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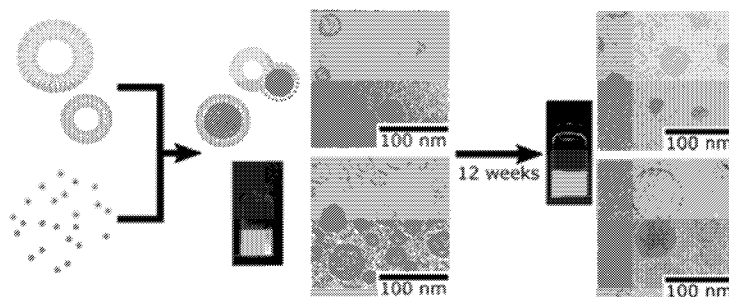


FIG. 87

(57) **Abstract:** Disclosed herein are compositions comprising an aqueous solution comprising a composite nanocarrier, the composite nanocarrier comprising a nanoparticle at least partially encapsulated within a vesicle, the vesicle comprising a sterol and a surfactant. In some examples, the vesicle consists of a sterol and a surfactant. In some examples, the sterol comprises cholesterol or a derivative thereof. In some examples, the surfactant comprises a quaternary ammonium surfactant, such as cetyl trimethylammonium bromide. In some examples, the nanoparticle comprises Si. The compositions described herein can be substantially stable. Also disclosed herein are methods of making and methods of use of the composition disclosed herein.

COMPOSITIONS COMPRISING COMPOSITE NANOCARRIERS AND METHODS OF MAKING AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/299,939, filed February 25, 2016, which is hereby incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with Government support under grant CHE1308813 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND

Nanoparticles have been studied for a variety of applications, many of which can require the nanoparticles to be dispersible in water. One approach to achieve dispersability in water for hydrophobic nanoparticles is to use colloidal vehicles, such as micelles, vesicles and polymer nanoparticles, for their stabilization in aqueous media. Vesicular structures like liposomes, which mimic cell membranes and are formed by bilayers of amphiphilic molecules, offer hydrophobic regions in the bilayer which can interact with hydrophobic particles. Despite their versatility, the translation of liposomal formulations to real-world applications have been hindered by the tendency of these lipid self-assemblies to aggregate and by their low degree of structural homogeneity. The compositions and methods disclosed herein address these and other needs.

SUMMARY

In accordance with the purposes of the disclosed compositions and methods, as embodied and broadly described herein, the disclosed subject matter relates to compositions and methods of making and using the compositions.

Disclosed herein are compositions comprising an aqueous solution comprising a composite nanocarrier, the composite nanocarrier comprising a nanoparticle at least partially encapsulated within a vesicle. In some examples, the vesicle can have an average size of from 25 to 500 nm as measured by dynamic light scattering (e.g., from 50 nm to 300 nm). In some examples, the vesicle can be substantially spherical in shape.

The vesicle can comprise a sterol and a surfactant. The vesicle can, for example, be formed by self-assembly of the sterol and the surfactant. As used herein, a lipid is not a surfactant, such that the vesicles are not liposomes. In some examples, the sterol and the surfactant can be present in the vesicle in a molar ratio of from 10:1 to 1:5 (e.g., from 2:1 to 1:2).

In some examples, the sterol and the surfactant can be present in the vesicle in a molar ratio of 1:1.

In some examples, the surfactant comprises a quaternary ammonium surfactant, such as cetyl trimethylammonium bromide. In some examples, the vesicle comprises a sterol and a quaternary ammonium surfactant. In some examples, the vesicle consists of a sterol and a quaternary ammonium surfactant. In some examples, the sterol comprises cholesterol or a derivative thereof. In some examples, the sterol consists of cholesterol or a derivative thereof.

The nanoparticle that can be used in the compositions disclosed herein can comprise, for example, a semiconductor. In some examples, the nanoparticle can comprise a semiconductor such as Fe_2O_3 , WO_3 , Ta_3N_5 , TaON , TiO_2 , ZnO , CdS , CdSe , Si , or combinations thereof. In some examples, the nanoparticle can comprise Si .

The nanoparticle that can be used in the compositions disclosed herein can comprise, for example, a metal. In some examples, the nanoparticle can comprise a metal selected from the group consisting of Be, Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, Ba, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and combinations thereof.

In some examples, the nanoparticle can comprise a magnetic nanoparticle. The magnetic nanoparticle can comprise any suitable material, for example Fe, Co, Zn, Ni, Mn, Ag, Au, C, Cd, or a combination thereof. In some examples, the magnetic nanoparticle can comprise any suitable metal compound, such as an oxide of Fe, Co, Zn, Ni, Mn, or a combination thereof. In some examples, the magnetic nanoparticle comprise an iron oxide, for example Fe_3O_4 .

In some examples, the nanoparticle can comprise a plasmonic nanoparticle. The plasmonic nanoparticle can any suitable material, for example Au, Ag, Pd, and combinations thereof.

In some examples, the nanoparticle can comprise a fluorescent nanoparticle. In some examples, the nanoparticle can be biocompatible. In some examples, the nanoparticle can be substantially spherical in shape. The nanoparticle can, for example, further comprise a capping layer comprising a plurality of ligands.

The average particle size of the nanoparticle as measured by transmission electron microscopy can, for example, be from 1 nm to 20 nm (e.g., from 1 nm to 5 nm). In some examples, the number of nanoparticles at least partially encapsulated within each vesicle can be 1 or more (e.g., from 1 to 200).

The nanoparticle and the sterol can be present in the composition, for example, in a molar

ratio of from 1:10000 to 1:50 (e.g., from 1:1800 to 1:100). In some examples, the nanoparticle and the surfactant can be present in the composition in a molar ratio of from 1:10000 to 1:50 (e.g., from 1:1800 to 1:100).

5 The average size of the composite nanocarrier as measured by dynamic light scattering can, for example, be from 25 to 500 nm (e.g., from 50 nm to 300 nm).

The compositions described herein can be substantially stable. In some examples, the compositions can be substantially stable upon dilution. In some examples, the average size of the composite nanocarriers can change by 35% or less over a period of time (e.g., 8 weeks or more) and/or upon dilution.

10 In certain examples, wherein the nanoparticle comprises a fluorescent nanoparticle, the composition can comprise a fluorescent composition. In certain examples, the fluorescence of the composition can be stable upon dilution of the composition. In some examples, the peak wavelength of the photoluminescence spectrum of the fluorescent composition can shift by 60 nm or less over a period of time (e.g., 12 weeks or more) and/or upon dilution.

15 The compositions can, in some examples, be biocompatible. In some examples, the compositions can be stable under physiological conditions.

The compositions can comprise, in some examples, water and a cosolvent. In other words, in some examples the aqueous solutions can further comprise a cosolvent. Examples of cosolvents include, but are not limited to, alcohols (e.g., methanol, ethanol, n-butanol, isopropanol, n-propanol), carboxylic acids (e.g., acetic acid), chloroform, and combinations thereof. In some examples, the aqueous solutions are substantially free of precipitate.

Also disclosed herein are methods of making the compositions disclosed herein. For example, also disclosed herein are methods of making the compositions described herein, the method comprising contacting the nanoparticle and the vesicle, thereby forming a mixture; and mixing the mixture, thereby at least partially encapsulating the nanoparticle within the vesicle (e.g., thereby forming the composite nanocarrier). Mixing can be accomplished by mechanical stirring, shaking, vortexing, sonication (e.g., bath sonication, probe sonication), and the like. In some examples, the mixture is bath sonicated. For example, the mixture can be bath sonicated for from 10 seconds to 5 hours (e.g., from 1 minute to 30 minutes, or for 5 minutes). In some examples, the method can further comprise forming the vesicle. In some examples, the method can further comprise forming the nanoparticle.

Also disclosed are pharmaceutical compositions that comprise the compositions disclosed herein in combination with a pharmaceutically acceptable excipient.

Also disclosed herein are methods of use of the composition disclosed herein, wherein the composition is used as a sensor to detect the presence or amount of a biological moiety; the structure, composition, and conformation of a biological moiety; the localization of a biological moiety in an environment; interactions of biological moieties; alterations in structures of biological compounds; alterations in biological processes; or combinations thereof. In certain examples, the compositions can further comprise a targeting moiety that has an affinity for a biological target.

Also disclosed herein are methods of use of the compositions disclosed herein in devices. In other words, also disclosed herein are devices comprising the compositions disclosed herein. Examples of devices include, but are not limited to, electronic devices, energy storage devices, energy conversion devices (e.g., solar cells, fuel cells, photovoltaic cells), optical devices (e.g., light emitting diodes), optoelectronic devices, bioanalytical devices, chemical sensors, biosensors, and combinations thereof.

Also disclosed herein are methods of imaging a cell or a population of cells within or about a subject. The methods can comprise administering to the subject an amount of a composition as described herein; and detecting the composition. Such imaging methods can be used, for example, for assessing the extent of a disease and/or the target of a therapeutic agent. In some examples, the cells are indicative of a disease, such as cancer.

Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE FIGURES

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosure and together with the description, serve to explain the principles of the disclosure.

Figure 1 is a transmission electron microscopy (TEM) image of octene-coated silicon nanocrystals used in the silicon nanocrystal and nanovesicle assembly experiments.

Figure 2 is a TEM image of octene-coated silicon nanocrystals used in the silicon nanocrystal and nanovesicle assembly experiments.

Figure 3 is a TEM image of octene-coated silicon nanocrystals used in the silicon nanocrystal and nanovesicle assembly experiments.

Figure 4 is a histogram of a sample of 150 nanocrystals measured from the TEM images of octene-coated silicon nanocrystals used in the silicon nanocrystal and nanovesicle assembly experiments shows the average size of the silicon nanocrystals is 2.8 nm with a standard deviation of 0.6 nm.

Figure 5 shows the results of thermogravimetric analysis of silicon nanocrystals coated with octene ligand.

Figure 6 is a schematic representation of the process for incorporating silicon nanocrystals into nanovesicles through bath sonication.

Figure 7 is a cryo-TEM image of nanovesicles (left) as well as photographs (upper right) of the vial containing the nanovesicles under ambient light (left) and under a 365 nm ultraviolet lamp (right) and a diagram of the nanovesicle structures (lower right).

Figure 8 is a TEM image of silicon nanocrystals (left) as well as photographs (upper right) of the vial containing the silicon nanocrystals under ambient light (left) and under a 365 nm ultraviolet lamp (right) and a diagram of the silicon nanocrystal structures (lower right).

Figure 9 is a Cryo-TEM images of the nanovesicle-silicon nanocrystal assemblies (left), as well as photographs (upper right) of the vial containing the nanovesicle-silicon nanocrystal assemblies under ambient light (left) and under a 365 nm ultraviolet lamp (right) and a diagrams of the nanovesicle-silicon nanocrystal assemblies (lower right).

Figure 10 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for spherical structures of silicon nanocrystals with similar sizes as plain nanovesicles (arrow) and empty nanovesicles with no silicon nanocrystals incorporated.

Figure 11 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for spherical structures of silicon nanocrystals with similar sizes as plain nanovesicles (upper arrow), nanovesicles with silicon nanocrystals on one side (lower arrow), and empty nanovesicles with no silicon nanocrystals incorporated.

Figure 12 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for nanovesicles with silicon nanocrystals on one side (arrows) and empty nanovesicles with no silicon nanocrystals incorporated.

Figure 13 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for spherical structures of silicon nanocrystals with similar

sizes as plain nanovesicles (rightmost arrow), nanovesicles with silicon nanocrystals on one side (left two arrows), and empty nanovesicles with no silicon nanocrystals incorporated.

Figure 14 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for nanovesicles with silicon nanocrystals on one side (lower
5 arrow), nanovesicles connected by silicon nanocrystal aggregates (upper arrow), and empty nanovesicles with no silicon nanocrystals incorporated.

Figure 15 is a Cryo-TEM image of fluorescent dispersions of nanovesicles and silicon nanocrystals. Examples are shown for nanovesicles connected by silicon nanocrystal aggregates (arrow) and empty nanovesicles with no silicon nanocrystals incorporated.

10 Figure 16 is a diagram of the radius of an average surface of both membranes (R_s) used to estimate the number of cholesterol-CTAB pairs in a typical nanovesicle.

Figure 17 is a diagram of the dimensions of the headgroup of the CTAB surfactant used to estimate the number of cholesterol and CTAB molecules per nanovesicles.

Figure 18 is a normalized absorbance (solid lines), PL (320 nm excitation) (dashed lines),
15 and PLE (660 nm emission for silicon nanocrystal in chloroform, 670 nm emission for silicon nanocrystal in nanovesicles) (dotted lines) spectra for octene coated silicon nanocrystals dispersed in chloroform or incorporated into nanovesicles in an aqueous media.

Figure 19 shows photographs of vials of silicon nanocrystals incorporated into nanovesicles and control solutions under ambient light (top) and under a 365 nm UV lamp
20 (bottom) taken immediately after preparation. The five vials monitored were (i) silicon nanocrystals in 7 mM cholesterol-CTAB nanovesicles (with CTAB concentration above cmc), (ii) silicon nanocrystals in 0.7 mM cholesterol-CTAB nanovesicles (with CTAB concentration bellow cmc), (iii) silicon nanocrystals in 7 mM CTAB micelles, (iv) silicon nanocrystals in 10% EtOH, and (v) chloroform in 7 mM CTAB nanovesicles. Vials were prepared by adding 20 μ l
25 silicon nanocrystal (in chloroform) to 0.75 ml of solution, and then bath sonicated for 5 minutes.

Figure 20 shows photographs of vials of silicon nanocrystals incorporated into nanovesicles and control solutions under ambient light (top) and under a 365 nm UV lamp
(bottom) taken one day after preparation. The five vials monitored were (i) silicon nanocrystals in 7 mM cholesterol-CTAB nanovesicles (with CTAB concentration above cmc), (ii) silicon
30 nanocrystals in 0.7 mM cholesterol-CTAB nanovesicles (with CTAB concentration bellow cmc), (iii) silicon nanocrystals in 7 mM CTAB micelles, (iv) silicon nanocrystals in 10% EtOH, and (v) chloroform in 7 mM CTAB nanovesicles. Vials were prepared by adding 20 μ l silicon nanocrystal (in chloroform) to 0.75 ml of solution, and then bath sonicated for 5 minutes.

Figure 21 shows photographs of vials of silicon nanocrystals incorporated into nanovesicles and control solutions under ambient light (top) and under a 365 nm UV lamp (bottom) taken 2 weeks after preparation. The five vials monitored were (i) silicon nanocrystals in 7 mM cholesterol-CTAB nanovesicles (with CTAB concentration above cmc), (ii) silicon nanocrystals in 0.7 mM cholesterol-CTAB nanovesicles (with CTAB concentration below cmc), (iii) silicon nanocrystals in 7 mM CTAB micelles, (iv) silicon nanocrystals in 10% EtOH, and (v) chloroform in 7 mM CTAB nanovesicles. Vials were prepared by adding 20 μ l silicon nanocrystal (in chloroform) to 0.75 ml of solution, and then bath sonicated for 5 minutes.

Figure 22 shows photographs of vials of silicon nanocrystals incorporated into nanovesicles and control solutions under ambient light (top) and under a 365 nm UV lamp (bottom) taken 8 weeks after preparation. The five vials monitored were (i) silicon nanocrystals in 7 mM cholesterol-CTAB nanovesicles (with CTAB concentration above cmc), (ii) silicon nanocrystals in 0.7 mM cholesterol-CTAB nanovesicles (with CTAB concentration below cmc), (iii) silicon nanocrystals in 7 mM CTAB micelles, (iv) silicon nanocrystals in 10% EtOH, and (v) chloroform in 7 mM CTAB nanovesicles. Vials were prepared by adding 20 μ l silicon nanocrystal (in chloroform) to 0.75 ml of solution, and then bath sonicated for 5 minutes.

Figure 23 shows photographs of vials of silicon nanocrystals incorporated into nanovesicles and control solutions under ambient light (top) and under a 365 nm UV lamp (bottom) taken 12 weeks after preparation. The five vials monitored were (i) silicon nanocrystals in 7 mM cholesterol-CTAB nanovesicles (with CTAB concentration above cmc), (ii) silicon nanocrystals in 0.7 mM cholesterol-CTAB nanovesicles (with CTAB concentration below cmc), (iii) silicon nanocrystals in 7 mM CTAB micelles, (iv) silicon nanocrystals in 10% EtOH, and (v) chloroform in 7 mM CTAB nanovesicles. Vials were prepared by adding 20 μ l silicon nanocrystal (in chloroform) to 0.75 ml of solution, and then bath sonicated for 5 minutes.

Figure 24 shows the absorbance measured at 320 nm for samples with silicon nanocrystals demonstrate the stability of the nanoparticle fluorescence in nanovesicles.

Figure 25 shows the average PL wavelength for samples with silicon nanocrystals (excited at 320 nm) demonstrate the stability of the nanoparticle fluorescence in nanovesicles.

Figure 26 is a Cryo-TEM image of silicon nanocrystals in low concentration of nanovesicles (below CMC). 0.75 ml of 0.7 mM cholesterol-CTAB nanovesicles was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals in chloroform, and the mixture was bath sonicated for 5 minutes.

Figure 27 is a Cryo-TEM image of silicon nanocrystals in low concentration of

nanovesicles (below CMC). 0.75 ml of 0.7 mM cholesterol-CTAB nanovesicles was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals in chloroform, and the mixture was bath sonicated for 5 minutes.

Figure 28 is a Cryo-TEM image of silicon nanocrystals in low concentration of nanovesicles (below CMC). 0.75 ml of 0.7 mM cholesterol-CTAB nanovesicles was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals in chloroform, and the mixture was bath sonicated for 5 minutes.

Figure 29 is a Cryo-TEM image of silicon nanocrystals in low concentration of nanovesicles (below CMC). 0.75 ml of 0.7 mM cholesterol-CTAB nanovesicles was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals in chloroform, and the mixture was bath sonicated for 5 minutes.

Figure 30 is a Cryo-TEM image taken on the day the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 31 is a Cryo-TEM image taken on the day the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 32 is a Cryo-TEM image taken on the day the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 33 is a Cryo-TEM image taken 2 days after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 34 is a Cryo-TEM image taken 2 days after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 35 is a Cryo-TEM image taken 2 days after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 36 is a Cryo-TEM image taken 7 weeks after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 37 is a Cryo-TEM image taken 7 weeks after the sample was prepared of the

structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 38 is a Cryo-TEM image taken 7 weeks after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 39 is a Cryo-TEM image taken 12 weeks after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 40 is a Cryo-TEM image taken 12 weeks after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 41 is a Cryo-TEM image taken 12 weeks after the sample was prepared of the structures observed after incorporation of silicon nanocrystals into nanovesicles (7 mM cholesterol-CTAB) through 5 minutes of bath sonication.

Figure 42 is a Cryo-TEM image of CTAB stabilized silicon nanocrystals. 0.75 ml of 7 mM CTAB micelles in water was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals (in chloroform), and the mixture was bath sonicated for 5 minutes.

Figure 43 is a Cryo-TEM image of CTAB stabilized silicon nanocrystals. 0.75 ml of 7 mM CTAB micelles in water was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals (in chloroform), and the mixture was bath sonicated for 5 minutes.

Figure 44 is a Cryo-TEM image of CTAB stabilized silicon nanocrystals. 0.75 ml of 7 mM CTAB micelles in water was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals (in chloroform), and the mixture was bath sonicated for 5 minutes.

Figure 45 is a Cryo-TEM image of CTAB stabilized silicon nanocrystals. 0.75 ml of 7 mM CTAB micelles in water was combined with 20 μ l of 6.75 mg/ml silicon nanocrystals (in chloroform), and the mixture was bath sonicated for 5 minutes.

Figure 46 shows the size distribution of 20 μ l of silicon nanocrystals in chloroform incorporated into 750 μ l nanovesicles (sample (i) from stability trials), measured by dynamic light scattering (DLS), following 5 minutes of bath sonication. Measurements were done in week 0 and in week 8. Sizes measured by DLS (each measured in triplicate) were: a z-ave of 54.34 nm with a polydispersity index (PDI) of 0.262 at week 0; and a z-ave of 65.17 nm with a PDI of 0.157 at week 8.

Figure 47 shows the size distribution of 20 μ l of chloroform with 750 μ l nanovesicles

(sample (v) from stability trials), measured by DLS, following 5 minutes of bath sonication. Measurements were done in week 0 and in week 8. Sizes measured by DLS (each measured in triplicate) were: a z-ave of 58.43 nm with a PDI of 0.235 at week 0; and a z-ave of 79.34 nm with a PDI of 0.096 at week 0.

5 Figure 48 is a Cryo-TEM image showing incorporation of silicon nanocrystals into nanovesicles formed with only water (no ethanol).

 Figure 49 is a Cryo-TEM image showing incorporation of silicon nanocrystals into nanovesicles formed with only water (no ethanol).

10 Figure 50 is a Cryo-TEM image showing incorporation of silicon nanocrystals into nanovesicles formed with only water (no ethanol).

 Figure 51 shows photographs of vials containing the samples formed by incorporating silicon nanocrystals into nanovesicles formed with only water (no ethanol) under ambient light (left) and under a 366 nm ultraviolet lamp (right), showing the characteristic fluorescence from the silicon nanocrystals.

15 Figure 52 is a Cryo-TEM image of the sample bath sonicated for 1 minute to incorporate silicon nanocrystals into nanovesicles.

 Figure 53 is a Cryo-TEM image of the sample bath sonicated for 5 minutes to incorporate silicon nanocrystals into nanovesicles.

20 Figure 54 is a Cryo-TEM image of the sample bath sonicated for 15 minutes to incorporate silicon nanocrystals into nanovesicles.

 Figure 55 is a Cryo-TEM image of the sample bath sonicated for 30 minutes to incorporate silicon nanocrystals into nanovesicles.

25 Figure 56 shows the dynamic light scattering (DLS) data results from using different bath sonication times to incorporate silicon nanocrystals into nanovesicles showed that at the longer time points of 15 and 30 minutes, nanovesicles appear smaller in size. Sizes measured by DLS (each measured in triplicate) were: a z-ave of 82.44 nm with a PDI of 0.248 at 1 min; a z-ave of 69.02 nm with a PDI of 0.232 at 5 min; a z-ave of 60.71 nm with a PDI of 0.154 at 15 min; and a z-ave of 64.61 nm with a PDI of 0.139 at 30 min.

30 Figure 57 shows the results from preparing DOPC (neutral) liposomes separately and then adding 20 μ l silicon nanocrystals in chloroform and bath sonicating for 5 minutes. Photographs of vials of DOPC were taken under ambient light (top row) and under a 365 nm UV lamp (bottom row) (i) before bath sonication, (ii) immediately after sonication, (iii) after 1 day, and (iv) after 3 days, showing that DOPC precipitated out of solution.

Figure 58 is a Cryo-TEM image of the DOPC liposomes before adding nanocrystals showed that the DOPC liposomes formed small unilamellar vesicles.

Figure 59 is a Cryo-TEM image of the DOPC liposomes after bath sonication showed that silicon nanocrystals incorporated with the lipid and formed large complexes.

5 Figure 60 is a Cryo-TEM image of the DOPC liposomes after bath sonication showed that silicon nanocrystals incorporated with the lipid and formed large complexes.

Figure 61 shows the results from preparing DOPG (anionic) liposomes separately and then adding 20 μ l silicon nanocrystals in chloroform and bath sonicating for 5 minutes.

10 Photographs of vials of the DOPG sample were taken under ambient light (upper row) and under a 365 nm UV lamp (bottom row) (i) before bath sonication, (ii) immediately after sonication, (iii) after 1 day, and (iv) after 3 days, showing that the DOPG solution remained turbid.

Figure 62 is a Cryo-TEM image of the DOPG liposomes before adding nanocrystals showed that the DOPG liposomes formed small unilamellar vesicles.

15 Figure 63 is a Cryo-TEM image of the DOPG liposomes after bath sonication showed that the silicon nanocrystals aggregated outside of empty liposomes, and no incorporation with the liposomes was observed.

Figure 64 is a Cryo-TEM image of the DOPG liposomes after bath sonication showed that the silicon nanocrystals aggregated outside of empty liposomes, and no incorporation with the liposomes was observed.

20 Figure 65 shows photographs of vials containing (i) 7 mM CTAB micelles and (ii) 7 mM cholesterol-CTAB nanovesicles one day after preparation with 5 minutes of bath sonication, prior to addition into dialysis tubing, under ambient light (top) and on 254 nm UV lamp (bottom).

25 Figure 66 shows photographs of dialysis tubing with solutions containing (i) 7 mM CTAB micelles and (ii) 7 mM cholesterol-CTAB nanovesicles after 1 round of dialysis, under ambient light (top) and under a 254 nm UV lamp (bottom).

Figure 67 shows photographs of dialysis tubing with solutions containing (i) 7 mM CTAB micelles and (ii) 7 mM cholesterol-CTAB nanovesicles after 3 rounds of dialysis, under ambient light (top) and under a 254 nm UV lamp (bottom).

