Title: BUBBLE ARCHITECTURES AND METHODS OF MAKING AND USING THEREOF

Abstract: Bubble architectures are formed using biologically-derived surfactant, for example, the protein Ranaspumin-2 and other biologically derived surfactants, to create functional materials that mimic cellular physiological processes. In one embodiment, the bubble architecture is used to form an artificial photosynthesis platform for converting light and CO2 to a value-added product, for example, simple sugar.

FIG. 1
BUBBLE ARCHITECTURES AND METHODS OF MAKING AND USING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 61/286,578, filed 12/15/2009, which is expressly incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the invention

The present invention relates to bubble architectures and methods of making and using such bubble architectures, wherein the bubble architectures are formed using biologically derived surfactant, for example, the protein Ranaspumin-2 and other biologically derived surfactants to creation functional materials that mimic cellular physiological processes. In one embodiment, the bubble architecture is used to form an artificial photosynthesis platform for converting light and CO2 to a value-added product, for example, simple sugar.

2. Related Art

Harvesting and converting biomass to combustible fuel has been suggested as a renewable energy solution to the ongoing depletion of fossil fuels. Nature has acquired means for solar energy capture in the photo-reactive centers of plants for the purpose of synthesizing and storing such biomass. However, the near 100 percent quantum efficiency of the photo-reactive centers is reduced to approximately 5 percent of that value in usable energy due to limited wavelength sensitivities and necessary cellular processes, including growth, repair, and maintenance. Converting plant sugars to ethanol has been proposed as a renewable energy source, particularly from sugar cane, corn stover and switchgrass; however, this requires that limited land and water resources be diverted, in part, to biomass production.

Processes which evolve liquid fuels, such as ethanol, from biomass have been widely developed. Due to only marginal energy yield from the production of bio-ethanol, 2,5-dimethylfuran (DMF) is considered a prudent alternative to ethanol given its higher energy density, boiling point, and insolubility in water. In addition to attractive liquid fuel properties, DMF has been synthesized from simple carbohydrates, such as glucose or fructose, harvested from biomass.

Engineered biological solar energy conversion has produced a variety of electrical and chemical energy storage strategies. Of the latter, ATP serves as the most important natural
energy molecule and has been formed artificially by coupling \( F_0F_1 \) ATP synthase to a photon induced proton motive force. In photo-synthetic organisms, long term energy storage is accomplished through biomass synthesis through ATP dependent carbon-fixation providing a foundation for liquid biofuel production.

To date, in vitro carbon fixation experiments have been limited to the examination of CBB cycle intermediates and cell extracts using radiometric and spectrophotometric techniques. Photosynthesis, carbon sequestration and carbohydrate generation involve several complex and well-studied processes; among these, a suite of 8 enzymes make up the portion of the CBB cycle responsible for converting the energy of ATP into 6-carbon sugars such as glucose and fructose. The chief products of the light-dependent reaction of photosynthesis are NADPH and ATP. The thermophilic \( F_0F_1 \) ATP synthase has been purified and reconstituted in both liposomes and ABA triblock polymersomes, along with the photoactivated proton pump bacteriorhodopsin (BR) to form ATP producing vesicles. Recently, an improvement in the artificial synthesis of ATP was demonstrated using Tween-20 (T20)-based bubbles/foam and polymersomes.

Bubbles are natural structures that are encountered in everyday life such as dishwashing foam or beer foam. While bubbles are common, they are deceptively complex structures, typically composed of a water layer sandwiched between two- surfactant monolayers. Despite their everyday appearance, bubbles and foams have been interesting research topics to scientists for the past several centuries, where many have sought to understand and utilize the chemical, physical, and mechanical properties of bubbles. Their applications, however, have been limited by their innate properties of drainage and uncontrollable size distributions. Recently, it has become possible to produce a microfoam, having no vertical drainage, from monodisperse stable microbubbles (Garstecki et al, *Appl Phys Lett* 2004, 85:2649). And several techniques have been proposed for formation of micro-scale droplets (Sugiura et al, *Langmuir* 2001, 17:5562; Thorsen et al, *Phys Rev Lett* 2001, 86:4163; Anna et al, *Appl Phys Lett* 2003, 82:364). Engineering complex biochemical cascades in vitro can be difficult because of an inability to locally contain chemical distributions within a defining nanostructure. Therefore, producing locally high concentrations of biochemicals in vitro is regarded as a major challenge in creating "life-like" function in engineered systems. Further, the recent technical developments in semiconductor device technology (MEMS) have been promising for use in nano-packaging. However, considering the time, cost, complexity, and biocompatibility of silicon technology, MEMS
are not expected to serve equally well for all kinds of hybrid organic/inorganic bioelectronic devices and sensors.

The International Published Application WO 2006/089245 is directed to a bubble architecture and method of making such a bubble, the contents of which are expressly incorporated herein by reference in its entirety. Although this document lists a variety of surfactants that may be used in making the bubble, the Examples are primarily directed to the use of TWEEN-20™. As explained in detail below, bubbles using such a surfactant have various drawbacks.

Because of drainage, evaporation, and hydrophilicity water-based foams are inherently fragile and relatively short-lived. The fact that foam nests are used by a variety of organisms (e.g., fish, amphibians and insects) is quite impressive, given the biological necessities required to persist in the environment. These include resistance to microbial and insect assault, resilience to changes in heat, humidity and desiccation, but continue to be compatible with exposed eggs and sperm. The latter requirement presents a remarkable paradox, since surfactant used to produce stable bubble films would by its very nature also destabilize and destroy the cell membranes and proteins necessary for reproduction.

The foam nest produced by the Tungara frog is one of the largest found in nature. It is used to protect developing tadpoles in terrestrial areas of tropical and subtropical Central America, until maturation or greater water availability. The creation and maintenance of the Tungara frog’s foam nest can be attributable to a small but astonishing suite of six proteins called ranaspumins (Rsnl-6). Of these, it is Rsn2 which is responsible for the reduction in water surface tension allowing foam creation upon liquid agitations. The other ranaspumins offer an arsenal of microbiob and insecticides, as well as carbohydrate binding proteins which help stabilize the foams to drainage and desiccation. Rsn-2 plays the surfactant role very economically at 0.1 g/ml, but also has the ability to exist in two conformational states (see Mackenzie, CD., et al., Ranaspumin-2; Structure and Function of a Surfactant Protein from the Foam Nests of a Tropical Frog, Biophysical Journal, 2009. 96(12); p.4984-4992, the contents of which are expressly incorporated herein in its entirety). When agitated, the protein denatures slightly, allowing the single hydrophobic alpha helix to extend into the air while the hydrophilic beta sheet remains in the water phase. Normally these two regions are folded onto each other, so without agitation or continued bridging of the air-water interface, the protein is most likely to exist as an invert water soluble protein. The blue foam nests of the Tungara frog offer an excellent example of a protein based foam, which is compatible with lipid membranes, yet resistant to environmental factors and can persist for the time
required for tadpole maturation, usually three days or more (see Downie, J.R., *Functions of the foam in foam-nesting Leptodactylids: the nest as aposthatching refuge in Physalaemus pustulosus*. Herperol, J 1993. 3:p. 35-42.)

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SUMMARY OF THE INVENTION

In accordance with the purposes of the disclosed materials, compositions, articles, devices, and methods, as embodied and broadly described herein, the disclosed subject matter, in one aspect, relates to bubble and anti-bubble compounds and compositions and methods for preparing and using such compounds and compositions wherein the surfactant used includes biologically derived surfactant, for example, the protein Ranaspumin-2 (Rsn2). The term "bubble" as used herein also expressly refers to "anti-bubble" compounds.

In one embodiment of the present invention, the bubble compound is used as an artificial photosynthesis platform combining two technology platforms to yield value-added products: bubble/foam architecture and proteopolymersomes. One such value-added product is simple sugar prepared by carbon fixation accomplished by integrating biosolar proteopolymersomes and a plurality of enzymes into the microchannels of inflatable foam. BR-ATPase polymersomes may be used to convert light into ATP, which powers a rubisco substrate-enzyme reaction of carbon synthesis, and eventually the formation of hydrocarbon for biofuels. This artificial photosynthesis platform produces glyceraldehyde-3-phosphate (G3P) and/or simple sugars that can be used to make a variety of useful organic compounds like HMF, DMF, methanol, ethanol or even sugars for human consumption.

Additional advantages will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below 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.

BRIEF DESCRIPTION OF THE FIGURES

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

FIG. 1 is a schematic of a portion of a bubble wall where a secondary component (3) is incorporated in the aqueous layer (2) between two surfactant monolayers (1 and 4).

FIG. 2(a) is a photograph of a foam; the bubbles are polyhedral.
FIG 2(b) is a schematic showing the geometry of a single foam polyhedron. Almost all liquid is concentrated in the Plateau borders, shown in the expanded view. FIG. 3(a) is a schematic of a bubble and an antibubble. FIG. 3(b) is a magnified structure of a portion of an antibubble wall where two surfactant monolayers (62 and 63) define an air layer (61) between two aqueous layers (64 and 65).

FIG. 4(a) is a schematic of a single bubble structure. FIG. 4(b) is a schematic of a cross-sectional view of a bubble. FIG. 5 is an illustration of the BR/FoFi ATP synthase vesicle solar conversion system coupled to the RuBisCO CBB cycle enzymes and trapped within the foam channels in accordance with one embodiment of the present invention.

FIG. 6 is a schematic of the encapsulating method from a mixture of bubble solution and secondary component.

FIG. 6(a) is a schematic showing a bubble solution containing surfactant (71) and a secondary component (70) (shown here as already-made functional polymersomes).

FIG. 6(b) is a schematic showing a cross-sectional view of bubble containing secondary component (70) inside the water channel (72) after the blowing process.

FIG. 7 is a schematic of the encapsulating method using coalescence between bubbles; (a) preparation of bubbles (one with polymersomes (shown as dots), the other without) under different conditions, (b) coalescence process by contacting bubbles, and (c) after coalescence process.

FIG. 8 is an illustration of a sol-gel design for a foam encasement in accordance with one embodiment of the present invention.

FIG. 9(a) is a synthetic scheme of PEtOz-PDMS-PEtOz triblock copolymer. FIG. 9(b) is a 1H NMR spectrum of PEtOz-PDMS-PEtOz in DMSO-d6.

FIG. 10 is a graph showing Control Group for ATP synthesis in Bulk Solution.

FIG. 11 is a graph showing Control Group for ATP synthesis in Foam Architecture.

FIG. 12 is a graph showing Control Group for ATP synthesis in Deflated Foam Solution.

FIG. 13 is a graph showing G3P Production with RuBisCO and ATP stock in bulk solution.

FIG. 14 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay with Artificial ATP Source and various components removed.
FIG. 15 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay Control with Bulk Vesicle ATP Source and various components removed.

FIG. 16 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay Control Group with Foam Vesicle ATP Source.

FIG. 17 is a graph showing an Absorbance Plot for glucose oxidase assay showing the oxidation of o-dianisidine to a pink colored product absorbing at 540 nm from various glucose concentrations.

FIG. 18 is a graph showing DLS Size Distribution Plot for polymer vesicles.

FIG. 19 is a graph showing DLS Size Distribution Plot for lipid vesicles.

FIG. 20 shows TEM Micrographs of BR/FoFi ATP Synthase Proteopolymersomes.

FIG. 21 shows TEM Micrographs of BR/FoFi ATP Synthase Liposomes.

FIG. 22 shows fluorescent images of foam vesicle solutions.

FIG. 23 is a graph showing the production of ATP with BR/ATP synthase lipid vesicles in Rsn-2 foam (A), in bulk (■), in deflated Rsn-2 foam (-4) in T20 foam (T) and a control experiment in the dark (●) for comparison. Inset is the light intensity standard curve created with ATP stock dilutions.

FIG. 24 is a graph showing BR/ATP synthase function in a lipid membrane was limited to the Rsn-2 based foam since the T20 adversely affected coupled FiFo-Atpase/BR vesicle function.

FIG. 25 is a graph showing ATP synthesis using BR/ATP synthase polymersomes in T20 foam (■), in bulk (●), deflated T20 foam (T), and a control experiment in the dark (A) for comparison (n=3 for each). Inset is the light intensity standard curve created with ATP stock dilutions.

FIG. 26 is a graph showing the foam system containing BR/ATP synthase vesicles, RuBisCO, PGK, GAPDH, NADH, which is converting CO2 and RuBP to G3P using phototoderived ATP wherein the RuBisCO dependent carbon fixation reaction is fueled by lipid photophosphorylation vesicles fuels within the Rsn-2 foam (black, n=3), and in bulk (brown, n=3); and the proteopolymersomes within Two foam (red, n=3), and in bulk (blue, n=3).

FIG. 27(a) is a TEM image of polymersomes after bacteriorhodopsin/ATP synthase incorporation.

FIG. 27(b) is a size distribution histogram derived from direct measurement of polymersome sizes by TEM micrographs.
FIG. 28(a) is a graph showing internal pH change for bacteriorhodopsin polymersomes (●) and bacteriorhodopsin-ATP synthase-polymersomes (■) together with a dark-incubated control (○) in buffer solution.

FIG. 28(b) is a graph showing photo-induced ATP synthesis in bacteriorhodopsin-ATP synthase-polymersomes in a foam.

DETAILED DESCRIPTION OF THE INVENTION

The materials, compounds, compositions, articles, 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 included therein and to the Figures.

Before the present materials, compounds, compositions, articles, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific 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 aspects 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.

