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(54) **AMPHIPHILIC SUBSTANCES AND FUNCTIONALIZED LIPID VESICLES INCLUDING THE SAME**

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(57) **ABSTRACT**

An amphiphilic substance includes a hydrophobic group, and a polynucleotide group attached to the hydrophobic group. The polynucleotide group includes a first polynucleotide segment and a second polynucleotide segment. The first and second polynucleotide segments are at least partially complementary and are bound together by interactions including base pairing. At least one of the first and second segments includes at least one of an aptamer and a nucleic acid-based enzyme. A lipid vesicle may include the amphiphilic substance, a first polar lipid that is an unstable vesicle former, and a polar liquid. Upon exposure to a rupture agent, the vesicle may rupture, releasing the contents of the vesicle. Substances that may be released from the vesicle include bioactive agents, such as drug agents.

13 Claims, 7 Drawing Sheets

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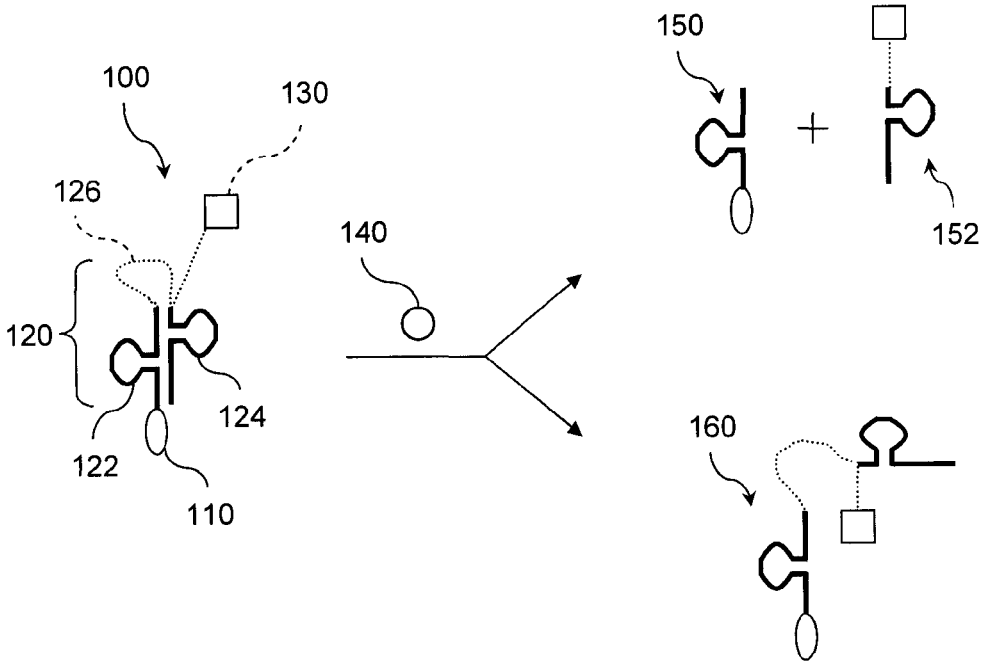
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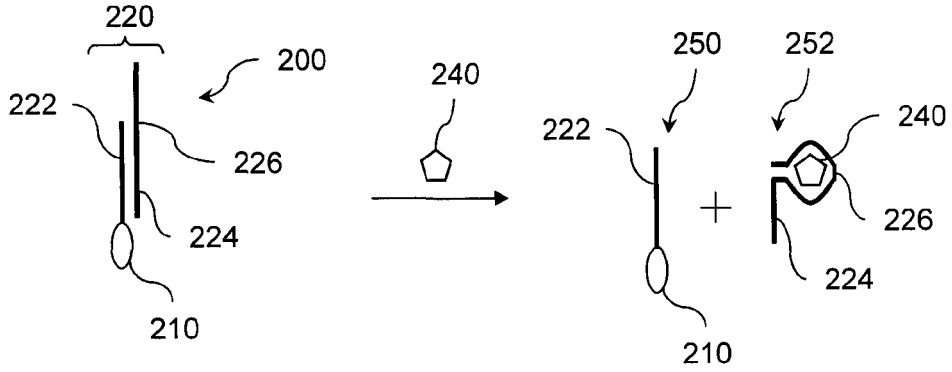
130
126
120
124
122
110
140
150
152
160

FIG. 1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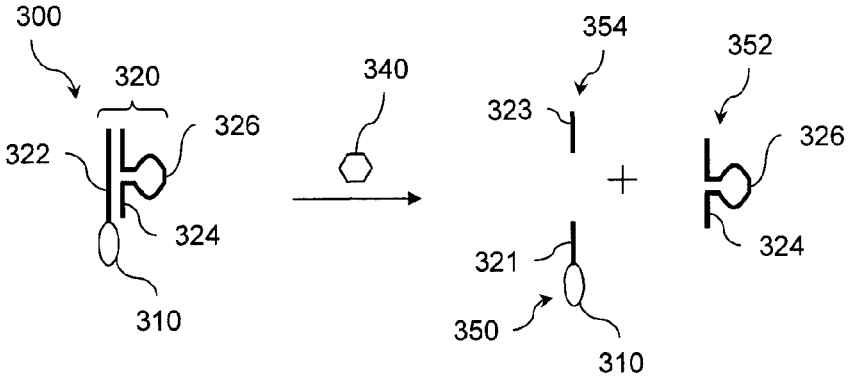
240
226
224
210

FIG. 2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320
326
322
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321
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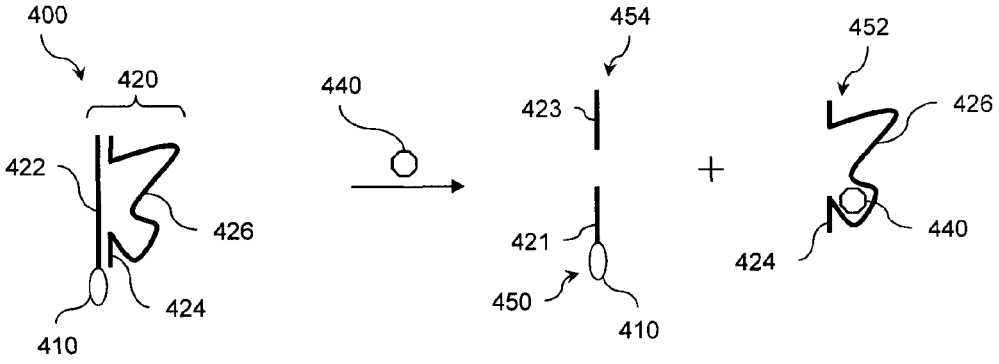
310

FIG. 3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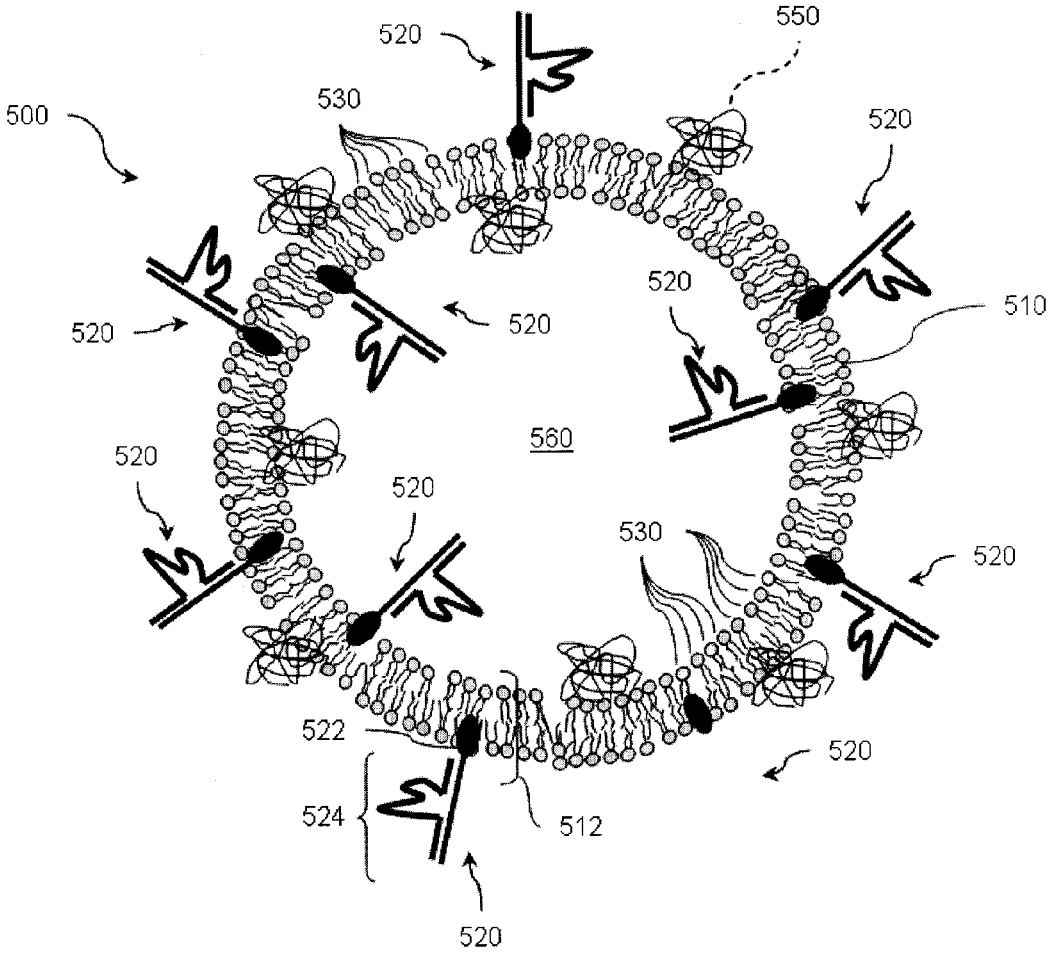
424

