkenyl, optionally substituted C2-50 alkynyl, optionally substituted C3-10 carbocyclyl, optionally substituted 3-14 membered heterocyclyl, optionally substituted C6-14 aryl, optionally substituted 5-14 membered heteroaryl or halogen. In certain embodiments, the compositions include a cationic lipid, “Target 23”, having a compound structure of:
##STR00117##
and pharmaceutically acceptable salts thereof.
[0530] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2016/004202, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00118##
or a pharmaceutically acceptable salt thereof.
[0531] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00119##
or a pharmaceutically acceptable salt thereof.
[0532] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00120##
or a pharmaceutically acceptable salt thereof.
[0533] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in J. McClellan, M. C. King, Cell 2010, 141, 210-217 and in Whitehead et al., Nature Communications (2014) 5:4277, which is incorporated herein by reference. In certain embodiments, the cationic lipids of the compositions include a cationic lipid having a compound structure of:
##STR00121##
and pharmaceutically acceptable salts thereof.
[0534] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2015/199952, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00122##
and pharmaceutically acceptable salts thereof.
[0535] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00123##
and pharmaceutically acceptable salts thereof.
[0536] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00124##
and pharmaceutically acceptable salts thereof.
[0537] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00125##
and pharmaceutically acceptable salts thereof.
[0538] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00126##
and pharmaceutically acceptable salts thereof.
[0539] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00127##
and pharmaceutically acceptable salts thereof.
[0540] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00128##
and pharmaceutically acceptable salts thereof.
[0541] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00129##
and pharmaceutically acceptable salts thereof.
[0542] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00130##
and pharmaceutically acceptable salts thereof.
[0543] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00131##

and pharmaceutically acceptable salts thereof.
[0544] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00132##
and pharmaceutically acceptable salts thereof.
[0545] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00133##
and pharmaceutically acceptable salts thereof.
[0546] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00134##
and pharmaceutically acceptable salts thereof.
[0547] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2017/004143, which is incorporated herein by reference.
[0548] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00135##
and pharmaceutically acceptable salts thereof.
[0549] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00136##
and pharmaceutically acceptable salts thereof.
[0550] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00137##
and pharmaceutically acceptable salts thereof.
[0551] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00138##
and pharmaceutically acceptable salts thereof.
[0552] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00139##
and pharmaceutically acceptable salts thereof.
[0553] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00140##
and pharmaceutically acceptable salts thereof.
[0554] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00141##
and pharmaceutically acceptable salts thereof.
[0555] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00142##
and pharmaceutically acceptable salts thereof.
[0556] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00143##
and pharmaceutically acceptable salts thereof.
[0557] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00144##
and pharmaceutically acceptable salts thereof.
[0558] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00145##
and pharmaceutically acceptable salts thereof.
[0559] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00146##
and pharmaceutically acceptable salts thereof.
[0560] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00147##
and pharmaceutically acceptable salts thereof.
[0561] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00148##
and pharmaceutically acceptable salts thereof.
[0562] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00149##
and pharmaceutically acceptable salts thereof.
[0563] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00150##
and pharmaceutically acceptable salts thereof.
[0564] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00151##
and pharmaceutically acceptable salts thereof.
[0565] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in

International Patent Publication WO 2017/075531, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid of the following formula:
##STR00152##
or a pharmaceutically acceptable salt thereof, wherein one of L.sup.1 or L.sup.2 is —O(C═O)—, —(C═O)O—, —C(═O)—, —O—, —S(O).sub.x, —S—S—, —C(═O)S—, —SC(═O)—, —NR.sup.aC(═O)—, —C(═O)NR.sup.a—, NR.sup.aC(═O)NR.sup.a—, —OC(═O)NR.sup.a—, or —NR.sup.aC(═O)O—; and the other of L.sup.1 or L.sup.2 is —O(C═O)—, —(C═O)O—, —C(═O)—, —O—, —S(O).sub.x, —S—S—, —C(═O)S—, SC(═O)—, —NR.sup.aC(═O)—, —C(═O)NR.sup.a—, NR.sup.aC(═O)NR.sup.a—, —OC(═O)NR.sup.a— or —NR.sup.aC(═O)O— or a direct bond; G.sup.1 and G.sup.2 are each independently unsubstituted C.sub.1-C.sub.12 alkylene or C.sub.1-C.sub.12 alkenylene; G.sup.3 is C.sub.1-C.sub.24 alkylene, C.sub.1-C.sub.24 alkenylene, C.sub.3-C.sub.8 cycloalkylene, C.sub.3-C.sub.8 cycloalkenylene; R.sup.a is H or C.sub.1-C.sub.12 alkyl; R.sup.1 and R.sup.2 are each independently C.sub.6-C.sub.24 alkyl or C.sub.6-C.sub.24 alkenyl; R.sup.3 is H, OR′, CN, —C(═O)OR.sup.4, —OC(═O)R.sup.4 or —NR.sup.5C(═O)R.sup.4; R.sup.4 is C.sub.1-C.sub.12 alkyl; R.sup.5 is H or C.sub.1-C.sub.6 alkyl; and x is 0, 1 or 2.
[0566] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2017/117528, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00153##
and pharmaceutically acceptable salts thereof.
[0567] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00154##
and pharmaceutically acceptable salts thereof.
[0568] In some embodiments, the compositions include a cationic lipid having the compound structure:
##STR00155##
and pharmaceutically acceptable salts thereof.
[0569] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2017/049245, which is incorporated herein by reference. In some embodiments, the cationic lipids of the compositions and methods of the present invention include a compound of one of the following formulas:
##STR00156##
and pharmaceutically acceptable salts thereof. For any one of these four formulas, R.sup.4 is independently selected from —(CH.sub.2).sub.nQ and —(CH.sub.2).sub.nCHQR; Q is selected from the group consisting of —OR, —OH, —O(CH.sub.2).sub.nN(R).sub.2, —OC(O)R, —CX.sub.3, —CN, —N(R)C(O)R, —N(H)C(O)R, —N(R)S(O).sub.2R, —N(H)S(O).sub.2R, —N(R)C(O)N(R).sub.2, —N(H)C(O)N(R).sub.2, —N(H)C(O)N(H)(R), —N(R)C(S)N(R).sub.2, —N(H)C(S)N(R).sub.2, —N(H)C(S)N(H)(R), and a heterocycle; R is independently selected from the group consisting of C.sub.1-3 alkyl, C.sub.2-3 alkenyl, and H; and n is 1, 2, or 3.
[0570] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00157##
and pharmaceutically acceptable salts thereof.
[0571] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00158##
and pharmaceutically acceptable salts thereof.
[0572] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00159##
and pharmaceutically acceptable salts thereof.
[0573] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00160##
and pharmaceutically acceptable salts thereof.
[0574] Other suitable additional cationic lipids for use in the compositions include the cationic lipids as described in International Patent Publication WO 2017/173054 and WO 2015/095340, each of which is incorporated herein by reference.
[0575] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00161##
and pharmaceutically acceptable salts thereof.
[0576] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00162##
and pharmaceutically acceptable salts thereof.
[0577] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00163##
and pharmaceutically acceptable salts thereof.
[0578] In certain embodiments, the compositions include a cationic lipid having a compound structure of:
##STR00164##
and pharmaceutically acceptable salts thereof.
[0579] Other suitable additional cationic lipids for use in the compositions include cholesterol-based cationic lipids. In certain embodiments, the compositions include imidazole cholesterol ester or “ICE”, having a compound structure of:
##STR00165##
and pharmaceutically acceptable salts thereof.

[0580] Other suitable additional cationic lipids for use in the compositions include cleavable cationic lipids as described in International Patent Publication WO 2012/170889, which is incorporated herein by reference. In some embodiments, the compositions include a cationic lipid of the following formula:
##STR00166##
wherein R.sub.1 is selected from the group consisting of imidazole, guanidinium, amino, imine, enamine, an optionally-substituted alkyl amino (e.g., an alkyl amino such as dimethylamino) and pyridyl; wherein R.sub.2 is selected from the group consisting of one of the following two formulas:
##STR00167##
and wherein R.sub.3 and R.sub.4 are each independently selected from the group consisting of an optionally substituted, variably saturated or unsaturated C.sub.6-C.sub.20 alkyl and an optionally substituted, variably saturated or unsaturated C.sub.6-C.sub.20 acyl; and wherein n is zero or any positive integer (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty or more).
[0581] In certain embodiments, the compositions include a cationic lipid, “HGT4001”, having a compound structure of:
##STR00168##
and pharmaceutically acceptable salts thereof.
[0582] In certain embodiments, the compositions include a cationic lipid, “HGT4002”, having a compound structure of:
##STR00169##
and pharmaceutically acceptable salts thereof.
[0583] In certain embodiments, the compositions include a cationic lipid, “HGT4003”, having a compound structure of:
##STR00170##
and pharmaceutically acceptable salts thereof.
[0584] In certain embodiments, the compositions include a cationic lipid, “HGT4004”, having a compound structure of:
##STR00171##
and pharmaceutically acceptable salts thereof.
[0585] In certain embodiments, the compositions include a cationic lipid “HGT4005”, having a compound structure of:
##STR00172##
and pharmaceutically acceptable salts thereof.
[0586] In some embodiments, the compositions include the cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (“DOTMA”). Feigner et al. (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355, each of which is incorporated herein by reference. DOTMA can be formulated alone or can be combined with a neutral lipid (e.g., dioleoylphosphatidyl-ethanolamine or “DOPE”) or still other cationic or non-cationic lipids into a liposomal transfer vehicle or a lipid nanoparticle, and such liposomes can be used to enhance the delivery of nucleic acids into target cells. Other cationic lipids suitable for the compositions include, for example, 5-carboxyspermylglycinedioctadecylamide (“DOGS”); 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (“DOSPA”) (Behr et al. Proc. Nat.'l Acad. Sci. 86, 6982 (1989), U.S. Pat. Nos. 5,171,678; 5,334,761); 1,2-Dioleoyl-3-Dimethylammonium-Propane (“DODAP”); 1,2-Dioleoyl-3-Trimethylammonium-Propane (“DOTAP”).
[0587] Additional exemplary cationic lipids suitable for the compositions also include: 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (“DSDMA”); 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (“DODMA”); 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (“DLinDMA”); 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (“DLenDMA”); N-dioleyl-N,N-dimethylammonium chloride (“DODAC”); N,N-distearyl-N,N-dimethylarnmonium bromide (“DDAB”); N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (“DMRIE”); 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane (“CLinDMA”); 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethyl-1-(cis,cis-9′,1-2′-octadecadienoxy)propane (“CpLinDMA”); N,N-dimethyl-3,4-dioleyloxybenzylamine (“DMOBA”); 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (“DOcarbDAP”); 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (“DLinDAP”); 1,2-N,N′-Dilinoleylcarbamyl-3-dimethylaminopropane (“DLincarbDAP”); 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane (“DLinCDAP”); 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (“DLin-K-DMA”); 2-((8-[(3P)-cholest-5-en-3-yloxy]octyl)oxy)-N, N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien-1-yloxy]propane-1-amine (“Octyl-CLinDMA”); (2R)-2-((8-[(3beta)-cholest-5-en-3-yloxy]octyl)oxy)-N, N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien-1-yloxy]propan-1-amine (“Octyl-CLinDMA (2R)”); (2S)-2-((8-[(3P)-cholest-5-en-3-yloxy]octyl)oxy)-N, fsl-dimethyh3-[(9Z, 12Z)-octadeca-9, 12-dien-1-yloxy]propan-1-amine (“Octyl-CLinDMA (2S)”); 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (“DLin-K-XTC2-DMA”); and 2-(2,2-di((9Z,12Z)-octadeca-9,12-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethanamine (“DLin-KC2-DMA”) (see, WO 2010/042877, which is incorporated herein by reference; Semple et al., Nature Biotech. 28: 172-176 (2010)). (Heyes, J., et al., J Controlled Release 107: 276-287 (2005); Morrissey, D V., et al., Nat. Biotechnol. 23(8): 1003-1007 (2005); International Patent Publication WO 2005/121348). In some embodiments, one or more of the cationic lipids comprise at least one of an imidazole, dialkylamino, or guanidinium moiety.
[0588] In some embodiments, one or more cationic lipids suitable for the compositions include 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (“XTC”); (3aR,5s,6aS)—N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (“ALNY-100”) and/or 4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide (“NC98-5”).
[0589] In some embodiments, the percentage of total cationic lipids in a composition (e.g., a liposomal composition) may be no more than 10%, no more than 20%, no more than 30%, no more than 40%, no more than 50%, no more than 60%, no more than 70%, no more than 80%, no more than 90%, or no more than 95% of total lipids as measured by molar ratios (mol %) or by weight (wt %).

[0590] In some embodiments, the percentage of total cationic lipids in a composition (e.g., a liposomal composition) may be greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, or greater than 95% of total lipids as measured by molar ratios (mol %) or by weight (wt %).
[0591] In some embodiments, total cationic lipid(s) constitute(s) about 30-50% (e.g., about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%) of the liposome by weight. In some embodiments, the cationic lipid constitutes about 30%, about 35%, about 40%, about 45%, or about 50% of a composition (e.g., a liposomal composition) by molar ratio. In some embodiments, total cationic lipid(s) constitute(s) about 30-50% (e.g., about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%) of the liposome by weight. In some embodiments, the cationic lipid constitutes about 30%, about 35%, about 40%, about 45%, or about 50% of a composition (e.g., a liposomal composition) by weight.
Non-cationic/Helper Lipids
[0592] Compositions (e.g., liposomal compositions) may also comprise one or more non-cationic ("helper") lipids. As used herein, the phrase "non-cationic lipid" refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase "anionic lipid" refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), or a mixture thereof.
[0593] In embodiments, a non-cationic or helper lipid is dioleoylphosphatidylethanolamine (DOPE).
[0594] In some embodiments, a non-cationic lipid is a neutral lipid, i.e., a lipid that does not carry a net charge in the conditions under which the composition is formulated and/or administered.
[0595] In some embodiments, a non-cationic lipid may be present in a molar ratio (mol %) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10% to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a molar ratio (mol %) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10% to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 mol %, greater than about 10 mol %, greater than about 20 mol %, greater than about 30 mol %, or greater than about 40 mol %. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 mol %, greater than about 10 mol %, greater than about 20 mol %, greater than about 30 mol %, or greater than about 40 mol %. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 mol %, no more than about 10 mol %, no more than about 20 mol %, no more than about 30 mol %, or no more than about 40 mol %. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than about 5 mol %, no more than about 10 mol %, no more than about 20 mol %, no more than about 30 mol %, or no more than about 40 mol %.
[0596] In some embodiments, a non-cationic lipid may be present in a weight ratio (wt %) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10% to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a weight ratio (wt %) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10% to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 wt %, greater than about 10 wt %, greater than about 20 wt %, greater than about 30 wt %, or greater than about 40 wt %. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 wt %, greater than about 10 wt %, greater than about 20 wt %, greater than about 30 wt %, or greater than about 40 wt %. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 wt %, no more than about 10 wt %, no more than about 20 wt %, no more than about 30 wt %, or no more than about 40 wt %. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than about 5 wt %, no more than about 10 wt %, no more than about 20 wt %, no more than about 30 wt %, or no more than about 40 wt %.
Cholesterol-Based Lipids
[0597] In some embodiments, a composition (e.g., a liposomal composition) comprises one or more cholesterol-based lipids. For example, suitable cholesterol-based lipids include cholesterol and, for example, DC-Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or imidazole cholesterol ester (ICE), which has the following structure,
##STR00173##
[0598] In embodiments, a cholesterol-bsaed lipid is cholesterol.
[0599] In some embodiments, a cholesterol-based lipid may be present in a molar ratio (mol %) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 mol %, greater than about 10 mol %, greater than about 20 mol %, greater than about 30 mol %, or greater than about 40 mol %. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 mol %, no more than about 10 mol %, no more than about 20 mol %, no more than about 30 mol %, or no more than about 40 mol %.

[0600] In some embodiments, a cholesterol-based lipid may be present in a weight ratio (wt %) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 wt %, greater than about 10 wt %, greater than about 20 wt %, greater than about 30 wt %, or greater than about 40 wt %. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 wt %, no more than about 10 wt %, no more than about 20 wt %, no more than about 30 wt %, or no more than about 40 wt %.

PEGylated Lipids

[0601] In some embodiments, a composition (e.g., a liposomal composition) comprises one or more PEGylated lipids.

[0602] For example, the use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-octanoyl-sphingosine-1-[succinyl(methoxy polyethylene glycol)-2000](C8 PEG-2000 ceramide) is also contemplated by the present invention in combination with one or more of the cationic and, in some embodiments, other lipids together which comprise the liposome. In some embodiments, particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C.sub.14 or C.sub.18).

[0603] In embodiments, a PEG-modified lipid is 1,2-dimyristoyl-sn-glycerol, methoxypolyethylene glycol (DMG-PEG2000).

[0604] Contemplated PEG-modified lipids (also referred to herein as a PEGylated lipid, which term is interchangeable with PEG-modified lipid) include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C.sub.6-C.sub.20 length. In some embodiments, a PEG-modified or PEGylated lipid is PEGylated cholesterol or PEG-2K. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target cell, (Klibanov et al. (1990) FEBS Letters, 268 (1): 235-37), or they may be selected to rapidly exchange out of the formulation in vivo (see U.S. Pat. No. 5,885,613).

[0605] A PEG-modified phospholipid and derivatized lipids of the present invention may be present in a molar ratio (mol %) from about 0% to about 15%, about 0.5% to about 15%, about 1% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the composition (e.g., a liposomal composition).

[0606] A PEG-modified phospholipid and derivatized lipids of the present invention may be present in a weight ratio (wt %) from about 0% to about 15%, about 0.5% to about 15%, about 1% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the composition (e.g., a liposomal composition).

Pharmaceutical Formulations and Therapeutic Uses

[0607] Cationic lipids described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) may be used in the preparation of compositions (e.g., to construct liposomal compositions) that facilitate or enhance the delivery and release of encapsulated materials (e.g., one or more therapeutic polynucleotides) to one or more target cells (e.g., by permeating or fusing with the lipid membranes of such target cells).

[0608] For example, when a liposomal composition (e.g., a lipid nanoparticle) comprises or is otherwise enriched with one or more of the compounds disclosed herein, the phase transition in the lipid bilayer of the one or more target cells may facilitate the delivery of the encapsulated materials (e.g., one or more therapeutic polynucleotides encapsulated in a lipid nanoparticle) into the one or more target cells.

[0609] Similarly, in certain embodiments cationic lipids described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) may be used to prepare liposomal vehicles that are characterized by their reduced toxicity in vivo. In certain embodiments, the reduced toxicity is a function of the high transfection efficiencies associated with the compositions disclosed herein, such that a reduced quantity of such composition may administered to the subject to achieve a desired therapeutic response or outcome.

[0610] Thus, pharmaceutical formulations comprising a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) and nucleic acids provided by the present invention may be used for various therapeutic purposes. To facilitate delivery of nucleic acids in vivo, a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ia)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ia)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ia)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ia)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) and nucleic acids can be formulated in combination with one or more additional pharmaceutical carriers, targeting ligands or stabilizing reagents. In some embodiments, a cationic lipid described herein (e.g., a cationic lipid of Formula (I) or (II), such as the cationic lipid of Formula (Ia), (IIa), compound (1) and/or compound (2)) can be formulated via pre-mixed lipid solution. In other embodiments, a composition comprising a cationic lipid described herein (e.g., a cationic lipid of any of Formulas A (e.g., (A-I)-(A-II) and (A-Ja)-(A-IIa)), D (e.g., (D-A), (D-I)-(D-III), (D-Ja)-(D-Id), (D-IIa), and (D-IIIa)-(D-IIId)), E (e.g., (E-I)-(E-II) and (E-Ja)-(E-IIa)), and K (e.g., (K-I)-(K-II) and (K-Ja)-(K-IIa)) such as any of Compounds (A1)-(A4), (D1)-(D7), (E1)-(E5) and (K1)-(K4)) can be formulated using post-insertion techniques into the lipid membrane of the nanoparticles. Techniques for formulation and administration of drugs may be found in “Remington's Pharmaceutical Sciences,” Mack Publishing Co., Easton, Pa., latest edition.

[0611] Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous,

intraperitoneal, or intranasal. In particular embodiments, the intramuscular administration is to a muscle selected from the group consisting of skeletal muscle, smooth muscle and cardiac muscle. In some embodiments the administration results in delivery of the nucleic acids to a muscle cell. In some embodiments the administration results in delivery of the nucleic acids to a hepatocyte (i.e., liver cell). In embodiments, administration is intramuscular. In embodiments, administration is intravenous. In embodiments, administration is intratracheal.

[0612] Alternatively or additionally, pharmaceutical formulations of the invention may be administered in a local rather than systemic manner, for example, via injection of the pharmaceutical formulation directly into a targeted tissue, preferably in a sustained release formulation. Local delivery can be affected in various ways, depending on the tissue to be targeted. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include, but are not limited to the liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid. In embodiments, the tissue to be targeted in the liver. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection.

[0613] In embodiments, administration is via pulmonary delivery. As used herein, pulmonary delivery refers to delivery to lung via, e.g., nasal cavity, trachea, bronchi, bronchioles, and/or other pulmonary system. In embodiments, a composition described herein is formulated for nebulization. In embodiments, the delivery vehicle may be in an aerosolized composition which can be inhaled. In embodiments, pulmonary delivery involves inhalation (e.g., for nasal, tracheal, or bronchial delivery). In embodiments, a composition is nebulized prior to inhalation.

[0614] The present invention provides methods for delivering a composition having full-length mRNA molecules encoding a peptide or polypeptide of interest for use in the treatment of a subject, e.g., a human subject or a cell of a human subject or a cell that is treated and delivered to a human subject.

[0615] Accordingly, in certain embodiments the present invention provides a method for producing a therapeutic composition comprising full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for cystic fibrosis transmembrane conductance regulator (CFTR) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ATP-binding cassette sub-family A member 3 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for dynein axonemal intermediate chain 1 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for dynein axonemal heavy chain 5 (DNAH5) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for alpha-1-antitrypsin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for forkhead box P3 (FOXP3) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes one or more surfactant protein, e.g., one or more of surfactant A protein, surfactant B protein, surfactant C protein, and surfactant D protein.