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 compound" includes mixtures of two or more such compounds, reference to "an agent" includes mixtures of two or more such agents, reference to "the moiety" includes mixtures of two or more such moieties, 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. 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 also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value," and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed, then "less than or equal to 10" as well as "greater than or equal to 10" is also disclosed. It is also understood that throughout the application data is provided in a number of different formats and that these data represent endpoints and starting points and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point "15" are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed, as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

References in the specification and concluding claims to parts by weight of a particular element or component in a composition denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.
I. Compositions

Disclosed herein are materials, compounds, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a compound is disclosed and a number of modifications that can be made to a number of components or residues of the compound are discussed, each and every combination and permutation that are possible are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of components or residues A, B, and C are disclosed as well as a class of components or residues D, E, and F, and an example of a combination compound A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated.

Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific aspect or combination of aspects of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

Certain materials, compounds, compositions, and components disclosed herein can be obtained commercially or readily synthesized using techniques generally known to those of skill in the art. For example, the starting materials and reagents used in preparing the disclosed compounds and compositions are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wis.), Acros Organics (Morris Plains, N.J.), Fisher Scientific (Pittsburgh, Pa.), or Sigma (St. Louis, Mo.) or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and

a. Bubbles

In one aspect, described herein is a bubble, comprising a wall, wherein the wall comprises a liquid layer between two layers of surfactant; and at least one secondary component, wherein the at least one secondary component is substantially present or completely present in the liquid layer. In one embodiment, the liquid layer is an aqueous layer. Such bubbles can be used as a biological system that serves as a synthesis chamber to produce biological products.

Figure 1 provides a general structure of a portion of a wall of the bubbles described herein (further examples are shown in Figures 2-6). Referring to Figure 1, the wall of the bubble is composed of one or more surfactants, where the surfactant(s) forms a layer defining the outer wall (1) and a layer defining the inner wall (4) of the bubble. The wall structure created by surfactant layers (1 and 4) creates a channel, which is depicted as (2) in Figure 1. The channel can be filled with a liquid. In one aspect, the channel can be filled with water alone or water in combination with one or more liquid solvents such as, for example, an organic solvent. The channel with and without organic solvent is referred to herein as the "aqueous layer." The bubbles described herein can be any shape such as, for example, spherical, elliptical, or polyhedral. In other aspects, the bubbles can be a thin film with an aqueous layer sandwiched between two layers of surfactant. Alternatively, the bubbles can exist as a foam. Foam formation takes place when bubbles come together and they share the same water layer to form a polyhedron. As shown in Figure 2, the edges of the polyhedron are connected to form channel-like structures known as Plateau borders. The froth of bubbles begins to drain under gravity, removing much of the water between the bubbles. Most of the water resides in the Plateau borders. Some of the bubbles merge into larger bubbles, which is called coarsening (Aubert et al, *Scientific American* 1986, 254:74-82; Isenberg, The science of soap films and soap bubbles. Dover, New York, 1992, pp. 17-21; Weaire and Hutzler, The physics of foams. Oxford, 2000, pp. 6-12; Stone et al, *J Phys Condens Matter* 2003, 15:S283-S290; Hilgenfeldt et al, *Europhys Lett*, 2004, 67(3):484-90, which are each incorporated by reference herein at least for their teachings of bubbles and bubble structures). The width of the channel created by the surfactant (i.e., the thickness of the bubble wall; e.g., as shown as (2) in Figure 1, (61) in Figure 3, and (72) in Figure 4) can typically be from about 1 nm to
about 10 μη (for spherical bubbles) and from about 10 nm to about 600 μη (for foams). In
still other examples, the width of the channel distance can be about 1 nm, 5 nm, 10 nm, 15
nm, 20 nm, 25 nm, 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm,
80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 225 nm, 250 nm,
275 nm, 300 nm, 325 nm, 350 nm, 375 nm, 400 nm, 425 nm, 450 nm, 475 nm, 500 nm, 525
nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, 725 nm, 750 nm, 775 nm,
800 nm, 825 nm, 850 nm, 875 nm, 900 nm, 925 nm, 950 nm, 975 nm, 1000 nm (1 μη), 1.1
μη, 1.2 μη, 1.3 μη, 1.4 μη, 1.5 μη, 1.6 μη, 1.7 μη, 1.8 μη, 1.9 μη, 2.0 μη, 2.1 μη, 2.2
μη, 2.3 μη, 2.4 μη, 2.5 μη, 2.6 μη, 2.7 μη, 2.8 μη, 2.9 μη, 3.0 μη, 3.1 μη, 3.2 μη, 3.3
μη, 3.4 μη, 3.5 μη, 3.6 μη, 3.7 μη, 3.8 μη, 3.9 μη, 4.0 μη, 4.1 μη, 4.2 μη, 4.3 μη, 4.4
μη, 4.5 μη, 4.6 μη, 4.7 μη, 4.8 μη, 4.9 μη, 5.0 μη, 5.1 μη, 5.2 μη, 5.3 μη, 5.4 μη, 5.5
μη, 5.6 μη, 5.7 μm, 5.8 μm, 5.9 μm, 6.0 μm, 6.1 μm, 6.2 μm, 6.3 μm, 6.4 μm, 6.5 μm, 6.6
μm, 6.7 μm, 6.8 μm, 6.9 μm, 7.0 μm, 7.1 μm, 7.2 μm, 7.3 μm, 7.4 μm, 7.5 μm, 7.6 μm, 7.7
μm, 7.8 μm, 7.9 μm, 8.0 μm, 8.1 μm, 8.2 μm, 8.3 μm, 8.4 μm, 8.5 μm, 8.6 μm, 8.7 μm, 8.8
μm, 8.9 μm, 9.0 μm, 9.1 μm, 9.2 μm, 9.3 μm, 9.4 μm, 9.5 μm, 9.6 μm, 9.7 μm, 9.8 μm, 9.9
μm, 10 μm, 15 μm, 20 μm, 25 μm, 30 μm, 35 μm, 40 μm, 45 μm, 50 μm, 55 μm, 60 μm, 65
μm, 70 μm, 75 μm, 80 μm, 85 μm, 90 μm, 95 μm, 100 μm, 101 μm, 102 μm, 103 μm, 104
μm, 105 μm, 106 μm, 107 μm, 108 μm, 109 μm, 110 μm, 111 μm, 112 μm, 113 μm, 114 μm,
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157 μm, 158 μm, 159 μm, 160 μm, 161 μm, 162 μm, 163 μm, 164 μm, 165 μm, 166 μm, 167
μm, 168 μm, 169 μm, 170 μm, 171 μm, 172 μm, 173 μm, 174 μm, 175 μm, 176 μm, 177 μm,
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μm, 189 μm, 190 μm, 191 μm, 192 μm, 193 μm, 194 μm, 195 μm, 196 μm, 197 μm, 198 μm,
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220 μm, 221 μm, 222 μm, 223 μm, 224 μm, 225 μm, 226 μm, 227 μm, 228 μm, 229 μm, 230
μm, 231 μm, 232 μm, 233 μm, 234 μm, 235 μm, 236 μm, 237 μm, 238 μm, 239 μm, 240 μm,
241 μm, 242 μm, 243 μm, 244 μm, 245 μm, 246 μm, 247 μm, 248 μm, 249 μm, 250 μm, 251
μm, 252 μm, 253 μm, 254 μm, 255 μm, 256 μm, 257 μm, 258 μm, 259 μm, 260 μm, 261 μm,
262 μm, 263 μm, 264 μm, 265 μm, 266 μm, 267 μm, 268 μm, 269 μm, 270 μm, 271 μm, 272
μm, 273 μm, 274 μm, 275 μm, 276 μm, 277 μm, 278 μm, 279 μm, 280 μm, 281 μm, 282 μm,
component (3) is substantially or completely present in the channel (2) created by the surfactant layers (1 and 4).

Referring to Figure 1 (and also Figure 2), the secondary component (3) is substantially or completely present in the channel (2) created by the surfactant layers (1 and 4).
4). By "substantially present" is meant that the secondary component is mostly present in the aqueous layer; however, it is contemplated that some amount of the secondary component can also be present, either entirely or partially, in either or both of the surfactant layers (1 or 4 in Figure 1). It is also contemplated that the secondary component can partially extend out from either or both surfactant layers into the gas (e.g., air) space. The phrase "incorporated into the bubble wall" is also used synonymously herein with the phrase "substantially present."

b. Anti-bubbles
In another aspect, described herein is a bubble comprising a wall, wherein the wall comprises an inner wall and an outer wall, wherein the inner wall comprises an inner surface and an outer surface and the outer wall comprises an inner surface and an outer surface, wherein the inner wall and the outer wall comprises a surfactant, wherein the inner wall and the outer wall comprises a gas between two layers of surfactant; an aqueous layer, wherein the aqueous layer is adjacent to the outer surface of the inner wall of the bubble; and a secondary component, wherein the secondary component is substantially present in the aqueous layer.

In this aspect, the bubble is also referred to herein as "an anti-bubble." The term bubble as used herein includes the bubbles described above in section (a) and anti-bubbles. Techniques for producing anti-bubbles are known (Hughes and Hughes, *Nature* 1932, 129:599). In one aspect, the anti-bubble can have a spherical air shell surrounding a liquid. This aspect is depicted in Figure 3, wherein a gas layer (61) (e.g., air) is sandwiched between two surfactant layers (62 and 63). In one aspect, an aqueous layer (64) is adjacent to the outer surface of inner wall (62). It is also contemplated that a second aqueous layer (65) can be adjacent to the outer surface of the outer wall (63). The term "adjacent" is defined herein as any solvent (e.g., water) that is in contact with the surfactant, which also includes penetration of the solvent into the surfactant layer. Similar to the bubbles described above, the secondary component can be substantially present in the aqueous layer. For example, referring to Figure 3, the secondary component can be present in the aqueous layers (64) and/or (65).

Additionally, the dimensions, shapes, and sizes of the anti-bubbles can be the same as those described above as for the bubbles described in section (a).

Described below are the different surfactants and secondary components useful in producing the bubbles described herein.
i. Surfactant

A "surfactant" as used herein is a molecule composed of hydrophilic and hydrophobic groups (i.e., an amphiphile). Because of solubility differences in water, when a bubble is formed, the hydrophobic ends of the surfactant molecules accumulate at an air/water interface, thereby reducing the surface tension (Wearie and Hutzler, The physics of foams, Oxford, 2000, Ch. 1-2). Thus, the surfactant forms a monolayer on the inside and a monolayer on the outside of the water. A schematic of a surfactant bubble composed of a several micrometer-thick water layer sandwiched between two surfactant monolayers is shown in Figure 4 (a close up of a portion of the bubble wall is shown in Figure 1). Because the hydrophobic end of the surfactant molecule sticks out from the surface of the bubble, the surfactant film is somewhat protected from evaporation which can prolong the life of the bubble. A closed container saturated with water vapor also slows evaporation and can allow surfactant films to last even longer.

Bubbles suitable for the compositions and methods disclosed herein can be made from biologically-derived surfactants. In one embodiment of the present invention, the biologically-derived surfactant may be a natural protein surfactant. In yet another embodiment of the present invention, the natural protein surfactant is Ranaspumin (Rsn) protein surfactant, such as Rsn-2. In one aspect, a bubble can be prepared from mixtures of two or more surfactants.

The expression of the Rsn2 gene factor may be accomplished using a variety of known techniques. For example, the expression of the Rsn2 gene has been demonstrated in bacteria (see Mackenzie, CD., et al., Ranaspumin-2; Structure and Function of a Surfactant Protein from the Foam Nests of a Tropical Frog. Biophysical Journal, 2009. 96(12); p.4984-4992, the contents of which are expressly incorporated herein in its entirety). As a result, for example, a gene containing idealized E.Coli codon usage can be constructed for more efficient bacterial expression. Using this example, once the synthetic gene has been completed, the next step is to transform the gene into an inducible expression host and purify the protein. In a preferred embodiment, the Rsn2 gene may include two affinity tags. However, one must ensure that the protein retains its natural surfactant and foam forming capabilities. As a rudimentary test, one may vary Rsn2 concentration in an aqueous solution and measure the resulting contact angle of the water droplet. In one aspect, a Langmuir Blodgett film may be used to acquire a more precise quantification of the protein's surfactant properties.
Foam topology arises from surprisingly uniform physical principles and structural
elements. The surface of the bubbles of aqueous foams are mediated by surfactants which are
necessary to stabilize the air-water interface and provide an energetic (both electrostatic and
steric) barrier to rupture and collapse. These form tetrahedral structures commonly referred
to as Plateau junctions. The legs and nodes of the junction contain the trapped liquid phase
typically 0.01-1 mm wide. The drainage of these channels is a primary concern for the foam
stability and functionality.

ii. Secondary Component

As used herein the secondary component can be anything (e.g., molecule,
compositions, device) that can be substantially present in the channel (e.g., aqueous layer) of
the bubble wall. In one aspect, the bubble can comprise two or more different secondary
components. In another aspect, the secondary component can have a width greater than, equal
to, or less than the width of the bubble wall, as described herein. For example, the secondary
component can have a width greater, equal to, or less than about 600 μm, 500 μm, 400 μm,
300 μm, 200 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, 40 μm, 30 μm, 20 μm, 10
μm, 9 μm, 8 μm, 7 μm, 6 μm, 5 μm, 4 μm, 3 μm, 2 μm, 1 μm, 500 nm, 100 nm, or 1nm. In
one aspect, the secondary component can be a biomolecule. Examples of biomolecules
include, but are not limited to, a small molecule (e.g., a drag), a peptide, a protein, an enzyme
(e.g., a kinase, a phosphatase, a methylating agent, a protease, a transcriptase, an
endonuclease, a ligase, and the like), an antibody and/or fragment thereof, a nucleic acid (e.g.,
an oligonucleotide, a prime, a probe, an aptamer, a ribozyme, etc.), a lipid, a carbohydrate, a
steroid, a hormone, a vitamin, a potential therapeutic agent. "Small molecule" as used herein,
is meant to refer to a composition, which has a molecular weight of less than about 5 kD, for
example, less than about 4 kD. Small molecules can be nucleic acids (e.g., DNA, RNA),
peptides, polypeptides, peptidomimetics, carbohydrates, lipids, factors, cofactors, hormones,
vitamins, steroids, trace elements, pharmaceutical drugs, or other organic (carbon containing)
or inorganic molecules.