FIG. 4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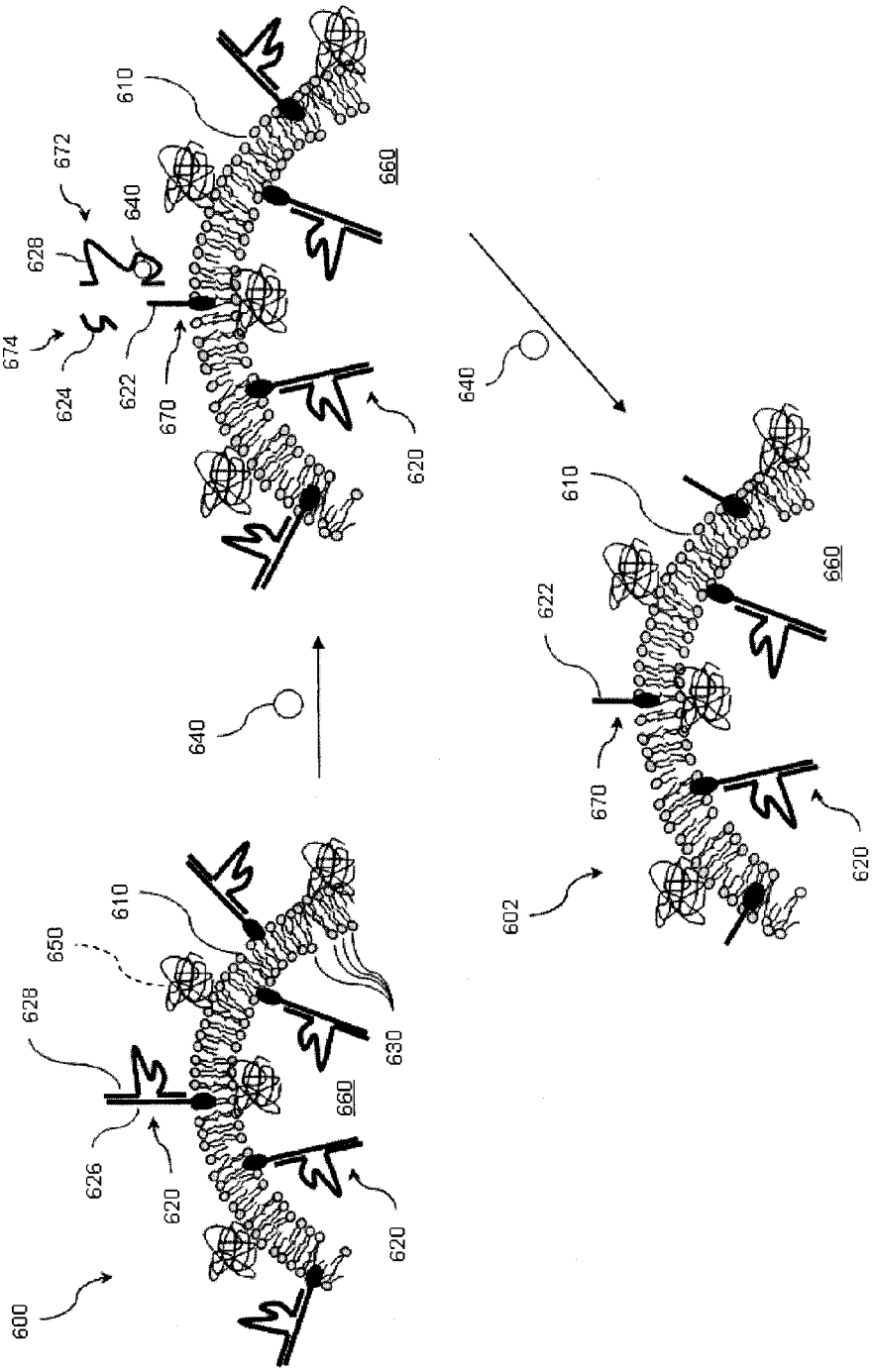
512
520

FIG. 5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628
626
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620
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610
622
670
620
602

FIG. 6

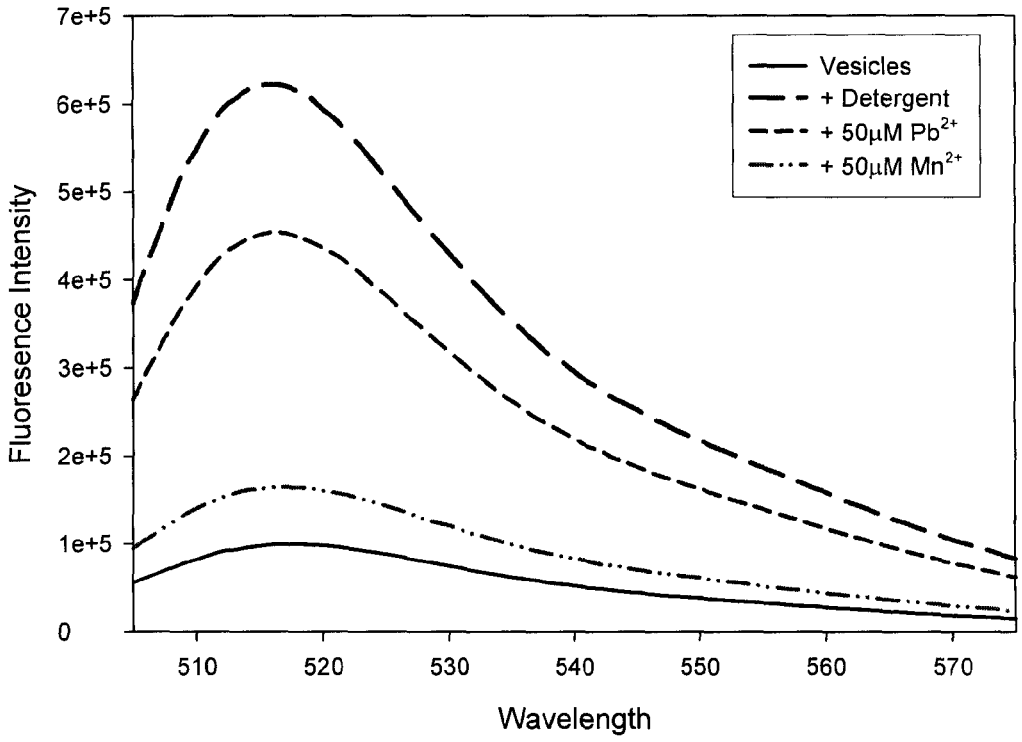
7e+5
6e+5
5e+5
4e+5
3e+5
2e+5
1e+5
0
Fluoresence Intensity
510
520
530
540
550
560
570
Wavelength
Vesicles
+ Detergent
+ 50μM Pb2+
+ 50μM Mn2+

FIG. 7

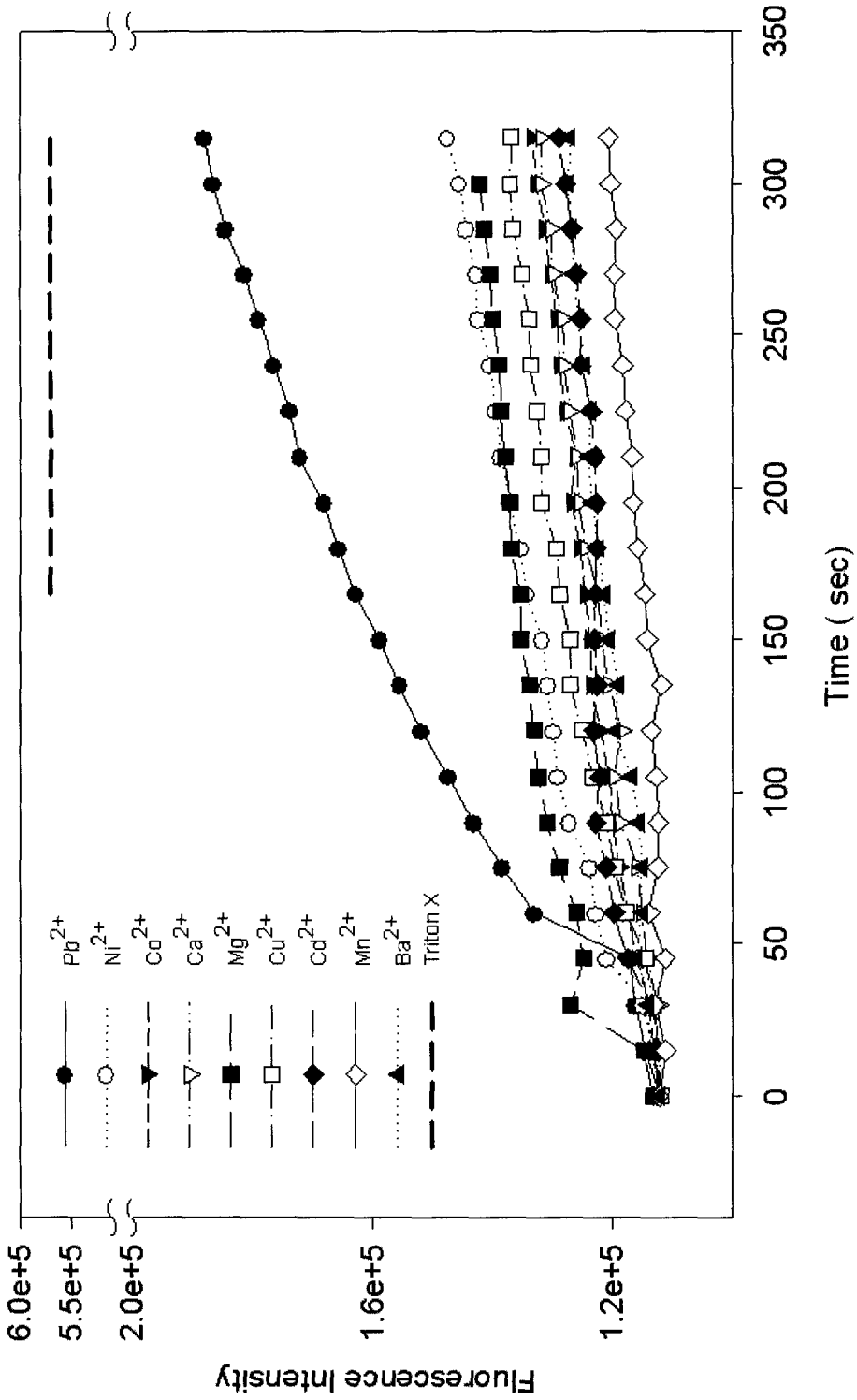
Fluorescence Intensity
6.0e+5
5.5e+5
2.0e+5
1.6e+5
1.2e+5
Time (sec)
0
50
100
150
200
250
300
350
Pb2+
Ni2+
Co2+
Ca2+
Mg2+
Cu2+
Cd2+
Mn2+
Ba2+
Triton X

