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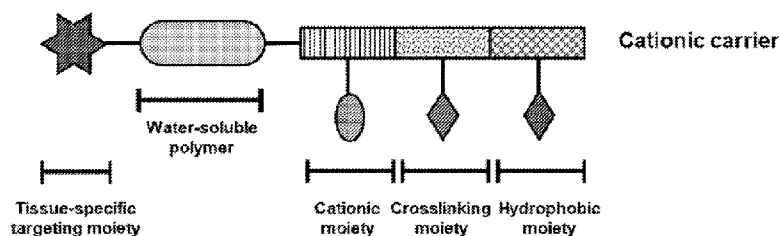
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(54) Title: MICELLAR NANOPARTICLES AND USES THEREOF

FIG. 1A



(57) Abstract: The present disclosure includes cationic carrier units comprising (i) a water soluble polymer, (ii) a positively charged carrier, (iii) a hydrophobic moiety, and (iv) a crosslinking moiety, wherein when the cationic carrier unit is mixed with an anionic payload (e.g., an RNA and/or DNA) that electrostatically interacts with the cationic carrier unit, the resulting composition self-organizes into a micelle encapsulating the anionic payload in its core. The cationic carrier units can also comprise a tissue specific targeting moiety, which would be displayed on the surface of the micelle. The disclosure also includes micelles comprising the cationic carrier units of the disclosure, methods of manufacture of cationic carrier units and micelles, pharmaceutical compositions comprising the micelles, and also methods of treating diseases or conditions comprising administering the micelles to a subject in need thereof.

MICELLAR NANOPARTICLES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 63/199,470, filed on December 30, 2020, which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in ASCII text file (Name: 4366_040PC01_Seqlisting_ST25; Size: 3,414 Bytes; Date of Creation: December 27, 2021) filed with the application is incorporated herein by reference in its entirety.

FIELD

[0003] The present disclosure provides cationic carrier units and micelle systems, which can be used to deliver anionic payloads (*e.g.*, RNA and/or DNA) across physiological permeation barriers, *e.g.*, the brain blood barrier.

BACKGROUND ART

[0004] Intracellular drug delivery is often challenging, because to reach the cytosol, exogenous molecules must first traverse the cell membrane. The cell membrane is selectively permeable to non-polar therapeutic agents, which are lipid soluble and can pass through the cell membrane. On the other hand, highly charged therapeutic agents such as mRNA are effectively excluded by the cell membrane.

[0005] Polynucleotides do not readily permeate the cellular membrane due to the charge repulsion between the negatively charged membrane and the high negative charge on the polynucleotide. As a result, polynucleotides have poor bioavailability and uptake into cells, typically less than 1% (Dheur et al, *Nucleic Acid Drug Dev.*, 9:522 (1999); Park et al, *J Controlled Release*, 93:188 (2003)). Since most polynucleotides are generally above 5,000 Da, they cannot readily diffuse through cellular membranes and uptake into cells is limited primarily to pinocytotic

or endocytotic processes. Once inside the cell, polynucleotides can accumulate in lysosomal compartments, limiting their access to the cytoplasm or the nucleus. Parenterally administered polynucleotides are also highly susceptible to rapid nuclease degradation both inside and outside the cytoplasm. Studies show rapid degradation of polynucleotides in blood after *i.v.* administration, with a half-life of about 30 minutes (Geary et al, J. Pharmacol. Exp. Ther. 296:890-897 (2001)).

[0006] Thus, the problems facing the delivery of polynucleotide, *e.g.*, mRNA, can roughly be divided into two parts. First, the therapeutic polynucleotide must be formulated in such a way that it can be delivered to the cytoplasm and second, the polynucleotide must reach the cell nucleus intact and fully functional. Despite the advances in application of nucleotides, *e.g.*, gene therapy, as therapeutics, the need exists for delivery systems providing improved pharmacological properties, *e.g.*, serum stability, delivery to the right organ, tissue, or cell, and transmembrane delivery.

[0007] Efforts aimed at improving the transmembrane delivery of nucleic acids have utilized protein carriers, antibody carriers, liposomal delivery systems, electroporation, direct injection, cell fusion, viral vectors, and calcium phosphate-mediated transformation. However, many of these techniques are limited by the types of cells in which transmembrane transport is enabled and by the conditions needed for achieving such transport. Accordingly, there is a need for delivery systems that can selectively direct charged therapeutic agents (*e.g.*, mRNAs) to specific target cells or tissues, and across permeation barriers (*e.g.*, the plasma membrane or the BBB), while improving serum stability and/or resistance to endogenous lytic enzymes (*e.g.*, RNases).

BRIEF SUMMARY

[0008] The present disclosure provides a cationic carrier unit comprising

[CC]-L1-[CM]-L2-[HM] (Schema I);

[CC]-L1-[HM]-L2-[CM] (Schema II);

[HM]-L1-[CM]-L2-[CC] (Schema III);

[HM]-L1-[CC]-L2-[CM] (Schema IV);

[CM]-L1-[CC]-L2-[HM] (Schema V); or

[CM]-L1-[HM]-L2-[CC] (Schema VI);

wherein

CC is a positively charged carrier moiety;

CM is a crosslinking moiety;

HM is a hydrophobic moiety; and,

L1 and L2 are independently optional linkers, and

wherein the number of HM is less than 40% relative to [CC] and [CM].

[0009] In some aspects, the number of HM is less than 39%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or about 1% relative to [CC] and [CM]. In some aspects, the number of HM is between about 35% and about 1%, about 35% and about 5%, about 35% and about 10%, about 35% and about 15%, about 35% and about 20%, about 35% and about 25%, about 35% and about 30%, about 30% and about 1%, about 30% and about 5%, about 30% and about 10%, about 30% and about 15%, about 30% and about 20%, about 30% and about 25%, about 25% and about 1%, about 25% and about 5%, about 25% and about 10%, about 25% and about 15%, about 25% and about 20%, about 20% and about 1%, about 20% and about 5%, about 20% and about 10%, about 20% and about 15%, about 15% and about 1%, about 15% and about 5%, about 15% and about 10%, about 10% and about 1%, or about 10% and about 5% relative to [CC] and [CM]. In some aspects, the number of HM is between about 39% and about 30%, about 30% and about 20%, about 20% and about 10%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM]. In some aspects, the number of HM is about 39%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, or about 1% relative to [CC] and [CM]. In some aspects, the cationic carrier unit is capable of interacting with an anionic payload.

[0010] In some aspects, the anionic payload comprises a nucleotide sequence having less than 4000 nucleotides, less than about 3500, less than about 3000, less than about 2500, less than about 2000, less than about 1500, less than about 1000, less than about 900, less than about 800, less than about 700, less than about 600, less than about 500, less than about 400, less than about 200, or less than about 150 in length. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 20, about 1 and about 19, about 1 and about 18, about 1 and about 17, about 1 and about 16, , about 1 and about 15, about 1 and about 14, about 1 and about 13, about 1 and about 12, about 1 and about 11, about 1 and about 10, about 1 and about 9, about 1 and about 8, about 1 and about 7, about 1 and about 6, or about 1 and about 5. In some aspects, the anionic payload and the cationic carrier unit

are capable of forming a micelle when mixed together in a solution, wherein N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10.

[0011] In some aspects, the anionic payload comprises a nucleotide sequence having about 100 nucleotides to about 1000 nucleotides in length. In some aspects, the number of HM is between 39% and about 30%, about 30% and about 20%, about 20% and about 10%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM]. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 10 or about 3 and about 7. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 2, about 2 and about 3, about 3 and about 4, or about 4 and about 5. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 1, about 2, about 3, about 4, or about 5.

[0012] In some aspects, the anionic payload comprises a nucleotide sequence having about 1000 nucleotides to about 2000 nucleotides in length. In some aspects, the number of HM is less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1% relative to [CC] and [CM]. In some aspects, the number of HM is between about 30% and about 20%, about 30% and about 25%, about 25% and about 20%, about 25% and about 15%, about 20% and about 10%, about 20% and about 5%, about 10% and about 1%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM]. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 4 and about 7. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 4 and about 5 or about 5 and about 6. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 4, about 5, about 6, or about 7.

[0013] In some aspects, the anionic payload comprises a nucleotide sequence having about 2000 nucleotides to about 3000 nucleotides in length. In some aspects, the number of HM is less than about 20%, less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% relative to [CC] and [CM]. In some aspects, the number of HM is less than about 20% to about 1%, from about 20% to about 5%, from about 20% to about 10%, from about 20% to about 15%, from about 15% to about 1%, from about 15% to about 5%, from about 15% to 10%, from about 10% to about 1%, from about 10% to about 5%, or from about 5% to about 1% nucleotides relative to [CC] and [CM]. In some aspects, the number of HM is about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1% relative to [CC] and [CM]. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 6 and about 9. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 6 and about 7 or about 7 and about 8. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 6, about 7, about 8, or about 9.

[0014] In some aspects, the anionic payload comprises a nucleotide sequence having about 3000 nucleotides to about 4000 nucleotides in length. In some aspects, the number of HM is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% relative to [CC] and [CM]. In some aspects, the number of HM is from about 10% to about 1%, from about 10% to about 5%, or from about 5% to about 1% nucleotides relative to [CC] and [CM]. In some aspects, the number of HM is about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1% relative to [CC] and [CM]. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 10. In some aspects, the anionic payload and the

cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 8 or about 8 and about 9. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 7, about 8, about 9, or about 10.

[0015] In some aspects, the anionic payload comprises mRNA, cDNA, or any combination thereof.

[0016] In some aspects, the cationic carrier further comprises a water-soluble polymer (WP). In some aspects, the water-soluble polymer is attached to [CC], [HM], or [CM]. In some embodiments, the water-soluble polymer is attached to the N terminus of [CC], [HM], or [CM]. In some embodiments, the water-soluble polymer is attached to the C terminus of [CC], [HM], or [CM].

[0017] In some embodiments the carrier unit comprises:

[WP]-L3]-[CC]-L1-[CM]-L2-[HM] (Schema I');

[WP]-L3]-[CC]-L1-[HM]-L2-[CM] (Schema II');

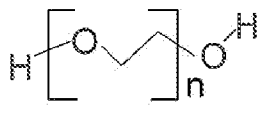
[WP]-L3]-[HM]-L1-[CM]-L2-[CC] (Schema III');

[WP]-L3]-[HM]-L1-[CC]-L2-[CM] (Schema IV');

[WP]-L3]-[CM]-L1-[CC]-L2-[HM] (Schema V'); or

[WP]-L3]-[CM]-L1-[HM]-L2-[CC] (Schema VI').

[0018] In some aspects, the water-soluble polymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof. In some aspects, the water-soluble polymer comprises polyethylene glycol ("PEG"), polyglycerol, or poly(propylene glycol) ("PPG"). In some aspects, the water-soluble polymer comprises:



wherein n is 1-1000.

[0019] In some aspects, n is at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, or at least about 141. In some aspects, n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 140 to about 150, or about 150 to about 160.

[0020] In some aspects, the water-soluble polymer is linear, branched, or dendritic. In some aspects, the cationic carrier moiety comprises one or more amino acids. In some aspects, the cationic carrier moiety comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50 basic amino acids, at least about 51, at least about 52, at least about 53, at least about 54, at least about 55, at least about 56, at least about 57, at least about 58, at least about 59, at least about 60, at least about 61, at least about 62, at least about 63, at least about 64, at least about 65, at least about 66, at least about 67, at least about 68, at least about 69, at least about 70, at least about 71, at least about 72, at least about 73, at least about 74, at least about 75, at least about 76, at least about 77, at least about 78, at least about 79, or at least about 80 amino acids. In some aspects, the cationic carrier moiety comprises at least 20, at least 30, at least 40, at least 50, at least 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, or at least about 150 amino acids. In some aspects, the the cationic carrier moiety comprises about 10 to about 60, about 15 to about 60, about 20 to about 60, about 25 to about 60, about 30 to about 60, about 35 to about 60, about 40 to about 60, about 10 to about 55, about 15 to about 55, about 20 to about 55, about 25 to about 55, about 30 to about 55, about 35 to about 55, about 40 to about 55, about 10 to about 50, about 15 to about 50, about 20 to about 50, about 25 to about 50, about 30 to about 50, about 35 to about 50, about 40 to about 50, about 10 to about 45, about 15 to about 45, about 20 to about 45, about 25 to about 45, about 30 to about 45, about 35 to about 45, about 40 to about 45, about 10 to about 40, about

15 to about 40, about 20 to about 40, about 25 to about 40, about 30 to about 40, about 35 to about 40, about 10 to about 35, about 15 to about 35, about 20 to about 35, about 25 to about 35, about 30 to about 35, about 35 to about 35, about 40 to about 35, or about 40 to about 35 amino acids.

[0021] In some aspects, the cationic carrier moiety comprises about 10, about 20, about 30, about 40, about 50, or about 60 amino acids. In some aspects, the amino acid comprises arginine, lysine, histidine, or any combination thereof. In some aspects, the cationic carrier moiety comprises about 20, about 30, about 40, about 50, or about 60 lysines. In some aspects, the cationic carrier moiety comprises about 40 lysine monomers.

[0022] In some aspects, the crosslinking moiety comprises one or more amino acids linked to a crosslinking agent. In some aspects, the crosslinking agent comprises a thiol group, a thiol derivative, or any combination thereof. In some aspects, the crosslinking agent comprises a thiol group. In some aspects, the amino acids in the crosslinking moiety comprise at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 amino acids. In some aspects, the amino acids in the crosslinking moiety comprise about 1 to about 40, about 5 to about 40, about 10 to about 40, about 15 to about 40, about 20 to about 40, about 1 to about 35, about 5 to about 35, about 10 to about 35, about 15 to about 35, about 20 to about 35, about 10 to about 50, about 15 to about 50, about 20 to about 50, about 25 to about 50, about 30 to about 40, about 10 to about 45, about 15 to about 45, about 20 to about 45, about 25 to about 45, about 30 to about 45, about 10 to about 40, about 15 to about 40, about 20 to about 40, about 25 to about 40, or about 30 to about 40 amino acids. In some aspects, the amino acids in the crosslinking moiety comprise about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 amino acids. In some aspects, the amino acids in the crosslinking moiety comprise arginine, lysine, histidine, or any combination thereof. In some aspects, the amino acids in the crosslinking moiety comprise about 35 lysines. In some aspects, the amino acids in the crosslinking moiety comprise about 23 lysines. In some aspects, wherein the amino acids in the crosslinking moiety comprises about 16 lysines.

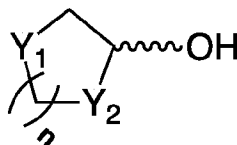
[0023] In some aspects, the hydrophobic moiety is capable of modulating an immune response, an inflammatory response, and/or a tissue microenvironment. In some aspects, the

hydrophobic moiety is capable of modulating an immune response. In some aspects, the hydrophobic moiety is capable of modulating a tumor microenvironment in a subject with a tumor.

[0024] In some aspects, the hydrophobic moiety is capable of inhibiting or reducing hypoxia in the tumor microenvironment. In some aspects, the hydrophobic moiety comprises one or more amino acids linked to an imidazole derivative, an amino acid, a vitamin, or any combination thereof.

[0025] In some aspects, the hydrophobic moiety is capable of inhibiting or reducing an inflammatory response. In some aspects, the hydrophobic moiety is one or more amino acids linked to a vitamin. In some aspects, the vitamin comprises a cyclic ring or cyclic heteroatom ring and a carboxyl group or hydroxyl group.

[0026] In some aspects, the vitamin comprises:



wherein each of Y₁ and Y₂ are independently selected from C, N, O, and S, and wherein n is 1 or 2.

[0027] In some aspects, the vitamin is selected from the group consisting of vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D2, vitamin D3, vitamin E, vitamin M, vitamin H, and any combination thereof. In some aspects, the vitamin is vitamin B3.

[0028] In some aspects, the hydrophobic moiety comprises at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, or at least 32 amino acids, each linked to a vitamin. In some aspects, the hydrophobic moiety comprises from about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 5 to about 10, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 10 to about 15, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 15 to

about 20, about 20 to about 35, about 20 to about 30, about 20 to about 25, about 25 to about 30, or about 25 to about 30 amino acids, each linked to a vitamin. In some aspects, the hydrophobic moiety comprises the hydrophobic moiety comprises about 2 vitamin B3, about 3 vitamin B3, about 4 vitamin B3, about 5 vitamin B3, about 6 vitamin B3, about 7 vitamin B3, about 8 vitamin B3, about 9 vitamin B3, about 10 vitamin B3, about 11 vitamin B3, about 12 vitamin B3, about 13 vitamin B3, about 14 vitamin B3, about 15 vitamin B3, about 16 vitamin B3, about 17 vitamin B3, about 18 vitamin B3, about 19 vitamin B3, about 20 vitamin B3, about 21 vitamin B3, about 22 vitamin B3, about 23 vitamin B3, about 24 vitamin B3, about 25 vitamin B3, about 26 vitamin B3, about 27 vitamin B3, about 28 vitamin B3, about 29 vitamin B3, about 30, about 31, about 32, about 33, about 34, or about 35 amino acids, each linked to vitamin B3.

[0029] In some aspects, the cationic carrier unit comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 20 to about 40 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3. In some aspects, the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 10 to about 20 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3. In some aspects, the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 10 to about 30 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3. In some aspects, the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 13 to about 25 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 20 lysine-vitamin B3. In some aspects, the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 13 to about 25 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 20 lysine-vitamin B3.

[0030] In some aspects, the cationic carrier unit comprises a water-soluble biopolymer moiety comprising about 120 to about 130 PEG units. In some aspects, the cationic carrier unit further comprises a targeting moiety (TM). In some aspects, the targeting moiety is capable of targeting a tissue. In some aspects, the tissue liver, brain, kidney, lung, ovary, pancreas, thyroid, breast, stomach, or any combination thereof. In some aspects, the targeting moiety is capable of being transported by large neutral amino acid transporter 1 (LAT1). In some aspects, the targeting moiety is an amino acid. In some aspects, the targeting moiety comprises a branched-chain or aromatic amino acid. In some aspects, the targeting moiety is phenylalanine, valine, leucine, and/or isoleucine. In some aspects, the amino acid is phenylalanine. In some aspects, the targeting moiety

is linked to the water-soluble polymer. In some aspects, the targeting moiety is linked to the water-soluble polymer by a linker.

[0031] The present disclosure also provides a micelle comprising the cationic carrier unit disclosed herein and an anionic payload, wherein the cationic carrier moiety of the cationic carrier complex and the anionic payload are associated with each other. In some aspects, the association is a covalent bond. In other aspects, the association is a non-covalent bond. In some aspects, the association is an ionic bond.

[0032] In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together and wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 1 and about 20. In some aspects, the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20. In some aspects, the positive charge of the cationic carrier moiety of the cationic carrier unit is sufficient to form a micelle when mixed with an anionic payload in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 3, about 4, about 5, about 6, about 7, about 8, or about 9. In some aspects, the cationic carrier unit is capable of protecting the anionic payload from degradation by a DNase and/or an RNase. In some aspects, the anionic payload is not conjugated to the cationic carrier unit by a covalent bond and/or the anionic payload interacts with the cationic carrier moiety of the cationic carrier unit only via an ionic interaction.

[0033] In some aspects, the half-life of the anionic payload is extended compared to the half-life of a free anionic payload not incorporated into a micelle.

[0034] In some aspects, the anionic payload that can be mixed with a cationic carrier unit to form a micelle that comprises a nucleotide sequence having about 1000 nucleotides to about 2000 nucleotides in length. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 4 and about 7. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 4 and about 5 or about 5 and about 6. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 4, about 5, about 6, or about 7.

[0035] In some aspects, the anionic payload that can be mixed with a cationic carrier unit to form a micelle comprises a nucleotide sequence having about 2000 nucleotides to about 3000 nucleotides in length. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, and wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 6 and about 9. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 6 and about 7 or about 7 and about 8. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the the N/P ratio of the cationic carrier unit and the anionic payload is about 6, about 7, about 8, or about 9.

[0036] In some aspects, the anionic payload that can be mixed with a cationic carrier unit to form a micelle comprises a nucleotide sequence having about 3000 nucleotides to about 4000 nucleotides in length. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 7 and about 10. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 7 and about 8 or about 8 and about 9. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 7, about 8, about 9, or about 10.

[0037] In some aspects, the diameter of the micelle is between about 10 nm and about 200 nm, between about 20 nm and about 200 nm, between about 1nm and 100nm, between about 10nm and about 100nm, between about 10nm and about 90nm, between about 10nm and about 80nm, between about 10nm and about 70nm, between about 20nm and about 100nm, between about 20nm and about 90nm, between about 20nm and about 80nm, between about 20nm and about 70nm, between about 30nm and about 100nm, between about 30nm and about 90nm, between about 30nm and about 80nm, between about 30nm and about 70nm, between about 40nm and about 100nm, between about 40nm and about 90nm, between about 40nm and about 80nm, or between about 40nm and about 70nm.

[0038] In some aspects, the anionic payload comprises a nucleic acid. In some aspects, the nucleic acid comprises mRNA, miRNA sponge, tough decoy miRNA, cDNA, pDNA, PNA, BNA, (ASO), aptamer, or any combination thereof. In some aspects, the nucleic acid comprises at least one nucleoside analog. In some aspects, the nucleoside analog comprises Locked Nucleic Acid

(LNA); 2'-O-alkyl-RNA; 2'-amino-DNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA, hexitol nucleic acid (HNA), intercalating nucleic acid (INA), constrained ethyl nucleoside (cEt), 2'-O-methyl nucleic acid (2'-OMe), 2'-O-methoxyethyl nucleic acid (2'-MOE), or any combination thereof.

[0039] In some aspects, the nucleic acid comprises a nucleotide sequence having at least about 100, at least about 500, at least about 1000, at least about 1500, at least about 2000, at least about 2500, at least about 3000, at least about 3500, or at least about 4000 nucleotides in length. In some aspects, the nucleotide sequence has a backbone, which comprises a phosphodiester linkage, a phosphotriester linkage, a methylphosphonate linkage, a phosphoramidate linkage, a phosphorothioate linkage, and combinations thereof. In some aspects, the cationic carrier unit further comprises a targeting moiety, which is linked to the water-soluble polymer optionally via a linker.

[0040] The present disclosure also provides a composition comprising the cationic carrier unit disclosed herein and an anionic payload. Also provided is a pharmaceutical composition comprising a cationic carrier unit, composition, or micelle disclosed herein, and a pharmaceutically acceptable carrier.

[0041] The present disclosure also provides a method of preparing the cationic carrier unit disclosed herein comprising linking the cationic carrier moiety to the crosslinking moiety and the hydrophobic moiety. In some aspects, the method further comprises linking a water-soluble polymer and a targeting moiety. In some aspects, the method of preparing a micelle disclosed herein comprises mixing the cationic carrier unit with the anionic payload in solution. In some aspects, the method further comprises purifying the micelle.

[0042] The present disclosure also provides a method of treating a disease or condition in a subject in need thereof comprising administering a micelle or pharmaceutical composition of the present disclosure to the subject. In some aspects, the anionic payload in the core of the micelle exhibits a longer half-life than a corresponding anionic payload not integrated into a micelle. In some aspects, the subject is a mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] **FIGs. 1A-1C** show exemplary architectures of carrier units and micelles of the present disclosure. The exemplary carrier units comprise an optional tissue-specific targeting moiety, water-soluble polymer, and cationic carrier unit (which can, respectively, interact with

anionic payloads) (**FIG. 1A**). In some aspects, the cationic carrier and anionic payload are not tethered and interact electrostatically. **FIG. 1B** shows a schematic diagram of an anionic payload. In some aspects, the cationic carrier and anionic payload are tethered and interact electrostatically. **FIG. 1C** shows a schematic diagram of a micelle comprising a cationic carrier and anionic payload of **FIGs. 1A** and **1B**.

[0044] **FIGs. 2A-2E** show exemplary compositions of cationic carrier units. **FIG 2A** shows a carrier unit comprising a targeting moiety, water-soluble polymer (*e.g.*, polyethylene glycol), and a cationic carrier unit comprising 80 lysine residues, wherein 64 lysine residues are unmodified (*e.g.*, contain positively charged -NH_3^+) and wherein 16 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol). **FIG. 2B** shows a carrier unit comprising a targeting moiety, water soluble polymer (*e.g.*, polyethylene glycol), and a cationic carrier unit comprising 80 lysine residues, wherein 40 lysine residues are unmodified (*e.g.*, contain a positively charged amine group, *e.g.*, -NH_3^+) and wherein 35 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 5 lysine residues are modified to contain a hydrophobic moiety (*e.g.*, a vitamin). **FIG. 2C** shows a carrier unit comprising a targeting moiety, water soluble polymer (*e.g.*, polyethylene glycol), and a cationic carrier unit comprising 80 lysine residues, wherein 38 lysine residues are unmodified (*e.g.*, contain a positively charged amine group, *e.g.*, -NH_3^+) and wherein 23 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 19 lysine residues are modified to contain a hydrophobic moiety (*e.g.*, a vitamin). **FIG. 2D** shows a carrier unit comprising a targeting moiety, water soluble polymer (*e.g.*, polyethylene glycol), and a cationic carrier unit comprising 80 lysine residues, wherein 32 lysine residues are unmodified (*e.g.*, contain a positively charged amine group, *e.g.*, -NH_3^+) and wherein 16 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 32 lysine residues are modified to contain a hydrophobic moiety (*e.g.*, a vitamin). **FIG. 2E** shows a carrier unit comprising a targeting moiety, water-soluble polymer (*e.g.*, polyethylene glycol), and a cationic carrier unit comprising 80 lysine residues, wherein 63 lysine residues are unmodified (*e.g.*, contain a positively charged amine group, *e.g.*, -NH_3^+) and wherein 17 lysine residues are modified to contain a hydrophobic moiety (*e.g.*, a vitamin).

[0045] **FIG. 3** shows a tissue specific targeting polymer structure for nucleotide micelle delivery and ^1H -NMR characteristics of a carrier unit. The ^1H -NMR chart corresponding to the targeting moiety (labeled "target molecule") shows that that the targeting moiety (an amino acid moiety containing a ring structure that binds to the LAT1 target on the brain endothelium) was

successfully synthesized. A second ^1H -NMR chart (labeled "polymer") shows that the cationic PEG block copolymer (comprising also the cationic carrier moiety and hydrophobic moiety) was also synthesized.

[0046] **FIGs. 4A-4D** show particle size and count rate at increasing N/P ratios for Compound A and nucleotide micelles measured by Zeta-sizer. **FIG. 4A** shows a schematic representation of Compound A (FIG. 2A). **FIG. 4B-4D** shows a the count rate and size of micelles comprising 800 nucleotides, 1,800 nucleotides, and 3,800 nucleotides, respectively, (e.g., anionic payload) and Compound A as the cationic carrier unit from an N/P ratio of 1-10.

[0047] **FIGs. 5A-5D** show particle size and count rate at increasing N/P ratios for Compound B and nucleotide micelles measured by Zeta-sizer. **FIG. 5A** shows a schematic representation of Compound B (FIG. 2B). **FIG. 5B-5D** shows a the count rate and size of micelles comprising 800 nucleotides, 1,800 nucleotides, and 3,800 nucleotides, respectively, (e.g., anionic payload) and Compound A as the cationic carrier unit from an N/P ratio of 1-10.

[0048] **FIGs. 6A-6D** show particle size and count rate at increasing N/P ratios for Compound C and nucleotide micelles measured by Zeta-sizer. **FIG. 6A** shows a schematic representation of Compound C (FIG. 2C). **FIG. 6B-6D** shows a the count rate and size of micelles comprising 800 nucleotides, 1,800 nucleotides, and 3,800 nucleotides, respectively, (e.g., anionic payload) and Compound A as the cationic carrier unit from an N/P ratio of 1-10.

[0049] **FIGs. 7A-7D** show particle size and count rate at increasing N/P ratios for Compound D and nucleotide micelles measured by Zeta-sizer. **FIG. 7A** shows a schematic representation of Compound D (FIG. 2D). **FIG. 7B-7D** shows a the count rate and size of micelles comprising 800 nucleotides, 1,800 nucleotides, and 3,800 nucleotides, respectively, (e.g., anionic payload) and Compound D as the cationic carrier unit from an N/P ratio of 1-10.

[0050] **FIGs. 8A-8D** show particle size and count rate at increasing N/P ratios for Compound E and nucleotide micelles measured by Zeta-sizer. **FIG. 8A** shows a schematic representation of Compound E (FIG. 2E). **FIG. 8B-8D** shows a the count rate and size of micelles comprising 800 nucleotides, 1,800 nucleotides, and 3,800 nucleotides, respectively, (e.g., anionic payload) and Compound E as the cationic carrier unit from an N/P ratio of 1-10.

[0051] **FIGs. 9A-9B** show particle size of mRNA micelle after micelle formulation between polymer (Compounds B, C, D) and mRNA (800 nt, 1800 nt, 3800 nt) individually at optimized N/P ratios (**FIG. 9A**) and fixed N/P ratios (**FIG. 9B**).

[0052] **FIGs. 10A-10C** show particle size distribution of mRNA containing micelles prepared with compound B and mRNA nucleotide lengths of 800 (**FIG. 10A**), 1,800 (**FIG. 10B**), and 3,800 (**FIG. 10C**).

[0053] **FIG. 11** shows relative mRNA expression level after transfection of PBS, mRNA encapsulated micelle and Lipofectamine 2000 with mRNA in HEK 293T cells. 5 μ g of mRNA formulated with PBS, polymeric carrier or Lipofectamine 2000 were transfected into the HEK-293T cells for 30 min. The mRNA level of gene was normalized on the basis of hGAPDH gene expression. The relative mRNA expression levels of the gene were calculated using the $2^{-\Delta\Delta Ct}$ method.

[0054] **FIGs. 12A-12D** show the protein expression levels of LAT1 in the muscles of BALB/c and DBA/2J mice. Western blot assays were performed to measure the LAT1 expression in BALB/c (**FIG. 12A**) and DBA/2J mice (**FIG. 12B**). Relative expression levels of LAT1 in BALB/c (**FIG. 12C**) and DBA/2J mice (**FIG. 12D**) were quantified, and expression levels of LAT1 were normalized with respect to GAPDH levels. Gastrocnemius; GAS, Quadriceps femoris; QF, Biceps femoris; BF, Tibialis anterior; TA.

[0055] **FIGs. 13A-13B** show bioluminescence images of mice after administration of Luc-mRNA encapsulated in polymeric micelles (**FIG. 13A**) or Lipofectamine (**FIG. 13B**), respectively. The luminescence images were taken up to 8 days after intramuscular injection.

DETAILED DESCRIPTION

[0056] The present disclosure is directed to carrier units comprising a water-soluble biopolymer moiety (*e.g.*, PEG), a charged moiety (*e.g.*, a polylysine), a crosslinking moiety, and a hydrophobic moiety. The cationic carrier units can be packaged into micelles when the units interact with anionic payloads, wherein the payload is located in the core of the micelle and the water-soluble biopolymer moiety is facing the solvent, and wherein the crosslinking moiety crosslinks one unit to the other carrier units. Non-limiting examples of various aspects are shown in the present disclosure.

[0057] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to the particular compositions or process steps described, as such can, of course, vary. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual aspects described and illustrated herein has discrete components and features which can be readily separated from or combined with the features of any of the other several

aspects without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

[0058] The headings provided herein are not limitations of the various aspects of the disclosure, which can be defined by reference to the specification as a whole. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[0059] Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

I. Definitions

[0060] In order that the present description can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0061] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. It is further noted that the claims can be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a negative limitation.

[0062] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0063] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0064] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999,

Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0065] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Where a range of values is recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed, along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither or both limits are included is also encompassed within the disclosure. Thus, ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 10 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

[0066] Where a value is explicitly recited, it is to be understood that values that are about the same quantity or amount as the recited value are also within the scope of the disclosure. Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the disclosure. Conversely, where different elements or groups of elements are individually disclosed, combinations thereof are also disclosed. Where any element of a disclosure is disclosed as having a plurality of alternatives, examples of that disclosure in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of a disclosure can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

[0067] Nucleotides are referred to by their commonly accepted single-letter codes. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Nucleotides are referred to herein by their commonly known one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Accordingly, 'a' represents adenine, 'c' represents cytosine, 'g' represents guanine, 't' represents thymine, and 'u' represents uracil.

[0068] Amino acid sequences are written left to right in amino to carboxy orientation. Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0069] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the

term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower).

[0070] The terms "administration," "administering," and grammatical variants thereof refer to introducing a composition, such as a micelle of the present disclosure, into a subject via a pharmaceutically acceptable route. The introduction of a composition, such as a micelle of the present disclosure, into a subject is by any suitable route, including intratumorally, orally, pulmonarily, intranasally, parenterally (intravenously, intra-arterially, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, intrathecally, periorcularly or topically. Administration includes self-administration and the administration by another. A suitable route of administration allows the composition or the agent to perform its intended function. For example, if a suitable route is intravenous, the composition is administered by introducing the composition or agent into a vein of the subject.

[0071] As used herein, the term "approximately," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain aspects, the term "approximately" refers to a range of values that fall within 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).