30 Figure 68 shows photographs of dialysis tubing with solutions containing (i) 7 mM CTAB micelles and (ii) 7 mM cholesterol-CTAB nanovesicles after 6 rounds of dialysis, under ambient light (top) and under a 254 nm UV lamp (bottom).

Figure 69 shows photographs of solutions (i) 7 mM CTAB micelles or (ii) 7 mM

cholesterol-CTAB nanovesicles removed from dialysis tubing after 6 rounds of dialysis, under ambient light (top) and on 254 nm UV lamp (bottom).

Figure 70 is a Cryo-TEM image of sample with cholesterol-CTAB nanovesicles and silicon nanocrystals following 6 rounds of dialysis.

5 Figure 71 is a Cryo-TEM image of sample with cholesterol-CTAB nanovesicles and silicon nanocrystals following 6 rounds of dialysis.

Figure 72 is a Cryo-TEM image of sample with cholesterol-CTAB nanovesicles and silicon nanocrystals following 6 rounds of dialysis.

10 Figure 73 is a Cryo-TEM image of sample with cholesterol-CTAB nanovesicles and silicon nanocrystals following 6 rounds of dialysis.

Figure 74 shows photographs of samples prepared by adding different volume ratios of silicon nanocrystals (in chloroform) : Nanovesicles (in water), under ambient light (top row) and under a 254 nm UV lamp (bottom row) before sonication. In each sample 135 µg of silicon nanocrystals was added, though in different concentrations, and thus volumes. Samples from left
15 to right are: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200 µl of 0.675 mg/ml silicon nanocrystal in chloroform.

Figure 75 shows photographs of sample prepared by adding different volume ratios of
20 silicon nanocrystals (in chloroform) : Nanovesicles (in water), under ambient light (top row) and under a 254 nm UV lamp (bottom row), immediately after sonication. In each sample 135 µg of silicon nanocrystals was added, though in different concentrations, and thus volumes. Samples from left to right are: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon
25 nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200 µl of 0.675 mg/ml silicon nanocrystal in chloroform.

Figure 76 shows photographs of samples prepared by adding different volume ratios of silicon nanocrystals (in chloroform) : Nanovesicles (in water), under ambient light (top row) and under a 254 nm UV lamp (bottom row), 1 day after sonication. In each sample 135 µg of silicon
30 nanocrystals was added, though in different concentrations, and thus volumes. Samples from left to right are: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200

µl of 0.675 mg/ml silicon nanocrystal in chloroform.

Figure 77 shows photographs of samples prepared by adding different volume ratios of silicon nanocrystals (in chloroform) : Nanovesicles (in water), under ambient light (top row) and under a 254 nm UV lamp (bottom row), 1 week after sonication. In each sample 135 µg of silicon nanocrystals was added, though in different concentrations, and thus volumes. Samples from left to right are: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200 µl of 0.675 mg/ml silicon nanocrystal in chloroform.

Figure 78 shows photographs of samples prepared by adding different volume ratios of silicon nanocrystals (in chloroform) : Nanovesicles (in water), under ambient light (top row) and under a 254 nm UV lamp (bottom row) 6 weeks after sonication. In each sample 135 µg of silicon nanocrystals was added, though in different concentrations, and thus volumes. Samples from left to right are: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200 µl of 0.675 mg/ml silicon nanocrystal in chloroform.

Figure 79 shows photographs of sample prepared by adding 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform under ambient light 1 day after sonication, showing an orange-brown precipitate consistent with silicon nanocrystals.

Figure 80 shows photographs of vial under 365 nm UV light of sample that was probe sonicated for 3 minutes to incorporate silicon nanocrystals into nanovesicles. The photographs show precipitation from solution and loss of fluorescence: (i) immediately after sonication, (ii) 1 day after preparation, and (iii) 1.5 months after preparation.

Figure 81 shows photographs of samples prepared using (i) 5 minute bath sonication to (ii) 5 minute vortex to incorporate silicon nanocrystals into nanovesicles, under ambient light. Vortexed vial appeared cloudy.

Figure 82 shows photographs of samples prepared using (i) 5 minute bath sonication to (ii) 5 minute vortex to incorporate silicon nanocrystals into nanovesicles under 365 nm UV light. Vortexed vial did not fluoresce.

Figure 83 is a Cryo-TEM image of the sample that used vortexing to incorporate silicon nanocrystals into the nanovesicles showed no silicon nanocrystals incorporated into the nanovesicles.

Figure 84 is a Cryo-TEM image of the sample that used vortexing to incorporate silicon nanocrystals into the nanovesicles showed no silicon nanocrystals incorporated into the nanovesicles.

Figure 85 shows the DLS data for silicon nanocrystals incorporated into the nanovesicles via bath sonication or vortexing indicated that vortexing changed the size distribution. Sizes measured by DLS (each measured in triplicate) were: a z-average of 108.4 nm with a PDI of 0.168 for bath sonication; and a z-average of 71.58 nm with a PDI of 0.214 for vortexing.

Figure 86 is a schematic diagram of the interactions of silicon particles and nanovesicles, and the formation of the stable silicon nanocrystal covered with a monolayer of nanovesicle.

Figure 87 is a schematic representation of the process of the formation of the silicon nanocrystal-nanovesicle assemblies, their long term structural stability, and long term fluorescence stability.

DETAILED DESCRIPTION

The compositions and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples and Figures included therein.

Before the present compositions and methods are disclosed and described, it is to be understood that this disclosure is not limited to specific synthetic methods or to particular reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

General Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

Throughout the description and claims of this specification the word “comprise” and other forms of the word, such as “comprising” and “comprises,” means including but not limited

to, and is not intended to exclude, for example, other additives, components, integers, or steps.

As used in the description and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a composition” includes mixtures of two or more such compositions, reference to “the vesicle” includes mixtures of two or more such vesicles, reference to “a nanoparticle” includes mixture of two or more such nanoparticles, and the like.

“Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. By “about” is meant within 5% of the value, e.g., within 4, 3, 2, or 1% of the value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

It is understood that throughout this specification the identifiers “first” and “second” are used solely to aid in distinguishing the various components and steps of the disclosed subject matter. The identifiers “first” and “second” are not intended to imply any particular order, amount, preference, or importance to the components or steps modified by these terms.

As used herein, by a “subject” is meant an individual. Thus, the “subject” can include domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.), and birds. “Subject” can also include a mammal, such as a primate or a human. Thus, the subject can be a human or veterinary patient. The term “patient” refers to a subject under the treatment of a clinician, e.g., physician.

The term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

Reference will now be made in detail to specific aspects of the disclosed materials, compounds, compositions, articles, and methods, examples of which are illustrated in the accompanying Examples and Figures.

Compositions

Disclosed herein are compositions comprising an aqueous solution comprising a composite nanocarrier, the composite nanocarrier comprising a nanoparticle at least partially encapsulated within a vesicle.

5 The vesicle can have an average size. “Average size” and “mean size” are used interchangeably herein with respect to the vesicles, and generally refer to the Z-average particle size of a population of vesicles as measured by dynamic light scattering (DLS). For example, the average size for a plurality of vesicles with a substantially spherical shape can comprise the average diameter of the plurality of vesicles as measured by dynamic light scattering.

10 In some examples, the vesicle can have an average size of 25 nanometers (nm) or more as measured by dynamic light scattering (e.g., 30 nm or more, 35 nm or more, 40 nm or more, 45 nm or more, 50 nm or more, 55 nm or more, 60 nm or more, 65 nm or more, 70 nm or more, 75 nm or more, 80 nm or more, 85 nm or more, 90 nm or more, 95 nm or more, 100 nm or more, 110 nm or more, 120 nm or more, 130 nm or more, 140 nm or more, 150 nm or more, 160 nm or
15 more, 170 nm or more, 180 nm or more, 190 nm or more, 200 nm or more, 210 nm or more, 220 nm or more, 230 nm or more, 240 nm or more, 250 nm or more, 260 nm or more, 270 nm or more, 280 nm or more, 290 nm or more, 300 nm or more, 325 nm or more, 350 nm or more, 375 nm or more, 400 nm or more, 425 nm or more, 450 nm or more, or 475 nm or more).

 In some examples, the vesicle can have an average size of 500 nm or less as measured by
20 dynamic light scattering (e.g., 475 nm or less, 450 nm or less, 425 nm or less, 400 nm or less, 375 nm or less, 350 nm or less, 325 nm or less, 300 nm or less, 290 nm or less, 280 nm or less, 270 nm or less, 260 nm or less, 250 nm or less, 240 nm or less, 230 nm or less, 220 nm or less, 210 nm or less, 200 nm or less, 190 nm or less, 180 nm or less, 170 nm or less, 160 nm or less, 150 nm or less, 140 nm or less, 130 nm or less, 120 nm or less, 110 nm or less, 100 nm or less,
25 95 nm or less, 90 nm or less, 85 nm or less, 80 nm or less, 75 nm or less, 70 nm or less, 65 nm or less, 60 nm or less, 55 nm or less, 50 nm or less, 45 nm or less, 40 nm or less, 35 nm or less, or 30 nm or less).

 The average size of the vesicle as measured by dynamic light scattering can range from any of the minimum values described above to any of the maximum values described above. For
30 example, the vesicle can have an average size of from 25 to 500 nm as measured by dynamic light scattering (e.g., from 25 nm to 250 nm, from 250 nm to 500 nm, from 25 nm to 100 nm, from 100 nm to 200 nm, from 200 nm to 300 nm, from 300 nm to 400 nm, from 400 nm to 500 nm, from 30 nm to 450 nm, from 35 nm to 400 nm, from 40 nm to 350 nm, or from 50 nm to

300 nm).

In some examples, the vesicle can comprise a plurality of vesicles that can be substantially monodisperse. "Monodisperse" and "homogeneous size distribution," as used interchangeably herein, and generally describe a population of vesicles where all of the vesicles are the same or nearly the same size. As used herein, a monodisperse distribution of vesicles refers to vesicle distributions in which the polydispersity index (PDI) as measured by dynamic light scattering is 0.35 or less (e.g., 0.30 or less, 0.25 or less, 0.20 or less, 0.15 or less, 0.10 or less, or 0.05 or less).

In some examples, the vesicle can be substantially spherical in shape. As used herein, substantially spherical in shape can include spherical, elliptical, ovular, or any other spheroidal shape.

The vesicle can comprise a sterol and a surfactant. The vesicle can, for example, be formed by self-assembly of the sterol and the surfactant. As used herein, a lipid is not a surfactant, such that the vesicles are not liposomes. Suitable surfactants can be anionic, cationic, amphoteric, or nonionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions. Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxy)-sulfosuccinate; and alkyl sulfates such as sodium lauryl sulfate. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glyceryl monostearate, glyceryl stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000 cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer[®] 401, stearyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl- β -alanine, sodium N-lauryl- β -iminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

Cationic surfactants include, but are not limited to, quaternary ammonium surfactants. As used herein, quaternary ammonium surfactants are quaternary ammonium salts in which at least one nitrogen substituent is a long chain alkyl group. Examples of quaternary ammonium surfactants include, but are not limited to, cetyl trimethylammonium bromide (CTAB), cetyl trimethylammonium chloride (CTAC), cetylpyridinium chloride (CPC), benzalkonium chloride

(BAC), menzethonium chloride (BZT), myristalkonium chloride (MKC), 5-bromo-5-nitro-1,3-dioxane, dimethyldioctadecylammonium chloride, dioctadecylmethylammonium bromide (DODAB), and combinations thereof. In some examples, the quaternary ammonium surfactant can comprise cetyl trimethylammonium bromide. In some examples, the quaternary ammonium surfactant can consist of cetyl trimethylammonium bromide.

In some examples, the vesicle comprises a sterol and a quaternary ammonium surfactant. In some examples, the vesicle consists of a sterol and a quaternary ammonium surfactant.

Examples of sterols include, but are not limited to, adosterol, avenasterol, azacosterol, blazein, campesterol, cerevisterol, cholesterol, cycloartenol, dinosterol, episterol, ergosterol, fecosterol, fucosterol, fungisterol, ganoderiol, anodermadiol, gandoderenic acid, inotodiol, lanosterol, lathosterol, lichesterol, lucidadiol, lumisterol, sitosterol, spinasterol, stigmasterol, trametenolic acid, zhankuid acid, derivatives thereof, and combinations thereof. In some examples, the sterol comprises cholesterol or a derivative thereof. In some examples, the sterol consists of cholesterol or a derivative thereof.

In some examples, the sterol and the surfactant can be present in the vesicle in a molar ratio of 10:1 or less (e.g., 9.5:1 or less, 9:1 or less, 8.5:1 or less, 8:1 or less, 7.5:1 or less, 7:1 or less, 6.5:1 or less, 6:1 or less, 5.5:1 or less, 5:1 or less, 4.5:1 or less, 4:1 or less, 3.5:1 or less, 3:1 or less, 2.5:1 or less, 2:1 or less, 1.9:1 or less, 1.8:1 or less, 1.7:1 or less, 1.6:1 or less, 1.5:1 or less, 1.4:1 or less, 1.3:1 or less, 1.2:1 or less, 1.1:1 or less, 1:1 or less, 1:1.1 or less, 1:1.2 or less, 1:1.3 or less, 1:1.4 or less, 1:1.5 or less, 1:1.6 or less, 1:1.7 or less, 1:1.8 or less, 1:1.9 or less, 1:2 or less, 1:2.5 or less, 1:3 or less, 1:3.5 or less, 1:4 or less, or 1:4.5 or less).

In some examples, the sterol and the surfactant can be present in the vesicle in a molar ratio of 1:5 or more (e.g., 1:4.5 or more, 1:4 or more, 1:3.5 or more, 1:3 or more, 1:2.5 or more, 1:2 or more, 1:1.9 or more, 1:1.8 or more, 1:1.7 or more, 1:1.6 or more, 1:1.5 or more, 1:1.4 or more, 1:1.3 or more, 1:1.2 or more, 1:1.1 or more, 1:1 or more, 1.1:1 or more, 1.2:1 or more, 1.3:1 or more, 1.4:1 or more, 1.5:1 or more, 1.6:1 or more, 1.7:1 or more, 1.8:1 or more, 1.9:1 or more, 2:1 or more, 2.5:1 or more, 3:1 or more, 3.5:1 or more, 4:1 or more, 4.5:1 or more, 5:1 or more, 5.5:1 or more, 6:1 or more, 6.5:1 or more, 7:1 or more, 7.5:1 or more, 8:1 or more, 8.5:1 or more, 9:1 or more, or 9.5:1 or more).

The molar ratio of the sterol and the surfactant present in the vesicle can range from any of the minimum values described above to any of the maximum values described above. For example, the sterol and the surfactant can be present in the vesicle in a molar ratio of from 10:1 to 1:5 (e.g., from 10:1 to 1:1, from 1:1 to 1:5, from 5:1 to 1:5, from 4:1 to 1:4, from 3:1 to 1:3,

from 2:1 to 1:2, or from 1.5:1 to 1:1.5). In some examples, the sterol and the surfactant can be present in the vesicle in a molar ratio of 1:1.

The nanoparticle that can be used in the compositions disclosed herein can comprise, for example, a semiconductor. In some embodiments, the nanoparticle can include a semiconductor that comprises a wide band gap. The band gap can be greater than 1 eV. In some embodiments, the band gap can be an indirect band gap. In some examples, the nanoparticle can comprise a semiconductor such as Fe_2O_3 , WO_3 , Ta_3N_5 , TaON , TiO_2 , ZnO , CdS , CdSe , Si , or combinations thereof. In some examples, the nanoparticle can comprise Si .

In some examples, the nanoparticle can comprise a nanocrystal. In some examples, the nanocrystal can comprise Si .

The nanoparticle that can be used in the compositions disclosed herein can comprise, for example, a metal. In some examples, the nanoparticle can comprise a metal selected from the group consisting of Be , Mg , Al , Ca , Sc , Ti , V , Cr , Mn , Fe , Co , Ni , Cu , Zn , Ga , Sr , Y , Zr , Nb , Mo , Tc , Ru , Rh , Pd , Ag , Cd , In , Sn , Ba , Hf , Ta , W , Re , Os , Ir , Pt , Au , Hg , Tl , Pb , Bi , La , Ce , Pr , Nd , Pm , Sm , Eu , Gd , Tb , Dy , Ho , Er , Tm , Yb , and combinations thereof.

In some examples, the nanoparticle can comprise a magnetic nanoparticle. The magnetic nanoparticle can comprise any suitable material, for example Fe , Co , Zn , Ni , Mn , Ag , Au , C , Cd , or a combination thereof. In some examples, the magnetic nanoparticle can comprise any suitable metal compound, such as an oxide of Fe , Co , Zn , Ni , Mn , or a combination thereof. In some examples, the magnetic nanoparticle comprise an iron oxide, for example Fe_3O_4 .

In some examples, the nanoparticle that can be used in the compositions disclosed herein can be capable of absorbing energy of a first wavelength. The nanoparticle (e.g., size, shape, composition) can, for example, be selected to tune the optical and/or electrical properties of the composition.

In some examples, the nanoparticle can comprise a plasmonic nanoparticle. The plasmonic nanoparticle can any suitable material, for example Au , Ag , Pd , and combinations thereof.

In some examples, the nanoparticle can be capable of absorbing energy of a first wavelength and emitting electromagnetic radiation (such as near infrared and visible light). In some examples, the nanoparticle can comprise a fluorescent nanoparticle. The optical properties can be, for example, a function of both the nanoparticle composition and physical size. Both the absorption and the photoluminescent wavelength are a function of the nanoparticle size and composition. The narrower the size distribution of the nanoparticles, the narrower the full-width

half max (FWHM) of the resultant photoluminescent spectra. The nanoparticle size, shape, and/or composition can, for example, be selected to tune the fluorescent properties of the composition.

In some examples, the nanoparticle can be biocompatible, such that the system is suitable for use in a variety of biological applications. “Biocompatible” or “biologically compatible”, as used herein, generally refer to compounds or particles that are, along with any metabolites or degradation products thereof, generally non-toxic to cells and tissues, and which do not cause any significant adverse effects to cells and tissues when cells and tissues are incubated (*e.g.*, cultured) in their presence. In some embodiments, the biocompatible nanoparticle can be degradable. In some embodiments, the biocompatible nanoparticle can be inert, that is, stable in biological environments. In some embodiments, the nanoparticle can be coated with a biocompatible material. The biocompatible material can be a lipid, a carbohydrate, a polysaccharide, a protein, an antibody, a glycoprotein, a glycolipid, silica, alumina, titanium oxide or combinations thereof. Examples of a suitable biocompatible nanoparticle include a silicon containing nanoparticle.

The nanoparticle can have an average particle size. “Average particle size,” “mean particle size,” and “median particle size” are used interchangeably herein with respect to the nanoparticles, and generally refer to the statistical mean particle size of the nanoparticles in a population of nanoparticles. For example, the average particle size for a plurality of nanoparticles with a substantially spherical shape can comprise the average diameter of the plurality of nanoparticles. Mean particle size can be measured using methods known in the art, such as evaluation by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and/or small angle X-ray scattering (SAXS).

The nanoparticle can, for example, have an average particle size that is less than the average particle size of the vesicle. In some examples, the nanoparticle can have an average particle size of 1 nm or more as measured by transmission electron microscopy (*e.g.*, 2 nm or more, 3 nm or more, 4 nm or more, 5 nm or more, 6 nm or more, 7 nm or more, 8 nm or more, 9 nm or more, 10 nm or more, 11 nm or more, 12 nm or more, 13 nm or more, 14 nm or more, 15 nm or more, 16 nm or more, 17 nm or more, 18 nm or more, or 19 nm or more). In some examples, the nanoparticle can have an average particle size of 20 nm or less as measured by transmission electron microscopy (*e.g.*, 19 nm or less, 18 nm or less, 17 nm or less, 16 nm or less, 15 nm or less, 14 nm or less, 13 nm or less, 12 nm or less, 11 nm or less, 10 nm or less, 9 nm or less, 8 nm or less, 7 nm or less, 6 nm or less, 5 nm or less, 4 nm or less, 3 nm or less, or 2

nm or less).

The average particle size of the nanoparticle as measured by transmission electron microscopy can range from any of the minimum values described above to any of the maximum values described above. For example the nanoparticle can have an average particle size of from 1
5 nm to 20 nm as measured by transmission electron microscopy (e.g., from 1 nm to 10 nm, from 10 nm to 20 nm, from 1 nm to 5 nm, from 5 nm to 10 nm, from 10 nm to 15 nm, from 15 nm to 20 nm, or from 5 nm to 15 nm).

In some examples, the nanoparticle can comprise a plurality of nanoparticles that can be substantially monodisperse. "Monodisperse" and "homogeneous size distribution," as used
10 interchangeably herein, and generally describe a population of nanoparticles where all of the nanoparticles are the same or nearly the same size. As used herein, a monodisperse distribution refers to nanoparticles distributions in which 70% of the distribution (e.g., 75% of the distribution, 80% of the distribution, 85% of the distribution, 90% of the distribution, or 95% of the distribution) lies within 25% of the median particle size (e.g., within 20% of the median
15 particle size, within 15% of the median particle size, within 10% of the median particle size, or within 5% of the median particle size).

The nanoparticle can be of any shape (e.g., sphere, rod, cube, rectangle, octahedron, truncated octahedron, plate, cone, prism, ellipse, triangle, etc.). In some examples, the nanoparticle can be substantially spherical in shape. In some examples, the nanoparticle can
20 comprise a core-shell nanoparticle.

The nanoparticle can, for example, further comprise a capping layer comprising a plurality of ligands. Suitable ligands for capping layers for nanoparticles are known in the art.

In some examples, the ligands can be attached to the nanoparticle, for example, by coordination bonds. Ligands can also be associated with the nanoparticle via non-covalent
25 interactions. In some examples, the ligands can individually be selected to be a hydrophilic, hydrophobic, or amphiphilic. In addition, the plurality of ligands can, in combination, be selected so as to provide a shell surrounding the nanoparticle which is hydrophilic, hydrophobic, or amphiphilic. The ligands can comprise coordinating or bonding functional groups, such as thiol, amine, phosphine, CO, N₂, alkene, chloride, hydride, alkyl, and derivatives thereof, and
30 combinations thereof.

The nanoparticle and the sterol can be present in the composition, for example, in a molar ratio of 1:10000 or more (e.g., 1:9000 or more, 1:8000 or more, 1:7000 or more, 1:6000 or more, 1:5000 or more, 1:4000 or more, 1:3000 or more, 1:2000 or more, 1:1500 or more, 1:1000 or

more, 1:500 or more, or 1:100 or more). In some examples, the nanoparticle and the sterol can be present in the composition in a molar ratio of 1:50 or less (e.g., 1:100 or less, 1:500 or less, 1:1000 or less, 1:1500 or less, 1:2000 or less, 1:3000 or less, 1:4000 or less, 1:5000 or less, 1:6000 or less, 1:7000 or less, 1:8000 or less, or 1:9000 or less).

5 The molar ratio at which the nanoparticle and the sterol are present in the composition can range from any of the minimum values described above to any of the maximum values described above. For example, the nanoparticle and the sterol can be present in the composition in a molar ratio of from 1:10000 to 1:50 (e.g., from 1:10000 to 1:5000, from 1:5000 to 1:50, from 1:10000 to 1:8000, from 1:8000 to 1:6000, from 1:6000 to 1:4000, from 1:4000 to 1:2000, 10 from 1:2000 to 1:50, or from 1:1800 to 1:100).

 In some examples, the nanoparticle and the surfactant can be present in the composition in a molar ratio of 1:10000 or more (e.g., 1:9000 or more, 1:8000 or more, 1:7000 or more, 1:6000 or more, 1:5000 or more, 1:4000 or more, 1:3000 or more, 1:2000 or more, 1:1500 or more, 1:1000 or more, 1:500 or more, or 1:100 or more). In some examples, the nanoparticle and 15 the surfactant can be present in the composition in a molar ratio of 1:50 or less (e.g., 1:100 or less, 1:500 or less, 1:1000 or less, 1:1500 or less, 1:2000 or less, 1:3000 or less, 1:4000 or less, 1:5000 or less, 1:6000 or less, 1:7000 or less, 1:8000 or less, or 1:9000 or less).

 The molar ratio at which the nanoparticle and the surfactant are present in the composition can range from any of the minimum values described above to any of the maximum 20 values described above. For example, the nanoparticle and the surfactant can be present in the composition in a molar ratio of from 1:10000 to 1:50 (e.g., from 1:10000 to 1:5000, from 1:5000 to 1:50, from 1:10000 to 1:8000, from 1:8000 to 1:6000, from 1:6000 to 1:4000, from 1:4000 to 1:2000, from 1:2000 to 1:50, or from 1:1800 to 1:100).

 In some examples, the number of nanoparticles at least partially encapsulated within 25 each vesicle can be 1 or more (e.g., 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 15 or more, 20 or more, 25 or more, 30 or more, 35 or more, 40 or more, 45 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 125 or more, 150 or more, 175 or more, or 200 or more).