[0616] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. Such peptides and polypeptides can include those associated with a urea cycle disorder, associated with a lysosomal storage disorder, with a glycogen storage disorder, associated with an amino acid metabolism disorder, associated with a lipid metabolism or fibrotic disorder, associated with methylmalonic acidemia, or associated with any other metabolic disorder for which delivery to or treatment of the liver or a liver cell with enriched full-length mRNA provides therapeutic benefit.

[0617] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with a urea cycle disorder. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ornithine transcarbamylase (OTC) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for arginosuccinate synthetase 1 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for carbamoyl phosphate synthetase I protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for arginosuccinate lyase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for arginase protein.

[0618] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with a lysosomal storage disorder. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for alpha galactosidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for glucocerebrosidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for iduronate-2-sulfatase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for iduronidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for N-acetyl-alpha-D-glucosaminidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for heparan N-sulfatase protein. In certain embodiments the present invention provides a method for producing a

therapeutic composition having full-length mRNA that encodes for galactosamine-6 sulfatase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for beta-galactosidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for lysosomal lipase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for arylsulfatase B (N-acetylgalactosamine-4-sulfatase) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for transcription factor EB (TFEB).

[0619] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with a glycogen storage disorder. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for acid alpha-glucosidase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for glucose-6-phosphatase (G6PC) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for liver glycogen phosphorylase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for muscle phosphoglycerate mutase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for glycogen debranching enzyme.

[0620] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with amino acid metabolism. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for phenylalanine hydroxylase enzyme. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for glutaryl-CoA dehydrogenase enzyme. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for propionyl-CoA caboxylase enzyme. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for oxalase alanine-glyoxylate aminotransferase enzyme.

[0621] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with a lipid metabolism or fibrotic disorder. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a mTOR inhibitor. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ATPase phospholipid transporting 8B1 (ATP8B1) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for one or more NF-kappa B inhibitors, such as one or more of I-kappa B alpha, interferon-related development regulator 1 (IFRD1), and Sirtuin 1 (SIRT1). In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for PPAR-gamma protein or an active variant.

[0622] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein associated with methylmalonic acidemia. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for methylmalonyl CoA mutase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for methylmalonyl CoA epimerase protein.

[0623] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA for which delivery to or treatment of the liver can provide therapeutic benefit. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ATP7B protein, also known as Wilson disease protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for porphobilinogen deaminase enzyme. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for one or clotting enzymes, such as Factor VIII, Factor IX, Factor VII, and Factor X. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for human hemochromatosis (HFE) protein.

[0624] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the cardiovasculature of a subject or a cardiovascular cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for vascular endothelial growth factor A protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for relaxin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for bone morphogenetic protein-9 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for bone morphogenetic protein-2 receptor protein.

[0625] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the muscle of a subject or a muscle cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for dystrophin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for frataxin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the cardiac muscle of a subject or a cardiac muscle cell. In certain

embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein that modulates one or both of a potassium channel and a sodium channel in muscle tissue or in a muscle cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein that modulates a Kv7.1 channel in muscle tissue or in a muscle cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a protein that modulates a Nav1.5 channel in muscle tissue or in a muscle cell.

[0626] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the nervous system of a subject or a nervous system cell. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for survival motor neuron 1 protein. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for survival motor neuron 2 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for frataxin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ATP binding cassette subfamily D member 1 (ABCD1) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for CLN3 protein.

[0627] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the blood or bone marrow of a subject or a blood or bone marrow cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for beta globin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for Bruton's tyrosine kinase protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for one or clotting enzymes, such as Factor VIII, Factor IX, Factor VII, and Factor X.

[0628] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the kidney of a subject or a kidney cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for collagen type IV alpha 5 chain (COL4A5) protein.

[0629] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery to or treatment of the eye of a subject or an eye cell. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for ATP-binding cassette sub-family A member 4 (ABCA4) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for retinoschisin protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for retinal pigment epithelium-specific 65 kDa (RPE65) protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for centrosomal protein of 290 kDa (CEP290).

[0630] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or polypeptide for use in the delivery of or treatment with a vaccine for a subject or a cell of a subject. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from an infectious agent, such as a virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from influenza virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from respiratory syncytial virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from rabies virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from cytomegalovirus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from rotavirus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from a hepatitis virus, such as hepatitis A virus, hepatitis B virus, or hepatis C virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from human papillomavirus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from a herpes simplex virus, such as herpes simplex virus 1 or herpes simplex virus 2. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from a human immunodeficiency virus, such as human immunodeficiency virus type 1 or human immunodeficiency virus type 2. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from a human metapneumovirus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from a human parainfluenza virus, such as human parainfluenza virus type 1, human parainfluenza virus type 2, or human parainfluenza virus type 3. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from malaria virus. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from zika virus. In certain embodiments the present invention provides a method for producing a therapeutic composition

having full-length mRNA that encodes for an antigen from chikungunya virus.
[0631] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen associated with a cancer of a subject or identified from a cancer cell of a subject. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen determined from a subject's own cancer cell, i.e., to provide a personalized cancer vaccine. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen expressed from a mutant KRAS gene.
[0632] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody. In certain embodiments, the antibody can be a bi-specific antibody. In certain embodiments, the antibody can be part of a fusion protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody to OX40. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody to VEGF. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody to tissue necrosis factor alpha. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody to CD3. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antibody to CD19.
[0633] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an immunomodulator. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for Interleukin 12. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for Interleukin 23. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for Interleukin 36 gamma. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a constitutively active variant of one or more stimulator of interferon genes (STING) proteins.
[0634] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an endonuclease. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an RNA-guided DNA endonuclease protein, such as Cas 9 protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a meganuclease protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a transcription activator-like effector nuclease protein. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for a zinc finger nuclease protein.
[0635] In embodiments, exemplary therapeutic uses result from the delivery of mRNA encoding a secreted protein. Accordingly, in embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding a secreted protein. In some embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding one or more secreted proteins listed in Table 1; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein
TABLE-US-00001 TABLE 1 Secreted Proteins Uniprot ID Protein Name Gene Name A1E959 Odontogenic ameloblast-associated ODAM protein A1KZ92 Peroxidasin-like protein PXDNL A1L453 Serine protease 38 PRSS38 A1L4H1 Soluble scavenger receptor cysteine-rich SSC5D domain-containing protein SSC5D A2RUU4 Colipase-like protein 1 CLPSL1 A2VDF0 Fucose mutarotase FUOM A2VEC9 SCO-spondin SSPO A3KMH1 von Willebrand factor A domain- VWA8 containing protein 8 A4D0S4 Laminin subunit beta-4 LAMB4 A4D1T9 Probable inactive serine protease 37 PRSS37 A5D8T8 C-type lectin domain family 18 member A CLEC18A A6NC86 phospholipase A2 inhibitor and PINLYP Ly6/PLAUR domain-containing protein A6NCI4 von Willebrand factor A domain- VWA3A containing protein 3A A6ND01 Probable folate receptor delta FOLR4 A6NDD2 Beta-defensin 108B-like A6NE02 BTB/POZ domain-containing protein 17 BTBD17 A6NEF6 Growth hormone 1 GH1 A6NF02 NPIP-like protein LOC730153 A6NFB4 HCG1749481, isoform CRA_k CSH1 A6NFZ4 Protein FAM24A FAM24A A6NG13 Glycosyltransferase 54 domain-containing protein A6NGN9 IgLON family member 5 IGLON5 A6NHN0 Otolin-1 OTOL1 A6NHN6 Nuclear pore complex-interacting protein- NPIPL2 like 2 A6NI73 Leukocyte immunoglobulin-like receptor LILRA5 subfamily A member 5 A6NIT4 Chorionic somatomammotropin hormone CSH2 2 isoform 2 A6NJ69 IgA-inducing protein homolog IGIP A6NKQ9 Choriogonadotropin subunit beta variant 1 CGB1 A6NMZ7 Collagen alpha-6(VI) chain COL6A6 A6NNS2 Dehydrogenase/reductase SDR family DHRS7C member 7C A6XGL2 Insulin A chain INS A8K0G1 Protein Wnt WNT7B A8K2U0 Alpha-2-macroglobulin-like protein 1 A2ML1 A8K7I4 Calcium-activated chloride channel CLCA1 regulator 1 A8MTL9 Serpin-like protein HMSD HMSD A8MV23 Serpin E3 SERPINE3 A8MZH6 Oocyte-secreted protein 1 homolog OOSP1 A8TX70 Collagen alpha-5(VI) chain COL6A5 B0ZBE8 Natriuretic peptide NPPA B1A4G9 Somatotropin GH1 B1A4H2 HCG1749481, isoform CRA_d CSH1 B1A4H9 Chorionic somatomammotropin hormone CSH2 B1AJZ6 Protein Wnt WNT4 B1AKI9 Isthmin-1 ISM1 B2RNN3 Complement C1q and tumor necrosis C1QTNF9B factor-related protein 9B B2RUY7 von Willebrand factor C domain- VWC2L containing protein 2-like B3GLJ2 Prostate and testis expressed protein 3 PATE3 B4DI03 SEC11-like 3 (*S. cerevisiae*), isoform SEC11L3 CRA_a B4DJF9 Protein Wnt WNT4 B4DUL4 SEC11-like 1 (*S. cerevisiae*), isoform SEC11L1 CRA_d B5MCC8 Protein Wnt WNT10B B8A595 Protein Wnt WNT7B B8A597 Protein Wnt WNT7B B8A598 Protein Wnt WNT7B B9A064 Immunoglobulin lambda-like polypeptide IGLL5 5 C9J3H3 Protein Wnt WNT10B C9J8I8 Protein Wnt WNT5A C9JAF2

Insulin-like growth factor II Ala-25 Del IGF2 C9JCI2 Protein Wnt WNT10B C9JL84 HERV-H LTR-associating protein 1 HHLA1 C9JNR5 Insulin A chain INS C9JUI2 Protein Wnt WNT2 D6RF47 Protein Wnt WNT8A D6RF94 Protein Wnt WNT8A E2RYF7 Protein PBMUCL2 HCG22 E5RFR1 PENK(114-133) PENK E7EML9 Serine protease 44 PRSS44 E7EPC3 Protein Wnt WNT9B E7EVP0 Nociceptin PNOC E9PD02 Insulin-like growth factor I IGF1 E9PH60 Protein Wnt WNT16 E9PJL6 Protein Wnt WNT11 F5GYM2 Protein Wnt WNT5B F5H034 Protein Wnt WNT5B F5H364 Protein Wnt WNT5B F5H7Q6 Protein Wnt WNT5B F8WCM5 Protein INS-IGF2 INS-IGF2 F8WDR1 Protein Wnt WNT2 H0Y663 Protein Wnt WNT4 H0YK72 Signal peptidase complex catalytic SEC11A subunit SEC11A H0YK83 Signal peptidase complex catalytic SEC11A subunit SEC11A H0YM39 Chorionic somatomammotropin hormone CSH2 H0YMT7 Chorionic somatomammotropin hormone CSH1 H0YN17 Chorionic somatomammotropin hormone CSH2 H0YNA5 Signal peptidase complex catalytic SEC11A subunit SEC11A H0YNG3 Signal peptidase complex catalytic SEC11A subunit SEC11A H0YNX5 Signal peptidase complex catalytic SEC11A subunit SEC11A H7BZB8 Protein Wnt WNT10A H9KV56 Choriogonadotropin subunit beta variant 2 CGB2 I3L0L8 Protein Wnt WNT9B J3KNZ1 Choriogonadotropin subunit beta variant 1 CGB1 J3KP00 Choriogonadotropin subunit beta CGB7 J3QT02 Choriogonadotropin subunit beta variant 1 CGB1 O00175 C-C motif chemokine 24 CCL24 O00182 Galectin-9 LGALS9 O00187 Mannan-binding lectin serine protease 2 MASP2 O00230 Cortistatin CORT O00253 Agouti-related protein AGRP O00270 12-(S)-hydroxy-5,8,10,14-eicosatetraenoic GPR31 acid receptor O00292 Left-right determination factor 2 LEFTY2 O00294 Tubby-related protein 1 TULP1 O00295 Tubby-related protein 2 TULP2 O00300 Tumor necrosis factor receptor TNFRSF11B superfamily member 11B O00339 Matrilin-2 MATN2 O00391 Sulfhydryl oxidase 1 QSOX1 O00468 Agrin AGRN O00515 Ladinin-1 LAD1 O00533 Processed neural cell adhesion molecule CHL1 L1-like protein O00584 Ribonuclease T2 RNASET2 O00585 C-C motif chemokine 21 CCL21 O00602 Ficolin-1 FCN1 O00622 Protein CYR61 CYR61 O00626 MDC(5-69) CCL22 O00634 Netrin-3 NTN3 O00744 Protein Wnt-10b WNT10B O00755 Protein Wnt-7a WNT7A O14498 Immunoglobulin superfamily containing ISLR leucine-rich repeat protein O14511 Pro-neuregulin-2, membrane-bound NRG2 isoform O14594 Neurocan core protein NCAN O14625 C-X-C motif chemokine 11 CXCL11 O14638 Ectonucleotide ENPP3 pyrophosphatase/phosphodiesterase family member 3 O14656 Torsin-1A TOR1A O14657 Torsin-1B TOR1B O14786 Neuropilin-1 NRP1 O14788 Tumor necrosis factor ligand superfamily TNFSF11 member 11, membrane form O14791 Apolipoprotein L1 APOL1 O14793 Growth/differentiation factor 8 MSTN O14904 Protein Wnt-9a WNT9A O14905 Protein Wnt-9b WNT9B O14944 Proepiregulin EREG O14960 Leukocyte cell-derived chemotaxin-2 LECT2 O15018 Processed PDZ domain-containing protein PDZD2 2 O15041 Semaphorin-3E SEMA3E O15072 A disintegrin and metalloproteinase with ADAMTS3 thrombospondin motifs 3 O15123 Angiopoietin-2 ANGPT2 O15130 Neuropeptide FF NPFF O15197 Ephrin type-B receptor 6 EPHB6 O15204 ADAM DEC1 ADAMDEC1 O15230 Laminin subunit alpha-5 LAMA5 O15232 Matrilin-3 MATN3 O15240 Neuroendocrine regulatory peptide-1 VGF O15263 Beta-defensin 4A DEFB4A O15335 Chondroadherin CHAD O15393 Transmembrane protease serine 2 TMPRSS2 catalytic chain O15444 C-C motif chemokine 25 CCL25 O15467 C-C motif chemokine 16 CCL16 O15496 Group 10 secretory phospholipase A2 PLA2G10 O15520 Fibroblast growth factor 10 FGF10 O15537 Retinoschisin RS1 O43157 Plexin-B1 PLXNB1 O43184 Disintegrin and metalloproteinase ADAM12 domain-containing protein 12 O43240 Kallikrein-10 KLK10 O43278 Kunitz-type protease inhibitor 1 SPINT1 O43320 Fibroblast growth factor 16 FGF16 O43323 Desert hedgehog protein C-product DHH O43405 Cochlin COCH O43508 Tumor necrosis factor ligand superfamily TNFSF12 member 12, membrane form O43555 Progonadoliberin-2 GNRH2 O43557 Tumor necrosis factor ligand superfamily TNFSF14 member 14, soluble form O43692 Peptidase inhibitor 15 PI15 O43699 Sialic acid-binding Ig-like lectin 6 SIGLEC6 O43820 Hyaluronidase-3 HYAL3 O43827 Angiopoietin-related protein 7 ANGPTL7 O43852 Calumenin CALU O43854 EGF-like repeat and discoidin I-like EDIL3 domain-containing protein 3 O43866 CD5 antigen-like CD5L O43897 Tolloid-like protein 1 TLL1 O43915 Vascular endothelial growth factor D FIGF O43927 C-X-C motif chemokine 13 CXCL13 O60218 Aldo-keto reductase family 1 member AKR1B10 B10 O60235 Transmembrane protease serine 11D TMPRSS11D O60258 Fibroblast growth factor 17 FGF17 O60259 Kallikrein-8 KLK8 O60383 Growth/differentiation factor 9 GDF9 O60469 Down syndrome cell adhesion molecule DSCAM O60542 Persephin PSPN O60565 Gremlin-1 GREM1 O60575 Serine protease inhibitor Kazal-type 4 SPINK4 O60676 Cystatin-8 CST8 O60687 Sushi repeat-containing protein SRPX2 SRPX2 O60844 Zymogen granule membrane protein 16 ZG16 O60882 Matrix metalloproteinase-20 MMP20 O60938 Keratocan KERA O75015 Low affinity immunoglobulin gamma Fc FCGR3B region receptor III-B O75077 Disintegrin and metalloproteinase ADAM23 domain-containing protein 23 O75093 Slit homolog 1 protein SLIT1 O75094 Slit homolog 3 protein SLIT3 O75095 Multiple epidermal growth factor-like MEGF6 domains protein 6 O75173 A disintegrin and metalloproteinase with ADAMTS4 thrombospondin motifs 4 O75200 Nuclear pore complex-interacting protein- NPIPL1 like 1 O75339 Cartilage intermediate layer protein 1 C1 CILP O75354 Ectonucleoside triphosphate ENTPD6 diphosphohydrolase 6 O75386 Tubby-related protein 3 TULP3 O75398 Deformed epidermal autoregulatory factor DEAF1 1 homolog O75443 Alpha-tectorin TECTA O75445 Usherin USH2A O75462 Cytokine receptor-like factor 1 CRLF1 O75487 Glypican-4 GPC4 O75493 Carbonic anhydrase-related protein 11 CA11 O75594 Peptidoglycan recognition protein 1 PGLYRP1 O75596 C-type lectin domain family 3 member A CLEC3A O75610 Left-right determination factor 1 LEFTY1 O75629 Protein CREG1 CREG1 O75636 Ficolin-3 FCN3 O75711 Scrapie-responsive protein 1 SCRG1 O75715 Epididymal secretory glutathione GPX5 peroxidase O75718 Cartilage-associated protein CRTAP O75829 Chondrosurfactant protein LECT1 O75830 Serpin 12 SERPINI2 O75882 Attractin ATRN O75888 Tumor necrosis factor ligand superfamily TNFSF13 member 13 O75900 Matrix metalloproteinase-23 MMP23A O75951 Lysozyme-like protein 6 LYZL6 O75973 C1q-related factor C1QL1 O76038 Secretagogin SCGN O76061 Stanniocalcin-2 STC2 O76076 WNT1-inducible-signaling pathway WISP2 protein 2 O76093 Fibroblast growth factor 18 FGF18 O76096 Cystatin-F CST7 O94769 Extracellular matrix protein 2 ECM2 O94813 Slit homolog 2 protein C-product SLIT2 O94907 Dickkopf-related protein 1 DKK1 O94919 Endonuclease domain-containing 1 ENDOD1 protein O94964 N-terminal form SOGA1 O95025 Semaphorin-3D SEMA3D O95084 Serine protease 23 PRSS23 O95150 Tumor necrosis factor ligand superfamily TNFSF15 member 15 O95156 Neurexophilin-2 NXPH2 O95157 Neurexophilin-3 NXPH3 O95158

Neurexophilin-4 NXPH4 O95388 WNT1-inducible-signaling pathway WISP1 protein 1 O95389 WNT1-inducible-signaling pathway WISP3 protein 3 O95390 Growth/differentiation factor 11 GDF11 O95393 Bone morphogenetic protein 10 BMP10 O95399 Urotensin-2 UTS2 O95407 Tumor necrosis factor receptor TNFRSF6B superfamily member 6B O95428 Papilin PAPLN O95445 Apolipoprotein M APOM O95450 A disintegrin and metalloproteinase with ADAMTS2 thrombospondin motifs 2 O95460 Matrilin-4 MATN4 O95467 LHAL tetrapeptide GNAS O95631 Netrin-1 NTN1 O95633 Follistatin-related protein 3 FSTL3 O95711 Lymphocyte antigen 86 LY86 O95715 C-X-C motif chemokine 14 CXCL14 O95750 Fibroblast growth factor 19 FGF19 O95760 Interleukin-33 IL33 O95813 Cerberus CER1 O95841 Angiopoietin-related protein 1 ANGPTL1 O95897 Noelin-2 OLFM2 O95925 Eppin EPPIN O95965 Integrin beta-like protein 1 ITGBL1 O95967 EGF-containing fibulin-like extracellular EFEMP2 matrix protein 2 O95968 Secretoglobin family 1D member 1 SCGB1D1 O95969 Secretoglobin family 1D member 2 SCGB1D2 O95970 Leucine-rich glioma-inactivated protein 1 LGI1 O95972 Bone morphogenetic protein 15 BMP15 O95994 Anterior gradient protein 2 homolog AGR2 O95998 Interleukin-18-binding protein IL18BP O96009 Napsin-A NAPSA O96014 Protein Wnt-11 WNT11 P00450 Ceruloplasmin CP P00451 Factor VIIIa light chain F8 P00488 Coagulation factor XIII A chain F13A1 P00533 Epidermal growth factor receptor EGFR P00709 Alpha-lactalbumin LALBA P00734 Prothrombin F2 P00738 Haptoglobin beta chain HP P00739 Haptoglobin-related protein HPR P00740 Coagulation factor IXa heavy chain F9 P00742 Factor X heavy chain F10 P00746 Complement factor D CFD P00747 Plasmin light chain B PLG P00748 Coagulation factor XIIa light chain F12 P00749 Urokinase-type plasminogen activator PLAU long chain A P00750 Tissue-type plasminogen activator PLAT P00751 Complement factor B Ba fragment CFB P00797 Renin REN P00973 2′-5′-oligoadenylate synthase 1 OAS1 P00995 Pancreatic secretory trypsin inhibitor SPINK1 P01008 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P05113 Interleukin-5 IL5 P05120 Plasminogen activator inhibitor 2 SERPINB2 P05121 Plasminogen activator inhibitor 1