[0072] As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, which are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[0073] In some aspects, two or more sequences are said to be "completely conserved" or "identical" if they are 100% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. In some aspects, two or more sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some aspects, two or more sequences are said to be "conserved" if they are about 30% identical, about 40% identical, about

50% identical, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of a polynucleotide or polypeptide or may apply to a portion, region or feature thereof.

[0074] The term "derived from," as used herein, refers to a component that is isolated from or made using a specified molecule or organism, or information (*e.g.*, amino acid or nucleic acid sequence) from the specified molecule or organism. For example, a nucleic acid sequence that is derived from a second nucleic acid sequence can include a nucleotide sequence that is identical or substantially similar to the nucleotide sequence of the second nucleic acid sequence. In the case of nucleotides or polypeptides, the derived species can be obtained by, for example, naturally occurring mutagenesis, artificial directed mutagenesis or artificial random mutagenesis. The mutagenesis used to derive nucleotides or polypeptides can be intentionally directed or intentionally random, or a mixture of each. The mutagenesis of a nucleotide or polypeptide to create a different nucleotide or polypeptide derived from the first can be a random event (*e.g.*, caused by polymerase infidelity) and the identification of the derived nucleotide or polypeptide can be made by appropriate screening methods, *e.g.*, as discussed herein. Mutagenesis of a polypeptide typically entails manipulation of the polynucleotide that encodes the polypeptide. In some aspects, a nucleotide or amino acid sequence that is derived from a second nucleotide or amino acid sequence has a sequence identity of at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% to the second nucleotide or amino acid sequence, respectively, wherein the first nucleotide or amino acid sequence retains the biological activity of the second nucleotide or amino acid sequence.

[0075] The terms "complementary" and "complementarity" refer to two or more oligomers (*i.e.*, each comprising a nucleobase sequence), or between an oligomer and a target gene, that are

related with one another by Watson-Crick base-pairing rules. For example, the nucleobase sequence "T-G-A (5'→3')," is complementary to the nucleobase sequence "A-C-T (3'→ 5')." Complementarity may be "partial," in which less than all of the nucleobases of a given nucleobase sequence are matched to the other nucleobase sequence according to base pairing rules. For example, in some aspects, complementarity between a given nucleobase sequence and the other nucleobase sequence may be about 70%, about 75%, about 80%, about 85%, about 90% or about 95%. On the other hand, there may be "complete" or "perfect" (100%) complementarity between a given nucleobase sequence and the other nucleobase sequence to continue the example. The degree of complementarity between nucleobase sequences has significant effects on the efficiency and strength of hybridization between the sequences.

[0076] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain aspects, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0077] The terms "excipient" and "carrier" are used interchangeably and refer to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound.

[0078] As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Generally, the term "homology" implies an evolutionary relationship between two molecules. Thus, two molecules that are homologous will have a common evolutionary ancestor. In the context of the present disclosure, the term homology encompasses both identity and similarity.

[0079] In some aspects, polymeric molecules are considered to be "homologous" to one another if at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the monomers in the molecule are identical (exactly the same monomer) or are similar (conservative substitutions). The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences).

[0080] As used herein, the term "identity" refers to the overall monomer conservation between polymeric molecules, *e.g.*, between polypeptide molecules or polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules). The term "identical" without any additional

qualifiers, *e.g.*, protein A is identical to protein B, implies the sequences are 100% identical (100% sequence identity). Describing two sequences as, *e.g.*, "70% identical," is equivalent to describing them as having, *e.g.*, "70% sequence identity."

[0081] Calculation of the percent identity of two polypeptide or polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second polypeptide or polynucleotide sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain aspects, the length of a sequence aligned for comparison purposes is at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% of the length of the reference sequence. The amino acids at corresponding amino acid positions, or bases in the case of polynucleotides, are then compared.

[0082] When a position in the first sequence is occupied by the same amino acid as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

[0083] Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is *bl2seq*, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). *BL2seq* performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa.

[0084] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[0085] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent of sequence identity value is rounded to the nearest

tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0086] In certain aspects, the percentage identity (%ID) or of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as $\%ID = 100 \times (Y/Z)$, where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0087] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[0088] As used herein, the terms "isolated," "purified," "extracted," and grammatical variants thereof are used interchangeably and refer to the state of a preparation of desired composition of the present disclosure, that has undergone one or more processes of purification. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of a composition of the present disclosure from a sample containing contaminants. In some aspects, an isolated composition has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In other aspects, an isolated composition has an amount and/or concentration of desired composition of the present disclosure, at or above an acceptable amount and/or concentration and/or activity. In other aspects, the isolated composition is enriched as compared to the starting material from which the composition is obtained. This enrichment can be by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least

about 98%, at least about 99%, at least about 99.9%, at least about 99.99%, at least about 99.999%, at least about 99.9999%, or greater than 99.9999% as compared to the starting material. In some aspects, isolated preparations are substantially free of residual biological products. In some aspects, the isolated preparations are 100% free, at least about 99% free, at least about 98% free, at least about 97% free, at least about 96% free, at least about 95% free, at least about 94% free, at least about 93% free, at least about 92% free, at least about 91% free, or at least about 90% free of any contaminating biological matter. Residual biological products can include abiotic materials (including chemicals) or unwanted nucleic acids, proteins, lipids, or metabolites.

[0089] The term "linked" as used herein refers to a first amino acid sequence or polynucleotide sequence covalently or non-covalently joined to a second amino acid sequence or polynucleotide sequence, respectively. The first amino acid or polynucleotide sequence can be directly joined or juxtaposed to the second amino acid or polynucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first polynucleotide sequence to a second polynucleotide sequence at the 5'-end or the 3'-end, but also includes insertion of the whole first polynucleotide sequence (or the second polynucleotide sequence) into any two nucleotides in the second polynucleotide sequence (or the first polynucleotide sequence, respectively). The first polynucleotide sequence can be linked to a second polynucleotide sequence by a phosphodiester bond or a linker. The linker can be, *e.g.*, a polynucleotide.

[0090] The terms "mismatch" or "mismatches" refer to one or more nucleobases (whether contiguous or separate) in an oligomer nucleobase sequence that are not matched to a target pre-mRNA according to base pairing rules. While perfect complementarity is often desired, some aspects can include one or more but preferably 6, 5, 4, 3, 2, or 1 mismatches with respect to the target pre-mRNA. Variations at any location within the oligomer are included. In certain aspects, antisense oligomers of the disclosure include variations in nucleobase sequence near the termini, variations in the interior, and if present are typically within about 6, 5, 4, 3, 2, or 1 subunits of the 5' and/or 3' terminus. In certain aspects, one, two, or three nucleobases can be removed and still provide on-target binding.

[0091] As used herein, the terms "modulate," "modify," and grammatical variants thereof, generally refer when applied to a specific concentration, level, expression, function or behavior, to the ability to alter, by increasing or decreasing, *e.g.*, directly or indirectly promoting/stimulating/up-regulating or interfering with/inhibiting/down-regulating the specific concentration, level, expression, function or behavior, such as, *e.g.*, to act as an antagonist or

agonist. In some instances, a modulator can increase and/or decrease a certain concentration, level, activity or function relative to a control, or relative to the average level of activity that would generally be expected or relative to a control level of activity.

[0092] "Nucleic acid," "nucleic acid molecule," "nucleotide sequence," "polynucleotide," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA, and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or more nucleic acids as described herein.

[0093] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0094] The terms "pharmaceutically-acceptable carrier," "pharmaceutically-acceptable excipient," and grammatical variations thereof, encompass any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in animals, including humans, as well as any carrier or diluent that does not cause the production of undesirable physiological effects to a degree that prohibits administration of the composition to a

subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[0095] As used herein, the term "pharmaceutical composition" refers to one or more of the compounds described herein, such as, *e.g.*, a micelle of the present disclosure, mixed or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically acceptable carriers and excipients. One purpose of a pharmaceutical composition is to facilitate administration of preparations of micelles to a subject.

[0096] The term "polynucleotide" as used herein refers to polymers of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. This term refers to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide.

[0097] More particularly, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, hRNA, siRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0098] In some aspects of the present disclosure a polynucleotide can be, *e.g.*, an RNA, *e.g.*, mRNA, or DNA. In some aspects, the RNA is a synthetic RNA. In some aspects, the synthetic RNA comprises at least one unnatural nucleobase. In some aspects, all nucleobases of a certain class have been replaced with unnatural nucleobases (*e.g.*, all uridines in a polynucleotide disclosed herein can be replaced with an unnatural nucleobase, *e.g.*, 5-methoxyuridine).

[0099] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or

more analogs of an amino acid (including, for example, unnatural amino acids such as homocysteine, ornithine, p-acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art. The term "polypeptide," as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogues, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide can be a single polypeptide or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multichain polypeptides. Most commonly, disulfide linkages are found in multichain polypeptides. The term polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some aspects, a "peptide" can be less than or equal to 50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[0100] Any amino acids, *e.g.*, lysines, used in the context of the cationic carrier possess a positive charge by having its natural side group (*e.g.*, -NH_3^+ for lysine) or by having a modified side group. Any amino acids, *e.g.*, lysines, used in the context of the crosslinking moiety or the hydrophobic moiety may not possess any positive charges and can be linked to a crosslinking agent (*e.g.*, thiol) or a hydrophobic agent (*e.g.*, vitamin B3), respectively, by an amide bond or a linker.

[0101] As used herein, the term "N/P ratio" as used herein means the molar ratio of protonated amine in a cationic carrier moiety of a cationic carrier unit to phosphate in an anionic payload when the cationic carrier unit and anionic payload are mixed together in solution.

[0102] The terms "prevent," "preventing," and variants thereof as used herein, refer partially or completely delaying onset of an disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular disease, disorder, and/or condition; partially or completely delaying progression from a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some aspects, preventing an outcome is achieved through prophylactic treatment.

[0103] As used herein, "prophylactic" refers to a therapeutic or course of action used to prevent the onset of a disease or condition, or to prevent or delay a symptom associated with a disease or condition.

[0104] As used herein, a "prophylaxis" refers to a measure taken to maintain health and prevent or delay the onset of a bleeding episode, or to prevent or delay symptoms associated with a disease or condition.

[0105] As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art. It is understood that percentage of similarity is contingent on the comparison scale used, *i.e.*, whether the amino acids are compared, *e.g.*, according to their evolutionary proximity, charge, volume, flexibility, polarity, hydrophobicity, aromaticity, isoelectric point, antigenicity, or combinations thereof.

[0106] The terms "subject," "patient," "individual," and "host," and variants thereof are used interchangeably herein and refer to any mammalian subject, including without limitation, humans, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like), and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the like) for whom diagnosis, treatment, or therapy is desired, particularly humans. The methods described herein are applicable to both human therapy and veterinary applications.

[0107] As used herein, the phrase "subject in need thereof" includes subjects, such as mammalian subjects, that would benefit from administration of a micelle of the disclosure, *e.g.*, to improve hemostasis.

[0108] The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[0109] As used herein the term "therapeutically effective amount" is the amount of reagent or pharmaceutical compound comprising a micelle of the present disclosure that is sufficient to produce a desired therapeutic effect, pharmacologic and/or physiologic effect on a subject in need thereof. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

[0110] The terms "treat," "treatment," or "treating," as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course;

the amelioration or elimination of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition. The term also include prophylaxis or prevention of a disease or condition or its symptoms thereof. In one aspect, the term "treating" or "treatment" means inducing an immune response in a subject against an antigen.

[0111] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence.

II. Carrier Units

[0112] The present disclosure provides carrier units that can self-assemble into micelles or be incorporated into micelles. Carrier units of the present disclosure comprise a water-soluble biopolymer moiety (*e.g.*, PEG), a charged carrier moiety, a crosslinking moiety, and a hydrophobic moiety. In some aspects, the charged carrier moiety is cationic (*e.g.*, a polylysine), as exemplified in **FIG. 1**.

[0113] Carrier units of the present disclosure can be used to deliver negatively payloads (*e.g.*, therapeutic or diagnostic agents). In some aspects, negatively payloads that can be delivered by micelles comprises at least about 100, at least about 1000, at least about 2000, at least about 3000, or at least about 4000 nucleotides in length. Carrier units with a cationic charged carrier moiety can be used for the delivery of anionic payloads, *e.g.*, polynucleotides. Carrier units with an anionic charged carrier moiety can be used for the delivery of cationic payloads, *e.g.*, positively charged small molecule drugs. See **FIG. 1**. In some aspects, the cationic charged carrier moiety and the anionic payloads can electrostatically interact with each other.

[0114] The resulting carrier unit:payload complex can have a "head" comprising the water-soluble biopolymer moiety and a "tail" comprising the cationic carrier moiety electrostatically bound to the payload.

[0115] Carrier unit:payload complexes can self-associate, alone or in combination with other molecules, to yield micelles in which the anionic payload is located in the core of the micelle and the water-soluble biopolymer moiety is facing the solvent. The term "micelles of the present disclosure" encompasses not only classic micelles but also small particles, small micelles, micelles, rod-like structures, or polymersomes. Given that polymersomes comprise a luminal space, it is to be understood that all the disclosures related to the "core" of classic micelles are equally applicable to the luminal space in polymersomes comprising carrier units of the present disclosure.

[0116] The carrier units of the present disclosure can also comprise a targeting moiety (e.g., a targeting ligand) covalently linked to the water-soluble biopolymer moiety via one or more optional linkers. Once a micelle is formed, the targeting moiety can be located on the surface of the micelle and can deliver the micelle to a specific target tissue, to a specific cell type, and/or facilitate transport across a physiological barrier (e.g., cell plasma membrane). In some aspects, the micelles of the present disclosure can comprises more than one type of targeting moiety.

[0117] The carrier units of the present disclosure can also comprise moiety hydrophobic moiety (HM) covalently linked to the charged cationic carrier moiety. The hydrophobic moiety can have, e.g., a therapeutic, a co-therapeutic effect, or positively affect the homeostasis of the target cell or target tissue. In some aspects, the HM comprises one or more amino acids. In some aspects, the HM comprises one or more amino acids linked to a hydrophobic molecule (e.g., a vitamin). In some aspects, the Hm comprises one or more lysine residues covalently bound to a hydrophobic molecule (e.g., a vitamin).

[0118] In some aspects, the anionic payload is not covalently linked to the carrier unit. However, in other aspects, the anionic payload can be covalently linked to the cationic carrier unit, e.g., a linker such as cleavable linker.

[0119] Non-limiting examples of various aspects are shown in the present disclosure. The disclosure refers in particular to the use of cationic carrier units, e.g., to deliver anionic payloads such as nucleic acids. However, it would be apparent to a person of ordinary skill in the art that the disclosures can be equally applied to the delivery of cationic payloads or to the delivery of neutral payloads by reversing the charges of the carrier moiety and payload (i.e., using an anionic carrier moiety in the carrier unit to deliver a cationic payload), or by using a neutral payload linked to a cationic or anionic adapter that would electrostatically interact with an anionic or cationic carrier moiety, respectively.

[0120] Accordingly, in one aspect, the present disclosure provides cationic carrier units of Schema I through Schema VI

[CC]-L1-[CM]-L2-[HM] (Schema I);
[CC]-L1-[HM]-L2-[CM] (Schema II);
[HM]-L1-[CM]-L2-[CC] (Schema III);
[HM]-L1-[CC]-L2-[CM] (Schema IV);
[CM]-L1-[CC]-L2-[HM] (Schema V); or
[CM]-L1-[HM]-L2-[CC] (Schema VI);

wherein

CC is a cationic carrier moiety, e.g., a polylysine;

CM is a crosslinking moiety;

HM is a hydrophobic moiety, e.g., vitamin, e.g., vitamin B3; and,

L1 and L2 are independently optional linkers.

[0121] In some aspects, the cationic carrier unit further comprises a water-soluble polymer (WP). In some aspects, the water-soluble polymer is attached to [CC], [HM], and/or [CM]. In some aspects, the water-soluble polymer is attached to the N terminus of [CC], [HM], or [CM]. In some aspects, the water-soluble polymer is attached to the N terminus of [CC]. In some aspects, the water-soluble polymer is attached to the C terminus of [CC], [HM], or [CM]. In some aspects, the water-soluble polymer is attached to the C terminus of [CC].

[0122] In some aspects, the cationic carrier unit comprises:

[WP]-L3-[CC]-L1-[CM]-L2-[HM] (Schema I');

[WP]-L3-[CC]-L1-[HM]-L2-[CM] (Schema II');

[WP]-L3-[HM]-L1-[CM]-L2-[CC] (Schema III');

[WP]-L3-[HM]-L1-[CC]-L2-[CM] (Schema IV');

[WP]-L3-[CM]-L1-[CC]-L2-[HM] (Schema V');

[WP]-L3-[CM]-L1-[HM]-L2-[CC] (Schema VI').

[0123] In some aspects of the constructs of Schema I' to VI' shown above, the [WP] component can be connected to at least one targeting moiety, i.e., [T]_n-[WP]-... wherein n is an integer, e.g., 1, 2 or 3.

[0124] **FIGs. 2A-2E** present a schematic representation of a cationic carrier unit of the present disclosure. For simplicity, the units in **FIG. 2A-2E** have been represented linearly. However, in some aspects, the carrier units can comprises the CC, CM, and HM moieties organized in a branched scaffold arrangement (see **FIG. 2** and **FIG. 3**), for example, with (i) a polymeric CC moiety comprising positively charged units (e.g., polylysines) and (ii) a CMs (e.g., lysine linked to a crosslinking agent, e.g., lysine-thiol) attached to the N or C terminus of the CC moiety and (iii) a HM (e.g., lysine linked to a hydrophobic agent, e.g., lysine linked to Vitamin B3) attached to the N or C terminus of the CM moiety.

[0125] When cationic carrier units of the present disclosure are mixed with an anionic payload (e.g., a nucleic acid) at an ionic ratio of about 20:about 1, i.e., the number of negative charges in the anionic payload is about 20 times higher than the number of positive charges in the cationic carrier moiety, to about 20:1, i.e., the number of positive charges in the cationic carrier moiety is about ten times higher than the number of negative charges in the anionic payload, the

neutralization of negative charges in the anionic payload by positive charges in the cationic carrier moiety mainly via electrostatic interaction leads to the formation of a cationic carrier unit:anionic payload complex having an unaltered hydrophilic portion (comprising the WP moiety) and a substantially more hydrophobic portion (resulting from the association between the cationic carrier moiety plus hydrophobic moiety and the anionic payload).

[0126] In some aspects, the hydrophobic moiety can contribute its own positive charges to the positive charges of the cationic carrier moiety, which would interact with the negative charges of the anionic payload. It is to be understood that references to the interactions (*e.g.*, electrostatic interactions) between a cationic carrier moiety and an anionic payload also encompass interactions between the charges of a cationic carrier moiety plus hydrophobic moiety and the charges of an anionic payload.

[0127] The increase in the hydrophobicity of the cationic carrier moiety of the cationic carrier unit due to the neutralization of its positive charges via electrostatic interaction with the negative charges of the anionic payload results in an amphipathic complex. Such amphipathic complexes can self-organize, alone or combination with other amphipathic components, into micelles. The resulting micelles comprise the WP moieties facing the solvent (*i.e.*, the WP moieties are facing the external surface of the micelle), whereas the CC and HM moieties as well as the associate payload (*e.g.*, a nucleotide sequence, *e.g.*, RNA, DNA, or any combination thereof) are in the core of the micelle.

[0128] In some specific aspects, the cationic carrier unit comprises:

- (a) a WP moiety, wherein the water-soluble biopolymer is a polyethylene glycol (PEG) of formula III (see below), wherein n is between about 120 to about PEG 130 (*e.g.*, PEG is a PEG5000 or a PEG6000);
- (b) a CC moiety, wherein the cationic carrier moiety comprises, *e.g.*, about 20 to about 100 lysines (*e.g.*, a linear poly(L-lysine) n wherein n is between about 30 and about 40), a polyethyleneimine (PEI), or chitosan;
- (c) a CM moiety, wherein the crosslinking moiety comprises about about 10 to about 50 lysines, each of which is linked to a crosslinking agent, *e.g.*, 10-40 lysine-thiol, and
- (d) an HM moiety, wherein the hydrophobic moiety has about 1 to about 20 lysines, each of which is linked to a vitamin B3 unit.

[0129] In some specific aspects, the cationic carrier unit comprises:

- (a) a WP moiety, wherein the water-soluble biopolymer is a polyethylene glycol (PEG) of formula III (see below), wherein n is between about 120 to about PEG 130 (e.g., PEG is a PEG5000 or a PEG6000);
- (b) a CC moiety, wherein the cationic carrier moiety comprises, e.g., about 20 to about 100 lysines (e.g., a linear poly(L-lysine) n wherein n is between about 30 and about 40), a polyethyleneimine (PEI), or chitosan;
- (c) a CM moiety, wherein the crosslinking moiety comprises about about 10 to about 50 lysines, each of which is linked to a crosslinking agent, e.g., 10-40 lysine-thiol, and
- (d) an HM moiety, wherein the hydrophobic moiety has about 1 to about 10 lysines, each of which is linked to a vitamin B3 unit.

[0130] In some specific aspects, the cationic carrier unit comprises:

- (a) a WP moiety, wherein the water-soluble biopolymer is a polyethylene glycol (PEG) of formula III (see below), wherein n is between about 120 to about PEG 130 (e.g., PEG is a PEG5000 or a PEG6000);
- (b) a CC moiety, wherein the cationic carrier moiety comprises, e.g., about 20 to about 100 lysines (e.g., a linear poly(L-lysine) n wherein n is between about 30 and about 40), a polyethyleneimine (PEI), or chitosan;
- (c) a CM moiety, wherein the crosslinking moiety comprises about about 10 to about 50 lysines, each of which is linked to a crosslinking agent, e.g., 10-40 lysine-thiol, and
- (d) an HM moiety, wherein the hydrophobic moiety has about 5 to about 10 lysines, each of which is linked to a vitamin B3 unit.

[0131] In some specific aspects, the cationic carrier unit comprises:

- (a) a WP moiety, wherein the water-soluble biopolymer is a polyethylene glycol (PEG) of formula III (see below), wherein n is between about 120 to about PEG 130 (e.g., PEG is a PEG5000 or a PEG6000);
- (b) a CC moiety, wherein the cationic carrier moiety comprises, e.g., about 20 to about 100 lysines (e.g., a linear poly(L-lysine) n wherein n is between about 30 and about 40, e.g., about 40), a polyethyleneimine (PEI), or chitosan;
- (c) a CM moiety, wherein the crosslinking moiety comprises about about 10 to about 50 lysines, each of which is linked to a crosslinking agent, e.g., 10-40 lysine-thiol, e.g., 35 lysine-thiol, and
- (d) an HM moiety, wherein the hydrophobic moiety has about 1 to about 5 lysines, each of which is linked to a vitamin B3 unit.

[0132] In some aspects, the cationic carrier unit further comprises at least one targeting moiety attached to the WP moiety of the cationic carrier unit. In some aspects, the number and/or density of targeting moieties displayed on the surface of the micelle can be modulated by using a specific ratio of cationic carrier units having targeting moieties to cationic carrier units not having targeting moieties. In some aspects, the ratio of cationic carrier units having a targeting moiety to cationic carrier units not having a targeting moiety is at least about 1:5, at least about 1:10, at least about 1:20, at least about 1:30, at least about 1:40, at least about 1:50, at least about 1:60, at least about 1:70, at least about 1:80, at least about 1:90, at least about 1:100, at least about 1:120, at least about 1:140, at least about 1:160, at least about 1:180, at least about 1:200, at least about 1:250, at least about 1:300, at least about 1:350, at least about 1:400, at least about 1:450, at least about 1:500, at least about 1:600, at least about 1:700, at least about 1:800, at least about 1:900, or at least about 1:1000.

[0133] In some aspects, the cationic carrier unit comprises

- (i) a targeting moiety (A) which targets the transporter LAT1 (*e.g.*, phenylalanine),
- (ii) a water soluble polymer which is PEG,
- (iii) a cationic carrier moiety comprising cationic polymer blocks which are lysine
- (iv) a crosslinking moiety comprising crosslinking polymer blocks which are lysines linked to crosslinking moieties, and
- (v) a hydrophobic moiety comprising hydrophobic polymer blocks which are lysines linked to vitamin B3.

[0134] In some aspects, the cationic carrier unit comprises

- (i) a targeting moiety (A) which targets the transporter LAT1 (*e.g.*, phenylalanine),
- (ii) a water soluble polymer which is PEG, wherein $n = 100 - 200$, *e.g.*, $100 - 150$, *e.g.*, 120-130,
- (iii) a cationic carrier moiety comprising cationic polymer blocks, *e.g.*, polylysine,
- (iv) a crosslinking moiety comprising crosslinking polymer blocks which are lysines linked to crosslinking moieties, and
- (iv) a hydrophobic moiety comprising hydrophobic polymer blocks which are lysines linked to vitamin B3.

[0135] In some aspects, the cationic carrier unit comprises

- (i) a targeting moiety (A) which targets the transporter LAT1 (*e.g.*, phenylalanine),
- (ii) a water soluble polymer which is PEG, wherein $n = 100 - 200$, *e.g.*, $100 - 150$, *e.g.*, 120-130,
- (iii) a cationic carrier moiety comprising cationic polymer blocks, *e.g.*, 10-100 lysines, *e.g.*, 10-50 lysines, *e.g.*, 30-40 lysines,

(iv) a crosslinking moiety comprising crosslinking polymer blocks which are lysines linked to crosslinking moieties, and

(iv) a hydrophobic moiety comprising hydrophobic polymer blocks which are lysines linked to vitamin B3.

[0136] In some aspects, the number (percentage) of HM is less than 39%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or about 1% relative to [CC] and [CM]. In some aspects, the number (percentage) of HM is between about 35% and about 1%, about 35% and about 5%, about 35% and about 10%, about 35% and about 15%, about 35% and about 20%, about 35% and about 25%, about 35% and about 30%, about 30% and about 1%, about 30% and about 5%, about 30% and about 10%, about 30% and about 15%, about 30% and about 20%, about 30% and about 25%, about 25% and about 1%, about 25% and about 5%, about 25% and about 10%, about 25% and about 15%, about 25% and about 20%, about 20% and about 1%, about 20% and about 5%, about 20% and about 10%, about 20% and about 15%, about 15% and about 1%, about 15% and about 5%, about 15% and about 10%, about 10% and about 1%, or about 10% and about 5% relative to [CC] and [CM]. In some aspects, the number (percentage) of HM is between about 39% and about 30%, about 30% and about 20%, about 20% and about 10%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM]. In some aspects, the number (percentage) of HM is about 39%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, or about 1% relative to [CC] and [CM]. In some aspects, the the number of HM is expressed as the percentage of [HM] relative to [CC] and [CM].

[0137] In some aspects, the cationic carrier unit of the present disclosure interacts with a nucleotide payload having about 100 to about 1000 nucleotides in length. In some aspects, the nucleotide payload having about 100 to about 1000 nucleotides in length encodes one or more proteins or fragments thereof, e.g., PAPD5/7 or any fragments thereof. In some aspects, the carrier unit complexed the nucleotide payload having about 100 to about 1000 nucleotides in length forms a micelle.

[0138] In some aspects, the cationic carrier unit of the present disclosure interacts with a nucleotide payload having about 1000 to about 2000 nucleotides in length. In some aspects, the nucleotide payload having about 1000 to about 2000 nucleotides in length encodes one or more proteins or fragments thereof, e.g., G protein or any fragments thereof. In some aspects, the carrier

unit complexed the nucleotide payload having about 1000 to about 2000 nucleotides in length forms a micelle.

[0139] In some aspects, the cationic carrier unit of the present disclosure interacts with a nucleotide payload having about 2000 to about 3000 nucleotides in length. In some aspects, the nucleotide payload having about 2000 to about 3000 nucleotides in length encodes one or more proteins or fragments thereof, e.g., Gephyrin or any fragments thereof. In some aspects, the carrier unit complexed the nucleotide payload having about 2000 to about 3000 nucleotides in length forms a micelle.

[0140] In some aspects, the cationic carrier unit of the present disclosure interacts with a nucleotide payload having about 3000 to about 4000 nucleotides in length. In some aspects, the nucleotide payload having about 3000 to about 4000 nucleotides in length encodes one or more proteins or fragments thereof, e.g., MDA5 (IFIH1) or any fragments thereof. In some aspects, the carrier unit complexed the nucleotide payload having about 3000 to about 4000 nucleotides in length forms a micelle.

[0141] In some aspects, the vitamin B3 unit are introduced into the side chains of the HM moiety, e.g., by a coupling reaction between NH₂ groups in the lysines and COOH groups of vitamin B3, in the presence of suitable conjugation reagents, for example, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxy succinimide (NHS).

[0142] The present disclosure provides composition comprising a carrier unit (e.g., a cationic carrier unit) of the present disclosure. In other aspects, the present disclosure provides complexes comprising a carrier unit (e.g., a cationic carrier unit) of the present disclosure non-covalently attached to a payload (e.g., an anionic payload such a nucleotide sequence, e.g., an RNA, DNA, or any combination thereof), wherein the carrier unit and the payload interact electrostatically. In other aspects, the present disclosure provides conjugates comprising a carrier unit (e.g., a cationic carrier unit) of the present disclosure covalently attached to a payload (e.g., an anionic payload such a nucleotide sequence, e.g., an RNA, DNA, or any combination thereof), wherein the carrier unit and the payload interact electrostatically. In some aspects, the carrier unit and the payload can be linked via a cleavable linker. In some aspects, the carrier unit and the payload, in addition to interacting electrostatically, can interact covalently (e.g., after electrostatic interaction the carrier unit and the payload can be "locked" via a disulfide bond or a cleavable bond).

[0143] In some specific aspects, the cationic carrier unit comprises a water-soluble polymer comprising a PEG with about 120 to about 130 units, a cationic carrier moiety comprising a

polylysine with about 20 to about 60 lysine units, a crosslinking moiety comprising about 3 to about 40 lysine-thiol units, and a hydrophobic moiety comprising about 1 to about 20 lysines linked to a vitamin B3 units.

[0144] In some aspects, the cationic carrier unit is associated with a negatively charged payload (*e.g.*, a nucleotide sequence, *e.g.*, an RNA, DNA, or any combination thereof), which interacts with the cationic carrier unit via at least one ionic bond (*i.e.*, via electrostatic interaction) with the cationic carrier moiety of the cationic carrier unit.

[0145] The specific components of the cationic carrier units of the present disclosure are disclosed in detail below.

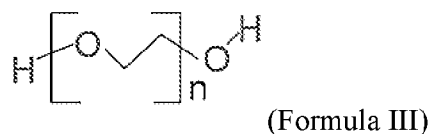
a. Water-soluble biopolymer

[0146] In some aspects, the cationic carrier units of the present disclosure comprise at least one water-soluble biopolymer. The term "water-soluble biopolymer" as used herein refers to a biocompatible, biologically inert, non-immunogenic, non-toxic, and hydrophilic polymer, *e.g.*, PEG.

[0147] In some aspects, the water-soluble polymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof. In some aspects, the water-soluble biopolymer is linear, branched, or dendritic.

[0148] In some aspects, the water-soluble biopolymer comprises polyethylene glycol ("PEG"), polyglycerol ("PG"), or poly(propylene glycol) ("PPG"). PPG is less toxic than PEG, so many biological products are now produced in PPG instead of PEG.

[0149] In some aspects, the water-soluble biopolymer comprises a PEG characterized by a formula $R^3-(O-CH_2-CH_2)_n-$ or $R^3-(O-CH_2-CH_2)_n-O-$ with R^3 being hydrogen, methyl or ethyl and n having a value from 2 to 200. In some aspects, the PEG has the formula



wherein n is 1 to 1000.

[0150] In some aspects, the n of the PEG has a value of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39,

40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or 200.

[0151] In some aspects, n is at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, at least about 150, at least about 160, at least about 170, at least about 180, at least about 190, at least about 200, at least about 210, at least about 220, at least about 230, at least about 240, at least about 250, at least about 260, at least about 270, at least about 280, at least about 290, at least about 300, at least about 310, at least about 320, at least about 330, at least about 340, at least about 350, at least about 360, at least about 370, at least about 380, at least about 390, at least about 400, at least about 410, at least about 420, at least about 430, at least about 440, at least about 450, at least about 460, at least about 470, at least about 480, at least about 490, at least about 500, at least about 510, at least about 520, at least about 530, at least about 540, at least about 550, at least about 560, at least about 570, at least about 580, at least about 590, at least about 600, at least about 610, at least about 620, at least about 630, at least about 640, at least about 650, at least about 660, at least about 670, at least about 680, at least about 690, at least about 700, at least about 710, at least about 720, at least about 730, at least about 740, at least about 750, at least about 760, at least about 770, at least about 780, at least about 790, at least about 800, at least about 810, at least about 820, at least about 830, at least about 840, at least about 850, at least about 860, at least about 870, at least about 880, at least about 890, at least about 900, at least about 910, at least about 920, at least about 930, at least about 940, at least about 950, at least about 960, at least about 970, at least about 980, at least about 990, or about 1000.