 The composite nanocarrier can have an average size. “Average size” and “mean size” are 30 used interchangeably herein with respect to the composite nanocarriers, and generally refer to the Z-average particle size of a population of composite nanocarriers as measured by dynamic light scattering. For example, the average size for a plurality of composite nanocarriers with a substantially spherical shape can comprise the average diameter of the plurality of composite

nanocarriers as measured by dynamic light scattering.

In some examples, the composite nanocarrier can have an average size of 25 nanometers (nm) or more as measured by dynamic light scattering (e.g., 30 nm or more, 35 nm or more, 40 nm or more, 45 nm or more, 50 nm or more, 55 nm or more, 60 nm or more, 65 nm or more, 70 nm or more, 75 nm or more, 80 nm or more, 85 nm or more, 90 nm or more, 95 nm or more, 100 nm or more, 110 nm or more, 120 nm or more, 130 nm or more, 140 nm or more, 150 nm or more, 160 nm or more, 170 nm or more, 180 nm or more, 190 nm or more, 200 nm or more, 210 nm or more, 220 nm or more, 230 nm or more, 240 nm or more, 250 nm or more, 260 nm or more, 270 nm or more, 280 nm or more, 290 nm or more, 300 nm or more, 325 nm or more, 350 nm or more, 375 nm or more, 400 nm or more, 425 nm or more, 450 nm or more, or 475 nm or more).

In some examples, the composite nanocarrier can have an average size of 500 nm or less as measured by dynamic light scattering (e.g., 475 nm or less, 450 nm or less, 425 nm or less, 400 nm or less, 375 nm or less, 350 nm or less, 325 nm or less, 300 nm or less, 290 nm or less, 280 nm or less, 270 nm or less, 260 nm or less, 250 nm or less, 240 nm or less, 230 nm or less, 220 nm or less, 210 nm or less, 200 nm or less, 190 nm or less, 180 nm or less, 170 nm or less, 160 nm or less, 150 nm or less, 140 nm or less, 130 nm or less, 120 nm or less, 110 nm or less, 100 nm or less, 95 nm or less, 90 nm or less, 85 nm or less, 80 nm or less, 75 nm or less, 70 nm or less, 65 nm or less, 60 nm or less, 55 nm or less, 50 nm or less, 45 nm or less, 40 nm or less, 35 nm or less, or 30 nm or less).

The average size of the composite nanocarrier as measured by dynamic light scattering can range from any of the minimum values described above to any of the maximum values described above. For example, the composite nanocarrier can have an average size of from 25 to 500 nm as measured by dynamic light scattering (e.g., from 25 nm to 250 nm, from 250 nm to 500 nm, from 25 nm to 100 nm, from 100 nm to 200 nm, from 200 nm to 300 nm, from 300 nm to 400 nm, from 400 nm to 500 nm, from 30 nm to 450 nm, from 35 nm to 400 nm, from 40 nm to 350 nm, or from 50 nm to 300 nm).

In some examples, the composite nanocarrier can comprise a plurality of composite nanocarriers that can be substantially monodisperse. “Monodisperse” and “homogeneous size distribution,” as used interchangeably herein, and generally describe a population of composite nanocarriers where all of the composite nanocarriers are the same or nearly the same size. As used herein, a monodisperse distribution of composite nanocarriers refers to composite nanocarrier the polydispersity index (PDI) as measured by dynamic light scattering is 0.35 or

less (e.g., 0.30 or less, 0.25 or less, 0.20 or less, 0.15 or less, 0.10 or less, or 0.05 or less).

The compositions described herein can be substantially stable. In some examples, the compositions can be substantially stable upon dilution.

In some examples, substantially stable can refer to the stability of the average size of the composite nanocarriers over time and/or upon dilution. For example, the average size of the composite nanocarriers can change by 35% or less (e.g., 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, or 5% or less) over a period of time (e.g., 12 hours or more, 1 day or more, 1 week or more, 4 weeks or more, 8 weeks or more, or 12 weeks or more) and/or upon dilution.

In certain examples, wherein the nanoparticle comprises a plasmonic nanoparticle, the composition can comprise a plasmonic composition. In certain examples, substantially stable can refer to the stability of the plasmonic properties of the composition. For example, the plasmonic composition can be stable over a period of time and/or upon dilution.

In certain examples, wherein the nanoparticle comprises a magnetic nanoparticle, the composition can comprise a magnetic composition. In certain examples, substantially stable can refer to the stability of the magnetic properties of the composition. For example, the magnetic composition can be stable over a period of time and/or upon dilution. In certain examples, wherein the nanoparticle comprises a fluorescent nanoparticle, the composition can comprise a fluorescent composition. In certain examples, substantially stable can refer to the stability of the fluorescence of the composition. For example, the fluorescence of the composition can be stable upon dilution of the composition. In some examples, the peak wavelength of the photoluminescence spectrum of the fluorescent composition can shift by 60 nm or less (e.g., 50 nm or less, 40 nm or less, 30 nm or less, 20 nm or less, or 10 nm or less) over a period of time (e.g., 1 hour or more, 12 hours or more, 1 day or more, 1 week or more, 4 weeks or more, 8 weeks or more, or 12 weeks or more) and/or upon dilution.

The compositions can, in some examples, be biocompatible. In some examples, the compositions can be stable under physiological conditions.

The compositions can comprise, in some examples, water and a cosolvent. In other words, in some examples the aqueous solutions can further comprise a cosolvent. Examples of cosolvents include, but are not limited to, alcohols (e.g., methanol, ethanol, n-butanol, isopropanol, n-propanol), carboxylic acids (e.g., acetic acid), chloroform, and combinations thereof. In some examples, the aqueous solutions are substantially free of precipitate.

Methods of Making

Also disclosed herein are methods of making the compositions disclosed herein. For example, also disclosed herein are methods of making the compositions described herein, the method comprising contacting the nanoparticle and the vesicle, thereby forming a mixture; and
5 mixing the mixture, thereby at least partially encapsulating the nanoparticle within the vesicle (e.g., thereby forming the composite nanocarrier). Mixing can be accomplished by mechanical stirring, shaking, vortexing, sonication (e.g., bath sonication, probe sonication), and the like.

In some examples, the mixture is bath sonicated. The mixture can be bath sonicated, for example, for 10 seconds or more (e.g., 20 seconds or more, 30 seconds or more, 40 seconds or
10 more, 50 seconds or more, 1 minute or more, 2 minutes or more, 3 minutes or more, 4 minutes or more, 5 minutes or more, 6 minutes or more, 7 minutes or more, 8 minutes or more, 9 minutes or more, 10 minutes or more, 15 minutes or more, 20 minutes or more, 25 minutes or more, 30 minutes or more, 40 minutes or more, 50 minutes or more, 1 hour or more, 1.5 hours or more, 2 hours or more, 2.5 hours or more, 3 hours or more, 3.5 hours or more, 4 hours or more, or 4.5
15 hours or more). In some examples, the mixture can be bath sonicated for 5 hours or less (e.g., 4.5 hours or less, 4 hours or less, 3.5 hours or less, 3 hours or less, 2.5 hours or less, 2 hours or less, 1.5 hours or less, 1 hour or less, 50 minutes or less, 40 minutes or less, 30 minutes or less, 25 minutes or less, 20 minutes or less, 15 minutes or less, 10 minutes or less, 9 minutes or less, 8 minutes or less, 7 minutes or less, 6 minutes or less, 5 minutes or less, 4 minutes or less, 3
20 minutes or less, 2 minutes or less, 1 minute or less, 50 seconds or less, 40 seconds or less, 30 seconds or less, or 20 seconds or less).

The amount of time for which the mixture is bath sonicated can range from any of the minimum values described above to any of the maximum values described above. For example, the mixture can be bath sonicated for from 10 seconds to 5 hours (e.g., from 10 seconds to 2.5
25 hours, from 2.5 hours to 5 hours, from 10 seconds to 1 hour, from 1 hour to 2 hours, from 2 hours to 3 hours, from 3 hours to 4 hours, from 4 hours to 5 hours, from 30 seconds to 40 minutes, from 1 minute to 30 minutes, or from 1 minute to 10 minutes).

In some examples, the method can further comprise forming the vesicle. The vesicle can, for example, be formed by self-assembly of the sterol and the surfactant. In certain examples,
30 forming the vesicle can comprise a one-step scalable method using CO₂ expanded solvents called Depressurization of an Expanded Liquid Organic Solution-suspension (DELOS-susp) (Cano-Sarabia M et al. *Langmuir* **2008**, 24, 2433–2437; Elizondo E et al. *Nanomed.* **2012**, 7, 1391–1408).

In some examples, the method can further comprise forming the nanoparticle. Methods of making nanoparticles are known in the art. In certain examples, wherein the nanoparticle comprises a silicon nanocrystal, the silicon nanocrystal can be synthesized according to previously reported methods (Hessel CM et al. *Chem. Mater.* **2012**, 24, 393–401). In certain examples, forming the silicon nanocrystal can include drying an organosilicone compound, such as silsesquioxane followed by heating the compound in a furnace. The silicon containing compound can be heated up to 1200°C at a heating rate of 18°C/min. The temperature can be held for about an hour. The reaction product can be etched with concentrated acid, such as hydrofluoric and hydrochloric acid, in the dark for about 4 to 6 hours. The mixture can then be purified by centrifugation for example at about 8000 rpm for 5 min then rinsed to yield the silicon containing nanoparticles.

Methods of Use

The compositions disclosed herein have potential applications in a number of fields such as imaging (e.g., biological imaging, fluorescence imaging, biomedical imaging), sensing (e.g., chemical sensing, biological sensing), medical applications (e.g., imaging, therapy, diagnostics, photothermal therapy, combinations thereof). In some examples, the compositions disclosed herein can be used in bioanalytical devices such as DNA chips, miniaturized biosensors and microfluidic devices. In some examples, the compositions can be used in gene expression profiling, drug discovery, and clinical diagnostics. In certain examples, the compositions can be used in applications benefiting from fluorescent labeling including medical and non-medical fluorescence microscopy, histology, flow cytometry, fundamental cellular and molecular biology protocols, fluorescence *in situ* hybridization, DNA sequencing, immuno assays, binding assays and separation.

Also disclosed herein are methods of use of the composition disclosed herein, wherein the composition is used as a sensor to detect the presence or amount of a biological moiety; the structure, composition, and conformation of a biological moiety; the localization of a biological moiety in an environment; interactions of biological moieties; alterations in structures of biological compounds; alterations in biological processes; or combinations thereof. In certain examples, the compositions can further comprise a targeting moiety that has an affinity for a biological target.

Also disclosed herein are methods of use of the compositions disclosed herein in devices. In other words, also disclosed herein are devices comprising the compositions disclosed herein.

Examples of devices include, but are not limited to, electronic devices, energy storage devices, energy conversion devices (e.g., solar cells, fuel cells, photovoltaic cells), optical devices (e.g., light emitting diodes), optoelectronic devices, bioanalytical devices, chemical sensors, biosensors, and combinations thereof.

Also disclosed herein are methods of imaging a cell or a population of cells within or about a subject. The methods can comprise administering to the subject an amount of a composition as described herein; and detecting the composition. The detecting can involve methods known in the art, for example, positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), X-ray, microscopy, computed tomography (CT). In some examples, the composition can further comprise a detectable label, such as a radiolabel, fluorescent label, enzymatic label, and the like. In some examples, the detectable label can comprise the nanoparticle. Such imaging methods can be used, for example, for assessing the extent of a disease and/or the target of a therapeutic agent. In some examples, the cells are indicative of a disease, such as cancer.

Compositions, Formulations and Methods of Administration

In vivo application of the disclosed compositions, can be accomplished by any suitable method and technique presently or prospectively known to those skilled in the art. For example, the disclosed compositions can be formulated in a physiologically- or pharmaceutically-acceptable form and administered by any suitable route known in the art including, for example, oral, nasal, rectal, topical, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, and intrasternal administration, such as by injection. Administration of the disclosed compositions can be a single administration, or at continuous or distinct intervals as can be readily determined by a person skilled in the art.

The compositions disclosed herein can be formulated according to known methods for preparing pharmaceutically acceptable compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin (1995) describes formulations that can be used in connection with the disclosed methods. In general, the compositions disclosed herein can be formulated such that an effective amount of the composition is combined with a suitable excipient in order to facilitate effective administration of the composition. The compositions used can also be in a variety of forms. These include, for example, solid, semi-

solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and application. The compositions also preferably include conventional pharmaceutically-acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the compositions include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, saline, and equivalent carriers and diluents. To provide for the administration of such compositions, the compositions disclosed herein can advantageously comprise between 0.1% and 100% by weight of the total of one or more of the subject composite nanocarriers based on the weight of the total composition including carrier or diluent.

Formulations suitable for administration include, for example, aqueous sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous sterile suspensions, which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the excipients particularly mentioned above, the compositions disclosed herein can include other agents conventional in the art having regard to the type of formulation in question.

For the treatment of disorders, the compositions disclosed herein can be administered to a patient in need of treatment in combination with other substances and/or treatments. These other substances or treatments can be given at the same as or at different times from the compositions disclosed herein.

In certain examples, the compositions disclosed herein can be locally administered at one or more anatomical sites, such as sites of unwanted cell growth (such as a tumor site or benign skin growth, *e.g.*, injected or topically applied to the tumor or skin growth), optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent. The compositions disclosed herein can be systemically administered, such as intravenously or orally, optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent, or an assimilable edible carrier for oral delivery. They can be enclosed in hard or soft shell gelatin capsules, can be compressed into tablets, or can be incorporated directly with the food of the

patient's diet. For oral therapeutic administration, the compositions can be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, aerosol sprays, and the like.

The tablets, troches, pills, capsules, and the like can also contain the following: binders
5 such as gum tragacanth, acacia, corn starch or gelatin; diluents such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring can be added.

When the unit dosage form is a capsule, it can contain, in addition to materials of the above type,
10 a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir can contain the composition, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of
15 course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the compositions can be incorporated into sustained-release preparations and devices.

The compositions disclosed herein can be administered intravenously, intramuscularly, or intraperitoneally by infusion or injection. Solutions of the compositions can be prepared in
20 water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile
25 aqueous solutions or dispersions or sterile powders comprising the active ingredient, which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and
30 suitable mixtures thereof. The proper fluidity can be maintained, for example, by the maintenance of the average size of the composite nanocarrier or by the use of surfactants. Optionally, the prevention of the action of microorganisms can be brought about by various

other antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the inclusion of agents that delay absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the compositions disclosed herein in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the compositions can be dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers, for example.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the subject.

Also disclosed are pharmaceutical compositions that comprise the compositions disclosed herein in combination with a pharmaceutically acceptable excipient.

Also disclosed are kits that comprise a composition disclosed herein in one or more containers. The disclosed kits can optionally include pharmaceutically acceptable carriers and/or diluents. In one embodiment, a kit includes one or more other components, adjuncts, or adjuvants as described herein. In another embodiment, a kit includes one or more anti-cancer agents, such as those agents described herein. In one embodiment, a kit includes instructions or packaging materials that describe how to administer a composition of the kit. Containers of the kit can be of any suitable material, *e.g.*, glass, plastic, metal, *etc.*, and of any suitable size, shape, or configuration. In one embodiment, a composition disclosed herein is provided in the kit as a solid, such as a tablet, pill, or powder form. In another embodiment, a composition disclosed herein is provided in the kit as a liquid or solution. In one embodiment, the kit comprises an

ampoule or syringe containing a composition disclosed herein in liquid or solution form.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

5 The examples below are intended to further illustrate certain aspects of the systems and methods described herein, and are not intended to limit the scope of the claims.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles,
10 devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature,
15 and pressure is at or near atmospheric.

Example 1

Nanoparticles have been studied for biomedical imaging applications due to their fluorescence properties and photostability, though many systems utilize heavy metals such as
20 cadmium (Michalet X et al. *Science* **2005**, 307, 538–544; Kovalenko MV et al. *ACS Nano* **2015**, 9, 1012–1057). Silicon nanocrystals (SiNCs) offer a biocompatible alternative to toxic quantum dots that exhibit emission in the visible to near-infrared region (NIR) (Hessel CM et al. *Chem. Mater.* **2012**, 24, 393–401; Park JH et al. *Nat. Mater.* **2009**, 8, 331–336; Henderson EJ et al. *Small* **2011**, 7, 2507–2516; O’Farrell N et al. *Int. J. Nanomedicine* **2006**, 1, 451–472). Although
25 silicon is an indirect band gap material, at the nanoscale the emission spectrum can be tuned according to the silicon nanocrystal size, with 2.8 nm silicon nanocrystals emitting at orange wavelengths (O’Farrell N et al. *Int. J. Nanomedicine* **2006**, 1, 451–472; Canham LT. *Appl. Phys. Lett.* **1990**, 57, 1046–1048; Cullis AG et al. *J. Appl. Phys.* **1997**, 82, 909–965). These silicon nanocrystals can be stably dispersed in organic solvents following the covalent attachment of
30 capping ligands, but such silicon nanocrystals have not been stably dispersed in water (Hessel CM et al. *Chem. Mater.* **2012**, 24, 393–401; Henderson EJ et al. *Small* **2011**, 7, 2507–2516; O’Farrell N et al. *Int. J. Nanomedicine* **2006**, 1, 451–472; Yu Y et al. *Langmuir* **2013**, 29, 1533–

1540; Erogbogbo F et al. *ACS Nano* **2008**, 2, 873–878). Silicon nanocrystals for use in medical applications should be dispersible in water.

Non-liposomal nanovesicular structures can be formed for example using equimolar amounts of sterols, such as cholesterol (chol), and quaternary ammonium surfactants, such as CTAB (Ferrer-Tasies L et al. *Langmuir* **2013**, 29, 6519–6528). None of the individual components of a nanovesicle self-assemble to form vesicular structures, since in water quaternary ammonium surfactants form micelles and the insoluble sterol species form crystals, though in combination the components form amphiphilic bimolecular building blocks that assemble into closed bilayers. These nanovesicles do not tend to aggregate, and they keep their structure for periods as long as several years. These vesicular structures exhibit a high vesicle homogeneity regarding size, lamellarity, and membrane supramolecular organization, which are all properties that can impact the use of such formulations in medical or diagnosis applications (Elizondo E et al. *J. Am. Chem. Soc.* **2012**, 134, 1918–1921; Cabrera I et al. *Nano Lett.* **2013**, 13, 3766–3774). Contrary to micellar structures formed exclusively with quaternary ammonium surfactants, the morphology of these vesicular nanostructures is substantially unaffected upon increasing the temperature or by dilution, making them attractive candidates for use *in-vivo*. The nanovesicles can be prepared by a one-step scalable method using CO₂ expanded solvents called Depressurization of an Expanded Liquid Organic Solution-suspension (DELOS-susp) (Cano-Sarabia M et al. *Langmuir* **2008**, 24, 2433–2437; Elizondo E et al. *Nanomed.* **2012**, 7, 1391–1408). The nanovesicles are stable aqueous colloidal structures and they have antibacterial and anti-biofilm properties. These results make them promising nanocarriers in the development of new nanovesicle-nanocrystal hybrids.

Herein, the integration of fluorescent silicon nanocrystals into cholesterol-CTAB nanovesicles is discussed. The long term stability in aqueous environments was also investigated. Comparisons to other colloidal systems used to disperse nanoparticles were explored, as well as the effects of varying concentrations and methods for integration, to determine how those factors affect the final stable structures.

Silicon nanocrystals were synthesized according to previously reported methods (Hessel CM et al. *Chem. Mater.* **2012**, 24, 393–401). Briefly, hydrogen silsesquioxane (HSQ, Dow Corning) was degassed and then heated at 1100°C for 60 minutes in a tube furnace under forming gas flow. The resulting brown material was then ground with a mortar and pestle, followed by further size reduction by mechanical shaking in a wrist action shaker with borosilicate beads for 9 hours. The final brown powder comprised crystalline silicon embedded

in a SiO₂ matrix. To liberate the silicon nanocrystals from the matrix, 0.6 g of the silicon nanocrystal (SiNC) powder was etched in the dark with 2 ml hydrochloric acid (HCl, Fisher) and 20 ml hydrofluoric acid (HF, 48%, Macron Fine Chemicals) for 3.5 hours. After etching, the material was precipitated by centrifugation for 5 minutes at 8000 rpm. The HF was removed and the precipitate was redispersed in ethanol (Pharmco-Aaper), and then centrifuged again. This washing process was repeat once more with ethanol and then once with chloroform ($\geq 99.8\%$, Fisher), resulting in –H terminated silicon nanocrystals. Surface passivation was achieved by redispersing the precipitated silicon nanocrystals with 20 ml of 1-octene (98%, Sigma-Aldrich) and then injecting the solution into a 3-neck flask under vacuum. The solution was quickly frozen under vacuum, and then thawed under nitrogen flow. The freeze-thaw process was repeat three more times. Finally, the flask was left stirring at 400 rpm under nitrogen flow at 120°C for 12 hours. Unpassivated silicon nanocrystals were precipitated out by centrifugation at 8000 rpm for 5 minutes. The 1-octene solution was removed on a rotary evaporated at 760 mmHg and 60°C, after which the silicon nanocrystals were redispersed in hexanes ($\geq 98.5\%$, Fisher). The silicon nanocrystals were then washed using ethanol as an antisolvent and hexanes as the solvent 4 times through centrifugation. The final silicon nanocrystals were dispersed in chloroform, and imaged using transmission electron microscopy (TEM) (Figure 1-Figure 3). Conventional TEM samples were prepared by drop casting silicon nanocrystals from chloroform dispersions onto 200 mesh carbon-coated copper grids (Electron Microscopy Science). TEM images were taken using an FEI Tecnai Biotwin TEM operated at 80 kV accelerating voltage, and acquired digitally. Results from measuring 150 silicon nanocrystals from TEM images determined that the average size was 2.8 nm with a standard deviation of 0.6 nm (Figure 4).

Thermogravimetric analysis (TGA) was conducted on silicon nanocrystals to determine the mass per nanocrystal of silicon vs. ligand (Figure 5). Thermal gravimetric analysis data were acquired on a Mettler Toledo TGA/DSC 1. Silicon nanocrystals dispersed in chloroform were drop cast and dried into a 70 μ l alumina crucible (Mettler Toledo). The sample was then heat under 50 ml per minute air flow at a rate of 10°C per minute from 25°C to 800°C and then held at 800°C for 30 minutes. It was assumed that at the beginning of the thermogravimetric analysis cycle the sample comprised a silicon core and octene ligands, while at the end of the cycle there was no remaining ligands and all the silicon had been converted to SiO₂. From the thermogravimetric analysis data and the known size of silicon nanocrystal core (2.8 ± 0.6 nm), it was determined that the total mass per silicon nanocrystal is 7.24×10^{-17} mg, of which approximately 37% is the silicon core and 63% is ligand (corresponding to a ligand coverage of

9.93 ligands/nm²).

Nanovesicles were synthesized using the DELOS-susp procedure, with cholesterol (chol, Anatrace) as the sterol and cetyl trimethylammonium bromide (CTAB, high purity grade, Amresco) as the quaternary ammonium surfactant (Cano-Sarabia M et al. *Langmuir* **2008**, *24*, 2433–2437; Cabrera I et al. *Nano Lett.* **2013**, *13*, 3766–3774). Briefly, a 7.5 ml high-pressure vessel was loaded with a solution containing 76 mg of cholesterol in 2.88 ml of ethanol at atmospheric pressure and 35°C. Carbon dioxide was then added to the solution using a syringe pump, until reaching a working pressure of 10 MPa and a solution CO₂ content of $X_{CO_2} = 0.62$. The system was kept at 35°C and 10 MPa for approximately 1 hour to achieve complete homogenization and to attain thermal equilibration. Finally, the CO₂-expanded cholesterol solution was depressurized over 24 ml of an aqueous solution containing 72 mg of CTAB, creating the desired nanovesicles. In this final step, a flow of N₂ is used as a plunger to push down the CO₂-expanded solution from the vessel and to maintain a constant pressure inside the vessel during depressurization. The molar ratio between the CTAB and the cholesterol in the final formulation was 1 to 1, which has been shown to be the correct proportion in order to have a pure vesicular phase (Ferrer-Tasies L et al. *Langmuir* **2013**, *29*, 6519–6528). The final concentration of nanovesicles in this vesicular phase, 7 mM cholesterol-CTAB nanovesicles, included 7 mM cholesterol and 7 mM CTAB in high purity water containing 10% ethanol. Nanovesicles with a final concentration of 0.7 mM CTAB and 0.7 mM in cholesterol were prepared by dilution of the 7 mM cholesterol-CTAB nanovesicles with water containing 10% ethanol.