SERPINE1 P05154 Plasma serine protease inhibitor SERPINA5 P05155 Plasma protease C1 inhibitor SERPING1 P05156 Complement factor I heavy chain CFI P05160 Coagulation factor XIII B chain F13B P05161 Ubiquitin-like protein ISG15 ISG15 P05230 Fibroblast growth factor 1 FGF1 P05231 Interleukin-6 IL6 P05305 Big endothelin-1 EDN1 P05408 C-terminal peptide SCG5 P05451 Lithostathine-1-alpha REG1A P05452 Tetranectin CLEC3B P05543 Thyroxine-binding globulin SERPINA7 P05814 Beta-casein CSN2 P05997 Collagen alpha-2(V) chain COL5A2 P06276 Cholinesterase BCHE P06307 Cholecystokinin-12 CCK P06396 Gelsolin GSN P06681 Complement C2 C2 P06702 Protein S100-A9 S100A9 P06727 Apolipoprotein A-IV APOA4 P06734 Low affinity immunoglobulin epsilon Fc FCER2 receptor soluble form P06744 Glucose-6-phosphate isomerase GPI P06850 Corticoliberin CRH P06858 Lipoprotein lipase LPL P06881 Calcitonin gene-related peptide 1 CALCA P07093 Glia-derived nexin SERPINE2 P07098 Gastric triacylglycerol lipase LIPF P07225 Vitamin 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FREM3 protein 3 P0COL4 C4d-A C4A P0COL5 Complement C4-B alpha chain C4B P0COP6 Neuropeptide S NPS P0C7L1 Serine protease inhibitor Kazal-type 8 SPINK8 P0C862 Complement C1q and tumor necrosis C1QTNF9 factor-related protein 9A P0C8F1 Prostate and testis expressed protein 4 PATE4 P0CG01 Gastrokine-3 GKN3P P0CG36 Cryptic family protein 1B CFC1B P0CG37 Cryptic protein CFC1 P0CJ68 Humanin-like protein 1 MTRNR2L1 P0CJ69 Humanin-like protein 2 MTRNR2L2 P0CJ70 Humanin-like protein 3 MTRNR2L3 P0CJ71 Humanin-like protein 4 MTRNR2L4 P0CJ72 Humanin-like protein 5 MTRNR2L5 P0CJ73 Humanin-like protein 6 MTRNR2L6 P0CJ74 Humanin-like protein 7 MTRNR2L7 P0CJ75 Humanin-like protein 8 MTRNR2L8 P0CJ76 Humanin-like protein 9 MTRNR2L9 P0CJ77 Humanin-like protein 10 MTRNR2L10 P0DJD7 Pepsin A-4 PGA4 P0DJD8 Pepsin A-3 PGA3 P0DJD9 Pepsin A-5 PGA5 P0DJI8 Amyloid protein A SAA1 P0DJI9 Serum amyloid A-2 protein SAA2 P10082 Peptide YY(3-36) PYY P10092 Calcitonin gene-related peptide 2 CALCB P10124 Serglycin SRGN 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HTN3 P15692 Vascular endothelial growth factor A VEGFA P15814 Immunoglobulin lambda-like polypeptide IGLL1 1 P15907 Beta-galactoside alpha-2,6- ST6GAL1 sialyltransferase 1 P15941 Mucin-1 subunit beta MUC1 P16035 Metalloproteinase inhibitor 2 TIMP2 P16112 Aggrecan core protein 2 ACAN P16233 Pancreatic triacylglycerol lipase PNLIP P16442 Histo-blood group ABO system ABO transferase P16471 Prolactin receptor PRLR P16562 Cysteine-rich secretory protein 2 CRISP2 P16619 C-C motif chemokine 3-like 1 CCL3L1 P16860 BNP(3-29) NPPB P16870 Carboxypeptidase E CPE P16871 Interleukin-7 receptor subunit alpha IL7R P17213 Bactericidal permeability-increasing BPI protein P17538 Chymotrypsinogen B CTRB1 P17931 Galectin-3 LGALS3 P17936 Insulin-like growth factor-binding protein IGFBP3 3 P17948 Vascular endothelial growth factor FLT1 receptor 1 P18065 Insulin-like growth factor-binding protein IGFBP2 2 P18075 Bone morphogenetic protein 7 BMP7 P18428 Lipopolysaccharide-binding protein LBP P18509 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COL5A3 P25942 Tumor necrosis factor receptor CD40 superfamily member 5 P26022 Pentraxin-related protein PTX3 PTX3 P26927 Hepatocyte growth factor-like protein beta MST1 chain P27169 Serum paraoxonase/arylesterase 1 PON1 P27352 Gastric intrinsic factor GIF P27487 Dipeptidyl peptidase 4 membrane form DPP4 P27539 Embryonic growth/differentiation factor 1 GDF1 P27658 Vastatin COL8A1 P27797 Calreticulin CALR P27918 Properdin CFP P28039 Acyloxyacyl hydrolase AOAH P28300 Protein-lysine 6-oxidase LOX P28325 Cystatin-D CST5 P28799 Granulin-1 GRN P29122 Proprotein convertase subtilisin/kexin PCSK6 type 6 P29279 Connective tissue growth factor CTGF P29320 Ephrin type-A receptor 3 EPHA3 P29400 Collagen alpha-5(IV) chain COL4A5 P29459 Interleukin-12 subunit alpha IL12A P29460 Interleukin-12 subunit beta IL12B P29508 Serpin B3 SERPINB3 P29622 Kallistatin SERPINA4 P29965 CD40 ligand, soluble form CD40LG P30990 Neurotensin/neuromedin N NTS P31025 Lipocalin-1 LCN1 P31151 Protein S100-A7 S100A7 P31371 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TFPI2 P48357 Leptin receptor LEPR P48594 Serpin B4 SERPINB4 P48645 Neuromedin-U-25 NMU P48740 Mannan-binding lectin serine protease 1 MASP1 P48745

Protein NOV homolog NOV P48960 CD97 antigen subunit beta CD97 P49223 Kunitz-type protease inhibitor 3 SPINT3 P49747 Cartilage oligomeric matrix protein COMP P49763 Placenta growth factor PGF P49765 Vascular endothelial growth factor B VEGFB P49767 Vascular endothelial growth factor C VEGFC P49771 Fms-related tyrosine kinase 3 ligand FLT3LG P49862 Kallikrein-7 KLK7 P49863 Granzyme K GZMK P49908 Selenoprotein P SEPP1 P49913 Antibacterial protein FALL-39 CAMP P50607 Tubby protein homolog TUB P51124 Granzyme M GZMM P51512 Matrix metalloproteinase-16 MMP16 P51654 Glypican-3 GPC3 P51671 Eotaxin CCL11 P51884 Lumican LUM P51888 Prolargin PRELP P52798 Ephrin-A4 EFNA4 P52823 Stanniocalcin-1 STC1 P53420 Collagen alpha-4(IV) chain COL4A4 P53621 Coatomer subunit alpha COPA P54108 Cysteine-rich secretory protein 3 CRISP3 P54315 Pancreatic lipase-related protein 1 PNLIPRP1 P54317 Pancreatic lipase-related protein 2 PNLIPRP2 P54793 Arylsulfatase F ARSF P55000 Secreted Ly-6/uPAR-related protein 1 SLURP1 P55001 Microfibrillar-associated protein 2 MFAP2 P55056 Apolipoprotein C-IV APOC4 P55058 Phospholipid transfer protein PLTP P55075 Fibroblast growth factor 8 FGF8 P55081 Microfibrillar-associated protein 1 MFAP1 P55083 Microfibril-associated glycoprotein 4 MFAP4 P55107 Bone morphogenetic protein 3B GDF10 P55145 Mesencephalic astrocyte-derived MANF neurotrophic factor P55259 Pancreatic secretory granule membrane GP2 major glycoprotein GP2 P55268 Laminin subunit beta-2 LAMB2 P55773 CCL23(30-99) CCL23 P55774 C-C motif chemokine 18 CCL18 P55789 FAD-linked sulfhydryl oxidase ALR GFER P56703 Proto-oncogene Wnt-3 WNT3 P56704 Protein Wnt-3a WNT3A P56705 Protein Wnt-4 WNT4 P56706 Protein Wnt-7b WNT7B P56730 Neurotrypsin PRSS12 P56851 Epididymal secretory protein E3-beta EDDM3B P56975 Neuregulin-3 NRG3 P58062 Serine protease inhibitor Kazal-type 7 SPINK7 P58215 Lysyl oxidase homolog 3 LOXL3 P58294 Prokineticin-1 PROK1 P58335 Anthrax toxin receptor 2 ANTXR2 P58397 A disintegrin and metalloproteinase with ADAMTS12 thrombospondin motifs 12 P58417 Neurexophilin-1 NXPH1 P58499 Protein FAM3B FAM3B P59510 A disintegrin and metalloproteinase with ADAMTS20 thrombospondin motifs 20 P59665 Neutrophil defensin 1 DEFA1B P59666 Neutrophil defensin 3 DEFA3 P59796 Glutathione peroxidase 6 GPX6 P59826 BPI fold-containing family B member 3 BPIFB3 P59827 BPI fold-containing family B member 4 BPIFB4 P59861 Beta-defensin 131 DEFB131 P60022 Beta-defensin 1 DEFB1 P60153 Inactive ribonuclease-like protein 9 RNASE9 P60827 Complement C1q tumor necrosis factor- C1QTNF8 related protein 8 P60852 Zona pellucida sperm-binding protein 1 ZP1 P60985 Keratinocyte differentiation-associated KRTDAP protein P61109 Kidney androgen-regulated protein KAP P61278 Somatostatin-14 SST P61366 Osteocrin OSTN P61626 Lysozyme C LYZ P61769 Beta-2-microglobulin B2M P61812 Transforming growth factor beta-2 TGFB2 P61916 Epididymal secretory protein E1 NPC2 P62502 Epididymal-specific lipocalin-6 LCN6 P62937 Peptidyl-prolyl cis-trans isomerase A PPIA P67809 Nuclease-sensitive element-binding YBX1 protein 1 P67812 Signal peptidase complex catalytic SEC11A subunit SEC11A P78310 Coxsackievirus and adenovirus receptor CXADR P78333 Secreted glypican-5 GPC5 P78380 Oxidized low-density lipoprotein receptor OLR1 1 P78423 Processed fractalkine CX3CL1 P78509 Reelin RELN P78556 CCL20(2-70) CCL20 P80075 MCP-2(6-76) CCL8 P80098 C-C motif chemokine 7 CCL7 P80108 Phosphatidylinositol-glycan-specific GPLD1 phospholipase D P80162 C-X-C motif chemokine 6 CXCL6 P80188 Neutrophil gelatinase-associated lipocalin LCN2 P80303 Nucleobindin-2 NUCB2 P80511 Calcitermin S100A12 P81172 Hepcidin-25 HAMP P81277 Prolactin-releasing peptide PRLH P81534 Beta-defensin 103 DEFB103A P81605 Dermcidin DCD P82279 Protein crumbs homolog 1 CRB1 P82987 ADAMTS-like protein 3 ADAMTSL3 P83105 Serine protease HTRA4 HTRA4 P83110 Serine protease HTRA3 HTRA3 P83859 Orexigenic neuropeptide QRFP QRFP P98088 Mucin-5AC MUC5AC P98095 Fibulin-2 FBLN2 P98160 Basement membrane-specific heparan HSPG2 sulfate proteoglycan core protein P98173 Protein FAM3A FAM3A Q00604 Norrin NDP Q00796 Sorbitol dehydrogenase SORD Q00887 Pregnancy-specific beta-1-glycoprotein 9 PSG9 Q00888 Pregnancy-specific beta-1-glycoprotein 4 PSG4 Q00889 Pregnancy-specific beta-1-glycoprotein 6 PSG6 Q01523 HD5(56-94) DEFA5 Q01524 Defensin-6 DEFA6 Q01955 Collagen alpha-3(IV) chain COL4A3 Q02297 Pro-neuregulin-1, membrane-bound NRG1 isoform Q02325 Plasminogen-like protein B PLGLB1 Q02383 Semenogelin-2 SEMG2 Q02388 Collagen alpha-1(VII) chain COL7A1 Q02505 Mucin-3A MUC3A Q02509 Otoconin-90 OC90 Q02747 Guanylin GUCA2A Q02763 Angiopoietin-1 receptor TEK Q02817 Mucin-2 MUC2 Q02985 Complement factor H-related protein 3 CFHR3 Q03167 Transforming growth factor beta receptor TGFBR3 type 3 Q03403 Trefoil factor 2 TFF2 Q03405 Urokinase plasminogen activator surface PLAUR receptor Q03591 Complement factor H-related protein 1 CFHR1 Q03692 Collagen alpha-1(X) chain COL10A1 Q04118 Basic salivary proline-rich protein 3 PRB3 Q04756 Hepatocyte growth factor activator short HGFAC chain Q04900 Sialomucin core protein 24 CD164 Q05315 Eosinophil lysophospholipase CLC Q05707 Collagen alpha-1(XIV) chain COL14A1 Q05996 Processed zona pellucida sperm-binding ZP2 protein 2 Q06033 Inter-alpha-trypsin inhibitor heavy chain ITIH3 H3 Q06141 Regenerating islet-derived protein 3-alpha REG3A Q06828 Fibromodulin FMOD Q07092 Collagen alpha-1(XVI) chain COL16A1 Q07325 C-X-C motif chemokine 9 CXCL9 Q07507 Dermatopontin DPT Q075Z2 Binder of sperm protein homolog 1 BSPH1 Q07654 Trefoil factor 3 TFF3 Q07699 Sodium channel subunit beta-1 SCN1B Q08345 Epithelial discoidin domain-containing DDR1 receptor 1 Q08380 Galectin-3-binding protein LGALS3BP Q08397 Lysyl oxidase homolog 1 LOXL1 Q08431 Lactadherin MFGE8 Q08629 Testican-1 SPOCK1 Q08648 Sperm-associated antigen 11B SPAG11B Q08830 Fibrinogen-like protein 1 FGL1 Q10471 Polypeptide N- GALNT2 acetylgalactosaminyltransferase 2 Q10472 Polypeptide N- GALNT1 acetylgalactosaminyltransferase 1 Q11201 CMP-N-acetylneuraminate-beta- ST3GAL1 galactosamide-alpha-2,3-sialyltransferase 1 Q11203 CMP-N-acetylneuraminate-beta-1,4-ST3GAL3 galactoside alpha-2,3-sialyltransferase Q11206 CMP-N-acetylneuraminate-beta- ST3GAL4 galactosamide-alpha-2,3-sialyltransferase 4 Q12794 Hyaluronidase-1 HYAL1 Q12805 EGF-containing fibulin-like extracellular EFEMP1 matrix protein 1 Q12836 Zona pellucida sperm-binding protein 4 ZP4 Q12841 Follistatin-related protein 1 FSTL1 Q12904 Aminoacyl tRNA synthase complex- AIMP1 interacting multifunctional protein 1 Q13018 Soluble secretory phospholipase A2 PLA2R1 receptor Q13072 B melanoma antigen 1 BAGE Q13093 Platelet-activating factor acetylhydrolase PLA2G7 Q13103 Secreted phosphoprotein 24 SPP2 Q13162 Peroxiredoxin-4 PRDX4 Q13201 Platelet glycoprotein Ia* MMRN1 Q13214 Semaphorin-3B SEMA3B Q13219 Pappalysin-1 PAPPA Q13231 Chitotriosidase-1 CHIT1 Q13253 Noggin NOG Q13261 Interleukin-15 receptor subunit alpha IL15RA Q13275 Semaphorin-3F SEMA3F Q13291 Signaling lymphocytic activation SLAMF1

molecule Q13316 Dentin matrix acidic phosphoprotein 1 DMP1 Q13361 Microfibrillar-associated protein 5 MFAP5 Q13410 Butyrophilin subfamily 1 member A1 BTN1A1 Q13421 Mesothelin, cleaved form MSLN Q13429 Insulin-like growth factor I IGF-I Q13443 Disintegrin and metalloproteinase ADAM9 domain-containing protein 9 Q13519 Neuropeptide 1 PNOC Q13751 Laminin subunit beta-3 LAMB3 Q13753 Laminin subunit gamma-2 LAMC2 Q13790 Apolipoprotein F APOF Q13822 Ectonucleotide ENPP2 pyrophosphatase/phosphodiesterase family member 2 Q14031 Collagen alpha-6(IV) chain COL4A6 Q14050 Collagen alpha-3(IX) chain COL9A3 Q14055 Collagen alpha-2(IX) chain COL9A2 Q14112 Nidogen-2 NID2 Q14114 Low-density lipoprotein receptor-related LRP8 protein 8 Q14118 Dystroglycan DAG1 Q14314 Fibroleukin FGL2 Q14393 Growth arrest-specific protein 6 GAS6 Q14406 Chorionic somatomammotropin hormone- CSHL1 like 1 Q14507 Epididymal secretory protein E3-alpha EDDM3A Q14508 WAP four-disulfide core domain protein 2 WFDC2 Q14512 Fibroblast growth factor-binding protein 1 FGFBP1 Q14515 SPARC-like protein 1 SPARCL1 Q14520 Hyaluronan-binding protein 2 27 kDa HABP2 light chain Q14563 Semaphorin-3A SEMA3A Q14623 Indian hedgehog protein IHH Q14624 Inter-alpha-trypsin inhibitor heavy chain ITIH4 H4 Q14667 UPF0378 protein KIAA0100 KIAA0100 Q14703 Membrane-bound transcription factor site- MBTPS1 1 protease Q14766 Latent-transforming growth factor beta- LTBP1 binding protein 1 Q14767 Latent-transforming growth factor beta- LTBP2 binding protein 2 Q14773 Intercellular adhesion molecule 4 ICAM4 Q14993 Collagen alpha-1(XIX) chain COL19A1 Q14CN2 Calcium-activated chloride channel CLCA4 regulator 4, 110 kDa form Q15046 Lysine--tRNA ligase KARS Q15063 Periostin POSTN Q15109 Advanced glycosylation end product- AGER specific receptor Q15113 Procollagen C-endopeptidase enhancer 1 PCOLCE Q15166 Serum paraoxonase/lactonase 3 PON3 Q15195 Plasminogen-like protein A PLGLA Q15198 Platelet-derived growth factor receptor- PDGFRL like protein Q15223 Poliovirus receptor-related protein 1 PVRL1 Q15238 Pregnancy-specific beta-1-glycoprotein 5 PSG5 Q15363 Transmembrane emp24 domain- TMED2 containing protein 2 Q15375 Ephrin type-A receptor 7 EPHA7 Q15389 Angiopoietin-1 ANGPT1 Q15465 Sonic hedgehog protein SHH Q15485 Ficolin-2 FCN2 Q15517 Corneodesmosin CDSN Q15582 Transforming growth factor-beta-induced TGFBI protein ig-h3 Q15661 Tryptase alpha/beta-1 TPSAB1 Q15726 Metastin KISS1 Q15782 Chitinase-3-like protein 2 CHI3L2 Q15828 Cystatin-M CST6 Q15846 Clusterin-like protein 1 CLUL1 Q15848 Adiponectin ADIPOQ Q16206 Protein disulfide-thiol oxidoreductase ENOX2 Q16270 Insulin-like growth factor-binding protein IGFBP7 7 Q16363 Laminin subunit alpha-4 LAMA4 Q16378 Proline-rich protein 4 PRR4 Q16557 Pregnancy-specific beta-1-glycoprotein 3 PSG3 Q16568 CART(42-89) CARTPT Q16610 Extracellular matrix protein 1 ECM1 Q16619 Cardiotrophin-1 CTF1 Q16623 Syntaxin-1A STX1A Q16627 HCC-1(9-74) CCL14 Q16651 Prostasin light chain PRSS8 Q16661 Guanylate cyclase C-activating peptide 2 GUCA2B Q16663 CCL15(29-92) CCL15 Q16674 Melanoma-derived growth regulatory MIA protein Q16769 Glutaminyl-peptide cyclotransferase QPCT Q16787 Laminin subunit alpha-3 LAMA3 Q16842 CMP-N-acetylneuraminate-beta- ST3GAL2 galactosamide-alpha-2,3-sialyltransferase 2 Q17RR3 Pancreatic lipase-related protein 3 PNLIPRP3 Q17RW2 Collagen alpha-1(XXIV) chain COL24A1 Q17RY6 Lymphocyte antigen 6K LY6K Q1L6U9 Prostate-associated microseminoprotein MSMP Q1W4C9 Serine protease inhibitor Kazal-type 13 SPINK13 Q1ZYL8 Izumo sperm-egg fusion protein 4 IZUMO4 Q29960 HLA class I histocompatibility antigen, HLA-C Cw-16 alpha chain Q210M5 R-spondin-4 RSPO4 Q2L4Q9 Serine protease 53 PRSS53 Q2MKA7 R-spondin-1 RSPO1 Q2MV58 Tectonic-1 TCTN1 Q2TAL6 Brorin VWC2 Q2UY09 Collagen alpha-1(XXVIII) chain COL28A1 Q2VPA4 Complement component receptor 1-like CR1L protein Q2WEN9 Carcinoembryonic antigen-related cell CEACAM16 adhesion molecule 16 Q30KP8 Beta-defensin 136 DEFB136 Q30KP9 Beta-defensin 135 DEFB135 Q30KQ1 Beta-defensin 133 DEFB133 Q30KQ2 Beta-defensin 130 DEFB130 Q30KQ4 Beta-defensin 116 DEFB116 Q30KQ5 Beta-defensin 115 DEFB115 Q30KQ6 Beta-defensin 114 DEFB114 Q30KQ7 Beta-defensin 113 DEFB113 Q30KQ8 Beta-defensin 112 DEFB112 Q30KQ9 Beta-defensin 110 DEFB110 Q30KR1 Beta-defensin 109 DEFB109P1 Q32P28 Prolyl 3-hydroxylase 1 LEPRE1 Q3B7J2 Glucose-fructose oxidoreductase domain- GFOD2 containing protein 2 Q3SY79 Protein Wnt WNT3A Q3T906 N-acetylglucosamine-1- GNPTAB phosphotransferase subunits alpha/beta Q495T6 Membrane metallo-endopeptidase-like 1 MMEL1 Q49AH0 Cerebral dopamine neurotrophic factor CDNF Q4G0G5 Secretoglobin family 2B member 2 SCGB2B2 Q4G0M1 Protein FAM132B FAM132B Q4LDE5 Sushi, von Willebrand factor type A, EGF SVEP1 and pentraxin domain-containing protein 1 Q4QY38 Beta-defensin 134 DEFB134 Q4VAJ4 Protein Wnt WNT10B Q4W5P6 Protein TMEM155 TMEM155 Q4ZHG4 Fibronectin type III domain-containing FNDC1 protein 1 Q53H76 Phospholipase A1 member A PLA1A Q53RD9 Fibulin-7 FBLN7 Q53S33 BolA-like protein 3 BOLA3 Q5BLP8 Neuropeptide-like protein C4orf48 C4orf48 Q5DT21 Serine protease inhibitor Kazal-type 9 SPINK9 Q5EBL8 PDZ domain-containing protein 11 PDZD11 Q5FYB0 Arylsulfatase J ARSJ Q5FYB1 Arylsulfatase I ARSI Q5GAN3 Ribonuclease-like protein 13 RNASE13 Q5GAN4 Ribonuclease-like protein 12 RNASE12 Q5GAN6 Ribonuclease-like protein 10 RNASE10 Q5GFL6 von Willebrand factor A domain- VWA2 containing protein 2 Q5H8A3 Neuromedin-S NMS Q5H8C1 FRAS1-related extracellular matrix FREM1 protein 1 Q5IJ48 Protein crumbs homolog 2 CRB2 Q5J5C9 Beta-defensin 121 DEFB121 Q5JS37 NHL repeat-containing protein 3 NHLRC3 Q5JTB6 Placenta-specific protein 9 PLAC9 Q5JU69 Torsin-2A TOR2A Q5JXM2 Methyltransferase-like protein 24 METTL24 Q5JZY3 Ephrin type-A receptor 10 EPHA10 Q5K4E3 Polyserase-2 PRSS36 Q5SRR4 Lymphocyte antigen 6 complex locus LY6G5C protein G5c Q5T1H1 Protein eyes shut homolog EYS Q5T4F7 Secreted frizzled-related protein 5 SFRP5 Q5T4W7 Artemin ARTN Q5T7M4 Protein FAM132A FAM132A Q5TEH8 Protein Wnt WNT2B Q5TIE3 von Willebrand factor A domain- VWA5B1 containing protein 5B1 Q5UCC4 ER membrane protein complex subunit 10 EMC10 Q5VST6 Abhydrolase domain-containing protein FAM108B1 FAM108B1 Q5VTL7 Fibronectin type III domain-containing FNDC7 protein 7 Q5VUM1 UPF0369 protein C6orf57 C6orf57 Q5VV43 Dyslexia-associated protein KIAA0319 KIAA0319 Q5VWW1 Complement C1q-like protein 3 C1QL3 Q5VXI9 Lipase member N LIPN Q5VXJ0 Lipase member K LIPK Q5VXM1 CUB domain-containing protein 2 CDCP2 Q5VYX0 Renalase RNLS Q5VYY2 Lipase member M LIPM Q5W186 Cystatin-9 CST9 Q5W5W9 Regulated endocrine-specific protein 18 RESP18 Q5XG92 Carboxylesterase 4A CES4A Q63HQ2 Pikachurin EGFLAM Q641Q3 Meteorin-like protein METRNL Q66K79 Carboxypeptidase Z CPZ Q685J3 Mucin-17 MUC17 Q68BL7 Olfactomedin-like protein 2A OLFML2A Q68BL8 Olfactomedin-like protein 2B OLFML2B Q68DV7 E3 ubiquitin-protein ligase RNF43 RNF43