FIG. 8

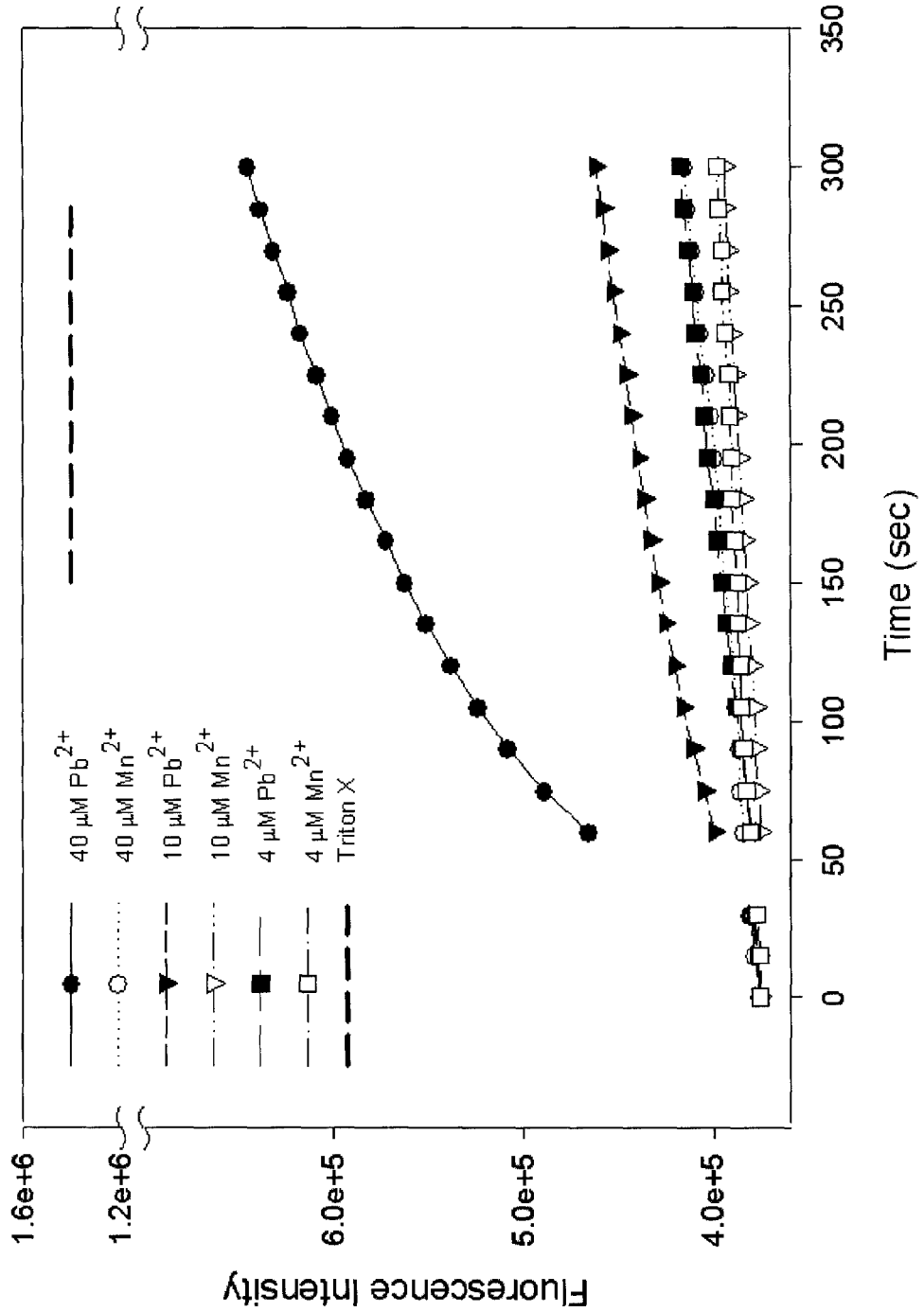
Fluorescence Intensity
1.6e+6
1.2e+6
6.0e+5
5.0e+5
4.0e+5
0
50
100
150
200
250
300
350
Time (sec)
40 µM Pb2+
40 µM Mn2+
10 µM Pb2+
10 µM Mn2+
4 µM Pb2+
4 µM Mn2+
Triton X

FIG. 9

AMPHIPHILIC SUBSTANCES AND FUNCTIONALIZED LIPID VESICLES INCLUDING THE SAME

REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/885,744 entitled "AMPHIPHILIC SUBSTANCES AND FUNCTIONALIZED LIPID VESICLES INCLUDING THE SAME" filed Jan. 19, 2007, the entire contents of which are hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The subject matter of this application may have been funded in part under a research grant from the National Science Foundation, under Grant Numbers DMR-0117792 and DMR-0409369. The U.S. Government may have rights in this invention.

BACKGROUND

Lipid vesicles have unilamellar or multilamellar exterior walls that enclose an internal space. The walls of lipid vesicles are bimolecular layers (bilayers) of one or more polar lipid components having polar heads and non-polar tails. In an aqueous liquid, or other polar liquid, the polar heads of one layer may orient outwardly to extend into the surrounding medium, the non-polar tail portions of the lipids thus associating with each other. This orientation provides a layer having a polar surface on either side of the layer, and having a non-polar core between the surfaces. In a lipid vesicle, the bilayer forms a closed structure, where both the exterior and the interior of the structure have a polar surface. Unilamellar vesicles have one such bilayer, whereas multilamellar vesicles typically have multiple, concentric bilayers.

One application of lipid vesicles is in the delivery of bioactive agents within an organism. Examples of bioactive agents include drug agents and imaging agents. Lipid vesicles may be used to isolate a bioactive agent, so as to direct the agent away from certain tissues and to deliver the agent to other tissues. Lipid vesicles also can be used to release drugs over a prolonged period of time, allowing for less frequent administration. In addition, lipid vesicles can allow for delivery of hydrophobic or amphiphilic drugs that would otherwise be difficult to administer by injection.

In one example of bioactive agent delivery, lipid vesicles have been researched for use with chemotherapeutic agents. Tumor-specific drug delivery has become an important area of research in cancer therapy. Application of chemotherapeutics for cancer treatment is often limited by severe side effects and poor systemic efficacy. Lipid vesicle delivery systems have decreased these pharmacokinetic drawbacks, resulting in a number of delivery systems for cancer treatment that have been approved by the U.S. Food & Drug Administration (FDA). These approved liposome delivery systems, however, release the encapsulated agent through passive diffusion from the vesicle or through slow, non-specific degradation of the vesicle. These mechanisms can lead to systemic toxicity and also lack the ability to actively release the encapsulated agent at a specific disease site and/or at a specific time.

At present, most lipid vesicle mediated bioactive agent delivery is either untargeted or passively targeted. Passive targeting involves stabilizing the lipid vesicle against degradation in the circulatory system, providing for an increase in blood circulation times. A typical approach to this stabilization is to coat the lipid vesicle with a hydrophilic polymer, such as poly(ethylene glycol) (PEG). A common drawback to the untargeted or passively targeted strategies is the lack of accuracy in delivering the bioactive agent to a specific tissue type or a specific area of the organism.

It would be desirable to provide a lipid vesicle system in which the contents of the vesicle are released when the vesicle is in contact with a specific environment, such as a specific type of tissue within an organism. Preferably, such a system would protect the vesicle contents until contacted with an agent that is present specifically at the targeted environment, at which point the contents would be released. For biological applications, it would be desirable for the vesicle contents to be released when the vesicle is in contact with a biomarker specific for the targeted tissue.

SUMMARY

In one aspect, the invention provides an amphiphilic substance including a hydrophobic group, and a polynucleotide group attached to the hydrophobic group. The polynucleotide group includes a first polynucleotide segment and a second polynucleotide segment. The first and second polynucleotide segments are at least partially complementary and are bound together by interactions including base pairing. At least one of the first and second segments includes at least one of an aptamer and a nucleic acid-based enzyme.

In another aspect, the invention provides a lipid vesicle including an amphiphilic substance as described above, a first polar lipid that is an unstable vesicle former, and a polar liquid.

In yet another aspect, the invention provides a method of forming a lipid vesicle including combining ingredients including an amphiphilic substance as described above, a first polar lipid that is an unstable vesicle former, and a polar liquid. The first polar lipid and the amphiphilic substance form a vesicle wall.

In yet another aspect, the invention provides a controlled delivery system including a lipid vesicle as described above, and a bioactive agent in the lipid vesicle.

In yet another aspect, the invention provides a method of delivering a bioactive agent including administering a controlled delivery system, as described above, to an organism.

The following definitions are included to provide a clear and consistent understanding of the specification and claims.

The term "amphiphilic substance" means a substance that includes both a hydrophilic group and a hydrophobic group.

The term "group" means a linked collection of atoms within a molecular entity.

The term "hydrophobic group" means a group that, if separate from any other group, is insoluble in water but is soluble in nonpolar organic solvents.

The term "polynucleotide group" means a group that, if separate from any other group, would be a polynucleotide radical.

The term "polynucleotide" means a sequence of at least two mononucleotides. Nucleic acids that may be incorporated into a polynucleotide as mononucleotides include natural nucleic acids, unnatural nucleic acids, modified nucleic acids, and peptide nucleic acids (PNAs).