Silicon nanocrystals with core diameters of approximately 2.8 nm and 1-octene capping ligands were incorporated into pre-formed cholesterol-CTAB nanovesicles using a 5 minute bath sonication procedure, as outlined in Figure 6. Unless otherwise noted, the following proportions were used: 750 µl of 7 mM cholesterol-CTAB nanovesicles were added to a 2 ml glass vial. 20 µl of 6.75 mg/ml silicon nanocrystals (0.135 mg) was added. As discussed above, from the thermogravimetric analysis data and the known size of silicon nanocrystal core (2.8 ± 0.6 nm), it was determined that the total mass per silicon nanocrystal is 7.24×10^{-17} mg, of which approximately 37% is the silicon core and 63% is ligand (corresponding to a ligand coverage of 9.93 ligands/nm²). Thus, when 135 µg of silicon nanocrystals are used in a preparation with 5.25 µmol each of CTAB and cholesterol (750 µl of 7 mM solution), the molar ratio of CTAB : Cholesterol : silicon nanocrystal is 1695 : 1695 : 1.

The vial with the 750 µl of 7 mM cholesterol-CTAB nanovesicles and 20 µl of 6.75

mg/ml silicon nanocrystals (0.135 mg) was then bath sonicated for 5 minutes to incorporate the silicon nanocrystals (Misonix 1510R-MT or Branson M1800 bath sonicator, 40 kHz, 1/2 gallon tank). All experiments were conducted under room temperature conditions. The nanovesicle dispersion, initially cloudy blue/grey, became light yellow following addition of the silicon nanocrystals and bath sonication, with no visible precipitate or phase separation (Figure 7- Figure 9). Under 365 nm ultraviolet lamp illumination the colloidal dispersion exhibited orange fluorescence, which is characteristic of the silicon nanocrystals.

Cryogenic transmission electron microscopy (cryo-TEM) images were taken using an FEI Tecnai Biotwin TEM operated at 80 kV accelerating voltage, and acquired digitally. Cryo-TEM samples were prepared using a Leica EM GP by dropping 3 µl of sample onto Quantifoil R1.2/1.3 holey carbon on 300 mesh copper grids (Electron Microscopy Science) inside the environmental control chamber set to 25°C and 90% humidity. After a blotting time of 3.5 seconds, the grid was plunged into liquid ethane to vitrify the sample. The grid was then transferred to a Gatan 626 Cryo-Transfer Holder under liquid nitrogen. FEI low dose software was used to obtain images of cryo-TEM samples.

Figure 10-Figure 15 show cryo-TEM images of the silicon nanocrystals incorporated into nanovesicles. Although many of the nanovesicles are free of silicon nanocrystals, there was still a significant amount of association between the silicon nanocrystals and the nanovesicles. The silicon nanocrystals formed aggregates that were either on one side of one or multiple nanovesicles or that appeared to form spherical structures with similar sizes as the nanovesicles without silicon nanoparticles.

Nanovesicles are composed of two component building blocks, cholesterol and CTAB, such that geometric calculations could be used to estimate the number of each molecule in a spherical nanovesicle. In this approximation, the radius of an average surface of both membranes (R_s) was used to estimate the number of cholesterol-CTAB pairs in a typical nanovesicle (Figure 16). This radius is given by:

$$R_s = \frac{d}{2} - \frac{h}{2}$$

In the calculation, d is the vesicle diameter and could be estimated from dynamic light scattering (DLS) data of nanovesicles (assuming spherical geometry and unimodal size distribution) that had been bath sonicated with 20 µl of chloroform (no silicon nanocrystals) as seen in Figure 7, which was found to be 113.7 ± 0.4 nm. The value for h , the total bilayer thickness, was calculated in previous work using molecular dynamics simulations was approximately 4.4 nm

(Ferrer-Tasies L et al. *Langmuir* **2013**, 29, 6519–6528). To know the number of cholesterol-CTAB pairs (N_s) that there are in a membrane of an average nanovesicle, the following equation was applied:

$$N_s = \frac{4\pi R_s^2}{a}$$

5 In this equation, a is the area of the polar head group of a cholesterol-CTAB pair, and can be estimated from just the headgroup of the CTAB surfactant, 0.64 nm^2 (Figure 17) (Ferrer-Tasies L et al. *Langmuir* **2013**, 29, 6519–6528). Using all these parameters the estimated number of bimolecular building-block cholesterol-CTAB (N_s) in a single layer typical nanovesicle is 58,643, and thus in a bilayer nanovesicle there are approximately 117,286 cholesterol-CTAB
10 pairs.

To calculate the number of silicon nanocrystals per nanovesicle, knowing that there are 117,286 cholesterol-CTAB pairs per nanovesicle and 1695 CTAB or cholesterol units per silicon nanocrystal, it was determined that there are approximately 69 silicon nanocrystals per nanovesicle. Cryogenic transmission electron microscopy (cryo-TEM) images show that not all
15 nanovesicles have silicon nanocrystals associated with them, though when the silicon nanocrystals do assemble into nanovesicles they form aggregates with many nanoparticles, so this estimate appears to be reasonable.

Figure 18 shows the absorbance (Varian Cary 50 Bio ultraviolet-visible spectrophotometer or Varian Cary 500 ultraviolet-visible-near infrared spectrophotometer),
20 photoluminescence excitation (PL) and photoluminescence emission (PLE) spectra (Varian Cary Eclipse fluorescence spectrophotometer) collected from silicon nanoparticles dispersed in chloroform and incorporated into nanovesicles. Spectra were measured at room temperature by diluting nanovesicle-silicon nanocrystal dispersions by a factor of 20 with water in glass cuvettes, and the average photoluminescence emission wavelength was calculated from the
25 photoluminescence curve. The spectra of each sample are similar (Figure 18), indicating that the incorporation of the nanocrystals into the nanovesicles, in an aqueous media, did not change the fluorescence properties of the nanocrystals.

Samples of silicon nanocrystals dispersed with cholesterol-CTAB nanovesicles in water with 10% ethanol were prepared through 5 minutes of bath sonication and monitored for 12
30 weeks, along with control samples prepared with only CTAB, no nanovesicles, or no silicon nanocrystals, as outlined in Table 1 and Figure 19-Figure 23).

Table 1. Description of samples i-v monitored in stability testing.

Sample	Silicon nanocrystals (SiNCs)	Dispersant	Molar ratio of components
i	20 μ l of 6.75 mg/ml SiNCs in chloroform	750 μ l of 7 mM cholesterol-CTAB nanovesicles (7 mM CTAB and 7 mM cholesterol) in 10% ethanol in water	1 SiNC : 1695 CTAB : 1695 cholesterol
ii	20 μ l of 6.75 mg/ml SiNCs in chloroform	750 μ l of 0.7 mM cholesterol-CTAB nanovesicles (0.7 mM CTAB and 0.7 mM cholesterol) in 10% ethanol in water	1 SiNC : 170 CTAB : 170 cholesterol
iii	20 μ l of 6.75 mg/ml SiNCs in chloroform	750 μ l of 7 mM CTAB in 10% ethanol in water	1 SiNC : 1695 CTAB
iv	20 μ l of 6.75 mg/ml SiNCs in chloroform	750 μ l of 10% ethanol in water	-
v	20 μ l of chloroform	750 μ l of 7 mM cholesterol-CTAB nanovesicles (7 mM CTAB and 7 mM cholesterol) in 10% ethanol in water	-

Immediately following bath sonication the solutions containing cholesterol-CTAB nanovesicles (samples i and ii) and CTAB micelles (sample iii) appeared cloudy, however after one day the turbidity dissipated. The initial turbidity likely occurred as a result of the high energy applied, causing changes in the nanovesicle structures. In the case where silicon nanocrystals were directly added to water, the nanoparticles precipitated to the bottom of the vial after 5 minutes of bath sonication, which was anticipated for hydrophobic nanocrystals added to an aqueous solution.

Samples prepared with cholesterol-CTAB nanovesicles (i) above (7 mM) and (ii) below (0.7 mM) the 1 mM CTAB critical micelle concentration (CMC). To prepare the 0.7 mM cholesterol-CTAB nanovesicle sample (sample ii), the nanovesicles were diluted in 10% EtOH in water to 0.7 mM cholesterol-CTAB (chol-CTAB) nanovesicles, which is below the CTAB critical micelle concentration of 1 mM, and 750 μ l of nanovesicles with 20 μ l silicon nanocrystals in chloroform were bath sonicated to form sample ii.

Samples prepared with chol-CTAB nanovesicles (i) above (7 mM) and (ii) below (0.7 mM) the 1 mM CTAB critical micelle concentration (CMC) were both observed to maintain similar absorbance and PL over time (Figure 24 and Figure 25). The lack of a significant blue

shift (only 56 nm over twelve weeks) in the PL spectra indicates that the nanoparticles are protected from oxidation or degradation that would normally occur if the nanocrystals were directly dispersed into aqueous solutions, as seen in vial (iv). Further, Cryo-TEM images (Figure 26-Figure 29) of sample ii indicate that the structures formed are similar to those when 7 mM cholesterol-CTAB nanovesicles was used (sample i), including one sided and full nanovesicle aggregates. Silicon nanocrystals appear to incorporate into nanovesicles similarly to when nanovesicles were at concentrations about the CTAB CMC of 1 mM (Figure 30-Figure 41). The fact that the nanovesicles both above and below the 1 mM CTAB CMC have similar stability suggests that the nanovesicles have a high ability to stabilize nanocrystals even when working with diluted systems.

Silicon nanocrystals could also be dispersed with 7 mM CTAB micelles (with no cholesterol) with good dispersion and fluorescence stability, though the shape of the nanocrystal aggregates were quite different than those stabilized by the nanovesicles. Figure 42-Figure 45 shows cryo-TEM images of CTAB-stabilized nanocrystals with irregularly shaped aggregates that were both very small and very large. For medical applications consistent size and concentration of load delivery is important, and thus the wide size distribution observed with CTAB micelles would not be ideal for those applications.

The nanovesicle-silicon nanocrystal assemblies were monitored for twelve weeks by cryo-TEM. Figure 30-Figure 41 shows images of sample (i) over a period of 12 weeks. There were no observed changes in size or shape during this time period. Over all time points, silicon nanocrystals were observed aggregated into one side of some nanovesicles and forming spherical structures with the same size as plain nanovesicles. Dynamic light scattering data were also acquired to monitor the size distribution in the mixtures over time.

Dynamic light scattering (DLS) data were acquired on a Zetasizer Nano ZS (Malvern Instruments). Samples were measured in 40 μ l disposable cuvettes at 173°, with temperature set to 25°C. All measurements were conducted in triplicate. Zetasizer software (Malvern Instruments) was used to analyze data and determine the average size (z-ave) and polydispersity index (PDI), with the dispersant set depending on the medium, as follows: 10% ethanol in water by volume had 8.06% by weight ethanol in water with viscosity of 1.2814 cP and refractive index of 1.335, while 17% ethanol in water by volume had 13.9% by weight ethanol in water with a viscosity of 1.6554 cP and refractive index of 1.3396.

Dynamic light scattering data of the nanovesicle-silicon nanocrystal assemblies confirmed the cryo-TEM findings, as the size distribution for the mixtures did not change

significantly over 8 weeks, although the distributions did become narrower over time (Figure 46). A similar trend was observed in the peak narrowing for nanovesicles bath sonicated without any silicon nanocrystals (sample v) (Figure 47), suggesting that the narrowing of the size distribution is associated with the nanovesicles and not the nanoparticles incorporated into the nanovesicles. A similar peak narrowing phenomena was observed in vesicle assemblies formed by cholesterol and zwitterionic surfactants (Alenaizi R et al. *Physicochem. Eng. Aspects* 482 (2015) 662-669) However over time the distribution narrows as the nanovesicles return to a thermodynamically stable size range, as expected for self-assembled liposomal systems (Antonietti M and Förster S. *Adv. Mater.* **2003**, *15*, 1323–1333).

To assess the impact of the ethanol present in the nanovesicle solution, nanovesicles were prepared with no ethanol using a sonication methodology previously described (Ferrer-Tasies L et al. *Langmuir* **2013**, *29*, 6519–6528). Briefly: 39 mg of cholesterol and 36 mg of CTAB were weighed into a glass bottle and suspended in 10 ml of deionized water. The dispersion was then sonicated at room temperature (25°C) for 4 minutes (Sonic and Materials Corporation, 20 kHz) to form a homogeneous dispersion. After allowing the mixture to stabilize for one week at 25°C, silicon nanocrystals were incorporated using the standard incorporation process (20 µl silicon nanocrystals in chloroform added to 750 µl nanovesicles, then bath sonicated for 5 minutes).

The samples appeared consistent with silicon nanocrystal – nanovesicle assemblies prepared using nanovesicles that were prepared using the DELOS-susp method with 10% ethanol. Cryo-TEM images of the samples showed the nanocrystals incorporated into some of the nanovesicles (Figure 48-Figure 50). Under 366 nm ultraviolet lamp illumination the colloidal dispersion exhibited fluorescence, which is characteristic of the silicon nanoparticles (Figure 51). This data suggests that the 10% ethanol in water used to form DELOS-susp nanovesicles does not have an impact on the incorporation of silicon nanocrystals into nanovesicles.

To explore the effects of bath sonication on the nanovesicle structures, different bath sonication times were used following addition of 20 µl silicon nanocrystals in chloroform to 750 µl of cholesterol-CTAB nanovesicles. Cryo-TEM images indicated that while the nanovesicles maintained similar size distributions after bath sonication for 1 minute (Figure 52) or 5 minutes (Figure 53), sonication times of 15 minutes (Figure 54) or 30 minutes (Figure 55) decreased the size of the nanovesicles in solution, which was also confirmed by the dynamic light scattering data (Figure 56).

Stabilization of silicon nanocrystal with charge-neutral 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes and anionic 1,2-dioleoyl-sn-glycero-3-(1'-rac-glycerol)

(DOPG) liposomes was attempted, for comparison purposes with silicon nanocrystal stabilized with cholesterol-CTAB nanovesicles. Neutral 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes were prepared using the DELOS-susp process. DOPC (35 mg, in chloroform, Avanti Polar Lipids) was dissolved in 1.9 ml of ethanol and loaded into a 7.5 ml high-pressure vessel at atmospheric pressure and 35°C. The solution was then volumetrically expanded with compressed CO₂, until reaching a molar fraction of CO₂ of $X_{CO_2} = 0.76$ at a working pressure of 10 MPa. The system was kept at 35°C and 10 MPa for approximately 1 hour to achieve homogenization and to attain thermal equilibration. Finally, the CO₂-expanded DOPC solution was depressurized over 9 ml of an aqueous solution to create the liposomes. The final concentration of DOPC in the liposomal system was 3.6 mM in high purity water containing 17% ethanol.

Anionic 1,2-dioleoyl-sn-glycero-3-(1'-*rac*-glycerol) (DOPG) liposomes were formed by drying 7 µmol DOPG (in chloroform, Avanti Polar Lipids) from chloroform into a film with a rotary evaporator for 15 minutes. Residual solvent was removed by placing the film in a vacuum oven for 2 hours. The film was then hydrated with 1 ml of 10 mM HEPES, 10 mM NaCl and bath sonicated for 30 minutes. Finally, the suspension was extruded 21 times through a 100 nm pore size polycarbonate filter on an Avanti MiniExtruder (Avanti Polar Lipids).

The bath sonication approach to silicon nanocrystal incorporation was also carried out for the preparation of the silicon nanocrystal-liposomes dispersions. Briefly, 20 µl of 6.75 mg/ml silicon nanocrystals (0.135 mg) was added to 750 µl of either 3.6 mM DOPC or 7 mM DOPG liposomes and then bath sonicated for 5 minutes.

As the cryo-TEM images in Figure 57-Figure 60 show, the charge-neutral DOPC liposomes become multilamellar and large silicon nanocrystal aggregates were observed. Dynamic light scattering of the DOPC liposomes showed that they had a z-average size of 58.08 nm (polydispersity index 0.230) before sonication and grew to a z-average of 463.7 nm (polydispersity index 0.135) after sonication with the silicon nanocrystals. The vial containing the DOPC and silicon nanocrystals also had a white precipitate at the bottom vial that was likely large lipid structures.

The anionic DOPG liposomes retained their initial size, but no silicon nanocrystals were observed to associate with the liposomes, as shown in Figure 61-Figure 64. The dispersion, however, was fluorescent and separate lipid-stabilized aggregates of silicon nanocrystals were observed. These dispersions lost their fluorescence after the third day. These results indicate that silicon nanocrystals do not incorporate into size-monodisperse DOPC or DOPG liposomes

through the bath sonication process that has been demonstrated for nanovesicles.

Medical diagnostic or theranostic applications where fluorescent nanoparticle dispersions are used as contrast agents, usually involve the dilution of the fluorescent formulations.

Therefore, it can be important for this kind of formulations maintain their physico-chemical and fluorescent properties upon dilution. The stability of silicon nanocrystals dispersed with cholesterol-CTAB nanovesicles and CTAB micelles at equal CTAB concentrations was assessed by dialysis into water.

For dialysis, 20 μ l of 6.75 mg/ml silicon nanocrystals (0.135 mg) were incorporated into 0.75 ml of either 7 mM cholesterol-CTAB nanovesicles or 7 mM CTAB micelles, both in 10% ethanol in water. Following 5 minutes of bath sonication to incorporate the silicon nanocrystals, the samples were kept in glass vials for 1 day prior to starting dialysis (Figure 65). Samples were then inserted into SnakeSkin Dialysis Tubing, 22 mm 10K molecular weight cutoff (Life Technologies), and sealed at both ends with locking closures. The dialysis bags were then immersed in 1 L of DI water under magnetic stirring. After 24 hours the water was changed, and this process was repeated for a total of six water changes. Following completion of the dialysis the remaining solutions inside the dialysis tubing were transferred to glass vials.

Both the 7 mM cholesterol-CTAB nanovesicles and 7 mM CTAB micelles in 10% ethanol dispersions were initially fluorescent, but only the nanovesicle dispersion remained fluorescent after dialysis (Figure 65-Figure 69). This suggests that the CTAB surfactant is only weakly associated with the silicon nanocrystals in the micelles, and that with repeated dilutions the structures disassemble. In contrast, nanovesicle-silicon nanocrystal assemblies imaged by cryo-TEM after six rounds of dialysis still showed structures similar to the freshly prepared materials, which indicates that even after multiple dilutions these assemblies are stable (Figure 70-Figure 73). This finding shows the benefit of the cholesterol-CTAB nanovesicle assemblies as compared to the CTAB micelles: while both maintain fluorescence for 12 weeks (Figure 24-Figure 25), only the nanovesicles could be used for *in-vivo* applications where particles injected into the anatomy would be diluted into the blood stream.

As discussed above, 750 μ l of nanovesicles at either 7 mM cholesterol-CTAB or 0.7 mM cholesterol-CTAB were both able to incorporate with 20 μ l silicon nanocrystals in chloroform with no visible precipitate forming in the vial (Figure 19-Figure 23). Next, the volumes and concentrations of silicon nanocrystals added to nanovesicles were explored to maximize incorporation of nanocrystals while not disrupting the nanovesicle structures.

To each of 5 vials, 750 μ l of nanovesicles (7 mM cholesterol-CTAB) was added. Then,

135 µg of silicon nanocrystals was added to each vial, though at different concentrations and thus in different volumes of chloroform. The amounts added were: (i) 10 µl of 13.5 mg/ml silicon nanocrystal in chloroform, (ii) 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform (typical conditions), (iii) 40 µl of 3.375 mg/ml silicon nanocrystal in chloroform, (iv) 80 µl of 1.6875 mg/ml silicon nanocrystal in chloroform, (v) 200 µl of 0.675 mg/ml silicon nanocrystal in chloroform. Images of the vials taken over several days after preparation indicate that while all exhibited fluorescence from silicon nanocrystal incorporation (Figure 74-Figure 78, bottom row), only the vial prepared with 20 µl of 6.75 mg/ml silicon nanocrystal in chloroform did not have any precipitate (Figure 74-Figure 78, top row). In vial (i) an orange-brown precipitate was observed after one day, likely containing silicon nanocrystals that were not incorporated into the nanovesicles (Figure 79). In vials (iii), (iv), and (v) the solutions became cloudy white and the bottoms of the vials had white precipitate (Figure 74-Figure 78, top row). The white precipitate suggests that the chloroform destabilized the cholesterol-CTAB nanovesicle structures and caused them to break from their typically sized dispersions. This suggests that the high volume fraction of chloroform added with the silicon nanocrystals had a destabilizing effect on the nanovesicles and caused the structures to break apart. Thus, the experiments to determine how the chloroform may interact with the nanovesicles found that the volume ratio of nanovesicles to silicon nanocrystals in chloroform influenced the stability of the assemblies.

A small amount of chloroform can enhance the incorporation of dodecanethiol-capped gold nanocrystals into liposomes (Rasch MR et al. *Langmuir* **2012**, 28, 12971–12981) and a similar interaction may be occurring with nanovesicles, resulting in successful incorporation without destroying the nanovesicles when 20 µl of chloroform is added. This is further confirmed by sample (v) in Figure 19-Figure 23, where 20 µl of chloroform without silicon nanocrystals was added to nanovesicles and bath sonication was performed with no resulting precipitation of material, suggesting that that amount of chloroform could not destabilize the nanovesicle structure.

Additional experiments were performed to investigate different routes for incorporating silicon nanocrystals into the nanovesicles. The bath sonication approach was the only technique that resulted in fluorescent samples with assemblies in the same size range as the nanovesicles. Attempts were made to add silicon nanocrystals directly into the DELOS-susp process to form the nanovesicles, and the result was a non-fluorescent dispersion. Most likely, the silicon nanocrystals degraded during the process due to the pressure and temperature used in the DELOS-susp procedure, or perhaps the nanocrystals were not incorporated into the nanovesicles

and instead became stuck in the DELOS-susp setup.

In other attempts to assemble silicon nanocrystals with pre-made cholesterol-CTAB nanovesicles, probe sonication and vortexing were both used to provide energy to the system, however neither approach was successful. A probe sonicator (Branson Sonifier 250, Output control: 2, constant duty cycle) was used for 3 minutes with the cholesterol-CTAB nanovesicles and silicon nanocrystals, and within one day aggregates were observed (Figure 80). The probe sonicated sample lost fluorescence over a period of a month and a half following probe sonication, likely indicating that the nanoparticles had degraded. Attempts to use 5 minutes of vortexing to incorporate 20 μ l silicon nanocrystals in chloroform to 750 μ l of cholesterol-CTAB nanovesicles resulted in very limited fluorescence in solution (Figure 81 and Figure 82), with cryo-TEM images showing no incorporation of silicon nanocrystals into the nanovesicles (Figure 83 and Figure 84). Further, after vortexing the nanovesicles appeared to have a wider size distribution skewed to smaller sizes compared to assemblies prepared through 5 minutes of bath sonication (Figure 85). Thus, compared to probe sonication and vortexing, 5 minutes of bath sonication resulted in the best structures of silicon nanocrystals assembled into cholesterol-CTAB nanovesicles, as observed through cryo-TEM and fluorescence stability.

The silicon nanocrystals have a hydrophobic coating, so they tend to aggregate when dispersed in water due to the strong attractive hydrophobic interaction. As the aggregates or clusters of particles form, they interact with other clusters of particles and with the hydrophilic surfaces of nanovesicles. In the case of the silicon nanocrystals (Figure 86), the capping layer is compact and the interaction of a nanovesicle with a cluster of silicon particles is reminiscent of the interaction of a vesicle with a hydrophobic solid surface. In this case, the layers of the nanovesicles were spread onto the surface of the clusters of silicon particles. This spreading process requires first the breaking of vesicles and the input of energy. Vesicles are dynamic structures that can be reformed or reduced in size with the addition of sonication energy (Marsh D. *Handbook of Lipid Bilayers, Second Edition*; CRC Press, 2013; Szoka F and Papahadjopoulos D. *Annu. Rev. Biophys. Bioeng.* **1980**, 9, 467–508). When cholesterol-CTAB nanovesicles with silicon nanocrystals were sonicated for 15 or 30 min, they were smaller than those sonicated for 1 minute or 5 minutes (Figure 52-Figure 56). Thus, it can be hypothesized that when nanovesicles are sonicated they are broken down through a redistribution of membrane molecules. When the sonication is performed in the presence of hydrophobic silicon nanocrystals with a “compact organic layer” coverage, this redistribution would allow small aggregates of silicon nanocrystals to become stabilized by the hydrophobic part of cholesterol-CTAB

monolayers (Figure 86). The resulting self-assembled structure (clusters of silicon particles covered by a nanovesicle monolayer) is now stable in water and acquires the radius corresponding to the spontaneous curvature of the nanovesicle building blocks (the bimolecular entities made by the association between CTAB and cholesterol) (Ferrer-Tasies L et al.

5 *Langmuir* **2013**, 29, 6519–6528). The result of this process is the coexistence between the nanovesicles and these self-assembled clusters of silicon covered by CTAB and cholesterol with a diameter similar to that of the nanovesicles.