The secondary component can also be a macromolecule such as a polymer, a vesicle,
or a dendrimer, or a cell or a microbe (e.g., a detoxifying organism), including mixtures
thereof.

There are a variety of compositions disclosed herein where the secondary component
(e.g., biomolecule) can comprise an amino acid based molecule, including for example
enzymes and antibodies. Thus, as used herein, "amino acid," means the typically encountered
twenty amino acids which make up polypeptides. In addition, it further includes less typical constituents which are both naturally occurring, such as, but not limited to formylmethionine and selenocysteine, analogs of typically found amino acids, and mimetics of amino acids or amino acid functionalities. Non-limiting examples of these and other molecules are discussed herein.

As used herein, the terms "peptide" and "protein" refer to a class of compounds composed of amino acids chemically bound together. Non-limiting examples of these and other molecules are discussed herein. In general, the amino acids are chemically bound together via amide linkages (CONH); however, the amino acids can be bound together by other chemical bonds known in the art. For example, the amino acids can be bound by amine linkages. "Peptide" as used herein includes oligomers of amino acids and small and large peptides, including naturally occurring or engineered polypeptides and proteins. It is understood that the terms "peptide" and "protein" can be used interchangeably herein.

It is also understood that there are numerous amino acid and peptide analogs that can be used as the secondary component. For example, there are numerous D amino acids or amino acids which have a different functional substituent than the typically encountered amino acids. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. Additionally, molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include -CH$_2$NH-, -CH$_2$S-, -CH$_2$CH$_2$-, -CH=CH- (cis and trans), -COCH$_2$-, -CH(OH)CH$_2$-, and -CHH$_2$SO-. These and others can be found in Spatola, in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, B. Weinstein, eds., Marcel Dekker, New York, 1983, p. 267; Spatola, Vega Data 1983, Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, *Trends Pharm Sci*, 1980, pp. 463-68; Hudson et al, *Int J Pept Prot Res* 1979, 14:177-85 (-CH$_2$NH-, -CH$_2$CH$_2$-); Spatola et al, *Life Sci* 1986, 38:1243-9 (-CH H$_2$-S); Hann, *JChem Soc Perkin Trans I* 1982, 307-14 (-CH=CH- cis and trans); Almquist et al, *J Med Chem* 1980, 23:1392-8 (-COCH$_2$-); Jennings- White et al, *Tetrahedron Lett* 1982, 23:2533 (-COCH$_2$-); Szelke et al, European Appln, EP 45665 CA (1982): 97:3940 (-CH(OH)CH$_2$-); Holladay et al, *Tetrahedron Lett* 1983, 24:4401-4 (-C(OH)CH$_2$-) and Hruby, *Life Sci* 1982, 31: 189-99 (-CH$_2$S -) each of which is incorporated herein by reference herein for at least their teachings of amino acid analogs. It is understood that peptide analogs can have more than one atom between the bond atoms, such as beta-alanine, gama- aminobutyric acid, and the like. Such analogs are contemplated within the meaning of the terms peptide and protein.
In addition, peptides and proteins contemplated herein as biomolecules can be derivatives and variants of the disclosed peptides and proteins that also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, and deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Substitutions, deletions, insertions, or any combination thereof maybe combined to arrive at a final construct.

Also, certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco 1983, pp. 79-86, which is incorporated herein at least for its teachings of peptide and protein modifications), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl. It is also possible to link peptides and proteins to other molecules (e.g., to form conjugates). For example, carbohydrates (e.g., glycoproteins) can be linked to a protein or peptide. Such derivatives, variants, and analogs of peptides and proteins are contemplated herein within the meaning of the terms peptide and protein.

Methods for producing such peptides and proteins are well known. One method of producing the disclosed proteins is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethoxycarbonyl) or Boc (tert-butoxycarbonyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group
which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant, Synthetic Peptides: A User Guide. W.H. Freeman and Co., N. Y. 1992; Bodansky and Trost, Ed. Principles of Peptide Synthesis. Springer-Verlag Inc., N. Y., 1993, which are incorporated by reference herein at least for their teachings of peptide synthesis).

Alternatively, a peptide or polypeptide can be independently synthesized in vivo. For example, advances in recombinant glycoprotein production methods, which allow more cost effective production of human glycoproteins by colonies of transgenic rabbits or by yeast strains carrying human N-glycosylation system enzymes can be used (Hamilton et al, Science 2003, 301:1244-6; Gerngross, Nature Biotechnology 2004, 22:1409, which are incorporated by reference herein at least for their teachings of peptide and protein synthesis).

Once isolated, independent peptides or polypeptides may be linked, if needed, to form a peptide or fragment thereof via similar peptide condensation reactions. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen et al, Biochemistry 1991, 30:4151, which is incorporated by reference herein at least for its teachings of peptide and protein synthesis). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. (See e.g., Dawson et al, Science 1994, 266:776-9; Baggionli et al, FEBS Lett 1992, 307:97-101; Clark-Lewis et al, J Biol Chem 1994, 269:16075; Clark-Lewis et al, Biochemistry 1991, 30:3128; Rajarathnam et al, Biochemistry 1994, 33:6623-30, which are incorporated by reference herein at least for their teachings of peptide and protein synthesis). Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer et al, Science 1992, 256:221, which is incorporated by reference herein at least for its teachings of peptide and protein synthesis). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton, et al, 1992 Techniques in Protein Chemistry IV. Academic Press, N. Y., pp. 257-67, which is incorporated by reference herein at least for its teachings of peptide and protein synthesis).

In another aspect, the secondary component (e.g., biomolecule) can comprise an antibody. As used herein, the term "antibody" encompasses, but is not limited to, whole immunoglobulin (i.e., an intact antibody) of any class. Native antibodies are usually
heterotetrameric glycoproteins, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V(H)) followed by a number of constant domains. Each light chain has a variable domain at one end (V(L)) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains. The light chains of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. One skilled in the art would recognize the comparable classes for mouse. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The term "variable" is used herein to describe certain portions of the variable domains that differ in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not usually evenly distributed through the variable domains of antibodies. It is typically concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the b-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat E. A. et al, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1987)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.
The term "antibody" as used herein is meant to include intact molecules as well as fragments thereof, such as, for example, Fab and F(\(\text{ab}'\))\(_2\), which are capable of binding the epitopic determinant. The term "antibody" also includes monoclonal and polyclonal antibodies, anti-idiopathic, and humanized antibodies.

As used herein, the term "antibody or fragments thereof" encompasses chimeric antibodies and hybrid antibodies, with dual or multiple antigen or epitope specificities, and fragments, such as F(\(\text{ab}'\))\(_2\), Fab', Fab and the like, including hybrid fragments. Such antibodies and fragments can be made by techniques known in the art (see Harlow and Lane. *Antibodies, A Laboratory Manual*. Cold Spring Harbor Publications, N. Y., 1988). Such antibodies and fragments thereof can be screened for specificity and activity according to the methods disclosed herein.

Also included within the meaning of "antibody or fragments thereof" are conjugates of antibody fragments and antigen binding proteins (single chain antibodies) as described, for example, in U.S. Patent No. 4,704,692, the contents of which are hereby incorporated by reference for at least its teaching of antibody conjugates. The fragments, whether attached to other sequences or not, include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues. Methods of producing and/or isolating antibodies as disclosed herein are well known. There are also a variety of compositions disclosed herein where the secondary component can comprise a nucleic acid based molecule. Thus, as used herein, "nucleic acid" means a molecule made up of, for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. A nucleic acid can be double stranded or single stranded. Nucleic acid is also meant to include oligonucleotides.

As used herein, "nucleotide" is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenine-9-yl (A), cytosine-1-yl (C), guanine-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

"Nucleotide analog," as used herein, is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-
hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

"Nucleotide substitutes," as used herein, are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

It is also possible to link other types of molecules to nucleotides or nucleotide analogs to make conjugates that can enhance, for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al, \textit{Proc Natl Acad Sd USA}, 1989, 86:6553-6, which is incorporated by reference herein at least for its teachings of nucleic acid conjugates). As used herein, the term nucleic acid includes such conjugates, analogs, and variants of nucleic acids.

Nucleic acids, such as those described herein, can be made using standard chemical synthetic methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook et al, \textit{Molecular Cloning: A Laboratory Manual}, 3d Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 2001, Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System IPlus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta et al, \textit{Ann Rev Biochem} 1984, 53:323-56 (phosphotriester and phosphite-triester methods), and Narang et al, \textit{Methods Enzymol} 1980, 65:610-20 (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen et al., \textit{Bioconjg Chem} 1994, 5:3-7. Each of these references is incorporated by reference herein at least for their teachings of nucleic acid synthesis.

"Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.
"Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

"Aptamers" are also contemplated herein and are molecules that interact with a target molecule, preferably in a specific way. Typically aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP (U.S. Patent No. 5,631,146) and theophiline (U.S. Patent No. 5,580,737), as well as large molecules, such as reverse transcriptase (U.S. Patent No. 5,786,462) and thrombin (U.S. Patent No. 5,543,293). Representative examples of how to make and use aptamers to bind a variety of different target molecules can be found in the following non-limiting list of U.S. Patents: 5,476,766, 5,503,978, 5,631,146, 5,731,424, 5,780,228, 5,792,613, 5,795,721, 5,846,713, 5,858,660, 5,861,254, 5,864,026, 5,869,641, 5,958,691, 6,001,988, 6,013,443, 6,020,130, 6,028,186, 6,030,776, and 6,051,698, which are incorporated by reference herein for at least their teachings of aptamers.

"Ribozymes" are also contemplated herein and are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecurarily or intermolecurarily.

Ribozymes are thus catalytic nucleic acid. There are a number of different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions which are based on ribozymes found in natural systems, such as hammerhead ribozymes (for example, but not limited to the following U.S. Patents: 5,334,711, 5,436,330, 5,616,466, 5,633,133, 5,646,020, 5,652,094, 5,712,384, 5,770,715, 5,856,463, 5,861,288, 5,891,683, 5,891,684, 5,985,621, 5,989,908, 5,998,193, 5,998,203, WO 9858058 by Ludwig and Sproat, WO 9858057 by Ludwig and Sproat, and WO 9718312 by Ludwig and Sproat) hairpin ribozymes (for example, but not limited to the following U.S. Patent Nos.: 5,631,115, 5,646,031, 5,683,902, 5,712,384, 5,856,188, 5,866,701, 5,869,339, and 6,022,962), and tetrahymena ribozymes (for example, but not limited to the following U.S. Patents: 5,595,873, and 5,652,107). There are also a number of ribozymes that are not found in natural systems, but which have been engineered to catalyze specific reactions de novo (for example, but not limited to the following U.S. Patents: 5,580,967, 5,688,670, 5,807,718, and 5,910,408). Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. This recognition is often based mostly on canonical or non-
canonical base pair interactions. This property makes ribozymes particularly good candidates for target specific cleavage of nucleic acids because recognition of the target substrate is based on the target substrates sequence. Representative examples of how to make and use ribozymes to catalyze a variety of different reactions can be found in the following non-limiting list of U.S. Patents: 5,646,042, 5,693,535, 5,731,295, 5,811,300, 5,837,855, 5,869,253, 5,877,021, 5,877,022, 5,972,699, 5,972,704, 5,989,906, and 6,017,756. These patents are all incorporated by reference herein at least for their teachings of ribozymes.

In another aspect, the secondary component can be an artificial or natural organelle (e.g., chloroplasts, mitochondria for energy production, etc.), including mixtures thereof. An example of an artificial organelle that can be incorporated into the bubble wall is disclosed in U.S. Application Publication No. 2004-0049230, which is incorporated by reference herein for its teachings of artificial organelles.

In one aspect, the biomolecule can be a protein, such as a membrane protein or enzyme. In other specific examples, the biomolecule can be a receptor, a channel, a signal transducer, or an ion pump. In still other example, the biomolecule can be an energy converting protein (e.g., bacteriorhodopsin), an aquaporin, MscL, a cytochrome oxidase, hemoglobin, hemerythrin, hemocyanin, GutR, VR15 CMR1, connexin, calreticulin, microtubule, S 100 proteins, heat shock proteins (hsp), OmpA, Omp F, FhuA, FecA, BtuB, OMPLA, OpcA, FadL, NspA, light-harvesting complex (LHC) proteins, fumarate reductase, succinate dehydrogenase, formate dehydrogenase, nitrate reductase, or an ATPase.

In alternative aspects, the secondary component can be an indicator (e.g., pH, fluorescence, etc.), a carbon based nanostructure (e.g., buckyballs and nano tubes), a dendrimer, a nanoscale device, a microelectric machine (MEMs), an organic or inorganic compound, a non-water liquid, a gas (e.g., hydrogen), and mixtures thereof.

It is contemplated that any of the secondary components described herein can be imbedded into a polymer matrix prior to bubble formation. By "imbedded into a polymer matrix" is meant that the secondary component is chemically attached (e.g., covalently, ionically, electrostatically, or by hydrogen bonding) to the polymer matrix or physically attached with the polymer matrix (e.g., wholly or partially encapsulated within the matrix).

This is also referred to herein as a "polymersome." In one aspect, the secondary component comprises a biomolecule imbedded into a polymer matrix.

The polymer matrix can comprise any polymer. Suitable polymers include, but are not limited to, homopolymers or copolymers. In some examples, the polymer can be a block, random, or graft copolymer. Suitable polymers for the polymer matrix are readily available
from commercial sources and/or can be prepared by methods known to those of ordinary skill in the art.