[0152] In some aspects, n is between about 50 and about 100, between about 100 and about 150, between about 150 and about 200, between about 200 and about 250, between about 250 and about 300, between about 300 and about 350, between about 350 and about 400, between about 400 and about 450, between about 450 and about 500, between about 500 and about 550, between about 550 and about 600, between about 600 and about 650, between about 650 and about 700,

between about 700 and about 750, between about 750 and about 800, between about 800 and about 850, between about 850 and about 900, between about 900 and about 950, or between about 950 and about 1000.

[0153] In some aspects, n is at least about 80, at least about 81, at least about 82, at least about 83, at least about 84, at least about 85, at least about 86, at least about 87, at least about 88, at least about 89, at least about 90, at least about 91, at least about 92, at least about 93, at least about 94, at least about 95, at least about 96, at least about 97, at least about 98, at least about 99, at least about 100, at least about 101, at least about 102, at least about 103, at least about 104, at least about 105, at least about 106, at least about 107, at least about 108, at least about 109, at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, at least about 141, at least about 142, at least about 143, at least about 144, at least about 145, at least about 146, at least about 147, at least about 148, at least about 149, at least about 150, at least about 151, at least about 152, at least about 153, at least about 154, at least about 155, at least about 156, at least about 157, at least about 158, at least about 159, or at least about 160.

[0154] In some aspects, n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150, about 150 to about 160, about 85 to about 95, about 95 to about 105, about 105 to about 115, about 115 to about 125, about 125 to about 135, about 135 to about 145, about 145 to about 155, about 155 to about 165, about 80 to about 100, about 100 to about 120, about 120 to about 140, about 140 to about 160, about 85 to about 105, about 105 to about 125, about 125 to about 145, or about 145 to about 165.

[0155] In some aspects, n is about 100 to about 150. In some aspects, n is about 100 to about 140. In some aspects, n is about 100 to about 130. In some aspects, n is about 110 to about 150. In some aspects, n is about 110 to about 140. In some aspects, n is about 110 to about 130. In some aspects, n is about 110 to about 120. In some aspects, n is about 120 to about 150. In some aspects, n is about 120 to about 140. In some aspects, n is about 120 to about 130. In some aspects, n is about 130 to about 150. In some aspects, n is about 130 to about 140.

[0156] Thus, in some aspects, the PEG is a branched PEG. Branched PEGs have three to ten PEG chains emanating from a central core group. In certain aspects, the PEG moiety is a monodisperse polyethylene glycol. In the context of the present disclosure, a monodisperse polyethylene glycol (mdPEG) is a PEG that has a single, defined chain length and molecular weight. mdPEGs are typically generated by separation from the polymerization mixture by chromatography. In certain formulae, a monodisperse PEG moiety is assigned the abbreviation mdPEG.

[0157] In some aspects, the PEG is a Star PEG. Star PEGs have 10 to 100 PEG chains emanating from a central core group. In some aspects, the PEG is a Comb PEGs. Comb PEGs have multiple PEG chains normally grafted onto a polymer backbone.

[0158] In certain aspects, the PEG has a molar mass between about 1000 g/mol and about 2000 g/mol, between about 2000 g/mol and about 3000 g/mol, between about 3000 g/mol to about 4000 g/mol, between about 4000 g/mol and about 5000 g/mol, between about 5000 g/mol and about 6000 g/mol, between about 6000 g/mol and about 7000 g/mol, or between 7000 g/mol and about 8000 g/mol.

[0159] In some aspects, the PEG is PEG₁₀₀, PEG₂₀₀, PEG₃₀₀, PEG₄₀₀, PEG₅₀₀, PEG₆₀₀, PEG₇₀₀, PEG₈₀₀, PEG₉₀₀, PEG₁₀₀₀, PEG₁₁₀₀, PEG₁₂₀₀, PEG₁₃₀₀, PEG₁₄₀₀, PEG₁₅₀₀, PEG₁₆₀₀, PEG₁₇₀₀, PEG₁₈₀₀, PEG₁₉₀₀, PEG₂₀₀₀, PEG₂₁₀₀, PEG₂₂₀₀, PEG₂₃₀₀, PEG₂₄₀₀, PEG₂₅₀₀, PEG₂₆₀₀, PEG₂₇₀₀, PEG₂₈₀₀, PEG₂₉₀₀, PEG₃₀₀₀, PEG₃₁₀₀, PEG₃₂₀₀, PEG₃₃₀₀, PEG₃₄₀₀, PEG₃₅₀₀, PEG₃₆₀₀, PEG₃₇₀₀, PEG₃₈₀₀, PEG₃₉₀₀, PEG₄₀₀₀, PEG₄₁₀₀, PEG₄₂₀₀, PEG₄₃₀₀, PEG₄₄₀₀, PEG₄₅₀₀, PEG₄₆₀₀, PEG₄₇₀₀, PEG₄₈₀₀, PEG₄₉₀₀, PEG₅₀₀₀, PEG₅₁₀₀, PEG₅₂₀₀, PEG₅₃₀₀, PEG₅₄₀₀, PEG₅₅₀₀, PEG₅₆₀₀, PEG₅₇₀₀, PEG₅₈₀₀, PEG₅₉₀₀, PEG₆₀₀₀, PEG₆₁₀₀, PEG₆₂₀₀, PEG₆₃₀₀, PEG₆₄₀₀, PEG₆₅₀₀, PEG₆₆₀₀, PEG₆₇₀₀, PEG₆₈₀₀, PEG₆₉₀₀, PEG₇₀₀₀, PEG₇₁₀₀, PEG₇₂₀₀, PEG₇₃₀₀, PEG₇₄₀₀, PEG₇₅₀₀, PEG₇₆₀₀, PEG₇₇₀₀, PEG₇₈₀₀, PEG₇₉₀₀, or PEG₈₀₀₀. In some aspects, the PEG is PEG₅₀₀₀. In some aspects, the PEG is PEG₆₀₀₀. In some aspects, the PEG is PEG₄₀₀₀.

[0160] In some aspects, the PEG is monodisperse, *e.g.*, mPEG₁₀₀, mPEG₂₀₀, mPEG₃₀₀, mPEG₄₀₀, mPEG₅₀₀, mPEG₆₀₀, mPEG₇₀₀, mPEG₈₀₀, mPEG₉₀₀, mPEG₁₀₀₀, mPEG₁₁₀₀, mPEG₁₂₀₀, mPEG₁₃₀₀, mPEG₁₄₀₀, mPEG₁₅₀₀, mPEG₁₆₀₀, mPEG₁₇₀₀, mPEG₁₈₀₀, mPEG₁₉₀₀, mPEG₂₀₀₀, mPEG₂₁₀₀, mPEG₂₂₀₀, mPEG₂₃₀₀, mPEG₂₄₀₀, mPEG₂₅₀₀, mPEG₂₆₀₀, mPEG₂₇₀₀, mPEG₂₈₀₀, mPEG₂₉₀₀, mPEG₃₀₀₀, mPEG₃₁₀₀, mPEG₃₂₀₀, mPEG₃₃₀₀, mPEG₃₄₀₀, mPEG₃₅₀₀, mPEG₃₆₀₀, mPEG₃₇₀₀, mPEG₃₈₀₀, mPEG₃₉₀₀, mPEG₄₀₀₀, mPEG₄₁₀₀, mPEG₄₂₀₀, mPEG₄₃₀₀, mPEG₄₄₀₀, mPEG₄₅₀₀,

mPEG₄₆₀₀, mPEG₄₇₀₀, mPEG₄₈₀₀, mPEG₄₉₀₀, mPEG₅₀₀₀, mPEG₅₁₀₀, mPEG₅₂₀₀, mPEG₅₃₀₀, mPEG₅₄₀₀, mPEG₅₅₀₀, mPEG₅₆₀₀, mPEG₅₇₀₀, mPEG₅₈₀₀, mPEG₅₉₀₀, mPEG₆₀₀₀, mPEG₆₁₀₀, mPEG₆₂₀₀, mPEG₆₃₀₀, mPEG₆₄₀₀, mPEG₆₅₀₀, mPEG₆₆₀₀, mPEG₆₇₀₀, mPEG₆₈₀₀, mPEG₆₉₀₀, mPEG₇₀₀₀, mPEG₇₁₀₀, mPEG₇₂₀₀, mPEG₇₃₀₀, mPEG₇₄₀₀, mPEG₇₅₀₀, mPEG₇₆₀₀, mPEG₇₇₀₀, mPEG₇₈₀₀, mPEG₇₉₀₀, or mPEG₈₀₀₀. In some aspects, the mPEG is mPEG₅₀₀₀. In some aspects, the mPEG is mPEG₆₀₀₀. In some aspects, the mPEG is mPEG₄₀₀₀.

[0161] In some aspects, the water-soluble biopolymer moiety is a polyglycerol (PG) described by the formula $((R^3-O-(CH_2-CHOH-CH_2O)_n-)$ with R^3 being hydrogen, methyl or ethyl, and n having a value from 3 to 200. In some aspects, the water-soluble biopolymer moiety is a branched polyglycerol described by the formula $(R^3-O-(CH_2-CHOR^5-CH_2-O)_n-)$ with R^5 being hydrogen or a linear glycerol chain described by the formula $(R^3-O-(CH_2-CHOH-CH_2-O)_n-)$ and R^3 being hydrogen, methyl or ethyl. In some aspects, the water-soluble biopolymer moiety is a hyperbranched polyglycerol described by the formula $(R^3-O-(CH_2-CHOR^5-CH_2-O)_n-)$ with R^5 being hydrogen or a glycerol chain described by the formula $(R^3-O-(CH_2-CHOR^6-CH_2-O)_n-)$, with R^6 being hydrogen or a glycerol chain described by the formula $(R^3-O-(CH_2-CHOR^7-CH_2-O)_n-)$, with R^7 being hydrogen or a linear glycerol chain described by the formula $(R^3-O-(CH_2-CHOH-CH_2-O)_n-)$ and R^3 being hydrogen, methyl or ethyl. Hyperbranched glycerol and methods for its synthesis are described in Oudshorn et al. (2006) *Biomaterials* 27:5471-5479; Wilms et al. (2010) *Acc. Chem. Res.* 43, 129-41, and references cited therein.

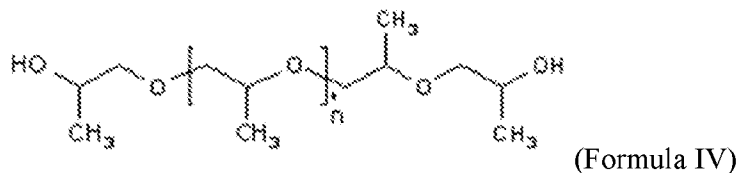
[0162] In certain aspects, the PG has a molar mass between about 1000 g/mol and about 2000 g/mol, between about 2000 g/mol and about 3000 g/mol, between about 3000 g/mol to about 4000 g/mol, between about 4000 g/mol and about 5000 g/mol, between about 5000 g/mol and about 6000 g/mol, between about 6000 g/mol and about 7000 g/mol, or between 7000 g/mol and about 8000 g/mol.

[0163] In some aspects, the PG is PG₁₀₀, PG₂₀₀, PG₃₀₀, PG₄₀₀, PG₅₀₀, PG₆₀₀, PG₇₀₀, PG₈₀₀, PG₉₀₀, PG₁₀₀₀, PG₁₁₀₀, PG₁₂₀₀, PG₁₃₀₀, PG₁₄₀₀, PG₁₅₀₀, PG₁₆₀₀, PG₁₇₀₀, PG₁₈₀₀, PG₁₉₀₀, PG₂₀₀₀, PG₂₁₀₀, PG₂₂₀₀, PG₂₃₀₀, PG₂₄₀₀, PG₂₅₀₀, PG₂₆₀₀, PG₂₇₀₀, PG₂₈₀₀, PG₂₉₀₀, PG₃₀₀₀, PG₃₁₀₀, PG₃₂₀₀, PG₃₃₀₀, PG₃₄₀₀, PG₃₅₀₀, PG₃₆₀₀, PG₃₇₀₀, PG₃₈₀₀, PG₃₉₀₀, PG₄₀₀₀, PG₄₁₀₀, PG₄₂₀₀, PG₄₃₀₀, PG₄₄₀₀, PG₄₅₀₀, PG₄₆₀₀, PG₄₇₀₀, PG₄₈₀₀, PG₄₉₀₀, PG₅₀₀₀, PG₅₁₀₀, PG₅₂₀₀, PG₅₃₀₀, PG₅₄₀₀, PG₅₅₀₀, PG₅₆₀₀, PG₅₇₀₀, PG₅₈₀₀, PG₅₉₀₀, PG₆₀₀₀, PG₆₁₀₀, PG₆₂₀₀, PG₆₃₀₀, PG₆₄₀₀, PG₆₅₀₀, PG₆₆₀₀, PG₆₇₀₀, PG₆₈₀₀, PG₆₉₀₀, PG₇₀₀₀, PG₇₁₀₀, PG₇₂₀₀, PG₇₃₀₀, PG₇₄₀₀,

PG₇₅₀₀, PG₇₆₀₀, PG₇₇₀₀, PG₇₈₀₀, PG₇₉₀₀, or PG₈₀₀₀. In some aspects, the PG is PG₅₀₀₀. In some aspects, the PG is PG₆₀₀₀. In some aspects, the PG is PG₄₀₀₀.

[0164] In some aspects, the PG is monodisperse, *e.g.*, mPG₁₀₀, mPG₂₀₀, mPG₃₀₀, mPG₄₀₀, mPG₅₀₀, mPG₆₀₀, mPG₇₀₀, mPG₈₀₀, mPG₉₀₀, mPG₁₀₀₀, mPG₁₁₀₀, mPG₁₂₀₀, mPG₁₃₀₀, mPG₁₄₀₀, mPG₁₅₀₀, mPG₁₆₀₀, mPG₁₇₀₀, mPG₁₈₀₀, mPG₁₉₀₀, mPG₂₀₀₀, mPG₂₁₀₀, mPG₂₂₀₀, mPG₂₃₀₀, mPG₂₄₀₀, mPG₂₅₀₀, mPG₂₆₀₀, mPG₂₇₀₀, mPG₂₈₀₀, mPG₂₉₀₀, mPG₃₀₀₀, mPG₃₁₀₀, mPG₃₂₀₀, mPG₃₃₀₀, mPG₃₄₀₀, mPG₃₅₀₀, mPG₃₆₀₀, mPG₃₇₀₀, mPG₃₈₀₀, mPG₃₉₀₀, mPG₄₀₀₀, mPG₄₁₀₀, mPG₄₂₀₀, mPG₄₃₀₀, mPG₄₄₀₀, mPG₄₅₀₀, mPG₄₆₀₀, mPG₄₇₀₀, mPG₄₈₀₀, mPG₄₉₀₀, mPG₅₀₀₀, mPG₅₁₀₀, mPG₅₂₀₀, mPG₅₃₀₀, mPG₅₄₀₀, mPG₅₅₀₀, mPG₅₆₀₀, mPG₅₇₀₀, mPG₅₈₀₀, mPG₅₉₀₀, mPG₆₀₀₀, mPG₆₁₀₀, mPG₆₂₀₀, mPG₆₃₀₀, mPG₆₄₀₀, mPG₆₅₀₀, mPG₆₆₀₀, mPG₆₇₀₀, mPG₆₈₀₀, mPG₆₉₀₀, mPG₇₀₀₀, mPG₇₁₀₀, mPG₇₂₀₀, mPG₇₃₀₀, mPG₇₄₀₀, mPG₇₅₀₀, mPG₇₆₀₀, mPG₇₇₀₀, mPG₇₈₀₀, mPG₇₉₀₀, or mPG₈₀₀₀.

[0165] In some aspects, the water-soluble biopolymer comprises poly(propylene glycol) ("PPG"). In some aspects, PPG is characterized by the following formula, with n having a value from 1 to 1000.



[0166] In some aspects, the n of the PPG has a value of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or 200.

[0167] In some aspects, n of the PPG is at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, at least about 150, at least about 160, at least about 170, at least about 180, at least about 190, at

least about 200, at least about 210, at least about 220, at least about 230, at least about 240, at least about 250, at least about 260, at least about 270, at least about 280, at least about 290, at least about 300, at least about 310, at least about 320, at least about 330, at least about 340, at least about 350, at least about 360, at least about 370, at least about 380, at least about 390, at least about 400, at least about 410, at least about 420, at least about 430, at least about 440, at least about 450, at least about 460, at least about 470, at least about 480, at least about 490, at least about 500, at least about 510, at least about 520, at least about 530, at least about 540, at least about 550, at least about 560, at least about 670, at least about 580, at least about 590, at least about 600, at least about 610, at least about 620, at least about 630, at least about 640, at least about 650, at least about 660, at least about 670, at least about 680, at least about 690, at least about 700, at least about 710, at least about 720, at least about 730, at least about 740, at least about 750, at least about 760, at least about 770, at least about 780, at least about 790, at least about 800, at least about 810, at least about 820, at least about 830, at least about 840, at least about 850, at least about 860, at least about 870, at least about 880, at least about 890, at least about 900, at least about 910, at least about 920, at least about 930, at least about 940, at least about 950, at least about 960, at least about 970, at least about 980, at least about 990, or about 1000.

[0168] In some aspects, the n of the PPG is between about 50 and about 100, between about 100 and about 150, between about 150 and about 200, between about 200 and about 250, between about 250 and about 300, between about 300 and about 350, between about 350 and about 400, between about 400 and about 450, between about 450 and about 500, between about 500 and about 550, between about 550 and about 600, between about 600 and about 650, between about 650 and about 700, between about 700 and about 750, between about 750 and about 800, between about 800 and about 850, between about 850 and about 900, between about 900 and about 950, or between about 950 and about 1000.

[0169] In some aspects, the n of the PPG is at least about 80, at least about 81, at least about 82, at least about 83, at least about 84, at least about 85, at least about 86, at least about 87, at least about 88, at least about 89, at least about 90, at least about 91, at least about 92, at least about 93, at least about 94, at least about 95, at least about 96, at least about 97, at least about 98, at least about 99, at least about 100, at least about 101, at least about 102, at least about 103, at least about 104, at least about 105, at least about 106, at least about 107, at least about 108, at least about 109, at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125,

at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, at least about 141, at least about 142, at least about 143, at least about 144, at least about 145, at least about 146, at least about 147, at least about 148, at least about 149, at least about 150, at least about 151, at least about 152, at least about 153, at least about 154, at least about 155, at least about 156, at least about 157, at least about 158, at least about 159, or at least about 160.

[0170] In some aspects, the n of the PPG is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 130 to about 140, about 140 to about 150, about 150 to about 160, about 85 to about 95, about 95 to about 105, about 105 to about 115, about 115 to about 125, about 125 to about 135, about 135 to about 145, about 145 to about 155, about 155 to about 165, about 80 to about 100, about 100 to about 120, about 120 to about 140, about 140 to about 160, about 85 to about 105, about 105 to about 125, about 125 to about 145, or about 145 to about 165.

[0171] Thus, in some aspects, the PPG is a branched PPG. Branched PPGs have three to ten PPG chains emanating from a central core group. In certain aspects, the PPG moiety is a monodisperse polyethylene glycol. In the context of the present disclosure, a monodisperse polyethylene glycol (mdPPG) is a PPG that has a single, defined chain length and molecular weight. mdPEGs are typically generated by separation from the polymerization mixture by chromatography. In certain formulae, a monodisperse PPG moiety is assigned the abbreviation mdPPG.

[0172] In some aspects, the PPG is a Star PPG. Star PPGs have 10 to 100 PPG chains emanating from a central core group. In some aspects, the PPG is a Comb PPGs. Comb PPGs have multiple PPG chains normally grafted onto a polymer backbone.

[0173] In certain aspects, the PPG has a molar mass between about 1000 g/mol and about 2000 g/mol, between about 2000 g/mol and about 3000 g/mol, between about 3000 g/mol to about 4000 g/mol, between about 4000 g/mol and about 5000 g/mol, between about 5000 g/mol and about 6000 g/mol, between about 6000 g/mol and about 7000 g/mol, or between 7000 g/mol and about 8000 g/mol.

[0174] In some aspects, the PPG is PPG₁₀₀, PPG₂₀₀, PPG₃₀₀, PPG₄₀₀, PPG₅₀₀, PPG₆₀₀, PPG₇₀₀, PPG₈₀₀, PPG₉₀₀, PPG₁₀₀₀, PPG₁₁₀₀, PPG₁₂₀₀, PPG₁₃₀₀, PPG₁₄₀₀, PPG₁₅₀₀, PPG₁₆₀₀, PPG₁₇₀₀, PPG₁₈₀₀, PPG₁₉₀₀, PPG₂₀₀₀, PPG₂₁₀₀, PPG₂₂₀₀, PPG₂₃₀₀, PPG₂₄₀₀, PPG₂₅₀₀, PPG₁₆₀₀, PPG₁₇₀₀, PPG₁₈₀₀, PPG₁₉₀₀, PPG₂₀₀₀, PPG₂₁₀₀, PPG₂₂₀₀, PPG₂₃₀₀, PPG₂₄₀₀, PPG₂₅₀₀, PPG₂₆₀₀, PPG₂₇₀₀, PPG₂₈₀₀, PPG₂₉₀₀, PPG₃₀₀₀,

PPG₃₁₀₀, PPG₃₂₀₀, PPG₃₃₀₀, PPG₃₄₀₀, PPG₃₅₀₀, PPG₃₆₀₀, PPG₃₇₀₀, PPG₃₈₀₀, PPG₃₉₀₀, PPG₄₀₀₀, PPG₄₁₀₀, PPG₄₂₀₀, PPG₄₃₀₀, PPG₄₄₀₀, PPG₄₅₀₀, PPG₄₆₀₀, PPG₄₇₀₀, PPG₄₈₀₀, PPG₄₉₀₀, PPG₅₀₀₀, PPG₅₁₀₀, PPG₅₂₀₀, PPG₅₃₀₀, PPG₅₄₀₀, PPG₅₅₀₀, PPG₅₆₀₀, PPG₅₇₀₀, PPG₅₈₀₀, PPG₅₉₀₀, PPG₆₀₀₀, PPG₆₁₀₀, PPG₆₂₀₀, PPG₆₃₀₀, PPG₆₄₀₀, PPG₆₅₀₀, PPG₆₆₀₀, PPG₆₇₀₀, PPG₆₈₀₀, PPG₆₉₀₀, PPG₇₀₀₀, PPG₇₁₀₀, PPG₇₂₀₀, PPG₇₃₀₀, PPG₇₄₀₀, PPG₇₅₀₀, PPG₇₆₀₀, PPG₇₇₀₀, PPG₇₈₀₀, PPG₇₉₀₀, or PPG₈₀₀₀. In some aspects, the PPG is PPG₅₀₀₀. In some aspects, the PPG is PPG₆₀₀₀. In some aspects, the PPG is PPG₄₀₀₀.

[0175] In some aspects, the PPG is monodisperse, *e.g.*, mPPG₁₀₀, mPPG₂₀₀, mPPG₃₀₀, mPPG₄₀₀, mPPG₅₀₀, mPPG₆₀₀, mPPG₇₀₀, mPPG₈₀₀, mPPG₉₀₀, mPPG₁₀₀₀, mPPG₁₁₀₀, mPPG₁₂₀₀, mPPG₁₃₀₀, mPPG₁₄₀₀, mPPG₁₅₀₀, mPPG₁₆₀₀, mPPG₁₇₀₀, mPPG₁₈₀₀, mPPG₁₉₀₀, mPPG₂₀₀₀, mPPG₂₁₀₀, mPPG₂₂₀₀, mPPG₂₃₀₀, mPPG₂₄₀₀, mPPG₂₅₀₀, mPPG₁₆₀₀, mPPG₁₇₀₀, mPPG₁₈₀₀, mPPG₁₉₀₀, mPPG₂₀₀₀, mPPG₂₁₀₀, mPPG₂₂₀₀, mPPG₂₃₀₀, mPPG₂₄₀₀, mPPG₂₅₀₀, mPPG₂₆₀₀, mPPG₂₇₀₀, mPPG₂₈₀₀, mPPG₂₉₀₀, mPPG₃₀₀₀, mPPG₃₁₀₀, mPPG₃₂₀₀, mPPG₃₃₀₀, mPPG₃₄₀₀, mPPG₃₅₀₀, mPPG₃₆₀₀, mPPG₃₇₀₀, mPPG₃₈₀₀, mPPG₃₉₀₀, mPPG₄₀₀₀, mPPG₄₁₀₀, mPPG₄₂₀₀, mPPG₄₃₀₀, mPPG₄₄₀₀, mPPG₄₅₀₀, mPPG₄₆₀₀, mPPG₄₇₀₀, mPPG₄₈₀₀, mPPG₄₉₀₀, mPPG₅₀₀₀, mPPG₅₁₀₀, mPPG₅₂₀₀, mPPG₅₃₀₀, mPPG₅₄₀₀, mPPG₅₅₀₀, mPPG₅₆₀₀, mPPG₅₇₀₀, mPPG₅₈₀₀, mPPG₅₉₀₀, mPPG₆₀₀₀, mPPG₆₁₀₀, mPPG₆₂₀₀, mPPG₆₃₀₀, mPPG₆₄₀₀, mPPG₆₅₀₀, mPPG₆₆₀₀, mPPG₆₇₀₀, mPPG₆₈₀₀, mPPG₆₉₀₀, mPPG₇₀₀₀, mPPG₇₁₀₀, mPPG₇₂₀₀, mPPG₇₃₀₀, mPPG₇₄₀₀, mPPG₇₅₀₀, mPPG₇₆₀₀, mPPG₇₇₀₀, mPPG₇₈₀₀, mPPG₇₉₀₀, or mPPG₈₀₀₀. In some aspects, the mPPG is mPPG₅₀₀₀. In some aspects, the mPPG is mPPG₆₀₀₀. In some aspects, the mPPG is mPPG₄₀₀₀.

b. Cationic carrier

[0176] In some aspects, the cationic carrier units of the present disclosure comprise at least one cationic carrier moiety. The term "cationic carrier" refers to a moiety or portion of a cationic carrier unit of the present disclosure comprising a plurality of positive charges that can interact and bind electrostatically an anionic payload (or an anionic carrier attached to a payload). In some aspects, the number of positive charges or positively charged groups on the cationic carrier is similar to the number of negative charges or negatively charged groups on the anionic payload (or an anionic carrier attached to a payload). In some aspects, the cationic carrier comprises a biopolymer, *e.g.*, a peptide (*e.g.*, a polylysine).

[0177] In some aspects, the cationic carrier comprises one or more basic amino acids (*e.g.*, lysine, arginine, histidine, or a combination thereof). In some aspects, the cationic carrier comprises at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least

about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, at least about 35, at least about 36, at least about 37, at least about 38, at least about 39, at least about 40, at least about 41, at least about 42, at least about 43, at least about 44, at least about 45, at least about 46, at least about 47, at least about 48, at least about 49, at least about 50, at least about 51, at least about 52, at least about 53, at least about 54, at least about 55, at least about 56, at least about 57, at least about 58, at least about 59, at least about 60, at least about 61, at least about 62, at least about 63, at least about 64, at least about 65, at least about 66, at least about 67, at least about 68, at least about 69, at least about 70, at least about 71, at least about 72, at least about 73, at least about 74, at least about 75, at least about 76, at least about 77, at least about 78, at least about 79, at least about 80 basic amino acids, *e.g.*, lysines, arginines, or combinations thereof.

[0178] In some aspects, the cationic carrier unit comprises at least about 40 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 45 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 50 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 55 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 60 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 65 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 70 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 75 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 80 basic amino acids, *e.g.*, lysines.

[0179] In some aspects, the cationic carrier unit comprises about 30 to about 1000, about 30 to about 900, about 30 to about 800, about 30 to about 700, about 30 to about 600, about 30 to about 500, about 30 to about 400, about 30 to about 300, about 30 to about 200, about 30 to about 100, about 40 to about 1000, about 40 to about 900, about 40 to about 800, about 40 to about 700, about 40 to about 600, about 40 to about 500, about 40 to about 400, about 40 to about 300, about 40 to about 200, or about 40 to about 100 basic amino acids, *e.g.*, lysines. In some aspects, the basic amino acids, *e.g.*, lysines, are not modified such that they possess -NH_3^+ (*e.g.*, positive charge).

[0180] In some aspects, the cationic carrier unit comprises about 30 to about 100, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 30 to about 40, about 40 to about 100, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 70 to about 80, about 75 to about 85, about 65 to about 75, about 65 to about 80, about 60 to about 85, or about 40 to about 500 basic amino acids, *e.g.*, lysines.

[0181] In some aspects, the cationic carrier unit comprises about 100 to about 1000, about 100 to about 900, about 100 to about 800, about 100 to about 700, about 100 to about 600, about 100 to about 500, about 100 to about 400, about 100 to about 300, about 100 to about 200, about 200 to about 1000, about 200 to about 900, about 200 to about 800, about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 1000, about 300 to about 900, about 300 to about 800, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 1000, about 400 to about 900, about 400 to about 800, about 400 to about 700, about 400 to about 600, about 400 to about 500, about 500 to about 1000, about 500 to about 900, about 500 to about 800, about 500 to about 700, about 500 to about 600, about 600 to about 1000, about 600 to about 900, about 600 to about 800, about 600 to about 700, about 700 to about 1000, about 700 to about 900, about 700 to about 800, about 800 to about 1000, about 800 to about 900, or about 900 to about 1000 basic amino acids, *e.g.*, lysines.

[0182] In some aspects, the number of basic amino acids, *e.g.*, lysines, arginines, histidines, or combinations thereof, can be adjusted based on the length of the anionic payload. For example, an anionic payload with a longer sequence can be paired with higher number of basic amino acids, *e.g.*, lysines. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit can be calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, *e.g.*, mRNA (N/P ratio) is at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, or at least about 20. In some aspects, the molar ratio of protonated amine in polymer to phosphate in an anionic payload, *e.g.*, mRNA (N/P ratio) is between about 1 to about 20, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in

polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 1 to about 10, *e.g.*, about 3 to about 4, about 4 to about 5, about 5 to about 6, about 6 to about 7, or about 7 to about 8. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 1 to about 2. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 3 to about 4. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 2 to about 3. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 4 to about 5. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 5 to about 6. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 6 to about 7. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 7 to about 8. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 8 to about 9. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., mRNA (N/P ratio) is about 9 to about 10.

[0183] A person of skill in the art would understand that since a role of the cationic carrier moiety is to neutralize negative charges on the payload (*e.g.*, negative charges in the phosphate backbone of an mRNA) via electrostatic interaction, in some aspects (*e.g.*, when the payload is a nucleic acid such as an antimir), the length of the cationic carrier, number of positively charged groups on the cationic carrier, and distribution and orientation of charges present on the cationic carrier will depend on the length and charge distribution on the payload molecule.

[0184] In some aspects, the cationic carrier comprises between about 5 and about 10, between about 10 and about 15, between about 15 and about 20, between about 20 and about 25,

between about 25 and about 30, between about 30 and about 35, between about 35 and about 40, between about 40 and about 45, between about 45 and about 50, between about 50 and about 55, between about 55 and about 60, between about 60 and about 65, between about and about 70, between about 70 and about 75, or between about 75 and about 80 basic amino acids. In some specific aspects, the positively charged carrier comprises between 30 and about 50 basic amino acids. In some specific aspects, the positively charged carrier comprises between 70 and about 80 basic amino acids.

[0185] In some aspects, the basic amino acid comprises arginine, lysine, histidine, or any combination thereof. In some aspects, the basic amino acid is a D-amino acid. In some aspects, the basic amino acid is an L-amino acid. In some aspects, the positively charged carrier comprises D-amino acids and L-amino acids. In some aspects, the basic amino comprises at least one unnatural amino acid or a derivative thereof. In some aspects, the basic amino acid is arginine, lysine, histidine, L-4-aminomethyl-phenylalanine, L-4-guanidine-phenylalanine, L-4-aminomethyl-N-isopropyl-phenylalanine, L-3-pyridyl-alanine, L-trans-4-aminomethylcyclohexyl-alanine, L-4-piperidinyl-alanine, L-4-aminocyclohexyl-alanine, 4-guanidinobutyric acid, L-2-amino-3-guanidinopropionic acid, DL-5-hydroxylysine, pyrrolysine, 5-hydroxy-L-lysine, methyllysine, hypusine, or any combination thereof. In a particular aspect, the positively charged carrier comprises about 40 lysines. In a particular aspect, the positively charged carrier comprises about 50 lysines. . In a particular aspect, the positively charged carrier comprises about 60 lysines. . In a particular aspect, the positively charged carrier comprises about 70 lysines. In a particular aspect, the positively charged carrier comprises about 80 lysines.