Although the nanoparticles are aggregated in the nanovesicles, it is still apparent that the synergy between cholesterol and CTAB, found before in plain nanovesicles (Ferrer-Tasies L et al. *Langmuir* **2013**, 29, 6519–6528), can lead to the stable interaction between the nanovesicles and silicon nanocrystals. When CTAB micelles were sonicated with silicon nanocrystals, the silicon nanocrystals appeared as either very small or very large, irregularly shaped clusters (Figure 42-Figure 45). Additionally, cryo-TEM images of silicon nanocrystals incorporated into cholesterol-CTAB nanovesicles in Figure 10-Figure 15 show that the silicon nanocrystal
15 aggregations are approximately the same size as the nanovesicles without any silicon nanocrystals. Since the size of the nanovesicles is influenced by the interaction between cholesterol and CTAB, the size of silicon nanocrystals aggregated suggests that they are associated with the same cholesterol-CTAB units. Further, as shown in the dilution experiment in Figure 65 – Figure 73, only silicon nanocrystal dispersed in nanovesicles remain stable after
20 several rounds of dilution, while silicon nanocrystal dispersed in CTAB micelles result in precipitation of the silicon nanocrystals after multiple dilutions. Thus, it is specifically the nanovesicles where cholesterol and CTAB interact as bimolecular building blocks that contribute to stabilizing the silicon nanocrystals.

A method to disperse fluorescent silicon nanocrystals in aqueous media with long-term
25 stability was developed, utilizing cholesterol-CTAB nanovesicles, which are non-liposomal vesicular structures. The stable silicon nanocrystal-nanovesicle aqueous dispersions can be made with five minutes of bath sonication. The silicon nanocrystal-nanovesicle assemblies remain dispersed in water for several weeks and maintain the fluorescence properties of the silicon nanocrystals for several weeks (Figure 87). The silicon nanocrystal-nanovesicle assemblies also
30 maintain the fluorescence properties of the silicon nanocrystals after dilution with additional water. Cryogenic transmission electron microscopy (cryo-TEM) imaging further confirmed that these assemblies retained their structure for several weeks. Conditions for incorporating the nanocrystals into nanovesicles were investigated, including the use of a bath sonication

incorporation process.

The long-term stability of the silicon nanocrystal-nanovesicle assemblies, their fluorescence and biocompatibility makes them attractive candidates for medical applications. Nanovesicles, which have been shown to have long term stability (e.g., at least three years in aqueous solutions) (Ferrer-Tasies L et al. *Langmuir* **2013**, 29, 6519–6528), provide a vehicle for the dispersion of hydrophobic silicon nanocrystals into *in vitro* or *in vivo* environments, even under dilute conditions. The biocompatibility of nanovesicles and near infrared emitting silicon nanocrystals make these structures excellent candidates for biomedical imaging applications. Furthermore, nanovesicles have been shown to enhance protein activity and to protect proteins against premature degradation in topical pharmaceutical formulations, as well as to treat biofilms (Thomas N et al. *J. Mater. Chem. B* **2015**, 3, 2770–2777; WO 2014/019555). Therefore, the incorporation of both biomolecules and silicon nanocrystals into nanovesicle structures offers the possibility to explore the behavior of these systems in biological environments and their use for theranostics.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

CLAIMS

What is claimed is:

1. A composition comprising: an aqueous solution comprising a composite nanocarrier, the composite nanocarrier comprising a nanoparticle at least partially encapsulated within a vesicle, the vesicle comprising a sterol and a surfactant.
2. The composition of claim 1, wherein the sterol comprises cholesterol or a derivative thereof.
3. The composition of claim 1 or claim 2, wherein the surfactant comprises a quaternary ammonium surfactant.
4. The composition of claim 3, wherein the surfactant comprises cetyl trimethylammonium bromide.
5. The composition of any one of claims 1-4, wherein the sterol and the surfactant are present in the vesicle in a molar ratio of from 10:1 to 1:5.
6. The composition of claim 5, wherein the sterol and the surfactant are present in the vesicle in a molar ratio of from 2:1 to 1:2.
7. The composition of claim 5 or claim 6, wherein the sterol and the surfactant are present in the vesicle in a molar ratio of 1:1.
8. The composition of any one of claim 1-7, wherein the vesicle has an average size of from 25 nanometers (nm) to 500 nm as measured by dynamic light scattering.
9. The composition of claim 8, wherein the vesicle has an average size of from 50 nm to 300 nm as measured by dynamic light scattering.
10. The composition of any one of claims 1-9, wherein the vesicle is substantially spherical in shape.
11. The composition of any one of claims 1-10, wherein the vesicle consists of a sterol and a quaternary ammonium surfactant.
12. The composition of any one of claims 1-11, wherein the nanoparticle comprises a

plasmonic nanoparticle.

13. The composition of claim 12, wherein the plasmonic nanoparticle comprises Au, Ag, Pd, and combinations thereof.

14. The composition of any one of claims 1-13, wherein the nanoparticle comprises a magnetic nanoparticle.

15. The composition of claim 14, wherein the magnetic nanoparticle comprises Fe, Co, Zn, Ni, Mn, Ag, Au, C, Cd, or a combination thereof.

16. The composition of claim 14 or claim 15, wherein the magnetic nanoparticle comprises an oxide of Fe, Co, Zn, Ni, Mn, or a combination thereof.

17. The composition of any one of claims 1-16, wherein the nanoparticle comprises a metal.

18. The composition of claim 17, wherein the metal comprises Be, Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, Ba, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, , or combinations thereof.

19. The composition of any one of claims 1-18, wherein the nanoparticle comprises a semiconductor.

20. The composition of claim 19, wherein the semiconductor comprises Fe_2O_3 , WO_3 , Ta_3N_5 , TaON, TiO_2 , ZnO, CdS, CdSe, Si, or combinations thereof.

21. The composition of claim 19 or claim 20, wherein the nanoparticle comprises Si.

22. The composition of any one of claims 1-21, wherein the nanoparticle comprises a fluorescent nanoparticle.

23. The composition of any one of claims 1-22, wherein the nanoparticle is biocompatible.

24. The composition of any one of claims 1-23, wherein the nanoparticle has an average particle size of from 1 nm to 20 nm as measured by transmission electron microscopy.

25. The composition of claim 24, wherein the nanoparticle has an average particle size of

from 1 nm to 5 nm as measured by transmission electron microscopy.

26. The composition of any one of claims 8-25, wherein the nanoparticle has an average particle size that is less than the average size of the vesicle.

27. The composition of any one of claims 1-26, wherein the nanoparticle is substantially spherical in shape.

28. The composition of any one of claims 1-27, wherein the nanoparticle further comprises a capping layer comprising a plurality of ligands.

29. The composition of any one of claims 1-28, wherein the nanoparticle and the sterol are present in the composition in a molar ratio of from 1:10000 to 1:50.

30. The composition of claim 29, wherein the nanoparticle and the sterol are present in the composition in a molar ratio of from 1:1800 to 1:100.

31. The composition of any one of claims 1-30, wherein the nanoparticle and the surfactant are present in the composition in a molar ratio of from 1:10000 to 1:50.

32. The composition of claim 31, wherein the nanoparticle and the surfactant are present in the composition in a molar ratio of from 1:1800 to 1:100. The composition of any one of claims 1-30, wherein the composite nanocarrier has an average size of from 25 nm to 500 nm as measured by dynamic light scattering.

33. The composition of any one of claims 1-32, wherein the composite nanocarrier has an average size of from 50 nanometers (nm) to 300 nm as measured by dynamic light scattering.

34. The composition of any one of claims 1-33, wherein the composite nanocarrier has an average size that changes by 35% or less over a period of 8 weeks.

35. The composition of any one of claims 1-34, wherein the composite nanocarrier has an average size that changes by 35% or less upon dilution

36. The composition of any one of claims 1-35, wherein the nanoparticle comprises a fluorescent nanoparticle such that the composition is fluorescent.

37. The composition of claim 36, wherein the fluorescence of the composition is stable upon

dilution of the composition.

38. The composition of claim 36 or claim 37, wherein the peak wavelength of the photoluminescence spectrum of the fluorescent composition shifts by 60 nm or less over a period of 12 weeks.

39. The composition of any one of claims 1-38, wherein the composition is biocompatible.

40. The composition of any one of claims 1-39, wherein the composition is stable under physiological conditions.

41. The composition of any one of claims 1-40, wherein the aqueous solution further comprises a cosolvent selected from the group consisting of ethanol, methanol, butanol, isopropanol, propanol, acetic acid, chloroform, or combinations thereof.

42. The composition of any one of claims 1-41, wherein the aqueous solution is substantially free of precipitate.

43. A pharmaceutical composition comprising the composition of any one of claims 1-42; and a pharmaceutically acceptable excipient.

44. A method of making the composition of any one of claims 1-42, the method comprising contacting the nanoparticle and the vesicle, thereby forming a mixture; and mixing the mixture, thereby forming the composite nanocarrier.

45. The method of claim 44, wherein mixing the mixture comprises mechanical stirring, shaking, vortexing, bath sonication, probe sonication, or a combination thereof.

46. The method of claim 45, wherein mixing the mixture comprises bath sonicating the mixture.

47. The method of claim 46, wherein the mixture is bath sonicated for from 10 seconds to 5 hours.

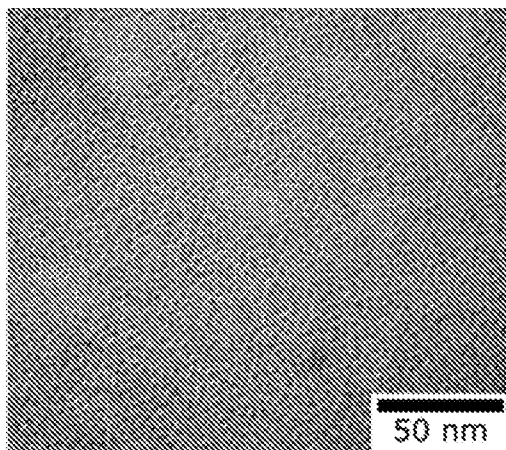
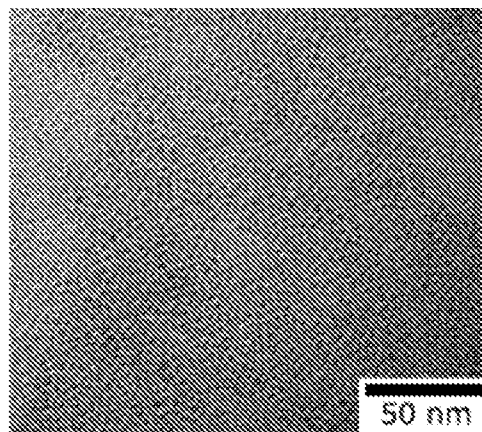
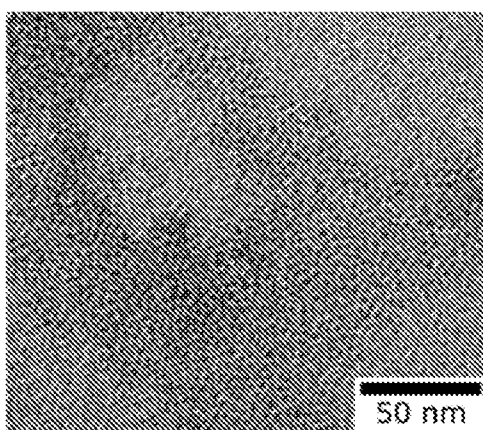
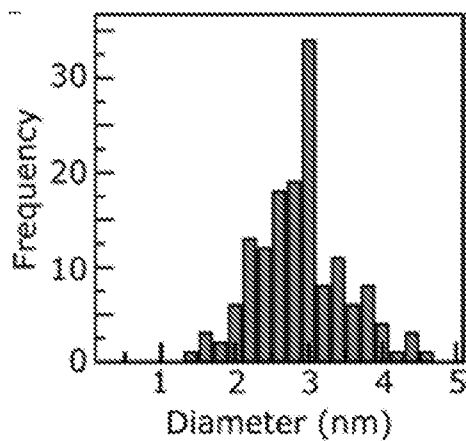
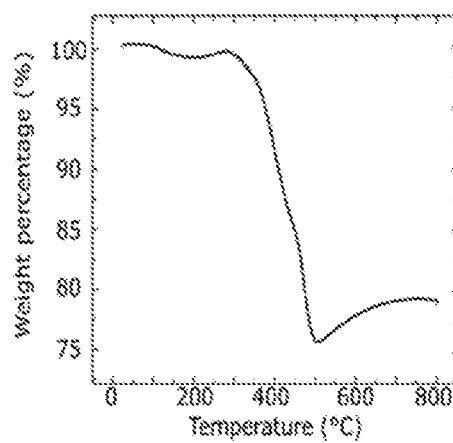
48. The method of claim 46 or claim 47, wherein the mixture is bath sonicated for from 1 minute to 30 minutes.

49. The method of any one of claims 46-48, wherein the mixture is bath sonicated for 5

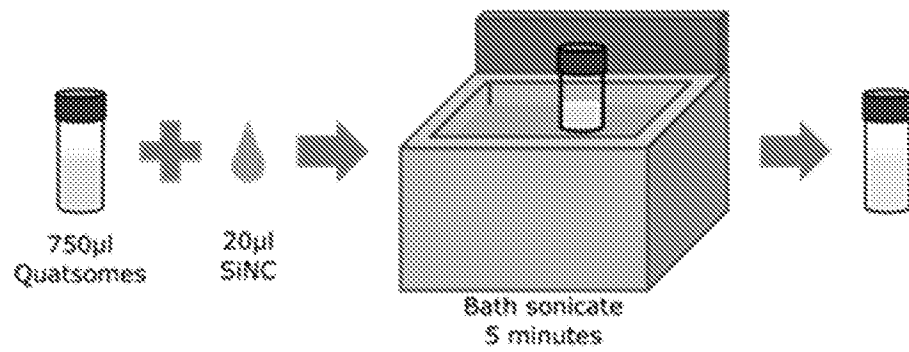
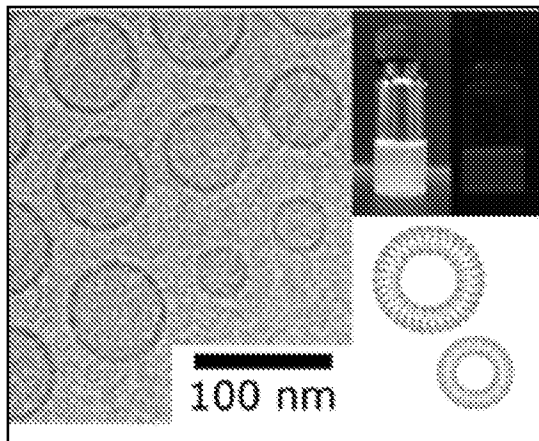
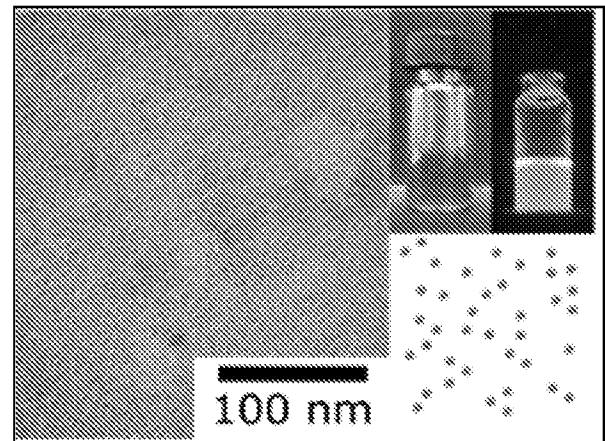
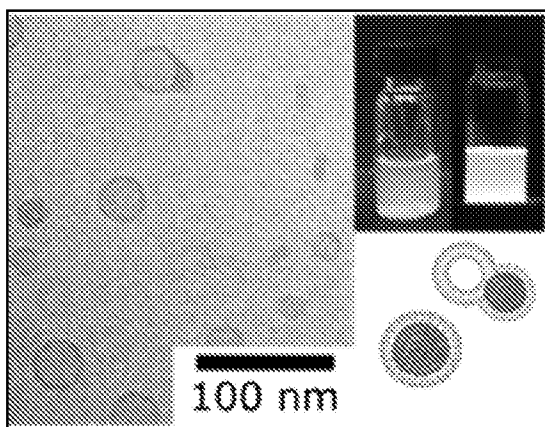
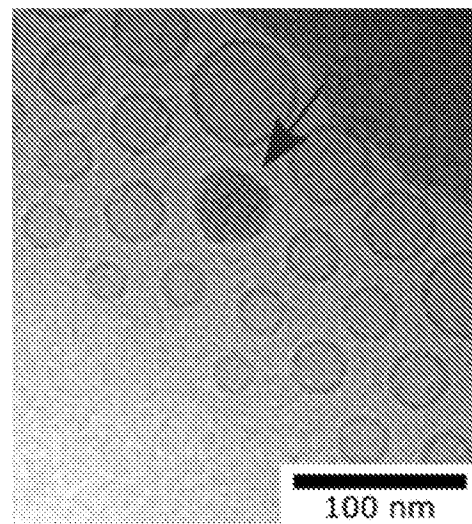
minutes.

50. The method of any one of claims 44-49, further comprising forming the vesicle.
51. The method of any one of claims 44-50, further comprising forming the nanoparticle.
52. A method of imaging a cell or a population of cells within or about a subject, the method comprising administering to the subject an amount of the composition of any one of claims 1-42; and detecting the composition of any one of claims 1-42.
53. The method of claim 52, wherein the cells are indicative of a disease.
54. The method of claim 53, wherein the disease is cancer.
55. A method of use of the composition of any one of claims 1-42, wherein the composition is used as a sensor to detect the presence or amount of a biological moiety; the structure, composition, and conformation of a biological moiety; the localization of a biological moiety in an environment; interactions of biological moieties; alterations in structures of biological compounds; alterations in biological processes; or combinations thereof.
56. A device comprising the composition of any one of claims 1-42, wherein the device comprises an electronic device, an energy storage device, an energy conversion device, an optical device, an optoelectronic device, a bioanalytical device, a chemical sensor, a biosensor, or combinations thereof.
57. The device of claim 56, wherein the energy conversion device comprises a solar cell, a fuel cell, a photovoltaic cell, or a combination thereof.
58. The device of claim 56, wherein the optical device comprises a light emitting diode.

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**FIG. 1****FIG. 2****FIG. 3****FIG. 4****FIG. 5**

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**FIG. 6****FIG. 7****FIG. 8****FIG. 9****FIG. 10**

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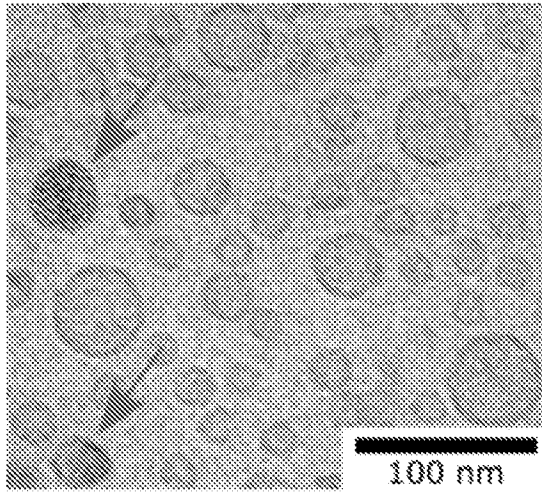


FIG. 11

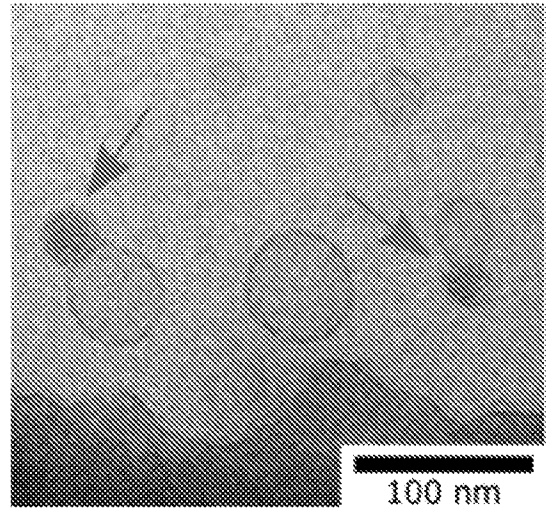


FIG. 12

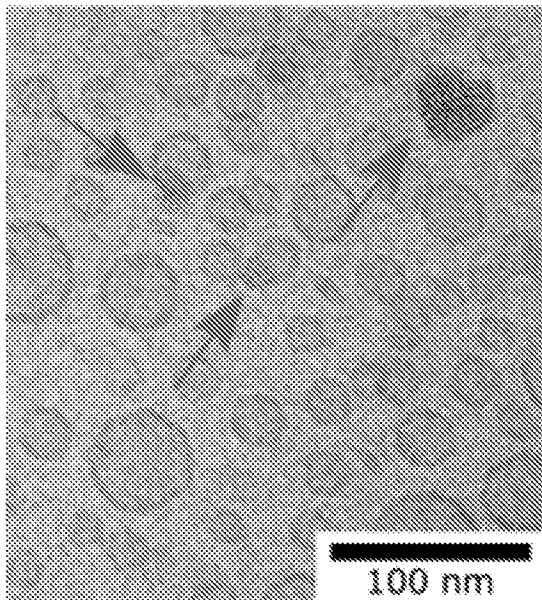


FIG. 13

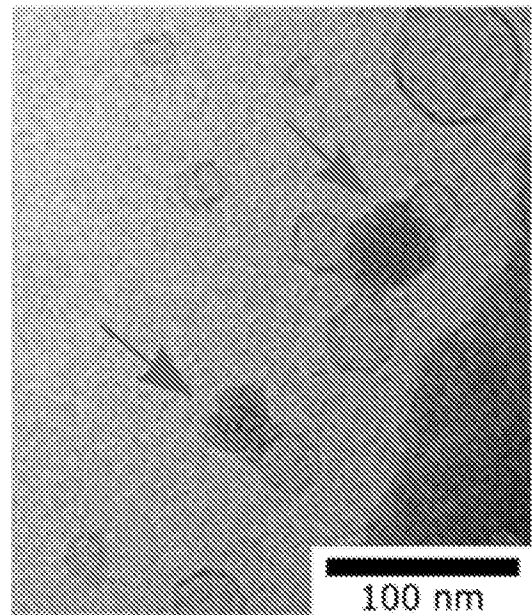


FIG. 14

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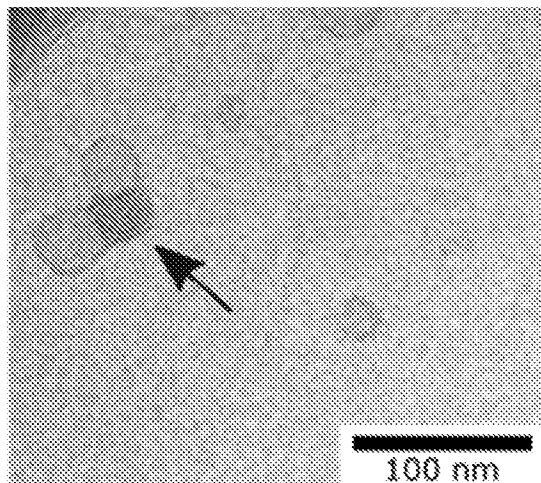


FIG. 15

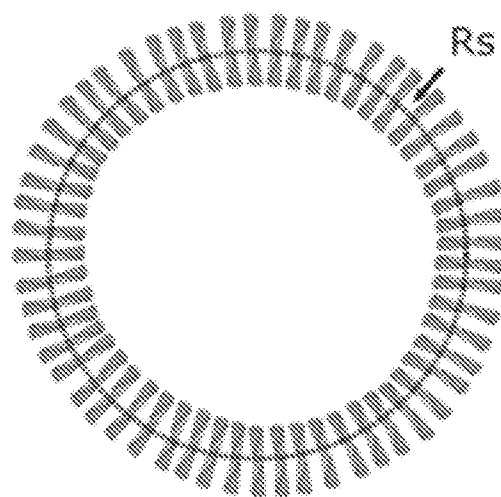


FIG. 16

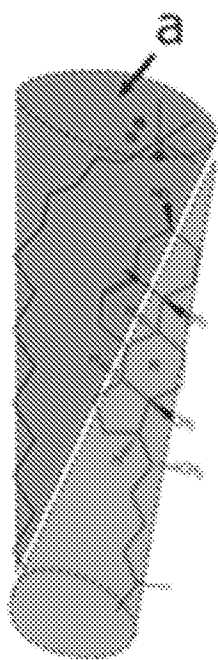


FIG. 17

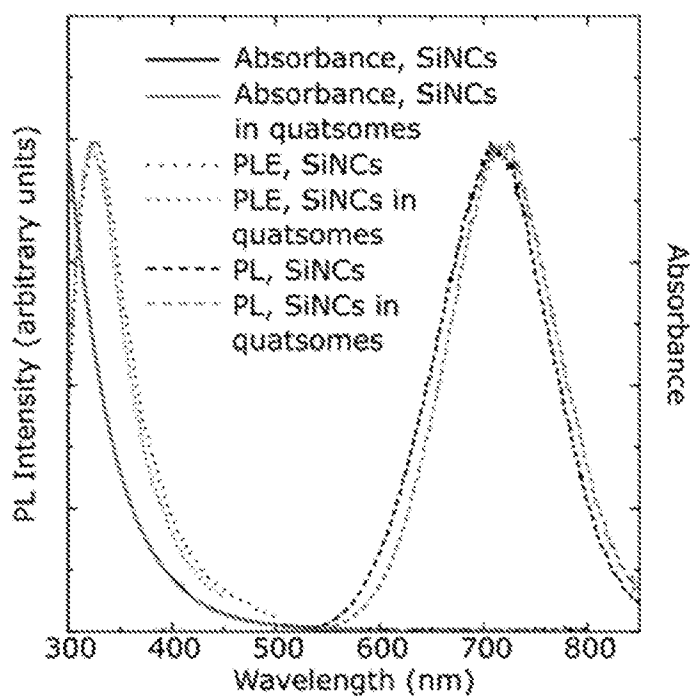


FIG. 18

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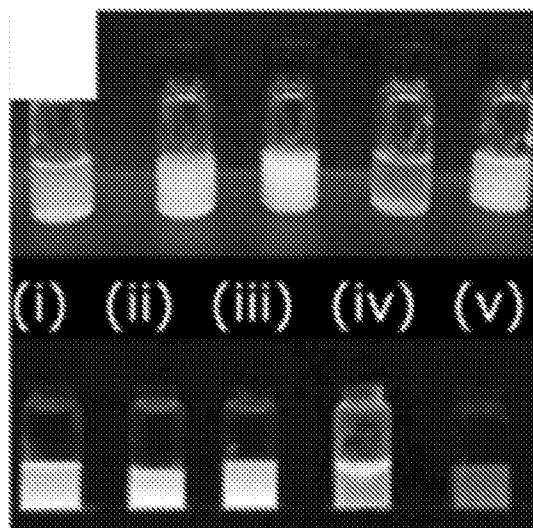


FIG. 19

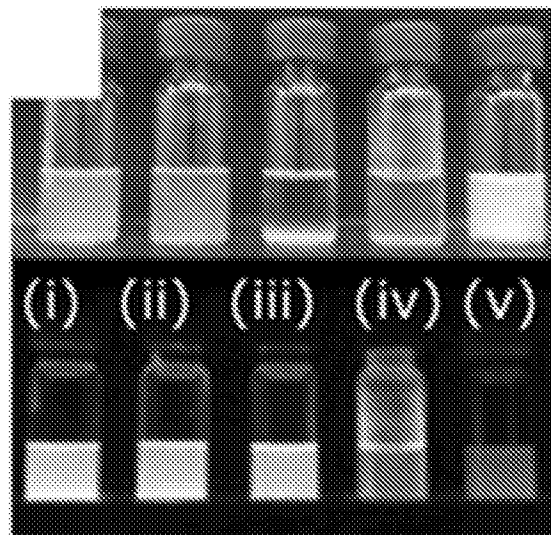


FIG. 20

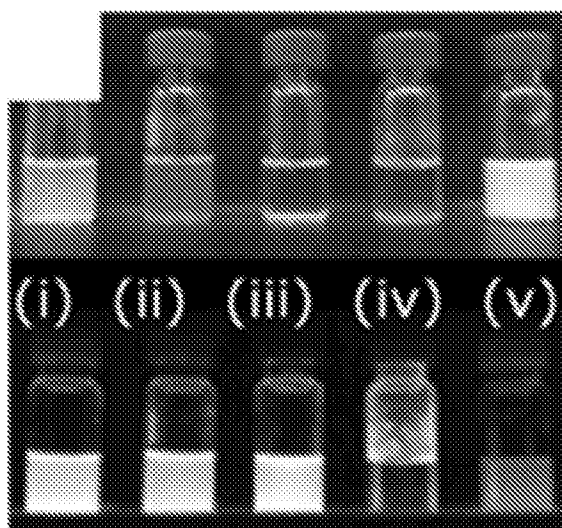


FIG. 21

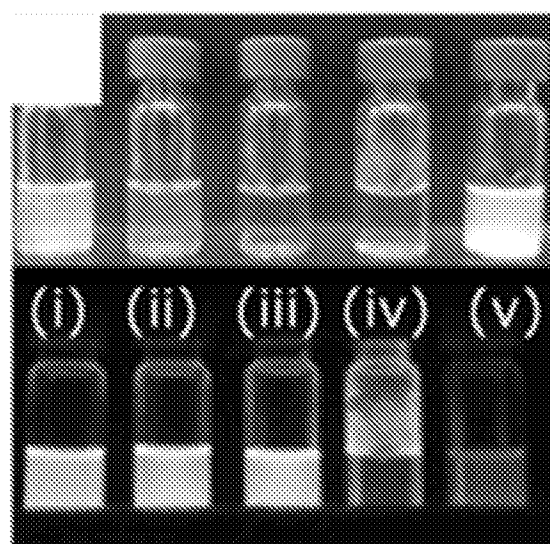


FIG. 22

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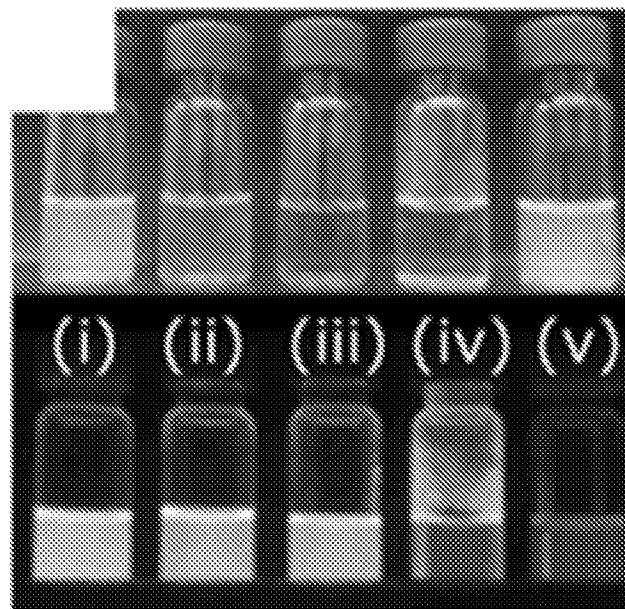


FIG. 23

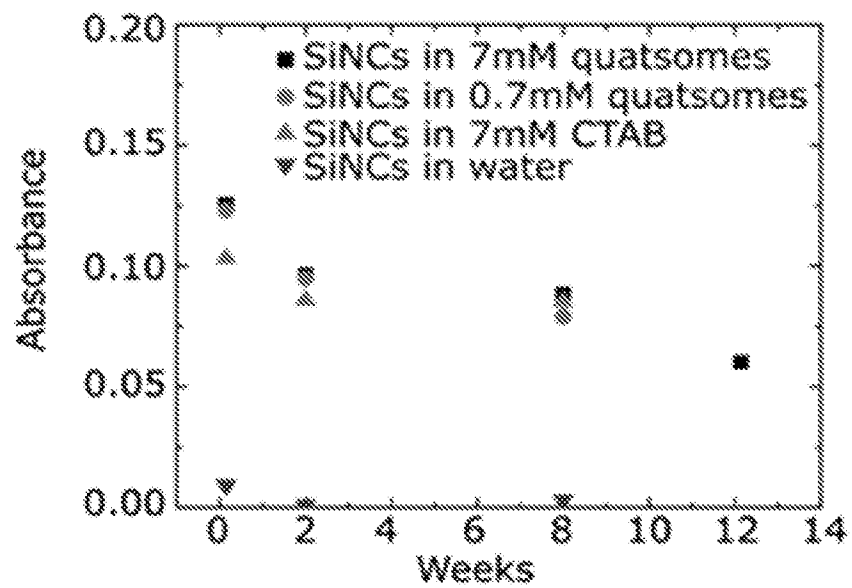


FIG. 24

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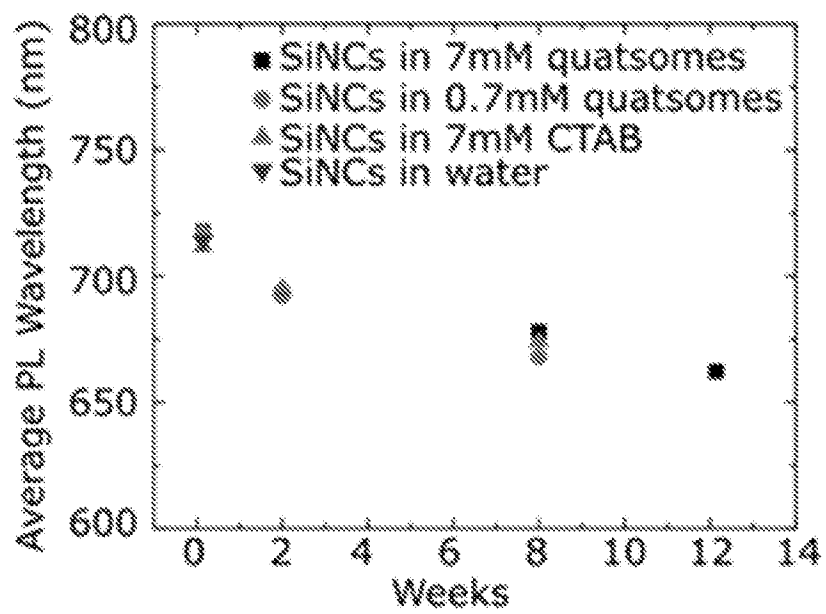


FIG. 25

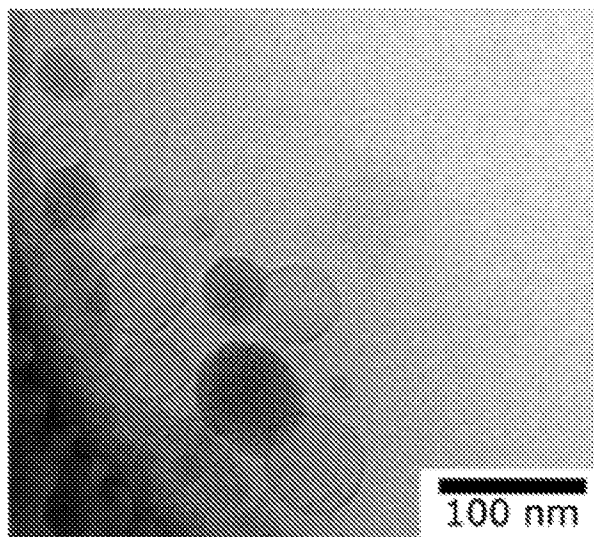


FIG. 26

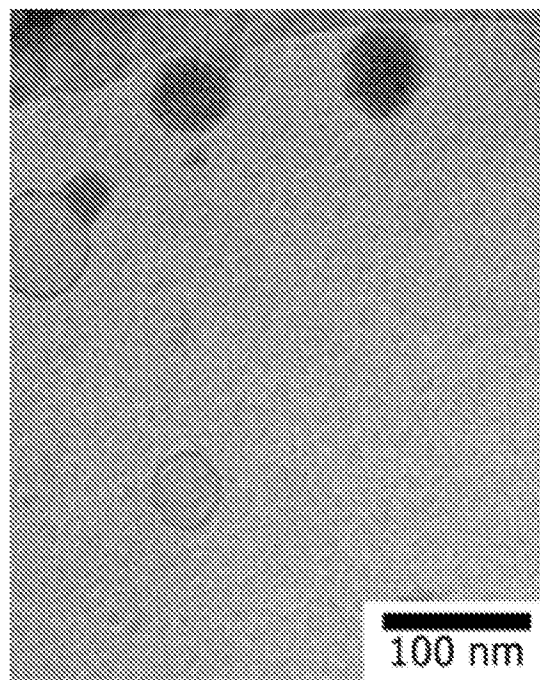


FIG. 27

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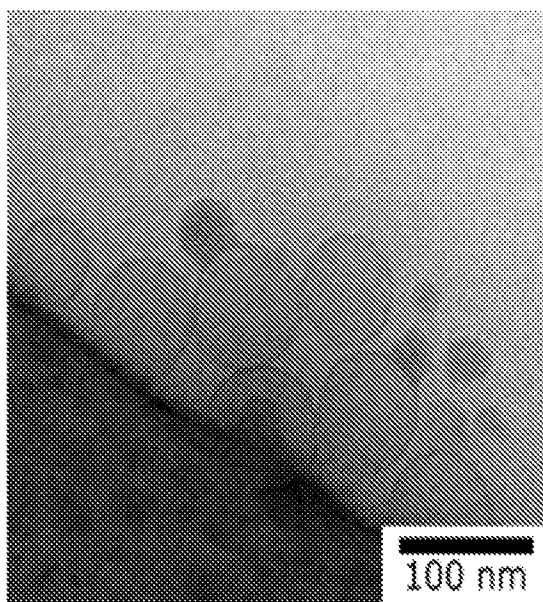


FIG. 28

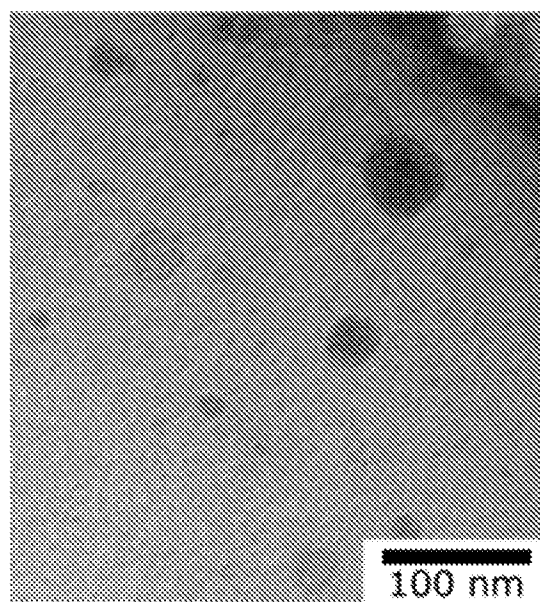


FIG. 29

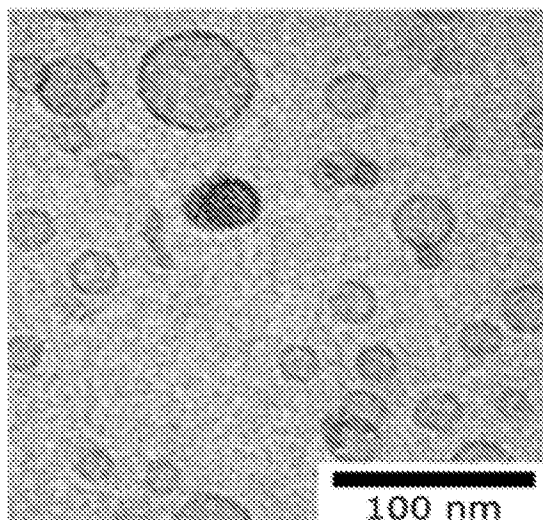


FIG. 30

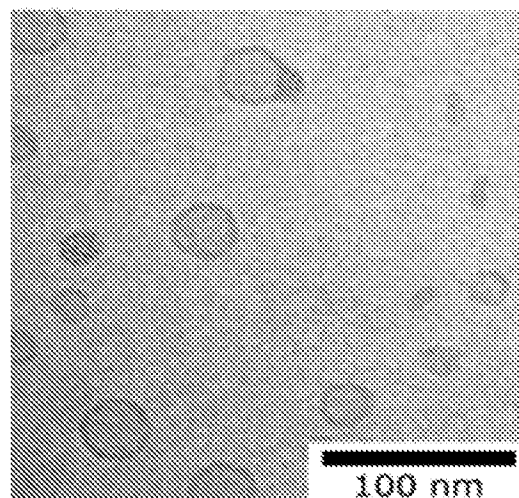


FIG. 31

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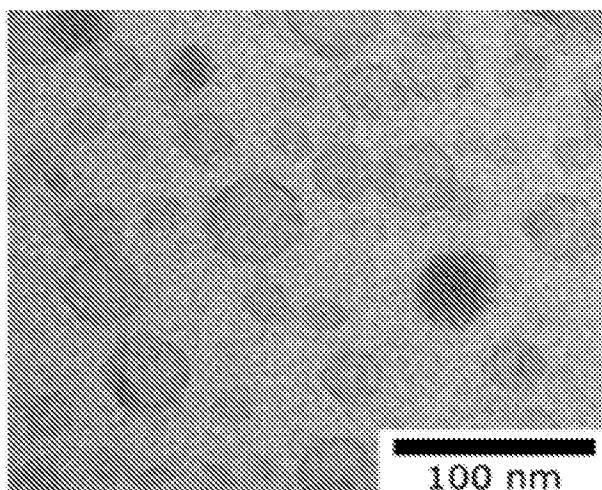


FIG. 32

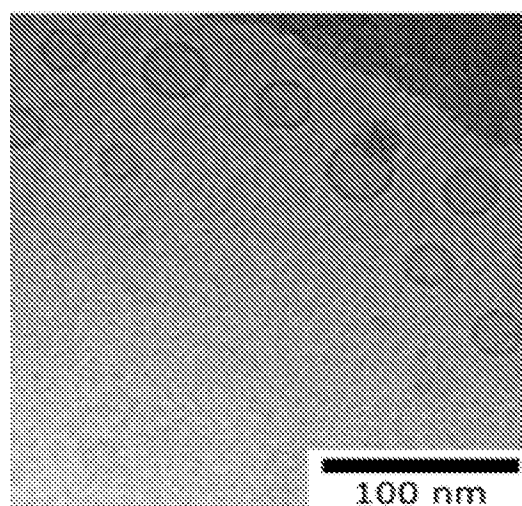


FIG. 33

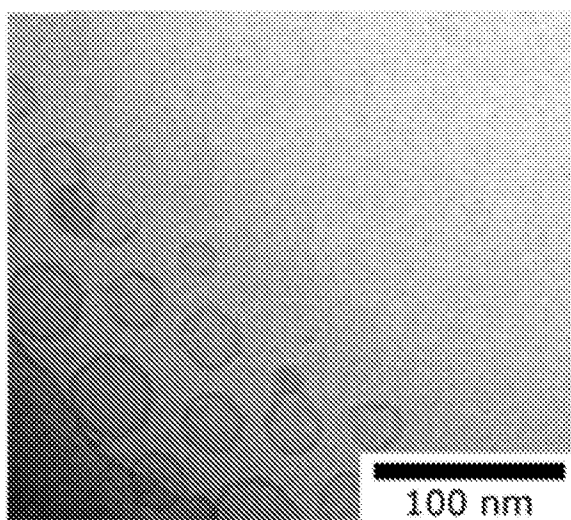


FIG. 34

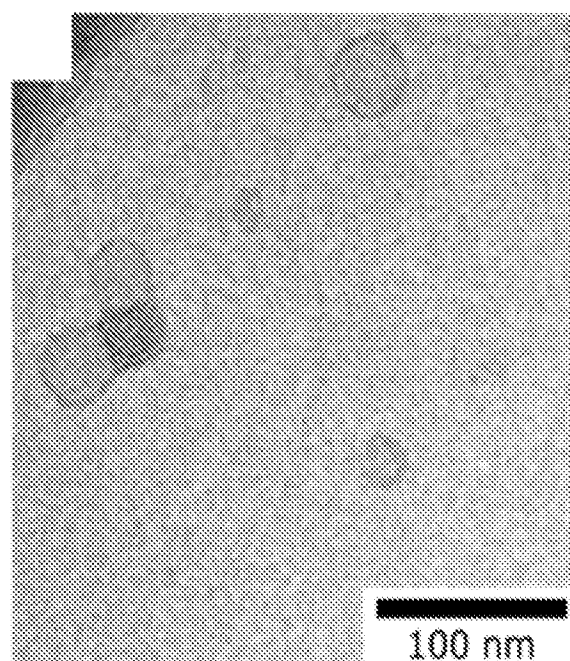


FIG. 35

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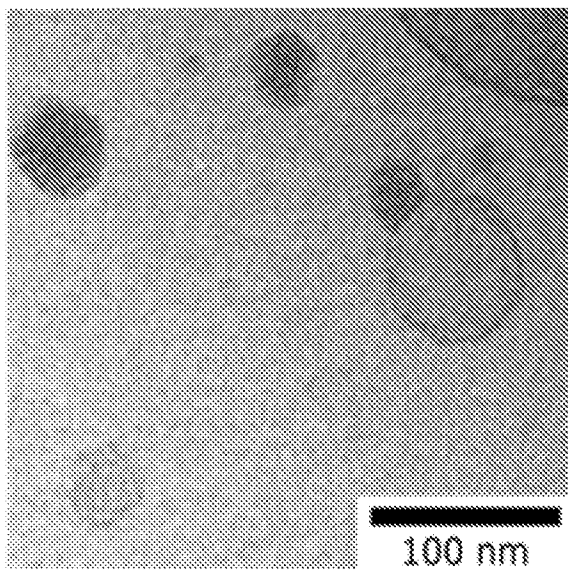


FIG. 36

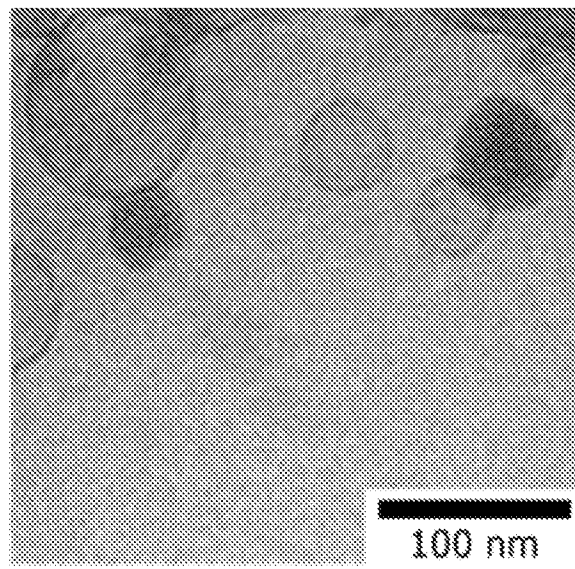


FIG. 37

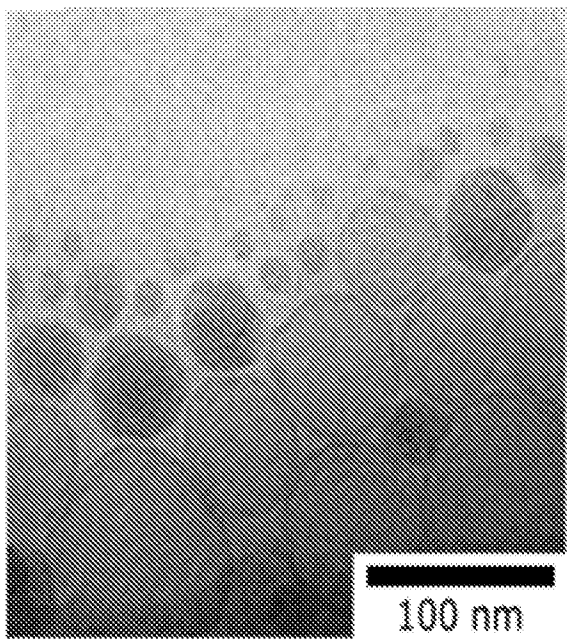


FIG. 38

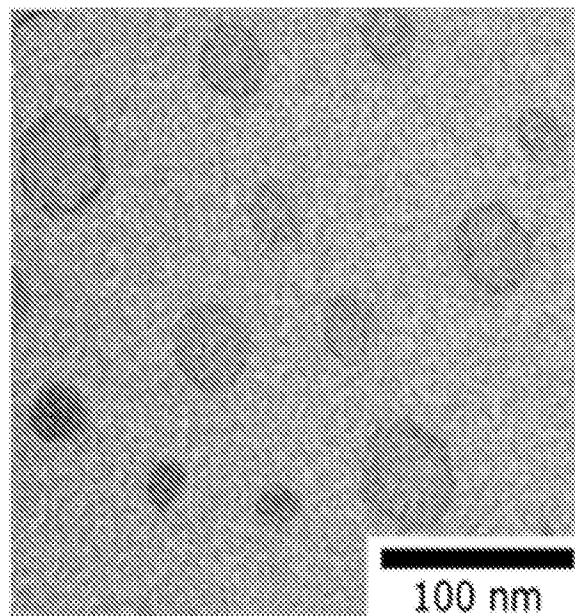


FIG. 39

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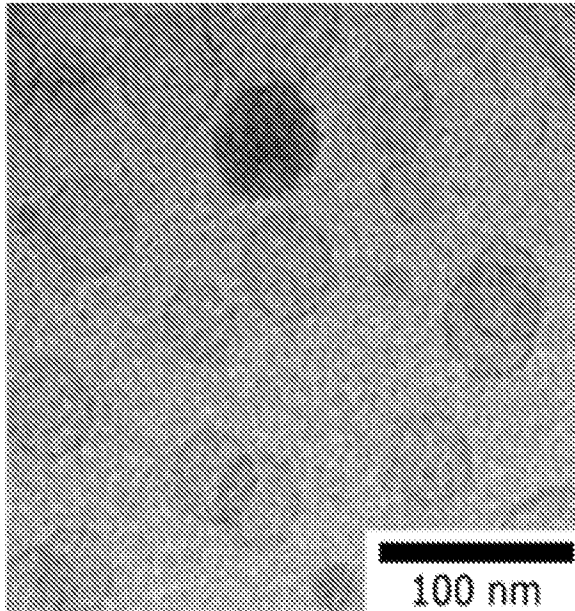