The term "complementary sequences" means two mononucleotide sequences that can form base pairing. The complementary sequences may be present in a single polynucleotide, or they may be present in separate polynucleotides.

The term "base pairing" means the ability of two nucleobases to form one or more hydrogen bonds. For example, in Watson-Crick base pairing, base pairing between adenosine

and uridine or thymidine involves two hydrogen bonds, whereas base pairing between guanosine and cytosine involves three hydrogen bonds.

The terms "complementary base pairing" and "complementarity" refer to the ability of a polynucleotide to form a secondary structure containing at least one nucleotide base-pair. For example, complementarity between sequences of two polynucleotides refers to the ability of the two sequences to form intermolecular base pairing (for example, Watson-Crick base pairing), resulting in a hybridized structure between the two polynucleotides. Complementarity may be complete, meaning that no unpaired or mismatched nucleobases exist within the hybrid structure formed. Complementarity may be partial, meaning that mismatches or internal bulges or loops may arise within the hybrid structure.

The term "aptamer" means a polynucleotide that has been selected from a random pool of polynucleotides based on its ability to bind another substance, referred to as a target. Aptamers have been selected that bind ions, polynucleotides, proteins, organic compounds, or entire organisms. The mononucleotides of an aptamer may adopt a particular conformation upon binding to its target.

The term "effector" means a molecule that, when bound to an aptamer induces a specific structure or function in the aptamer. Examples of effectors include metal ions, polynucleotides, proteins, and organic compounds.

An "effector binding site" is at least a portion of an aptamer that can bind the effector for that aptamer. An effector binding site may be specific, binding only one effector in the presence of other possible effectors. For example, an aptamer may have a binding site that specifically binds adenosine, even in the presence of many other molecules such as guanosine, thymidine, cytidine. An effector binding site may be partially specific, binding only a class of substances, or it may be non-specific.

The term "nucleic acid-based enzyme" means an enzyme that principally contains mononucleotides. Examples of nucleic acid-based enzymes include ribozymes (RNAzymes), deoxyribozymes (DNAzymes), and aptazymes. Nucleic acids that may be incorporated into a nucleic acid-based enzyme as mononucleotides include natural nucleic acids, unnatural nucleic acids, modified nucleic acids, and peptide nucleic acids (PNAs). A nucleic acid-based enzyme may require a co-factor for efficient substrate cleavage and/or specific effector binding. Examples of co-factors include metals such as Mg(II) and Pb(II).

The term "aptazyme", also referred to as "allosteric DNA/RNAzyme" or "allosteric (deoxy)ribozyme," is a DNA/RNAzyme in which the enzymatic activity is regulated by an effector. An aptazyme typically contains an aptamer domain, which recognizes an effector, and a catalytic domain. See, for example, Hesselberth, J., et al., *Reviews in Molecular Biotechnology*, vol. 74, pp. 15-25, (2000); Soukup, G. A., et al., *Current Opinion in Structural Biology*, vol. 10, pp. 318-325, (2000); and Tang, J., et al., *Chemistry & Biology*, vol. 4, no. 6, pp. 453-459, (1997).

BRIEF DESCRIPTION OF THE DRAWINGS

The invention can be better understood with reference to the following drawings and description. The components in the figures are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

FIG. **1** is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes an aptamer and/or a nucleic acid-based enzyme.

FIG. **2** is a scheme representing an example of an interaction of a rupture agent with an amphiphilic substance that includes an aptamer.

FIG. **3** is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes a nucleic acid-based enzyme.

FIG. **4** is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes an aptazyme.

FIG. **5** is a scheme representation of a lipid vesicle that includes a vesicle wall, an amphiphilic substance and optionally a stabilizing group.

FIG. **6** is a scheme representing the interaction of a rupture agent with a vesicle that contains an amphiphilic substance including an aptamer and/or a nucleic acid-based enzyme.

FIG. **7** is a collection of fluorescence spectra for vesicles exposed to 50 micromolar (μM) Pb^{2+} ion, to 50 μM Mn^{2+} ion, to Triton-X detergent, and to no added ions or detergent.

FIG. **8** is a graph of normalized fluorescence intensity over time after exposure of the vesicles to 40 μM of different divalent metal ions, or to a detergent.

FIG. **9** is a graph of normalized fluorescence intensity over time after exposure of the vesicles to 40 μM, 10 μM or 4 μM of either Mn^{2+} or Pb^{2+} ions, or to a detergent.

DETAILED DESCRIPTION

The present invention makes use of the discovery that the formation, stabilization and/or rupture of lipid vesicles can be controlled by the incorporation of an amphiphilic substance containing an aptamer and/or a nucleic acid-based enzyme in the vesicle wall. In the absence of a rupture agent, the amphiphilic substance can facilitate the formation and stabilization of a vesicle. In the presence of a rupture agent, the vesicle can be destabilized, causing the vesicle to rupture and release its contents. These vesicles may be used in a variety of applications, including controlled delivery of bioactive agents in an organism.

The amphiphilic substance for incorporation in a vesicle wall may include a hydrophobic group and a polynucleotide group, attached to the hydrophobic group, where the polynucleotide group includes an aptamer and/or a nucleic acid-based enzyme. The rupture agent may be, for example, an effector for an aptamer of the polynucleotide group, or a co-factor for a nucleic acid-based enzyme of the polynucleotide group. In one example, the aptamer can bind the rupture agent, causing the aptamer to undergo a conformational change and/or to release a segment of the polynucleotide group that was bound together with the aptamer by interactions including base pairing. In another example, the nucleic acid-based enzyme can be activated in the presence of the rupture agent, causing the enzyme to cleave the polynucleotide group, releasing a portion of the group. The amphiphilic substance also may include a stabilizing group connected to the polynucleotide group. The interaction of the aptamer and/or nucleic acid-based enzyme with the rupture agent may then allow the stabilizing group to dissociate from the amphiphilic substance and the vesicle wall, causing the vesicle to rupture.

FIG. **1** is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes an aptamer and/or a nucleic acid-based enzyme. Amphiphilic substance **100** includes a hydrophobic group **110**, a polynucleotide group **120**, and optionally a stabilizing group **130**. The polynucleotide group **120** includes a first polynucleotide segment **122** and a second polynucleotide segment **124**, where the first and second segments are at least partially complementary and are bound together by interactions including base pairing. At

least one of the first and second segments includes an aptamer and/or a nucleic acid-based enzyme. The polynucleotide group **120** optionally may include a linking group **126** between the first and second segments.

Exposure of the amphiphilic substance **100** to a rupture agent **140** disrupts the base pairing between the first and second polynucleotide segments, resulting in a dissociation of the first and second polynucleotide segments. The polynucleotide group **120** thus undergoes a change in its size and/or conformation. If the optional linking group **126** is absent, the dissociation may produce a first product **150** that includes the hydrophobic group and at least a portion of the first polynucleotide segment, and a second product **152** that includes at least a portion of the second polynucleotide segment and optionally includes the stabilizing group. If the optional linking group **126** is present, the dissociation may produce a product **160** that has a polynucleotide group conformation different from that of amphiphilic substance **100**.

The hydrophobic group **110** may be any group that, if separate from the amphiphilic substance, would be insoluble in water but soluble in nonpolar organic solvents. Preferably, the hydrophobic group can be incorporated into a lipid vesicle wall, for example by association with the non-polar core of the lipid bilayer. Examples of hydrophobic molecules that can be linked to a polynucleotide to form an amphiphilic substance include waxes, fatty acids and salts, triglycerides, phosphoglycerides, sphingolipids, prostaglandins, terpenes, and steroids.

Examples of sphingolipids that may be linked to a polynucleotide to form an amphiphilic substance include sphingosines, such as D-erythro-sphingosine, sphingomyelin, ceramides, cerebrosides, brain sulfatides and glucosylceramide; gangliosides, such as ovine brain gangliosides and porcine brain gangliosides; and phytosphingosines, such as phytosphingosine, D-ribo-phytosphingosine-1-phosphate, and N-acyl phytosphingosines (i.e. C2, C8 or C18). Examples of phosphoglycerides that may be linked to a polynucleotide to form an amphiphilic substance include phosphatidylcholines, phosphocolines (such as platelet-activation factor (PAF), phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidylserines (including sodium salts), cardiolipins (including sodium salts), phosphatidic acids, lysophosphatides, and diglycerides. These phosphoglycerides may include one or more saturated or unsaturated hydrocarbon groups, such as lauroyl, myristoyl, palmitoyl, stearoyl, arachidoyl, oleyl, linoleoyl, and erucoyl. Mixtures of these hydrophobic molecules, such as mixtures present in tissue extractions, may also be used. These and other hydrophobic molecules, which may be linked to a polynucleotide to form an amphiphilic substance, may be purchased from a commercial supplier, such as Avanti Polar Lipids (Alabaster, Ala.).

Preferably, the hydrophobic group **110** is a group formed from a steroid, such as a sterol, a bile acid, or a hormone. More preferably the hydrophobic group is formed from a sterol, such as cholesterol, cholestanol, coprostanol, stigmasterol, sitosterol, ergosterol or calciferol. More preferably the hydrophobic group is formed from chlolesterol. See, for example, Cram, D. J. and Hammond, G. S. "Organic Chemistry" McGraw-Hill, 1959, pp. 560-569.

The polynucleotide group **120** includes a first polynucleotide segment **122** and a second polynucleotide segment **124**. The first and second polynucleotide segments are at least partially complementary, and can bind together by interactions including base pairing. This base pairing may be disrupted when the polynucleotide group is contacted with a rupture agent **140**, resulting in a dissociation of the first and second polynucleotide segments from each other. Other interactions besides base pairing may exist between the first and second polynucleotide segments, such as covalent bonding. Optional linking group **126** may extend between the first and second polynucleotide segments.