Q6B9Z1 Insulin growth factor-like family member IGFL4 4 Q6BAA4 Fc receptor-like B FCRLB Q6E0U4 Dermokine DMKN Q6EMK4 Vasorin VASN Q6FHJ7 Secreted frizzled-related protein 4 SFRP4 Q6GPI1 Chymotrypsin B2 chain B CTRB2 Q6GTS8 Probable carboxypeptidase PM20D1 PM20D1 Q6H9L7 Isthmin-2 ISM2 Q6IE36 Ovostatin homolog 2 OVOS2 Q6IE37 Ovostatin homolog 1 OVOS1 Q6IE38 Serine protease inhibitor Kazal-type 14 SPINK14 Q6ISS4 Leukocyte-associated immunoglobulin- LAIR2 like receptor 2 Q6JVE5 Epididymal-specific lipocalin-12 LCN12 Q6JVE6 Epididymal-specific lipocalin-10 LCN10 Q6JVE9 Epididymal-specific lipocalin-8 LCN8 Q6KF10 Growth/differentiation factor 6 GDF6 Q6MZW2 Follistatin-related protein 4 FSTL4 Q6NSX1 Coiled-coil domain-containing protein 70 CCDC70 Q6NT32 Carboxylesterase 5A CES5A Q6NT52 Choriogonadotropin subunit beta variant 2 CGB2 Q6NUI6 Chondroadherin-like protein CHADL Q6NUJ1 Saposin A-like PSAPL1 Q6P093 Arylacetamide deacetylase-like 2 AADACL2 Q6P4A8 Phospholipase B-like 1 PLBD1 Q6P5S2 UPF0762 protein C6orf58 C6orf58 Q6P988 Protein notum homolog NOTUM Q6PCB0 von Willebrand factor A domain- VWA1 containing protein 1 Q6PDA7 Sperm-associated antigen 11A SPAG11A Q6PEW0 Inactive serine protease 54 PRSS54 Q6PEZ8 Podocan-like protein 1 PODNL1 Q6PKH6 Dehydrogenase/reductase SDR family DHRS4L2 member 4-like 2 Q6Q788 Apolipoprotein A-V APOA5 Q6SPF0 Atherin SAMD1 Q6UDR6 Kunitz-type protease inhibitor 4 SPINT4 Q6URK8 Testis, prostate and placenta-expressed TEPP protein Q6UW01 Cerebellin-3 CBLN3 Q6UW10 Surfactant-associated protein 2 SFTA2 Q6UW15 Regenerating islet-derived protein 3- REG3G gamma Q6UW32 Insulin growth factor-like family member IGFL1 1 Q6UW78 UPF0723 protein C11orf83 C11orf83 Q6UW88 Epigen EPGN Q6UWE3 Colipase-like protein 2 CLPSL2 Q6UWF7 NXPE family member 4 NXPE4 Q6UWF9 Protein FAM180A FAM180A Q6UWM5 GLIPR1-like protein 1 GLIPR1L1 Q6UWN8 Serine protease inhibitor Kazal-type 6 SPINK6 Q6UWP2 Dehydrogenase/reductase SDR family DHRS11 member 11 Q6UWP8 Suprabasin SBSN Q6UWQ5 Lysozyme-like protein 1 LYZL1 Q6UWQ7 Insulin growth factor-like family member IGFL2 2 Q6UWR7 Ectonucleotide ENPP6 pyrophosphatase/phosphodiesterase family member 6 soluble form Q6UWT2 Adropin ENHO Q6UWU2 Beta-galactosidase-1-like protein GLB1L Q6UWW0 Lipocalin-15 LCN15 Q6UWX4 HHIP-like protein 2 HHIPL2 Q6UWY0 Arylsulfatase K ARSK Q6UWY2 Serine protease 57 PRSS57 Q6UWY5 Olfactomedin-like protein 1 OLFML1 Q6UX06 Olfactomedin-4 OLFM4 Q6UX07 Dehydrogenase/reductase SDR family DHRS13 member 13 Q6UX39 Amelotin AMTN Q6UX46 Protein FAM150B FAM150B Q6UX73 UPF0764 protein C16orf89 C16orf89 Q6UXB0 Protein FAM131A FAM131A Q6UXB1 Insulin growth factor-like family member IGFL3 3 Q6UXB2 VEGF co-regulated chemokine 1 CXCL17 Q6UXF7 C-type lectin domain family 18 member B CLEC18B Q6UXH0 Hepatocellular carcinoma-associated C19orf80 protein TD26 Q6UXH1 Cysteine-rich with EGF-like domain CRELD2 protein 2 Q6UXH8 Collagen and calcium-binding EGF CCBE1 domain-containing protein 1 Q6UXH9 Inactive serine protease PAMR1 PAMR1 Q6UXI7 Vitrin VIT Q6UXI9 Nephronectin NPNT Q6UXN2 Trem-like transcript 4 protein TREML4 Q6UXS0 C-type lectin domain family 19 member A CLEC19A Q6UXT8 Protein FAM150A FAM150A Q6UXT9 Abhydrolase domain-containing protein ABHD15 15 Q6UXV4 Apolipoprotein O-like APOOL Q6UXX5 Inter-alpha-trypsin inhibitor heavy chain ITIH6 H6 Q6UXX9 R-spondin-2 RSPO2 Q6UY14 ADAMTS-like protein 4 ADAMTSL4 Q6UY27 Prostate and testis expressed protein 2 PATE2 Q6W4X9 Mucin-6 MUC6 Q6WN34 Chordin-like protein 2 CHRDL2 Q6WRI0 Immunoglobulin superfamily member 10 IGSF10 Q6X4U4 Sclerostin domain-containing protein 1 SOSTDC1 Q6X784 Zona pellucida-binding protein 2 ZPBP2 Q6XE38 Secretoglobin family 1D member 4 SCGB1D4 Q6XPR3 Repetin RPTN Q6XZB0 Lipase member I LIPI Q6ZMM2 ADAMTS-like protein 5 ADAMTSL5 Q6ZMP0 Thrombospondin type-1 domain- THSD4 containing protein 4 Q6ZNF0 Iron/zinc purple acid phosphatase-like PAPL protein Q6ZRI0 Otogelin OTOG Q6ZRP7 Sulfhydryl oxidase 2 QSOX2 Q6ZWJ8 Kielin/chordin-like protein KCP Q75N90 Fibrillin-3 FBN3 Q765I0 Urotensin-2B UTS2D Q76B58 Protein FAM5C FAM5C Q76LX8 A disintegrin and metalloproteinase with ADAMTS13 thrombospondin motifs 13 Q76M96 Coiled-coil domain-containing protein 80 CCDC80 Q7L1S5 Carbohydrate sulfotransferase 9 CHST9 Q7L513 Fc receptor-like A FCRLA Q7L8A9 Vasohibin-1 VASH1 Q7RTM1 Otopetrin-1 OTOP1 Q7RTW8 Otoancorin OTOA Q7RTY5 Serine protease 48 PRSS48 Q7RTY7 Ovochymase-1 OVCH1 Q7RTZ1 Ovochymase-2 OVCH2 Q7Z304 MAM domain-containing protein 2 MAMDC2 Q7Z3S9 Notch homolog 2 N-terminal-like protein NOTCH2NL Q7Z4H4 Intermedin-short ADM2 Q7Z4P5 Growth/differentiation factor 7 GDF7 Q7Z4R8 UPF0669 protein C6orf120 C6orf120 Q7Z4W2 Lysozyme-like protein 2 LYZL2 Q7Z5A4 Serine protease 42 PRSS42 Q7Z5A7 Protein FAM19A5 FAM19A5 Q7Z5A8 Protein FAM19A3 FAM19A3 Q7Z5A9 Protein FAM19A1 FAM19A1 Q7Z5J1 Hydroxysteroid 11-beta-dehydrogenase 1- HSD11B1L like protein Q7Z5L0 Vitelline membrane outer layer protein 1 VMO1 homolog Q7Z5L3 Complement C1q-like protein 2 C1QL2 Q7Z5L7 Podocan PODN Q7Z5P4 17-beta-hydroxysteroid dehydrogenase 13 HSD17B13 Q7Z5P9 Mucin-19 MUC19 Q7Z5Y6 Bone morphogenetic protein 8A BMP8A Q7Z7B7 Beta-defensin 132 DEFB132 Q7Z7B8 Beta-defensin 128 DEFB128 Q7Z7C8 Transcription initiation factor TFIID TAF8 subunit 8 Q7Z7H5 Transmembrane emp24 domain- TMED4 containing protein 4 Q86SG7 Lysozyme g-like protein 2 LYG2 Q86SI9 Protein CEI C5orf38 Q86TE4 Leucine zipper protein 2 LUZP2 Q86TH1 ADAMTS-like protein 2 ADAMTSL2 Q86U17 Serpin A11 SERPINA11 Q86UU9 Endokinin-A TAC4 Q86UW8 Hyaluronan and proteoglycan link protein HAPLN4 4 Q86UX2 Inter-alpha-trypsin inhibitor heavy chain ITIH5 H5 Q86V24 Adiponectin receptor protein 2 ADIPOR2 Q86VB7 Soluble CD163 CD163 Q86VR8 Four-jointed box protein 1 FJX1 Q86WD7 Serpin A9 SERPINA9 Q86WN2 Interferon epsilon IFNE Q86WS3 Placenta-specific 1-like protein PLAC1L Q86X52 Chondroitin sulfate synthase 1 CHSY1 Q86XP6 Gastrokine-2 GKN2 Q86XS5 Angiopoietin-related protein 5 ANGPTL5 Q86Y27 B melanoma antigen 5 BAGE5 Q86Y28 B melanoma antigen 4 BAGE4 Q86Y29 B melanoma antigen 3 BAGE3 Q86Y30 B melanoma antigen 2 BAGE2 Q86Y38 Xylosyltransferase 1 XYLT1 Q86Y78 Ly6/PLAUR domain-containing protein 6 LYPD6 Q86YD3 Transmembrane protein 25 TMEM25 Q86YJ6 Threonine synthase-like 2 THNSL2 Q86YW7 Glycoprotein hormone beta-5 GPHB5 Q86Z23 Complement C1q-like protein 4 C1QL4 Q8IU57 Interleukin-28 receptor subunit alpha IL28RA Q8IUA0 WAP four-disulfide core domain protein 8 WFDC8 Q8IUB2 WAP four-disulfide core domain protein 3 WFDC3 Q8IUB3 Protein WFDC10B WFDC10B Q8IUB5 WAP four-disulfide core domain protein WFDC13 13 Q8IUH2 Protein CREG2 CREG2 Q8IUK5 Plexin domain-containing protein 1 PLXDC1 Q8IUL8 Cartilage intermediate layer protein 2 C2 CILP2 Q8IUX7 Adipocyte enhancer-binding protein 1 AEBP1 Q8IUX8 Epidermal growth factor-like protein 6 EGFL6 Q8IVL8

Carboxypeptidase O CPO Q8IVN8 Somatomedin-B and thrombospondin SBSPON type-1 domain-containing protein Q8IVW8 Protein spinster homolog 2 SPNS2 Q8IW75 Serpin A12 SERPINA12 Q8IW92 Beta-galactosidase-1-like protein 2 GLB1L2 Q8IWL1 Pulmonary surfactant-associated protein SFTPA2 A2 Q8IWL2 Pulmonary surfactant-associated protein SFTPA1 A1 Q8IWV2 Contactin-4 CNTN4 Q8IWY4 Signal peptide, CUB and EGF-like SCUBE1 domain-containing protein 1 Q8IX30 Signal peptide, CUB and EGF-like SCUBE3 domain-containing protein 3 Q8IXA5 Sperm acrosome membrane-associated SPACA3 protein 3, membrane form Q8IXB1 DnaJ homolog subfamily C member 10 DNAJC10 Q8IXL6 Extracellular serine/threonine protein FAM20C kinase Fam20C Q8IYD9 Lung adenoma susceptibility protein 2 LAS2 Q8IYP2 Serine protease 58 PRSS58 Q8IYS5 Osteoclast-associated immunoglobulin- OSCAR like receptor Q8IZC6 Collagen alpha-1(XXVII) chain COL27A1 Q8IZJ3 C3 and PZP-like alpha-2-macroglobulin CPAMD8 domain-containing protein 8 Q8IZN7 Beta-defensin 107 DEFB107B Q8N0V4 Leucine-rich repeat LGI family member 2 LGI2 Q8N104 Beta-defensin 106 DEFB106B Q8N119 Matrix metalloproteinase-21 MMP21 Q8N129 Protein canopy homolog 4 CNPY4 Q8N135 Leucine-rich repeat LGI family member 4 LGI4 Q8N145 Leucine-rich repeat LGI family member 3 LGI3 Q8N158 Glypican-2 GPC2 Q8N1E2 Lysozyme g-like protein 1 LYG1 Q8N2E2 von Willebrand factor D and EGF VWDE domain-containing protein Q8N2E6 Prosalusin TOR2A Q8N2S1 Latent-transforming growth factor beta- LTBP4 binding protein 4 Q8N302 Angiogenic factor with G patch and FHA AGGF1 domains 1 Q8N307 Mucin-20 MUC20 Q8N323 NXPE family member 1 NXPE1 Q8N387 Mucin-15 MUC15 Q8N3Z0 Inactive serine protease 35 PRSS35 Q8N436 Inactive carboxypeptidase-like protein X2 CPXM2 Q8N474 Secreted frizzled-related protein 1 SFRP1 Q8N475 Follistatin-related protein 5 FSTL5 Q8N4F0 BPI fold-containing family B member 2 BPIFB2 Q8N4T0 Carboxypeptidase A6 CPA6 Q8N5W8 Protein FAM24B FAM24B Q8N687 Beta-defensin 125 DEFB125 Q8N688 Beta-defensin 123 DEFB123 Q8N690 Beta-defensin 119 DEFB119 Q8N6C5 Immunoglobulin superfamily member 1 IGSF1 Q8N6C8 Leukocyte immunoglobulin-like receptor LILRA3 subfamily A member 3 Q8N6G6 ADAMTS-like protein 1 ADAMTSL1 Q8N6Y2 Leucine-rich repeat-containing protein 17 LRRC17 Q8N729 Neuropeptide W-23 NPW Q8N8U9 BMP-binding endothelial regulator BMPER protein Q8N907 DAN domain family member 5 DAND5 Q8NAT1 Glycosyltransferase-like domain- GTDC2 containing protein 2 Q8NAU1 Fibronectin type III domain-containing FNDC5 protein 5 Q8NB37 Parkinson disease 7 domain-containing PDDC1 protein 1 Q8NBI3 Draxin DRAXIN Q8NBM8 Prenylcysteine oxidase-like PCYOX1L Q8NBP7 Proprotein convertase subtilisin/kexin PCSK9 type 9 Q8NBQ5 Estradiol 17-beta-dehydrogenase 11 HSD17B11 Q8NBV8 Synaptotagmin-8 SYT8 Q8NCC3 Group XV phospholipase A2 PLA2G15 Q8NCF0 C-type lectin domain family 18 member C CLEC18C Q8NCW5 NAD(P)H-hydrate epimerase APOA1BP Q8NDA2 Hemicentin-2 HMCN2 Q8NDX9 Lymphocyte antigen 6 complex locus LY6G5B protein G5b Q8NDZ4 Deleted in autism protein 1 C3orf58 Q8NEB7 Acrosin-binding protein ACRBP Q8NES8 Beta-defensin 124 DEFB124 Q8NET1 Beta-defensin 108B DEFB108B Q8NEX5 Protein WFDC9 WFDC9 Q8NEX6 Protein WFDC11 WFDC11 Q8NF86 Serine protease 33 PRSS33 Q8NFM7 Interleukin-17 receptor D IL 17RD Q8NFQ5 BPI fold-containing family B member 6 BPIFB6 Q8NFQ6 BPI fold-containing family C protein BPIFC Q8NFU4 Follicular dendritic cell secreted peptide FDCSP Q8NFW1 Collagen alpha-1(XXII) chain COL22A1 Q8NG35 Beta-defensin 105 DEFB105B Q8NG41 Neuropeptide B-23 NPB Q8NHW6 Otospiralin OTOS Q8NI99 Angiopoietin-related protein 6 ANGPTL6 Q8TAA1 Probable ribonuclease 11 RNASE11 Q8TAG5 V-set and transmembrane domain- VSTM2A containing protein 2A Q8TAL6 Fin bud initiation factor homolog FIBIN Q8TAT2 Fibroblast growth factor-binding protein 3 FGFBP3 Q8TAX7 Mucin-7 MUC7 Q8TB22 Spermatogenesis-associated protein 20 SPATA20 Q8TB73 Protein NDNF NDNF Q8TB96 T-cell immunomodulatory protein ITFG1 Q8TC92 Protein disulfide-thiol oxidoreductase ENOX1 Q8TCV5 WAP four-disulfide core domain protein 5 WFDC5 Q8TD06 Anterior gradient protein 3 homolog AGR3 Q8TD33 Secretoglobin family 1C member 1 SCGB1C1 Q8TD46 Cell surface glycoprotein CD200 receptor CD200R1 1 Q8TDE3 Ribonuclease 8 RNASE8 Q8TDF5 Neuropilin and tolloid-like protein 1 NETO1 Q8TDL5 BPI fold-containing family B member 1 BPIFB1 Q8TE56 A disintegrin and metalloproteinase with ADAMTS17 thrombospondin motifs 17 Q8TE57 A disintegrin and metalloproteinase with ADAMTS16 thrombospondin motifs 16 Q8TE58 A disintegrin and metalloproteinase with ADAMTS15 thrombospondin motifs 15 Q8TE59 A disintegrin and metalloproteinase with ADAMTS19 thrombospondin motifs 19 Q8TE60 A disintegrin and metalloproteinase with ADAMTS18 thrombospondin motifs 18 Q8TE99 Acid phosphatase-like protein 2 ACPL2 Q8TER0 Sushi, nidogen and EGF-like domain-SNED1 containing protein 1 Q8TEU8 WAP, kazal, immunoglobulin, kunitz and WFIKKN2 NTR domain-containing protein 2 Q8WTQ1 Beta-defensin 104 DEFB104B Q8WTR8 Netrin-5 NTN5 Q8WTU2 Scavenger receptor cysteine-rich domain-SRCRB4D containing group B protein Q8WU66 Protein TSPEAR TSPEAR Q8WUA8 Tsukushin TSKU Q8WUF8 Protein FAM172A FAM172A Q8WUJ1 Neuferricin CYB5D2 Q8WUY1 UPF0670 protein THEM6 THEM6 Q8WVN6 Secreted and transmembrane protein 1 SECTM1 Q8WVQ1 Soluble calcium-activated nucleotidase 1 CANT1 Q8WWA0 Intelectin-1 ITLN1 Q8WWG1 Neuregulin-4 NRG4 Q8WWQ2 Inactive heparanase-2 HPSE2 Q8WWU7 Intelectin-2 ITLN2 Q8WWY7 WAP four-disulfide core domain protein WFDC12 12 Q8WWY8 Lipase member H LIPH Q8WWZ8 Oncoprotein-induced transcript 3 protein OIT3 Q8WX39 Epididymal-specific lipocalin-9 LCN9 Q8WXA2 Prostate and testis expressed protein 1 PATE1 Q8WXD2 Secretogranin-3 SCG3 Q8WXF3 Relaxin-3 A chain RLN3 Q8WXI7 Mucin-16 MUC16 Q8WXQ8 Carboxypeptidase A5 CPA5 Q8WXS8 A disintegrin and metalloproteinase with ADAMTS14 thrombospondin motifs 14 Q92484 Acid sphingomyelinase-like SMPDL3A phosphodiesterase 3a Q92485 Acid sphingomyelinase-like SMPDL3B phosphodiesterase 3b Q92496 Complement factor H-related protein 4 CFHR4 Q92520 Protein FAM3C FAM3C Q92563 Testican-2 SPOCK2 Q92583 C-C motif chemokine 17 CCL17 Q92626 Peroxidasin homolog PXDN Q92743 Serine protease HTRA1 HTRA1 Q92752 Tenascin-R TNR Q92765 Secreted frizzled-related protein 3 FRZB Q92819 Hyaluronan synthase 2 HAS2 Q92820 Gamma-glutamyl hydrolase GGH Q92824 Proprotein convertase subtilisin/kexin PCSK5 type 5 Q92832 Protein kinase C-binding protein NELL1 NELL1 Q92838 Ectodysplasin-A, membrane form EDA Q92874 Deoxyribonuclease-1-like 2 DNASE1L2 Q92876 Kallikrein-6 KLK6 Q92913 Fibroblast growth factor 13 FGF13 Q92954 Proteoglycan 4 C-terminal part PRG4 Q93038 Tumor necrosis factor receptor TNFRSF25 superfamily member 25 Q93091 Ribonuclease K6 RNASE6 Q93097 Protein Wnt-2b WNT2B Q93098 Protein Wnt-8b WNT8B Q95460 Major histocompatibility complex class