Specific examples of polymers suitable for use in the polymeric matrix include, but are not limited to, modified or unmodified polyolefins, polyethers, and polyalkylene oxides. More specific examples of suitable polymers can include, but are not limited to, modified or unmodified polyethylene, polypropylene, polystyrene, polybutylene, poly(meth)acrylate, polymethylmethacrylate, polyacrylonitrile, ABS, polyethylene oxide, polypropylene oxide, polybutylene oxide, polyterephthalate, polyamide, nylon, polysiloxane, polyvinylacetate, polyvinylethers, polyoxazoline, polyacrylic acid, polyacyl alkylene imine, polyhydroxyalkylacrylates, copolymers, and mixtures thereof.

The term "modified" is used herein to describe polymers and means that a particular monomeric unit that would typically make up the pure polymer has been replaced by another monomeric unit that shares a common polymerization capacity with the replaced monomeric unit. Thus, for example, it is possible to substitute diol residues for glycol in poly(ethylene glycol), in which case the polyethylene glycol) will be "modified" with the diol. In one aspect, the polymer used to prepare the polymer matrix comprises a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

In one aspect, secondary component comprises a protein such as, for example, bacteriorhodopsin, imbedded in a polymer matrix comprising a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

In one aspect, the secondary component may be a polymer vesicle, or polymersome, embedded with biomolecules, such as proteins, in a manner which retains the functionality of the biomolecule. In one aspect, the biomolecule is embedded within the wall of the polymersome such that a portion of the biomolecule extends outside the polymersome and a portion of the biomolecule extends inside the polymersome. These types of polymer vesicles, or polymersomes, are referred to as "proteopolymersomes". In one embodiment, block copolymer BR/ATPase polymersomes may be used as the secondary component. Such nanoscale polymersomes can produce ATP from light while in foam scaffold which contains moderate levels of detergent. It is known that a diblock copolymer, poly(ethylene oxide-b-polyethylene) (OE) of a particular molecular weight and composition, can form bilayer membranes and enclosed vesicles can range in size from hundreds of nanometers to tens of microns in diameter. (Discher, B.M., et al., Polymersomes: tough, giant vesicles made from
diblock copolymers. Science, 1999. 284: p. 1143-1146 the entire contents of which are expressly incorporated herein by reference.) The family of di-block copolymers that can be used to make polymersomes is known to those of skill in the art. A series of poly(1,2 butadiene-b-polyethylene oxide) polymers (OB), most notably OB-2 (MW= 3600 g/mol, ethylene oxide block fraction (fEO)=0.28); OB-29 (MW=3800 g/mol, fEO=0.34); OB-9 (MW=5200 g/mol, fEO=0.37); and OB-18 (MW=10,400 g/mol, fEO=0.39). (Bermudez, H. et al., Molecular weight dependence of polymersome membrane elasticity and stability. Macromolecules, 2002. 35: p. 8203-8202, the entire contents of which are expressly incorporated herein by reference.) These polymers have pendant side unsaturation that can be used for crosslinking and further stabilization. Further increases in stability can be achieved by crosslinking polymer vesicles if there is a pendant side group that allows linking among vesicles. One strategy to modulate the toughness and stability of polymersomes is to cross-link the membrane to form a robust polymer network (Discher, B.M., et al., Cross-linked polymersome membranes: Vesicles with broadly adjustable properties. Journal of Physical Chemistry B, 2002. 106(11): p. 2848-2854, the entire contents of which are expressly incorporated herein by reference.) Using OB polymers with unsaturated side groups, polymer vesicles can be crosslinked to form solid networks that, if the concentration of crosslinkable polymer is sufficiently high, can greatly increase the critical tension required to cause the vesicle to fail. Crosslinking may be achieved using a chemical electron donor that facilitates the saturation of opposing unsaturated side chains, and the degree of crosslinking is adjusted using mixtures of crosslinkable (OB) and non-crosslinkable (OE) polymers. Alternatively, the degree of crosslinking may be adjusted by mixing fully crosslinkably polymers, but adjusting the extent of crosslinking by using a solute that can crosslink pendant unsaturated side chains using UV radiation of tunable duration and intensity. Once crosslinkied, a vesicle made with 100% crosslinkable OB polymer has a 100-fold greater critical tension than an OE-21 fluid vesicle. The strength of the membrane can be tuned by changing the percentage of crosslinkable polymer in the membrane, with the strength increasing monotonically with % crosslinkable polymer beyond 10%. Furthermore, crosslinked vesicles can be either air dried or lyophilized, are stable, and can be rehydrated later. A broad family of polymers may be used to make proteopolymersomes. In one aspect, as described above, a functional BR-containing proteopolymersome membrane may be formed. Foams maybe formed using biodegradable BR-PEO-PCL vesicles or non-biodegradable BR-PEO-PB vesicles.
iii. Additional components

The bubbles disclosed herein can also comprise additional components. For example, additional components can be added to make the bubble more stable. Suitable additional components can include, but are not limited to, preservatives, antioxidants, stabilizers, and the like. For example, by adding glycerine, long-lasting bubbles can be made.

c. Artificial Photosynthesis Platform

In one aspect, the present invention may be used to create an artificial photosynthesis platform for converting light into value-added products. In one embodiment, light energy is converted to chemical energy by providing the microchannels of the bubble and/or foam with at least one biosolar component that is capable of converting light energy to chemical energy. The chemical energy is then converted to a value-added product using a plurality of enzymes selected from the Calvin cycle enzymes, or CBB enzymes, such enzymes are also provided within the microchannels of the bubble and/or foam. In one embodiment whereby the bubble and/or foam provides an artificial photosynthesis platform, the secondary components include proteopolymersomes replete with biomolecules capable of producing ATP, and several plant and yeast enzymes representing the requisite components of the Calvin cycle. In one aspect, the biomolecules may include a biological proton pump and an ATP generator. The ATP generator produces ATP when activated by protons produced by the biological proton pump.

The proton pump may be a photoactivated proton pump which produces protons when subject to light energy. For example, the biomolecules may be bacteriorhodopsin (BR) and FoFi-ATP synthase (herein referred to as "ATP synthase") entirely embedded in the wall of a polymer vesicle to form the proteopolymersomes in a manner which retains their biological functionality (Choi, H-J.; Montemagno, C. Nanotechnology, 2006, 17, 2198-2202, the content of which is expressly incorporated herein by reference in its entirety).

Fig 5. illustrates one embodiment of the artificial photosynthesis platform. Foam is fabricated such that the secondary components are retained in microchannels formed from the aqueous layer provided between the two layers of surfactant. One of the secondary components is a proteopolymersome including bacteriorhodopsin (BR) and ATP synthase entirely embedded in the wall of the polymer vesicle. BR utilizes light to create a proton gradient, which is subsequently used by ATP synthase to produce chemical energy. It is desirable to widen the excitation bandwidth of BR and further increase the efficiency of BR. In one embodiment, a purified form of BR is attached to quantum dots (QD's). Quantum dots are UV sensitive semiconductor nanoparticles which emit visible and
IR wavelength photons in a size dependent manner. QD’s can be attached to BR via nickel
nitrilotriacetic acid (Ni-NTA) using the c-terminal Histidine (His) tag engineered onto the BR.
The chelated Ni ion of Ni-NTA is capable of orthogonal attachment to the His-tag on the c-
terminus, which in the correct orientation, would be on the outside of the vesicles. Once the
QD is attached, the protein is separated from the unbound QD’s using gel-filtration. By
widening the absorption spectra of BR to include a greater portion of solar radiation, the
photoconversion efficiency and overall output of the system may be improved. Other
proteins may be used instead of or in conjunction with BR as a proton pump for the
conversion of light to ATP as described herein. For example, the proton pump
Xanthorhodopsin may also be used (Balashov, S.P., et al., Xanthorhodopsin: A Proton Pump
contents of which are expressly incorporated herein in its entirety).

Referring again to Fig. 5, the other important secondary components of the artificial
photosynthesis platform are the enzymes 32 required to convert ATP to glyceraldehyde-3-
phosphate (G3P) and/or sugar using ribulose 1,5-biphosphate (RuBP) and CO2 from the air.
In the conversion to sugar, there are eight enzymes that are used for the conversion to hexose,
such as fructose and/or glucose. RuBisCO, phosphoglycerate kinase (PGK) and
glyceraldehyde phosphate dehydrogenase (GAPDH) are enzymes used to form
glyceraldehyde-3-phosphate (G3P). The conversion of G3P to hexose is accomplished using
triosephosphate isomerase, fructose-1,6-biphosphate aldolase, fructose-1,6-bisphosphatase,
phosphoglucose isomerase and glucose-6-phosphatase.

In order to reduce the cost of the artificial photosynthesis platform, an inexpensive
source of a majority of the enzymes is desired. All the proteins except the solar conversion
system proteins (BR and ATP synthase) can be engineered to coexpress in an Algae based
biofuel system, which the former could be cheaply obtained from bacteria. If a commercial
Algae biofuel system like Algaeanol is used, where the product is excreted, the algae biomass
could then be used to supply the proteins. It is understood that those of skill in the art would
understand alternate means for obtaining the listed enzymes, proteins and bacteria.

In the above-described embodiment, the three necessary substrates for conversion of
light and CO₂ to glyceraldehyde-3-phosphate (G3P) and/or hexose are ATP, NADH, and
ribulose-1,5-bisphosphate (RuBP). Bisphosphatase (RuBP) and carbon dioxide (CO₂) are
catalysed by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) using
the energy in ATP. As described above, in a preferred embodiment ATP is provided by the
BR/ATP synthase proteopolymersome. NADH can be provided to the artificial
photosynthesis platform in a variety of ways. During the process of carbon fixation, NADH is oxidized into NAD⁺ by the enzyme GAPDH. Since the described embodiment synthesizes simple sugars, NADH is used up and must be replenished. In one embodiment, the NADH may be photocatalytically regenerated. NAD⁺ can readily be converted back into NADH via P-doped Titanium Oxide nanoparticles using light and a Ru charge carrier. (Shi, Q., Yang, D., Jiang, Z. and Jian Li (2006). Visible-light photocatalytic regeneration of NADH using P-Doped TiO₂ nanoparticles. J. Mol. Cat. B, 43: 44-48, the entire contents of which are expressly incorporated herein by reference). The nanoparticles are available from Reade Inc. (Providence, RI) and could be added to the foam's aqueous phase and inflated alongside the ATP synthesizing vesicles. In one embodiment, RuBP is regenerated within the system as well. The shortest path to recycle a portion of the fixed carbon back to RuBP involves three enzymes: transketolase, phosphopentose epimerase and phosphoribulose kinase at a cost of 6 ATP molecules. This will convert fructose back into RuBP with an intermediate product either in the form of ribose-5-phosphate or xylulose-5-phosphate by way of the versatility of transketolase, and adolase already present. Since there is a net gain from the atmosphere, this regeneration cycle could be balanced with sugar output to produce a completely renewable system while still maximizing yield. One of skill in the art will appreciate that NADH and RuBP may be added to the artificial photosynthesis platform using a variety of known processes or each may be regenerated within the system using known techniques.

II. Methods of Making

In one aspect, the bubbles disclosed can be prepared by various methods. In one aspect, the bubbles can be prepared by admixing an aqueous solution comprising one or more secondary components and one or more biologically-derived surfactants, and blowing a gas into the mixture. The term "admixing" is defined as mixing two or more components together. Depending upon the components to be admixed, there may or may not be a chemical or physical interaction between two or more components. Figure 6 shows a schematic of one possible process for constructing a bubble as disclosed herein. In this aspect, the secondary component (70) as shown is encapsulated within a polymer matrix. The bubble solution can be admixed with pre-formed functional polymersomes comprising the secondary component and polymer matrix, as shown in Figure 6(a). While being blown with gas, the biologically-derived surfactant molecules (71) can self-assemble to form monolayers on the inside and
outside surface of the water channel (72) (see Figure 6(b)). As a result, biologically-derived surfactant molecules can form two layers that sandwich a layer of water-containing secondary component (e.g., in Figure 6, a polymersome) in between. It is also contemplated to form two or more bubble compositions comprising different secondary components and admixing the bubble compositions.

In another aspect, the bubbles described herein can be prepared by admixing an aqueous solution comprising one or more secondary components and one or more surfactants, and blowing a gas into the mixture. Figure 7 shows a schematic of a procedure of constructing the polymersome-incorporated bubbles using a coalescence process that occurs between bubbles. First, bubbles are blown with bubble solutions containing no or small amounts of secondary components (e.g., vesicles containing secondary components). Also, other bubbles blown from bubble solutions containing polymersomes can be prepared. This bubble solution can have a different composition (different pH, temperature, additives, surfactant molecules) compared with the first one. When these two different kinds of bubbles come in contact, this can lead to the growth of some bubbles at the expense of others. Eventually, all the bubbles merge into a single one to reduce the surface energy of the system. Using this method, the effect of biologically-derived surfactant molecules on the components (such as protein in polymersomes) can be minimized during the mixing process between the bubble solution and the polymersome solution. Especially, when it is desired to incorporate components incompatible with bubble solution, this method can be used. For example, bubbles using amphiphilic block copolymers as bubble surfactant can be made. However, these bubbles are typically not stable. Thus, for example, bubbles blown from the bubble solution by admixing a high concentration of the same amphiphilic copolymer with BR/ATP synthase reconstituted polymersomes can be merged with longer lasting surfactant bubbles.

As a result, biologically functional polymersomes can be incorporated inside strong biologically-derived surfactant bubbles without the side effects of detergent molecules.

It is also possible to prepare the bubbles disclosed herein with gases generated from chemical reactions. In this method, a manual bubble blowing process is not needed. The gases coming from various experimental conditions can automatically blow the bubbles with the presence of surfactant molecules.

**III. Methods of Using**

The compositions disclosed herein can be used for many varied uses. For example, the disclosed bubbles can be used for chemical and biochemical syntheses, chemical and biological assays, as biochemical sensors, drug delivery, purification in biology, specific gas
filters, environmental hazard monitoring systems, cosmetics, gas or liquid transporters, fluidic channels, fuel cells, to measure various properties, conditions, and/or interactions, and the like. It is contemplated that any molecular, nanoscale, or microscale chemical or biochemical analysis can be performed within the bubbles disclosed herein.