The polynucleotide group **120** may include an aptamer. One or both of the first and second polynucleotide segments **122** and **124** independently may include an aptamer. For a polynucleotide having an aptamer in each segment, these aptamers may be the same or different. Preferably only one of the first and second polynucleotide segments includes an aptamer. In one example of a polynucleotide group that includes an aptamer, the rupture agent **140** is an effector for the aptamer. In the absence of the effector, the two polynucleotide segments are bound together by interactions including base pairing of the complementary sequences. In the presence of the effector, the corresponding aptamer preferentially binds the effector, disrupting the base pairing between the two polynucleotide segments. An aptamer in a polynucleotide segment may include at least part of the complementary sequence of the segment. In this example, the aptamer may be selected for its ability to bind the other polynucleotide segment in the absence of an effector, but to preferentially bind the effector when the effector is present. An aptamer in a polynucleotide segment may include none of the complementary sequence of the segment. In this example, the aptamer may be selected for its ability to induce a conformational change in the complementary sequence when the effector is present.

FIG. **2** is a scheme representing an example of an interaction of a rupture agent with an amphiphilic substance that includes an aptamer. Amphiphilic substance **200** includes a hydrophobic group **210** and a polynucleotide group **220**. The polynucleotide group **220** includes a first polynucleotide segment **222** and includes a second polynucleotide segment **224**, including an aptamer **226**. The first and second segments are at least partially complementary, and are bound together by interactions including base pairing. Exposure of the amphiphilic substance **200** to an effector **240** for the aptamer **226** results in a dissociation of the first and second polynucleotide segments. This dissociation yields a first product **250** and a second product **252**. The first product **250** includes the hydrophobic group **210** and the first polynucleotide segment **222**. The second product **252** includes the second polynucleotide segment **224**, including aptamer **226** bound to effector **240**. If the polynucleotide group included an optional linking group (not shown), the dissociation of the first and second polynucleotide segments may yield a polynucleotide group having a conformation different from that present in amphiphilic substance **200**.

An aptamer that binds a particular effector may be isolated by in vitro selection from a library of polynucleotides having random sequences. The selection process may be performed using standard procedures, such as the technique of systematic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold 1990) or other approaches (Cadwell and Joyce 1992, Chapman and Szostak 1994, Joyce 1994, Cadwell and Joyce 1994, Tsang and Joyce 1996). A list of aptamers and their corresponding effectors is disclosed in U.S. Provisional Patent Application No. 60/821,043, to Yi Lu et al., filed Aug. 1, 2006, which is incorporated herein by reference.

A library for selection of an aptamer that binds a particular effector may include polynucleotides that have two regions. One of the regions has random sequences, and the other region has conserved sequences suitable for hybridizing to a second polynucleotide. The library may be pre-selected for hybrid formation with the second polynucleotide by incubat-

ing representatives from the library with the second polynucleotide under conditions favorable for duplex formation and in the absence of the effector. Polynucleotides that represent "non-binders" are removed from the pool at the outset, as these already adopt structures that preclude hybridization to the second polynucleotide, independent of the effector. The initial collection of bound polynucleotides represents the candidate polynucleotides of the library that may adopt a hybridized complex with the second polynucleotide.

These candidate polynucleotides may then be isolated, amplified under suitable conditions, and incubated in the presence of the effector molecule and the second polynucleotide. In this phase of selection, the "non-binders" are isolated, as these represent candidate sequences that have bound the effector rather than hybridizing with the second polynucleotide. The polynucleotides in the unbound fraction may be re-amplified, and the selection process may be repeated. The next selection may be carried out under more stringent conditions that are favorable to hybrid selection, or it may be carried out under conditions of lower effector concentrations. Another type of selection process involves gradient elution profiles, in which varying concentrations of the effector are present. This selection may isolate discrete populations of polynucleotides having different response profiles for the same effector, such as different concentration dependencies. One or more rounds of selection may be performed in the absence of the effector to ensure the enriched aptamer species retain the ability to hybridize to the second polynucleotide.

Hybrid selection may be performed using second polynucleotides immobilized on solid supports, or any other method that enables separation of bound and unbound candidate polynucleotides from second polynucleotides, such as gradient centrifugation, chromatography, or mobility-shift assay. Where gradient elution procedures are employed, the use of automated chromatography procedures, such as RPLC or HPLC, is preferred.

An aptamer may be constructed using rational design principles. An aptamer for a particular effector may be chosen from one of the several hundred examples known in the art. See, for example, Jennifer F. Lee, Jay R. Hesselberth, Lauren Ancel Meyers, and Andrew D. Ellington, "Aptamer Database," *Nucleic Acids Res.* 2004 Jan. 1; 32(Database issue): D95-D100. The folding of many aptamers is well characterized, and the nucleotide sequence of the second polynucleotide may be designed based on the aptamer sequence. For example, a region within an aptamer may be designed to adopt a particular single-stranded conformation upon binding the effector. The second polynucleotide may then be designed to have a sequence complementary to this region. Binding between the first and second polynucleotides may be favored in the absence of the effector, but may be disfavored in the presence of the effector.

Rational design principles can be confirmed in the laboratory through simple and rapid experimental tests. For example, candidate polynucleotides and second polynucleotides may be prepared that contain one or more probes, such as a radioisotope, a fluorophore, or a particle. The stabilities of the polynucleotide complexes in the presence or absence of the effector can be studied, for example by mobility-shift assay, gradient centrifugation, or hybrid selection. Polynucleotide complexes may also be studied spectrophotometrically, where the polynucleotides are labeled with substances having a spectral signature, such as gold particles. In this technique, complex formation is monitored by the formation of particle aggregates, which display a distinct color change relative to dispersed particles. Examples of this technique are described in U.S. Patent Application Publication No. US 2006/0166222 A1 to Lu et al., published Jul. 27, 2006, which is hereby incorporated by reference.

An aptamer may be provided in vitro from a random sequence library by first selecting with the desired effector. Candidate polynucleotides displaying high affinity for the effector are characterized with regard to their folded secondary structure. Species having high affinity for the effector may share nucleotide sequences, known as consensus sequences, which may conserved for functional reasons. These species also may adopt common secondary structures, giving rise to additional consensus structural elements. The predicted conformations may be generated using a folding program, such as RNAstructure, as implemented in the Vienna RNA Package. The predicted foldings may be confirmed experimentally by doping one or more selected sequences with random nucleotides in the regions containing putative primary and secondary structure consensus motifs, and then reselecting for efficient effector binding. Where a particular structural feature of the aptamer is required for efficient effector binding, aptamers containing the preserved features may be recovered during the reselection process. Once the desired aptamer species have been isolated and characterized, second polynucleotides that bind to the aptamer(s) may be designed based on structural considerations, such as classical Watson-Crick base pairing rules.

The polynucleotide group **120** may include a nucleic acid-based enzyme. One or both of the first and second polynucleotide segments **122** and **124** independently may include a nucleic acid-based enzyme. For a polynucleotide having a nucleic acid-based enzyme in each segment, these enzymes may be the same or different. Preferably only one of the first and second polynucleotide segments includes a nucleic acid-based enzyme, and the other of the first and second polynucleotide segments includes a substrate for the enzyme. In one example of a polynucleotide group that includes a nucleic acid-based enzyme, the rupture agent **140** is a co-factor for the enzyme. In the absence of the co-factor, the two polynucleotide segments are bound together by interactions including base pairing of the complementary sequences. In the presence of the co-factor, the nucleic acid-based enzyme of the one polynucleotide segment cleaves the substrate included in the other polynucleotide segment, disrupting the base pairing between the two polynucleotide segments. Preferably the two polynucleotide segments are bound together in an orientation that provides for the substrate of the nucleic acid-based enzyme to be close to the active site of the enzyme. A nucleic acid-based enzyme of a polynucleotide segment may include at least part of the complementary sequence of the segment, or the enzyme may be distinct from the complementary sequence.

FIG. **3** is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes a nucleic acid-based enzyme. Amphiphilic substance **300** includes a hydrophobic group **310** and a polynucleotide group **320**. Polynucleotide group **320** includes a second polynucleotide segment **324** including a nucleic acid-based enzyme **326**, and includes a first polynucleotide segment **322** including a substrate for the nucleic acid-based enzyme. The first and second segments are at least partially complementary, and are bound together by interactions including base pairing. Exposure of the amphiphilic substance **300** to a co-factor **340** for the nucleic acid-based enzyme **326** results in cleavage of the substrate of the enzyme and a dissociation of the first and second polynucleotide segments. This dissociation yields a first product **350**, a second product **352** and a third product **354**. The first product **350** includes the hydrophobic group

310 and a first portion 321 of the first polynucleotide segment. The second product 352 includes the second polynucleotide segment 324. The third product 354 includes a second portion 323 of the first polynucleotide segment.

In another example of a polynucleotide group 120 that includes a nucleic acid-based enzyme, the enzyme is present with an aptamer, and the two species together form an aptazyme. In this example, the rupture agent 140 may be an effector for the aptamer, where the interaction between the aptamer and the effector activates the enzyme. In the absence of the effector for the aptamer portion of the aptazyme, the two polynucleotide segments are bound together by interactions including base pairing of the complementary sequences. In the presence of the effector, the enzyme portion of the aptazyme included in the one polynucleotide segment cleaves the substrate included in the other polynucleotide segment, disrupting the base pairing between the two polynucleotide segments. Preferably the two polynucleotide segments are bound together in an orientation that provides for the substrate of the enzyme to be close to the active site of the enzyme. An aptazyme of a polynucleotide segment may include at least part of the complementary sequence of the segment, or the aptazyme may be distinct from the complementary sequence.

FIG. 4 is a scheme representing the interaction of a rupture agent with an amphiphilic substance that includes an aptazyme. Amphiphilic substance 400 includes a hydrophobic group 410 and a polynucleotide group 420. Polynucleotide group 420 includes a second polynucleotide segment 424 including an aptazyme 426, and includes a first polynucleotide segment 422 including a substrate for the aptazyme. The first and second segments are at least partially complementary, and are bound together by interactions including base pairing. Exposure of the amphiphilic substance 400 to an effector 440 for the aptazyme 426 results in cleavage of the substrate of the enzyme and a dissociation of the first and second polynucleotide segments. This dissociation yields a first product 450, a second product 452 and a third product 454. The first product 450 includes the hydrophobic group 410 and a first portion 421 of the first polynucleotide segment. The second product 452 includes the second polynucleotide segment 424. The third product 454 includes a second portion 423 of the first polynucleotide segment.