I- MR1 related gene protein Q969D9 Thymic stromal lymphopoietin TSLP Q969E1 Liver-expressed antimicrobial peptide 2 LEAP2 Q969H8 UPF0556 protein C19orf10 C19orf10 Q969Y0 NXPE family member 3 NXPE3 Q96A54 Adiponectin receptor protein 1 ADIPOR1 Q96A83 Collagen alpha-1(XXVI) chain EMID2 Q96A84 EMI domain-containing protein 1 EMID1 Q96A98 Tuberoinfundibular peptide of 39 residues PTH2 Q96A99 Pentraxin-4 PTX4 Q96BH3 Epididymal sperm-binding protein 1 ELSPBP1 Q96BQ1 Protein FAM3D FAM3D Q96CG8 Collagen triple helix repeat-containing CTHRC1 protein 1 Q96DA0 Zymogen granule protein 16 homolog B ZG16B Q96DN2 von Willebrand factor C and EGF VWCE domain-containing protein Q96DR5 BPI fold-containing family A member 2 BPIFA2 Q96DR8 Mucin-like protein 1 MUCL1 Q96DX4 RING finger and SPRY domain- RSPRY1 containing protein 1 Q96EE4 Coiled-coil domain-containing protein CCDC126 126 Q96GS6 Abhydrolase domain-containing protein FAM108A1 FAM108A1 Q96GW7 Brevican core protein BCAN Q96HF1 Secreted frizzled-related protein 2 SFRP2 Q96I82 Kazal-type serine protease inhibitor KAZALD1 domain-containing protein 1 Q96ID5 Immunoglobulin superfamily member 21 IGSF21 Q96II8 Leucine-rich repeat and calponin LRCH3 homology domain-containing protein 3 Q96IY4 Carboxypeptidase B2 CPB2 Q96JB6 Lysyl oxidase homolog 4 LOXL4 Q96JK4 HHIP-like protein 1 HHIPL1 Q96KN2 Beta-Ala-His dipeptidase CNDP1 Q96KW9 Protein SPACA7 SPACA7 Q96KX0 Lysozyme-like protein 4 LYZL4 Q96L15 Ecto-ADP-ribosyltransferase 5 ART5 Q96LB8 Peptidoglycan recognition protein 4 PGLYRP4 Q96LB9 Peptidoglycan recognition protein 3 PGLYRP3 Q96LC7 Sialic acid-binding Ig-like lectin 10 SIGLEC10 Q96LR4 Protein FAM19A4 FAM19A4 Q96MK3 Protein FAM20A FAM20A Q96MS3 Glycosyltransferase 1 domain-containing GLT1D1 protein 1 Q96NY8 Processed poliovirus receptor-related PVRL4 protein 4 Q96NZ8 WAP, kazal, immunoglobulin, kunitz and WFIKKN1 NTR domain-containing protein 1 Q96NZ9 Proline-rich acidic protein 1 PRAP1 Q96P44 Collagen alpha-1(XXI) chain COL21A1 Q96PB7 Noelin-3 OLFM3 Q96PC5 Melanoma inhibitory activity protein 2 MIA2 Q96PD5 N-acetylmuramoyl-L-alanine amidase PGLYRP2 Q96PH6 Beta-defensin 118 DEFB118 Q96PL1 Secretoglobin family 3A member 2 SCGB3A2 Q96PL2 Beta-tectorin TECTB Q96QH8 Sperm acrosome-associated protein 5 SPACA5 Q96QR1 Secretoglobin family 3A member 1 SCGB3A1 Q96QU1 Protocadherin-15 PCDH15 Q96QV1 Hedgehog-interacting protein HHIP Q96RW7 Hemicentin-1 HMCN1 Q96S42 Nodal homolog NODAL Q96S86 Hyaluronan and proteoglycan link protein HAPLN3 3 Q96SL4 Glutathione peroxidase 7 GPX7 Q96SM3 Probable carboxypeptidase X1 CPXM1 Q96T91 Glycoprotein hormone alpha-2 GPHA2 Q99062 Granulocyte colony-stimulating factor CSF3R receptor Q99102 Mucin-4 alpha chain MUC4 Q99217 Amelogenin, X isoform AMELX Q99218 Amelogenin, Y isoform AMELY Q99435 Protein kinase C-binding protein NELL2 NELL2 Q99470 Stromal cell-derived factor 2 SDF2 Q99542 Matrix metalloproteinase-19 MMP19 Q99574 Neuroserpin SERPINI1 Q99584 Protein S100-A13 S100A13 Q99616 C-C motif chemokine 13 CCL13 Q99645 Epiphycan EPYC Q99674 Cell growth regulator with EF hand CGREF1 domain protein 1 Q99715 Collagen alpha-1(XII) chain COL12A1 Q99727 Metalloproteinase inhibitor 4 TIMP4 Q99731 C-C motif chemokine 19 CCL19 Q99748 Neurturin NRTN Q99935 Proline-rich protein 1 PROL1 Q99942 E3 ubiquitin-protein ligase RNF5 RNF5 Q99944 Epidermal growth factor-like protein 8 EGFL8 Q99954 Submaxillary gland androgen-regulated SMR3A protein 3A Q99969 Retinoic acid receptor responder protein 2 RARRES2 Q99972 Myocilin MYOC Q99983 Osteomodulin OMD Q99985 Semaphorin-3C SEMA3C Q99988 Growth/differentiation factor 15 GDF15 Q9BPW4 Apolipoprotein L4 APOL4 Q9BQ08 Resistin-like beta RETNLB Q9BQ16 Testican-3 SPOCK3 Q9BQ51 Programmed cell death 1 ligand 2 PDCD1LG2 Q9BQB4 Sclerostin SOST Q9BQI4 Coiled-coil domain-containing protein 3 CCDC3 Q9BQP9 BPI fold-containing family A member 3 BPIFA3 Q9BQR3 Serine protease 27 PRSS27 Q9BQY6 WAP four-disulfide core domain protein 6 WFDC6 Q9BRR6 ADP-dependent glucokinase ADPGK Q9BS86 Zona pellucida-binding protein 1 ZPBP Q9BSG0 Protease-associated domain-containing PRADC1 protein 1 Q9BSG5 Retbindin RTBDN Q9BT30 Probable alpha-ketoglutarate-dependent ALKBH7 dioxygenase ABH7 Q9BT56 Spexin C12orf39 Q9BT67 NEDD4 family-interacting protein 1 NDFIP1 Q9BTY2 Plasma alpha-L-fucosidase FUCA2 Q9BU40 Chordin-like protein 1 CHRDL1 Q9BUD6 Spondin-2 SPON2 Q9BUN1 Protein MENT MENT Q9BUR5 Apolipoprotein O APOO Q9BV94 ER degradation-enhancing alpha- EDEM2 mannosidase-like 2 Q9BWP8 Collectin-11 COLEC11 Q9BWS9 Chitinase domain-containing protein 1 CHID1 Q9BX67 Junctional adhesion molecule C JAM3 Q9BX93 Group XIIB secretory phospholipase A2- PLA2G12B like protein Q9BXI9 Complement C1q tumor necrosis factor- C1QTNF6 related protein 6 Q9BXJ0 Complement C1q tumor necrosis factor- C1QTNF5 related protein 5 Q9BXJ1 Complement C1q tumor necrosis factor- C1QTNF1 related protein 1 Q9BXJ2 Complement C1q tumor necrosis factor- C1QTNF7 related protein 7 Q9BXJ3 Complement C1q tumor necrosis factor- C1QTNF4 related protein 4 Q9BXJ4 Complement C1q tumor necrosis factor- C1QTNF3 related protein 3 Q9BXJ5 Complement C1q tumor necrosis factor-C1QTNF2 related protein 2 Q9BXN1 Asporin ASPN Q9BXP8 Pappalysin-2 PAPPA2 Q9BXR6 Complement factor H-related protein 5 CFHR5 Q9BXS0 Collagen alpha-1(XXV) chain COL25A1 Q9BXX0 EMILIN-2 EMILIN2 Q9BXY4 R-spondin-3 RSPO3 Q9BY15 EGF-like module-containing mucin-like EMR3 hormone receptor-like 3 subunit beta Q9BY50 Signal peptidase complex catalytic SEC11C subunit SEC11C Q9BY76 Angiopoietin-related protein 4 ANGPTL4 Q9BYF1 Processed angiotensin-converting enzyme ACE2 2 Q9BYJ0 Fibroblast growth factor-binding protein 2 FGFBP2 Q9BYW3 Beta-defensin 126 DEFB126 Q9BYX4 Interferon-induced helicase C domain- IFIH1 containing protein 1 Q9BYZ8 Regenerating islet-derived protein 4 REG4 Q9BZ76 Contactin-associated protein-like 3 CNTNAP3 Q9BZG9 Ly-6/neurotoxin-like protein 1 LYNX1 Q9BZJ3 Tryptase delta TPSD1 Q9BZM1 Group XIIA secretory phospholipase A2 PLA2G12A Q9BZM2 Group IIF secretory phospholipase A2 PLA2G2F Q9BZM5 NKG2D ligand 2 ULBP2 Q9BZP6 Acidic mammalian chitinase CHIA Q9BZZ2 Sialoadhesin SIGLEC1 Q9C0B6 Protein FAM5B FAM5B Q9GZM7 Tubulointerstitial nephritis antigen-like TINAGL1 Q9GZN4 Brain-specific serine protease 4 PRSS22 Q9GZP0 Platelet-derived growth factor D, receptor- PDGFD binding form Q9GZT5 Protein Wnt-10a WNT10A Q9GZU5 Nyctalopin NYX Q9GZV7 Hyaluronan and proteoglycan link protein HAPLN2 2 Q9GZV9 Fibroblast growth factor 23 FGF23 Q9GZX9 Twisted gastrulation protein homolog 1 TWSG1 Q9GZZ7 GDNF family receptor alpha-4 GFRA4 Q9GZZ8 Extracellular glycoprotein lacritin LACRT Q9H0B8 Cysteine-rich secretory protein LCCL CRISPLD2 domain-containing 2 Q9H106 Signal-regulatory protein delta SIRPD Q9H114 Cystatin-like 1 CSTL1 Q9H173 Nucleotide exchange factor SIL1 SIL1 Q9H1E1 Ribonuclease 7 RNASE7 Q9H1F0 WAP four-disulfide

core domain protein WFDC10A 10A Q9H1J5 Protein Wnt-8a WNT8A Q9H1J7 Protein Wnt-5b WNT5B Q9H1M3 Beta-defensin 129 DEFB129 Q9H1M4 Beta-defensin 127 DEFB127 Q9H1Z8 Augurin C2orf40 Q9H239 Matrix metalloproteinase-28 MMP28 Q9H2A7 C-X-C motif chemokine 16 CXCL16 Q9H2A9 Carbohydrate sulfotransferase 8 CHST8 Q9H2R5 Kallikrein-15 KLK15 Q9H2X0 Chordin CHRD Q9H2X3 C-type lectin domain family 4 member M CLEC4M Q9H306 Matrix metalloproteinase-27 MMP27 Q9H324 A disintegrin and metalloproteinase with ADAMTS10 thrombospondin motifs 10 Q9H336 Cysteine-rich secretory protein LCCL CRISPLD1 domain-containing 1 Q9H3E2 Sorting nexin-25 SNX25 Q9H3R2 Mucin-13 MUC13 Q9H3U7 SPARC-related modular calcium-binding SMOC2 protein 2 Q9H3Y0 Peptidase inhibitor R3HDML R3HDML Q9H4A4 Aminopeptidase B RNPEP Q9H4F8 SPARC-related modular calcium-binding SMOC1 protein 1 Q9H4G1 Cystatin-9-like CST9L Q9H5V8 CUB domain-containing protein 1 CDCP1 Q9H6B9 Epoxide hydrolase 3 EPHX3 Q9H6E4 Coiled-coil domain-containing protein CCDC134 134 Q9H741 UPF0454 protein C12orf49 C12orf49 Q9H772 Gremlin-2 GREM2 Q9H7Y0 Deleted in autism-related protein 1 CXorf36 Q9H8L6 Multimerin-2 MMRN2 Q9H9S5 Fukutin-related protein FKRP Q9HAT2 Sialate O-acetylesterase SIAE Q9HB40 Retinoid-inducible serine SCPEP1 carboxypeptidase Q9HB63 Netrin-4 NTN4 Q9HBJ0 Placenta-specific protein 1 PLAC1 Q9HC23 Prokineticin-2 PROK2 Q9HC57 WAP four-disulfide core domain protein 1 WFDC1 Q9HC73 Cytokine receptor-like factor 2 CRLF2 Q9HC84 Mucin-5B MUC5B Q9HCB6 Spondin-1 SPON1 Q9HCQ7 Neuropeptide NPSF NPVF Q9HCT0 Fibroblast growth factor 22 FGF22 Q9HD89 Resistin RETN Q9NNX1 Tuftelin TUFT1 Q9NNX6 CD209 antigen CD209 Q9NP55 BPI fold-containing family A member 1 BPIFA1 Q9NP70 Ameloblastin AMBN Q9NP95 Fibroblast growth factor 20 FGF20 Q9NP99 Triggering receptor expressed on myeloid TREM1 cells 1 Q9NPA2 Matrix metalloproteinase-25 MMP25 Q9NPE2 Neugrin NGRN Q9NPH0 Lysophosphatidic acid phosphatase type 6 ACP6 Q9NPH6 Odorant-binding protein 2b OBP2B Q9NQ30 Endothelial cell-specific molecule 1 ESM1 Q9NQ36 Signal peptide, CUB and EGF-like SCUBE2 domain-containing protein 2 Q9NQ38 Serine protease inhibitor Kazal-type 5 SPINK5 Q9NQ76 Matrix extracellular phosphoglycoprotein MEPE Q9NQ79 Cartilage acidic protein 1 CRTAC1 Q9NR16 Scavenger receptor cysteine-rich type 1 CD163L1 protein M160 Q9NR23 Growth/differentiation factor 3 GDF3 Q9NR71 Neutral ceramidase ASAH2 Q9NR99 Matrix-remodeling-associated protein 5 MXRA5 Q9NRA1 Platelet-derived growth factor C PDGFC Q9NRC9 Otoraplin OTOR Q9NRE1 Matrix metalloproteinase-26 MMP26 Q9NRJ3 C-C motif chemokine 28 CCL28 Q9NRM1 Enamelin ENAM Q9NRN5 Olfactomedin-like protein 3 OLFML3 Q9NRR1 Cytokine-like protein 1 CYTL1 Q9NS15 Latent-transforming growth factor beta- LTBP3 binding protein 3 Q9NS62 Thrombospondin type-1 domain- THSD1 containing protein 1 Q9NS71 Gastrokine-1 GKN1 Q9NS98 Semaphorin-3G SEMA3G Q9NSA1 Fibroblast growth factor 21 FGF21 Q9NT22 EMILIN-3 EMILIN3 Q9NTU7 Cerebellin-4 CBLN4 Q9NVR0 Kelch-like protein 11 KLHL11 Q9NWH7 Spermatogenesis-associated protein 6 SPATA6 Q9NXC2 Glucose-fructose oxidoreductase domain-GFOD1 containing protein 1 Q9NY56 Odorant-binding protein 2a OBP2A Q9NY84 Vascular non-inflammatory molecule 3 VNN3 Q9NZ20 Group 3 secretory phospholipase A2 PLA2G3 Q9NZC2 Triggering receptor expressed on myeloid TREM2 cells 2 Q9NZK5 Adenosine deaminase CECR1 CECR1 Q9NZK7 Group IIE secretory phospholipase A2 PLA2G2E Q9NZP8 Complement C1r subcomponent-like C1RL protein Q9NZV1 Cysteine-rich motor neuron 1 protein CRIM1 Q9NZW4 Dentin sialoprotein DSPP Q9P0G3 Kallikrein-14 KLK14 Q9P0W0 Interferon kappa IFNK Q9P218 Collagen alpha-1(XX) chain COL20A1 Q9P2C4 Transmembrane protein 181 TMEM181 Q9P2K2 Thioredoxin domain-containing protein 16 TXNDC16 Q9P2N4 A disintegrin and metalloproteinase with ADAMTS9 thrombospondin motifs 9 Q9UBC7 Galanin-like peptide GALP Q9UBD3 Cytokine SCM-1 beta XCL2 Q9UBD9 Cardiotrophin-like cytokine factor 1 CLCF1 Q9UBM4 Opticin OPTC Q9UBP4 Dickkopf-related protein 3 DKK3 Q9UBQ6 Exostosin-like 2 EXTL2 Q9UBR5 Chemokine-like factor CKLF Q9UBS5 Gamma-aminobutyric acid type B GABBR1 receptor subunit 1 Q9UBT3 Dickkopf-related protein 4 short form DKK4 Q9UBU2 Dickkopf-related protein 2 DKK2 Q9UBU3 Ghrelin-28 GHRL Q9UBV4 Protein Wnt-16 WNT16 Q9UBX5 Fibulin-5 FBLN5 Q9UBX7 Kallikrein-11 KLK11 Q9UEF7 Klotho KL Q9UFP1 Protein FAM198A FAM198A Q9UGM3 Deleted in malignant brain tumors 1 DMBT1 protein Q9UGM5 Fetuin-B FETUB Q9UGP8 Translocation protein SEC63 homolog SEC63 Q9UHF0 Neurokinin-B TAC3 Q9UHF1 Epidermal growth factor-like protein 7 EGFL7 Q9UHG2 ProSAAS PCSK1N Q9UHI8 A disintegrin and metalloproteinase with ADAMTS1 thrombospondin motifs 1 Q9UHL4 Dipeptidyl peptidase 2 DPP7 Q9UI42 Carboxypeptidase A4 CPA4 Q9UIG4 Psoriasis susceptibility 1 candidate gene 2 PSORS1C2 protein Q9UIK5 Tomoregulin-2 TMEFF2 Q9UIQ6 Leucyl-cystinyl aminopeptidase, LNPEP pregnancy serum form Q9UJA9 Ectonucleotide ENPP5 pyrophosphatase/phosphodiesterase family member 5 Q9UJH8 Meteorin METRN Q9UJJ9 N-acetylglucosamine-1- GNPTG phosphotransferase subunit gamma Q9UJW2 Tubulointerstitial nephritis antigen TINAG Q9UK05 Growth/differentiation factor 2 GDF2 Q9UK55 Protein Z-dependent protease inhibitor SERPINA10 Q9UK85 Dickkopf-like protein 1 DKKL1 Q9UKJ1 Paired immunoglobulin-like type 2 PILRA receptor alpha Q9UKP4 A disintegrin and metalloproteinase with ADAMTS7 thrombospondin motifs 7 Q9UKP5 A disintegrin and metalloproteinase with ADAMTS6 thrombospondin motifs 6 Q9UKQ2 Disintegrin and metalloproteinase ADAM28 domain-containing protein 28 Q9UKQ9 Kallikrein-9 KLK9 Q9UKR0 Kallikrein-12 KLK12 Q9UKR3 Kallikrein-13 KLK13 Q9UKU9 Angiopoietin-related protein 2 ANGPTL2 Q9UKZ9 Procollagen C-endopeptidase enhancer 2 PCOLCE2 Q9UL52 Transmembrane protease serine 11E non- TMPRSS11E catalytic chain Q9ULC0 Endomucin EMCN Q9ULI3 Protein HEG homolog 1 HEG1 Q9ULZ1 Apelin-13 APLN Q9ULZ9 Matrix metalloproteinase-17 MMP17 Q9UM21 Alpha-1,3-mannosyl-glycoprotein 4-beta- MGAT4A N-acetylglucosaminyltransferase A soluble form Q9UM22 Mammalian ependymin-related protein 1 EPDR1 Q9UM73 ALK tyrosine kinase receptor ALK Q9UMD9 97 kDa linear IgA disease antigen COL17A1 Q9UMX5 Neudesin NENF Q9UN73 Protocadherin alpha-6 PCDHA6 Q9UNA0 A disintegrin and metalloproteinase with ADAMTS5 thrombospondin motifs 5 Q9UNI1 Chymotrypsin-like elastase family CELA1 member 1 Q9UNK4 Group IID secretory phospholipase A2 PLA2G2D Q9UP79 A disintegrin and metalloproteinase with ADAMTS8 thrombospondin motifs 8 Q9UPZ6 Thrombospondin type-1 domain- THSD7A containing protein 7A Q9UQ72 Pregnancy-specific beta-1-glycoprotein 11 PSG11 Q9UQ74 Pregnancy-specific beta-1-glycoprotein 8 PSG8 Q9UQC9 Calcium-activated chloride channel CLCA2 regulator 2 Q9UQE7 Structural maintenance of chromosomes SMC3 protein 3 Q9UQP3 Tenascin-N TNN Q9Y223 UDP-N-acetylglucosamine 2-epimerase

GNE Q9Y240 C-type lectin domain family 11 member A CLEC11A Q9Y251 Heparanase 8 kDa subunit HPSE Q9Y258 C-C motif chemokine 26 CCL26 Q9Y264 Angiopoietin-4 ANGPT4 Q9Y275 Tumor necrosis factor ligand superfamily member 13b, membrane form TNFSF13B Q9Y287 BRI2 intracellular domain ITM2B Q9Y2E5 Epididymis-specific alpha-mannosidase MAN2B2 Q9Y334 von Willebrand factor A domain- VWA7 containing protein 7 Q9Y337 Kallikrein-5 KLK5 Q9Y3B3 Transmembrane emp24 domain- TMED7 containing protein 7 Q9Y3E2 BolA-like protein 1 BOLA1 Q9Y426 C2 domain-containing protein 2 C2CD2 Q9Y4K0 Lysyl oxidase homolog 2 LOXL2 Q9Y4X3 C-C motif chemokine 27 CCL27 Q9Y5C1 Angiopoietin-related protein 3 ANGPTL3 Q9Y5I2 Protocadherin alpha-10 PCDHA10 Q9Y5I3 Protocadherin alpha-1 PCDHA1 Q9Y5K2 Kallikrein-4 KLK4 Q9Y5L2 Hypoxia-inducible lipid droplet- HILPDA associated protein Q9Y5Q5 Atrial natriuretic peptide-converting CORIN enzyme Q9Y5R2 Matrix metalloproteinase-24 MMP24 Q9Y5U5 Tumor necrosis factor receptor TNFRSF18 superfamily member 18 Q9Y5W5 Wnt inhibitory factor 1 WIF1 Q9Y5X9 Endothelial lipase LIPG Q9Y625 Secreted glypican-6 GPC6 Q9Y646 Carboxypeptidase Q CPQ Q9Y6C2 EMILIN-1 EMILIN1 Q9Y6F9 Protein Wnt-6 WNT6 Q9Y619 Testis-expressed sequence 264 protein TEX264 Q9Y6L7 Tolloid-like protein 2 TLL2 Q9Y6N3 Calcium-activated chloride channel CLCA3P regulator family member 3 Q9Y6N6 Laminin subunit gamma-3 LAMC3 Q9Y6R7 IgGFc-binding protein FCGBP Q9Y6Y9 Lymphocyte antigen 96 LY96 Q9Y6Z7 Collectin-10 COLEC10

[0636] In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more additional exemplary proteins listed in Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 2 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 2 (or a homolog thereof) along with other components set out herein.