In one aspect, disclosed herein are methods of assaying an interaction between a first compound and a second compound, wherein the method comprises providing a bubble as disclosed herein, wherein the secondary component of the bubble comprises the second compound; contacting the bubble with the first compound; and detecting an interaction between the first compound and the second compound. A detectable interaction can indicate that the first compound has an activity or specific affinity for the second compound or vice-versa.

**a. Interaction**

The term "interaction" means and is meant to include any measurable physical, chemical, or biological affinity between two or more molecules or between two or more moieties on the same or different molecules. As will be understood from the compositions and methods disclosed herein, any measurable interaction between molecules can be involved in and are suitable for the methods and compositions disclosed herein. General examples include interactions between small molecules, between proteins, between nucleic acids, between small molecules and proteins, between small molecules and nucleic acids, between proteins and nucleic acids, and the like.

An interaction can be characterized by a dissociation constant of at least about $1 \times 10^{-6}$ M, generally at least about $1 \times 10^{-7}$ M, usually at least about $1 \times 10^{-8}$ M, or at least about $1 \times 10^{-9}$ M, or at least about $1 \times 10^{-10}$ M or greater. An interaction generally is stable under physiological conditions, including, for example, conditions that occur in a living individual such as a human or other vertebrate or invertebrate, as well as conditions that occur in a cell culture such as used for maintaining mammalian cells or cells from another vertebrate organism or an invertebrate organism.

Examples of interactions that can be involved in and/or determined by the compositions and methods disclosed herein include, but are not limited to, an attraction, affinity, a binding specificity, an electrostatic interaction, a van der Waals interaction, a hydrogen bonding interaction, and the like.

One specific type of interaction that can be involved in and/or determined by the methods and compositions disclosed herein is an interaction between a ligand (e.g., a potential therapeutic agent, a small molecule, an agonist, an antagonist, an inhibitor, an activator, a suppressor, a stimulator, and the like) and a protein (e.g., a receptor, a channel, a
signal transducer, an enzyme, and the like). For example, an interaction between a potential therapeutic agent and a target protein can indicate a potential therapeutic activity for the agent. In another example, an interaction between a small molecule (e.g., a lipid, a carbohydrate, etc.) and an enzyme (e.g., a kinase, a phosphatase, a reductase, an oxidase, and the like) can indicate enzymatic activity or substrate specificity. In another example of a type of interaction that can be involved in and/or determined by the methods and compositions disclosed herein is an interaction between two proteins or fragments thereof (e.g., an enzyme and a protein substrate or an antibody and an antigen or an epitope of an antigen). An example of this interaction can include, but is not limited to, the binding of a kinase, a protease, a phosphatase, and the like to a substrate protein. Such interactions can, but need not, result in a reaction or chemical transformation (e.g., phosphorylation, cleavage, or dephosphorylation). Another example of an interaction includes the binding or affinity of an antibody for an antigen or epitope of an antigen.

Another type of interaction that can be involved in and/or determined by the methods and compositions disclosed herein is hybridization between two nucleic acid sequences (e.g., a primer, probe, aptamer, ribozyme, and the like hybridizing to a target sequence of a nucleic acid). The term "hybridization" typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. "Sequence driven interaction" means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide substitute in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

Another type of interaction that can be involved in and/or determined by the compositions and methods disclosed herein includes a Watson-Crick interaction, i.e., at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is another example and is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major
groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups
(NH$_2$ or O) at the C6 position of purine nucleotides.

Yet another type of interaction that can be involved in and/or detected by the
compositions and methods disclosed herein includes an interaction between a protein (e.g., a
polymerase, endonuclease, or ligase) and a nucleic acid.

**b. Detection**

Detecting an interaction in the methods disclosed herein can be performed by any
method, but will usually depend on the particular interaction being detected. For example, the
first compound and/or second compound may contain a fluorescent marker, and detection of
an interaction can be made by measuring fluorescence or changes in fluorescence. In another
aspect, detecting an interaction can involve identifying a particular product. For example, if
the first and second compound interact in such a way as to produce a reaction product (e.g., a
kinase phosphorylating a substrate protein, a protease cleaving a particular protein, an
endonuclease cleaving a particular nucleic acid, a ligase ligating nucleic acids, and the like),
detection can be accomplished by identifying a particular product (e.g., the phosphorylated or
cleaved product). Identifying a product can be done by known methods such as
chromatography (e.g., retention times or Rf), fluorescence detection, ionization, mass spectral
analysis, nuclear magnetic resonance imaging, immunohistological techniques, microscopy
(e.g., TEM, SEM, optical microscope, or AFM), XRD, XPS, AES, infrared spectroscopy,
kinetic analysis, circular dichroism, electrochemical analysis (e.g., cyclic voltametry or
impedance spectroscopy), dynamic light scattering, static light scattering, and the like.

**c. First and Second Compounds**

In the disclosed methods the first compound can be any molecule that one may desire
to measure a potential interaction with any other desired molecule. For example, the first
compound can be any of the secondary components disclosed herein, for example, amino
acid based molecules (e.g., peptide, proteins, enzymes, or antibodies, including variants,
derivatives, and analogs thereof), nucleic acid based molecules (e.g., primers, probes,
aptamers, or ribozymes, including variants, derivatives, and analogs thereof), small molecules
(e.g., biomolecules, drugs, potential therapeutics, or organic and inorganic compounds),
macromolecules (e.g., carbon based nanostructures, dendrimers, or polymers), cell, or
organelle (natural or artificial). The second compound, which is present in the secondary
component, can also be any molecule as described above for the first compound. It is
contemplated that the disclosed methods are not limited by the particular order, identity or
priority of the first or second component; the identifiers "first" and "second" are merely
arbitrary and are used herein to simply distinguish one compound from the other; no connotation of order of addition is intended as any order of the compounds is contemplated and can be used in the methods disclosed herein.

**d. Exemplary Assays**

In one example, the second compound can be a protein and the first compound can be a small molecule such as a potential therapeutic agent, a kinase, a phosphatase, a protease, a methylating agent, an antibody, or fragments thereof. Alternatively, the second compound can be a small molecule, a kinase, a protease, a methylating agent, an antibody, or fragment thereof and the first compound can be a target protein. When the second compound is a protein and the first compound is a potential therapeutic or vice-versa, the detectable interaction can indicate a potential therapeutic activity. In this example, the method can be used to screen for potential drugs against a particular protein.

When the second compound is a protein and the first compound is a kinase, a phosphatase, a protease, a methylating agent, or a fragment thereof, or vice-versa, the detectable interaction can indicate enzymatic activity. Thus, in this example, one can analyze the ability of a protease to cleave a particular protein, or the ability of a kinase to phosphorylate a particular protein, or the ability of a protein to be dephosphorylated by a particular phosphatase, and the like.

In another example, the second compound can be a protein, antigen, or epitope, and the first compound can be an antibody or fragment thereof, or vice-versa. Here, the method can be used to detect an interaction that indicates binding activity. Thus, one can use this method to screen antibodies to find those that bind to a particular antigen or epitope. Conversely, one can use the disclosed method to find particular antigens or epitopes recognized by a particular antibody. It can also be possible, when the first compound is a cell or microorganism and the second compound is an antibody or fragment thereof, to screen for particular surface antigens on the cell surface, or to screen for antibodies that recognize a given organism. These and other uses are contemplated herein.

Still further, the disclosed compositions can be used to detect a particular infection in a subject. For example, a bubble as disclosed herein, wherein the secondary component comprises second compound that is a particular antigen, can be contacted with an antibody-containing sample from a subject. Detecting an interaction of the antigen and the antibody specifically reactive therewith can indicate the presence of the antigen or previous infection in the subject.
In another example, the second compound can be a nucleic acid and the first compound can be a primer, a probe, a ligase, an endonuclease, a transcriptase, a ribozyme, or fragment thereof, or vice-versa, that is the second compound can be a primer, a probe, a ligase, an endonuclease, a transcriptase, a ribozyme, or fragment thereof and the first compound can be a target nucleic acid. When the second compound is a nucleic acid and the first compound is a ligase, an endonuclease, a transcriptase, a ribozyme, or a fragment thereof, or vice-versa, the interaction can indicate enzymatic activity. For example, one can use the disclosed method to analyze the ability of an endonuclease to recognize and/or cleave a particular nucleic acid sequence, or the ability of a particular nucleic acid (e.g., a primer) to initiate transcription with a particular transcriptase.

When the second compound is a nucleic acid and the first compound is a primer, probe, or aptamer, or vice-versa, the interaction can indicate hybridization. In this example, one can use the disclosed methods to analyze the ability of a primer or probe sequence to hybridize to a particular nucleic acid sequence.

In the methods disclosed herein, the methods can further comprise contacting the bubble with a third compound. This can be done to, for example, evaluate or analyze a particular interaction between a first compound and a second compound while a third compound is present. Also, it is contemplated that the methods disclosed herein can further comprise contacting the bubble with a fourth, fifth, six, etc. compound. Any number of additional compounds can be used in the methods and compositions disclosed herein.

In the methods disclosed herein, the third compound can be any molecule or group of molecules. For example, any of the molecules disclosed herein, such as amino acid based molecules, nucleic acid based molecules, small molecules, macromolecules, cells, etc. Specific examples of suitable third compounds include, but are not limited to, an antagonist, an agonist, a ligand, an inhibitor, an activator, a primer, a promoter, a transcription factor, an endonuclease, a ligase, a transcriptase, a protease, a kinase, a phosphatase, a methylating agent, or mixtures thereof.

In another aspect, disclosed herein are methods of assaying a condition, comprising subjecting a bubble as disclosed herein, wherein the secondary component comprises an indicator to a condition to be assayed, and detecting the indicator. By indicator is meant any molecule, compound, or composition, which when contacted with or subjected to a particular condition (e.g., pH, light intensity, temperature, ionic strength, electrochemical potential), provides a detectable signal. The detectable signal that a suitable indicator can provide can be, for example, a color change, fluorescence, phosphorescence, magnetic resonance, electric
potential, and the like. For example, an indicator can provide a change in color or emit light in response to being subjected to a particular pH condition.

e. Chemical and biochemical synthesis

In one specific aspect, the disclosed bubbles can be used to form a hybrid ATP generating bubble device. The protein bacteriorhodopsin (BR) and F_0F_1-ATP synthase were reconstituted into 4 nm thick polymersome membranes that can convert optical energy to electrochemical energy. BR transports protons across the cell membrane upon the absorption of a photon of green light. Because of the pumping of protons, a pH gradient forms across the cell membrane, forming an electrochemical potential. When coupled with F_0F_1-ATP synthase, this proton gradient drives the synthesis of ATP from ADP and inorganic phosphate (Pi). Next, these biologically active polymersomes were packaged into the thin water channel of the surfactant bubbles. The ATP production by BR-ATP synthase-polymersomes was demonstrated in the bubble architecture. This has significance both in the development of a hybrid organic/inorganic power source obtaining its energy from light and in using surfactant bubbles for packaging structures. Functional polymersomes incorporated into the water channel of bubble walls were able to provide useful amounts of electrochemical energy which can be used for other nano-bio applications.

In one aspect, the bubble architecture is used to form an artificial photosynthesis platform for converting light and CO_2 to a value-added product, for example, simple sugar. Carbon fixation for the production of sugar is achieved by incorporating biosolar proteopolymerosomes and a plurality of enzymes into the microchannels of an inflatable foam provided by the present invention. BR-ATPase polymersomes may be used to convert light into ATP, which powers a rubisco substrate-enzyme reaction of carbon synthesis, and eventually the formation of hydrocarbon for biofuels. This artificial photosynthesis platform produces simple sugars that can be used to make a variety of useful organic compounds like HMF, DMF, methanol, ethanol or even sugars for human consumption. There are a handful of technologies available that can be used for the production of liquid fuel from biomass. Recently, a 4-phase acid catalyzed dehydration/hydration of sugars for the production of long chained alkanes has been developed. (Blommel, P. and R. Cortright. Production of Conventional Liquid Fuels from Sugars. 2008: available from: http://www.virent.com/BioBorming/Virent _Technology_Whitepaper.pdf., the contents of which are expressly incorporated herein by reference in its entirety.) A similar 2-phase can be used to convert biomass sugars into 2,5-dimethylfuran (DMF). (Huber, G.W., et al.,
**Production of Liquid Alkanes by Aqueous-Phase Processing of Biomass-Derived Carbohydrates.** Science, 2005. 308 (5727): p. 1446-1450., the contents of which are expressly incorporated herein by reference in its entirety.) Similarly, DMF can be generated in low boiling point solvents, which are also good sources of energy rich compounds that can be used in fuel. The combination of free carbohydrates, exothermic reactions, and the absence of distillation would make energy rich fuels in an extremely efficient and inexpensive manner.