Aptazymes may be obtained using in vitro selection techniques or by rational design. See, for example Tang et al. 1997; Hesselberth et al. 2000; Soukup, G. A., et al., *Proc. Natl. Acad. Sci. USA*, vol. 96, pp. 3584-3589, (1999); Robertson, M. P., et al., *Nucleic Acids Research*, vol. 28, no. 8, pp. 1751-1759, (2000); Seetharaman, S., et al., *Nature Biotechnology*, vol. 19, pp. 336-341, (2001); Wang, D. Y., et al., *J. Mol. Biol.*, vol. 318, pp. 33-43, (2002). Aptazymes made using in vitro selection techniques include a nucleic acid-based enzyme specific for a particular substrate whose activity depends upon an effector. Numerous examples of nucleic acid-based enzymes have been identified, either by engineering naturally occurring ribozymes or by in vitro selection of ribozymes and deoxyribozymes.

A library population of candidate polynucleotides containing nucleic acid-based enzymes may be prepared in which the activity is compromised due to the insertion of a randomized sequence cassette. Catalytically active members of the library are then selected based upon having restored catalytic activity in the presence of a selected effector. Where appropriate, the requisite co-factors and ions are included in the selection protocols at the outset, so that the aptazymes become responsive to the selected effector in the form of the aptamer target, rather than to co-factors. Negative selections may be performed to improve specificity of aptazymes with regard to different effectors.

Aptazymes may also be obtained by a rational design approach. Candidate polynucleotides may be selected from a group of nucleic acid-based enzymes belonging to different classifications (for example, hairpin ribozyme, hammerhead ribozyme, group I intron, among others). Preferred nucleic acid-based enzymes include those in which an insertion of additional nucleotides or sequences disrupts catalytic activity. An aptamer for a particular effector may be selected from one of many aptamer sequences known in the art or made using in vitro selection techniques. The chosen aptamer may be introduced in the vicinity of the catalytic core for a nucleic acid-based enzyme, whereby the activity of the catalytic core becomes responsive upon binding the target effector. Examples of rationally designed aptazymes have been generated using this general approach.

Optional linking group 126 may include any group that can form a stable connection between the first and second polynucleotide segments. For example, an optional linking group may include a third polynucleotide segment that is not complementary to either of the first or second polynucleotide segments. In another example, the polynucleotide group may be a single stranded polynucleotide. In another example, an optional linking group may include a polypeptide containing two or more amino acid residues.

Optional stabilizing group 130 may include a polymer. Examples of polymeric stabilizing groups include hydrophilic polymers, including polyethers, such as poly(ethylene oxide), polyethylene glycol (PEG), and ethers, esters and amides of PEG; ionic polymers, such as poly(acrylic acids), poly(methacrylic acids), sulfonated polystyrene, and sulfonated polystyrene; polyacrylamides; poly(vinyl alcohol); poly(vinyl imidazoles), and poly(ethylene imine). Examples of polymeric stabilizing groups include biodegradable polymers, including poly(hydroxyalkanoates), such as poly(hydroxybutyrate) and poly(hydroxyvalerate); polyesters, such as poly(lactic acid), poly(glycolic acid) and poly(caprolactone); and poly(amidoamine) dendrons and dendrimers (PAMAM). Examples of polymeric stabilizing groups include polysaccharides, such as cellulose, cellulose esters, dextrans, starches, and glycogens. Examples of polymeric stabilizing groups include polypeptides, such as avidin, streptavidin and neutravidin. Examples of polymeric stabilizing groups include polynucleotides, such as DNA, RNA, nucleic acid-based enzymes, aptamers, and peptide nucleic acids. A polymeric stabilizing group may include two or more different polymeric groups, and may include a copolymer having units of two or more different polymers. Preferably the polymeric stabilizing group is PEG, avidin or streptavidin. Although the stabilizing group may by hydrophobic or may include a polynucleotide, the stabilizing group 130 is different from the hydrophobic group 110 and the polynucleotide group 120.

An amphiphilic substance 100 may be prepared by combining a hydrophobic group and a polynucleotide group. In one example, a first polynucleotide segment of a polynucleotide group is linked to a hydrophobic group, and this combined product is contacted with the second polynucleotide segment. The second polynucleotide segment may be linked to an optional stabilizing group, and this may occur before or after the second segment is combined with the first segment. In another example, a first and a second polynucleotide segment are combined to form a polynucleotide group, and this polynucleotide group is then linked at one end with a hydrophobic group. The second polynucleotide segment may be

linked to an optional stabilizing group, and this may occur before or after the second segment is combined with the first segment.

FIG. **5** is a representation of a lipid vesicle **500** that includes a vesicle wall **510**, an amphiphilic substance **520** and optionally a stabilizing group **550**. The vesicle wall **510** includes a lipid bilayer **512**, including polar lipid **530**. The amphiphilic substance **520** includes a hydrophobic group **522** and a polynucleotide group **524**, and the hydrophobic group **522** is at least partially in the lipid bilayer **512**. The polynucleotide group **524** includes an aptamer and/or a nucleic acid-based enzyme. The vesicle wall encloses an internal space **560**.

The polar lipid **530** includes a polar lipid that is an "unstable vesicle former." Unstable vesicle formers are polar lipids that will not form vesicles, at least 50% of which persist for at least one hour, when prepared by the following method. The polar lipid is dissolved in chloroform and placed in a glass test tube. The chloroform is then removed by evaporation under a steady stream of nitrogen, followed by vacuum for twelve hours. The dried lipid material is re-hydrated in 10 millimolar (mM) Na_2HPO_4 to give a 25 mg/mL concentration. The resultant aqueous mixture is maintained for 60 minutes at a temperature above the phase transition temperature of the lipid. The lipid vesicles are then reduced in size by any convenient means, such as by high pressure homogenization or by sonication with a micro-tip 450 watt sonicator used at a 40% duty cycle.

The polar lipid **530** may include a polar phospholipid that is an unstable vesicle former. Examples of polar phospholipids that may be used as an unstable vesicle former include Lyso-Phosphatidylcholine (Lyso-PC), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOPC-e), 1-palmitoyl-2-oleyl-3-glycerophosphorcholine (POPA), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), 1-steroyl-2-docosaheaxenoyl-3-phosphocholine (18:0, 22:6, PC), mixed chain phosphatidyl choline (MPC), phosphatidyl ethanol (PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (16:0-Lyso PC), and combinations thereof. Preferably the unstable vesicle former includes DOPE.

The polar lipid **530** also may include a polar lipid that is a "stable vesicle former." Stable vesicle formers are polar lipids that will form vesicles, at least 50% of which will persist for at least one hour, when prepared as described for unstable vesicle formers. The polar lipid may include a polar phospholipid that is a stable vesicle former. Examples of polar phospholipids that may be used as a stable vesicle former include Soy Phosphatidylcholine (SOYPC) (Structure (XIV), dioleoylphosphatidylcholine (DOPC) (Structure (XV)), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (16:0, 22:6 PC) (Structure (XVI)), 1-palmitoyl-2-oleoyl-phosphocholine (16:0, 18:1 PC), 1-palmitoyl-2-linolinoyl-3-phosphocholine (16:0, 18:3 PC), 1-palmitoyl-2-arachidonoyl-3-phosphocholine (16:0, 20:4, PC), and combinations thereof.

The internal space **560** may contain a polar liquid, such as water, an aqueous mixture and/or a polar organic liquid. The internal space may further contain one or more other substances. In one example, the internal space includes a bioactive agent, such as an organic molecule, an inorganic molecule, a pharmaceutical, a polypeptide, a polynucleotide, or an antibody that interacts with an intracellular antigen. The internal space **560** may contain a bioactive agent without a polar liquid.

A method of forming a lipid vesicle includes combining ingredients including a polar lipid, a polar liquid, and an amphiphilic substance that includes a hydrophobic group and a polynucleotide group. The polynucleotide group includes an aptamer and/or a nucleic acid-based enzyme. The polar lipid includes a polar lipid that is an unstable vesicle former. The ingredients may further include a second polar lipid that is a stable vesicle former. The ingredients may further include a bioactive agent.

The polar lipid and the amphiphilic substance may be as described above. More than one type of polar lipid and/or more than one type of amphiphilic substance may be present. The polar liquid may include a polar liquid, such as water, an aqueous mixture and/or a polar organic liquid. The polar liquid may further include one or more other substances, such as a bioactive agent.

The ingredients for forming the lipid vesicle optionally may further include a stabilizing substance. Examples of stabilizing substances include hydrophilic polymers, ionic polymers, biodegradable polymers, polysaccharides, polypeptides, and polynucleotides. A stabilizing substance may include two or more different polymers, and may include a copolymer having units of two or more different polymers. Preferred stabilizing substances include PEG, avidin and streptavidin. In one example, the stabilizing substance may be present as a stabilizing group connected to a second hydrophobic group. In this example, the vesicle wall may further include at least a portion of the second hydrophobic group. The second hydrophobic group may be as described for the hydrophobic group of the amphiphilic substance. Although the stabilizing substance may by hydrophobic or may include a polynucleotide, the stabilizing substance is different from the hydrophobic group and the polynucleotide group of the amphiphilic substance.

FIG. **6** is a scheme representing the interaction of a rupture agent with a vesicle that contains an amphiphilic substance including an aptamer and/or a nucleic acid-based enzyme. Vesicle **600** includes a vesicle wall **610** enclosing an internal space **660**, and an amphiphilic substance **620**. The vesicle wall includes a polar lipid **630** and an optional stabilizing substance **650**. The amphiphilic substance **620** includes a polynucleotide group having a first polynucleotide segment **626** and a second polynucleotide segment **628**. The vesicle is stable until the amphiphilic substance **620** is exposed to sufficient amounts of a rupture agent **640**. Exposure of the amphiphilic substance **620** to sufficient amounts of the rupture agent **640** results in a disruption of the base pairing between the first and second polynucleotide segments. This disruption leads to the dissociation of the first and second polynucleotide segments at the exterior of the vesicle wall, yielding at least one product **670**, which includes a modified polynucleotide group **672** that differs from the polynucleotide group of the amphiphilic substance **620** in its size and/or conformation. De-stabilized vesicle **602** may include product **670** at the exterior of the vesicle wall, and amphiphilic substance **620** at the interior of the vesicle wall. De-stabilized vesicle **602** cannot maintain its shape, and the vesicle ruptures.

In the example of FIG. **6**, the polynucleotide group **620** includes an aptazyme in the second polynucleotide group **628**, and includes a substrate for the aptazyme in the first polynucleotide group **626**. The dissociation of the first and second polynucleotide groups yields a first product **670**, a second product **672** and a third product **674**. The first product **670** includes the hydrophobic group and a first portion **622** of the first polynucleotide segment. The second product **672** includes the second polynucleotide segment **628**. The third product **674** includes a second portion **624** of the first polynucleotide segment. The contribution of first product **670** to the stability of the vesicle is less than the contribution of the

amphiphilic substance **620**, and the original shape of vesicle **600** cannot be maintained by de-stabilized vesicle **602**. The polynucleotide group **620** may include an aptazyme, as represented in FIG. **6**, or it may include either an aptamer or a nucleic acid-based enzyme. Thus, the at least one product of the dissociation of the first and second polynucleotide segments may be as illustrated in FIGS. **1-4**.

One possible explanation for the effect of the change in the amphiphilic substance on the stability of the lipid vesicle is that the steric bulk of the amphiphilic substance influences the geometry of the vesicle wall. Molecular shapes of polar lipids can strongly affect their phase behavior. For example, many amphiphilic molecules with high positive or negative curvatures are unstable vesicle formers, since non-zero curvatures do not allow for the self-assembly necessary for vesicle formation. Polar lipids having a high molecular curvature can be manipulated into forming lipid vesicles by imposing a steric force on the bilayer containing the lipids. This steric force can result from the incorporation in the bilayer of an amphiphilic substance that includes a polynucleotide group. Such lipid vesicles may be stabilized by the difference in the amount of steric bulk at the inner and outer surfaces of the lipid bilayer. When the steric bulk of the polynucleotide group at the outer surface is reduced, for example due to a change in the size and/or conformation of the polynucleotide group, the balance of forces between the inner and outer surfaces is disrupted, leading to loss of integrity of the vesicle wall.

The destabilization effect may be enhanced when the amphiphilic substance includes a stabilizing group. For example, a stabilizing group may be attached to the polynucleotide group in such a way that the dissociation of the first and second polynucleotide segments allows the stabilizing group to separate from the vesicle. The presence of the stabilizing group can affect the asymmetry between the inner and outer layers of the lipid bilayer, and consequently can function as a control parameter for the release of the contents from the vesicle. For example, the addition of a large molecular weight PEG can increase the sensitivity of functionalized vesicles, allowing them to rupture at lower analyte concentrations.

A vesicle that includes an amphiphilic substance, where the amphiphilic substance includes an aptamer and/or a nucleic acid-based enzyme, may be tailored to rupture at different rates. In one example, the diameter of the vesicle can be controlled to affect the rate of rupture when the vesicle is exposed to a rupture agent. For example, a smaller vesicle diameter may allow the vesicle to rupture more rapidly. The average diameter of the vesicles may be, for example, from 20 nanometers (nm) to 10 micrometers (μm). Preferably the average diameter of the vesicles is from 150 nm to 7 μm, more preferably from 200 nm to 5 μm.

Lipid vesicles that include an amphiphilic substance including an aptamer and/or a nucleic acid-based enzyme may be used as part of a controlled delivery system. For example, a controlled delivery system may include the lipid vesicle and a bioactive agent in the vesicle. The contents of the vesicle may be isolated from the surrounding environment until a rupture agent is encountered. The aptamer and/or nucleic acid-based enzyme may interact with the rupture agent, causing the vesicle to rupture and release its contents.

The controlled delivery system can be applied to any environment in which it is desired to release an agent when a particular substance is present. Aptamers and nucleic acid-based enzymes can be produced to have molecular recognition capabilities for a wide range of substances. Thus, if a particular substance is correlated with an environment in which it is desirable to release an agent, an aptamer and/or nucleic acid-based enzyme may be produced that will bind to or be activated by the substance. A lipid vesicle may then be formed from a polar lipid, a polar liquid, the agent to delivered, and an amphiphilic substance that includes the aptamer and/or nucleic acid-based enzyme. Moreover, the lipid vesicle may be programmed to rupture based on a threshold concentration of the substance. This selectivity may be provided, for example, by the properties of the aptamer and/or nucleic acid-based enzyme, by the presence or absence of a stabilizing group attached to the polynucleotide group, and/or by the composition of the lipid vesicle wall.

A controlled delivery system that includes a bioactive agent in a lipid vesicle, where the lipid vesicle includes an amphiphilic substance including an aptamer and/or a nucleic acid-based enzyme, may be used to deliver a bioactive agent. For example, a method of delivering a bioactive agent includes administering the controlled delivery system to an organism.

The controlled delivery system may be used to deliver drug agents to a particular type of tissue in an organism. In one example, an amphiphilic substance may be prepared that includes an aptamer for prostate-specific-membrane-antigen (PSMA). The amphiphilic substance may include a hydrophobic group and a polynucleotide group having two segments, where one segment includes the aptamer, and the other segment includes a sequence complementary to a sequence in that segment. A chemotherapeutic agent for prostate cancer can be encapsulated in a lipid vesicle that includes the amphiphilic substance. When the lipid vesicle comes into contact with prostate cancer tissue, the aptamer can bind the PSMA, causing a change in the polynucleotide group, inducing instability in the bilayer surface, and rupturing the vesicle. Thus, the chemotherapeutic agent is delivered specifically to the cancerous tissue.

The following examples are provided to illustrate one or more preferred embodiments of the invention. Numerous variations may be made to the following examples that lie within the scope of the invention.

EXAMPLES

Materials

All oligonucleotides were obtained from Integrated DNA Technologies Inc. (IDT) and Eurogentec Oligos. The following oligonucleotides were obtained from IDT:

(7)-17E-(-1):
(SEQ ID NO: 1)
5' AA AGA GAG ATC TCT TCT (CCGAGCCGGTCGAA) ATA GTG AG 3'

Biotin-(7)-17E-(-1) (biotin-TEG modification):
(SEQ ID NO: 2)
Biotin -5' AA AGA GAG ATC TCT TCT (CCGAGCCGGTCGAA) ATA GTG AG 3'

SH-(7)-17E-(-1)) (thiol modification):
(SEQ ID NO: 3)
Thiol-C6-5' AA AGA GAG ATC TCT TCT (CCGAGCCGGTCGA A) ATA GTG AG 3'

An oligonucleotide with cholesterol and RNA base modification, which was a 17E substrate, was obtained from Eurogentec Oligos:

(5)-17DS-(1)-ChoI

(SEQ ID NO: 4)
3' TCT CTG TAG AGA AGG (Ra) TAT CAC TC AA 5'-ChoI

Streptavidin was obtained from SouthernBiotech and used without any purification or dilution. Dioleoylphosphatidyl ethanolamine (DOPE) in powder form was obtained from Avanti Polar Lipids. Biotin was obtained from Sigma-Aldrich, dissolved in 50 mM pH 7.3 Hepes buffer, and diluted to 2.2 mM. A FluoroMax® fluorometer was used for measuring the changes in fluorescence intensity in bulk solution of liposomes.

Example 1

Synthesis of Amphiphilic Substance Including Polynucleotide Group Containing DNAzyme

The oligonucleotides (5)-17DS-(1)-Chol and (7)-17E-(−1) were combined to form an amphiphilic substance. The first polynucleotide segment of the amphiphilic substance included a cholesterol group as a hydrophobic group, and included a substrate for a nucleic acid-based enzyme based on DNA (DNAzyme). The second polynucleotide segment of the amphiphilic substance included the DNAzyme, and included a sequence complementary to a sequence in the first segment.

The amphiphilic substance was prepared using the following protocol. The oligonucleotides (7)-17E-(−1) (4 microliters (μL) of a 1 mM solution) and (5)-17DS-(1)-Chol (4 μL of a 1 mM solution) were added to 24 μL, of deionized water in a 500 μL PCR tube. To this mixture was added 8 μL of 1 M NaCl and 4 μL of 500 mM pH 7.3 Hepes buffer. The resulting mixture was brought to 80° C. by placing the tube in a beaker with hot water. The mixture then slowly cooled down to room temperature and was stored at 4° C. for half an hour in order to fully hybridize the DNA strands. The sequence of the resulting amphiphilic substance was:

(SEQ ID NO: 5)
5'-Cholesterol-TEG-AACTCACTAT R(a) GGAAGAGATGTCTC T-3'

(SEQ ID NO: 1)
5'-AAAGAGAGATCTCT TCTCCGAGCCGGTCGAAATAGTGAG-3'

Example 2

Formation of Stable Vesicle Including Amphiphilic Substance Containing Polynucleotide Group

The amphiphilic substance of Example 1 was combined with DOPE and a 2% PEG-lipid to form stable vesicles. The PEG had a molecular weight of 2,000 daltons. The stable vesicles had an average diameter of 1 micrometer.

Vesicles were prepared using the following protocol. The DOPE polar lipid (8 micromoles (μmol)) was dissolved in chloroform, dried in a glass vial under nitrogen, and desiccated under vacuum overnight. The amphiphilic substance of Example 1 and a 2% PEG-lipid (Avanti Polar Lipids) was added into the vial, and the mixture was incubated overnight at 35° C. The mixture was then hydrated by adding 1 ml of 50 mM fluorescein in 100 mM NaCl and 50 mM pH 7.3 Hepes buffer. The hydrated mixture was incubated at 35° C. for one day. The resultant solution was extruded 11 times in a mini-extruder through a 1 μm polycarbonate membrane. The resulting vesicles were isolated from the dye mixture using a PD-10 desalting column (Amersham Biosciences). The column was primarily washed with 50 mM pH 7.3 Hepes buffer and 100 mM NaCl. The vesicle solution (200 μL) was run through the column, and two clear reddish lanes were observed. The first fraction containing vesicles was collected in a 1.5 ml tube, and the remainder was washed out with buffer.

Example 3

Controlled Rupture of Vesicles

The stabilized vesicles of Example 2 were reconstituted in water containing 100 mM NaCl and 50 mM pH 7.4 Hepes buffer. The release of fluorescein was measured by fluorescence spectroscopy. The 50 mM fluorescein mixture in the vesicles was self-quenching. As the fluorescein is released into the environment, the total concentration of fluorescein in the solution after vesicle disruption is much less than the concentration of fluorescein within the vesicles before disruption, causing a cessation of the self-quenching effect of the high concentration of fluorescein in the vesicles. This was observed as an increase in fluorescence intensity.

The effect of three separate reagents on the vesicles was measured. FIG. **7** is a collection of fluorescence spectra for vesicles exposed to 50 micromolar (μM) Pb^{2+} ion, to 50 μM Mn^{2+} ion, to Triton-X detergent, and to no added ions or detergent (labeled "vesicles"), where the spectra were recorded 5 minutes after addition of the reagent. Upon addition of Pb^{2+}, the fluorescence intensity increased, indicating release of the contents of the vesicles. In contrast, addition of Mn^{2+} resulted in minimal increase in fluorescence intensity relative to the control sample. Addition of the detergent Triton-X, which is known to destabilize vesicles, resulted in an increase in fluorescence intensity greater than that observed with Pb^{2+}.

The kinetic effect of different metal ions on the vesicles also was measured. FIG. **8** is a graph of normalized fluorescence intensity over time after exposure of the vesicles to 40 μM either of Mn^{2+}, Pb^{2+} or other metal ions. The result demonstrated that Pb^{2+} ruptured the vesicles. Other metal ions had minor effects on dye release, which probably was due not to the affinity of metal ions, but rather to time dependent release of dye through natural leakage.

The kinetic effect of different metal ion concentrations on the vesicles also was measured. FIG. **9** is a graph of normalized fluorescence intensity over time after exposure of the vesicles to either Mn^{2+} or Pb^{2+} at different concentrations. The result demonstrated that Pb^{2+} ruptured the vesicles. Mn^{2+} had minor effects on dye release, which probably was due not to the affinity of metal ions, but rather to time dependent release of dye through natural leakage.

These results were compared with the release observed from two types of control vesicles. In one type of control vesicle, the amphiphilic substance included in the vesicle included a DNAzyme that did not require Pb^{2+} ion as a co-factor. This amphiphilic substance was prepared as described in Example 1, but using an inactive DNAzyme, resulting in a substance having the following sequence:

(SEQ ID NO: 5)
5'-Cholesterol-TEG-AACTCACTAT R(a) GGAAGAGATGTCTC T-3'

(SEQ ID NO: 1)
5'-AAAGAGAGATCTCT TCCCCGAGCCGGTCGAAATAGTGAG-3'

The release of fluorescein from these vesicles was higher with Pb^{2+} exposure than with exposure to the other metal ions. This may have been due to minor activity of the inactive DNAzyme with lead ion. When compared to active DNAzyme vesicles, however, the amount of release was insignificant.

Example 4

Synthesis of Amphiphilic Substance Including Polynucleotide Group Containing DNAzyme and Stabilizing Group

The oligonucleotides (5)-17DS-(1)-Choi and Biotin-(7)-17E-(−1) were combined to form an amphiphilic substance. The first polynucleotide segment of the amphiphilic substance included a cholesterol group as a hydrophobic group, and included a substrate for a nucleic acid-based enzyme based on DNA (DNAzyme). The second polynucleotide segment of the amphiphilic substance included the DNAzyme, and included a sequence complementary to a sequence in the first segment. The biotin was linked to streptavidin to form an amphiphilic substance that included a streptavidin group as a stabilizing group.

An initial amphiphilic substance was prepared using the protocol of Example 1. This initial amphiphilic substance was mixed with 6 μL of 2.2 mM biotin solution. Streptavidin (10.5 μL) was added to the mixture and mixed by gentle vortexing. The streptavidin addition was repeated three more times, for a total of four additions. The sequence of the resulting amphiphilic substance was:

(SEQ ID NO: 5)
5'-Cholesterol-TEG-AACTCACTAT R(a) GGAAGAGATGTCTC T-3'

(SEQ ID NO: 6)
Streptavidin-Biotin-TEG-5'-AAAGAGAGATCTCTTCTCCGAGC CGGTCGAAATA-GTGAG 3'

Example 5

Formation of Stable Vesicle Including Amphiphilic Substance Containing Polynucleotide Group and Stabilizing Group

DOPE was combined with the amphiphilic substance of Example 4 in water containing 100 mM NaCl and 50 mM pH 7.4 Hepes buffer containing 50 mM fluorescein. The mixture formed stable vesicles having an average diameter of 1 micrometer. In contrast, DOPE in buffer containing 50 mM fluorescein without the amphiphilic substance produced only an agglomeration of the lipid. Exposure of the stable vesicles to Pb^{2+} ruptured the vesicles and released the fluorescein. The results of this study were observed by optical microscopy.

While various embodiments of the invention have been described, it will be apparent to those of ordinary skill in the art that other embodiments and implementations are possible within the scope of the invention. Accordingly, the invention is not to be restricted except in light of the attached claims and their equivalents.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 6

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<211> LENGTH: 39
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 1

aaagagagat ctcttctccg agccggtcga aatagtgag 39

<210> SEQ ID NO 2
<211> LENGTH: 39
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Biotin on 5'-end of oligonucleotide

<400> SEQUENCE: 2

aaagagagat ctcttctccg agccggtcga aatagtgag 39

<210> SEQ ID NO 3
<211> LENGTH: 39
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<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
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-continued

<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Thiol on 5'-end of oligonucleotide.

<400> SEQUENCE: 3

aaagagagat ctcttctccg agccggtcga aatagtgag 39

<210> SEQ ID NO 4
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Cholesterol on 5'-end
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<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: A at position 11 is a ribonucleotide

<400> SEQUENCE: 4

aactcactat aggaagagat gtctct 26

<210> SEQ ID NO 5
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<220> FEATURE:
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<223> OTHER INFORMATION: Cholesterol-TEG on 5'-end
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: A at position 11 is a ribonucleotide

<400> SEQUENCE: 5

aactcactat aggaagagat gtctct 26

<210> SEQ ID NO 6
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<223> OTHER INFORMATION: oligonucleotide
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<223> OTHER INFORMATION: Streptavidin-Biotin-TEG on 5'-end

<400> SEQUENCE: 6

aaagagagat ctcttctccg agccggtcga aatagtgag 39

What is claimed is:

1. An amphiphilic substance, comprising:

a polynucleotide group comprising a first polynucleotide segment and a second polynucleotide segment that is at least partially complementary to the first polynucleotide segment, wherein the first polynucleotide segment comprises an aptamer, and wherein base pairing between the first polynucleotide segment and the second polynucleotide segment occurs in an absence of binding of an effector specific for the aptamer and base pairing between the first polynucleotide segment and the second polynucleotide segment is disrupted in the presence of the effector;

a hydrophobic group attached to only one of the first polynucleotide segment and the second polynucleotide segment; and

a stabilizing group, attached to the first or second polynucleotide segment that is not attached to the hydrophobic group, where the stabilizing group is different from the hydrophobic group and the first and second polynucleotide segments.

2. The amphiphilic substance of claim **1**, where the hydrophobic group comprises a member selected from the group consisting of a wax group, a fatty acyl, a triglyceride, a phosphoglyceride, a sphingolipid group, a prostaglandin group, a terpene group and a steroid group.

3. The amphiphilic substance of claim 1, further comprising a linking group between the first polynucleotide segment and the second polynucleotide segment.

4. A lipid vesicle, comprising:

a vesicle wall surrounding an internal space, the vesicle wall including a lipid bilayer comprising a first polar lipid that is an unstable vesicle former;

the amphiphilic substance of claim 1, wherein the hydrophobic group is at least partially within the lipid bilayer;

at least one bioactive agent within the internal space; and

a polar liquid.

5. The lipid vesicle of claim 4, where the lipid bilayer further comprises a second polar lipid that is a stable vesicle former.

6. The lipid vesicle of claim 4, where the first polar lipid comprises a phospholipid.

7. The lipid vesicle of claim 4, where the first polar lipid comprises DOPE.

8. The amphiphilic substance of claim 1, where the effector is a metal ion, polynucleotide, protein, or organic compound.

9. The amphiphilic substance of claim 1, where the effector is adenosine.

10. The lipid vesicle of claim 4, wherein the disruption of the base pairing between the first polynucleotide segment and the second polynucleotide segment allows the stabilizing group to separate from the vesicle.

11. The lipid vesicle of claim 4, where the disruption of the base pairing between the between the first polynucleotide segment and the second polynucleotide segment causes the vesicle to rupture.

12. The amphiphilic substance of claim 1 where the stabilizing group is polyethylene glycol, avidin, streptavidin, neutravidin, poly(acrylic acid), poly(methacrylic acid), sulfonated polystyrene, polyacrylamide, poly(vinyl alcohol), poly(vinyl imidazole), poly(ethylene imine), poly(hydroxybutyrate) poly(hydroxyvalerate), a polyester, poly(amidoamine) dendrons and dendrimers (PAMAM), or a combination thereof.

13. The amphiphilic substance of claim 1, where at least one of the first polynucleotide aptamer segment or the second polynucleotide segment further comprises a nucleic acid-based enzyme.

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