TABLE-US-00002 TABLE 2 Additional Exemplary Proteins Uniprot ID Protein Name Gene Name A6NGW2 Putative stereocilin-like protein STRCP1 A6NIE9 Putative serine protease 29 PRSS29P A6NJ16 Putative V-set and immunoglobulin IGHV4OR15-8 domain-containing-like protein IGHV4OR15-8 A6NJS3 Putative V-set and immunoglobulin IGHV1OR21-1 domain-containing-like protein IGHV1OR21-1 A6NMY6 Putative annexin A2-like protein ANXA2P2 A8MT79 Putative zinc-alpha-2-glycoprotein-like 1 A8MWS1 Putative killer cell immunoglobulin-like KIR3DP1 receptor like protein KIR3DP1 A8MXU0 Putative beta-defensin 108A DEFB108P1 C9JUS6 Putative adrenomedullin-5-like protein ADM5 P0C7V7 Putative signal peptidase complex SEC11B catalytic subunit SEC11B P0C854 Putative cat eye syndrome critical region CECR9 protein 9 Q13046 Putative pregnancy-specific beta-1- PSG7 glycoprotein 7 Q16609 Putative apolipoprotein(a)-like protein 2 LPAL2 Q2TV78 Putative macrophage-stimulating protein MST1P9 MSTP9 Q5JQD4 Putative peptide YY-3 PYY3 Q5R387 Putative inactive group IIC secretory PLA2G2C phospholipase A2 Q5VSP4 Putative lipocalin 1-like protein 1 LCN1P1 Q5W188 Putative cystatin-9-like protein CST9LP1 CST9LP1 Q6UXR4 Putative serpin A13 SERPINA13P Q86SH4 Putative testis-specific prion protein PRNT Q86YQ2 Putative latherin LATH Q8IVG9 Putative humanin peptide MT-RNR2 Q8NHM4 Putative trypsin-6 TRY6 Q8NHW4 C-C motif chemokine 4-like CCL4L2 Q9H7L2 Putative killer cell immunoglobulin-like KIR3DX1 receptor-like protein KIR3DX1 Q9NRI6 Putative peptide YY-2 PYY2 Q9UF72 Putative TP73 antisense gene protein 1 TP73-AS1 Q9UKY3 Putative inactive carboxylesterase 4 CES1P1

[0637] The Uniprot IDs set forth in Table 1 and Table 2 refer to the human versions the listed proteins and the sequences of each are available from the Uniprot database. Sequences of the listed proteins are also generally available for various animals, including various mammals and animals of veterinary or industrial interest. Accordingly, in some embodiments, compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more proteins chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of the secreted proteins listed in Table 1 and Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 and Table 2 along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 and Table 2 along with other components set out herein. In some embodiments, mammalian homologs are chosen from mouse, rat, hamster, gerbil, horse, pig, cow, llama, alpaca, mink, dog, cat, ferret, sheep, goat, or camel homologs. In some embodiments, the animal of veterinary or industrial interest is chosen from the mammals listed above and/or chicken, duck, turkey, salmon, catfish, or tilapia.

[0638] In embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a lysosomal protein chosen from Table 3. In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more lysosomal and/or related proteins listed in Table 3; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 3 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 3 (or a homolog thereof) along with other components set out herein.

TABLE-US-00003 TABLE 3 Lysosomal and Related Proteins α-fucosidase α-galactosidase α-glucosidase α-Iduronidase α-mannosidase α-N-acetylgalactosaminidase (α-galactosidase B) β-galactosidase β-glucuronidase β-hexosaminidase β-mannosidase 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase 3-methylcrotonyl-CoA carboxylase 3-O-sulfogalactosyl cerebroside sulfatase (arylsulfatase A) acetyl-CoA transferase acid alpha-glucosidase acid ceramidase acid lipase acid phosphatase acid sphingomyelinase alpha-galactosidase A arylsulfatase A beta-galactosidase beta-glucocerebrosidase beta-hexosaminidase Biotinidase cathepsin A cathepsin K CLN3 CLN5 CLN6 CLN8 CLN9 cystine transporter (cystinosin) cytosolic protein beta3A subunit of the adaptor protein-3 complex, AP3 formyl-Glycine generating enzyme (FGE) Galactocerebrosidase galactose-1-phosphate uridyltransferase (GALT) galactose 6-sulfate sulfatase (also known as N-acetylgalactosamine-6-sulfatase) Glucocerebrosidase glucuronate sulfatase glucuronidase glycoprotein cleaving enzymes glycosaminoglycan cleaving enzymes glycosylasparaginase (aspartylglucosaminidase) GM2-AP Heparan-alpha-glucosaminide

N-acetyltransferase (HGSNAT, TMEM76) Heparan sulfatase hexosaminidase A lysosomal proteases methylmalonyl-CoA mutase Hyaluronidase Iduronate sulfatase LAMP-2 lysosomal α-mannosidase Lysosomal p40 (C2orf18) Major facilitator superfamily domain containing 8 protein (MFSD8 or CLN7) N-acetylgalactosamine 4-sulfatase N-acetyl glucosamine 6-sulfatase N-acetyl glucosaminidase N-acetylglucosamine-1-phosphate transferase NPC1 NPC2 palmitoyl-protein thioesterase palmitoyl-protein thioesterase (CLN1) Saposin A (Sphingolipid activator protein A) Saposin B (Sphingolipid activator protein B) Saposin C (Sphingolipid activator protein C) Saposin D (Sphingolipid activator protein D) sialic acid transporter (sialin) Sialidase Sialin Sulfatase Transmembrane protein 74 (TMEM74) tripeptidyl-peptidase tripeptidyl-peptidase I (CLN2) UDP-N-acetylglucosamine- phosphotransferase

[0639] Information regarding lysosomal proteins is available from Lubke et al., "Proteomics of the Lysosome," *Biochim Biophys Acta*. (2009) 1793: 625-635. In some embodiments, the protein listed in Table 3 and encoded by mRNA in the compositions and methods of the invention is a human protein. Sequences of the listed proteins are also available for various animals, including various mammals and animals of veterinary or industrial interest as described above.

[0640] In some embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a therapeutic protein (e.g., cytosolic, transmembrane or secreted) such as those listed in Table 4. In some embodiments, the compositions and methods of the invention provide for the delivery of an mRNA encoding a therapeutic protein useful in treating a disease or disorder (i.e., indication) listed in Table 4; thus, compositions of the invention may comprise an mRNA encoding a therapeutic protein listed or not listed in Table 4 (or a homolog thereof, as discussed below) along with other components set out herein for treating a disease or disorder (i.e., indication) listed in Table 4, and methods ofthe invention may comprise preparing and/or administering a composition comprising an mRNA encoding a such a protein (or a homolog thereof, as discussed below) along with other components set out herein for treatment of a disease or disorder listed in Table 4.

TABLE-US-00004 TABLE 4 Exemplary Indications and Related Proteins Indication Therapeutic Protein 3-Methylcrotonyl-CoA carboxylase Methylcrotonoyl-CoA carboxylase deficiency 3-Methylglutaconic aciduria Methylglutaconyl-CoA hydratase Actinic keratosis Acute intermittent porphyria Porphobilinogen deaminase Acute lymphocytic leukemia Acute myeloid leukemia Addison's disease Adenosine deaminase deficiency Adenosine deaminase Adrenoleukodystrophy ABCD1 Adrenomyeloneuropathy AIDS/HIV Alcohol use disorders Alkaptonuria Homogentisate 1,2-dioxygenase Allergic asthma Anti-IgE mAb Allergies (dermatitis, rhinitis) Alopecia areata Alpers' disease POLG Alpers-Huttenlocher syndrome Alpha 1-antitrypsin deficiency Alpha 1 protease inhibitor Alpha-mannosidosis Alpha-D-mannosidase Alport syndrome Alzheimer's disease Amyloid light-chain amyloidosis Amyotrophic lateral sclerosis (ALS) Anemia Erythropoietin Aortic valve stenosis Argininemia Arginase Argininosuccinic acidemia Argininosuccinate lyase Arrhythmogenic right ventricular dysplasia Autism Autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions Autosomal recessive polycystic kidney ARPKD disease Bacterial infections Basal cell carcinoma Batten disease Battenin + others B-cell chronic lymphocytic leukemia Becker muscular dystrophy Dystrophin Beta-thalassemia Beta globin Binge eating disorder Bipolar disorder Bladder cancer Blepharospasm, Cervical dystonia, Chronic Botulinum toxin migraine, more Bronchiolitis obliterans Brugada syndrome Buerger's disease CACNA1A CACNB4-related Episodic Ataxia Type 2 Cancer and depression Cancer and sexual dysfunction Cancer in pregnancy Carbamylphosphate synthetase deficiency Carbamylphosphate synthetase Carcinoma of the gallbladder Cardiomyopathy (diabetic) Cardiomyopathy (hypertrophic) Carnitine uptake defect SLC22A5 Catecholaminergic polymorphic ventricular tachycardia CDKL5-related Atypical Rett Syndrome Celiac disease Cellulitis Cerebrovascular disease Cervix uteri cancer Chronic fatigue syndrome Chronic graft versus host disease Chronic idiopathic urticaria Chronic immune thrombocytopenia Thrombopoietin Chronic kidney kisease Chronic liver disease Chronic lymphocytic leukemia Chronic myeloid leukemia Chronic pancreatitis Cirrhosis of the liver Citrullinemia, type I Argininosuccinate synthase Classic Rett Syndrome Classical galactosemia Galactose-1-phosphate uridylyltransferase Clostridium difficile associated diarrhea Clotting disorders COAD/COPD Cocaine addiction COL4A5-related disorders Cold contact urticaria Contraception, female Coronary artery diseases Corpus uteri cancer Corticobasal degeneration Crigler-Najjar syndrome UDP-glucuronosyltransferase Critical limb ischemia CTNS-related cystinosis Cutaneous lupus erythematosus Cutaneous neuroendocrine carcinoma (Merkel Cell) Cystic fibrosis CFTR Cystic fibrosis Deoxyribonuclease I Cystinosis Cystinosin Cystinuria SLC7A9 Dementia (Lewy body) Depression Diabetic foot infections Diabetic foot ulcer Diabetic peripheral neuropathy Diabetic ulcers Diarrhoeal diseases Diffuse large B-cell lymphoma DiGeorge syndrome Diverticulitis Drug use disorders Duchenne muscular dystrophy Dystrophin Dysarthria Dyskinesia (levodopa-induced) Early-onset autosomal dominant Alzheimer's disease Eczema Ehlers-Danlos syndrome, type 1 EIF2B1 EIF2B2 EIF2B3 EIF2B4 EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter Eosinophilic esophagitis Epilepsy Erectile dysfunction Erythropoietic protoporphyria Ferrochelatase Esophageal carcinoma Essential tremor Fabry disease Alpha galactosidase Familial adenomatous polyposis APC Familial chylomicronemia Lipoprotein lipase Familial dysbetalipoproteinemia Apolipoprotein E Familial isolated dilated cardiomyopathy Familial mediterranean fever Pyrin (MEFV) Familial melanoma Female infertility Follicle stimulating hormone Female sexual dysfunction Fibromyalgia FMR1-related disorders Fracture healing Fragile X Premature Ovarian Failure Syndrome Fragile X syndrome FMRP Fragile X-Associated Tremor/Ataxia Syndrome Friedreich's ataxia Frontotemporal dementia Fryns syndrome Galactocerebrosidase deficiencies GALE deficiency Galactose epimerase GALK deficiency Galactokinase GALT-related galactosemia Gastric cancer Gastroesophageal reflux disease Gaucher disease Glucocerebrosidase Gilbert syndrome UDP-glucuronosyltransferase Glioblastoma multiforme Glomerulonephritis Glutaric acidemia, type I Glutaryl-CoA dehydrogenase GM2 gangliosidosis HEXA, HEXB Gout Urate oxidase Graft versus host disease Growth hormone deficiency Growth hormone 1/Growth hormone 2 Head and neck cancer, Metastatic colorectal Anti-EGFr mAb cancer Hearing loss, adult onset Heart failure Hemachromatosis HFE protein Hemifacial spasm Hemolytic uremic syndrome Anti-complement factor C5 mAb Hemophilia A Factor VIII Hemophilia A, Hemophilia B Factor VII Hemophilia B Factor IX Hepatitis B, Hepatitis C Interferon alpha HER2+ breast cancer, gastric cancer Anti-HER2 mAb Hereditary angioedema C1 esterase inhibitor Hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (AT) Hereditary spherocytosis Hidradenitis suppurativa Homocystinuria Cystathionine beta-synthase Homozygous familial hypercholesterolemia LDL receptor Hunter syndrome (MPS II) Iduronate-2-sulfatase Huntington disease Huntingtin Hurler syndrome (MPS I) Alpha-L iduronidase Hydrolethalus Hyperalgesia Hyperbilirubinemia Hyperhidrosis Hyperlipidemia Hypermethioninemia Methionine adenosyltransferase Hyperoxaluria, type I Serine-pyruvate aminotransferase Hypertension Hyperuricemia Hyponatremia Hypoparathyroidism Parathyroid hormone Hypophosphatasia TNSALP Idiopathic pulmonary fibrosis Iminoglycinuria Immunoglobulin deficiency Immunoglobulin Infection (adenovirus) Infection (anthrax prophylaxis) Infection (BK virus) Infection (Clostridium difficile prophylaxis) Infection (Dengue fever prophylaxis) Infection (Epstein-Barr virus) Infection (Hepatitis-D) Infection (Lyme disease prophylaxis) Infection (Smallpox virus) Infectious diseases vaccines Infectious antigen Inflammatory heart diseases Insomnia Interstitial cystitis Iron-deficiency anaemia Irritable bowel disease Ischaemic heart disease Isovaleric aciduria Isovaleric acid CoA dehydrogenase deficiency Jansky-Bielschowsky disease Juvenile Batten disease Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) Juvenile rheumatoid arthritis TNF-alpha inhibitors Kennedy's disease (SBMA) Keratoconus Krabbe disease Galactocerebrosidase Leber's hereditary optic neuropathy NADH dehydrogenase Leiomyosarcoma Lennox-Gastaut syndrome Lesch-Nyhan syndrome Hypoxanthine phosphoribosyltransferase 1 Leukaemia Li-Fraumeni syndrome TP53 Lipoma Liposarcoma Liver cancer Long-chain 3-OH acyl-CoA dehydrogenase Long-chain-3-hydroxyacyl-CoA deficiency dehydrogenase Lower respiratory infections Lysosomal acid lipase deficiency Lysosomal acid lipase Macular degeneration Major depressive disorder Malignant fibrous histiocytoma Mantle cell lymphoma Maple syrup urine disease 3-methyl-2-oxobutanoate dehydrogenase Marfan syndrome FBN1 Maroteaux-Lamy syndrome (MPS VI) N-acetylgalactosamine 4-sulfatase Mastocytosis McArdle disease Muscle glycogen phosphorylase MECP2-related disorders MECP2-related Severe Neonatal Encephalopathy Medium-chain acyl-CoA dehydrogenase Acyl-CoA dehydrogenase deficiency Melanoma Anti-CTLA4 mAb Metachromatic leukodystrophy Arylsulfatase A Metastatic colorectal cancer, NSCLC, others Anti-VEGF mAb Methylmalonyl-CoA mutase deficiency Methylmalonyl-CoA mutase Migraine Mitochondrial oxidative phosphorylation disorders Morquio syndrome, type A (MPS IVA) Galactose 6-sulfate sulfatase Morquio syndrome, type B (MPS IVB) Beta-galactosidase Mouth and oropharynx cancers Multiple carboxylase deficiency Biotin-methylcrotonoyl-CoA- carboxylase ligase Multiple myeloma Multiple sclerosis Anti-VLA-4 mAb Multiple sclerosis Interferon beta Multiple system atrophy Myasthenia gravis Myelofibrosis Narcolepsy Neonatal bronchopulmonary dysplasia Neonatal infections Nephritis and nephrosis Neurofibromatosis, type 1 NF-1 Neuronal ceroid lipofuscinoses-related diseases Neutropenia G-CSF Niemann Pick disease, type A/B SMPD1 Niemann Pick disease, type C NPC1 Niemann-Pick disease Type C1 Nocturia Non-alcoholic fatty liver disease Non-Hodgkin lymphoma Anti-CD20 mAb Non-small cell lung cancer Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) Obesity Ophthalmoparesis Opioid induced constipation Ornithine transcarbamylase deficiency Ornithine transcarbamylase Osteoarthritis Osteopetrosis Osteoporosis Anti-RANKL mAb Ovarian cancer Paget disease of bone Sequestosome 1 Pain Pancreatic carcinoma Panic disorder Parkinson disease Paroxysmal nocturnal hemoglobinuria Anti-complement factor C5 Mab Pediculosis capitis (head lice) Pelizaeus-Merzbacher disease Pemphigus vulgaris Peptic ulcer disease Peripheral neuropathy Peyronie's disease Phenylketonuria Phenylalanine hydroxylase Pneumococcal infection prophylaxis POLG-related sensory ataxic neuropathy Polycystic kidney disease Polycystic ovary syndrome Polycythaemia vera Polymerase G-related disorders Polymorphous light eruption Pompe disease Alpha glucosidase Porphyria cutanea tarda Uroporphyrinogen decarboxylase Post herpetic neuralgia Post-organ transplant Pouchitis PPM-X Syndrome Prader-Willi syndrome Preeclampsia Premature ejaculation Prematurity and low birth weight Primary ciliary dyskinesia Primary glomerular diseases Primary humoral immune deficiencies (e.g., Immunoglobulin CVID) Proctitis Progressive multifocal leukoencephalopathy Progressive supranuclear palsy Propionic acidemia Propionyl-CoA carboxylase Prostate cancer Psoriasis Anti-IL-12 & IL-23 mAb Psoriatic arthritis TNF-alpha inhibitors PTT-1 Pulmonary arterial hypertension Pulmonary arterial hypertension Raynaud's phenomenon Refractive errors Renal cell carcinoma Restless leg syndrome Retinitis pigmentosa Rheumatic heart disease Rheumatoid arthritis Anti-interleukin-6 (IL-6) mAb Rheumatoid arthritis T-cell costimulation blocker Rheumatoid arthritis TNF-alpha inhibitor Romano-Ward syndrome Rosacea Sanfilippo syndrome, type A (MPS IIIA) Heparan N-sulfatase Sanfilippo syndrome, type B (MPS IIIB) N-acetyl-alpha-D-glucosaminidase Santavuori-Haltia disease Schizophrenia Schnitzler syndrome Scleroderma SCN1A SCN1B-related seizure disorders Short-chain acyl-CoA dehydrogenase Butyryl-CoA dehydrogenase deficiency Sickle cell disease Hemoglobin SLC3A1-related disorders Small cell lung cancer SMN-1-related spinal muscular atrophy (SMA) Spinal muscular atrophy Survival motor neuron protein Squamous cell carcinoma of head and neck Stickler syndrome Stomach cancer Stroke prophylaxis Synovial sarcoma Systemic lupus erythematosus Anti-BAFF Systemic sclerosis Tetrahydrobiopterin-deficient Tetrahydrobiopterin hyperphenylalaninemia Thromboangiitis obliterans Thrombotic disorders Thyroid cancer TPP1 deficiencies Trachea, bronchus, lung cancers Tricuspid atresia TSC1 TSC2-related tuberous sclerosis Type 2 diabetes mellitus Glucagon-like peptide 1 (GLP-1) agonist Type 2 diabetes mellitus Insulin Tyrosinemia, type I Fumarylacetoacetase Ulcerative colitis Uterine fibroids Varicose veins Venous thromboembolism Very long-chain acyl-CoA dehydrogenase Long-chain-acyl-CoA dehydrogenase deficiency von Gierke's disease Glucose-6-phosphatase Von Hippel-Lindau disease pVHL Wegener granulomatosis Wilson disease Wilson disease protein X-Linked adrenal hypoplasia X-linked adrenoleukodystrophy X-linked agammaglobulinemia Bruton's tyrosine kinase

[0641] In some embodiments, the present invention is used to prevent, treat and/or cure a subject affected with a disease or disorder listed or associated with the proteins listed in Tables 1, 2, 3, or 4. In some embodiments, an mRNA encodes one or more of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), argininosuccinate synthetase (ASS1), Factor IX, survival motor neuron 1 (SMN1), or phenylalanine hydroxylase (PAH).

EXEMPLIFICATION

Example 1: Synthesis of Compound (A3)

##STR00174##

[0642] To a solution of Vitamin A or Retinol (0.7 g, 2.44 mmol) and 3-(4-methylpiperazin-1-yl)propanoic acid (0.50 g, 2.93 mmol) in DMF (20 mL) were added HOBt (0.49 g, 3.66 mmol), HBTU (1.40 g, 3.66 mmol), and DMAP (0.45 g, 3.66 mmol) followed by slow addition of DIPEA (2.13 mL, 12.2 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. Reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure to obtain Compound (3) as a dark brown oil (1.3 g), which was purified by column chromatography.

Example 2: Synthesis of Compound (A4)

##STR00175##

[0643] To a solution of Vitamin A or Retinol (0.25 g, 0.87 mmol) and 4-(Dimethylamino)butyric acid hydrochloride (0.17 g, 1.05 mmol) in DMF (20 mL) were added HOBt (0.18 g, 1.31 mmol), HBTU (0.49 g, 1.31 mmol), and DMAP (0.16 g, 1.31 mmol) followed by slow addition of DIPEA (0.76 mL, 4.36 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. Reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure to obtain Compound (4) as a dark brown oil (0.5 g).

Example 3: Synthesis of Compound (D5)

##STR00176##

[0644] To a solution of Vitamin-D3 (1.0 g, 2.60 mmol) and 4-(dimethylamino)butyric acid hydrochloride (0.52 g, 3.12 mmol) in DMF (20 mL) were added HOBt (0.53 g, 3.90 mmol), HBTU (1.5 g, 3.90 mmol), and DMAP (0.48 g, 3.90 mmol) followed by slow addition of DIPEA (2.3 mL, 13.0 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (5) as a sticky brown solid (0.78 g, 60%).

Example 4: Synthesis of Compound (D6)

##STR00177##

[0645] To a solution of Vitamin-D3 (1.0 g, 2.60 mmol) and 3-(4-methylpiperazin-1-yl)propanoic acid (0.54 g, 3.12 mmol) in DMF (20 mL) were added HOBt (0.53 g, 3.90 mmol), HBTU (1.5 g, 3.90 mmol), and DMAP (0.5 g, 3.90 mmol) followed by slow addition of DIPEA (2.3 mL, 12.9 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (6) as a sticky yellow solid (0.56 g, 40%).

Example 5: Synthesis of Compound (D7)

##STR00178##

[0646] To a solution of Vitamin D3 (1.0 g, 2.60 mmol) and 3-(bis(3-((8-methylnonyl)oxy)-3-oxopropyl)amino)propanoic acid (1.5 g, 2.86 mmol) in DMF (20 mL) were added HOBt (0.53 g, 3.90 mmol), HBTU (1.48 g, 3.90 mmol), and DMAP (0.48 g, 3.90 mmol) followed by slow addition of DIPEA (2.3 mL, 13.0 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring for another 24 h at room temperature. The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (7) as a sticky yellow solid (0.80 g, 35%).

Example 6: Synthesis of Compound (E3)

##STR00179##

[0647] To a solution of Vitamin E or DL-a-tocopherol (1.0 g, 2.32 mmol) and 3-(4-methylpiperazin-1-yl)propanoic acid (0.48 g, 2.79 mmol) in DMF (20 mL) were added HOBt (0.47 g, 3.50 mmol), HBTU (1.32 g, 3.50 mmol), and DMAP (0.42 g, 3.50 mmol) followed by slow addition of DIPEA (2.0 mL, 11.6 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. Reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (3) as a sticky yellow solid (0.45 g, 33%).

Example 7: Synthesis of Compound (E4)

Step 1

##STR00180##

[0648] Beta alanine (10.0 g, 112.2 mmol) was dissolved into DMSO/H.sub.2O (100 mL, 1:1 v/v ratio). Isodecyl acrylate (68.1 mL, 280.6 mmol) was added into it and the reaction mixture was heated at 85° C. for 3 days. The reaction was stopped after 3 days and cooled to room temperature. The organic layer was then separated, diluted with ethyl acetate (300 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 1.0-3.0% MeOH in DCM) to obtain 3-(bis(3-((8-methylnonyl)oxy)-3-oxopropyl)amino) propanoic acid as a colorless oil (12.0 g, 21%).

Step 2

##STR00181##

[0649] To a solution of Vitamin E or DL-a-tocopherol (0.6 g, 1.40 mmol) and 3-(bis(3-((8-methylnonyl)oxy)-3-oxopropyl)amino)propanoic acid (0.60 g, 1.17 mmol) in DMF (15 mL) were added HOBt (0.24 g, 1.75 mmol), HBTU (0.66 g, 1.75 mmol), and DMAP (0.19 g, 1.52 mmol) followed by slow addition of DIPEA (1.02 mL, 5.85 mmol). The reaction was

heated at 65° C. for 1 hour and continued stirring for another 24 h at room temperature. Reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (4) as a light brown oil (0.50 g, 46%).

Example 8: Synthesis of Compound (E5)

##STR00182##

[0650] To a solution of Vitamin E or DL-a-tocopherol (1.0 g, 2.32 mmol) and 4-(dimethylamino)butyric acid hydrochloride (0.47 g, 2.78 mmol) in DMF (20 mL) were added HOBt (0.47 g, 3.48 mmol), HBTU (1.32 g, 3.48 mmol), and DMAP (0.42 g, 3.48 mmol) followed by slow addition of DIPEA (2.0 mL, 11.6 mmol). The reaction was heated at 65° C. for 1 hour and continued stirring overnight at room temperature. Reaction mixture was then diluted with ethyl acetate (200 mL) and washed with brine solution (3×100 mL). After drying over anhydrous Na.sub.2SO.sub.4, the organic layer was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (eluent: 0.5-1.0% MeOH in DCM) to obtain Compound (5) as a sticky brown solid (0.78 g, 60%).

Example 9: Lipid Nanoparticle Formulation Using Vitamin Cationic Lipids and In Vivo Expression of FFLuc in CD1 Mice

[0651] Cationic lipids described herein can be used in the preparation of lipid nanoparticles according to methods known in the art. For example, suitable methods include methods described in International Publication No. WO 2018/089801, which is hereby incorporated by reference in its entirety.

[0652] One exemplary process for lipid nanoparticle formulation is Process A of WO 2018/089801 (see, e.g., Example 1 and FIG. 1 of WO 2018/089801). Process A (“A”) relates to a conventional method of encapsulating mRNA by mixing mRNA with a mixture of lipids, without first pre-forming the lipids into lipid nanoparticles. In an exemplary process, an ethanol lipid solution and an aqueous buffered solution of mRNA were prepared separately. A solution of mixture of lipids (cationic lipid, helper lipids, zwitterionic lipids, PEG lipids etc.) was prepared by dissolving lipids in ethanol. The mRNA solution was prepared by dissolving the mRNA in citrate buffer, resulting in mRNA at a concentration of 0.0833 mg/ml in citrate buffer with a pH of 4.5. The mixtures were then both heated to 65° C. prior to mixing. Then, these two solutions were mixed using a pump system. In some instances, the two solutions were mixed using a gear pump system. In certain embodiments, the two solutions were mixing using a ‘T’ junction (or “Y” junction). The mixture was then purified by diafiltration with a TFF process. The resultant formulation concentrated and stored at 2-8° C. until further use.

[0653] A second exemplary process for lipid nanoparticle formulation is Process B of WO 2018/089801 (see, e.g., Example 2 and FIG. 2 of WO 2018/089801). Process B (“B”) refers to a process of encapsulating messenger RNA (mRNA) by mixing pre-formed lipid nanoparticles with mRNA. A range of different conditions, such as varying temperatures (i.e., heating or not heating the mixture), buffers, and concentrations, may be employed in Process B. In an exemplary process, lipids dissolved in ethanol and citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in the formation of empty lipid nanoparticles, which was a self-assembly process. The resultant formulation mixture was empty lipid nanoparticles in citrate buffer containing alcohol. The formulation was then subjected to a TFF purification process wherein buffer exchange occurred. The resulting suspension of pre-formed empty lipid nanoparticles was then mixed with mRNA using a pump system. For certain cationic lipids, heating the solution post-mixing resulted in a higher percentage of lipid nanoparticles containing mRNA and a higher total yield of mRNA.

[0654] The nanoparticle formulations of Table 5 were prepared by Process A as described above for intratracheal administration via MicroSprayer®. All of the formulations comprised mRNA encoding firefly luciferase (FFLuc) protein and other components in the following mol % ratio: Cationic Lipid:DMG-PEG2000; Cholesterol:DOPE=40:5:25:30.

TABLE-US-00005 TABLE 5 Exemplary Lipid Nanoparticle Formulations Encapsulation mRNA Formulation Composition Process N/P Size PDI % FFLuc (D5): A 4 73.46 0.228 63.08 DMG-PEG2000:Cholesterol:DOPE FFLuc (D6): A 4 65.34 0.247 98.47 DMG-PEG2000:Cholesterol:DOPE

Example 10: In Vivo Expression of mRNA Encoding Firefly Luciferase (FFLuc) Protein

[0655] Intratracheal administration of lipid nanoparticle formulations comprising exemplary vitamin cationic lipids and mRNA encoding FFLuc protein (Table 1) was undertaken in order to study mRNA delivery and resultant protein expression. Male CD1 mice at 6-8 weeks old were dosed by a single intratracheal aerosol administration (50 ul/animal) while anesthetized with isoflurane (1% to 4%) via nose cone. The mice were sacrificed 24 hours post-dose and both lungs harvested for ex vivo IVIS imaging following perfusion. FFLuc protein was detected in the lungs of animals dosed with the formulations of Table 5. These studies demonstrate that the vitamin cationic lipids described herein are effective at delivering mRNA in vivo and result in expression of the protein or polypeptide encoded by the delivered mRNA.

[0656] While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same.

Exemplary Embodiments

First Set of Embodiments

[0657] 1. A liposome encapsulating an mRNA encoding a protein, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a cationic lipid having a structure according to Formula (A-I):

##STR00183## [0658] wherein [0659] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0660] X.sup.1 is an ionizable nitrogen-containing group; [0661] X.sup.2 is S, C═O, or C═S; [0662] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0663] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl,

C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0664] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0665] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. [0666] 2. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure according to Formula (A-Ia):

##STR00184## [0667] 3. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0668] 4. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene. [0669] 5. The liposome encapsulating an mRNA encoding a protein of embodiment 3, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0670] 6. The liposome encapsulating an mRNA encoding a protein of embodiment 4, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0671] 7. The liposome encapsulating an mRNA encoding a protein of embodiment 5, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—. [0672] 8. The liposome encapsulating an mRNA encoding a protein of embodiment 6, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0673] 9. The liposome encapsulating an mRNA encoding a protein of embodiment 5, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0674] 10. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0675] 11. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0676] 12. The liposome encapsulating an mRNA encoding a protein of embodiment 12, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0677] 13. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0678] 14. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0679] 15. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0680] 16. The liposome encapsulating an mRNA encoding a protein of embodiment 15, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0681] 17. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein X.sup.1 is dialkylamine. [0682] 18. The liposome encapsulating an mRNA encoding a protein of embodiment 17, wherein X.sup.1 is

##STR00185## [0683] wherein [0684] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6. [0685] 19. The liposome encapsulating an mRNA encoding a protein of embodiment 18, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0686] 20. The liposome encapsulating an mRNA encoding a protein of embodiment 19, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0687] 21. The liposome encapsulating an mRNA encoding a protein of embodiment 20, wherein R.sup.3a and R.sup.3b are each independently selected from C.sub.6-C.sub.30-alkyl. [0688] 22. The liposome encapsulating an mRNA encoding a protein of embodiment 18, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl. [0689] 23. The liposome encapsulating an mRNA encoding a protein of embodiment 22, wherein R.sup.3a and R.sup.3b are each independently selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0690] 24. The liposome encapsulating an mRNA encoding a protein of embodiment 22 or 23, wherein R.sup.3a and R.sup.3b

are each independently selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3

[0691] 25. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is

##STR00186## [0692] 26. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is

##STR00187## [0693] 27. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is

##STR00188## [0694] 28. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00189## [0695] 29. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00190## [0696] 30. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00191## [0697] 31. A composition comprising the liposome encapsulating an mRNA encoding a protein of any one of embodiment 1-30. [0698] 32. The composition of embodiment 31, comprising an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0699] 33. The composition of embodiment 31, comprising an mRNA encoding for ornithine transcarbamylase (OTC) protein. [0700] 34. A nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid having a structure according to Formula (A-I):

##STR00192## [0701] wherein [0702] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0703] X.sup.1 is an ionizable nitrogen-containing group; [0704] X.sup.2 is S, C═O, or C═S; [0705] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0706] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0707] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0708] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. [0709] 35. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure according to Formula (A-Ia):

##STR00193## [0710] 36. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0711] 37. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene. [0712] 38. The nucleic acid encapsulated within a liposome of embodiment 36, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0713] 39. The nucleic acid encapsulated within a liposome of embodiment 37, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0714] 40. The nucleic acid encapsulated within a liposome of embodiment 38, wherein R.sup.1 is selected from —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—. [0715] 41. The nucleic acid encapsulated within a liposome of embodiment 39, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0716] 42. The nucleic acid encapsulated within a liposome of embodiment 38, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0717] 43. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0718] 44. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.1i-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0719] 45. The nucleic acid encapsulated within a liposome of embodiment 43, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0720] 46. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —

(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0721] 47. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0722] 48. The nucleic acid encapsulated within a liposome of embodiment 47, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0723] 49. The nucleic acid encapsulated within a liposome of embodiment 48, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0724] 50. The nucleic acid encapsulated within a liposome of embodiment 47, wherein X.sup.1 is substituted dialkylamine. [0725] 51. The nucleic acid encapsulated within a liposome of embodiment 50, wherein X.sup.1 is ##STR00194## [0726] wherein [0727] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and [0728] each n is independently an integer having a value between about 1 and about 6. [0729] 52. The nucleic acid encapsulated within a liposome of embodiment 51, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0730] 53. The nucleic acid encapsulated within a liposome of embodiment 52, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0731] 54. The nucleic acid encapsulated within a liposome of embodiment 53, wherein R.sup.3a and R.sup.3b are each independently selected from —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51. [0732] 55. The nucleic acid encapsulated within a liposome of embodiment 51, wherein R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl. [0733] 56. The nucleic acid encapsulated within a liposome of embodiment 55, wherein R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0734] 57. The nucleic acid encapsulated within a liposome of embodiment 55 or 56, wherein R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3. [0735] 58. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is ##STR00195## [0736] 59. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is ##STR00196## [0737] 60. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, ##STR00197## [0738] 61. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of: ##STR00198## [0739] 62. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of: ##STR00199## [0740] 63. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of: ##STR00200## [0741] 64. A composition comprising a nucleic acid encapsulated within a liposome of any one of embodiments 34-63. [0742] 65. The composition of embodiment 64, further comprising one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids. [0743]

66. The composition of embodiment 64 or 65, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide. [0744] 67. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. [0745] 68. The composition of embodiment 67, wherein the mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0746] 69. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. [0747] 70. The composition of embodiment 69, wherein the mRNA encodes ornithine transcarbamylase (OTC) protein. [0748] 71. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in vaccine. [0749] 72. The composition of embodiment 71, wherein the mRNA encodes an antigen.

Second Set of Embodiments

[0750] 1. A liposome encapsulating an mRNA encoding a protein, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a cationic lipid having a structure according to Formula (D-A):

##STR00201## [0751] wherein [0752] custom-character represents a single or double bond; [0753] X.sup.1 is an ionizable nitrogen-containing group; [0754] X.sup.2 is O or S; [0755] Z is O or a covalent bond; [0756] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0757] R.sup.2 is H or C.sub.1-C.sub.4-alkyl. [0758] 2. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure according to Formula (D-I):

##STR00202## [0759] 3. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure according to Formula (D-III):

##STR00203## [0760] 4. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein the cationic lipid has the structure according to Formula (D-Ia):

##STR00204## [0761] 5. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 3, wherein the cationic lipid has the structure according to Formula (D-IIIa):

##STR00205## [0762] 6. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein the cationic lipid has the structure according to Formula (D-Ib):

##STR00206## [0763] 7. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 3, wherein the cationic lipid has the structure according to Formula (D-IIIb):

##STR00207## [0764] 8. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein the cationic lipid has the structure according to Formula (D-Ic):

##STR00208## [0765] 9. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 3, wherein the cationic lipid has the structure according to Formula (D-IIIc):

##STR00209## [0766] 10. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein the cationic lipid has the structure according to Formula (D-Id):

##STR00210## [0767] 11. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 3, wherein the cationic lipid has the structure according to Formula (D-IIId):

##STR00211## [0768] 12. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-11, wherein X.sup.2 is O. [0769] 13. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-12, wherein R.sup.1 is C.sub.1-C.sub.6-alkylene. [0770] 14. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-12, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0771] 15. The liposome encapsulating an mRNA encoding a protein of embodiment 13, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0772] 16. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0773] 17. The liposome encapsulating an mRNA encoding a protein of embodiment 15, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0774] 18. The liposome encapsulating an mRNA encoding a protein of embodiment 16, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—. [0775] 19. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0776] 20. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-12, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0777] 21. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-12, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0778] 22. The liposome encapsulating an mRNA encoding a protein of embodiment 21, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0779] 23. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-12, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—,

—(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH —, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH —, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2 —. [0780] 24. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-23, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0781] 25. The liposome encapsulating an mRNA encoding a protein of embodiment 24, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0782] 26. The liposome encapsulating an mRNA encoding a protein of embodiment 25, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0783] 27. The liposome encapsulating an mRNA encoding a protein of embodiment 24, wherein X.sup.1 is dialkylamine. [0784] 28. The liposome encapsulating an mRNA encoding a protein of embodiment 27, wherein X.sup.1 is ##STR00212## [0785] wherein [0786] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and each n is independently an integer having a value between about 1 and about 6. [0787] 29. The liposome encapsulating an mRNA encoding a protein of embodiment 28, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0788] 30. The liposome encapsulating an mRNA encoding a protein of embodiment 29, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0789] 31. The liposome encapsulating an mRNA encoding a protein of embodiment 30, wherein R.sup.3a and R.sup.3b are each independently —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51 [0790] 32. The liposome encapsulating an mRNA encoding a protein of embodiment 28, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl. [0791] 33. The liposome encapsulating an mRNA encoding a protein of embodiment 32, wherein R.sup.3a and R.sup.3b are each independently selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, Cis-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0792] 34. The liposome encapsulating an mRNA encoding a protein of embodiment 32 or 33, wherein R.sup.3a and R.sup.3b are each independently selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3 —, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3 [0793] 35. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-23, wherein X.sup.1 is ##STR00213## [0794] 36. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-23, wherein X.sup.1 is

##STR00214## [0795] 37. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-23, wherein X.sup.1 is

##STR00215## [0796] 38. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00216## ##STR00217## ##STR00218## ##STR00219## [0797] 39. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00220## ##STR00221## [0798] 40. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of:

##STR00222## ##STR00223## [0799] 41. A composition comprising the liposome encapsulating an mRNA encoding a protein of any one of embodiment 1-40. [0800] 42. The composition of embodiment 41, comprising an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0801] 43. The composition of embodiment 41,

comprising an mRNA encoding for ornithine transcarbamylase (OTC) protein. [0802] 44. A nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid having a structure according to Formula (D-A):
##STR00224## [0803] wherein [0804] custom-character represents a single or double bond; [0805] X.sup.1 is an ionizable nitrogen-containing group; [0806] X.sup.2 is O or S; [0807] Z is O or a covalent bond; [0808] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; and [0809] R.sup.2 is H or C.sub.1-C.sub.4-alkyl. [0810] 45. The nucleic acid encapsulated within a liposome of embodiment 44, wherein the cationic lipid has the structure according to Formula (D-I):
##STR00225## [0811] 46. The liposome encapsulating an mRNA encoding a protein of embodiment 44, wherein the cationic lipid has the structure according to Formula (D-III):
##STR00226## [0812] 47. The nucleic acid encapsulated within a liposome of embodiment 44 or 45, wherein the cationic lipid has the structure according to Formula (D-Ia):
##STR00227## [0813] 48. The nucleic acid encapsulated within a liposome of embodiment 44 or 46, wherein the cationic lipid has the structure according to Formula (D-IIIa):
##STR00228## [0814] 49. The nucleic acid encapsulated within a liposome of embodiment 44 or 45, wherein the cationic lipid has the structure according to Formula (D-Ib):
##STR00229##
[0815] 50. The nucleic acid encapsulated within a liposome of embodiment 44 or 46, wherein the cationic lipid has the structure according to Formula (D-IIIb):
##STR00230##
[0816] 51. The nucleic acid encapsulated within a liposome of embodiment 44 or 45, wherein the cationic lipid has the structure according to Formula (D-Ic):
##STR00231## [0817] 52. The nucleic acid encapsulated within a liposome of embodiment 44 or 46, wherein the cationic lipid has the structure according to Formula (D-IIIc):
##STR00232##
[0818] 53. The nucleic acid encapsulated within a liposome of embodiment 44 or 45, wherein the cationic lipid has the structure according to Formula (D-Id):
##STR00233##
[0819] 54. The nucleic acid encapsulated within a liposome of embodiment 44 or 46, wherein the cationic lipid has the structure according to Formula (D-IIId):
##STR00234## [0820] 55. The nucleic acid encapsulated within a liposome of any one of embodiments 44-54, wherein X.sup.2 is O. [0821] 56. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene. [0822] 57. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0823] 58. The nucleic acid encapsulated within a liposome of embodiment 56, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0824] 59. The nucleic acid encapsulated within a liposome of embodiment 57, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0825] 60. The nucleic acid encapsulated within a liposome of embodiment 58, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0826] 61. The nucleic acid encapsulated within a liposome of embodiment 59, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, and —C.sub.25H.sub.50—. [0827] 62. The nucleic acid encapsulated within a liposome of embodiment 57, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0828] 63. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0829] 64. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, Cis-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0830] 65. The nucleic acid encapsulated within a liposome of embodiment 64, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0831] 66. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —

(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0832] 67. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0833] 68. The nucleic acid encapsulated within a liposome of embodiment 67, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0834] 69. The nucleic acid encapsulated within a liposome of embodiment 68, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0835] 70. The nucleic acid encapsulated within a liposome of embodiment 67, wherein X.sup.1 is dialkylamine. [0836] 71. The nucleic acid encapsulated within a liposome of embodiment 70, wherein X.sup.1 is
##STR00235## [0837] wherein [0838] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6. [0839] 72. The nucleic acid encapsulated within a liposome of embodiment 69, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0840] 73. The nucleic acid encapsulated within a liposome of embodiment 72, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0841] 74. The nucleic acid encapsulated within a liposome of embodiment 73, wherein R.sup.3a and R.sup.3b are each independently —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51. [0842] 75. The nucleic acid encapsulated within a liposome of embodiment 69, wherein R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl. [0843] 76. The nucleic acid encapsulated within a liposome of embodiment 75, wherein R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0844] 77. The nucleic acid encapsulated within a liposome of embodiment 75 or 76, wherein R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3. [0845] 78. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein X.sup.1 is
##STR00236## [0846] 79. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein X.sup.1 is
##STR00237## [0847] 80. The nucleic acid encapsulated within a liposome of any one of embodiments 44-55, wherein X.sup.1 is
##STR00238## [0848] 81. The nucleic acid encapsulated within a liposome of embodiment 44, wherein the cationic lipid has the structure of:
##STR00239## ##STR00240## ##STR00241## ##STR00242## [0849] 82. The nucleic acid encapsulated within a liposome of embodiment 44, wherein the cationic lipid has the structure of:
##STR00243## ##STR00244## [0850] 83. The nucleic acid encapsulated within a liposome of embodiment 44, wherein the cationic lipid has the structure of:
##STR00245## ##STR00246## [0851] 84. A composition comprising a nucleic acid encapsulated within a liposome of any one of embodiments 44-83. [0852] 85. The composition of embodiment 84, further comprising one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids. [0853] 86. The composition of embodiment 84 or 85, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide. [0854] 87. The composition of any one of embodiments 82-83, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. [0855] 88. The composition of embodiment 87, wherein the mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0856] 89. The composition of any one of embodiments 84-86, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. [0857] 90. The composition of embodiment 89, wherein the mRNA encodes ornithine transcarbamylase (OTC) protein. [0858] 91. The composition of any one of embodiments 84-86, wherein the mRNA encodes a

peptide or polypeptide for use in vaccine. [0859] 92. The composition of embodiment 91, wherein the mRNA encodes an antigen.