[0186] In other aspects, the cationic carrier comprises a polymer or copolymer comprising at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 11, at least 12, at least 13, at least 14, at last 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at least 58, at least 59, at least 60, at least 61, at least 62, at least 63, at least 64, at least 65, at least 66, at least 67, at least 68, at least 69, at least 70, at least 71, at least 72, at least 73, at least 74, at least 75, at least 76, at least 77, at least 78, at least 79, or at least 80 cationic groups (*e.g.*, amino groups). In some aspects, the cationic carrier comprises a polymer or copolymer comprising between about 5 and about 10 cationic

groups, between about 10 and about 15 cationic groups, between about 15 and about 20 cationic groups, between about 20 and about 25 cationic groups, between about 25 and about 30 cationic groups, between about 30 and about 35 cationic groups, between about 35 and about 40 cationic groups, between about 40 and about 45 cationic groups, between about 45 and about 50 cationic groups, between about 50 and about 55 cationic groups, between about 55 and about 60 cationic groups, between about 60 and about 65 cationic groups, between about 65 and about 70 cationic groups, between about 70 and about 75 cationic groups, or between about 45 and about 50 cationic groups (*e.g.*, amino groups). In some specific aspects, the cationic carrier comprises a polymer or copolymer comprising between 30 and about 50 cationic groups (*e.g.*, amino groups). In some specific aspects, the cationic carrier comprises a polymer or copolymer comprising between 70 and about 80 cationic groups (*e.g.*, amino groups). In some aspects, the polymer or copolymer is an acrylate, a polyalcohol, or a polysaccharide.

[0187] In some aspects, the cationic carrier moiety binds to a single payload molecule. In other aspects, a cationic carrier moiety can bind to multiple payload molecules, which may be identical or different.

[0188] In some aspects, the positive charges of the cationic carrier moiety and negative charges of a nucleic acid payload are at an ionic ratio of about 20:1, about 19:1, about 18:1, about 17:1, about 16:1, about 15:1, about 14:1, about 13:1, about 12:1, about 11:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1. In some aspects, the negative charges of a nucleic acid payload and the positive charges of the cationic carrier moiety are at an ionic ratio of about 20:1, about 19:1, about 18:1, about 17:1, about 16:1, about 15:1, about 14:1, about 13:1, about 12:1, about 11:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1.

[0189] In some aspects, the anionic payload comprises a nucleotide sequence having about 10 to about 1000 (*e.g.*, about 100 to about 1000) in length, wherein the N/P ratio of the cationic carrier moiety and the anionic payload is about 2 to about 10, *e.g.*, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, *e.g.*, *e.g.*, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10. In some aspects, an N/P ratio of the cationic carrier moiety and the anionic payload of about 10 to about 1000 nucleotides in length is between about 1 and about 10, *e.g.*, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10.

[0190] In some aspects, the anionic payload comprises a nucleotide sequence having about 1000 to about 2000 in length, wherein the N/P ratio of the cationic carrier moiety and the anionic

payload is about 3 to about 12, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, or about 12. In some aspects, the N/P ratio of the cationic carrier moiety and the anionic payload is between about 4 and about 7, e.g., about 4, about 5, about 6, or about 7.

[0191] In some aspects, the anionic payload comprises a nucleotide sequence having about 2000 to about 3000 in length, wherein the N/P ratio of the cationic carrier moiety and the anionic payload is about 3 to about 16, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16. In some aspects, wherein the N/P ratio of the cationic carrier moiety and the anionic payload is between about 6 and about 9, e.g., about 6, about 7, about 8, or about 9.

[0192] In some aspects, the anionic payload comprises a nucleotide sequence having about 3000 to about 4000 in length, wherein the N/P ratio of the cationic carrier moiety and the anionic payload is about 3 to about 20, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20. In some aspects, wherein the N/P ratio of the cationic carrier moiety and the anionic payload is between about 7 and about 10, e.g., about 7, about 8, about 9, or about 10.

[0193] In some aspects, the cationic carrier moiety has a free terminus wherein the end group is a reactive group. In some aspects, the cationic carrier moiety has a free terminus (e.g., the C-terminus in a poly-lysine cationic carrier moiety) wherein the end group is an amino (-NH₂) group. In some aspects, the cationic carrier moiety has a free terminus wherein the end group is an sulfhydryl group. In some aspects, the reactive group of the cationic carrier moiety is attached to a hydrophobic moiety, e.g., a vitamin B3 hydrophobic moiety.

c. Crosslinking Moiety

[0194] In some aspects, the cationic carrier units of the present disclosure comprise at least one crosslinking moiety. The term "crosslinking moiety" refers to a moiety or portion of a polymer block comprising a plurality of agents that are capable of forming crosslinks. In some aspects, the number of agents that are capable of forming crosslinks comprises an amino acid with a side chain of a crosslinking agent. In some aspects, the CM comprises a biopolymer, e.g., a peptide (e.g., a polylysine) linked to a crosslinking agent.

[0195] In some aspects, the crosslinking moiety comprises one or more amino acids (e.g., lysine, arginine, histidine, or a combination thereof). In some aspects, the crosslinking moiety comprises at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least

about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, at least about 35, at least about 36, at least about 37, at least about 38, at least about 39, at least about 40, at least about 41, at least about 42, at least about 43, at least about 44, at least about 45, at least about 46, at least about 47, at least about 48, at least about 49, or at least about 50 amino acids, *e.g.*, lysines, arginines, or combinations thereof, each of which is linked to a crosslinking agent.

[0196] In some aspects, the crosslinking moiety comprises at least about 10 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 11 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 12 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 13 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 14 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 15 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 16 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 17 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 18 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 19 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 20 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent.

[0197] In some aspects, a crosslinking agent is a thiol. In some aspects, a crosslinking agent is a thiol derivative.

d. Hydrophobic moiety

[0198] In some aspects, the cationic carrier units of the present disclosure comprise at least one hydrophobic moiety. The term "hydrophobic moiety", as used herein, refers to a molecular entity that can, *e.g.*, (i) complement the therapeutic or prophylactic activity of the payload, (ii)

modulate the therapeutic or prophylactic activity of the payload, (iii) function as a therapeutic and/or prophylactic agent in the target tissue or target cells, (iv) facilitate the transport of the cationic carrier unit across a physiological barrier, *e.g.*, the BBB and/or the plasma membrane, (v) improve the homeostasis of the target tissue or target cell, (vi) contribute positively charged groups to the cationic carried moiety, or (vii) any combination thereof.

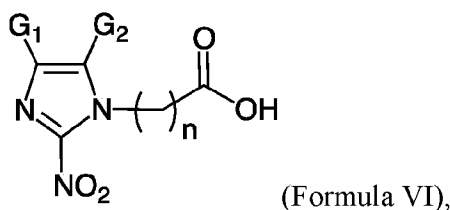
[0199] In some aspects, the hydrophobic moiety is capable of modulating, *e.g.*, an immune response, an inflammatory response, or a tissue microenvironment.

[0200] In some aspects, a hydrophobic moiety capable of modulating an immune response can comprise, *e.g.*, tyrosine or dopamine. Tyrosine can be transformed into L-DOPA, and then be converted to dopamine via 2-step enzymatic reaction. Normally, dopamine levels are low in the Parkinson's disease patients. Therefore, in some aspects, tyrosine is a hydrophobic moiety in cationic carrier units used for the treatment of Parkinson's disease. Tryptophan can be converted to serotonin, a neurotransmitter thought to play a role in appetite, emotions, and motor, cognitive, and autonomic functions. Accordingly, in some aspects, cationic carrier units of the present disclosure used for the treatment of disease or conditions related to low serotonin levels comprise tryptophan as a hydrophobic moiety.

[0201] In some aspects, a hydrophobic moiety can modulate a tumor microenvironment in a subject with a tumor, for example, by inhibiting or reducing hypoxia in the tumor microenvironment.

[0202] In some aspects, the hydrophobic moiety comprises, *e.g.*, an amino acid linked to an imidazole derivative, a vitamin, or any combination thereof.

[0203] In some aspects, the hydrophobic moiety comprises an amino acid (*e.g.*, lysine) linked to an imidazole derivative comprising:

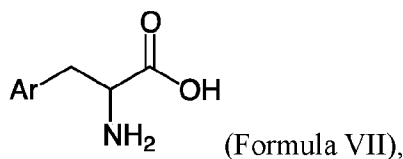


wherein each of G_1 and G_2 is independently H, an aromatic ring, or 1-10 alkyl, or G_1 and G_2 together form an aromatic ring, and wherein n is 1-10.

[0204] In some aspects, the hydrophobic moiety comprises an amino acid (*e.g.*, lysine) linked to nitroimidazole. Nitroimidazoles function as antibiotics. Nitroheterocycles in

nitroimidazoles can be reductively activated in hypoxic cells, and then undergo redox recycling or decompose to cytotoxic products. Reduction usually happens only in anaerobic bacteria or in anoxic tissues, therefore, they have relative little effect upon human cells or aerobic bacteria. In some aspects, the hydrophobic moiety comprises an amino acid (e.g., lysine) linked to metronidazole, tinidazole, nimorazole, dimetridazole, pretomanid, ornidazole, megazol, azanidazole, benznidazole, nitroimidazole, or any combination thereof.

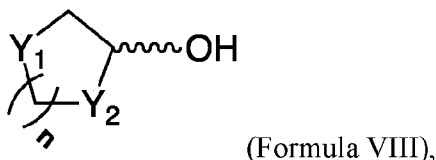
[0205] In some aspects, the hydrophobic moiety comprises



wherein Ar is or , and
wherein each of Z1 and Z2 is H or OH.

[0206] In some aspects, the hydrophobic moiety is capable of inhibiting or reducing an inflammatory response.

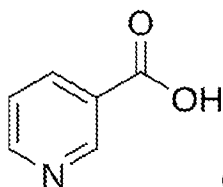
[0207] In some aspects, the hydrophobic moiety is an amino acid (e.g., lysine) linked to a vitamin. In some aspects, the vitamin comprises a cyclic ring or cyclic hetero atom ring and a carboxyl group or hydroxyl group. In some aspects, the vitamin comprises:



wherein each of Y1 and Y2 is C, N, O, or S, and wherein n is 1 or 2.

[0208] In some aspects, the vitamin is selected from the group consisting of vitamin A (retinol), vitamin B1 (Thiamine Chloride), vitamin B2 (Riboflavin), vitamin B3 (Niacinamide), vitamin B6 (Pyridoxal), vitamin B7 (Biotin), vitamin B9 (Folic acid), vitamin B12 (Cobalamin), vitamin C (Ascorbic acid), vitamin D2, vitamin D3, vitamin E (Tocopherol), vitamin M, vitamin H, a derivative thereof, and any combination thereof.

[0209] In some aspects, the vitamin is vitamin B3 (also known as niacin or nicotinic acid).



(Formula IX)

[0210] In some aspects, the hydrophobic moiety comprises at least about one, at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, or at least about 20 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 1 amino acid (e.g., lysine), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 2 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 3 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 4 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 5 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 6 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 7 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 8 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 9 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 10 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 11 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 12 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 13 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 14 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 15 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 16 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 17 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 18 amino acids (e.g., lysines), each of which is linked to

vitamin B3. In some aspects, the hydrophobic moiety comprises about 19 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the hydrophobic moiety comprises about 20 amino acids (e.g., lysines), each of which is linked to vitamin B3..

[0211] In some aspects the hydrophobic moiety comprises from about 1 to about 10 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 5 to about 10 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 10 to about 15 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 15 to about 20 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 1 to about 20 vitamin amino acids (e.g., lysines), each of which is linked to B3, about 1 to about 15 vitamin amino acids (e.g., lysines), each of which is linked to B3, about 1 to about 10 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 1 to about 5 amino acids (e.g., lysines), each of which is linked to vitamin B3

[0212] Niacin is a precursor of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in vivo. NAD converts to NADP by phosphorylation in the presence of the enzyme NAD⁺ kinase. NADP and NAD are coenzymes for many dehydrogenases, participating in many hydrogen transfer processes. NAD is important in catabolism of fat, carbohydrate, protein, and alcohol, as well as cell signaling and DNA repair, and NADP mostly in anabolism reactions such as fatty acid and cholesterol synthesis. High-energy requirements (brain) or high turnover rate (gut, skin) organs are usually the most susceptible to their deficiency.

[0213] Niacin produces marked anti-inflammatory effects in a variety of tissues – including the brain, gastrointestinal tract, skin, and vascular tissue – through the activation of NIACR1. Niacin has been shown to attenuate neuroinflammation and may have efficacy in treating neuroimmune disorders such as multiple sclerosis and Parkinson's disease. See Offermanns & Schwaninger (2015) Trends in Molecular Medicine 21:245-266; Chai et al (2013) Current Atherosclerosis Reports 15:325; Graff et al. (2016) Metabolism 65:102-13; and Wakade & Chong (2014) Journal of the Neurological Sciences 347:34-8, which are herein incorporated by reference in their entireties.

e. Targeting moiety

[0214] In some aspects, the cationic carrier unit comprises a targeting moiety, which is linked to the water-soluble polymer optionally via a linker. As used herein, the term "targeting moiety" refers to a biorecognition molecule that binds to a specific biological substance or site. In some aspects, the targeting moiety is specific for a certain target molecule (e.g., a ligand targeting

a receptor, or an antibody targeting a surface protein), tissue (*e.g.*, a molecule that would preferentially carry the micelle to a specific organ or tissue, *e.g.*, liver, brain, or endothelium), or facilitate transport through a physiological barrier (*e.g.*, a peptide or other molecule that may facilitate transport across the brain blood barrier or plasma membrane).

[0215] For targeting a payload (*e.g.*, a nucleotide molecule, *e.g.*, an mRNA) according to the present disclosure, a targeting moiety can be coupled to a cationic carrier unit, and therefore, to the external surface of a micelle, whereas the micelle has the payload entrapped within its core.

[0216] In some aspects, the targeting moiety is a targeting moiety capable of targeting the micelle of the present disclosure to a tissue. In some aspects, the tissue is liver, brain, kidney, lung, ovary, pancreas, thyroid, breast, stomach, or any combination thereof. In some aspects, the tissue is cancer tissue, *e.g.*, liver cancer, brain cancer, kidney cancer, lung cancer, ovary cancer, pancreas cancer, thyroid cancer, breast cancer, stomach cancer, or any combination thereof.

[0217] In a specific aspect, the tissue is liver. In a specific aspect, the targeting moiety targeting liver is cholesterol. In other aspects, the targeting moiety targeting liver is a ligand that binds an asialoglycoprotein receptor-targeting moiety. In some aspects, the asialoglycoprotein receptor-targeting moiety comprises a GalNAc cluster. In some aspects, the GalNAc cluster is a monovalent, divalent, trivalent, or tetravalent GalNAc cluster.

[0218] In another aspect, the tissue is pancreas. In some aspects, the targeting moiety targeting pancreas comprises a ligand targeting $\alpha v \beta 3$ integrin receptors on pancreatic cells. In some aspects, the targeting moiety comprises an arginylglycylaspartic acid (RGD) peptide sequence (L-Arginyl-Glycyl-L-Aspartic acid; Arg-Gly-Asp).

[0219] In some aspects, the tissue is a tissue in the central nervous system, *e.g.*, neural tissue. In some aspects, the targeting moiety targeting the central nervous system is capable being transported by Large-neutral Amino Acid Transporter 1 (LAT1). LAT1 (SLC7A5) is a transporter for both the uptake of large neutral amino acids and a number of pharmaceutical drugs. LAT1 can transport drugs such as L-dopa or gabapentin.

[0220] In some aspects, a targeting moiety comprises glucose, *e.g.*, D-glucose, which can bind to Glucose transporter 1 (or GLUT1) and cross BBB. GLUT1, also known as solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1), is a uniporter protein that in humans is encoded by the SLC2A1 gene. GLUT1 facilitates the transport of glucose across the plasma membranes of mammalian cells. This gene encodes a major glucose transporter in the mammalian blood-brain barrier.

[0221] In some aspects, a targeting moiety comprises galactose, e.g., D-galactose, which can bind to GLUT1 transporter to cross BBB. In some aspects, a targeting moiety comprises glutamic acid, which can bind to acetylcholinesterase inhibitor (AChEI) and/or EAATs inhibitors and cross BBB. Acetylcholinesterase is the enzyme that is the primary member of the cholinesterase enzyme family. An acetylcholinesterase inhibitor (AChEI) is the inhibitor that inhibits acetylcholinesterase from breaking down acetylcholine into choline and acetate, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine in the central nervous system, autonomic ganglia and neuromuscular junctions, which are rich in acetylcholine receptors. Acetylcholinesterase inhibitors are one of two types of cholinesterase inhibitors; the other being butyryl-cholinesterase inhibitors.

[0222] In some aspects, the tissue targeted by a targeting moiety is a skeletal muscle. In some aspects, the targeting moiety targeting skeletal muscle is capable being transported by Large-neutral Amino Acid Transporter 1 (LAT1).

[0223] It is expressed in numerous cell types including T-cells, cancer cells and brain endothelial cells. LAT1 is consistently expressed at high levels in brain microvessel endothelial cells. Being a solute carrier located primarily in the BBB, targeting the micelles of the present disclosure to LAT1 allows delivery through the BBB. In some aspects, the targeting moiety targeting a micelle of the present disclosure to the LAT1 transporter is an amino acid, e.g., a branched-chain or aromatic amino acid. In some aspects, the amino acid is valine, leucine, and/or isoleucine. In some aspects, the amino acid is tryptophan and/or tyrosine. In some aspects, the amino acid is tryptophan. In other aspects, the amino acid is tyrosine.

[0224] In some aspects, the targeting moiety is a LAT1 ligand selected from tryptophan, tyrosine, phenylalanine, tryptophan, methionine, thyroxine, melphalan, L-DOPA, gabapentin, 3,5-I-diiodotyrosine, 3-iodo-I-tyrosine, fenclonine, acivicin, leucine, BCH, methionine, histidine, valine, or any combination thereof.

[0225] See Singh & Ecker (2018) "Insights into the Structure, Function, and Ligand Discovery of the Large Neutral Amino Acid Transporter 1, LAT1," *Int. J. Mol. Sci.* 19:1278; Geier et al. (2013) "Structure-based ligand discovery for the Large-neutral Amino Acid Transporter 1, LAT-1," *Proc. Natl. Acad. Sci. USA* 110:5480-85; and Chien et al. (2018) "Reevaluating the Substrate Specificity of the L-type Amino Acid Transporter (LAT1)," *J. Med. Chem.* 61:7358-73, which are herein incorporated by reference in their entireties.

[0226] Non-limiting examples of targeting moieties are described below.

i. Ligands

[0227] A ligand functions as a type of targeting moiety defined as a selectively bindable material that has a selective (or specific), affinity for another substance. The ligand is recognized and bound by a usually, but not necessarily, larger specific binding body or "binding partner," or "receptor." Examples of ligands suitable for targeting are antigens, haptens, biotin, biotin derivatives, lectins, galactosamine and fucosylamine moieties, receptors, substrates, coenzymes and cofactors among others.

[0228] When applied to the micelles of the present disclosure a ligand includes an antigen or hapten that is capable of being bound by, or to, its corresponding antibody or fraction thereof. Also included are viral antigens or hemagglutinins and neuraminidases and nucleocapsids including those from any DNA and RNA viruses, AIDS, HIV and hepatitis viruses, adenoviruses, alphaviruses, arenaviruses, coronaviruses, flaviviruses, herpesviruses, myxoviruses, oncornaviruses, papovaviruses, paramyxoviruses, parvoviruses, picornaviruses, poxviruses, reoviruses, rhabdoviruses, rhinoviruses, togaviruses and viroids; any bacterial antigens including those of gram-negative and gram-positive bacteria, *Acinetobacter*, *Achromobacter*, *Bacteroides*, *Clostridium*, *Chlamydia*, enterobacteria, *Haemophilus*, *Lactobacillus*, *Neisseria*, *Staphylococcus*, or *Streptococcus*; any fungal antigens including those of *Aspergillus*, *Candida*, *Coccidioides*, mycoses, phycomycetes, and yeasts; any mycoplasma antigens; any rickettsial antigens; any protozoan antigens; any parasite antigens; any human antigens including those of blood cells, virus infected cells, genetic markers, heart diseases, oncoproteins, plasma proteins, complement factors, rheumatoid factors. Included are cancer and tumor antigens such as alpha-fetoproteins, prostate specific antigen (PSA) and CEA, cancer markers and oncoproteins, among others.

[0229] Other substances that can function as ligands for targeting a micelle of the present disclosure are certain vitamins (i.e. folic acid, B₁₂), steroids, prostaglandins, carbohydrates, lipids, antibiotics, drugs, digoxins, pesticides, narcotics, neuro-transmitters, and substances used or modified such that they function as ligands.

[0230] In some aspects, the targeting moiety comprises a protein or protein fragment (e.g., hormones, toxins), and synthetic or natural polypeptides with cell affinity. Ligands also include various substances with selective affinity for ligators that are produced through recombinant DNA, genetic and molecular engineering. Except when stated otherwise, ligands of the instant disclosure also include ligands as defined in U.S. Pat. No. 3,817,837, which is herein incorporated by reference in its entirety.

ii. Ligators

[0231] A ligator functions as a type of targeting moiety defined for this disclosure as a specific binding body or "partner" or "receptor," that is usually, but not necessarily, larger than the ligand it can bind to. For the purposes of this disclosure, it can be a specific substance or material or chemical or "reactant" that is capable of selective affinity binding with a specific ligand. A ligator can be a protein such as an antibody, a nonprotein binding body, or a "specific reactor."

[0232] When applied to this disclosure, a ligator includes an antibody, which is defined to include all classes of antibodies, monoclonal antibodies, chimeric antibodies, Fab fractions, fragments and derivatives thereof. The term "antibody" encompasses an immunoglobulin whether natural or partly or wholly synthetically produced, and fragments thereof. The term also covers any protein having a binding domain that is homologous to an immunoglobulin binding domain. "Antibody" further includes a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. Use of the term antibody is meant to include whole antibodies, polyclonal, monoclonal and recombinant antibodies, fragments thereof, and further includes single-chain antibodies, humanized antibodies, murine antibodies, chimeric, mouse-human, mouse-primate, primate-human monoclonal antibodies, anti-idiotypic antibodies, antibody fragments, such as, *e.g.*, scFv, scFab, (scFab)₂, (scFv)₂, Fab, Fab', and F(ab')₂, F(ab)₁, Fv, dAb, and Fd fragments, diabodies, and antibody-related polypeptides. Antibody includes bispecific antibodies and multispecific antibodies so long as they exhibit the desired biological activity or function. In some aspects of the present disclosure, the targeting moiety is an antibody or a molecule comprising an antigen binding fragment thereof. In some aspects, the antibody is a nanobody. In some aspects, the antibody is an ADC. The terms "antibody-drug conjugate" and "ADC" are used interchangeably and refer to an antibody linked, *e.g.*, covalently, to a therapeutic agent (sometimes referred to herein as agent, drug, or active pharmaceutical ingredient) or agents. In some aspects of the present disclosure, the targeting moiety is an antibody-drug conjugate.

[0233] Under certain conditions, the instant disclosure is also applicable to using other substances as ligators. For instance, other ligators suitable for targeting include naturally occurring receptors, any hemagglutinins and cell membrane and nuclear derivatives that bind specifically to hormones, vitamins, drugs, antibiotics, cancer markers, genetic markers, viruses, and histocompatibility markers. Another group of ligators includes any RNA and DNA binding substances such as polyethylenimine (PEI) and polypeptides or proteins such as histones and protamines.

[0234] Other ligators also include enzymes, especially cell surface enzymes such as neuraminidases, plasma proteins, avidins, streptavidins, chalones, cavitands, thyroglobulin, intrinsic factor, globulins, chelators, surfactants, organometallic substances, staphylococcal protein A, protein G, ribosomes, bacteriophages, cytochromes, lectins, certain resins, and organic polymers.

[0235] Targeting moieties also include various substances such as any proteins, protein fragments or polypeptides with affinity for the surface of any cells, tissues or microorganisms that are produced through recombinant DNA, genetic and molecular engineering. Thus, in some aspects, the targeting moiety directs a micelle of the present disclosure to a specific tissue (*i.e.*, liver tissue or brain tissue), to a specific type of cell (*e.g.*, a certain type of cancer cells), or to a physiological compartment or physiological barrier (*e.g.*, the BBB).

f. Linkers

[0236] As described above, a cationic carrier unit disclosed herein can comprise, as shown, *e.g.*, in FIG. 2, one or more linkers. As used herein, the term "linker" refers to a peptide or polypeptide sequence (*e.g.*, a synthetic peptide or polypeptide sequence), or a non-peptide linker for which its main function is to connect two moieties in a cationic carrier unit disclosed herein. In some aspects, cationic carrier units of the present disclosure can comprise at least one linker connecting a tissue-specific targeting moiety (TM) with a water soluble polymer (WS), at least one linker connecting a water-soluble biopolymer (WP) with cationic carrier (CC) or a hydrophobic moiety (HM) or a crosslinking moiety (CM), at least one linker connecting a cationic carrier (CC) with a hydrophobic moiety (HM), or any combination thereof. In some aspects, two or more linkers can be linked in tandem.

[0237] When multiple linkers are present in a cationic carrier unit disclosed herein, each of the linkers can be the same or different. Generally, linkers provide flexibility to the cationic carrier unit. Linkers are not typically cleaved; however, in certain aspects, such cleavage can be desirable. Accordingly, in some aspects a linker can comprise one or more protease-cleavable sites, which can be located within the sequence of the linker or flanking the linker at either end of the linker sequence.

[0238] In one aspect, the linker is a peptide linker. In some aspects, the peptide linker can comprise at least about two, at least about three, at least about four, at least about five, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least

about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, or at least about 100 amino acids.

[0239] In some aspects, the peptide linker can comprise at least about 110, at least about 120, at least about 130, at least about 140, at least about 150, at least about 160, at least about 170, at least about 180, at least about 190, or at least about 200 amino acids.

[0240] In other aspects, the peptide linker can comprise at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, or at least about 1,000 amino acids.

[0241] The peptide linker can comprise between 1 and about 5 amino acids, between 1 and about 10 amino acids, between 1 and about 20 amino acids, between about 10 and about 50 amino acids, between about 50 and about 100 amino acids, between about 100 and about 200 amino acids, between about 200 and about 300 amino acids, between about 300 and about 400 amino acids, between about 400 and about 500 amino acids, between about 500 and about 600 amino acids, between about 600 and about 700 amino acids, between about 700 and about 800 amino acids, between about 800 and about 900 amino acids, or between about 900 and about 1000 amino acids.

[0242] Examples of peptide linkers are well known in the art. In some aspects, the linker is a glycine/serine linker. In some aspects, the peptide linker is glycine/serine linker according to the formula $[(\text{Gly})_n\text{-Ser}]_m$ where n is any integer from 1 to 100 and m is any integer from 1 to 100. In other aspects the glycine/serine linker is according to the formula $[(\text{Gly})_x\text{-Sery}]_z$ (SEQ ID NO: 1) wherein x is an integer from 1 to 4, y is 0 or 1, and z is an integer from 1 to 50. In one aspect, the peptide linker comprises the sequence G_n , where n can be an integer from 1 to 100. In a specific aspect, the sequence of the peptide linker is GGGG (SEQ ID NO: 2).

[0243] In some aspects, the peptide linker can comprise the sequence $(\text{GlyAla})_n$ (SEQ ID NO: 3), wherein n is an integer between 1 and 100. In other aspects, the peptide linker can comprise the sequence $(\text{GlyGlySer})_n$ (SEQ ID NO: 4), wherein n is an integer between 1 and 100.

[0244] In other aspects, the peptide linker comprises the sequence $(\text{GGGS})_n$ (SEQ ID NO: 5). In still other aspects, the peptide linker comprises the sequence $(\text{GGS})_n(\text{GGGS})_n$ (SEQ ID NO: 6). In these instances, n can be an integer from 1-100. In other instances, n can be an integer from one to 20, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0245] Examples of linkers include, but are not limited to, GGG, SGGSGGS (SEQ ID NO: 7), GSGSGSGSGSGSGG (SEQ ID NO: 8), GSGSGSGSGSGSGSGG (SEQ ID NO: 9),

GGSGGSGGSGGSGGSGGS (SEQ ID NO: 10), or GGGGSGGGGSGGGGS (SEQ ID NO: 11). In other aspects, the linker is a poly-G sequence (GGGG)_n (SEQ ID NO: 12), where n can be an integer from 1-100.

[0246] In one aspect, the peptide linker is synthetic, *i.e.*, non-naturally occurring. In one aspect, a peptide linker includes peptides (or polypeptides) (*e.g.*, natural or non-naturally occurring peptides) which comprise an amino acid sequence that links or genetically fuses a first linear sequence of amino acids to a second linear sequence of amino acids to which it is not naturally linked or genetically fused in nature. For example, in one aspect the peptide linker can comprise non-naturally occurring polypeptides that are modified forms of naturally occurring polypeptides (*e.g.*, comprising a mutation such as an addition, substitution or deletion). In another aspect, the peptide linker can comprise non-naturally occurring amino acids. In another aspect, the peptide linker can comprise naturally occurring amino acids occurring in a linear sequence that does not occur in nature. In still another aspect, the peptide linker can comprise a naturally occurring polypeptide sequence.

[0247] In some aspects, the linker comprises a non-peptide linker. In other aspects, the linker consists of a non-peptide linker. In some aspects, the non-peptide linker can be, *e.g.*, maleimido caproyl (MC), maleimido propanoyl (MP), methoxyl polyethyleneglycol (MPEG), succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), succinimidyl 6-[3-(2-pyridyldithio)-propionamide]hexanoate (LC-SPDP), 4-succinimidylloxycarbonyl-alpha-methyl-alpha-(2-pyridyldithio)toluene (SMPT), etc. (see, *e.g.*, U.S. Pat. No. 7,375,078).

[0248] Linkers can be introduced into polypeptide sequences using techniques known in the art (*e.g.*, chemical conjugation, recombinant techniques, or peptide synthesis). Modifications can be confirmed by DNA sequence analysis. In some aspects, the linkers can be introduced using recombinant techniques. In other aspects, the linkers can be introduced using solid phase peptide synthesis. In certain aspects, a cationic carrier unit disclosed herein can contain simultaneously one or more linkers that have been introduced using recombinant techniques and one or more linkers that have been introduced using solid phase peptide synthesis or methods of chemical conjugation known in the art. In some aspects, the linker comprises a cleavage site.

III. Payloads

[0249] As used herein the term "payload" refers to a biologically active molecule, *e.g.*, a therapeutic agent that can interact by itself or via an adapter with a cationic carrier unit of the present disclosure, and be included within the core of a micelle of the present disclosure.

[0250] Other biologically active molecules are anti-viral drugs, nucleic acids and other anti-viral substances including those against any DNA and RNA viruses, AIDS, HIV and hepatitis viruses, adenoviruses, alphaviruses, arenaviruses, coronaviruses, flaviviruses, herpesviruses, myxoviruses, oncornaviruses, papovaviruses, paramyxoviruses, parvoviruses, picomaviruses, poxviruses, reoviruses, thaboviruses, rhinoviruses, togaviruses and virioids; any anti-bacterial drugs, nucleic acids and other anti-bacterial substances including those against gram-negative and grampositive bacteria, *Acinetobacter*, *Achromobacter*, *Bacteroides*, *Clostridium*, *Chlamydia*, enterobacteria, *Haemophilus*, *Lactobacillus*, *Neisseria*, *Staphylococcus*, or *Streptococcus*; any antifungal drugs, nucleic acids and other anti-fungal substances including those against *Aspergillus*, *Candida*, *Coccidioides*, mycoses, phycomycetes, and yeasts; any drugs, nucleic acids and other substances against mycoplasma and rickettsia; any anti-protozoan drugs, nucleic acids and other substances; any anti-parasitic drugs, nucleic acids and other substances; any drugs, nucleic acids and other substances against heart diseases, tumors, and virus infected cells, among others.

(a) Nucleic acids

[0251] In some aspects, the biologically active molecule (payload) is a nucleic acid, *e.g.*, an RNA or a DNA. Nucleic acid active agents suitable for delivery using the micelles of the present disclosure include all types of RNA and all types of DNA. In some aspects, the nucleic acid comprises mRNA, miRNA sponge, tough decoy miRNA (TD), cDNA, pDNA, PNA, BNA, aptamer, or any combination thereof.

[0252] In some aspects, the biologically active molecule (*e.g.*, anionic payload) comprises a nucleotide sequence having less than 4,000 nucleotides in length. In some aspects, the biologically active molecule (*e.g.*, anionic payload) comprises a nucleotide sequence having less than about 3,500, less than about 3,000, less than about 2,500, less than about 2,000, less than about 1,500, less than about 1,000, less than about 900, less than about 800, less than about 700, less than about 600, less than about 500, less than about 400, less than about 200, or less than about 150 in length.

[0253] In some aspects, the nucleic acids are phosphodiester nucleotides, and any nucleotides where the sugar-phosphate "backbone" has been derivatized or replaced with "backbone analogues" such as with phosphorothioate, phosphorodithioate, phosphoroamidate, alkyl phosphotriester, or methylphosphonate linkages. In some aspects, the nucleic acids have non-phosphorous backbone analogues such as sulfamate, 3'-thioformacetal, methylene (methylimino) (MMI), 3'-N-carbamate, or morpholino carbamate.

[0254] In some aspects, the anionic payload is a polynucleotide, also can be referred to as nucleotide or nucleic acid. The term "polynucleotide," in its broadest sense, includes any compound and/or substance that comprise a polymer of nucleotides. Exemplary polynucleotides of the disclosure include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β -D-ribo configuration, α -LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization) or hybrids thereof.

[0255] In some aspects, the synthetic polynucleotide is a synthetic messenger RNA (mRNA). As used herein, the term "messenger RNA" (mRNA) refers to any polynucleotide, which may be synthetic, which encodes a polypeptide, e.g., a G protein, and which is capable of being translated to produce the encoded polypeptide in vitro, in vivo, in situ or ex vivo.

[0256] The present disclosure expands the scope of functionality of traditional mRNA molecules by providing synthetic polynucleotides which comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the polynucleotides including, in some aspects, the lack of a substantial induction of the innate immune response of a cell into which the polynucleotide is introduced. As used herein, a "structural" feature or modification is one in which two or more linked nucleotides are inserted, deleted, duplicated, inverted or randomized in a synthetic polynucleotide, primary construct or mmRNA without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications can result in a different sequence of nucleotides. For example, the polynucleotide "ATCG" may be chemically modified to "AT-5meC-G". The same polynucleotide may be structurally modified from "ATCG" to "ATCCCG".

[0257] In some aspects, the anionic payload can include untranslated regions. Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5' UTR starts at the transcription

start site and continues to the start codon but does not include the start codon; whereas, the 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the polynucleotides, primary constructs and/or mmRNA of the present invention to enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites.

[0258] It should be understood that those listed are examples and that any UTR from any gene may be incorporated into the respective first or second flanking region of the primary construct. Furthermore, multiple wild-type UTRs of any known gene can be utilized. It is also within the scope of the present invention to provide artificial UTRs which are not variants of wild type genes. These UTRs or portions thereof can be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5' or 3' UTR can be inverted, shortened, lengthened, made chimeric with one or more other 5' UTRs or 3' UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR can be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

[0259] In some aspects, the nucleotide also include a poly A tail, a miRNA binding site, an AU element, or any combination thereof.

[0260] In some aspects, an anionic payload having about 100 to about 1000 nucleotides in length can be any protein or fragments thereof having about 30 amino acids or less in length. For example, an anionic payload having about 100 to about 1000 nucleotides in length encodes PAPD5/7 (0.8 kb), or any fragments thereof.

[0261] In some aspects, an anionic payload having about 1000 to about 2000 nucleotides in length can be any protein or fragments thereof having about 660 amino acids or less in length. For example, an anionic payload having about 1000 to about 2000 nucleotides in length can encode G protein (1.5 kb).

[0262] In some aspects, an anionic payload having about 2000 to about 3000 nucleotides in length can be any protein or fragments thereof having about 1000 amino acids or less in length.

For example, an anionic payload having about 2000 to about 3000 nucleotides in length can encode Gephyrin, protein anchor (2.2 kb).

[0263] In some aspects, an anionic payload having about 3000 to about 4000 nucleotides in length can be any protein or fragments thereof having about 1330 amino acids or less in length. For example, an anionic payload having about 3000 to about 4000 nucleotides in length can encode MDA5 (IFIH1) (3.0 kb).

i. Chemically Modified Polynucleotides

[0264] In some aspects, a polynucleotide of the present disclosure (*e.g.*, an mRNA) comprises at least one chemically modified nucleoside and/or nucleotide. When the polynucleotides of the present disclosure are chemically modified, the polynucleotides can be referred to as "modified polynucleotides."

[0265] A "nucleoside" refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase").

[0266] A "nucleotide" refers to a nucleoside including a phosphate group. Modified nucleotides can be synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides.

[0267] Polynucleotides can comprise a region or regions of linked nucleosides. Such regions can have variable backbone linkages. The linkages can be standard phosphodiester linkages, in which case the polynucleotides would comprise regions of nucleotides.

[0268] The modified polynucleotides disclosed herein can comprise various distinct modifications. In some aspects, the modified polynucleotides contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some aspects, a modified polynucleotide can exhibit one or more desirable properties, *e.g.*, improved thermal or chemical stability, reduced immunogenicity, reduced degradation, increased binding to the target gene or protein, reduced non-specific binding to other gene or other molecules, as compared to an unmodified polynucleotide.

[0269] In some aspects, a polynucleotide of the present disclosure is chemically modified. As used herein in reference to a polynucleotide, the terms "chemical modification" or, as appropriate, "chemically modified" refer to modification with respect to adenosine (A), guanosine (G), uridine (U), thymidine (T) or cytidine (C) ribo- or deoxyribonucleosides in one or more of

their position, pattern, percent or population, including, but not limited to, its nucleobase, sugar, backbone, or any combination thereof.

[0270] In some aspects, a polynucleotide of the present disclosure (*e.g.*, an mRNA) can have a uniform chemical modification of all or any of the same nucleoside type or a population of modifications produced by downward titration of the same starting modification in all or any of the same nucleoside type, or a measured percent of a chemical modification of all any of the same nucleoside type but with random incorporation. In another aspect, the polynucleotide of the present disclosure (*e.g.*, an mRNA) can have a uniform chemical modification of two, three, or four of the same nucleoside type throughout the entire polynucleotide (such as all uridines and/or all cytidines, etc. are modified in the same way).

[0271] Modified nucleotide base pairing encompasses not only the standard adenine-thymine, adenine-uracil, or guanine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures. One example of such non-standard base pairing is the base pairing between the modified nucleobase inosine and adenine, cytosine, or uracil. Any combination of base/sugar or linker can be incorporated into polynucleotides of the present disclosure.

[0272] The skilled artisan will appreciate that, except where otherwise noted, polynucleotide sequences set forth in the instant application will recite "T"s in a representative DNA sequence but where the sequence represents RNA, the "T"s would be substituted for "U"s. For example, TD's of the present disclosure can be administered as RNAs, as DNAs, or as hybrid molecules comprising both RNA and DNA units.

[0273] In some aspects, the polynucleotide (*e.g.*, an mRNA) includes a combination of at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, 20 or more) modified nucleobases.

1. Base Modifications

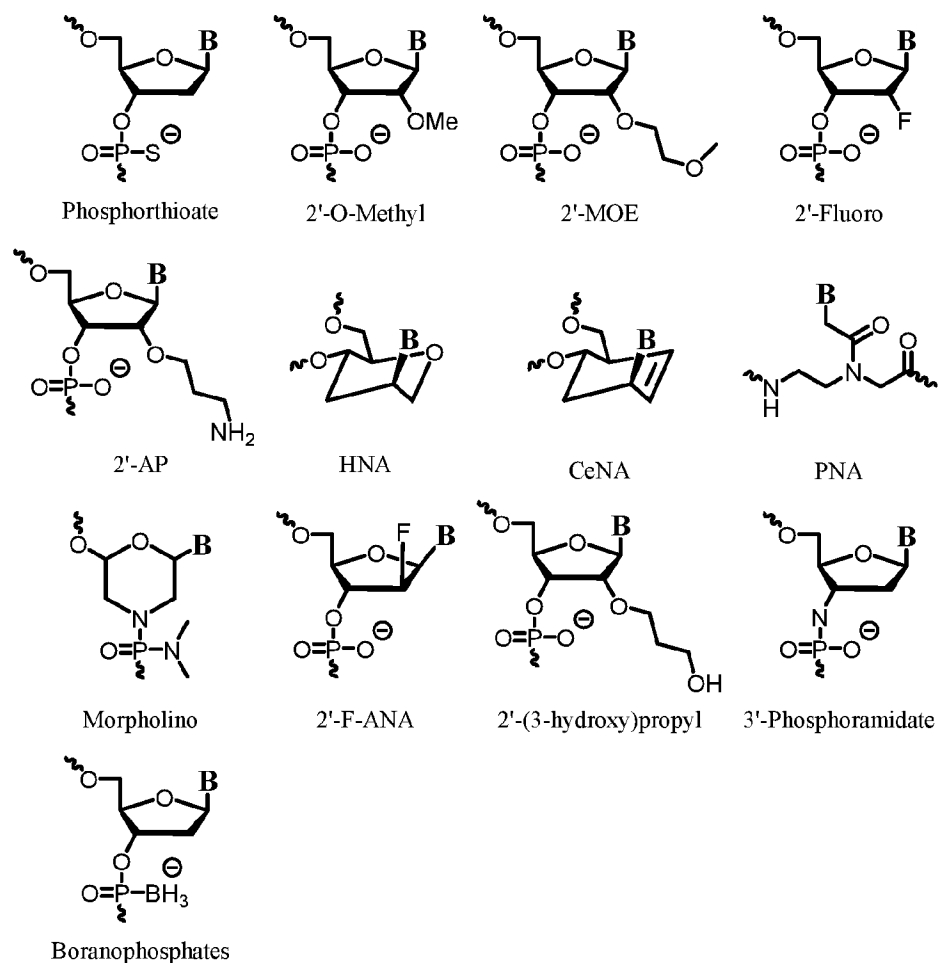
[0274] In certain aspects, the chemical modification is at nucleobases in a polynucleotide of the present disclosure (*e.g.*, an mRNA). In some aspects, the at least one chemically modified nucleoside is a modified uridine (*e.g.*, pseudouridine (ψ), 2-thiouridine (s2U), 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), or 5-methoxy-uridine (mo5U)), a modified cytosine (*e.g.*, 5-methyl-cytidine (m5C)) a modified adenosine (*e.g.*, 1-methyl-adenosine (m1A),

N6-methyl-adenosine (m6A), or 2-methyl-adenine (m2A)), a modified guanosine (*e.g.*, 7-methyl-guanosine (m7G) or 1-methyl-guanosine (m1G)), or a combination thereof.

[0275] In some aspects, the polynucleotide of the present disclosure (*e.g.*, an mRNA) is uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with the same type of base modification, *e.g.*, 5-methyl-cytidine (m5C), meaning that all cytosine residues in the polynucleotide sequence are replaced with 5-methyl-cytidine (m5C). Similarly, a polynucleotide can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified nucleoside such as any of those set forth above.

2. Backbone modifications

[0276] In some aspects, the payload can comprise a "polynucleotide of the present disclosure" (for example comprising an mRNA), wherein the polynucleotide includes any useful modification to the linkages between the nucleosides. Such linkages, including backbone modifications, that are useful in the composition of the present disclosure include, but are not limited to the following: 3'-alkylene phosphonates, 3'-amino phosphoramidate, alkene containing backbones, aminoalkylphosphoramidates, aminoalkylphosphotriesters, boranophosphates, -CH₂-O-N(CH₃)-CH₂-, -CH₂-N(CH₃)-N(CH₃)-CH₂-, -CH₂-NH-CH₂-, chiral phosphonates, chiral phosphorothioates, formacetyl and thioformacetyl backbones, methylene (methylimino), methylene formacetyl and thioformacetyl backbones, methyleneimino and methylenehydrazino backbones, morpholino linkages, -N(CH₃)-CH₂-CH₂-, oligonucleosides with heteroatom internucleoside linkage, phosphinates, phosphoramidates, phosphorodithioates, phosphorothioate internucleoside linkages, phosphorothioates, phosphotriesters, PNA, siloxane backbones, sulfamate backbones, sulfide sulfoxide and sulfone backbones, sulfonate and sulfonamide backbones, thionoalkylphosphonates, thionoalkylphosphotriesters, and thionophosphoramidates.



[0277] In some aspects, the presence of a backbone linkage disclosed above increase the stability (*e.g.*, thermal stability) and/or resistance to degradation (*e.g.*, enzyme degradation) of a polynucleotide of the present disclosure (*e.g.*, an mRNA). In some aspects, the stability and/or resistance to degradation increases by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% in the modified polynucleotide compared to a corresponding polynucleotide without the modification (reference or control polynucleotide)

[0278] In some aspects, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100%

of the backbone linkages in a polynucleotide of the present disclosure ((*e.g.*, an mRNA) are modified (*e.g.*, all of them are phosphorothioate).

[0279] In some aspects, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 backbone linkages in a polynucleotide of the present disclosure (*e.g.*, an mRNA) are modified (*e.g.*, phosphorothioate).

[0280] In some aspects, the backbone comprises linkages selected from the group consisting of phosphodiester linkage, phosphotriesters linkage, methylphosphonate linkage, phosphoramidate linkage, phosphorothioate linkage, and combinations thereof.

3. *Sugar Modifications*

[0281] The modified nucleosides and nucleotides which can be incorporated into a polynucleotide of the present disclosure (*e.g.*, an mRNA), can be modified on the sugar of the nucleic acid. Thus, in some aspects, the payload comprises a nucleic acid, wherein the nucleic acid comprises at least one nucleoside analog (*e.g.*, a nucleoside with a sugar modification).

[0282] Incorporating affinity-enhancing nucleotide analogues in the polynucleotide, such as LNA or 2'-substituted sugars can allow the length of polynucleotide to be reduced, and also may reduce the upper limit of the size a polynucleotide before non-specific or aberrant binding takes place.

[0283] In some aspects, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% of the nucleotides in a polynucleotide of the present disclosure (*e.g.*, an mRNA) contain sugar modifications (*e.g.*, LNA).

[0284] In some aspects, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 nucleotide units in a polynucleotide of the present disclosure (*e.g.*, an mRNA) are sugar modified (*e.g.*, LNA).

[0285] Generally, RNA includes the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary, non-limiting modified nucleotides include replacement of the oxygen in ribose (*e.g.*, with S, Se, or alkylene, such as methylene or ethylene); addition of a double bond (*e.g.*, to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of ribose (*e.g.*, to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (*e.g.*, to form a 6-

or 7-membered ring having an additional carbon or heteroatom, such as for anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino that also has a phosphoramidate backbone); multicyclic forms (*e.g.*, tricyclo; and "unlocked" forms, such as glycol nucleic acid (GNA) (*e.g.*, R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), threose nucleic acid (TNA, where ribose is replaced with α -L-threofuranosyl-(3' $\rightarrow$ 2')) , and peptide nucleic acid (PNA, where 2-amino-ethyl-glycine linkages replace the ribose and phosphodiester backbone). The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a polynucleotide molecule can include nucleotides containing, *e.g.*, arabinose, as the sugar.

[0286] The 2' hydroxyl group (OH) of ribose can be modified or replaced with a number of different substituents. Exemplary substitutions at the 2'-position include, but are not limited to, H, halo, optionally substituted C₁₋₆ alkyl; optionally substituted C₁₋₆ alkoxy; optionally substituted C₆₋₁₀ aryloxy; optionally substituted C₃₋₈ cycloalkyl; optionally substituted C₃₋₈ cycloalkoxy; optionally substituted C₆₋₁₀ aryloxy; optionally substituted C₆₋₁₀ aryl-C₁₋₆ alkoxy, optionally substituted C₁₋₁₂ (heterocyclyl)oxy; a sugar (*e.g.*, ribose, pentose, or any described herein); a polyethyleneglycol (PEG), -O(CH₂CH₂O)_nCH₂CH₂OR, where R is H or optionally substituted alkyl, and n is an integer from 0 to 20 (*e.g.*, from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20); "locked" nucleic acids (LNA) in which the 2'-hydroxyl is connected by a C₁₋₆ alkylene or C₁₋₆ heteroalkylene bridge to the 4'-carbon of the same ribose sugar, where exemplary bridges include methylene, propylene, ether, amino bridges, aminoalkyl, aminoalkoxy, amino, and amino acid.

[0287] In some aspects, nucleoside analogues present in a polynucleotide of the present disclosure (*e.g.*, an mRNA) comprise, *e.g.*, 2'-O-alkyl-RNA units, 2'-OMe-RNA units, 2'-O-alkyl-SNA, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid) units, 2'MOE units, or any combination thereof. In some aspects, the LNA is, *e.g.*, oxy-LNA (such as beta-D-oxy-LNA, or alpha-L-oxy-LNA), amino-LNA (such as beta-D-amino-LNA or alpha-L-amino-LNA), thio-LNA (such as beta-D-thio-LNA or alpha-L-thio-LNA), ENA (such a beta-D-ENA or alpha-L-ENA), or any combination thereof.

[0288] In some aspects, nucleoside analogs present in a polynucleotide of the present disclosure comprise Locked Nucleic Acid (LNA); 2'-O-alkyl-RNA; 2'-amino-DNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA, hexitol nucleic acid (HNA), intercalating nucleic acid (INA), constrained ethyl nucleoside (cEt), 2'-O-methyl nucleic acid (2'-OMe), 2'-O-methoxyethyl nucleic acid (2'-MOE), or any combination thereof.

IV. Micelles

[0289] The present disclosure also provides micelles comprising the cationic carrier units of the present disclosure. The micelles of the present disclosure comprise cationic carriers unit of the present disclosure and negatively charged payload, wherein the negatively charged payload and the cationic carrier unit are associate with each other. In some aspects, the association is comprises a covalent bond. In other aspects, the association does not comprise a covalent bond. In other aspects, the association is via an ionic bond, *i.e.*, via electrostatic interaction. In some aspects, the negatively charged payload (*e.g.*, a DNA and/or RNA) is not conjugated to the cationic carrier unit by a covalent bond and/or the negatively charged payload interacts with the cationic carrier moiety of the cationic carrier unit only via an ionic interaction.

[0290] In some aspects, the cationic carrier units and micelles of the present disclosure protect the payload (*e.g.*, a DNA and/or RNA) from degradation (*e.g.*, by a DNase and/or an RNase). First, the cationic carrier unit is capable of protecting the payload through electrostatic interaction. Secondly, the micelle sequesters the payload to the core of the micelle, *i.e.*, out of the reach of DNases and/or an RNases. In some aspects, the protection of the payload from circulating enzymes (*e.g.*, nucleases) can increase the half-life of the negatively charged payload (*e.g.*, a DNA and/or RNA) compared to the free payload. In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the plasma half-life of the payload at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, at least about 20-fold, at least about 21-fold, at least about 22-fold, at least about 23-fold, at least about 24-fold, at least about 25-fold, at least about 26-fold, at least about 27-fold, at least about 28-fold, at least about 29-fold, or at least about 30-fold compared to the free payload.

[0291] In some aspects, the positive charge of the cationic carrier unit, and in particular the charge of the cationic carrier moiety is sufficient to form a micelle when mixed with a negatively charged payload (*e.g.*, a nucleic acid) in a solution, wherein the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 20:1, about 19:1, about 18:1, about 17:1, about 16:1, about 15:1, about 14:1, about 13:1, about 12:1, about 11:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 1:20, about 1:19, about 1:18, about 1:17, about 1:16, about 1:15, about 1:14, about 1:13, about 1:12, about 1:11, about 1:10, about 1:9, about 1:8, about 1:7, about 1:6, about 1:5, about 1:4, about 1:3, about 1:2, or about 1:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 2:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 3:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 4:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 5:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 6:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 7:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 8:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 9:1. In some aspects, the overall ionic ratio between the cationic carrier unit, in particular its cationic carrier moiety, and the negatively charged payload (*e.g.*, a nucleic acid) is about 10:1.

[0292] In some aspects, the anionic payload for the micelles comprises a nucleotide sequence having about 10 to about 1000 (*e.g.*, about 100 to about 1000) in length, wherein the N/P ratio of the the cationic carrier unit and the anionic payload is about 2 to about 10, *e.g.*, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, *e.g.*, *e.g.*, about 2, about 3, about 4, about 5, about 6, about 7, about 8,

about 9, or about 10. In some aspects, an N/P ratio of the cationic carrier unit and the anionic payload of about 10 to about 1000 nucleotides in length is between about 1 and about 10, e.g., about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10.

[0293] In some aspects, the anionic payload for the micelles comprises a nucleotide sequence having about 1000 to about 2000 in length, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 3 to about 12, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, or about 12. In some aspects, the N/P ratio of the cationic carrier unit and the anionic payload is between about 4 and about 7, e.g., about 4, about 5, about 6, or about 7.

[0294] In some aspects, the anionic payload for the micelles comprises a nucleotide sequence having about 2000 to about 3000 in length, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 3 to about 16, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16. In some aspects, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 6 and about 9, e.g., about 6, about 7, about 8, or about 9.

[0295] In some aspects, the anionic payload for the micelles comprises a nucleotide sequence having about 3000 to about 4000 in length, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 3 to about 20, e.g., about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20. In some aspects, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 7 and about 10, e.g., about 7, about 8, about 9, or about 10.

[0296] In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 10. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 8 or about 8 and about 9. In some aspects, the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 7, about 8, about 9, or about 10.

[0297] In some aspects, upon combination with a suitable buffer (*e.g.*, PBS), the complexes formed between the cationic carrier units of the present disclosure and payload (*e.g.*, an mRNA), self-organize to yield micelles.

[0298] A micelle is a water soluble or colloidal structure or aggregate composed of one or more amphiphilic molecules. Amphiphilic molecules are those that contain at least one hydrophilic (polar) moiety and at least one hydrophobic (nonpolar) moiety. "Classic micelles" have a single, central and primarily hydrophobic zone or "core" surrounded by a hydrophilic layer or "shell." In aqueous solution, the micelle forms an aggregate with the hydrophilic "head" regions of the amphiphilic molecule in contact with the surrounding solvent, sequestering the hydrophobic single-tail regions of the amphiphilic molecule in the micelle core. Micelles are approximately spherical in shape. Other shapes, *e.g.*, ellipsoids, cylinders, rod-like structures, or polymersomes are also possible. The shape and size, and therefore loading capacity, of the micelles disclosed can be modified by altering the ratio between water-soluble biopolymer (*e.g.*, PEG) and cationic carrier (*e.g.*, poly lysine). Depending on the ratio, the carrier units can organize as small particles, small micelles, micelles, rod-like structures, or polymersomes. Thus, the term "micelles of the present disclosure" encompasses not only classic micelles but also small particles, small micelles, micelles, rod-like structures, or polymersomes.

[0299] The micelles of the present disclosure can be composed of either a single monomolecular polymer containing hydrophobic and hydrophilic moieties or an aggregate mixture containing many amphiphilic (*i.e.* surfactant) molecules formed at or above the critical micelle concentration (CMC), in a polar (*i.e.* aqueous) solution. The micelle is self-assembled from one or more amphiphilic molecules where the moieties are oriented to provide a primarily hydrophobic interior core and a primarily hydrophilic exterior.

[0300] Micelles of the present disclosure can range in size from 5 to about 2000 nanometers. In some aspects, the diameter of the micelle is between about 10 nm and about 200 nm. In some aspects, the diameter of the micelle is between about 1nm and about 100nm, between about 10nm and about 100nm, between about 10nm and about 90nm, between about 10nm and about 80nm, between about 10nm and about 70nm, between about 20nm and about 100nm, between about 20nm and about 90nm, between about 20nm and about 80nm, between about 20nm and about 70nm, between about 30nm and about 100nm, between about 30nm and about 90nm, between about 30nm and about 80nm, between about 30nm and about 70nm, between about 40nm and about 100nm, between about 40nm and about 90nm, between about 40nm and about 80nm, or between about 40nm and about 70nm. In some aspects, the diameter of the micelles of the present

disclosure is between about 30 nm and about 60 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 15 nm and about 90 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 15 nm and about 80 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 15 nm and about 70 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 15 nm and about 60 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 15 nm and about 50 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 20 nm and about 60 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 20 nm and about 50 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 20 nm and about 40 nm. In some aspects, the diameter of the micelles of the present disclosure is between about 25 nm and about 35 nm. In some aspects, the diameter of the micelles of the present disclosure is about 32 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 100 nm and about 200 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 40 nm and about 50 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 50 nm and about 60 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 60 nm and about 70 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 70 nm and about 80 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 80 nm and about 90 nm. In some aspects, the diameter of the micelles of the present disclosure is about about 90 nm and about 100 nm.

[0301] In some aspects, the micelles of the present disclosure comprise a single type of cationic carrier unit. In other aspects, the micelles of the present disclosure comprise more than one type of cationic carrier unit (*e.g.*, targeting different receptor on the surface of a target cell). In some aspects, micelles of the present disclosure can comprise cationic carrier units with different targeting moieties, different cationic carrier moieties (*e.g.*, to accommodate different payloads), and/or different hydrophobic and/or crosslinking units.

[0302] In order to form a micelle with a payload, different types of cationic or anionic carrier unit can be combined together. For example, in order to target blood brain barrier, the micelle of the present disclosure can comprise a cationic (or an anionic) carrier unit linked to a targeting moiety and a cationic (or an anionic) carrier unit not linked to a targeting moiety. In some aspects, a micelle comprises about 50 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 50 to about 150, about 50 to about 140, about 50 to about 130, about 50 to about 120, about 50 to about 110, or about 50 to about 100 cationic or anionic carrier units. In

some aspects, a micelle comprises about 60 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 60 to about 150, about 60 to about 140, about 60 to about 130, about 60 to about 120, about 60 to about 110, about 60 to about 100, about 60 to about 90, about 60 to about 80, or about 60 to about 70 cationic or anionic carrier units. In some aspects, a micelle comprises about 70 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 70 to about 150, about 70 to about 140, about 70 to about 130, about 70 to about 120, about 70 to about 110, about 70 to about 100, about 70 to about 90, or about 70 to about 80 cationic or anionic carrier units. In some aspects, a micelle comprises about 80 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 80 to about 150, about 80 to about 140, about 80 to about 130, about 80 to about 120, about 80 to about 110, about 80 to about 100, or about 80 to about 90 cationic or anionic carrier units. In some aspects, a micelle comprises about 90 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 90 to about 150, about 90 to about 140, about 90 to about 130, about 90 to about 120, about 90 to about 110, or about 90 to about 100 cationic or anionic carrier units. In some aspects, a micelle comprises about 100 to about 200 cationic or anionic carrier units. In other aspects, a micelle comprises about 100 to about 150, about 100 to about 140, about 100 to about 130, about 100 to about 120, about 100 to about 110, or about 100 to about 100 cationic or anionic carrier units.

[0303] The present disclosure also includes a micelle comprising (i) a nucleotide sequence (e.g., an mRNA having less than 4000 nucleotides in length) and (ii) a cationic carrier unit described herein. In some aspects, the disclosure is directed to a micelle comprising (i) a nucleotide sequence, e.g., mRNA having less than 4000 nucleotides in length), and (ii) about 80 to about 120 (e.g., about 85 to about 115, about 90 to about 110, about 95 to about 105) cationic carrier units described herein, e.g., Schemas I-VI, Schemas I'-VI', or a combination thereof (see **FIG. 2A-2E**). In some aspects, the micelle comprises (i) a nucleotide sequence, e.g., mRNA, and (ii) about 80 to about 120 (e.g., about 80, about 85, about 90, about 95, about 100, about 105, or about 110) of a cationic carrier unit described herein, e.g., optional [CC]-L1-[CM]-L2-[HM] (see **FIG. 2**). In some aspects, the micelle comprises (i) a nucleotide sequence, e.g., mRNA), and (ii) about 60 to about 110, e.g., about 80, cationic carrier units, wherein (a) about 45 to about 90, e.g., about 80 of the cationic carrier units comprise [CC]-L1-[CM]-L2-[HM] and (b) about 45 to about 55, e.g., about 50 of the cationic carrier units comprise TM-[CC]-L1-[CM]-L2-[HM], wherein TM is phenyl alanine, WP is (PEG)₅₀₀₀, and CC is about 40 to about 50 lysines, e.g., about 45, about 46, about

47, about 48, about 49, or about 50 lysines, and wherein each of about 5 to about 15 of lysines, about 5 lysines, is fused to Vitamin B3 (nicotinamide).

[0304] In some aspects, the micelle can comprise a single payload (e.g., a single mRNA). In other aspects, the micelle can comprise more than one payload (e.g., multiple mRNA).

V. Methods of manufacture

[0305] The present disclosure also provides methods of making the cationic carrier units and micelles of the present disclosure. In general, the present disclosure provides a method of preparing a cationic carrier unit of the present disclosure comprising synthesizing the cationic carrier unit as described, e.g., in the Examples section. As used herein, the term "synthesizing" refers to the assembling the cationic carrier unit using methods known in the art. For example, protein components (e.g., an antibody targeting moiety) can be prepared recombinantly and subsequently conjugated to the other components of the cationic carrier units. In some aspects, each one of the components of the cationic carrier unit can be prepared using methods known in the art, e.g., recombinant protein production, solid phase peptide or nucleic acid synthesis, chemical synthesis, enzymatic synthesis, or any combination thereof, and the resulting component can be conjugated using chemical and/or enzymatic methods known in the art.

[0306] The cationic carrier units of the present disclosure can be purified to remove contaminants. In some aspects, the cationic carrier unit comprises a uniform population of cationic carrier units. However, in other aspects, the cationic carrier unit can comprise multiple species (e.g., some of them comprising a targeting moiety, and some comprising the remaining moieties but without a targeting moiety). In some aspects, the manufacture of the cationic carrier units of the present disclosure comprise lyophilization or any other form of dry storage suitable for reconstitution. In some aspects, the preparation of the cationic carrier unit in a dry form takes place after combination of the cationic carrier units with the payload (e.g., a nucleic acid).

[0307] In some aspects, the method of preparing a micelle of the present disclosure comprises mixing the cationic carrier unit with the negatively charged payload (e.g., a nucleic acid such as mRNA) at an ionic ratio of 1:1. In some aspects, the cationic carrier unit and the negatively charged payload are combined in solution. In some aspects, after combination of the cationic carrier and the negative charged payload in solution, the resulting solution is lyophilized or dried. In some aspects, the combination of the cationic carrier and the negative charged payload is conducted in dry form.

[0308] The ratio of number n of monomer units in the water-soluble polymer (*e.g.*, PEG) to the number m of monomer units (*e.g.*, lysines) in the cationic carrier moiety (*e.g.*, poly lysine), wherein the number of units n or m in each case can be up to 1,000 units affects the size and shape of the resulting micelles. At $mB/(nA+mB)$ ratios of 0.5, the micelles obtained are classic micelles. If $mB/(nA+mB)$ is above 0.5, the micelles obtained are rod like micelles or polymersomes. If $mB/(nA+mB)$ is below 0.5, the micelles obtained are small micelles or small particles.

[0309] The micelles of the present disclosure can be generation using any of the techniques known in the art, for example, vortexing, extrusion, or sonication. The formation of micelles depends on applying conditions that are above the critical micelle concentration (CMC) of a solution comprising the cationic carrier units of the present disclosure. After they reach a certain value of concentration, surfactants begin to associate and to organize themselves into more complex units, such as micelles. The CMC of a solution comprising the cationic carriers of the present disclosure can be determined by any physical property (*e.g.*, surface tension) that shows a distinct transition around the CMC.

[0310] The well-known Smith-Ewart theory predicts that the number of particles nucleated leading to the formation of micelles above the CMC is proportional to the surfactant (in the present disclosure, the cationic carrier units complexed or associated to the anionic payload) concentration to the 0.6 power. This is so because for a given surfactant the number of micelles formed generally increases with an increase in the surfactant concentration.

[0311] In some aspects, the micelles of the present disclosure can be purified, *e.g.*, to remove contaminants and/or to generate an uniform population of micelles (*e.g.*, micelles having the same size, or micelles having the same payload or the same targeting moiety).

VI. Pharmaceutical Compositions

[0312] The present disclosure also provides pharmaceutical compositions comprising cationic carrier units and/or micelles of the present disclosure (*i.e.*, micelles comprising cationic carrier units of the present disclosure) that are suitable for administration to a subject. As discussed above, micelles of the present disclosure can be homogeneous (*i.e.*, all micelles comprises the same type of cationic carrier unit, with the same targeting moiety and the same payload). However, in other aspects, the micelles can comprise multiple targeting moieties, multiple payloads, etc.

[0313] The pharmaceutical compositions generally comprise a cationic carrier unit and/or micelle of the present disclosure and a pharmaceutically acceptable excipient or carrier in a form

suitable for administration to a subject. Pharmaceutically acceptable excipients or carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition.

[0314] There is a wide variety of suitable formulations of pharmaceutical compositions comprising micelles of the present disclosure (See, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. 18th ed. (1990)). The pharmaceutical compositions are generally formulated sterile and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. In some aspects, the pharmaceutical composition comprises one or more micelles described herein.

[0315] In certain aspects, the micelles described herein are co-administered with one or more additional therapeutic agents, in a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition comprising the micelles described herein is administered prior to administration of the additional therapeutic agent(s). In other aspects, the pharmaceutical composition comprising the micelles described herein is administered after the administration of the additional therapeutic agent(s). In further aspects, the pharmaceutical composition comprising the micelles described herein is administered concurrently with the additional therapeutic agent(s).

[0316] In some aspects, the pharmaceutical carrier is added following micelle formation. In other aspects, the pharmaceutical carrier is added before micelle formation.

[0317] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients (*e.g.*, animals or humans) at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0318] Examples of carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. The use of such media and

compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the cationic carrier units or micelles disclosed herein, use thereof in the compositions is contemplated.

[0319] Supplementary therapeutic agents can also be incorporated into the compositions of the present disclosure. Typically, a pharmaceutical composition is formulated to be compatible with its intended route of administration. The micelles described herein can be administered by parenteral, topical, intravenous, oral, subcutaneous, intra-arterial, intradermal, transdermal, rectal, intracranial, intraperitoneal, intranasal, intratumoral, intramuscular route or as inhalants. In certain aspects, the pharmaceutical composition micelles described herein is administered intravenously, *e.g.* by injection. The micelles described herein can optionally be administered in combination with other therapeutic agents that are at least partly effective in treating the disease, disorder or condition for which the micelles described herein are intended.

[0320] Solutions or suspensions can include the following components: a sterile diluent such as water, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0321] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (if water-soluble) or dispersions and sterile powders. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The composition is generally sterile and fluid to the extent that easy syringeability exists. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. If desired, isotonic compounds, *e.g.*, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride can be added to the composition. Prolonged

absorption of the injectable compositions can be brought about by including in the composition a compound that delays absorption, *e.g.*, aluminum monostearate and gelatin.

[0322] Pharmaceutical compositions of the present disclosure can be sterilized by conventional, well-known sterilization techniques. Aqueous solutions can be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration.

[0323] Sterile injectable solutions can be prepared by incorporating the micelles described herein in an effective amount and in an appropriate solvent with one or a combination of ingredients enumerated herein, as desired. Generally, dispersions are prepared by incorporating the micelles described herein into a sterile vehicle that contains a basic dispersion medium and any desired other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The micelles described herein can be administered in the form of a depot injection or implant preparation that can be formulated in such a manner to permit a sustained or pulsatile release of the micelles described herein.

[0324] Systemic administration of compositions comprising micelles described herein can also be by transmucosal means. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished using, *e.g.*, nasal sprays.

[0325] In certain aspects the pharmaceutical composition comprising micelles described herein is administered intravenously into a subject that would benefit from the pharmaceutical composition. In certain aspects, the composition is administered to the lymphatic system, *e.g.*, by intralymphatic injection or by intranodal injection (*see e.g.*, Senti *et al.*, PNAS 105(46): 17908 (2008)), or by intramuscular injection, by subcutaneous administration, by intratumoral injection, by direct injection into the thymus, or into the liver.

[0326] In certain aspects, the pharmaceutical composition comprising micelles described herein is administered as a liquid suspension. In certain aspects, the pharmaceutical composition is administered as a formulation that is capable of forming a depot following administration. In certain preferred aspects, the depot slowly releases the micelles described herein into circulation, or remains in depot form.

[0327] Typically, pharmaceutically acceptable compositions are highly purified to be free of contaminants, are biocompatible and not toxic, and are suited to administration to a subject. If water is a constituent of the carrier, the water is highly purified and processed to be free of contaminants, *e.g.*, endotoxins.

[0328] The pharmaceutically acceptable carrier can be lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginates, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and/or mineral oil, but is not limited thereto. The pharmaceutical composition can further include a lubricant, a wetting agent, a sweetener, a flavor enhancer, an emulsifying agent, a suspension agent, and/or a preservative.

[0329] The pharmaceutical compositions described herein comprise the micelles described herein and optionally a pharmaceutically active or therapeutic agent. The therapeutic agent can be a biological agent, a small molecule agent, or a nucleic acid agent.

[0330] Dosage forms are provided that comprise micelles described herein. In some aspects, the dosage form is formulated as a liquid suspension for intravenous injection.

[0331] The micelles disclosed herein or pharmaceutical composition comprising the micelles may be used concurrently with other drugs. To be specific, the micelles or pharmaceutical compositions of the present disclosure may be used together with medicaments such as hormonal therapeutic agents, chemotherapeutic agents, immunotherapeutic agents, medicaments inhibiting the action of cell growth factors or cell growth factor receptors and the like.

VII. Methods of Treatment and Use

[0332] The present disclosure also provides methods of treating a disease or condition in a subject in need thereof comprising administering a micelle of the present disclosure or a combination thereof to the subject, *e.g.*, a mammal, such as human subject. In some aspects, the present disclosure provides a method of treating a neurodegenerative disorder or cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a micelle of the present disclosure, or a pharmaceutical composition of the present disclosure.

[0333] In some aspects, the micelles of the present disclosure can administered via intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac,

intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0334] In some aspects, the micelles of the present disclosure can be used concurrently with other medicaments or treatment suitable for the treatment of the diseases and conditions disclosed herein.

[0335] The present disclosure also provides methods to encapsulate a payload for delivery, comprising incorporating the payload, *e.g.*, an anionic payload such as a nucleic acid (*e.g.*, an mRNA) into a micelle of the present disclosure.

[0336] The present disclosure also provides methods to increase the resistance of a payload to degradation (*e.g.*, nuclease-mediated degradation), comprising incorporating the payload, *e.g.*, an anionic payload such as a nucleic acid (*e.g.*, an mRNA) into a micelle of the present disclosure.

[0337] In some aspects, the present disclosure provides methods of crossing blood brain barrier (BBB) comprising administering the micelles disclosed herein, *e.g.*, micelles comprising tryptophan and/or tyrosine as a targeting moiety. A micelle of the present disclosure loaded with mRNA can be targeted to a BBB receptor, *e.g.*, LAT1, as disclosed above.

[0338] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the resistance of the payload to degradation at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% compared to the free payload (*i.e.*, not in a micelle, *e.g.*, free in solution).

[0339] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the resistance of the payload to degradation at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, at least about 20-fold, at least about 21-fold, at least about 22-fold, at least about 23-fold, at least about 24-fold, at least about 25-fold, at least about 26-fold, at least about 27-fold, at least about 28-fold, at least about 29-fold, or at least about 30-fold compared to the free payload.

[0340] The present disclosure also provides methods to increase the stability of a payload during administration (*e.g.*, while in the subject's bloodstream) comprising incorporating the

payload, *e.g.*, an anionic payload such as a nucleic acid (*e.g.*, an mRNA) into a micelle of the present disclosure.

[0341] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the stability (*e.g.*, increase the resistance to nucleases) of the payload at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% compared to the free payload.

[0342] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the stability (*e.g.*, increase the resistance to nucleases) of the payload at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, at least about 20-fold, at least about 21-fold, at least about 22-fold, at least about 23-fold, at least about 24-fold, at least about 25-fold, at least about 26-fold, at least about 27-fold, at least about 28-fold, at least about 29-fold, or at least about 30-fold compared to the free payload.

[0343] The present disclosure also provides methods to increase a payload's plasma half-life comprising incorporating the payload, *e.g.*, an anionic payload such as a nucleic acid (*e.g.*, an mRNA) into a micelle of the present disclosure.

[0344] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the plasma half-life of the payload at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, at least about 1900%, or at least about 2000%, compared to the free payload.

[0345] In some aspects, encapsulation of the payload in a micelle of the present disclosure can increase the plasma half-life of the payload at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold,

at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, at least about 20-fold, at least about 21-fold, at least about 22-fold, at least about 23-fold, at least about 24-fold, at least about 25-fold, at least about 26-fold, at least about 27-fold, at least about 28-fold, at least about 29-fold, or at least about 30-fold compared to the free payload.

[0346] In some aspects, encapsulation of a payload in a micelle of the present disclosure can increase the permeation, delivery, transit, or transport of the payload through a physiological barrier, *e.g.*, the BBB or the plasma membrane, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% compared to the free payload.

[0347] In some aspects, encapsulation of a payload in a micelle of the present disclosure can increase the permeation, delivery, transit, or transport of the payload through a physiological barrier, *e.g.*, the BBB or the plasma membrane, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, at least about 20-fold, at least about 21-fold, at least about 22-fold, at least about 23-fold, at least about 24-fold, at least about 25-fold, at least about 26-fold, at least about 27-fold, at least about 28-fold, at least about 29-fold, or at least about 30-fold compared to the free payload.

[0348] In some aspects, the micelles of the present disclosure can be used to target stem cells, *e.g.*, to deliver therapeutic molecules (*e.g.*, therapeutic polynucleotides) or gene therapy components. In other aspects, the micelles of the present disclosure can be used to treat cancer. For example, micelles of the present disclosure can target a marker specific for a certain type of cancer, *e.g.*, a glioma, breast cancer, pancreatic cancer, liver cancer, skin cancer, or cervical cancer, and carry as payload a therapeutic molecule (*e.g.*, a therapeutic polynucleotide, a peptide, or a small molecule).

[0349] In specific aspects, the micelles of the present disclosure can be used to treat pancreatic cancer. In some aspects, the targeting moiety directing the micelles of the present disclosure to pancreatic tissues is a cyclic RGD peptide. In other aspects, the targeting moiety

directing the micelles of the present disclosure to pancreatic tissues is a biomarker predominantly or exclusively expressed on the surface of normal or cancerous pancreatic cells.

VIII. Kits

[0350] The present disclosure also provides kits, or products of manufacture, comprising a cationic carrier unit, a micelle, or a pharmaceutical composition of the present disclosure and optionally instructions for use. In some aspects, the kit or product of manufacture comprises a cationic carrier unit, a micelle, or a pharmaceutical composition of the present disclosure in one or more containers. In some aspects, the kit or product of manufacture comprises a cationic carrier unit, a micelle, or a pharmaceutical composition of the present disclosure and a brochure. In some aspects, the kit or product of manufacture comprises a cationic carrier unit, a micelle, or a pharmaceutical composition of the present disclosure and instructions for use. One skilled in the art will readily recognize that a cationic carrier unit, a micelle, or a pharmaceutical composition of the present disclosure, or combinations thereof, can be readily incorporated into one of the established kit formats which are well known in the art.

[0351] In some aspects, the kit or product of manufacture comprises a cationic carrier unit of the present disclosure in dry form in a container (*e.g.*, a glass vial), and optionally a vial with a solvent suitable to hydrate the dry the cationic carrier unit, and optionally instructions for the hydration of the cationic carrier unit and the formation of micelles. In some aspects, the kit or product of manufacture further comprises at least one additional container (*e.g.*, a glass vial) with the micelle's anionic payload (*e.g.*, an mRNA). In some aspects, the kit or product of manufacture comprises a cationic carrier unit of the present disclosure in a dry form and the micelle's anionic payload also in dry form in the same container, or in different containers. In some aspects, the kit or product of manufacture comprises a cationic carrier unit of the present disclosure in solution and the micelle's anionic payload also in solution in the same container, or in different containers. In some aspects, the kit or product of manufacture comprises a micelle of the present disclosure in solution, and instructions for use. In some aspects, the kit or product of manufacture comprises a micelle of the present disclosure in dry form, and instructions for use (*e.g.*, instructions for reconstitution and administration).

[0352] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology,

microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook et al., ed. (1989) *Molecular Cloning A Laboratory Manual* (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook et al., ed. (1992) *Molecular Cloning: A Laboratory Manual*, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) *DNA Cloning*, Volumes I and II; Gait, ed. (1984) *Oligonucleotide Synthesis*; Mullis et al. U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) *Nucleic Acid Hybridization*; Hames and Higgins, eds. (1984) *Transcription And Translation*; Freshney (1987) *Culture Of Animal Cells* (Alan R. Liss, Inc.); *Immobilized Cells And Enzymes* (IRL Press) (1986); Perbal (1984) *A Practical Guide To Molecular Cloning*; the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) *Gene Transfer Vectors For Mammalian Cells*, (Cold Spring Harbor Laboratory); Wu et al., eds., *Methods In Enzymology*, Vols. 154 and 155; Mayer and Walker, eds. (1987) *Immunochemical Methods In Cell And Molecular Biology* (Academic Press, London); Weir and Blackwell, eds., (1986) *Handbook Of Experimental Immunology*, Volumes I-IV; *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986);); Crooke, *Antisense drug Technology: Principles, Strategies and Applications*, 2nd Ed. CRC Press (2007) and in Ausubel et al. (1989) *Current Protocols in Molecular Biology* (John Wiley and Sons, Baltimore, Md.).

[0353] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0354] The following examples are offered by way of illustration and not by way of limitation.

Examples

Example 1

[0355] **Synthesis of alkyne modified tyrosine:** A mixture of N-(tert-Butoxycarbonyl)-L-tyrosine methyl ester (Boc-Tyr-OMe) (0.5g, 1.69 mmol) and K₂CO₃ (1.5 equiv., 2.54 mmol) in acetonitrile (4.0 ml) was added dropwise to propargyl bromide (1.2 equiv., 2.03 mmol).

[0356] The reaction mixture was heated at 60 °C for 16 hours. After the reaction, the reaction mixture was extracted by water with Ethyl Acetate (EA). Then, the organic layer was washed with a brine solution. The crude material was purified by flash column (EA in hexane 10%). Next, the resulting product was dissolved in Tetrahydrofuran (7.0 ml) and 6.0 M HCl (7.0 ml), and heated at 65 °C for 16 hrs. Afterwards, the dioxane was removed and the product was extracted using EA. Then, an aqueous NaOH (1.0 M) solution was added to the mixture until the pH value

become 7. The reactant was concentrated by evaporator and centrifuged at 12,000 rpm at 0°C. The precipitate was washed with deionized water and lyophilized prior to use.

[0357] **Synthesis of (methoxy or) azido-poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-PLL):** Poly(ethylene glycol)-*b*-poly(L-lysine) was synthesized by ring opening polymerization of Lys(TFA)-NCA with azido PEG (N3-PEG) as a macroinitiator. In brief, N3-PEG (600 mg, 0.12 mmol) and Lys(TFA)-NCA (1447 mg, 5.4 mmol) were separately dissolved in DMF containing 1M thiourea and DMF. Lys(TFA)-NCA solution was dropped into the N3-PEG solution by micro syringe needle and the reaction mixture were stirred at 37 °C for 3 days. The reaction bottles were purged with Ar and vacuum. All reactions were conducted under Ar atmosphere.

[0358] After the reaction, the mixture was precipitated into an excess amount of diethyl ether. Then, the mixture was filtered and white powder was obtained after drying in vacuo. For the deprotection of the TFA group in PEG-PLL(TFA), N3-PEG-PLL (500 mg) was dissolved in methanol (60 mL), and 1N NaOH (6 mL) was dropped into the polymer solution with stirring. The mixture was maintained for 1 day with stirring at 37°C. The reaction mixture was dialyzed against 10 mM HEPES for 4 times and distilled water. White powder of N3-PEG-PLL(NH₂) was obtained after lyophilization.

[0359] **Synthesis of (Methoxy or) azide poly(ethylene glycol)-*b*-poly(L-lysine/mercaptopropanamide) (PEG_{5K}-PLL₈₀(SH₁₆) (Compound A):** In order to enhance the stability of the micelles, a thiolated end group was introduced into the polymer backbone as a hydrophobic moiety. First, (methoxy or) azido-Poly(ethylene glycol)-*b*-poly(L-lysine/mercaptopropanamide) ((MeO- or) N₃-PEG_{5K}-PLL₈₀(SH₁₆)) was synthesized by chemical modification of PEG_{5K}-PLL₈₀-NH₂ and 3,3'-dithiodipropionic acid in the presence of EDC/NHS: (a) PEG_{5K}-PLL₈₀-NH₂ (100 mg) was dissolved in a mixture of deionized water and methanol (1:1). (b) 3,3'-dithiodipropionic acid (65.6 mg, 0.17 equiv. to NH₂ of PEG-PLL) was dissolved in MeOH. (c) EDC (20.5 mg, 0.2 equiv. to NH₂ of PEG-PLL) and NHS (12.3 mg, 0.2 equiv. to NH₂ of PEG-PLL) were added into to 3,3'-dithiodipropionic acid solution of (b), which was the combined with the PEG_{5K}-PLL₈₀-NH₂ solution of (a).

[0360] After 4 hours of stirring the resulting mixture at 37 °C, the reactant was dialyzed (MWCO = 7,000~8,000) against MeOH for 2 hours, and 1,4-dithiotritol (DTT, 20.6 mg, 0.14 equiv. to NH₂ of PEG-PLL) was directly added into the membrane to cleave the disulfide bond of the polymer side chain.

[0361] The membrane was incubated for 30 min, and dialyzed against 50% MeOH for 2 hours and dialyzed against deionized water for 24 hours. The solution was filtered with syringe

filter (0.45 μ m) and lyophilized for 2 days. A schematic of (PEG_{5K}-PLL₈₀(SH₁₆)) is shown in **FIG. 2A**.

[0362] **Synthesis of (Methoxy or) azide poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) (PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) (Compound B):** In order to enhance the stability of the micelles, a thiolated end group comprising nicotinamide was introduced into the polymer backbone as a hydrophobic moiety. First of all, (methoxy or) azido-Poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) ((MeO- or) N₃-PEG_{5K}-PLL₈₀ (Nic₅/SH₃₅)) was synthesized by chemical modification of PEG_{5K}-PLL₈₀-NH₂ and nicotinic acid in the presence of EDC/NHS.

[0363] PEG_{5K}-PLL₈₀-NH₂ (200 mg) and nicotinic acid (88 mg, 0.7 equiv. to NH₂ of PEG-PLL) were separately dissolved in mixture of deionized water and methanol (1:1). EDC (205.2 mg, 1 equiv. to NH₂ of PEG-PLL) was added into nicotinic acid solution and NHS (123.2 mg, 1 equiv. to NH₂ of PEG-PLL) stepwise added into the mixture. After 30 min of post incubation at room temperature, the reaction mixture was added into the N₃-PEG-PLL(NH₂) solution.

[0364] The reaction mixture was maintained at 37 °C for 16 hours with stirring. Secondly, 3,3'-dithiodipropionic acid (65.6 mg, 0.17 equiv. to NH₂ of PEG-PLL), EDC (41.8 mg, 0.25 equiv. to NH₂ of PEG-PLL) and NHS (25.1 mg, 0.25 equiv. to NH₂ of PEG-PLL) were dissolved in MeOH and directly added into the reaction mixture. After 4 hours of stirring the mixture at 37 °C, the reactant was dialyzed (MWCO = 7,000~8,000) against MeOH for 2 hours, and 1,4-dithiotritol (DTT, 20.6 mg, 0.14 equiv. to NH₂ of PEG-PLL) was directly added into the membrane to cleavage the disulfide bond of polymer side chain. The membrane was incubated for 30 min, and dialyzed against 50% MeOH for 2 hours and dialyzed against deionized water for 24 hours. The solution was filtered with syringe filter (0.45 μ m) and lyophilized for 2 days. A schematic of (PEG_{5K}-PLL₈₀(Nic₅/SH₃₅)) is shown in **FIG. 2B**.

[0365] **Synthesis of (Methoxy or) azide poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) (PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) (Compound C):** In order to enhance the stability of the micelles, nicotinamide and a thiolated end group were introduced into the polymer backbone as a hydrophobic moiety. First, (methoxy or) azido-Poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) ((MeO- or) N₃-PEG_{5K}-PLL₈₀ (Nic₁₉/SH₂₃)) was synthesized by chemical modification of PEG_{5K}-PLL₈₀-NH₂ and nicotinic acid in the presence of EDC/NHS. PEG_{5K}-PLL₈₀-NH₂ (200 mg) and nicotinic acid (88 mg, 0.7 equiv. to NH₂ of PEG-PLL) were separately dissolved in mixture of deionized water and methanol (1:1). EDC (205.2 mg, 1 equiv. to NH₂ of PEG-PLL) was added into nicotinic acid

solution and NHS (123.2 mg, 1 equiv. to NH_2 of PEG-PLL) stepwise added into the mixture. After 30 min of post incubation at room temperature, the reaction mixture was added into the N3-PEG-PLL(NH_2) solution.

[0366] The reaction mixture was maintained at 37 °C for 16 hours with stirring. Secondly, 3,3'-dithiodipropionic acid (18.8 mg, 0.17 equiv. to NH_2 of PEG-PLL), EDC (41.8 mg, 0.25 equiv. to NH_2 of PEG-PLL) and NHS (25.1 mg, 0.25 equiv. to NH_2 of PEG-PLL) were dissolved in MeOH and directly added into the reaction mixture. After 4 hours of stirring the mixture at 37 °C, the reactant was dialyzed (MWCO = 7,000~8,000) against MeOH for 2 hours, and 1,4-dithiotritol (DTT, 20.5 mg, 0.14 equiv. to NH_2 of PEG-PLL) was directly added into the membrane to cleavage the disulfide bond of polymer side chain. The membrane was incubated for 30 min, and dialyzed against 50% MeOH for 2 hours and dialyzed against deionized water for 24 hours. The solution was filtered with syringe filter (0.45 μm) and lyophilized for 2 days. A schematic of (PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃)) is shown in **FIG. 2C**.

[0367] **Synthesis of (Methoxy or) azide poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) (PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆)) (Compound D):** In order to enhance the stability of the micelles, nicotinamide and a thiolated end group were introduced into the polymer backbone as a hydrophobic moiety. First, (methoxy or) azido-Poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) ((MeO- or) N3-PEG_{5K}-PLL₈₀ (Nic₃₂/SH₁₆)) was synthesized by chemical modification of PEG_{5K}-PLL₈₀-NH₂ and nicotinic acid in the presence of EDC/NHS. PEG_{5K}-PLL₈₀-NH₂ (200 mg) and nicotinic acid (131 mg, 1.0 equiv. to NH_2 of PEG-PLL) were separately dissolved in mixture of deionized water and methanol (1:1). EDC (307.8 mg, 1 equiv. to NH_2 of PEG-PLL) was added into nicotinic acid solution and NHS (184.8 mg, 1 equiv. to NH_2 of PEG-PLL) stepwise added into the mixture. After 30 min of post incubation at room temperature, the reaction mixture was added into the N₃-PEG_{5K}-PLL₈₀ (NH₂) solution.

[0368] The reaction mixture was maintained at 37 °C for 16 hours with stirring. Secondly, 3,3'-dithiodipropionic acid (9.4 mg, 0.17 equiv. to NH_2 of PEG-PLL) EDC (41.8 mg, 0.25 equiv. to NH_2 of PEG-PLL) and NHS (25.1 mg, 0.25 equiv. to NH_2 of PEG-PLL) were dissolved in MeOH and directly added into the reaction mixture. After 4 hours of stirring the mixture at 37 °C, the reactant was dialyzed (MWCO = 7,000~8,000) against MeOH for 2 hours, and 1,4-dithiotritol (DTT, 10.3 mg, 0.14 equiv. to NH_2 of PEG-PLL) was directly added into the membrane to cleavage the disulfide bond of polymer side chain. The membrane was incubated for 30 min, and dialyzed against 50% MeOH for 2 hours and dialyzed against deionized water for 24 hours. The solution

was filtered with syringe filter (0.45 μ m) and lyophilized for 2 days. A schematic of (PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆)) is shown in **FIG. 2D**.

[0369] **Synthesis of (Methoxy or) azide poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide) (PEG_{5K}-PLL₈₀(Nic₁₇)) (Compound E):** In order to enhance the stability of the micelle, nicotinamide and a thiolated end group were introduced into the polymer backbone as a hydrophobic moiety. First, (methoxy or) azido-Poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide) ((MeO- or) N₃- PEG_{5K}-PLL₈₀ (Nic₁₇)) was synthesized by chemical modification of PEG_{5K}-PLL₈₀-NH₂ and nicotinic acid in the presence of EDC/NHS. PEG_{5K}-PLL₈₀-NH₂ (150 mg) and nicotinic acid (79.1 mg, 0.8 equiv. to NH₂ of PEG-PLL) were separately dissolved in mixture of deionized water and methanol (1:1). EDC (184.9 mg, 1.2 equiv. to NH₂ of PEG-PLL) was added into nicotinic acid solution and NHS (111.0 mg, 1.2 equiv. to NH₂ of PEG-PLL) stepwise added into the mixture. After 30 min of post incubation at room temperature, the reaction mixture was added into the N₃-PEG-PLL(NH₂) solution.

[0370] The reaction mixture was maintained at 37 °C for 16 hours with stirring. The crude product was dialyzed against deionized water for 24 hours. The solution was filtered with syringe filter (0.45 μ m) and lyophilized for 2 days. A schematic of (PEG_{5K}-PLL₈₀(Nic₁₇)) is shown in **FIG. 2E**.

[0371] **Synthesis of Phenyl alanine-poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/ mercaptopropanamide) (Phe-PEG-PLL(Nic/SH)):** In order to target brain endothelial tissue in a blood stream, as a LAT1 targeting amino acid, phenylalanine was introduced by click reaction between N₃-PEG-PLL(Nic/ss) and alkyne modified tyrosine in the presence of copper catalyst.

[0372] In brief, N₃-PEG-PLL(Nic/ss) (30 mg, 3.2 μ mol) and alkyne modified phenyl alanine (1.31 mg, 6.4 μ mol) were dissolved in deionized water. Then, CuSO₄•H₂O (0.172 mg, 0.69 μ mol) and ascorbic acid (0.3 mg, 1.7 μ mol) were added into the mixture solution. The reaction mixture was maintained with stirring for 16 hours at room temperature. After the reaction, the mixture was transferred into a dialysis membrane (MWCO = 7,000) and dialyzed against deionized water for 1 days. The final product was obtained after lyophilization.

Example 2

Polyion Complex (PIC) micelle preparation

[0373] Once the cationic carrier units of the present disclosure were generated as described in Example 1, micelles were produced. The micelles described in the present example comprised cationic carrier units combined with mRNA payloads with different lengths.

[0374] **(a) mRNA 800 nucleotides with Compound B:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) and mRNA. PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 1.26 mg/mL. Then, an mRNA solution (0.5 μ M) in RNase free water was mixed with the polymer solution at a 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 3.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.33 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0375] **(b) mRNA 800 nucleotides with Compound C:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) and mRNA. PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 1.32 mg/mL. Then, an mRNA solution (0.38 μ M) in RNase free water was mixed with the polymer solution at a 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine- in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 4.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.25 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0376] **(c) mRNA 800 nucleotides with Compound D:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) and mRNA. PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 1.59 mg/mL. Then, an mRNA solution (0.3 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine- in-polymer (N) and phosphate-in-mRNA

(P). The optimal N to P ratio was 5.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.2 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0377] **(d) mRNA 1,800 nucleotides with Compound B:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) and mRNA. PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 2.73 mg/mL. Then, an mRNA solution (0.25 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 6.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.17 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0378] **(e) mRNA 1,800 nucleotides with Compound C:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) and mRNA. PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 2.85 mg/mL. Then, an mRNA solution (0.25 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 6.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.17 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0379] **(f) mRNA 1,800 nucleotides with Compound D:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) and mRNA. PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 3.44 mg/mL. Then, an mRNA solution (0.21 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine- in-polymer (N) and phosphate-in-mRNA

(P). The optimal N to P ratio was 7.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.14 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0380] **(g) mRNA 3,800 nucleotides with Compound B:** Nano-sized PIC micelle were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) and mRNA. PEG_{5K}-PLL₈₀(Nic₅/SH₃₅) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 6.03 mg/mL. Then, an mRNA solution (0.21 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 7.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.14 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0381] **(h) mRNA 3,800 nucleotides with Compound C:** Nano-sized PIC micelle were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) and mRNA. PEG_{5K}-PLL₈₀(Nic₁₉/SH₂₃) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 6.3 mg/mL. Then, an mRNA solution (0.19 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA (P). The optimal N to P ratio was 8.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.13 μ M of mRNA Conc.) were stored at 4 °C prior to use.

[0382] **(i) mRNA 3,800 nucleotides with Compound D:** Nano-sized PIC micelles were prepared by mixing of MeO- or Phe-PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) and mRNA. PEG_{5K}-PLL₈₀(Nic₃₂/SH₁₆) was dissolved in 200 mM DTT (in 10 mM HEPES buffer) at 7.59 mg/mL. Then, an mRNA solution (0.15 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of mRNA to polymer. The mixing ratios of polymer to mRNA were determined by optimization of the micelle-forming ratio between amine-in-polymer (N) and phosphate-in-mRNA

(P). The optimal N to P ratio was 10.0. The mixture of polymer and mRNA was vigorously mixed for 3 min by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles. Particle size distribution and scattering light intensity (SLI) were measured by Zeta-sizer with 634 nm wavelength. The resulting micelles (0.1 μ M of mRNA Conc.) were stored at 4 °C prior to use.

Example 3

In vitro mRNA expression

[0383] **Cell line and culture:** The human embryonic kidney cell line (HEK-293T) and human lung cancer cell line (A549) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Welgene, LM 001-05) and RPMI 1640 (Welgene, LM 011-01) medium supplemented with 10% FBS (Gibco 26140-079) and penicillin streptomycin (Gibco 15140-122). Cells were seeded into 6-well plates at a density of 1×10^6 cells/well. Cells were cultured for 24 h in a culture medium containing 2% FBS and 0.5% penicillin-streptomycin. mRNA-loaded micelles or Lipofectamine 2000/mRNA complexes as a positive control were transfected into the cells.

[0384] **Transfection of the cell line:** Cells were seeded into 6-well plates at a density of 1×10^6 cells/well. Cells were cultured for 24 h in medium containing 2% FBS and 0.5% penicillin-streptomycin and were transfected with PBS, mRNA-loaded micelles, or Lipofectamine 2000/mRNA complexes (as positive controls).

[0385] The Lipofectamine 2000 transfection reagent (Invitrogen, 11668019) was prepared by diluting 10 μ L of stock solution into 100 μ L of Opti-MEM. For mRNA formulation, Lipofectamine 2000 was mixed with 5 μ g of mRNA. The mixture was gently pipetted and incubated at room temperature for 15 min to form Lipofectamine/mRNA complexes.

[0386] mRNA micelles were diluted with Opti-MEM to adjust the concentration of mRNA so it was the same concentration as is the Lipofectamine/mRNA complexes.

[0387] 5 μ g of mRNA containing Lipofectamine/mRNA complexes or mRNA micelle were added into the cells respectively. After 30 min of post incubation, the cells were harvested, and RNA was isolated.

[0388] **RNA extraction and qRT-PCR:** Total RNA was isolated from the cells using TRIzol reagent (Thermofisher, 15596018) according to the manufacturer's protocol. 500 ng of RNA were reverse transcribed into cDNA using the TOPscript™ RT DryMIX (dN6 plus) (Enzynomics, RT210) according to the manufacturer's protocol. RT-qPCR was performed with the resultant cDNA as a template using TOPreal qPCR 2x PreMIX (SYBR Green with low ROX)

(Enzynomics, RT500M) according to the manufacturer's protocol in the Bio-Rad CFX96 cycler (Bio-Rad Laboratories, Inc.). The relative level of HA mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method with normalization to hGAPDH.

[0389] **Results:** After 30 min of post transfection, almost no mRNA was detected by qRT-PCR. The group treated with mRNA-loaded micelles it showed a mRNA level of approximately 1×10^3 . In contrast, a relative mRNA level of $1 \times 10^6 \sim 1 \times 10^7$ was observed in the group treated with Lipofectamine 2000/mRNA complex. Lipofectamine 2000 is a commercial transfection reagent that is used for in vitro experimentation. The mRNA-loaded micelles were uptaken by the cells *in vitro* despite being specifically designed for *in vivo* delivery. See FIG. 11.

Example 4

LAT-1 expression level *in vivo*

[0390] **LAT-1 distribution in mouse tissues:** The animals were anesthetized and muscles (Gastrocnemius; GAS, Quadriceps femoris; QF, Biceps femoris; BF, Tibialis anterior; TA) were collected. Homogenized tissues were lysed using RIPA lysis and extraction buffer containing protease inhibitors, and protein concentration was determined using a BCA protein assay kit.

[0391] Equal amounts of total protein were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membranes electrophoretically. The membranes were incubated with LAT1 (Santa Cruz, SC-374232) or GAPDH (Santa Cruz, SC-32233) primary antibody at 4°C overnight and then incubated with a horseradish peroxidase (HRP) – conjugated secondary antibody for 1 h at RT.

[0392] **Results:** To assess the LAT1 distribution level in mouse muscles, we performed Western blot assays. 50µg of protein was used per Western blot. As a result of the evaluation, it was showed that LAT1 was expressed in all the GAS, QF, BF, and TA sites. There was a difference in expression patterns in BALB/c mouse and DBA/2J mouse, but it was not significant. These results indicated that the muscles can be targeted using a LAT1-targeted polymeric vehicle. See FIGs. 12A-12D.

Example 5

In vivo mRNA expression

[0393] 5 µg of mRNA encoding firefly luciferase (Luc) was formulated with polymeric carrier, Lipofectamine 2000 (Thermo Fisher Scientific), and *in vivo*-jetRNA (Polyplus) in a total volume of 50 µl.

[0394] Luc mRNA was injected into BALB/c mice through intramuscular administration (bilateral). Mice were injected VivoGlo Luciferin (Promega) at a dose of 150mg/kg intraperitoneally. Bioluminescence imaging was performed with an IVIS imaging system (PerkinElmer).

[0395] **Results:** The luciferase signals of Luc-mRNA encapsulated in Lipofectamine2000 (Lipo+Luc) or *in vivo* jetRNA (jetRNA+Luc, not shown) started to be observed after approximately 6 hours post-injection. The level of the signals was reduced by the first day, and practically no signal remained after 2 or 3 days. **FIG. 13A.**

[0396] In contrast, although the maximum luciferase signal in the mRNA-loaded micelle treated group was not higher than the signal observed using other delivery reagents, the response over time was different. Signal intensity was similar to that observed with Luc-mRNA encapsulated in Lipofectamine2000 (Lipo+Luc) or *in vivo* jetRNA (jetRNA+Luc, not shown) at 6 hours, but it started to increase after 24 hours. High expression levels were observed for 3 to 5 days, and the intensity of the signal gradually decreased. Expression was maintained for up to 7 days. See **FIG. 13B.**

[0397] These results indicated that mRNA delivery using polymeric micelle system was more stable than delivery using other delivery reagents. The sustained mRNA expression indicated that the cationic carriers of the present disclosure, such as polymeric micelle, can be effectively used to deliver mRNA and release the attached mRNA for an extended period of time, which makes them suitable, for example, for mRNA vaccine delivery.

[0398] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections may set forth one or more but not all exemplary aspects of the present disclosure as contemplated by the inventor(s), and thus, are not intended to limit the present disclosure and the appended claims in any way.

[0399] The present disclosure has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0400] The foregoing description of the specific embodiments will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art,

readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0401] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A cationic carrier unit comprising
[CC]-L1-[CM]-L2-[HM] (Schema I);
[CC]-L1-[HM]-L2-[CM] (Schema II);
[HM]-L1-[CM]-L2-[CC] (Schema III);
[HM]-L1-[CC]-L2-[CM] (Schema IV);
[CM]-L1-[CC]-L2-[HM] (Schema V); or
[CM]-L1-[HM]-L2-[CC] (Schema VI);

wherein

CC is a positively charged carrier moiety;

CM is a crosslinking moiety;

HM is a hydrophobic moiety; and,

L1 and L2 are independently optional linkers, and

wherein the number of HM is less than 40% relative to [CC] and [CM].

2. The cationic carrier unit of claim 1, wherein the number of HM is less than 39%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or about 1% relative to [CC] and [CM].

3. The cationic carrier unit of claim 1, wherein the number of HM is between about 35% and about 1%, about 35% and about 5%, about 35% and about 10%, about 35% and about 15%, about 35% and about 20%, about 35% and about 25%, about 35% and about 30%, about 30% and about 1%, about 30% and about 5%, about 30% and about 10%, about 30% and about 15%, about 30% and about 20%, about 30% and about 25%, about 25% and about 1%, about 25% and about 5%, about 25% and about 10%, about 25% and about 15%, about 25% and about 20%, about 20% and about 1%, about 20% and about 5%, about 20% and about 10%, about 20% and about 15%, about 15% and about 1%, about 15% and about 5%, about 15% and about 10%, about 10% and about 1%, or about 10% and about 5% relative to [CC] and [CM].

4. The cationic carrier unit of claim 1, wherein the number of HM is between about 39% and about 30%, about 30% and about 20%, about 20% and about 10%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM].

5. The cationic carrier unit of claim 1, wherein the number of HM is about 39%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, or about 1% relative to [CC] and [CM].
6. The cationic carrier unit of any one of claims 1 to 5, wherein the cationic carrier unit is capable of interacting with an anionic payload.
7. The cationic carrier unit of claim 6, wherein the anionic payload comprises a nucleotide sequence having less than 4000 nucleotides, less than about 3500, less than about 3000, less than about 2500, less than about 2000, less than about 1500, less than about 1000, less than about 900, less than about 800, less than about 700, less than about 600, less than about 500, less than about 400, less than about 200, or less than about 150 in length.
8. The cationic carrier unit of any one of claims 1 to 7, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 20, about 1 and about 19, about 1 and about 18, about 1 and about 17, about 1 and about 16, about 1 and about 15, about 1 and about 14, about 1 and about 13, about 1 and about 12, about 1 and about 11, about 1 and about 10, about 1 and about 9, about 1 and about 8, about 1 and about 7, about 1 and about 6, or about 1 and about 5.
9. The cationic carrier unit of any one of claims 1 to 7, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10.
10. The cationic carrier unit of claim 6 or 7, wherein the anionic payload comprises a nucleotide sequence having about 100 nucleotides to about 1000 nucleotides in length.
11. The cationic carrier unit of claim 10, wherein the number of HM is between 39% and about 30%, about 30% and about 20%, about 20% and about 10%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM].
12. The cationic carrier unit of claim 10 or 11, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P

ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 10 or about 3 and about 7.

13. The cationic carrier unit of claim 10 or 11, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 1 and about 2, about 2 and about 3, about 3 and about 4, or about 4 and about 5.

14. The cationic carrier unit of claim 10 or 11, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 1, about 2, about 3, about 4, or about 5.

15. The cationic carrier unit of claim 6 or 7, wherein the anionic payload comprises a nucleotide sequence having about 1000 nucleotides to about 2000 nucleotides in length.

16. The cationic carrier unit of claim 15, wherein the number of HM is less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1% relative to [CC] and [CM].

17. The cationic carrier unit of claim 15, wherein the number of HM is between about 30% and about 20%, about 30% and about 25%, about 25% and about 20%, about 25% and about 15%, about 20% and about 10%, about 20% and about 5%, about 10% and about 1%, about 10% and about 5%, and about 5% and about 1% relative to [CC] and [CM].

18. The cationic carrier unit of any one of claims 15 to 17, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 4 and about 7.

19. The cationic carrier unit of any one of claims 15 to 17, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 4 and about 5 or about 5 and about 6.

20. The cationic carrier unit of any one of claims 15 to 17, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 4, about 5, about 6, or about 7.
21. The cationic carrier unit of claim 6 or 7, wherein the anionic payload comprises a nucleotide sequence having about 2000 nucleotides to about 3000 nucleotides in length.
22. The cationic carrier unit of claim 21, wherein the number of HM is less than about 20%, less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% relative to [CC] and [CM].
23. The cationic carrier unit of claim 21, wherein the number of HM is less than about 20% to about 1%, from about 20% to about 5%, from about 20% to about 10%, from about 20% to about 15%, from about 15% to about 1%, from about 15% to about 5%, from about 15% to 10%, from about 10% to about 1%, from about 10% to about 5%, or from about 5% to about 1% nucleotides relative to [CC] and [CM].
24. The cationic carrier unit of claim 21, wherein the number of HM is about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1% relative to [CC] and [CM].
25. The cationic carrier unit of any one of claims 21 to 24, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 6 and about 9.
26. The cationic carrier unit of any one of claims 21 to 24, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 6 and about 7 or about 7 and about 8.

27. The cationic carrier unit of any one of claims 21 to 24, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 6, about 7, about 8, or about 9.
28. The cationic carrier unit of claim 6 or 7, wherein the anionic payload comprises a nucleotide sequence having about 3000 nucleotides to about 4000 nucleotides in length.
29. The cationic carrier unit of claim 28, wherein the number of HM is less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% relative to [CC] and [CM].
30. The cationic carrier unit of claim 28, wherein the number of HM is from about 10% to about 1%, from about 10% to about 5%, or from about 5% to about 1% nucleotides relative to [CC] and [CM].
31. The cationic carrier unit of claim 28, wherein the number of HM is about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1% relative to [CC] and [CM].
32. The cationic carrier unit of any one of claims 28 to 31, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 10.
33. The cationic carrier unit of any one of claims 28 to 31, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is between about 7 and about 8 or about 8 and about 9.
34. The cationic carrier unit of any one of claims 28 to 31, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload in the solution is about 7, about 8, about 9, or about 10.

35. The cationic carrier unit of claims 6 to 34, wherein the anionic payload comprises mRNA, cDNA, or any combination thereof.
36. The cationic carrier unit of any one of claims 1 to 35, which further comprises a water-soluble polymer (WP).
37. The cationic carrier unit of claim 36, wherein the water-soluble polymer is attached to [CC], [HM], or [CM].
38. The cationic carrier unit of claim 36 or 37, wherein the water-soluble polymer is attached to the N terminus of [CC], [HM], or [CM].
39. The cationic carrier unit of claim 36 or 37, wherein the water-soluble polymer is attached to the C terminus of [CC], [HM], or [CM].
40. The cationic carrier unit of any one of claims 36 to 39, which comprises:
[WP]-L3-[CC]-L1-[CM]-L2-[HM] (Schema I');
[WP]-L3-[CC]-L1-[HM]-L2-[CM] (Schema II');
[WP]-L3-[HM]-L1-[CM]-L2-[CC] (Schema III');
[WP]-L3-[HM]-L1-[CC]-L2-[CM] (Schema IV');
[WP]-L3-[CM]-L1-[CC]-L2-[HM] (Schema V'); or
[WP]-L3-[CM]-L1-[HM]-L2-[CC] (Schema VI').
41. The cationic carrier unit of any one of claims 36 to 40, wherein the water-soluble polymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof.
42. The cationic carrier unit of any one of claims 36 to 41, wherein the water-soluble polymer comprises polyethylene glycol ("PEG"), polyglycerol, or poly(propylene glycol) ("PPG").
43. The cationic carrier unit of any one of claims 36 to 42, wherein the water-soluble polymer comprises:



wherein n is 1-1000.

44. The cationic carrier unit of claim 43, wherein the n is at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, or at least about 141.

45. The cationic carrier unit of claim 43, wherein the n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 140 to about 150, or about 150 to about 160.

46. The cationic carrier unit of any one of claims 36 to 45, wherein the water-soluble polymer is linear, branched, or dendritic.

47. The cationic carrier unit of any one of claims 1 to 46, wherein the cationic carrier moiety comprises one or more amino acids.

48. The cationic carrier unit of claim 47, wherein the cationic carrier moiety comprises at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, at least about 35, at least about 36, at least about 37, at least about 38, at least about 39, at least about 40, at least about 41, at least about 42, at least about 43, at least about 44, at least about 45, at least about 46, at least about 47, at least about 48, at least about 49, at least about 50, at least about 51, at least about 52, at least about 53, at least about 54, at least about 55, at least about 56, at least about 57, at least

about 58, at least about 59, at least about 60, at least about 61, at least about 62, at least about 63, at least about 64, at least about 65, at least about 66, at least about 67, at least about 68, at least about 69, at least about 70, at least about 71, at least about 72, at least about 73, at least about 74, at least about 75, at least about 76, at least about 77, at least about 78, at least about 79, or at least about 80 amino acids.

49. The cationic carrier unit of claim 47, wherein the cationic carrier moiety comprises at least 20, at least 30, at least 40, at least 50, at least 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, or at least about 150 amino acids.

50. The cationic carrier unit of claim 47, wherein the cationic carrier moiety comprises about 10 to about 60, about 15 to about 60, about 20 to about 60, about 25 to about 60, about 30 to about 60, about 35 to about 60, about 40 to about 60, about 10 to about 55, about 15 to about 55, about 20 to about 55, about 25 to about 55, about 30 to about 55, about 35 to about 55, about 40 to about 55, about 10 to about 50, about 15 to about 50, about 20 to about 50, about 25 to about 50, about 30 to about 50, about 35 to about 50, about 40 to about 50, about 10 to about 45, about 15 to about 45, about 20 to about 45, about 25 to about 45, about 30 to about 45, about 35 to about 45, about 40 to about 45, about 10 to about 40, about 15 to about 40, about 20 to about 40, about 25 to about 40, about 30 to about 40, about 35 to about 40, about 10 to about 35, about 15 to about 35, about 20 to about 35, about 25 to about 35, about 30 to about 35, about 35 to about 35, about 40 to about 35, or about 40 to about 35 amino acids.

51. The cationic carrier unit of claim 47, wherein the cationic carrier moiety comprises about 10, about 20, about 30, about 40, about 50, or about 60 amino acids.

52. The cationic carrier unit of any one of claims 47 to 51, wherein the amino acid comprises arginine, lysine, histidine, or any combination thereof.

53. The cationic carrier unit of any one of claims 1 to 52, wherein the cationic carrier moiety comprises about 20, about 30, about 40, about 50, or about 60 lysines.

54. The cationic carrier unit of any one of claims 1 to 52, wherein the cationic carrier moiety comprises about 40 lysines.

55. The cationic carrier unit of any one of claims 1 to 54, wherein the crosslinking moiety comprises one or more amino acids linked a crosslinking agent.
56. The cationic carrier unit of claim 55, wherein the crosslinking agent comprises a thiol group, a thiol derivative, or any combination thereof.
57. The cationic carrier unit of claim 55, wherein the crosslinking agent comprises a thiol group.
58. The cationic carrier unit of claim 55 to 57, wherein the amino acids in the crosslinking moiety comprise at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39 at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 amino acids.
59. The cationic carrier unit of any one of claims 55 to 57, wherein the amino acids in the crosslinking moiety comprise about 1 to about 40, about 5 to about 40, about 10 to about 40, about 15 to about 40, about 20 to about 40, about 1 to about 35, about 5 to about 35, about 10 to about 35, about 15 to about 35, about 20 to about 35, about 10 to about 50, about 15 to about 50, about 20 to about 50, about 25 to about 50, about 30 to about 40, about 10 to about 45, about 15 to about 45, about 20 to about 45, about 25 to about 45, about 30 to about 45, about 10 to about 40, about 15 to about 40, about 20 to about 40, about 25 to about 40, or about 30 to about 40 amino acids.
60. The cationic carrier unit of claim 55 to 57, wherein the amino acids in the crosslinking moiety comprise about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 amino acids.
61. The cationic carrier unit of any one of claims 55 to 60, wherein the amino acids in the crosslinking moiety comprise arginine, lysine, histidine, or any combination thereof.
62. The cationic carrier unit of any one of claims 55 to 61, wherein the amino acids in the crosslinking moiety comprise about 35 lysines.

63. The cationic carrier unit of any one of claims 55 to 61, wherein the amino acids in the crosslinking moiety comprise about 23 lysines.

64. The cationic carrier unit of any one of claims 55 to 61, wherein the amino acids in the crosslinking moiety comprises about 16 lysines.

65. The cationic carrier unit of any one of claims 1 to 64, wherein the hydrophobic moiety is capable of modulating an immune response, an inflammatory response, and/or a tissue microenvironment.

66. The cationic carrier unit of claim 65, wherein the hydrophobic moiety is capable of modulating an immune response.

67. The cationic carrier unit of claim 65, wherein the hydrophobic moiety is capable of modulating a tumor microenvironment in a subject with a tumor.

68. The cationic carrier unit of claim 65, wherein the hydrophobic moiety is capable of inhibiting or reducing hypoxia in the tumor microenvironment.

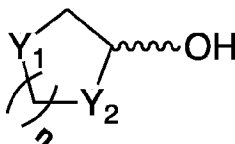
69. The cationic carrier unit of any one of claims 65 to 68, wherein the hydrophobic moiety comprises one or more amino acids linked to an imidazole derivative, an amino acid, a vitamin, or any combination thereof.

70. The cationic carrier unit of claim 65, wherein the hydrophobic moiety is capable of inhibiting or reducing an inflammatory response.

71. The cationic carrier unit of claim 70, wherein the hydrophobic moiety is one or more amino acids linked to a vitamin.

72. The cationic carrier unit of claim 71, wherein the vitamin comprises a cyclic ring or cyclic hetero atom ring and a carboxyl group or hydroxyl group.

73. The cationic carrier unit of claim 71, wherein the vitamin comprises:



wherein each of Y1 and Y2 is C, N, O, or S, and wherein n is 1 or 2.

74. The cationic carrier unit of any one of claims 71 to 73, wherein the vitamin is selected from the group consisting of vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D2, vitamin D3, vitamin E, vitamin M, vitamin H, and any combination thereof.

75. The cationic carrier unit of any one of claims 74, wherein the vitamin is vitamin B3.

76. The cationic carrier unit of any one of claims 1 to 75, wherein the hydrophobic moiety comprises at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, or at least about 32 amino acids, each linked to a vitamin.

77. The cationic carrier unit of any one of claims 1 to 75, wherein the hydrophobic moiety comprises from about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 5 to about 10, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 10 to about 15, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 15 to about 20, about 20 to about 35, about 20 to about 30, about 20 to about 25, about 25 to about 30, or about 25 to about 30 amino acids, each linked to a vitamin.

78. The cationic carrier unit of any one of claims 1 to 77, wherein the hydrophobic moiety comprises about 2 vitamin B3, about 3 vitamin B3, about 4 vitamin B3, about 5 vitamin B3, about 6 vitamin B3, about 7 vitamin B3, about 8 vitamin B3, about 9 vitamin B3, about 10 vitamin B3, about 11 vitamin B3, about 12 vitamin B3, about 13 vitamin B3, about 14 vitamin B3, about 15 vitamin B3, about 16 vitamin B3, about 17 vitamin B3, about 18 vitamin B3, about 19 vitamin B3, about 20 vitamin B3, about 21 vitamin B3, about 22 vitamin B3, about 23 vitamin B3, about 24 vitamin B3, about 25 vitamin B3, about 26 vitamin B3, about 27 vitamin B3, about 28 vitamin B3,

about 29 vitamin B3, about 30, about 31, about 32, about 33, about 34, or about 35 amino acids, each linked to vitamin B3.

79. The cationic carrier unit of claim 1 to 9, wherein the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 20 to about 40 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3.

80. The cationic carrier unit of claim 1 to 9, wherein the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 10 to about 20 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3.

81. The cationic carrier unit of claim 1 to 9, wherein the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 10 to about 30 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 10 lysine-vitamin B3.

82. The cationic carrier unit of any one of claims 1 to 9, wherein the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 13 to about 25 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 20 lysine-vitamin B3.

83. The cationic carrier unit of any one of claims 1 to 9, wherein the cationic carrier moiety comprises about 35 to about 45 lysines, the crosslinking moiety comprises about 13 to about 25 lysine-thiol, and the hydrophobic moiety comprises about 1 to about 20 lysine-vitamin B3.

84. The cationic carrier unit of any one of claims 1 to 83, wherein the water-soluble biopolymer moiety comprises about 120 to about 130 PEG units.

85. The cationic carrier unit of any one of claims 1 to 84, further comprising a targeting moiety (TM).

86. The cationic carrier unit of claim 85, wherein the targeting moiety is capable of targeting a tissue.

87. The cationic carrier unit of claim 86 wherein the tissue is liver, brain, kidney, lung, ovary, pancreas, thyroid, breast, stomach, or any combination thereof.

88. The cationic carrier unit of claim 87, wherein the targeting moiety is capable of being transported by large neutral amino acid transporter 1 (LAT1).

89. The cationic carrier unit of claim 88, wherein the targeting moiety is an amino acid.
90. The cationic carrier unit of claim 88, wherein the targeting moiety comprises a branched-chain or aromatic amino acid.
91. The cationic carrier unit of claim 90, wherein the targeting moiety is phenylalanine, valine, leucine, and/or isoleucine.
92. The cationic carrier unit of claim 91, wherein the amino acid is phenylalanine.
93. The cationic carrier unit of any one of claims 85 to 92, wherein the targeting moiety is linked to the water-soluble polymer.
94. The cationic carrier unit of claim 93, wherein the targeting moiety is linked to the water-soluble polymer by a linker.
95. A micelle comprising the cationic carrier unit of any one of claims 1 to 94 and an anionic payload, wherein the cationic carrier moiety of the cationic carrier complex and the anionic payload are associated with each other.
96. The micelle of claim 95, wherein the association is a covalent bond.
97. The micelle of claim 95, wherein the association is a non-covalent bond.
98. The micelle of claim 95, wherein the association is an ionic bond.
99. The micelle of any one of claims 95 to 98, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together and wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 1 and about 20.
100. The micelle of claim 99, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20.
101. The micelle of claim 99, wherein the positive charge of the cationic carrier moiety of the cationic carrier unit is sufficient to form a micelle when mixed with an anionic payload in a

solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 3, about 4, about 5, about 6, about 7, about 8, or about 9.

102. The micelle of any one of claims 95 to 101, wherein the cationic carrier unit is capable of protecting the anionic payload from degradation by a DNase and/or an RNase.

103. The micelle of any one of claims 95 to 102, wherein the anionic payload is not conjugated to the cationic carrier unit by a covalent bond and/or the anionic payload interacts with the cationic carrier moiety of the cationic carrier unit only via an ionic interaction.

104. The micelle of any one of claims 95 to 103, wherein the half-life of the anionic payload is extended compared to the half-life of a free anionic payload not incorporated into a micelle.

105. The micelle of any one of claims 95 to 104, wherein the anionic payload comprises a nucleotide sequence having about 1000 nucleotides to about 2000 nucleotides in length.

106. The micelle of claim 105, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 4 and about 7.

107. The micelle of claim 105 or 106, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 4 and about 5 or about 5 and about 6.

108. The micelle of any one of claims 105 to 107, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 4, about 5, about 6, or about 7.

109. The micelle of any one of claims 95 to 104, wherein the anionic payload comprises a nucleotide sequence having about 2000 nucleotides to about 3000 nucleotides in length.

110. The micelle of claim 109, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, and wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 6 and about 9.

111. The micelle of claim 109 or 110, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 6 and about 7 or about 7 and about 8.

112. The micelle of any one of claims 109 to 111, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 6, about 7, about 8, or about 9.

113. The micelle of any one of claims 95 to 104, wherein the anionic payload comprises a nucleotide sequence having about 3000 nucleotides to about 4000 nucleotides in length.

114. The micelle of claim 113, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 7 and about 10.

115. The micelle of claim 113 or 114, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is between about 7 and about 8 or about 8 and about 9.

116. The micelle of any one of claims 113 to 115, wherein the anionic payload and the cationic carrier unit are capable of forming a micelle when mixed together in a solution, wherein the N/P ratio of the cationic carrier unit and the anionic payload is about 7, about 8, about 9, or about 10.

117. The micelle of any one of claims 95 to 116, where the diameter of the micelle is between about 10 nm and about 200 nm, between about 20 nm and about 200 nm, between about 1nm and 100nm, between about 10nm and about 100nm, between about 10nm and about 90nm, between about 10nm and about 80nm, between about 10nm and about 70nm, between about 20nm and about 100nm, between about 20nm and about 90nm, between about 20nm and about 80nm, between about 20nm and about 70nm, between about 30nm and about 100nm, between about 30nm and about 90nm, between about 30nm and about 80nm, between about 30nm and about 70nm, between about 40nm and about 100nm, between about 40nm and about 90nm, between about 40nm and about 80nm, or between about 40nm and about 70nm.

118. The micelle of any one of claims 95 to 117, wherein the anionic payload comprises a nucleic acid.

119. The micelle of claim 118, wherein the nucleic acid comprises mRNA, miRNA sponge, tough decoy miRNA, gDNA, cDNA, pDNA, PNA, BNA, aptamer, or any combination thereof.

120. The micelle of claim 118 or 119, wherein the nucleic acid comprises at least one nucleoside analog.

121. The micelle of claim 120, wherein the nucleoside analog comprises Locked Nucleic Acid (LNA); 2'-O-alkyl-RNA; 2'-amino-DNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA, hexitol nucleic acid (HNA), intercalating nucleic acid (INA), constrained ethyl nucleoside (cEt), 2'-O-methyl nucleic acid (2'-OMe), 2'-O-methoxyethyl nucleic acid (2'-MOE), or any combination thereof.

122. The micelle of any one of claims 118 to 121, wherein the nucleic acid comprises a nucleotide sequence having 5 to 30 nucleotides in length.

123. The micelle of claim 122, wherein the nucleotide sequence is less than 4000, less than 3000, less than 2000, or less than 1000 nucleotides in length.

124. The micelle of claim 122 or 123, wherein the nucleotide sequence has a backbone, which comprises a phosphodiester linkage, a phosphotriester linkage, a methylphosphonate linkage, a phosphoramidate linkage, a phosphorothioate linkage, and combinations thereof.

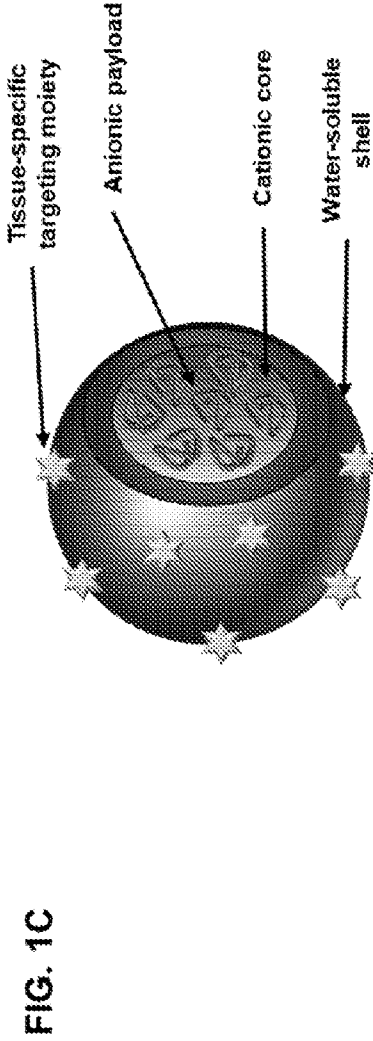
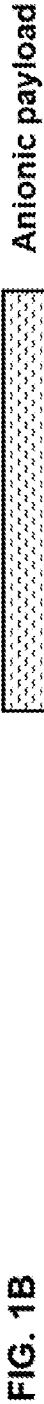
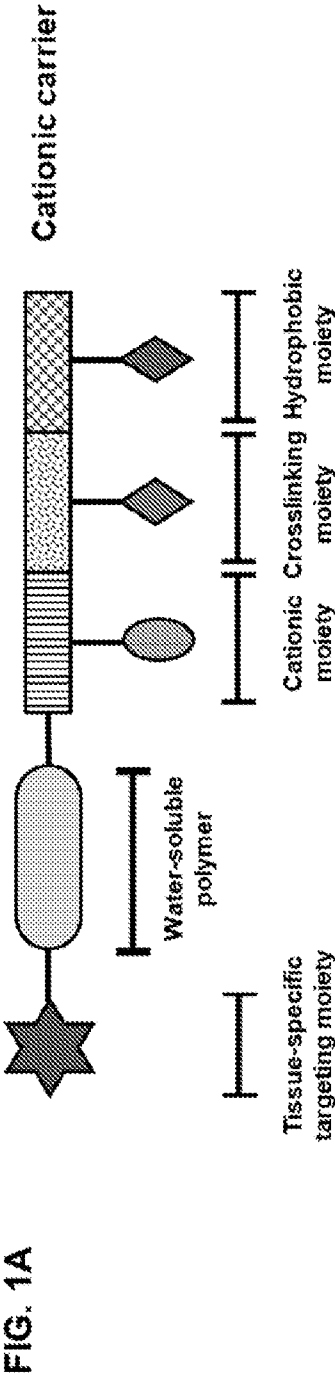
125. The micelle of claim 122, wherein the nucleic acid comprises the nucleotide sequence encoding PAPD5/7, G protein, Gephyrin, protein anchor, or MDA5 (IFIH1).

126. A composition comprising the cationic carrier unit of any one of claims 1 to 94 and an anionic payload.

127. A pharmaceutical composition comprising the cationic carrier unit of any one of claims 1 to 94, the micelle of any one of claims 95 to 125, the composition of claim 126, or and a pharmaceutically acceptable carrier.

128. A method of preparing the cationic carrier unit of any one of claims 1 to 94 comprising linking the cationic carrier moiety to the crosslinking moiety and the hydrophobic moiety.

129. The method of claim 128, further linking a water-soluble polymer and a targeting moiety.
130. The method of preparing the micelle of any one of claims 128 to 129, comprising mixing the cationic carrier unit with the anionic payload in solution.
131. The method of claim 130, further comprising purifying the micelle.
132. A method of treating a disease or condition in a subject in need thereof comprising administering the micelle of any one of claims 95 to 125 or the pharmaceutical composition of any one of claim 126 to the subject.
133. The method of claim 132 wherein the anionic payload in the core of the micelle exhibits a longer half-life than a corresponding anionic payload not integrated into a micelle.
134. The method of claim 132 or 133, wherein the subject is a mammal.



Composition of Cationic Carrier Unit

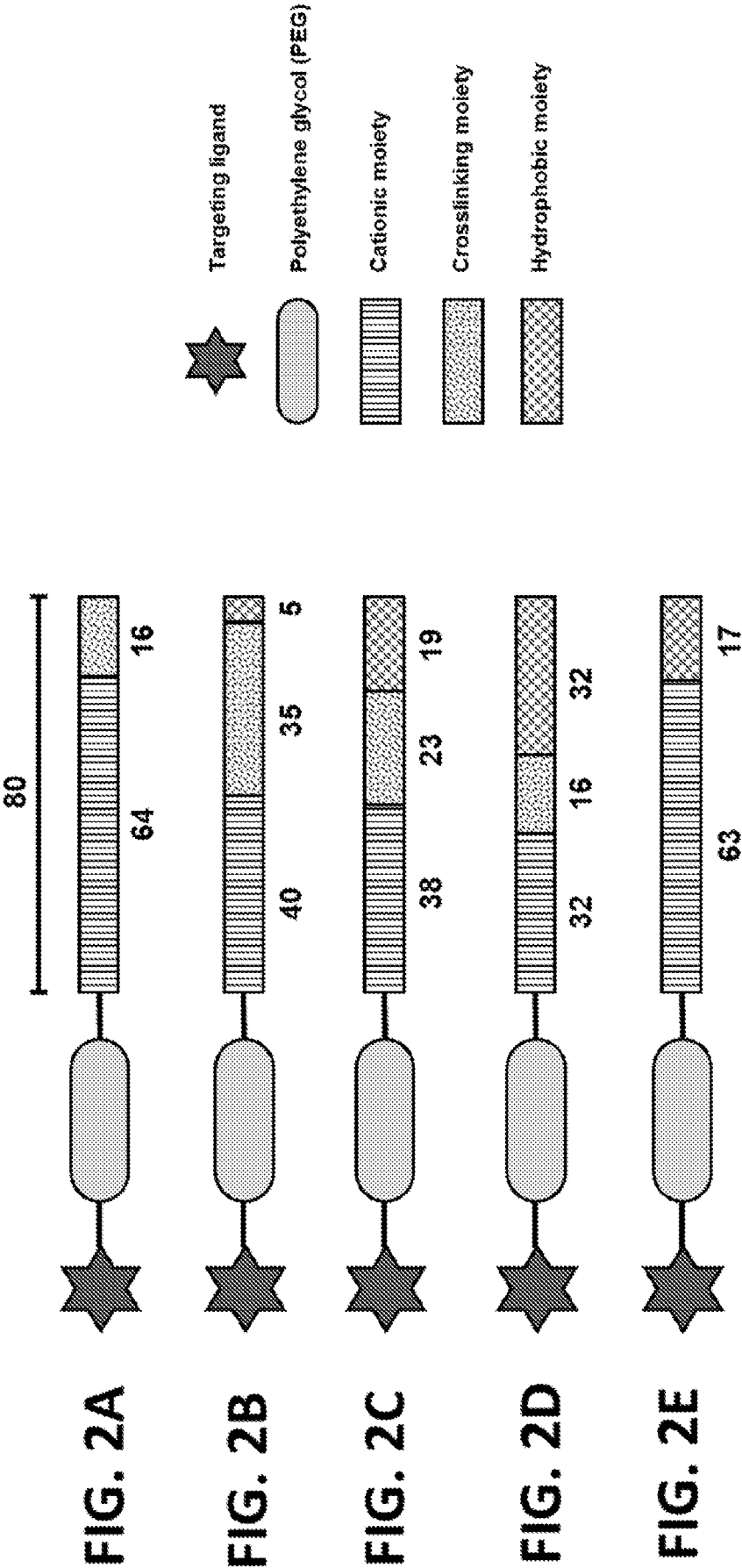
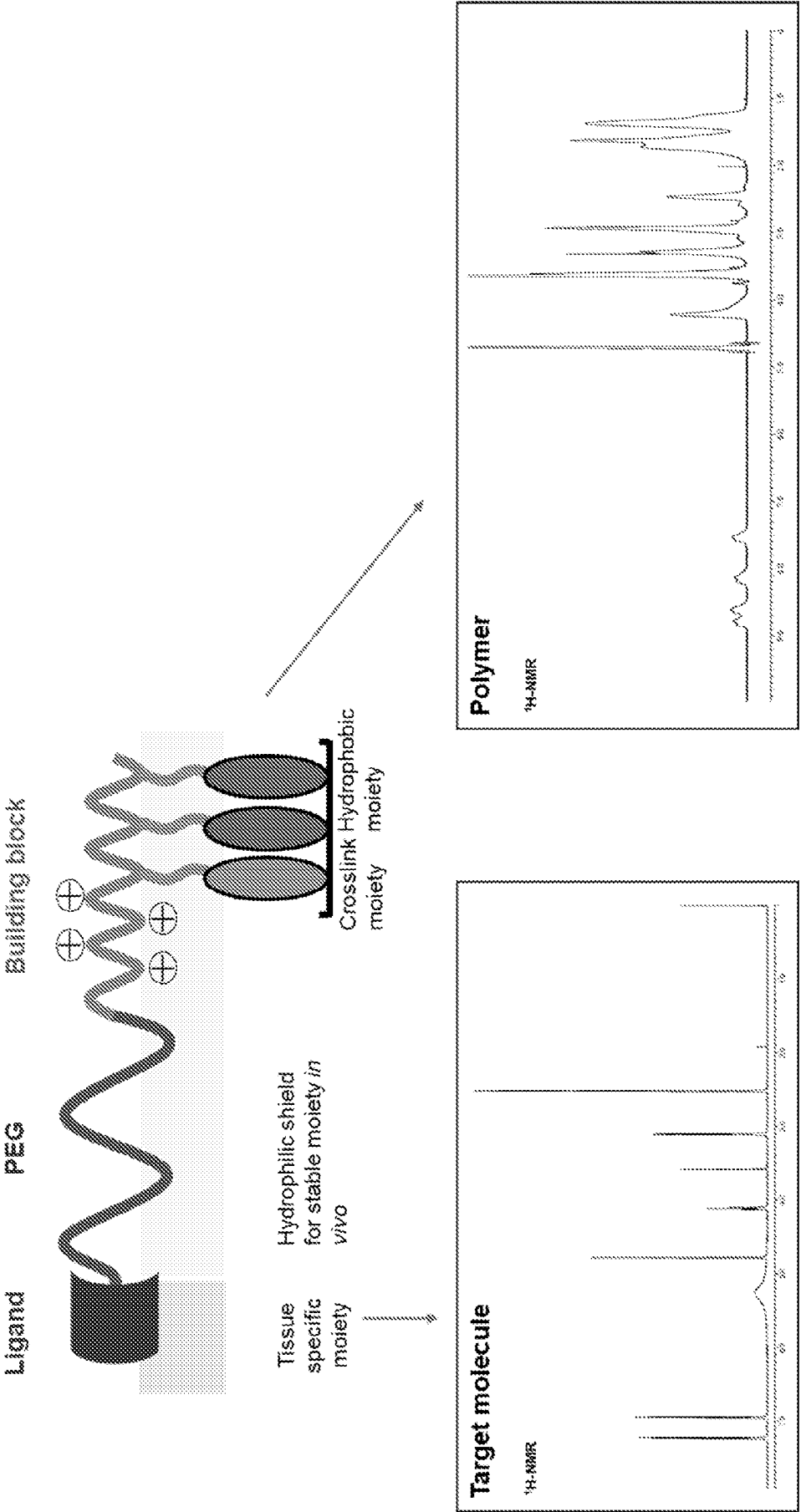


FIG. 3



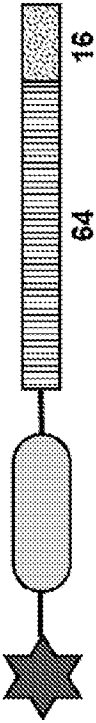


FIG. 4A

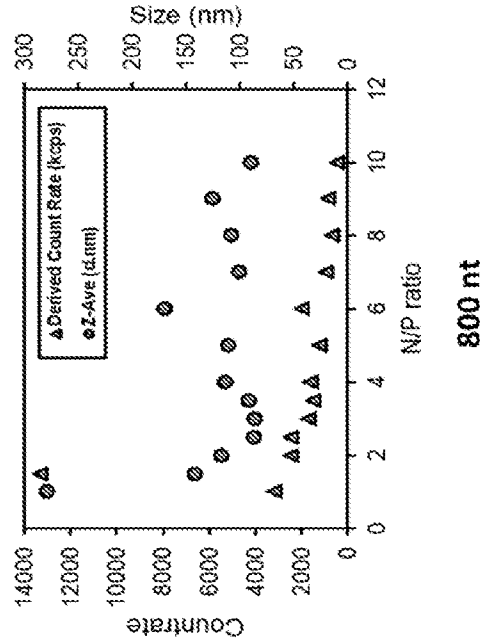


FIG. 4B

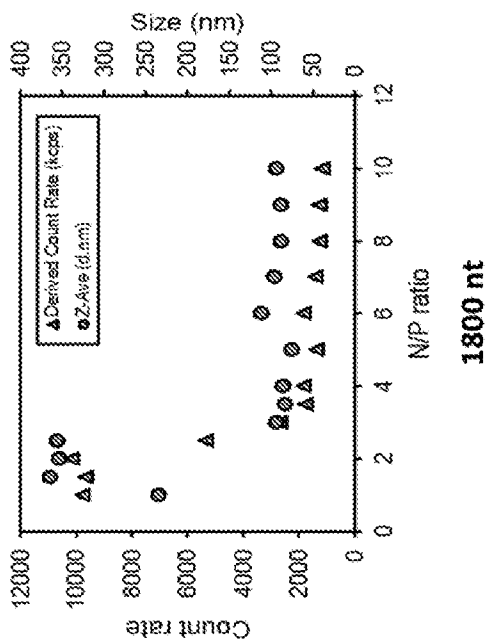


FIG. 4C

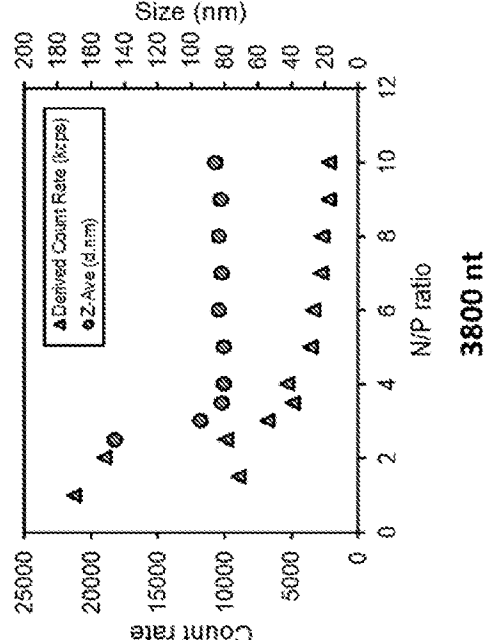


FIG. 4D

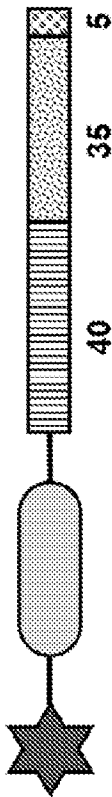


FIG. 5A

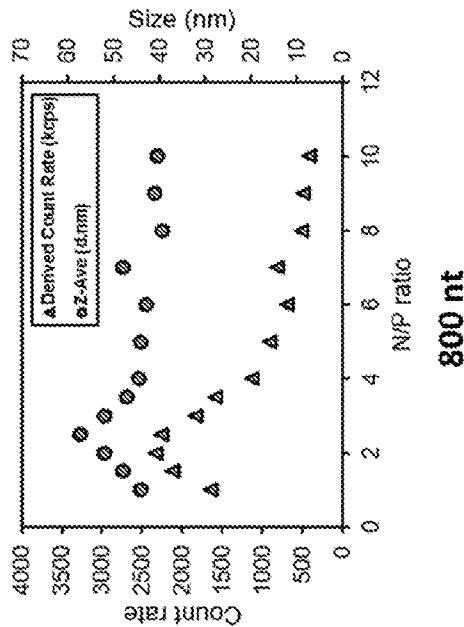


FIG. 5B

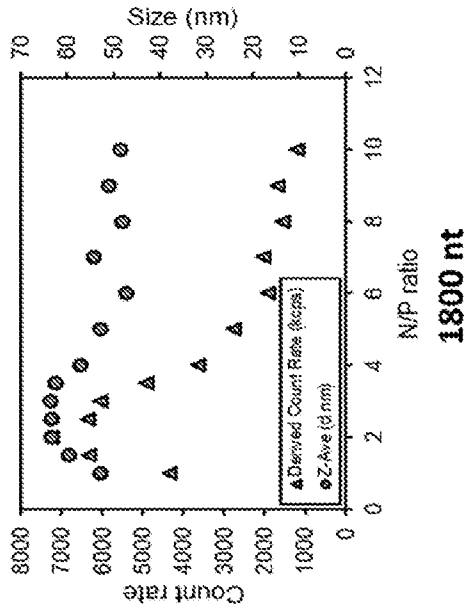


FIG. 5C

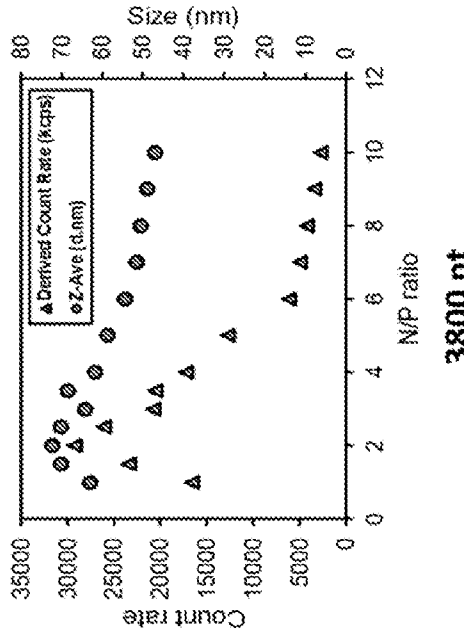


FIG. 5D

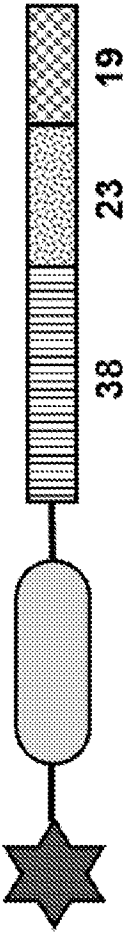


FIG. 6A

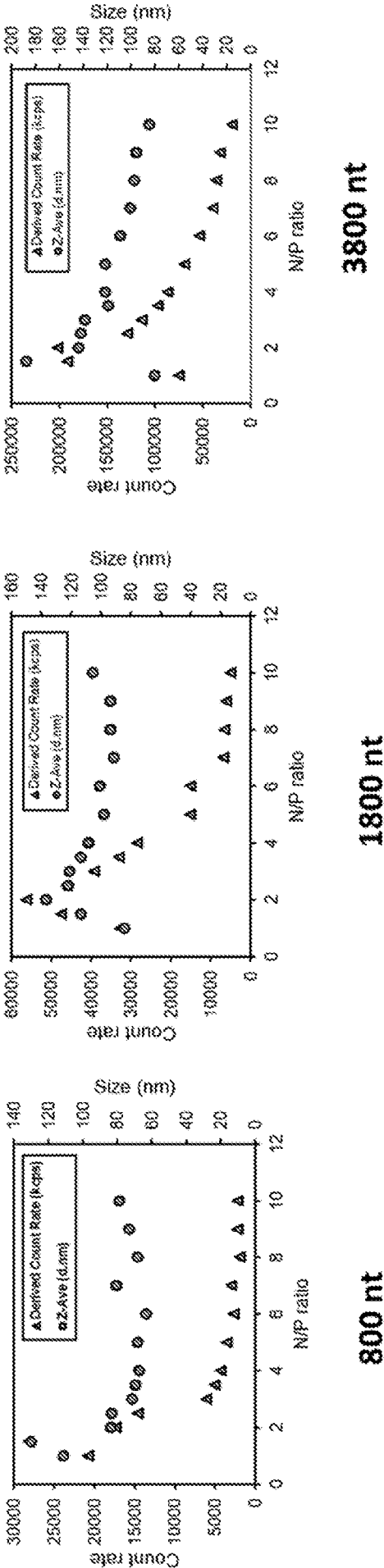
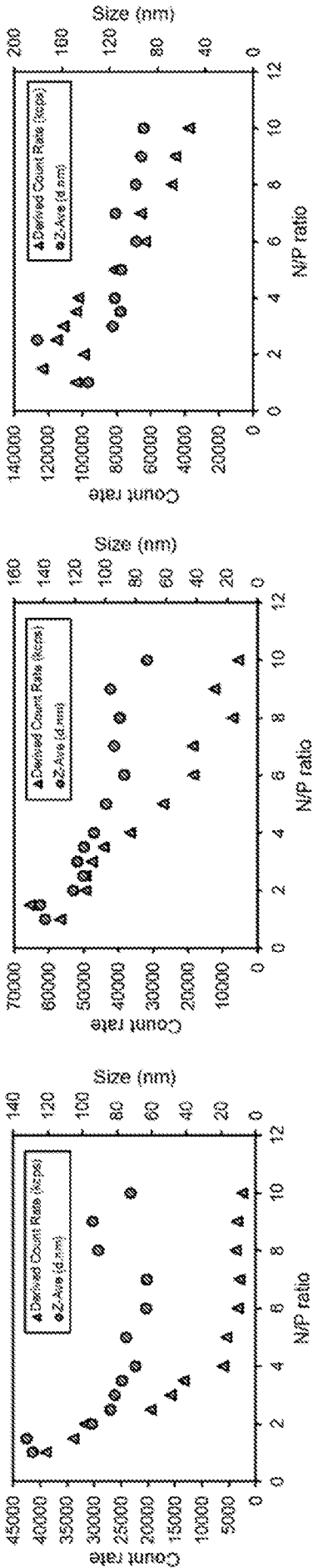
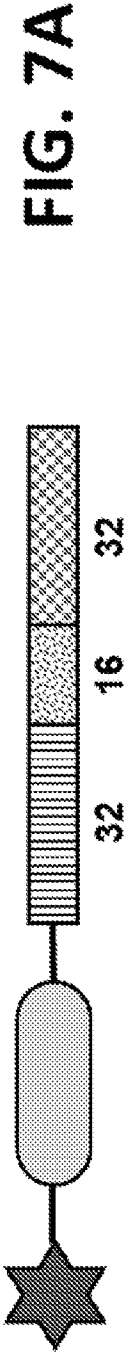


FIG. 6B

FIG. 6C

FIG. 6D



Optimization of N/P ratio (5)

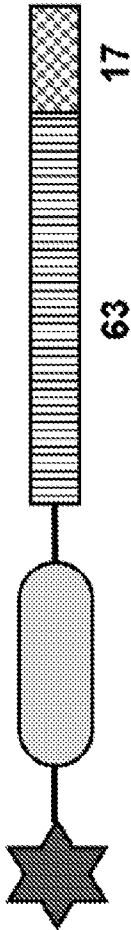


FIG. 8A

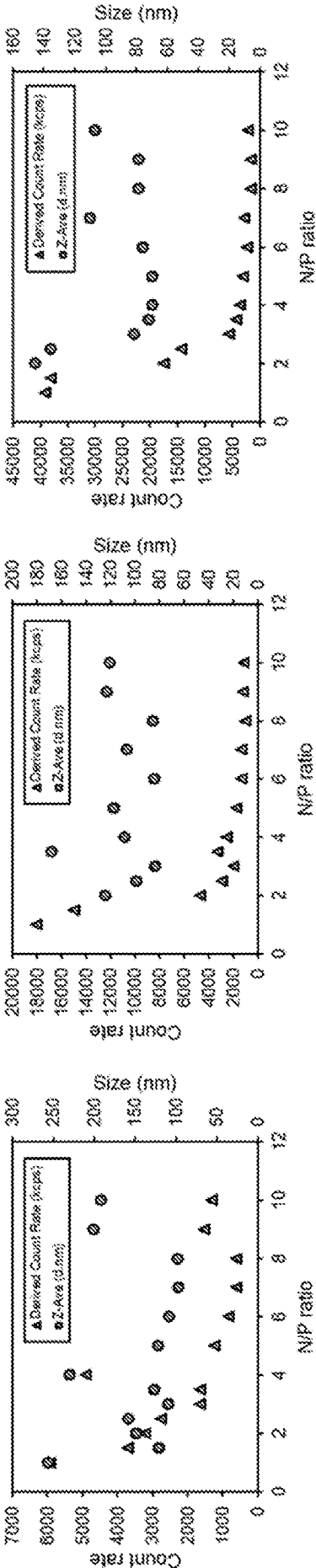


FIG. 8B

FIG. 8C

FIG. 8D

FIG. 9A

polymer		800 nt	1800 nt	3800 nt
Compound B	N/P ratio	3	6	7
	size	51.91	47.33	41.49
Compound C	N/P ratio	4	6	8
	size	67.28	100.9	97.33
Compound D	N/P ratio	5	7	10
	size	72.02	94.29	91.69

FIG. 9B

polymer		800 nt	1800 nt	3800 nt
Compound B	N/P ratio	5	5	5
	size	44.03	52.82	58.65
Compound C	N/P ratio	6	6	6
	size	63.23	100.9	108.9
Compound D	N/P ratio	7	7	7
	size	62.69	94.29	115.5

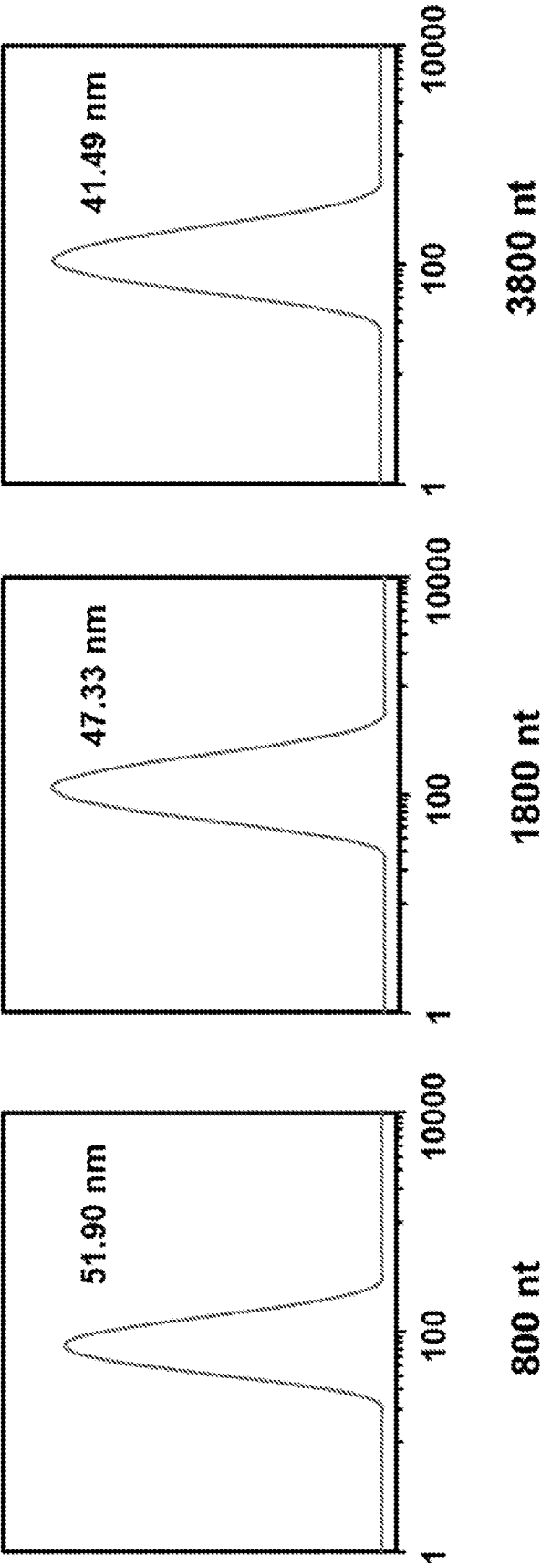


FIG. 10A

FIG. 10B

FIG. 10C

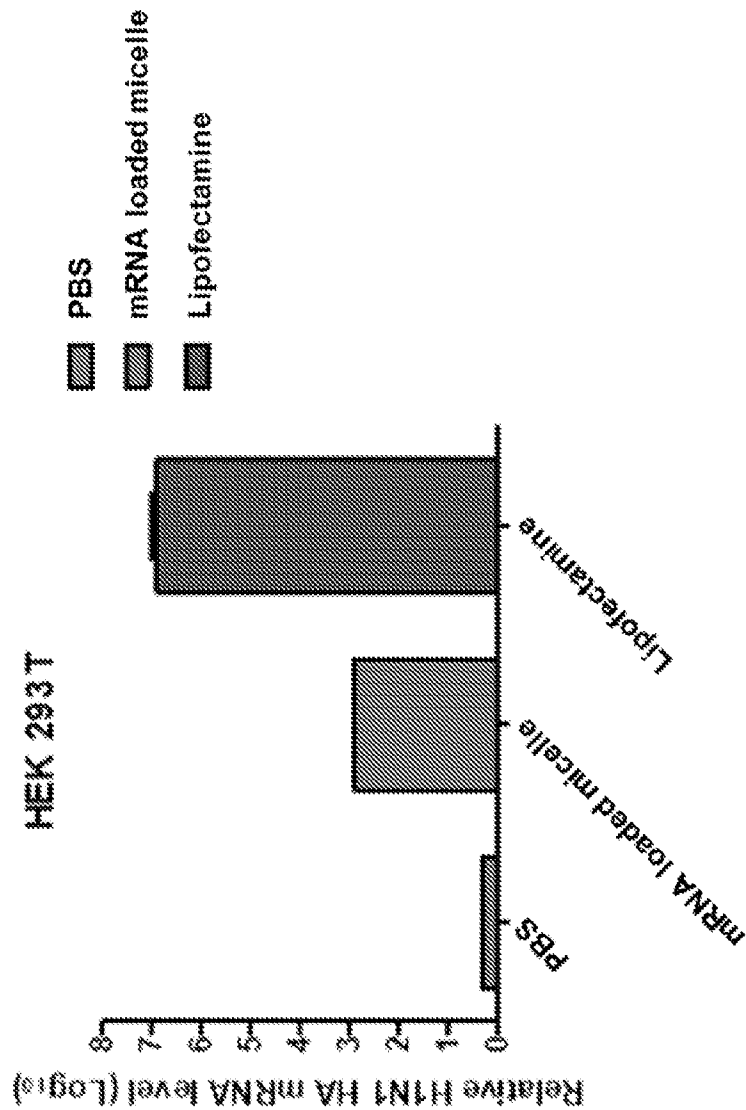


FIG. 11

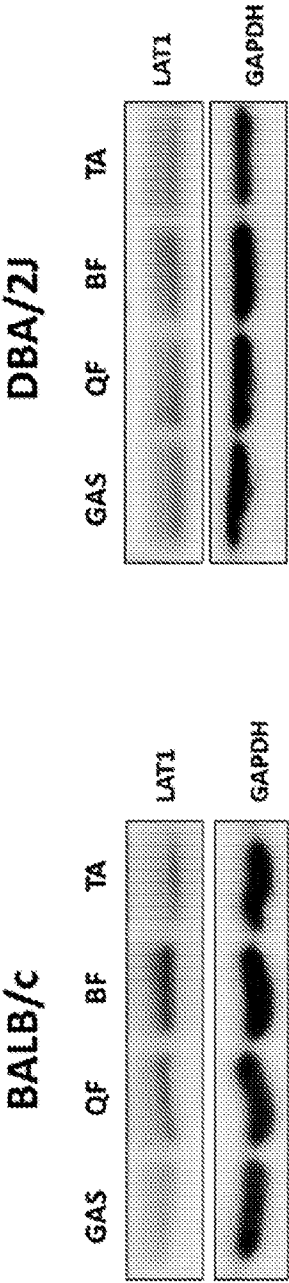


FIG. 12A

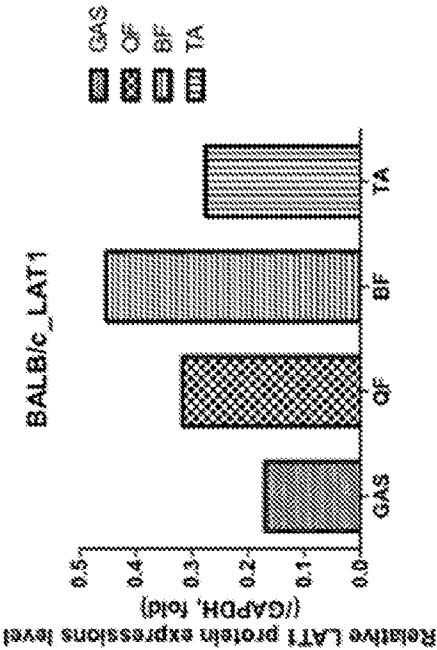


FIG. 12C

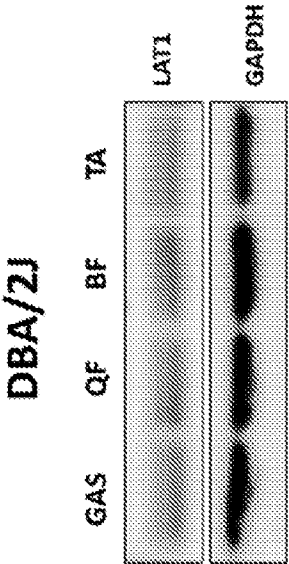


FIG. 12B

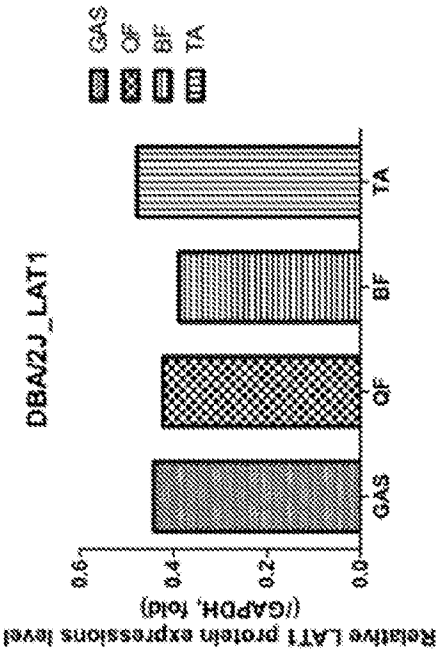
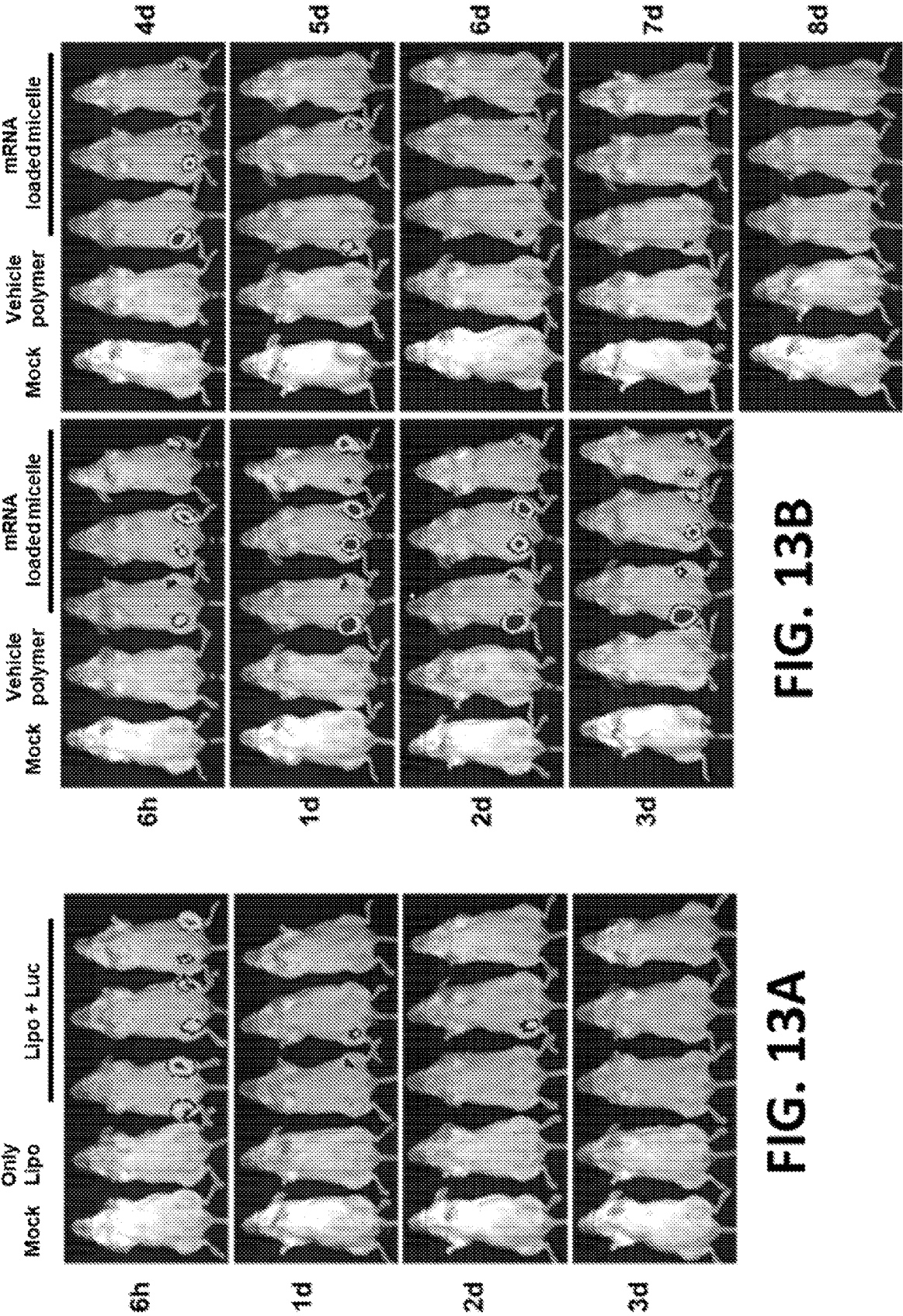


FIG. 12D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/062447

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/107(2006.01)i; **A61K 47/10**(2006.01)i; **A61K 47/18**(2006.01)i; **A61K 47/22**(2006.01)i; **A61K 48/00**(2006.01)i;
A61K 31/7088(2006.01)i; **C12N 15/113**(2010.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 9/107(2006.01); A61K 31/74(2006.01); A61K 31/765(2006.01); A61K 38/28(2006.01); A61K 47/30(2006.01);
A61K 47/68(2017.01); A61K 47/69(2017.01); A61K 48/00(2006.01); A61K 9/51(2006.01); C08G 63/664(2006.01);
C12N 15/85(2006.01)

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: cationic carrier, anionic payload, drug delivery, hydrophobic moiety, micellar nanoparticles, polynucleotides

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2019-0350870 A1 (OMID C. FAROKHZAD et al.) 21 November 2019 (2019-11-21) claims 1, 5; and paragraphs [0144], [0199], [0345]-[0348]	1-7
A	CN 111632153 A (NINGXIA MEDICAL UNIVERSITY) 08 September 2020 (2020-09-08) abstract; and claim 1	1-7
A	CN 111819217 A (SELECTION BIOSCIENCE LLC) 23 October 2020 (2020-10-23) abstract; and claim 1	1-7
A	US 2020-0095605 A1 (LIGANDAL, INC.) 26 March 2020 (2020-03-26) claim 1	1-7
A	WO 2019-204799 A1 (UNIVERSITY OF PITTSBURGH -OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 24 October 2019 (2019-10-24) abstract	1-7



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance
“D” document cited by the applicant in the international application
“E” earlier application or patent but published on or after the international filing date
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
“O” document referring to an oral disclosure, use, exhibition or other means
“P” document published prior to the international filing date but later than the priority date claimed

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

11 April 2022

Date of mailing of the international search report

12 April 2022

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/062447

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

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

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

1. ☒ Claims Nos.: **132-134**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 132-134 pertain to methods for treatment of the human body by surgery or therapy, as well as diagnostic methods (PCT Article 17(2)(a)(i) and Rule 39.1(iv)).
2. ☒ Claims Nos.: **11,16,17,22-24,29-31,37,44,45,48-51,56,57,66-68,70-73,75,86-92,94,96-98,100,101,106,110,114,119,121,123,125,129,131,133**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 11, 16, 17, 22-24, 29-31, 37, 44, 45, 48-51, 56, 57, 66-68, 70-73, 75, 86-92, 94, 96-98, 100, 101, 106, 110, 114, 119, 121, 123, 125, 129, 131, 133 refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a).
3. ☒ Claims Nos.: **8-10,12-15,18-21,25-28,32-36,38-43,46,47,52-55,58-65,69,74,76-85,93,95,99,102-105,107-109,111-113,115-118,120,122,124,126-128,130,132,134**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IB2021/062447

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)		Publication date (day/month/year)
US	2019-0350870	A1	21 November 2019	US	11123304 B2	21 September 2021
				WO	2018-144022 A1	09 August 2018
CN	111632153	A	08 September 2020	None		
CN	111819217	A	23 October 2020	CN	110156971 A	23 August 2019
				CN	111819217 B	01 October 2021
				EP	3753966 A1	23 December 2020
				JP	2021-509138 A	18 March 2021
				JP	6912048 B2	28 July 2021
				US	11225551 B2	18 January 2022
				US	2021-0047467 A1	18 February 2021
				WO	2019-158037 A1	22 August 2019
US	2020-0095605	A1	26 March 2020	AU	2017-378427 A1	20 June 2019
				AU	2017-378431 A1	20 June 2019
				CA	3045131 A1	21 June 2018
				CA	3045134 A1	21 June 2018
				CN	110582301 A	17 December 2019
				CN	110582302 A	17 December 2019
				EP	3554546 A1	23 October 2019
				EP	3554546 A4	14 April 2021
				IL	267033 D0	31 July 2019
				IL	267034 D0	31 July 2019
				JP	2020-502120 A	23 January 2020
				JP	2020-502124 A	23 January 2020
				KR	10-2021-0035021 A	31 March 2021
				KR	10-2021-0035022 A	31 March 2021
				RU	2019121801 A	15 January 2021
				RU	2019121801 A3	12 October 2021
				RU	2019121992 A	15 January 2021
				RU	2019121992 A3	26 January 2021
				US	10975388 B2	13 April 2021
				US	2018-161447 A1	14 June 2018
				WO	2018-112278 A1	21 June 2018
				WO	2018-112282 A1	21 June 2018
WO	2019-204799	A1	24 October 2019	US	2021-236645 A1	05 August 2021