In one aspect, the artificial photosynthesis platform is encased in a multifunctional material for the production of solar derived liquid fuels. The encasement provides a robust environment for the foam without compromising optical transparency and enables the separation of the liquid fuel (G3P) from the other constituents, while providing antifouling and antimicrobial protection. In one embodiment, the encasement will be synthesized via a sol-gel process which enables control of materials chemistry and microstructure. The sol-gel process is a chemical synthesis technique for preparing amorphous inorganic solids. The most common synthetic route involves the use of metal alkoxides which undergo hydrolysis and condensation polymerization reactions to give rigid solids (gels) of metal oxides such as SiO₂, TiO₂, A₁O₃, ZrO₂, etc. (Brinker, C.J. and G.W. Scherer, *Sol-Gel Science* 1990, New York: Academic Press, the contents of which are expressly incorporated herein by reference in its entirety). The microstructure of the resulting gel is determined by the synthesis conditions (pH, starting alkoxide, ratio of alkoxide: water, type of catalyst, etc.). Although the material is technically defined as a gel, it is a nanoporous glass that is rigid and dimensionally stable. Another important advantage of sol-gel processing is that the solution nature of the synthesis enables one to cast or form the sol-gel derived materials into a wide variety of shapes and sizes, including thin films or fibers. It has been shown that it is possible to immobilize biomolecules which retain their characteristic reactivities and spectroscopic properties in the pores of the sol-gel glass. (Rolison, D.R. and B. Dunn, *Electrically conductive oxide aerogels: new material in electrochemistry*. Journal of Materials Chemistry, 2001. 11(4): p.963-980, the contents of which are expressly incorporated herein in its entirety). As a result, a new generation of bioactive materials was created. (Ellerby, L.M., et al., *Encapsulation of Proteins in Transparent Porous Silicate-Glasses Prepared by the Sol-Gel Method*, Science, 1992. 555(5048): p. 1113-1115., the contents of which are expressly incorporated herein in its entirety). Relatively large biomolecules such as proteins and enzymes are trapped inside the pores of the inorganic matrix while small analytes can diffuse in and out. Important benefits of sol-gel technology include a marked improvement in the
stability of the biomolecules as well as protection from protease and microorganisms. In one aspect, the sol-gel encasement includes the following material requirements: 1) optical transparency in the 350-700 nm wavelength range; 2) good diffusion of CO2 through the encasement; 3) antifouling and antimicrobial properties; 4) good compatibility with the foam; and 5) ability to separate the G3P liquid fuel from the other constituents. FIG. 8 shows one design of the sol-gel encasement in accordance with the present invention. Due to the inherent optical transparency of S1O2 and high porosity of sol-gel derived materials, requirements 1) and 2) are fulfilled. In one aspect, to become antifouling/antimicrobial, S1O2 is functionalized with nanoparticle Ag and/or MgO. Nanoparticles MgO are known to be antibacterial, and nanoparticles Ag have demonstrated to be both antibacterial and antifungal. However, since S1O2 is able to prevent the protein protease from entering the sol-gel, unfunctionalized S1O2 will exclude bacteria and microbes from contact with the foam since bacteria and other microbes are larger than protease. Increased antifouling protection, however, can be afforded by addition of nanoparticles Ag and/or MgO. It is understood that other antimicrobial and/or antifouling materials may also be used subject the material requirements provided herein. To optimize the compatibility with the foam, the matrix and/or surface of the sol-gel may be functionalized with lipids. Another issue is to minimize water evaporation from the foam, which can be accomplished by incorporating lipids into the sol-gel matrix. By incorporating, for example, short chain diacylphosphatidylcholine into sol-gel derived S1O2, the hygroscopic nature of the lipid and their organization into the uniform SiC-ch-lipid structure suppresses overall water loss so that a water-rich microenvironment is retained. Water evaporation through the porous sol-gel encasement can also be minimized by incorporating polyethylene glycol (PEG) into the sol-gel starting solution to retain a water-rich environment in the foam. Another important function of the encasement is to separate the G3P or sugar from the rest of the constituents. The generated G3P can be captured by the foam by incorporating a "capture chamber" specifically designed to capture G3P. This capture chamber may also be sol-gel derived inorganic, porous matrix involving the use of metal alkoxides which undergo hydrolysis and condensation polymerization reactions to give rigid solids (gels) of metal oxides such as S1O2, TiO2, Al2O3, ZrC>2, etc., but molecularly imprinted with G3P. Molecular imprinting (MIP) allows the polymerization/cross-linking of the alkoxide monomers around template molecules, whereby removal of the template leaves behind a tailored pocked (an imprint) with greater affinity for the template over other structurally related compounds. (Diaz-Garcia, M.E. and R.B. Laino, *Molecular imprinting in sol-gel materials: Recent developments and applications.*
Microchimica Acta, 2005. 149 (1-2): p. 19-36, the contents of which are expressly incorporated herein by reference in its entirety.) The G3P, therefore, will be temporarily captured in the imprinted sol-gel but not covalently attached. Since the G3P is trapped in the matrix without covalent attachment or electrostatic interaction, it can be subsequently eluted from the capture chamber. Moreover, post-treatment on the MIP-sol-gel matrix can be performed to enlarge the imprinted size to more easily elute the G3P. An interesting phenomenon has been observed when working with the enzyme creatine kinase (CK), where mild heat treatment (only ~ 15° above room temperature) was able to slightly enlarge the pores of the sol-gel matrix. The sol-gel network typically forms around the biomolecule (CK in this case), creating a site-specific pore around the enzyme. With mild heat treatment, the pores enlarge -10%, providing more space around the enzyme. The ability to enlarge the pores can be utilized in MIP sol-gel, so that the G3P can be more readily eluted from the capture chamber. Alternatively, we can convert the G3P to sugar using isomerase and aldolase enzymes and elute the sugar. The G3P to sugar conversion can be accomplished by adding a "conversion chamber" to the encasement. The conversion chamber can be a sol-gel derived inorganic, porous matrix involving the use of metal alkoxides which undergo hydrolysis and condensation polymerization reactions to give rigid solids (gels) of metal oxides such as \( \text{SiO}_2 \), \( \text{TiO}_2 \), \( \text{Al}_2\text{O}_3 \), \( \text{Zr}(\text{IV}) \), etc. with immobilized aldolase and isomerase. Upon entering the conversion chamber, G3P will be converted to sugar due to the immobilized aldolase and isomerase, and the sugar can be subsequently eluted from the conversion chamber. The encasement will be fabricated with an inlet and outlet to facilitate the addition of foam and separation of the biofuel (G3P or sugar). There will be a semi-permeable membrane dividing the encasement from the sol-gel derived \( \text{SiO}_2 \) capture or conversion chamber, which will permit the diffusion of G3P. Once inside the capture or conversion chamber, the G3P or sugar can be eluted from the chamber by application of a gentle vacuum.

**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, devices, and/or methods described and claimed herein are made and evaluated, and are intended to be purely exemplary 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.
temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the methods described herein. Only reasonable and routine experimentation will be required to optimize such process conditions.

Example 1: Triblock copolymer synthesis

PEtOz-PDMS-PEtOz triblock copolymer (Mn = 7800, polydispersity index = 1.48) was synthesized by ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS in the presence of Nal (see Figure 9(a)). To utilize bis(hydroxyalkyl) terminated polydimethylsiloxane (PDMS) (Aldrich; Mn = 5600 gmol⁻¹) as a macroinitiator for oxazoline polymerization, the hydroxyl group must be converted to a functional group that can initiate the polymerization of oxazoline. First, bis(hydroxyalkyl) terminated PDMS was dehydrated under vacuum at 80-90 °C for 24 h and freeze-dried. After this drying process, cyclohexane (10 mL) (Aldrich; anhydrous) was added to 3.308 g of PDMS, and the mixture was stirred for 6 hours. To this reaction mixture, two molar excess volume of sec-butyl lithium (Aldrich; 1.4 M in cyclohexane) was added dropwise with a syringe at -20 °C, and the resulting solution was kept stirring until the temperature increased to room temperature under a nitrogen atmosphere. Then 0.58 g of ((chloromethyl)phenylethyl)dimethylchlorosilane (Gelest) was added, and the mixture was stirred for approximately 1 hour at room temperature to prepare bifunctional benzyl chloride-terminated PDMS. The resulting suspension was washed with methanol and sodium thiosulfate solution, then filtered under vacuum using a separatory funnel to remove LiCl salt. The solvent was evaporated at about 60 °C under high vacuum. The resulting product was dissolved in 40 mL of hexane (anhydrous; Aldrich), supplemented with activated charcoal, and then filtered again. After that, the solvent was removed in a vacuum evaporator a final time. EtOz (Aldrich; purity >99%) was dried over calcium hydride (Aldrich; powder 99.99%) followed by double distillation under a nitrogen atmosphere. To a solution of room temperature bifunctional PDMS in 30 mL of chlorobenzene, freshly distilled EtOz (2.4 g) and 0.408 g of Nal (Aldrich; >99.99%) were added successively. The reaction mixture was stirred under reflux for 2 hours at room temperature and next heated to 100 °C. The reaction was allowed to proceed until all the monomer was depleted, as monitored by ¹H-NMR. The end-capping of triblock copolymers by hydroxyl terminal groups was carried out by adding 2.5 mL of potassium hydroxide solution (Aldrich; 0.1N in methanol) to the system at room temperature,
yielding a solution color change from light yellow to colorless. The solution was diluted with chloroform (Aldrich; anhydrous) and washed with a 10% Na\textsubscript{2}S\textsubscript{O}\textsubscript{3} (Aldrich; >99.99%) solution, followed by a washing with water. After evaporation of the solvent to remove any unreacted PDMS oligomers, the products were dissolved in hexane supplemented with charcoal and MgSO\textsubscript{4}, then filtered. The hexane was evaporated under high vacuum and the remaining material was finally dehydrated using a freeze-dryer. The final product was a yellowish, fine powder. ABA triblock copolymer with hydroxyl terminal groups on the polyamide ends was confirmed by the \textsuperscript{1}H-NMR spectrum. Figure 9(b) shows the \textsuperscript{1}H-NMR spectrum of the obtained PEtO\textsubscript{2}-PDMS-PEtO\textsubscript{2} measured in DMSO-d\textsubscript{6}. It shows a sharp peak at 5=3.3 ppm (N-CH\textsubscript{2}-CH\textsubscript{2}-N) due to the PEtO\textsubscript{2} backbone and two broad peaks at \( \delta = 2.25 \) ppm (- C(0)C\textsubscript{3}-) and \( \delta = 0.93 \) ppm (CH\textsubscript{3}-CH\textsubscript{2}-), which represent the successful formation of PEtO\textsubscript{2} blocks. Gel permeation chromatography (GPC) analysis in THF revealed a molecular weight of \( M_n = 7800 \) g/mol and a polydispersity of \( M_M / M_n = 1.48 \).

**Example 2: TEM sample preparation**

For the TEM observation, the polymersome solution was dropped onto a 3 mm amorphous carbon coated Cu-grid by pipette. For faster drying, copper grids were placed on KIMWIPESTM, and, after 1 minute, excess solution was removed by blotting. The samples were transferred to the transmission electron microscope using a liquid-nitrogen cooled specimen stage, designed to maintain a temperature from about \(-160 \) °C to about \(-185 \) °C. Elevated temperatures that could cause structural changes of the specimen due to long electron beam exposure were minimized by performing TEM analysis under low electron beam density and also, by using the cooling stage during TEM observation.

**Example 3: Purple membrane and F\textsubscript{o}Fi-ATP synthase preparation and it's incorporation into polymersomes**

Bacteriorhodopsin (BR) was incorporated into the polymersomes in the form of purple membrane (PM). Purple membrane was obtained from *Halobacterium Salinarium* grown in high volume. The bacterial culture conditions and the procedure for isolation of PM mainly followed those described in Heyn et al., *Methods Enzymol*, 1982, 88:5-10. F\textsubscript{o}Fi-ATP synthase was purified from Bacillus PS3 cells as described in Hazard et al., *Arch Biochem Biophys* 2002, 407: 117-24). All samples were stored and prepared in the dark to preserve the maximum proton pumping activity during assays. To form protein- incorporated polymersomes, 3 mg of the polymer powder was first added to 68.5 \( \mu \)L of the PM (BR concentration: 4.8 mg/mL) with vigorous mixing for 1.5 hours. Then, 27.7 \( \mu \)L of FoFi-ATP
synthase (2.6 mg/ml) was added to the polymer/BR mixture. After stirring for 30 minutes, this protein-polymer mixture was added drop-wise to buffer solution (20 mM MOPS-Sigma, 50 mM Na₂SO₄, 50 mM K₂SO₄, 2.5 mM MgSO₄, 0.25 mM DTT-Fluka, 0.2 mM EDTA-Sigma, pH = 7.20-7.25) at the rate of 10 µL every 30 seconds. Syringe filtration through a membrane with a pore size of 0.2 µm was used to remove non-functional multi-lamellar vesicles and tube-like structures. This yielded functional BR/ATP synthase-reconstituted polymersomes (BR-ATP synthase- polymersomes) after overnight dialysis.

**Comparative Example 4 using TWEEN-20 Surfactant: Bubble solution preparation and polymersome incorporation into the water channel of bubble wall**

A bubble stock solution (pH = 6.5) was prepared by mixing glycerin, TWEEN-20™, and deionized water with a volume ratio of 2 : 1 : 2, respectively. To prepare samples with protein-incorporated polymersomes in bubbles and in detergent solution, bubble stock solution and polymersome solution were mixed with a 1 : 4 (bubble solution : polymersome solution) volume ratio for 30 seconds. Bubbles were blown outside the mixture solution using a 10-100 µL adjustable-volume pipette (EPPENDORF™) after dipping the tip into the solution by expelling air. Blown bubbles were transferred to fill a UV cuvette (12.5 mm x 12.5 mm x 45 mm). Before any measurements, the cuvette was kept inverted on top of KIMWIPES™ in the dark for 20 to 30 minutes in order to remove the excess polymersome solution not incorporated in the water channels. The cuvette entrance was sealed to prevent the liquid in the bubbles’ aqueous channels from evaporating and to increase the stability of the foam structure. Before taking any measurements, samples having similar density of bubbles (bubble size: 3.5 to 4 mm) were chosen; also the formation of dry foam where bubbles take the form of polyhedra with nanoscale liquid films and Plateau borders were confirmed (see Figure 2). During measurements, special care was taken not to break the bubbles. During incubations both in the dark and in light, cuvettes were rotated every 3 seconds to minimize the destabilization of the bubble architecture due to gravity-induced drainage.