FIG. 40

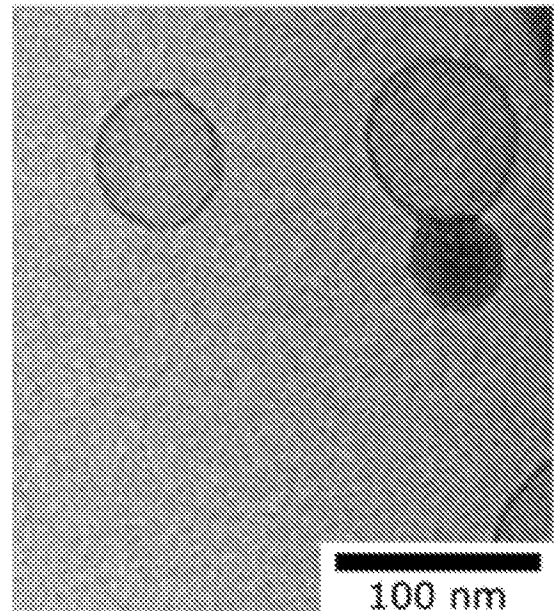


FIG. 41

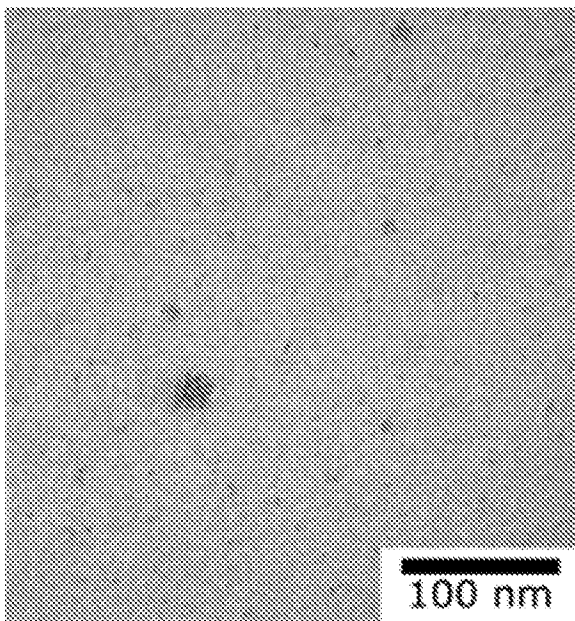


FIG. 42

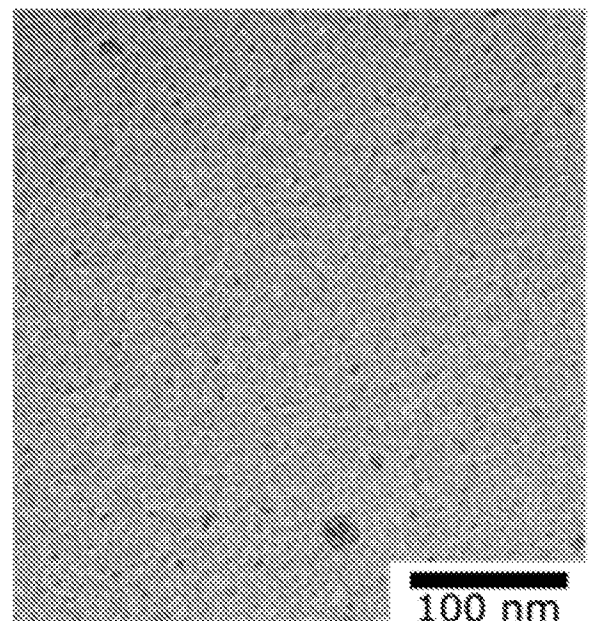
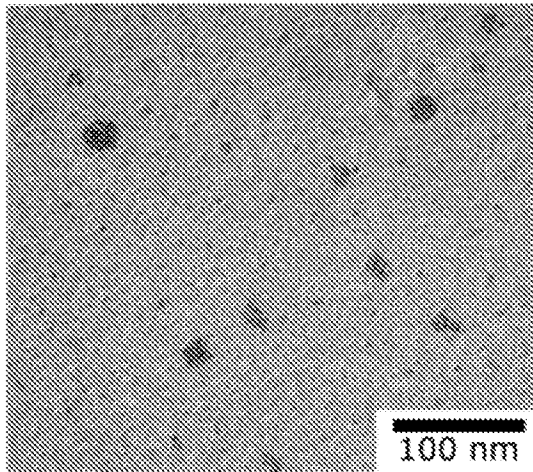
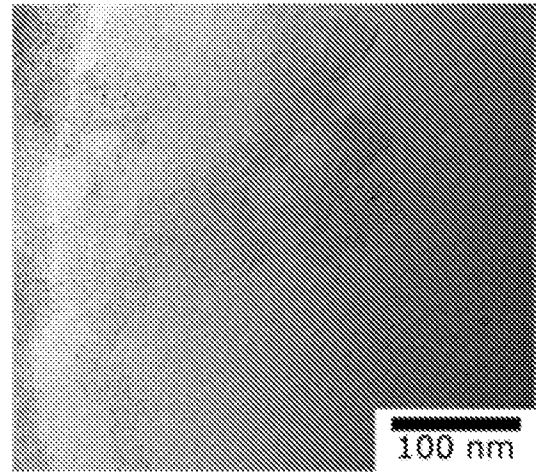
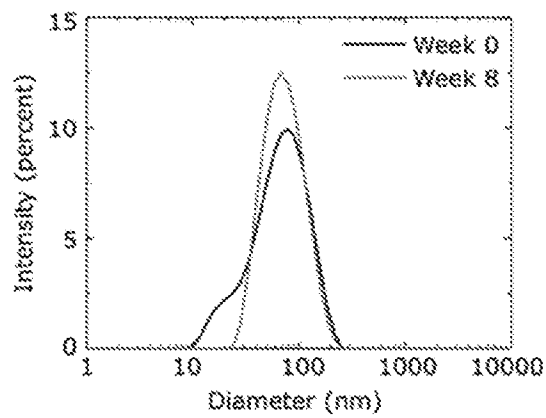
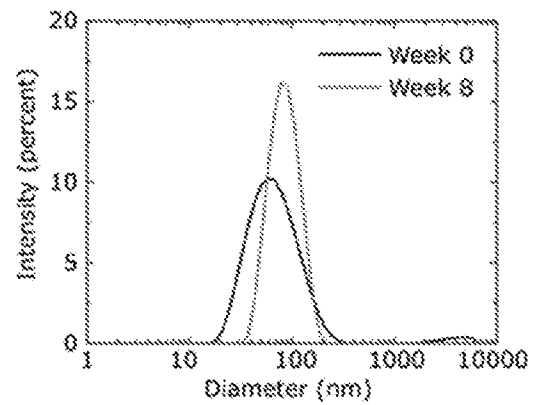
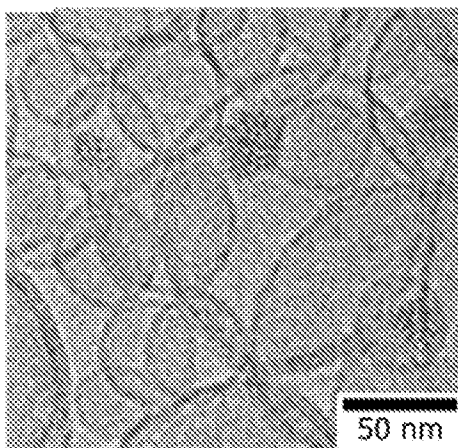
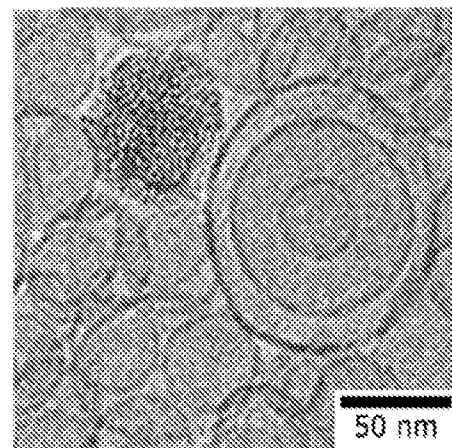


FIG. 43

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**FIG. 44****FIG. 45****FIG. 46****FIG. 47****FIG. 48****FIG. 49**

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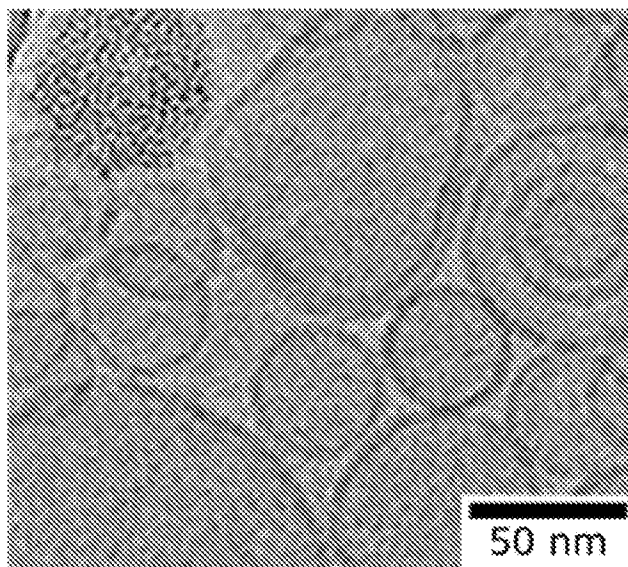


FIG. 50

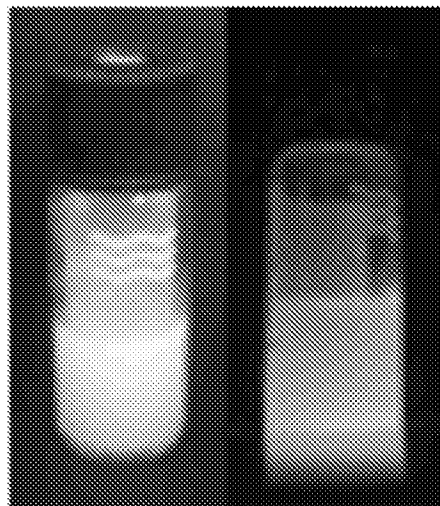


FIG. 51

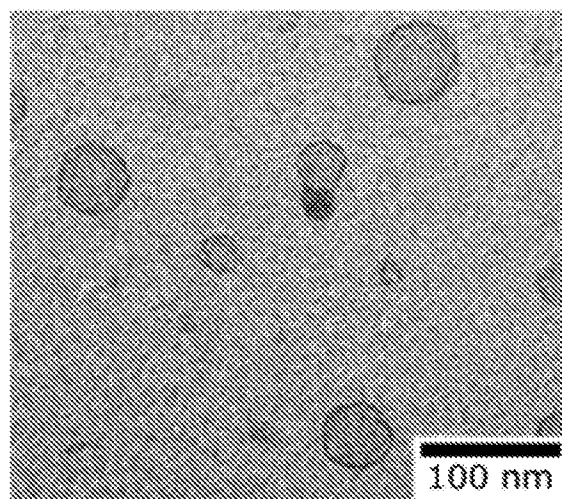


FIG. 52

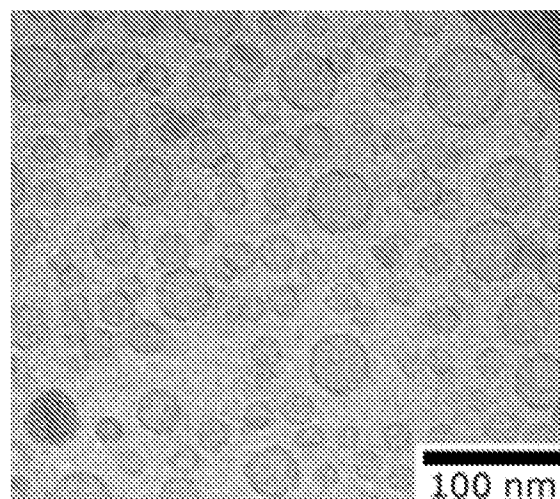
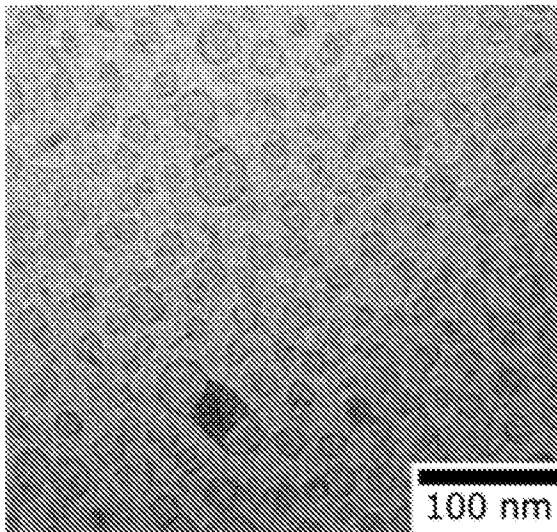
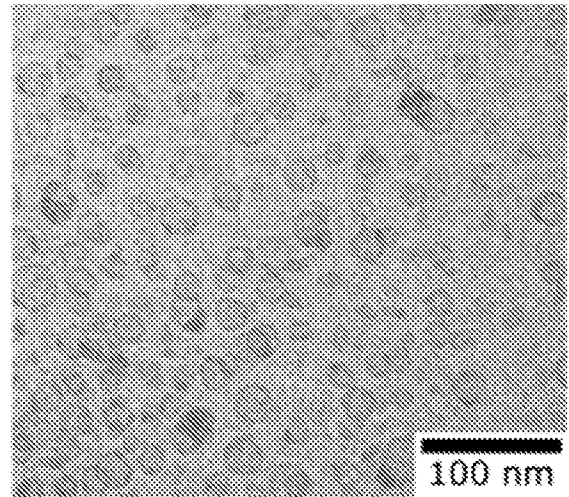
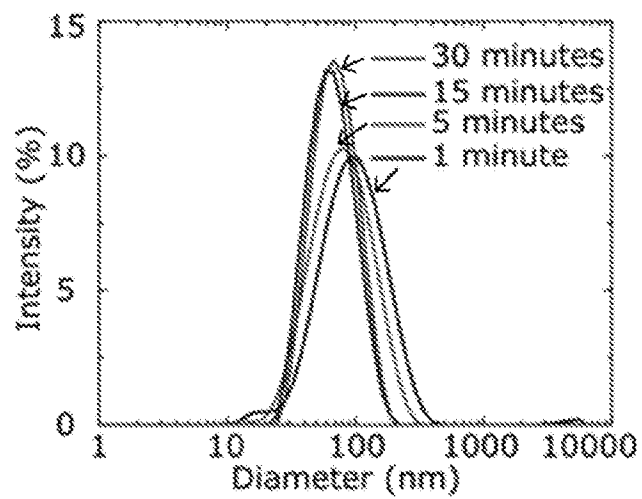
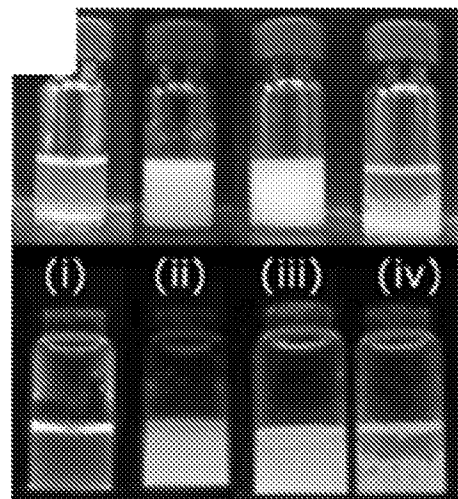


FIG. 53

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**FIG. 54****FIG. 55****FIG. 56****FIG. 57**

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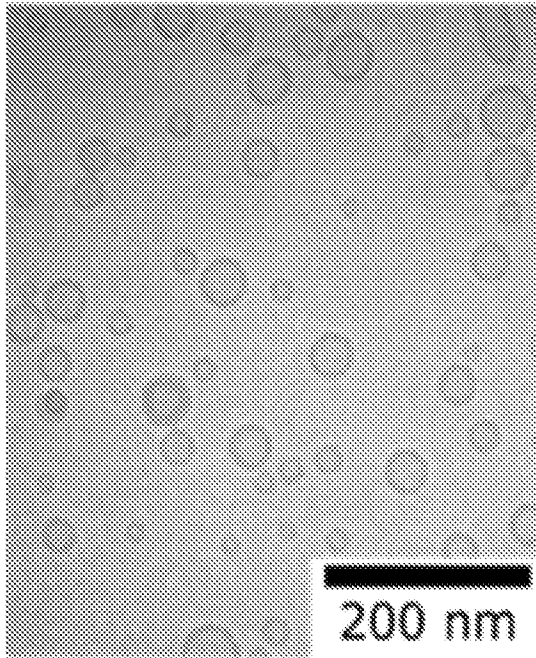


FIG. 58

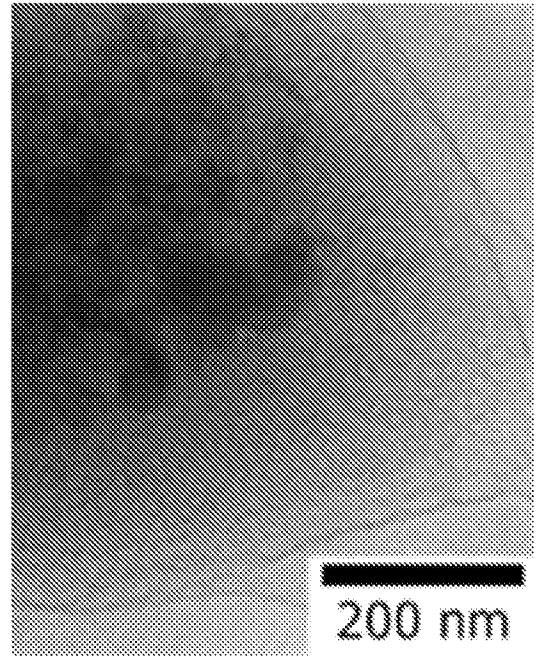


FIG. 59

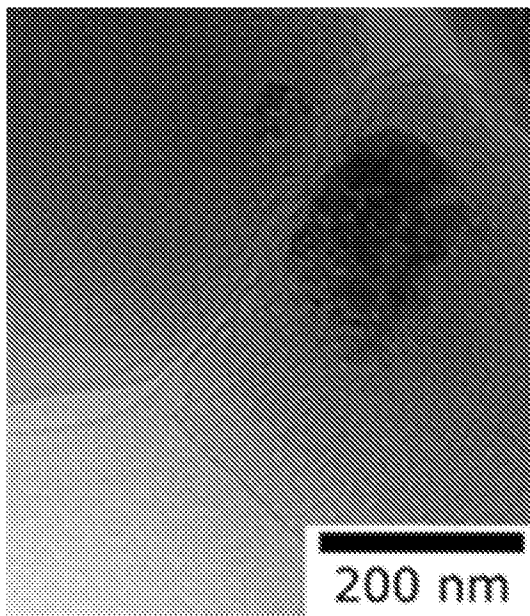


FIG. 60

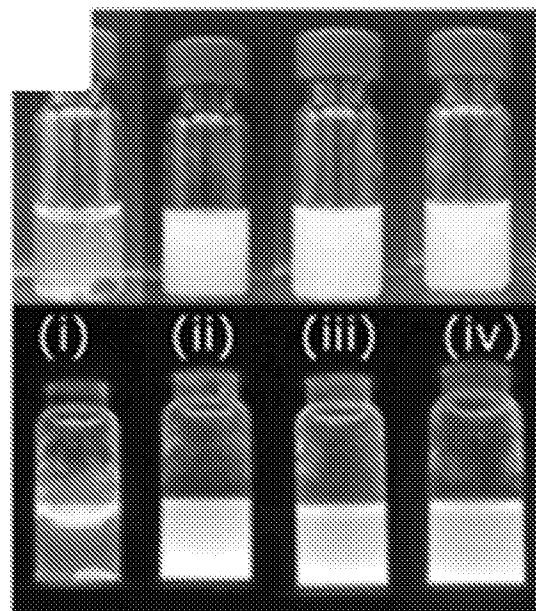


FIG. 61

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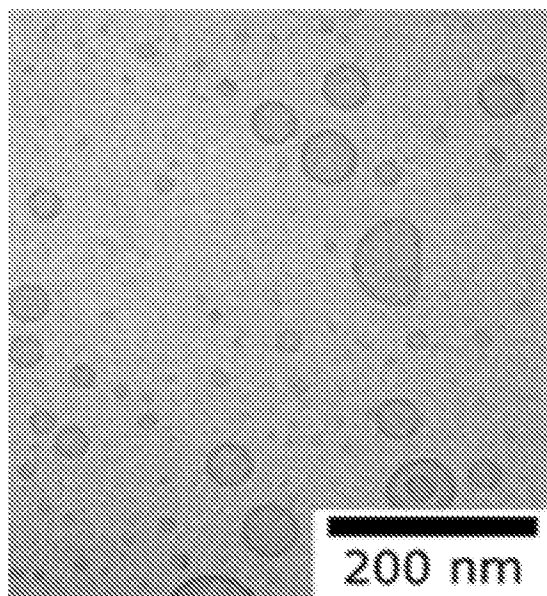


FIG. 62

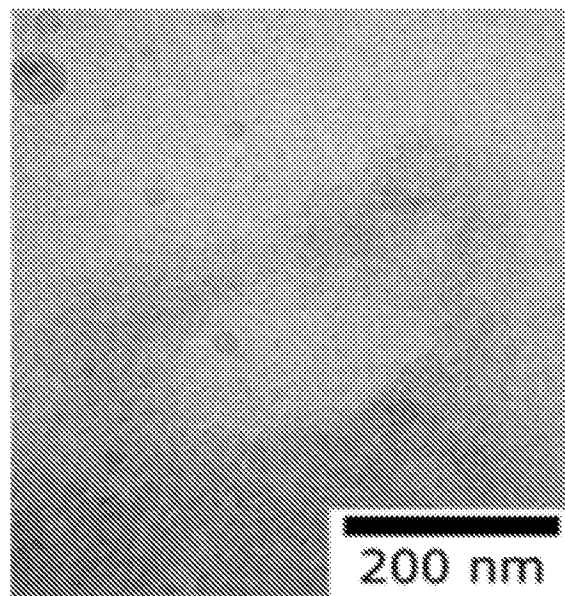


FIG. 63

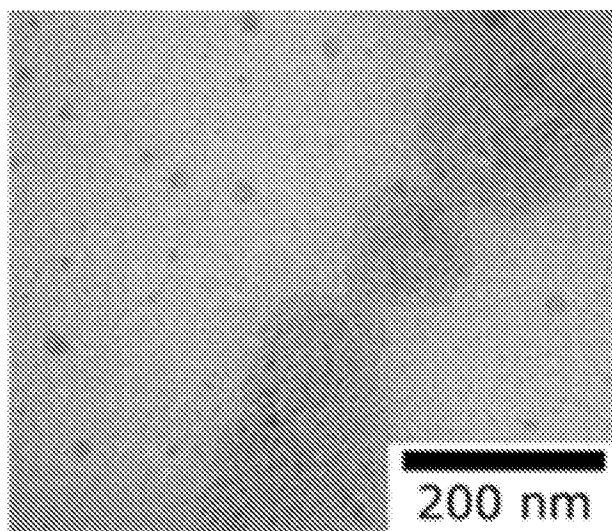


FIG. 64

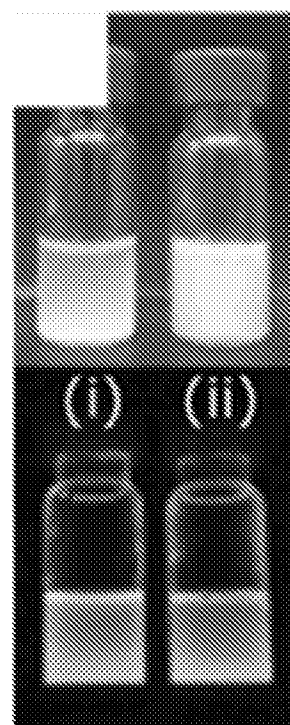


FIG. 65

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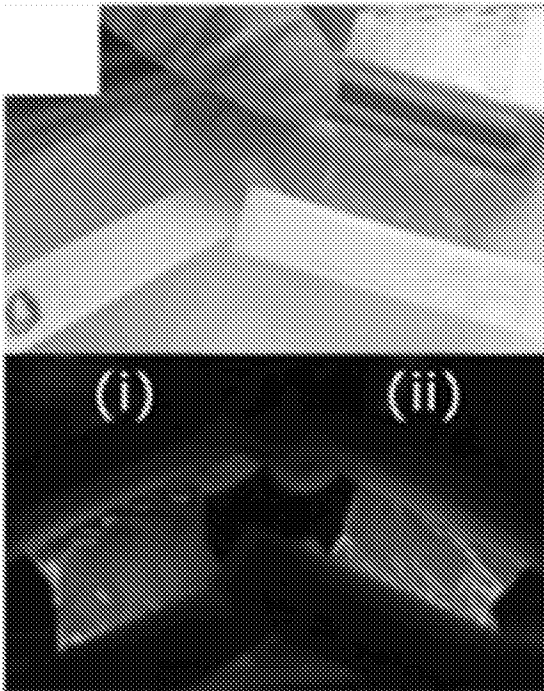


FIG. 66

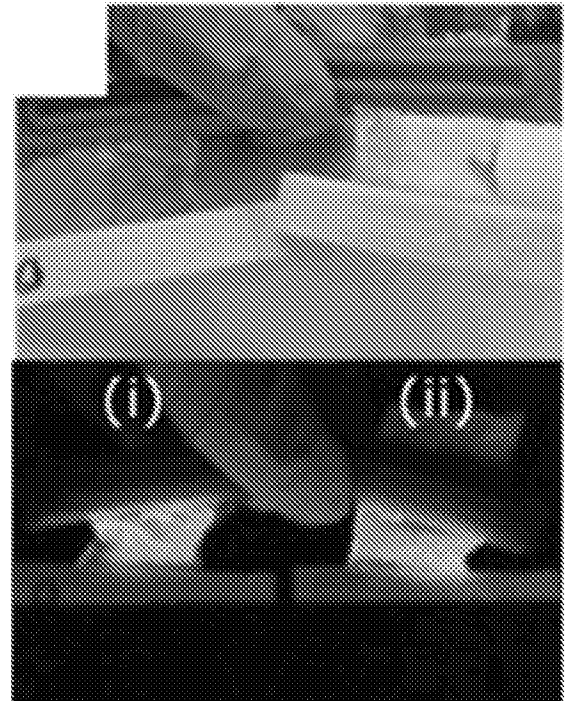


FIG. 67

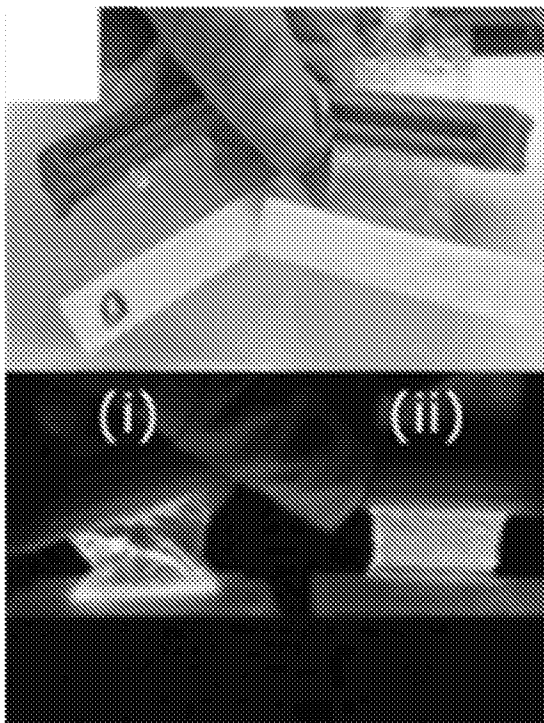


FIG. 68

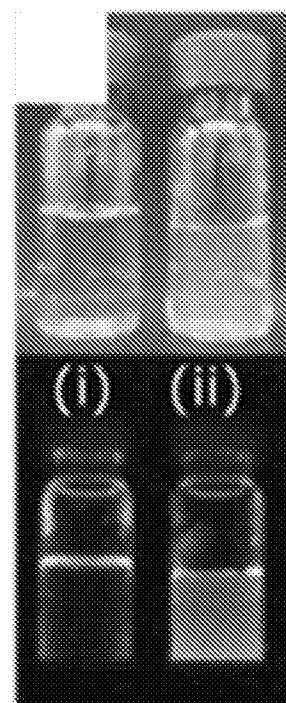


FIG. 69

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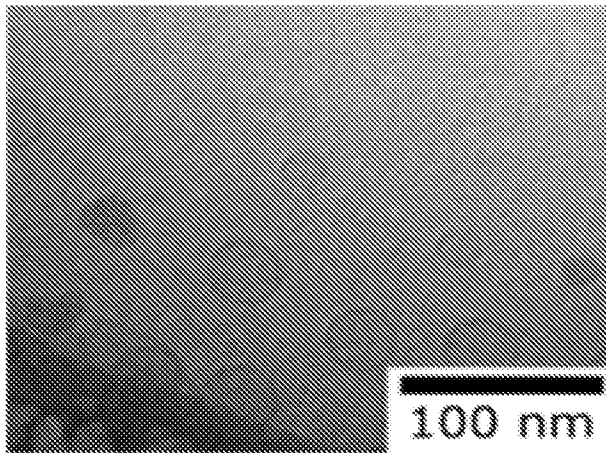


FIG. 70

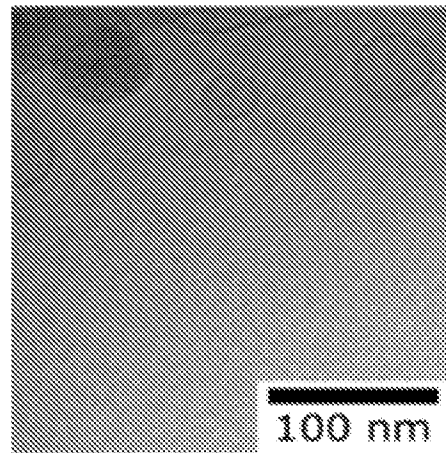


FIG. 71

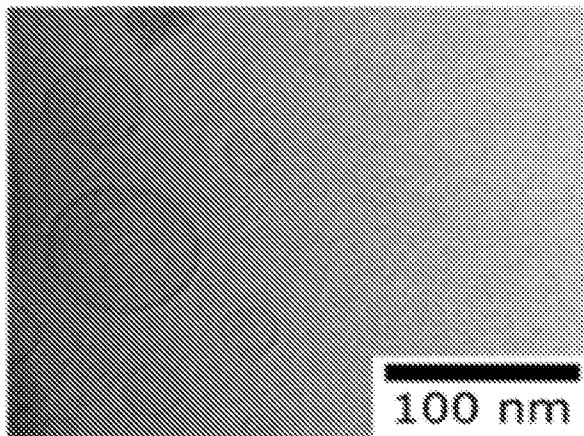


FIG. 72

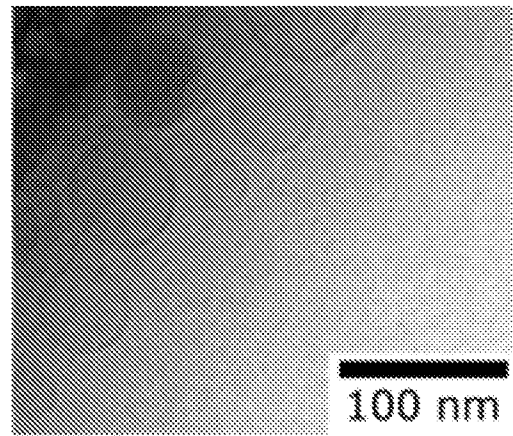


FIG. 73

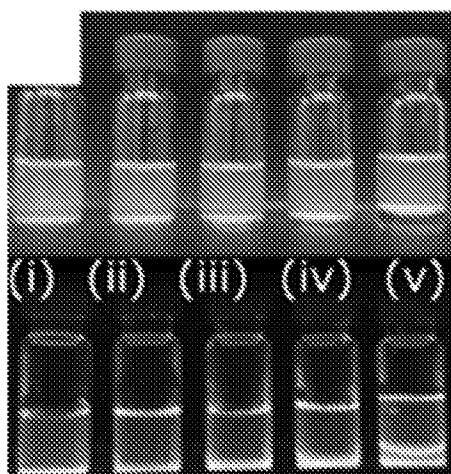


FIG. 74

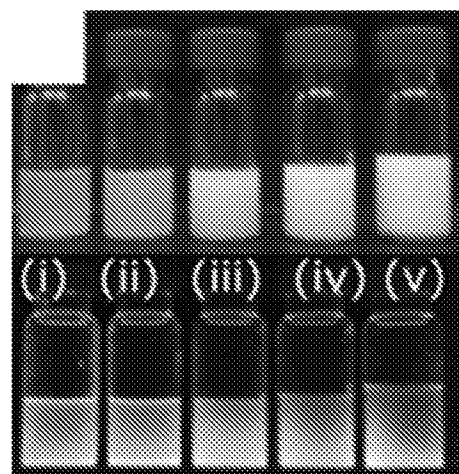


FIG. 75

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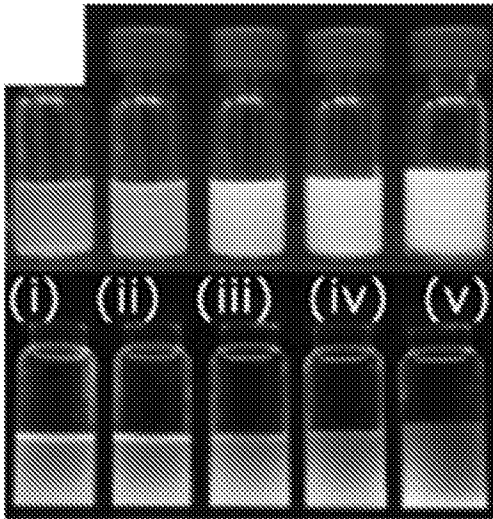


FIG. 76

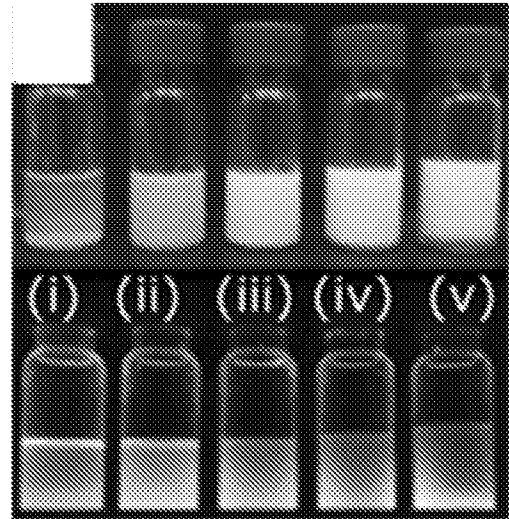


FIG. 77

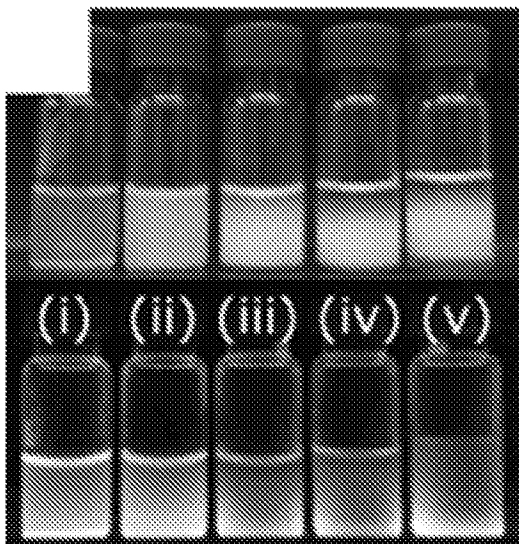


FIG. 78

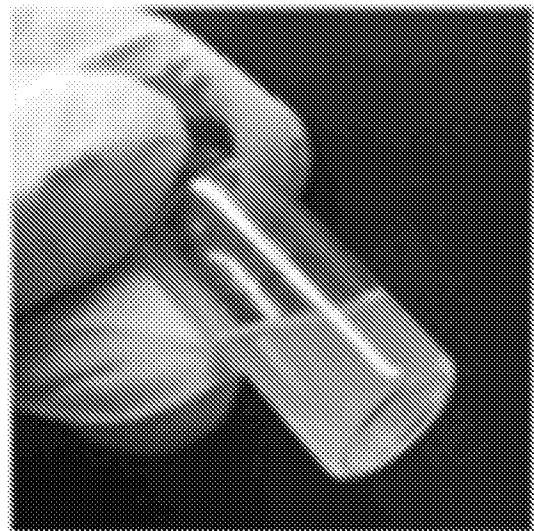


FIG. 79

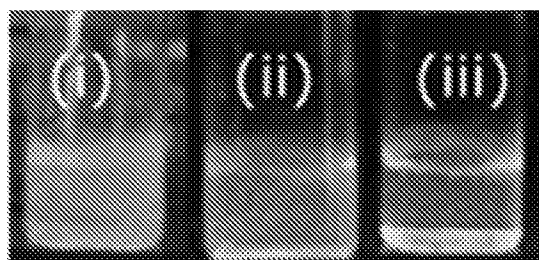
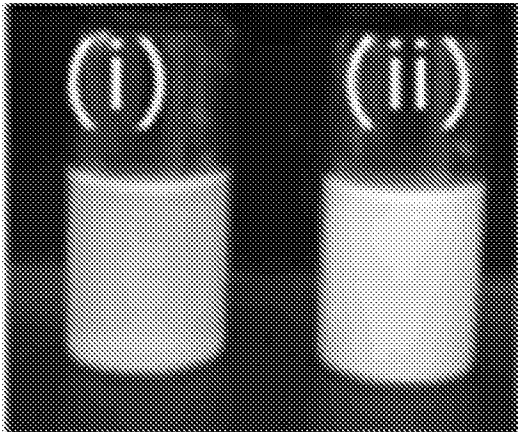
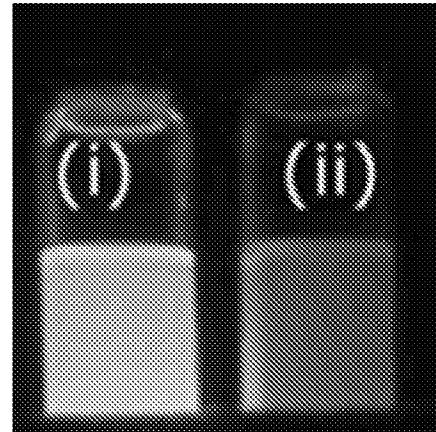
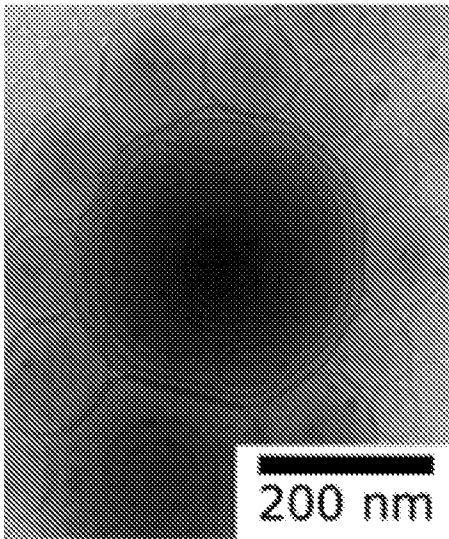
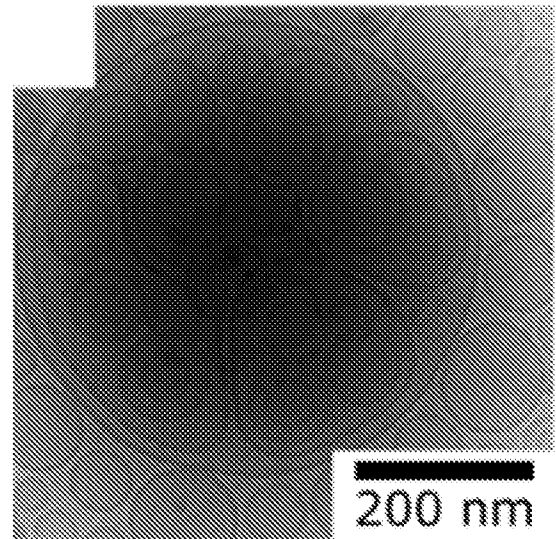
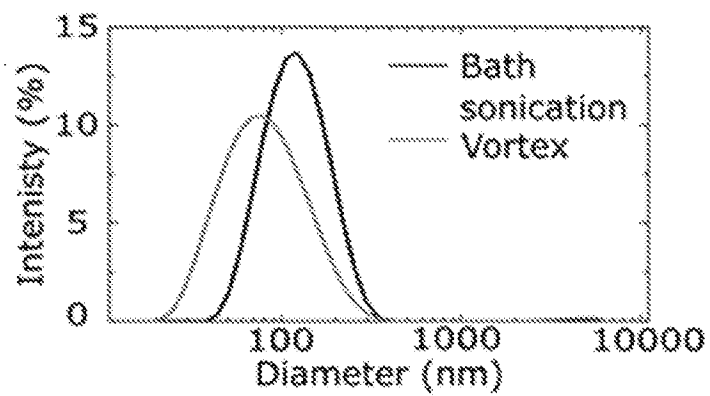


FIG. 80

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**FIG. 81****FIG. 82****FIG. 83****FIG. 84****FIG. 85**

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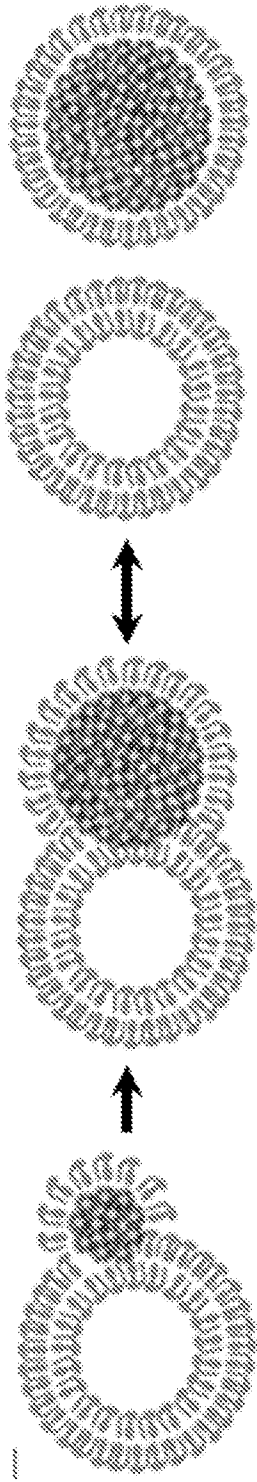


FIG. 86

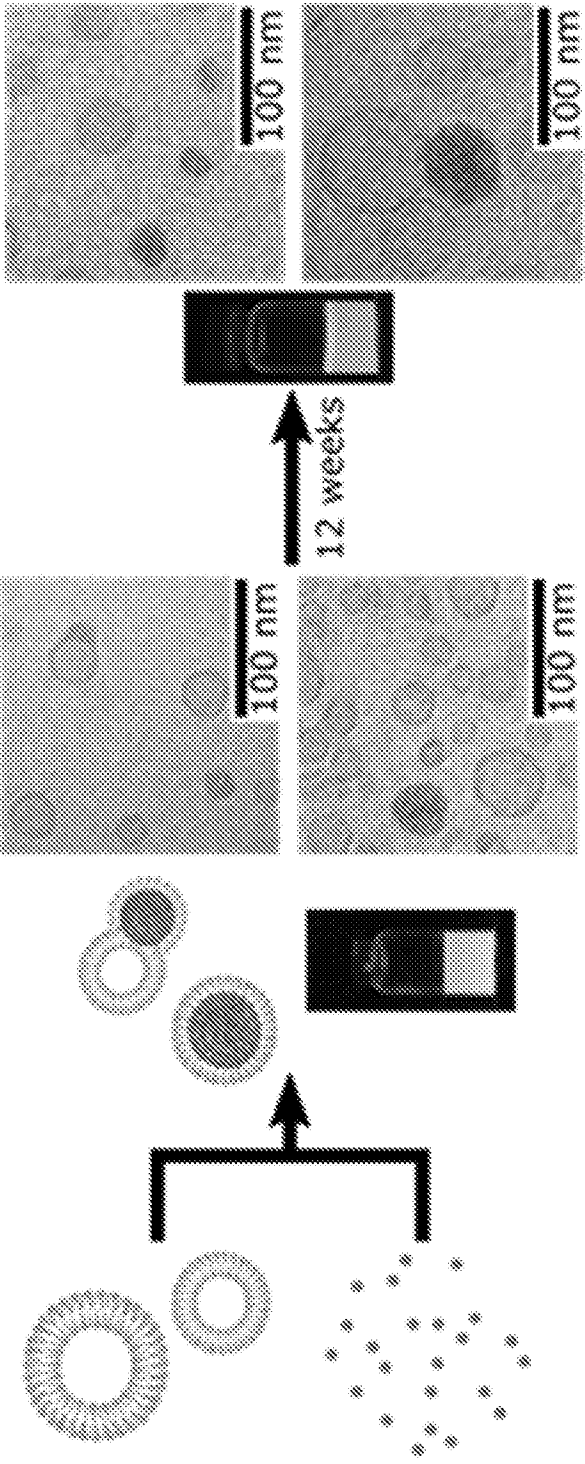


FIG. 87

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/019333

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/543 G01N33/58
ADD.

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)
G01N

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/094727 A1 (UNIV TORONTO [CA]; WALKER GILBERT C [CA]; MACLAUGHLIN CHRISTINA M [CA]) 19 July 2012 (2012-07-19) whole document, in particular p. 9, l. 8-29; p. 10, l. 3-7; p. 10, l. 21 - p. 12, l. 13; p. 19, l. 2-10; p. 23, l. 1-9; p. 21, l. 26 - p. 22, l. 16; p. 23, l. 13-22; p. 24, l. 16-34; p. 25, l. 11-34; p. 27, l. 3-25; fig. 1, 10; claims 23-30; 62-67 -----	1-42
X	WO 2014/089698 A1 (ZAMECNIK COLIN R [US]; WALKER GILBERT C [CA]) 19 June 2014 (2014-06-19) whole document, in particular p. p. 13, l. 25 - p. 15, l. 20 ----- -/--	1,2

☒ 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

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Date of the actual completion of the international search

24 May 2017

Date of mailing of the international search report

31/07/2017

Name and mailing address of the ISA/

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Authorized officer

Chrétien, Eva Maria

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/019333

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/61045 A1 (BIOCRYSTAL LTD [US]) 23 August 2001 (2001-08-23) whole document, in particular p. 5-7; example 2, 9 -----	1,2, 19-21
A	US 8 859 727 B2 (LIN JACKI [US] ET AL) 14 October 2014 (2014-10-14) the whole document -----	1-42
A	WO 02/29410 A2 (QUANTUM DOT CORP [US]; BRUCHEZ MARCEL P [US]; DANIELS R HUGH [US]; DIA) 11 April 2002 (2002-04-11) the whole document -----	1-42

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/019333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012094727 A1	19-07-2012	US 2013273561 A1 WO 2012094727 A1	17-10-2013 19-07-2012
WO 2014089698 A1	19-06-2014	EP 2932243 A1 JP 2016507726 A US 2015316481 A1 WO 2014089698 A1	21-10-2015 10-03-2016 05-11-2015 19-06-2014
WO 0161045 A1	23-08-2001	AT 383440 T AU 3705701 A DE 60132311 T2 EP 1266032 A1 US 2002001716 A1 US 2005019486 A1 US 2006099147 A1 WO 0161045 A1	15-01-2008 27-08-2001 02-01-2009 18-12-2002 03-01-2002 27-01-2005 11-05-2006 23-08-2001
US 8859727 B2	14-10-2014	AU 2006339072 A1 CA 2600370 A1 EP 1957045 A2 JP 2009501237 A KR 20080016793 A US 2006251726 A1 US 2013266634 A1 WO 2007149062 A2	25-10-2007 14-09-2006 20-08-2008 15-01-2009 22-02-2008 09-11-2006 10-10-2013 27-12-2007
WO 0229410 A2	11-04-2002	AT 425457 T AT 536550 T AU 2687602 A CA 2424817 A1 EP 1327145 A2 EP 2085781 A1 US 2002155507 A1 US 2004023261 A1 US 2007148634 A1 US 2016161496 A1 WO 0229410 A2	15-03-2009 15-12-2011 15-04-2002 11-04-2002 16-07-2003 05-08-2009 24-10-2002 05-02-2004 28-06-2007 09-06-2016 11-04-2002

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/019333

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-42

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-42

Composition comprising an aqueous solution comprising a composite nanocarrier the composite nanocarrier comprising a nanoparticle at least partially encapsulated within a vesicle, the vesicle comprising a sterol and a surfactant.

2. claim: 43

Pharmaceutical composition

3. claims: 44-51

Method of making the composition

4. claims: 52-54

Method of imaging a cell or a population of cells within or about a subject

5. claim: 55

Method of use of the composition as a sensor

6. claims: 56-58

Device comprising the composition