Third Set of Embodiments

[0860] 1. A liposome encapsulating an mRNA encoding a protein, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a cationic lipid having a structure according to Formula (E-I):

##STR00247## [0861] wherein [0862] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0863] X.sup.1 is an ionizable nitrogen-containing group; [0864] X.sup.2 is S, C═O, or C═S; [0865] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0866] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0867] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0868] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. [0869] 2. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure according to Formula (E-Ia):

##STR00248## [0870] 3. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0871] 4. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene. [0872] 5. The liposome encapsulating an mRNA encoding a protein of embodiment 3, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0873] 6. The liposome encapsulating an mRNA encoding a protein of embodiment 4, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0874] 7. The liposome encapsulating an mRNA encoding a protein of embodiment 5, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—. [0875] 8. The liposome encapsulating an mRNA encoding a protein of embodiment 6, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0876] 9. The liposome encapsulating an mRNA encoding a protein of embodiment 5, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0877] 10. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0878] 11. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.11-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0879] 12. The liposome encapsulating an mRNA encoding a protein of embodiment 12, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted C.sub.15-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0880] 13. The liposome encapsulating an mRNA encoding a protein of embodiment 1 or 2, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0881] 14. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0882] 15. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0883] 16. The liposome encapsulating an mRNA encoding a protein of embodiment 15, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0884] 17. The liposome encapsulating an mRNA encoding a protein of embodiment 14, wherein X.sup.1 is dialkylamine. [0885] 18. The liposome encapsulating an mRNA encoding a protein of embodiment 17, wherein X.sup.1 is

##STR00249## [0886] wherein [0887] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-

membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6. [0888] 19. The liposome encapsulating an mRNA encoding a protein of embodiment 18, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0889] 20. The liposome encapsulating an mRNA encoding a protein of embodiment 19, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0890] 21. The liposome encapsulating an mRNA encoding a protein of embodiment 20, wherein R.sup.3a and R.sup.3b are each independently selected from C.sub.6-C.sub.30-alkyl. [0891] 22. The liposome encapsulating an mRNA encoding a protein of embodiment 18, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkenyl or C.sub.5-C.sub.20-alkenyl. [0892] 23. The liposome encapsulating an mRNA encoding a protein of embodiment 22, wherein R.sup.3a and R.sup.3b are each independently selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0893] 24. The liposome encapsulating an mRNA encoding a protein of embodiment 22 or 23, wherein R.sup.3a and R.sup.3b are each independently selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3. [0894] 25. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is ##STR00250## [0895] 26. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is ##STR00251## [0896] 27. The liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-13, wherein X.sup.1 is ##STR00252## [0897] 28. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of: ##STR00253## [0898] 29. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of: ##STR00254## [0899] 30. The liposome encapsulating an mRNA encoding a protein of embodiment 1, wherein the cationic lipid has the structure of: ##STR00255## [0900] 31. A composition comprising the liposome encapsulating an mRNA encoding a protein of any one of embodiments 1-30. [0901] 32. The composition of embodiment 31, comprising an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0902] 33. The composition of embodiment 31, comprising an mRNA encoding for ornithine transcarbamylase (OTC) protein. [0903] 34. A nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid having a structure according to Formula (E-I): ##STR00256## [0904] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0905] X.sup.1 is an ionizable nitrogen-containing group; [0906] X.sup.2 is S, C═O, or C═S; [0907] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0908] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0909] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0910] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. [0911] 35. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure according to Formula (E-Ia): ##STR00257##

(E-Ia).

[0912] 36. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0913] 37. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene. [0914] 38. The nucleic acid encapsulated within a liposome of embodiment 36, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0915] 39. The nucleic acid encapsulated within a liposome of embodiment 37, wherein R.sup.1 is unsubstituted C.sub.1-C.sub.5-alkylene. [0916] 40. The nucleic acid encapsulated within a liposome of embodiment 38, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, and —C.sub.25H.sub.50—. [0917] 41. The nucleic acid encapsulated within a liposome of embodiment 39, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—. [0918] 42. The nucleic acid encapsulated within a liposome of

embodiment 38, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0919] 43. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenylene. [0920] 44. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.1i-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0921] 45. The nucleic acid encapsulated within a liposome of embodiment 44, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted C.sub.18-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0922] 46. The nucleic acid encapsulated within a liposome of embodiment 34 or 35, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0923] 47. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0924] 48. The nucleic acid encapsulated within a liposome of embodiment 47, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl. [0925] 49. The nucleic acid encapsulated within a liposome of embodiment 48, wherein X.sup.1 is substituted or unsubstituted pyrrolidinyl, piperidinyl, pyrazolidinyl, or piperazinyl. [0926] 50. The nucleic acid encapsulated within a liposome of embodiment 47, wherein X.sup.1 is substituted dialkylamine. [0927] 51. The nucleic acid encapsulated within a liposome of embodiment 50, wherein X.sup.1 is ##STR00258## [0928] wherein [0929] R.sup.3a and R.sup.3b are each independently C.sub.1-C.sub.30-alkyl, C.sub.2-C.sub.30-alkenyl, C.sub.2-C.sub.30-alkynyl, hetero-C.sub.1-C.sub.30-alkyl, hetero-C.sub.1-C.sub.30-alkenyl, hetero-C.sub.1-C.sub.30-alkynyl, a polymer, C.sub.5-C.sub.6-cycloalkyl, 5- to 6-membered heterocycloalkyl, C.sub.5-C.sub.6-aryl, or 5- to 6-membered heteroaryl; and each n is independently an integer having a value between about 1 and about 6. [0930] 52. The nucleic acid encapsulated within a liposome of embodiment 51, wherein R.sup.3a and R.sup.3b are each independently C.sub.6-C.sub.30-alkyl. [0931] 53. The nucleic acid encapsulated within a liposome of embodiment 52, wherein R.sup.3a and R.sup.3b are each independently unsubstituted C.sub.6-C.sub.30-alkyl. [0932] 54. The nucleic acid encapsulated within a liposome of embodiment 53, wherein R.sup.3a and R.sup.3b are each independently selected from —C.sub.6H.sub.13, —C.sub.7H.sub.15, —C.sub.8H.sub.17, —C.sub.9H.sub.19, —C.sub.10H.sub.21, —C.sub.11H.sub.23, —C.sub.12H.sub.25, —C.sub.13H.sub.27, —C.sub.14H.sub.29, —C.sub.15H.sub.31, —C.sub.16H.sub.33, —C.sub.17H.sub.35, —C.sub.18H.sub.37, —C.sub.19H.sub.39, —C.sub.20H.sub.41, —C.sub.21H.sub.43, —C.sub.22H.sub.45, —C.sub.23H.sub.47, —C.sub.24H.sub.49, or —C.sub.25H.sub.51. [0933] 55. The nucleic acid encapsulated within a liposome of embodiment 51, wherein R.sup.3a and R.sup.3b are each independently is C.sub.6-C.sub.30-alkenyl or C.sub.8-C.sub.20-alkenyl. [0934] 56. The nucleic acid encapsulated within a liposome of embodiment 55, wherein R.sup.3a and R.sup.3b are each independently is selected from C.sub.8-alkenyl, C.sub.9-alkenyl, C.sub.10-alkenyl, C.sub.11-alkenyl, C.sub.12-alkenyl, C.sub.13-alkenyl, C.sub.14-alkenyl, C.sub.15-alkenyl, C.sub.16-alkenyl, C.sub.17-alkenyl, C.sub.18-alkenyl, C.sub.19-alkenyl, and C.sub.20-alkenyl. [0935] 57. The nucleic acid encapsulated within a liposome of embodiment 55 or 56, wherein R.sup.3a and R.sup.3b are each independently is selected from —(CH.sub.2).sub.4CH═CH.sub.2, —(CH.sub.2).sub.5CH═CH.sub.2, —(CH.sub.2).sub.6CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH.sub.2, —(CH.sub.2).sub.8CH═CH.sub.2, —(CH.sub.2).sub.9CH═CH.sub.2, —(CH.sub.2).sub.10CH═CH.sub.2, —(CH.sub.2).sub.11CH═CH.sub.2, —(CH.sub.2).sub.12CH═CH.sub.2, —(CH.sub.2).sub.13CH═CH.sub.2, —(CH.sub.2).sub.14CH═CH.sub.2, —(CH.sub.2).sub.15CH═CH.sub.2, —(CH.sub.2).sub.16CH═CH.sub.2, —(CH.sub.2).sub.17CH═CH.sub.2, —(CH.sub.2).sub.18CH═CH.sub.2, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.3—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.3, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.3, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.3, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.3, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.3, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.3. [0936] 58. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is

##STR00259## [0937] 59. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is
##STR00260## [0938] 60. The nucleic acid encapsulated within a liposome of any one of embodiments 34-46, wherein X.sup.1 is
##STR00261## [0939] 61. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of:
##STR00262## [0940] 62. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of:
##STR00263## [0941] 63. The nucleic acid encapsulated within a liposome of embodiment 34, wherein the cationic lipid has the structure of:
##STR00264## [0942] 64. A composition comprising a nucleic acid encapsulated within a liposome of any one of embodiments 34-63. [0943] 65. The composition of embodiment 64, further comprising one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids. [0944] 66. The composition of embodiment 64 or 65, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide. [0945] 67. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. [0946] 68. The composition of embodiment 67, wherein the mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0947] 69. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. [0948] 70. The composition of embodiment 69, wherein the mRNA encodes ornithine transcarbamylase (OTC) protein. [0949] 71. The composition of any one of embodiments 64-66, wherein the mRNA encodes a peptide or polypeptide for use in vaccine. [0950] 72. The composition of embodiment 71, wherein the mRNA encodes an antigen.

Fourth Set of Embodiments

[0951] 1. A cationic lipid having a structure according to Formula (K-I):
##STR00265## [0952] wherein [0953] R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; [0954] X.sup.1 is an ionizable nitrogen-containing group; [0955] X.sup.2 is S, C═O, or C═S; [0956] X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; [0957] R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or [0958] R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and [0959] R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl. [0960] 2. The cationic lipid having a structure according to embodiment 1, having a structure according to Formula (K-Ia):
##STR00266##
(K-Ia).
[0961] 3. The cationic lipid having a structure according to embodiment 1, having a structure according to Formula (K-Ib):
##STR00267## [0962] 4. The cationic lipid of any one of embodiments 1-3, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene. [0963] 5. The cationic lipid of embodiment 4, wherein R.sup.1 is unsubstituted C.sub.6-C.sub.30-alkylene. [0964] 6. The cationic lipid of embodiment 5, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—. [0965] 7. The cationic lipid of embodiment 4, wherein R.sup.1 is substituted C.sub.6-C.sub.30-alkylene with one or substituents selected from halogen, hydroxyl, amino, thiol, ester, and thioester. [0966] 8. The cationic lipid of any one of embodiments 1-3, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene. [0967] 9. The cationic lipid of any one of embodiments 1-3, wherein R.sup.1 is selected from C.sub.8-alkenylene, C.sub.9-alkenylene, C.sub.10-alkenylene, C.sub.1i-alkenylene, C.sub.12-alkenylene, C.sub.13-alkenylene, C.sub.14-alkenylene, C.sub.15-alkenylene, C.sub.16-alkenylene, C.sub.17-alkenylene, C.sub.18-alkenylene, C.sub.19-alkenylene, and C.sub.20-alkenylene. [0968] 10. The cationic lipid of embodiment 9, wherein R.sup.1 is selected from unsubstituted C.sub.8-alkenylene, unsubstituted C.sub.9-alkenylene, unsubstituted C.sub.10-alkenylene, unsubstituted C.sub.11-alkenylene, unsubstituted C.sub.12-alkenylene, unsubstituted C.sub.13-alkenylene, unsubstituted C.sub.14-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.16-alkenylene, unsubstituted C.sub.17-alkenylene, unsubstituted Cis-alkenylene, unsubstituted C.sub.19-alkenylene, and unsubstituted C.sub.20-alkenylene. [0969] 11. The cationic lipid of any one of embodiments 1-3, wherein R.sup.1 is selected from —(CH.sub.2).sub.4CH═CH—, —(CH.sub.2).sub.5CH═CH—, —(CH.sub.2).sub.6CH═CH—, —(CH.sub.2).sub.7CH═CH—, —(CH.sub.2).sub.8CH═CH—, —(CH.sub.2).sub.9CH═CH—, —(CH.sub.2).sub.10CH═CH—, —(CH.sub.2).sub.11CH═CH—, —(CH.sub.2).sub.12CH═CH—, —(CH.sub.2).sub.13CH═CH—, —(CH.sub.2).sub.14CH═CH—, —(CH.sub.2).sub.15CH═CH—, —(CH.sub.2).sub.16CH═CH—, —(CH.sub.2).sub.17CH═CH—, —(CH.sub.2).sub.18CH═CH—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.3CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.5CH.sub.2—, —(CH.sub.2).sub.4CH═CH(CH.sub.2).sub.8CH.sub.2—, —(CH.sub.2).sub.7CH═CH(CH.sub.2).sub.7CH.sub.2—, —(CH.sub.2).sub.6CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.7CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —

(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CH(CH.sub.2).sub.4CH.sub.2—, —(CH.sub.2).sub.3CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—, —(CH.sub.2).sub.11CH═CH(CH.sub.2).sub.7CH.sub.2—, and —(CH.sub.2).sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH═CHCH.sub.2CH.sub.2—. [0970] 12. The cationic lipid of any one of embodiments 1-3, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl. [0971] 13. The cationic lipid of any one of embodiments 1-3 and 12, wherein X.sup.1 is ##STR00268## [0972] 14. The cationic lipid of embodiment 1, having the structure of: ##STR00269## [0973] 15. A composition comprising an mRNA encoding a protein, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids, wherein at least one cationic lipid is of any one of embodiments 1-14. [0974] 16. The composition of embodiment 15, comprising an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0975] 17. The composition of embodiment 15, comprising an mRNA encoding for ornithine transcarbamylase (OTC) protein. [0976] 18. A composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid of any one of embodiments 1-14. [0977] 19. The composition of embodiment 18, further comprising one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids. [0978] 20. The composition of embodiment 18 or 19, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide. [0979] 21. The composition of any one of embodiments 18-20, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the lung of a subject or a lung cell. [0980] 22. The composition of embodiment 21, wherein the mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. [0981] 23. The composition of any one of embodiments 18-20, wherein the mRNA encodes a peptide or polypeptide for use in the delivery to or treatment of the liver of a subject or a liver cell. [0982] 24. The composition of embodiment 23, wherein the mRNA encodes ornithine transcarbamylase (OTC) protein. [0983] 25. The composition of any one of embodiments 18-20, wherein the mRNA encodes a peptide or polypeptide for use in vaccine. [0984] 26. The composition of embodiment 25, wherein the mRNA encodes an antigen.

Claims

1. A liposome encapsulating an mRNA encoding a peptide or polypeptide, wherein the liposome comprises one or more cationic lipids, and optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids; and wherein the liposome comprises at least one cationic lipid having the structure according to Formula A-I: ##STR00270## wherein R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; X.sup.1 is an ionizable nitrogen-containing group; X.sup.2 is S, C═O, or C═S; X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3—C-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
2. (canceled)
3. (canceled)
4. The liposome of claim 1, wherein the cationic lipid of Formula A-I has the structure according to Formula (A-Ia): ##STR00271##
5-13. (canceled)
14. The liposome of claim 1, wherein R.sup.1 is C.sub.6-C.sub.30-alkylene.
15. The liposome of claim 1, wherein R.sup.1 is C.sub.1-C.sub.5-alkylene.
16. (canceled)
17. (canceled)
18. The liposome of claim 1, wherein R.sup.1 is —C.sub.6H.sub.12—, —C.sub.7H.sub.14—, —C.sub.8H.sub.16—, —C.sub.9H.sub.18—, —C.sub.10H.sub.20—, —C.sub.11H.sub.22—, —C.sub.12H.sub.24—, —C.sub.13H.sub.26—, —C.sub.14H.sub.28—, —C.sub.15H.sub.30—, —C.sub.16H.sub.32—, —C.sub.17H.sub.34—, —C.sub.18H.sub.36—, —C.sub.19H.sub.38—, —C.sub.20H.sub.40—, —C.sub.21H.sub.42—, —C.sub.22H.sub.44—, —C.sub.23H.sub.46—, —C.sub.24H.sub.48—, or —C.sub.25H.sub.50—.
19. The liposome of claim 1, wherein R.sup.1 is —C.sub.2H.sub.4—, —C.sub.3H.sub.6—, or C.sub.4H.sub.8—.
20. (canceled)
21. The liposome of claim 1, wherein R.sup.1 is C.sub.6-C.sub.30-alkenylene or C.sub.8-C.sub.20-alkenylene.
22-24. (canceled)
25. The liposome of claim 1, wherein X.sup.1 is NH.sub.2, guanidine, amidine, a mono- or dialkylamine, 5- to 6-membered heterocycloalkyl, or 5- to 6-membered nitrogen-containing heteroaryl.
26. The liposome of claim 25, wherein X.sup.1 is a 5- to 6-membered, nitrogen containing heterocycloalkyl.
27. (canceled)
28. The liposome of claim 25, wherein X.sup.1 is dialkylamine.

29-**35**. (canceled)
36. The liposome of claim 1, wherein X.sup.1 is ##STR00272##
37. (canceled)
38. The liposome of claim 1, wherein X.sup.1 is ##STR00273## or ##STR00274##
39. The liposome of claim 1, wherein the cationic lipid of Formula A-I is selected from the group consisting of: ##STR00275##
40. The liposome of claim 1, wherein the cationic lipid of Formula A-I is: ##STR00276##
41. The liposome of claim 1, wherein the cationic lipid of Formula A-I is: ##STR00277##
42-**48**. (canceled)
49. A composition comprising the liposome of claim 1.
50. (canceled)
51. The composition of claim 49, wherein the mRNA encodes for: cystic fibrosis transmembrane conductance regulator (CFTR) protein; ornithine transcarbamylase (OTC) protein; or an antigen from an infectious agent.
52-**56**. (canceled)
57. The composition of claim 49, formulated for intravenous (IV) administration intramuscular (IM) administration, or inhaled administration.
58-**121**. (canceled)
122. A liposome encapsulating an mRNA encoding a peptide or polypeptide, wherein the liposome comprises one or more cationic lipids, and optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids; and wherein the liposome comprises at least one cationic lipid having the structure according to Formula E-I: ##STR00278## wherein R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; X.sup.1 is an ionizable nitrogen-containing group; X.sup.2 is S, C═O, or C═S; X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.
123. A liposome encapsulating an mRNA encoding a peptide or polypeptide, wherein the liposome comprises one or more cationic lipids, and optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids and optionally one or more PEG-modified lipids; and wherein the liposome comprises at least one cationic lipid having the structure according to Formula K-I: ##STR00279## wherein R.sup.1 is C.sub.1-C.sub.30-alkylene, C.sub.2-C.sub.30-alkenylene, C.sub.2-C.sub.30-alkynylene, hetero-C.sub.1-C.sub.30-alkylene, hetero-C.sub.1-C.sub.30-alkenylene, hetero-C.sub.1-C.sub.30-alkynylene, a polymer, C.sub.5-C.sub.6-cycloalkylene, 5- to 6-membered heterocycloalkylene, C.sub.5-C.sub.6-arylene, or 5- to 6-membered heteroarylene; X.sup.1 is an ionizable nitrogen-containing group; X.sup.2 is S, C═O, or C═S; X.sup.3 is S, O, CR.sup.aR.sup.b, or NR.sup.c; R.sup.a and R.sup.b are each independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl; or R.sup.a and R.sup.b, together with the carbon atom through which they are connected, form a saturated or unsaturated C.sub.5-C.sub.6-cycloalkyl or 5- to 6-membered heterocyclic ring; and R.sup.c is independently H, C.sub.1-C.sub.6-alkyl, C.sub.1-C.sub.6-alkoxy, C.sub.3-C.sub.6-cycloalkyl, C.sub.2-C.sub.6-alkenyl, or C.sub.2-C.sub.6-alkynyl.