**Example 5 using Ranaspumin-2 (Rsn-2) Surfactant:**

**a. Preparation of BR-ATP synthase vesicles and foam solution**

BR was obtained from the purple membrane of Halobacterium and was purified using the method adapted from Pitard. (see Pitard, B.; Richard, P.; Dunach, M.; Girault, G.; Rigaud, J-L. Eur. J. Biochem. 1996, 235, 769-778, the entire contents of which are expressly incorporated herein by reference.) FoFi-ATP synthase was purified from Bacillus PS3 cells and photophosphorylating lipid vesicles were made as described in Hazard, (see Hazard, A;
Montemagno, C. Arch. Biochem. Biophys. 2002, 407, 177-124, the entire contents of which are expressly incorporated herein by reference.) The Rsn-2 gene was synthesized using PCR since the length was relatively short (338 bp). The sequence was designed for E.coli codon usage with a 6xHis c-terminal tag. Purification was performed with Ni-NTA resin as described by Mackenzie, (see Mackenzie, CD.; Smith, B.O.; Meister, A.; Blume, A.; Zhau, X.; Lu, J.R.; Kennedy, M.W.; Cooper, A. Biophysical Journal. 2009, 96(12), 4984-4992, the entire contents of which are expressly incorporated herein by reference.) Surfactant measurements were done with capillary tube rise (0.1 cm diameter) and a rame-hart goniometer with water droplets placed on a flat Teflon surface. The custom made ABA triblockpolymer 12:33:12 polydimethylsiloxane-methylloxazoline-polydimethylsioloxane (PMOXA-PDMS-PMOXA, -5200 MW) was purchased from Polymer Source Inc. (Montreal, Canada). BR-ATP synthase proteopolymersomes were formed and injected into a Tween-20 foam solution as described in Choi, (see Choi, H-J.; Montemagno, C. Nanotechnology. 2006,17, 2198-2202, the entire contents of which are expressly incorporated by reference.) This solution contained 2 ml of Tween-20® (non-ionic acid detergent), 1 ml of glycerin, and 2 ml of deionized water. The solution was vortexed for 2 minutes and stored at 4 °C. Rsn-2 foam solutions were created by diluting a 1 mg/ml stock solution 1:10 into the vesicle mixture and aspirating the liquid.

**b. ATP synthesis activity assays**

Photo-derived ATP synthesis activity was measured in bulk-vesicle solution, as well as in both inflated and deflated foam-vesicle solutions. A bioluminescence assay (FLAA Luciferin-Luciferase, Sigma-Aldrich, USA) was used for ATP measurement. The procedure used here was adapted from previous methods, (see Choi, H-J.; Montemagno, C. Nanotechnology, 2006,17, 2198-2202). The reaction mixture in bulk-vesicle solutions contained 200 µl BR-ATP synthase proteopolymersome solution, 20 µl of 0.2 M ADP, 20 µl of 0.5 M KH₂PO₄, and 60 µl deionized water. In the foam-vesicle solutions, 60 µl of foam stock solution was substituted for the deionized water. All solutions were illuminated using a Fostec xenon lamp using a yellow filter with maximum emission at a wavelength of 572 nm for a total time of one hour unless otherwise noted. Initially before exposure and at subsequent time points 10 µl aliquots were removed from the reaction to quantify the rate and amount of ATP production. After light incubation, the volume of the bubble solution was calculated using the weight of each sample with the density values. In this calculation, an assumption was made that the density does not change before and after blowing bubbles. Foams were then broken and ATP was measured by recording the intensity of light produced.
by the sample and comparing that with a standard calibration curve. All experiments were performed at room temperature.

c. Rubisco carbon-fixation Assay

Carbon-fixing activity and subsequent formation of G3P was assayed using an adapted protocol based on the method of Racker. (see Wu, R.; Racker, E. J. Biol. Chem. 1958, 234, 1029-1035, the entire contents of which are expressly incorporated herein by reference.) The change in absorbance at 340 nm due to the oxidation of NADH by GAPDH was monitored using a Beckman Coulter® DU® 720 spectrophotometer. The amount of G3P was calculated using the extinction coefficient of 6220 M⁻¹ for NADH. The final assay mix contained 625 µg of Rubisco, 5 units of GAPDH, 10 units of PGK, 250 nmol of NADH, 375 nmol of RuBP, and 750 nmol of ATP in a volume of 750 µL of reaction buffer (pH 7.8) containing 0.1 M Tris-HCL, 5 mM MgCl₂, 66 mM KHCO₃, and 5 mM DTT. The assay was allowed to react for 20 minutes at room temperature with absorbance being measured at 10 second intervals.

d. Glucose Assay

The formation of glucose was assayed using a colorimetric assay kit (GAGO20, Sigma-Aldrich, USA). Glucose oxidase forms gluconic acid and hydrogen peroxide from glucose. Hydrogen peroxide reacts with the o-dianisidine in the assay mixture forming a colored product. The oxidized o-dianisidine then reacts with sulfuric acid to form a more stable pink colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration. The amount of glucose present was quantified by comparison to a standard calibration curve obtained from samples of glucose of known concentration. Glucose was formed by the addition of 12.5 units each of TPI, fructose-1,6-biphosphatase, and PGI, 0.2 units of fructose-1,6-biphosphate aldolase, and 0.1 units of glucose-6-phosphatase, diluting the total volume to 1 ml using water and incubating for 4 hours.

e. Full Sugar Synthesis Process and Assay

Glucose was formed from starting components RuBP, ATP and NADH using the following CBB enzymes (Sigma-Aldrich, USA): Rubisco, GAPDH, PGK, triose phosphate isomerase (TPI), fructose 1,6-biphosphate aldolase, fructose-1,6-biphosphatase, phosphoglucone isomerase (PGI), and glucose-6-phosphatase, during a 4 hour incubation. A typical reaction mixture contained 250 µg of Rubisco, 2 units of GAPDH, 4 units of PGK, 125 nmol of NADH, 200 nmol of RuBP, 500 nmol of ATP, 5 units each of TPI, fructose-1,6-biphosphatase, and PGI, 0.1 units of fructose-1,6-biphosphate aldolase, and 0.05 units of
glucose-6-phosphatase in a volume of 260 µg of reaction buffer (pH 7.8) containing 0.1 M
Tris-HCL, 5 mM MgCl₂, 66 mM KHCO₃, and 5 mM DTT was prepared and incorporated in
a bubble architecture by the addition of 65 µl of foam stock solution. Comparative samples
with 65 µl of water in place of foam stock solution in order to give bulk vesicle solution
results were also prepared. The samples were then exposed to light for one hour and
monitored for absorbance at 350 and 540 nm.

t. Results

FIG. 10 is a graph showing Control Group for ATP synthesis in Bulk Solution. Blue
triangles are samples without vesicles, Black squares are samples with no ADP, Red circles
are samples with no KH2PO4.

FIG. 11 is a graph showing Control Group for ATP synthesis in Foam Architecture.
Blue triangles are samples without vesicles, Black squares are samples with no ADP, Red
circles are samples with no KH2PO4.

FIG. 12 is a graph showing Control Group for ATP synthesis in Deflated Foam
Solution. Blue triangles are samples without vesicles, Black squares are samples with no
ADP, Red circles are samples with no KH2PO4.

FIG. 13 is a graph showing G3P Production with RuBisCO and ATP stock in bulk
solution. The data shows that functional RubisCO reaction using RuBP to form G3P from
NADH, ATP and dissolved carbonate (red. n=3) and without ATP (black, n=3).

FIG. 14 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay
with Artificial ATP Source and various components removed. Lines overlap at 0, extended y-
axis scale is provided for direct comparison to non-control results.

FIG. 15 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay
Control with Bulk Vesicle ATP Source and various components removed. Lines overlap at 0,
extended y-axis scale is provided for direct comparison to non-control results.

FIG. 16 is a graph showing an Absorbance Plot for RuBisCO Carbon Fixation Assay
Control Group with Foam Vesicle ATP Source. Lines overlap at 0, extended y-axis scale is
provided for direct comparison to non-control results.

FIG. 17 is a graph showing an Absorbance Plot for glucose oxidase assay showing the
oxidation of o-dianisidine to a pink colored product absorbing at 540 nm from various
glucose concentrations.

FIG. 18 is a graph showing DLS Size Distribution Plot for polymer vesicles. Graph
displays particle size relative to particle number. TEM and Weighted DLS data was used to
determine percentage of functional proteopolymersomes with diameter ranging from 69-110 nm. This range was determined by directly observing vesicles with TEM.

FIG. 19 is a graph showing DLS Size Distribution Plot for lipid vesicles. Lipid vesicles were relatively abundant compared to polymersomes with a peak readily observed and close to 100 nm. Particle size versus particle number was not investigated because lipid vesicle appeared to form functional hollow vesicles at all sizes as observed under TEM.

FIG. 20 shows TEM Micrographs of BR/FoFi ATP Synthase Proteopolymersomes (average diameter = 91 nm ± 64, n= 38). Proteopolymer vesicle solutions were taken from working stocks and dried for analysis. Salts from the buffer can be seen as dark crystals in the background.

FIG. 21 shows TEM Micrographs of BR/FoFi ATP Synthase Liposomes (average diameter = 102 nm ± 108 nm, n = 69). Liposomes were diluted from working stocks 1:100 and placed on TEM grid.

FIG. 22 shows fluorescent images of foam vesicle solutions. Liposomes containing 1 nM quantum dots, 0QD565 (Quantum Dot Corporation, USA.) were added to 0.1 mg/mL Rsn-2 foam after size exclusion chromatography on a S-200 column (GE Biosciences) and observed to flow between nodes in the foam channels as the foam drained. Rapid reorganization and collection at foam nodes was observed throughout the imaging process.

Figure 23 is a graph showing the production of ATP with BR/ATP synthase lipid vesicles in Rsn-2 foam ( ), in bulk ( ), in deflated Rsn-2 foam ( ) in T20 foam ( ) and a control experiment in the dark ( ) for comparison. Inset is the light intensity standard curve created with ATP stock dilutions.

Figure 24 is a graph showing BR/ATP synthase function in a lipid membrane was limited to the Rsn-2 based foam since the T20 adversely affected coupled FiFo-Atpase/BR vesicle function. All error bars refer to standard deviation (n=3).

Figure 25 is a graph showing ATP synthesis using BR/ATP synthase polymersomes in T20 foam ( ), in bulk ( ), deflated T20 foam ( ), and a control experiment in the dark ( ) for comparison (n=3 for each). Inset is the light intensity standard curve created with ATP stock dilutions.

Figure 26 is a graph showing the foam system containing BR/ATP synthase vesicles, RuBisCO, PGK, GAPDH, NADH, which is converting C02 and RuBP to G3P using photodervived ATP wherein the RuBisCO dependent carbon fixation reaction is fueled by lipid photophosphorylation vesicles fuels within the Rsn-2 foam (black, n=3), and a in bulk (brown, n=3); and the proteopolymersomes within Two foam (red, n=3), and in bulk (blue,
n=3). Control experiments were also performed in the dark (green and purple overlapping at 0, n=3 for each) and with selectively removed individual components (S5-S7, without detectable G3P production).

**Proton pumping activity assays**

The generation of a photo-induced electrochemical proton gradient was measured by trapping the fluorescent probe, 8-hydroxypropene-1,3,6-trisulphonic acid (pyranine) outside the polymersomes. When the pyranine was trapped inside polymersomes, the relatively small concentration of polymersomes resulted in low fluorescence intensity. Therefore, in these experiments, pyranine was trapped outside polymersomes (inside bubble aqueous channels) allowing the monitoring of external pH. An excitation scan with a Luminescence Spectrometer (LS 50B Perkin Elmer) was performed from 350 nm to 475 nm at an emission wavelength of 511 nm. Small shifts in the excitation spectrum were corrected and the conversion from fluorescence to pH was performed as described in Hazard et al., *Arch Biochem Biophys* 2002, 407: 117-24.

**Morphology and size distribution of polymersomes after BR- incorporation**

The bright-field TEM images of BR-reconstituted polymersomes are shown in Figure 20. As seen in Figure 27(a), spherical polymersomes were observed distributed throughout the sample. Figure 27(b) shows the size distribution histogram derived from direct measurement of polymersome sizes by TEM micrographs. The size distributions with a mean polymersome diameter of 270 ± 156 nm are based on an analysis of 135 polymersomes from TEM images.

**Example 8: Bubble water channel thickness measurement using IR**

The planar bubble film thickness was measured following the procedures described in Wu et al., *Review of Scientific Instruments* 2001, 72(5):2467-71. Using IR, the thickness of the bubble wall was measured to be 1.23 μm.

**Example 9: Proton pumping activity of hybrid BR/ATP synthase incorporated polymersome system in buffer solution**

Figure 28(a) shows ΔpH as a function of time together with a control. Intravescicular pH measurements were performed in buffer solution using BR- polymersomes and BR-ATP synthase-polymersomes. Both systems in buffer solution showed an increase in the internal pH with illumination. That is, the generation of a photo-induced proton gradient resulted in alkalinization of the protein-incorporated polymer vesicles. This pH change over time indicates that more than 50% of BR is selectively oriented, allowing protons to be pumped primarily outward.
The kinetics of light-induced proton transport were affected by the presence of ATP synthase, which can be seen in the slower and slightly smaller pH change in the presence of ATP synthase. While the BR-ATP synthase-polymersome system showed a smaller increase in pH at the initial stages (first 20 minutes: 3.5x10^{-3} ΔpH min^{-1}), ultimately a level of photo-induced basicity was similar to that of the BR-polymersome system. All of these effects indicate the light-driven generation of a proton gradient. In other words, upon illumination, BR undergoes a series of conformational changes, resulting in the transfer of protons across the membrane. For both systems, a light-driven pH change occurred rapidly for the initial 20 minutes and then saturated to a ΔpH of about 0.08 units. Proton permeability through the polymer membrane as well as the backpressure effect experienced by BR account for the limitation on the maximum obtainable pH changes from both systems.

**Example 10: ATP synthesis activity of hybrid BR/ATP synthase incorporated polymersome system within bubble architecture**

ATP production, normalized to the amount of ATP synthase present in the polymersomes was plotted as a function of light incubation time (Figure 28(b)). Polymersomes in the bubble architecture showed stable light-driven ATP synthesis. Initially, the ATP synthesis rate was small then, increased rapidly to 1800 nmol/mg of ATP synthase after 60 minutes. Considering the fact that electrochemical proton gradient drives the synthesis of ATP from ADP and inorganic phosphate (Pi) with FoFi-ATP synthase, these measurements demonstrate that both BR and ATP synthase did not denature and retained their biological functionality in the PEtOz-PEMS-PEtOz polymersomes inside the bubble water channel.

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 bubble, comprising:
   a. a wall, wherein the wall comprises an aqueous layer between two layers of a biologically-derived surfactant; and
   b. at least one secondary component, wherein said at least one secondary component is substantially or completely present in the aqueous layer.

2. A bubble, comprising:
   a. a wall, wherein the wall comprises an inner wall and an outer wall, wherein the inner wall comprises an inner surface and an outer surface and the outer wall comprises an inner surface and an outer surface, wherein the inner wall and the outer wall comprises a biologically-derived surfactant, wherein the inner wall and the outer wall comprises a gas between two layers of the biologically-derived surfactant;
   b. an aqueous layer, wherein the aqueous layer is adjacent to the outer surface of the inner wall of the bubble; and
   c. a secondary component, wherein the secondary component is substantially or completely present in the aqueous layer.

3. The bubble of any one of claim 1 or 2, wherein said biologically-derived surfactant is a natural protein surfactant.

4. The bubble of claim 3, wherein said natural protein surfactant is a Ranaspumin protein surfactant.

5. The bubble of claim 4, wherein the Ranaspumin protein surfactant is a Ranaspumin-2 protein surfactant.

6. The bubble of any one of claims 1-5, wherein the bubble comprises two or more different secondary components.
7. The bubble of any one of claims 1-6, wherein the secondary component comprises a biomolecule.

8. The bubble of claim 7, wherein the biomolecule is selected from the group comprising a small molecule, a peptide, a protein, an enzyme, an antibody, a nucleic acid, a lipid, a carbohydrate, a steroid, a hormone, a vitamin, a potential therapeutic agent, a polymer, a vesicle, a cell, a microbe, a drug, an organelle, and mixtures thereof.

9. The bubble of claim 8, wherein said enzymes are Calvin cycle enzymes.

10. The bubble of claim 9, wherein the Calvin cycle enzymes are selected from the group comprising ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), phosphoglycerate kinase (PGK), glyceraldehyde phosphate dehydrogenase (GAPDH), triosephosphate isomerase, fructose-1,6-biphosphate aldolase, fructose-1,6-bisphosphatase, phosphoglucose isomerase and glucose-6-phosphatase.

11. The bubble of any one of the claims 8-10, wherein said enzymes are transketolase, phosphopentose epimerase and phosphoribulose kinase.

12. The bubble of any one of claims 1-11, wherein the secondary component comprises a component selected from the group comprising an indicator, a carbon based nanostructure, a dendrimer, a nanoscale device, microelectric machine (MEMs), a microbe, a non-water liquid, a gas, and mixtures thereof.

13. The bubble of any one of claims 1-12, wherein the secondary component comprises a protein.

14. The bubble of any one of claims 1-13, wherein the secondary component comprises a membrane protein.

15. The bubble of any one of claims 1-14, wherein the secondary component comprises a receptor, a channel, a signal transducer, or an ion pump.
16. The bubble of any one of claims 1-15 wherein the secondary component comprises bacteriorodopsin, cytochrome oxidase, aquaporin, or ATPase.

17. The bubble in claim 16, wherein the secondary component comprises bacteriorodopsin.

18. The bubble of any one of claims 1-17, wherein the secondary component comprises a polymer matrix and a biomolecule, wherein the biomolecule is imbedded in the polymer matrix.

19. The bubble of claim 18, wherein the polymer matrix comprises a homopolymer.

20. The bubble of claim 18, wherein the polymer matrix comprises a copolymer.

21. The bubble of claim 18, wherein the polymer matrix comprises polyvinyl alcohol, polyacrylamide, a sol-gel, or mixture thereof.

22. The bubble of claim 18, wherein the polymer matrix comprises modified or unmodified polyethylene, polypropylene, polystyrene, polybutylene, poly(meth)acrylate, polymethylmethacrylate, polyacrylonitrile, ABS, polyethylene oxide, polypropylene oxide, polybutylene oxide, polyterephthalate, polyamide, nylon, or a mixture thereof.

23. The bubble of claim 18, wherein the polymer matrix comprises a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

24. The bubble in any one of claims 1-23, wherein the secondary component comprises bacteriorhodopsin imbedded in a polymer matrix comprising a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

25. The bubble in any one of claims 1-24, wherein the secondary component is a polymersome embedded with functional biomolecules.
26. The bubble of claim 25, wherein the functional biomolecules are selected from the group including a biological proton pump and an ATP generator.

27. The bubble of claim 26, wherein the biological proton pump is selected from the family of rhodopsin proteins.

28. The bubble of claim 27, wherein the family of rhodopsin proteins is selected from the group comprising bacteriorhodopsin and xanthorhodopsin.

29. The bubble of any one of claims 26-28, wherein ATP generator is FoFi-ATP synthase.

30. The bubble of any one of claims 26-29, wherein a quantum dot is attached to said biological proton pump.

31. The bubble of claim 30, wherein said quantum dot is attached to bacteriorhodopsin.

32. The bubble of any one of claims 25-31, wherein said polymersome is a di-block copolymer.

33. The bubble of claim 32, wherein said di-block copolymer is selected from the group comprising poly(ethylene oxide-b-polyethylene) and poly(1,2 butadiene-b-polyethylene oxide).

34. The bubble of any one of claims 1-33, wherein the secondary component is ribulose 1,5-biphosphate or NADH.

35. The bubble of any one of claims 1-34, wherein said secondary component is a P-doped titanium oxide.

36. A sol-gel encasement comprising an inorganic matrix having a plurality of pores for retaining the bubble of any one of claims 1-35.

37. The sol-gel encasement of claim 36 wherein said matrix is selected from the group comprising at least one metal oxide matrix.
38. The sol-gel encasement of claim 37, wherein said at least one metal oxide matrix is selected from the group comprising SiO₂, TiO₂, Al₂O₃, and ZrO₂.

39. The sol-gel encasement of any one of claims 36-38, wherein said matrix is functionalized with an antimicrobial agent or an antifouling agent or both.

40. The sol-gel encasement of claim 39, wherein said matrix is functionalized with a nanoparticle including silver or magnesium oxide or a combination thereof.

41. The sol-gel encasement of claim 39 or 40, wherein said matrix is functionalized with a lipid.

42. The sol-gel encasement of any one of claims 41, wherein said lipid is diacylphosphotidylcholine.

43. The sol-gel encasement of any one of claims 36-42, further comprising a capture chamber for capturing glyceraldehyde-3-phosphate (G3P).

44. The sol-gel encasement of claim 43, wherein the capture chamber is a sol-gel having an inorganic matrix.

45. The sol-gel encasement of claim 44, wherein said inorganic matrix is selected from the group comprising at least one metal oxide matrix.

46. The sol-gel encasement of claim 45, wherein said at least one metal oxide matrix is selected from the group comprising SiO₂, TiO₂, Al₂O₃, and ZrO₂.

47. The sol-gel encasement of any one of claims 43-46, wherein said capture chamber is imprinted with glyceraldehyde-3-phosphate (G3P).

48. The sol-gel encasement of any one of claims 36-47, further including the enzyme creatine kinase.
49. The sol-gel encasement of any one of claims 36-48, further comprising a conversion chamber for converting glyceraldehyde-3-phosphate (G3P) to sugar.

50. The sol-gel encasement of claim 49, wherein the conversion chamber is a sol-gel having an inorganic matrix.

51. The sol-gel encasement of claim 50, wherein said inorganic matrix is selected from the group comprising at least one metal oxide matrix.

52. The sol-gel encasement of claim 51, wherein said at least one metal oxide matrix is selected from the group comprising SiO$_2$, TiO$_2$, Al$_2$O$_3$, and ZrO$_2$.

53. The sol-gel encasement of any one of claims 50-52, wherein said sol-gel includes immobilized aldolase and isomerase.

54. A method for converting light and carbon dioxide to glyceraldehyde-3-phosphate (G3P) or hexose comprising,

   a. providing a bubble of any one of claims 1-5 including a first secondary component having at least one polymersome embedded with at least one functional biomolecule and a second secondary component including a plurality of Calvin cycle enzymes.

   b. subjecting said bubble to light and carbon dioxide to produce glyceraldehyde-3-phosphate (G3P) or hexose.

55. The method of claim 54, wherein the Calvin cycle enzymes are selected from the group comprising ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), phosphoglycerate kinase (PGK), glyceraldehyde phosphate dehydrogenase (GAPDH), triosephosphate isomerase, fructose-1,6-bisphosphate aldolase, fructose-1,6-bisphosphatase, phosphoglucone isomerase and glucose-6-phosphatase.

56. The method of claim 54 or 55, wherein said bubble further includes enzymes selected from the group comprising transketolase, phosphopentose epimerase and phosphoribulose kinase.
57. The method of any one of claims 54-56, wherein the functional biomolecules are selected from the group including a biological proton pump and an ATP generator.

58. The method of claim 57, wherein the biological proton pump is selected from the family of rhodopsin proteins.

59. The method of claim 58, wherein the family of rhodopsin proteins is selected from the group comprising bacteriorhodopsin and xanthorhodopsin.

60. The method of any one of claims 57-59, wherein ATP generator is FoFi-ATP synthase.

61. The method of any one of claims 57-60, wherein a quantum dot is attached to said biological proton pump.

62. The method of claim 61, wherein said quantum dot is attached to bacteriorhodopsin.

63. The method of any one of claims 54-62, wherein said polymersome is a di-block copolymer.

64. The method of claim 63, wherein said di-block copolymer is selected from the group comprising poly(ethylene oxide-b-polyethylene) and poly(1,2 butadiene-b-polyethylene oxide).

65. The method of any one of claims 54-64, wherein another secondary component is provided to include ribulose 1,5-biphosphate or NADH.

66. The method of any one of claims 54-65, wherein another secondary component is provided including P-doped titanium oxide.

67. The method of any one of claims 54-66, wherein said hexose is fructose or glucose.
68. A method for harvesting and converting light energy to chemical energy comprising,
   a) providing the bubble of any one of claims 1-5 including a first secondary
      components include at least one biosolar component and a second secondary
      component including a plurality of Calvin cycle enzymes.
   b) subjecting said biosolar component in said bubble to light energy to produce
      chemical energy;
   c) subjecting said plurality of Calvin cycle enzymes in said bubble to carbon dioxide
      and the chemical energy of step b) to produce glyceraldehyde-3-phosphate (G3P) or hexose;
   d) harvesting said G3P or hexose to produce a biomass.

69. The method of claim 68, wherein said biosolar component is a polymersome embedded
    with at least one biological proton pump and at least one ATP generator.

70. The method of claim 69, wherein said biological proton pump is selected from the family
    of rhodopsin proteins.

71. The method of claim 70, wherein the family of rhodopsin proteins is selected from the
    group comprising bacteriorhodopsin and xanthorhodopsin.

72. The method of any one of claims 69-71, wherein ATP generator is FoFi-ATP synthase.

73. The method of any one of claims 69-72, wherein a quantum dot is attached to said
    biological proton pump.

74. The method of claim 73, wherein said quantum dot is attached to bacteriorhodopsin.

75. The method of any one of claims 69-74, wherein said polymersome is a di-block copolymer.

76. The method of claim 75, wherein said di-block copolymer is selected from the group
    comprising poly(ethylene oxide-b-polyethylene) and poly(1,2 butadiene-b-polyethylene oxide).
77. The method of any one of claims 68-76, wherein another secondary component is provided to include ribulose 1,5-biphosphate or NADH.

78. The method of any one of claims 68-77, wherein another secondary component is provided including P-doped titanium oxide.

79. The method of any one of claims 68-78, wherein said hexose is fructose or glucose.

80. The method of any one of claims 68-79, wherein said biomass is selected from the group comprising HMF, DMF, methanol, ethanol, and sugar.
FIG. 8
FIG. 9b
FIG. 10

FIG. 11
FIG. 16

FIG. 17

SUBSTITUTE SHEET (RULE 26)
FIG. 23

FIG. 24
FIG. 28a

FIG. 28b
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/60610

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) B01F 17/00; C07K 14/435 (201 1.01 )

USPC - 530/344; 530/41 2

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)

IPC(8) B01F 17/00; C07K 14/435 (201 1.01 )

USPC - 530/344; 530/412

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

IPC(8) B01F 17/00; C07K 14/435 (201 1.01 )

USPC - 530/344; 530/412 (keyword delimited) Patents, Non-Patent Literature

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

USPTO-PubWEST; Google, Google Scholar. Search terms used: active, activity, animal, between, bilayer, biologicals, bubble, cell, closed, double, extract, foam, froth, inclusion, layer, layer, micelle, natural, number, plant, pore, purificat$, purify, ranasupmin, resin, rsn, rsn-2, separate$, separation, solubility, structure, surface, surfact$,...

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2, 3(2-5(2), 5(1)</td>
</tr>
<tr>
<td>Y</td>
<td>W O 2004/101447 A1 (Fisenko) 25 November 2004 (25.1 1.2004), pg 2 In 32-35</td>
<td>2, 3(2-5(2)</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

* 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
23 February 201 1 (23.02.201 1)

Date of mailing of the international search report
18 March 201 1

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OIS: 571-272-7774

Form PCT/ISA/2 10 (second sheet) (July 2009)
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. 68,  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: