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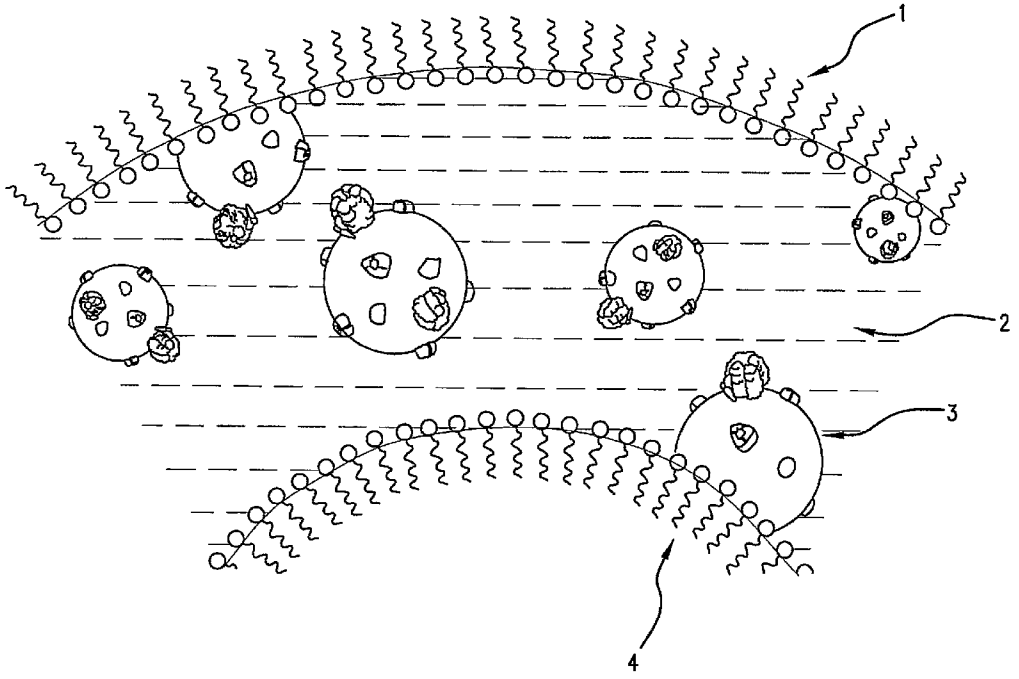

(57) **Abstract:** Disclosed are compositions and methods related to bubbles that can be used for chemical and biochemical analysis and synthesis.

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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. 60/653,354, filed February 16, 2005, which is incorporated by reference herein in its entirety.

FIELD

Disclosed herein are compositions and methods related to bubbles that can be used for chemical and biochemical analysis and synthesis.

BACKGROUND

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.

Therefore, there is a need for an easily producible packaging process that does not

resemble those used today in form or function for applications in future microscale and nanoscale hybrid devices. Also needed are compositions and processes that can allow complex biochemical synthesis by capitalizing on the architecture of bubbles and foams that mimics this behavior. The compositions and methods disclosed herein meet these needs.

SUMMARY

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 compounds and compositions (*e.g.*, bubbles and anti-bubbles) and methods for preparing and using such compounds and compositions.

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.

Figure 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**).

Figure 2(a) is a photograph of a foam; the bubbles are polyhedral. Figure 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.

Figure 3(a) is a schematic of a bubble and an antibubble. Figure 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**).

Figure 4(a) is a schematic of a single bubble structure. Figure 4(b) is a schematic of a cross-sectional view of a bubble.

Figure 5 is a schematic of the encapsulating method from a mixture of bubble solution and secondary component. Figure 5(a) is a schematic showing a bubble solution containing surfactant (**71**) and a secondary component (**70**) (shown here as already-made functional polymersomes). Figure 5(b) is a schematic showing a cross-sectional view of

bubble containing secondary component (**70**) inside the water channel (**72**) after the blowing process.

Figure 6 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.

Figure 7(a) is a synthetic scheme of PEtOz-PDMS-PEtOz triblock copolymer. Figure 7(b) is a ^{1}H NMR spectrum of PEtOz-PDMS-PEtOz in DMSO-d_6.

Figure 8(a) is a TEM image of polymersomes after bacteriorhodopsin/ATP synthase incorporation. Figure 8(b) is a size distribution histogram derived from direct measurement of polymersome sizes by TEM micrographs.

Figure 9(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. Figure 9(b) is a graph showing photo-induced ATP synthesis in bacteriorhodopsin-ATP synthase-polymersomes in a foam.

DETAILED DESCRIPTION

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 Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); March's Advanced Organic Chemistry, (John Wiley and Sons, 4th Edition); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989).

a. Bubbles

In one aspect, described herein is a bubble, comprising a wall, wherein the wall comprises an aqueous layer between two layers of surfactant; and a secondary component, wherein the secondary component is substantially present in the 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 solvent. In one aspect, the channel can be filled with water alone or water in combination with one or more 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 μm (for spherical bubbles) and from about 10 nm to about 600 μm (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, 1000nm (1.0 μm), 1.1 μm, 1.2 μm, 1.3 μm, 1.4 μm, 1.5 μm, 1.6 μm, 1.7 μm, 1.8 μm, 1.9 μm, 2.0 μm, 2.1 μm, 2.2 μm, 2.3 μm, 2.4 μm, 2.5 μm, 2.6 μm, 2.7 μm, 2.8 μm, 2.9 μm, 3.0 μm, 3.1 μm, 3.2 μm, 3.3 μm, 3.4 μm, 3.5 μm, 3.6 μm, 3.7 μm, 3.8 μm, 3.9 μm, 4.0 μm, 4.1 μm, 4.2 μm, 4.3 μm, 4.4 μm, 4.5 μm, 4.6 μm, 4.7 μm, 4.8 μm, 4.9 μm, 5.0 μm, 5.1 μm, 5.2 μm, 5.3 μm, 5.4 μm, 5.5 μm, 5.6 μm, 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, 115 μm, 116 μm, 117 μm, 118 μm, 119 μm, 120 μm, 121 μm, 122 μm, 123 μm, 124 μm, 125 μm, 126 μm, 127 μm, 128 μm, 129 μm, 130 μm, 131 μm, 132 μm, 133 μm, 134 μm, 135 μm, 136 μm, 137 μm, 138 μm, 139 μm, 140 μm, 141 μm, 142 μm, 143 μm, 144 μm, 145 μm, 146 μm, 147 μm, 148 μm, 149 μm, 150 μm, 151 μm, 152 μm, 153 μm,

154 μm, 155 μm, 156 μm, 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, 178 μm, 179 μm, 180 μm, 181 μm, 182 μm, 183 μm, 184 μm, 185 μm, 186 μm, 187 μm, 188 μm, 189 μm, 190 μm, 191 μm, 192 μm, 193 μm, 194 μm, 195 μm, 196 μm, 197 μm, 198 μm, 199 μm, 200 μm, 201 μm, 202 μm, 203 μm, 204 μm, 205 μm, 206 μm, 207 μm, 208 μm, 209 μm, 210 μm, 211 μm, 212 μm, 213 μm, 214 μm, 215 μm, 216 μm, 217 μm, 218 μm, 219 μm, 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, 283 μm, 284 μm, 285 μm, 286 μm, 287 μm, 288 μm, 289 μm, 290 μm, 291 μm, 292 μm, 293 μm, 294 μm, 295 μm, 296 μm, 297 μm, 298 μm, 299 μm, 300 μm, 301 μm, 302 μm, 303 μm, 304 μm, 305 μm, 306 μm, 307 μm, 308 μm, 309 μm, 310 μm, 311 μm, 312 μm, 313 μm, 314 μm, 315 μm, 316 μm, 317 μm, 318 μm, 319 μm, 320 μm, 321 μm, 322 μm, 323 μm, 324 μm, 325 μm, 326 μm, 327 μm, 328 μm, 329 μm, 330 μm, 331 μm, 332 μm, 333 μm, 334 μm, 335 μm, 336 μm, 337 μm, 338 μm, 339 μm, 340 μm, 341 μm, 342 μm, 343 μm, 344 μm, 345 μm, 346 μm, 347 μm, 348 μm, 349 μm, 350 μm, 351 μm, 352 μm, 353 μm, 354 μm, 355 μm, 356 μm, 357 μm, 358 μm, 359 μm, 360 μm, 361 μm, 362 μm, 363 μm, 364 μm, 365 μm, 366 μm, 367 μm, 368 μm, 369 μm, 370 μm, 371 μm, 372 μm, 373 μm, 374 μm, 375 μm, 376 μm, 377 μm, 378 μm, 379 μm, 380 μm, 381 μm, 382 μm, 383 μm, 384 μm, 385 μm, 386 μm, 387 μm, 388 μm, 389 μm, 390 μm, 391 μm, 392 μm, 393 μm, 394 μm, 395 μm, 396 μm, 397 μm, 398 μm, 399 μm, 400 μm, 401 μm, 402 μm, 403 μm, 404 μm, 405 μm, 406 μm, 407 μm, 408 μm, 409 μm, 410 μm, 411 μm, 412 μm, 413 μm, 414 μm, 415 μm, 416 μm, 417 μm, 418 μm, 419 μm, 420 μm, 421 μm, 422 μm, 423 μm, 424 μm, 425 μm, 426 μm, 427 μm, 428 μm, 429 μm, 430 μm, 431 μm, 432 μm, 433 μm, 434 μm, 435 μm, 436 μm, 437 μm, 438 μm, 439 μm, 440 μm, 441 μm, 442 μm, 443 μm, 444 μm, 445 μm, 446 μm, 447 μm, 448 μm, 449 μm, 450 μm, 451 μm, 452 μm, 453 μm, 454 μm, 455 μm, 456 μm, 457 μm, 458 μm, 459 μm, 460 μm, 461 μm, 462 μm, 463 μm, 464 μm, 465 μm, 466 μm, 467 μm, 468 μm, 469 μm, 470 μm, 471 μm, 472 μm, 473 μm, 474 μm, 475 μm, 476 μm, 477 μm, 478 μm, 479 μm, 480 μm, 481 μm, 482 μm, 483 μm, 484 μm, 485 μm, 486 μm, 487 μm, 488 μm, 489 μm, 490 μm, 491 μm, 492 μm, 493 μm,

494 μm, 495 μm, 496 μm, 497 μm, 498 μm, 499 μm, 500 μm, 501 μm, 502 μm, 503 μm, 504 μm, 505 μm, 506 μm, 507 μm, 508 μm, 509 μm, 510 μm, 511 μm, 512 μm, 513 μm, 514 μm, 515 μm, 516 μm, 517 μm, 518 μm, 519 μm, 520 μm, 521 μm, 522 μm, 523 μm, 524 μm, 525 μm, 526 μm, 527 μm, 528 μm, 529 μm, 530 μm, 531 μm, 532 μm, 533 μm, 534 μm, 535 μm, 536 μm, 537 μm, 538 μm, 539 μm, 540 μm, 541 μm, 542 μm, 543 μm, 544 μm, 545 μm, 546 μm, 547 μm, 548 μm, 549 μm, 550 μm, 551 μm, 552 μm, 553 μm, 554 μm, 555 μm, 556 μm, 557 μm, 558 μm, 559 μm, 560 μm, 561 μm, 562 μm, 563 μm, 564 μm, 565 μm, 566 μm, 567 μm, 568 μm, 569 μm, 570 μm, 571 μm, 572 μm, 573 μm, 574 μm, 575 μm, 576 μm, 577 μm, 578 μm, 579 μm, 580 μm, 581 μm, 582 μm, 583 μm, 584 μm, 585 μm, 586 μm, 587 μm, 588 μm, 589 μm, 590 μm, 591 μm, 592 μm, 593 μm, 594 μm, 595 μm, 596 μm, 597 μm, 598 μm, 599 μm, or 600 μm, where any of the stated values can form an upper or lower endpoint when appropriate.

Referring to Figure 1 (and also Figure 2), the secondary component **(3)** is substantially present in the channel **(2)** created by the surfactant layers **(1** and **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 (Weaire 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 any surfactant. In one aspect, a bubble can be prepared from mixtures of two or more surfactants. Suitable surfactants can be generally classified as ionic (anionic/cationic/dipolar) and nonionic. More specifically, polymeric surfactants, natural surfactants, silicon surfactants, fluorinated surfactants, oligomeric surfactants, dimeric surfactants, and the like, are suitable for the compositions and methods disclosed herein. In one aspect, the surfactants disclosed in U.S. Patent No. 6,849,426, which is incorporated by reference herein in its entirety, can be used.

In one aspect, the bubbles disclosed herein can comprise an anionic surfactant. Any anionic surfactants can be used. Suitable anionic surfactants are commonly used in detergents, shampoos, soaps, etc., and can be obtained commercially or prepared by methods known in the art. They include, but are not limited to, alkylbenzene sulfonates (detergent), fatty acid based surfactants, lauryl sulfate (*e.g.*, a foaming agent), di-alkyl sulfosuccinate (*e.g.*, a wetting agent), lignosulfonates (*e.g.*, a dispersant), and the like, including mixtures thereof. In other examples, linear alkylbenzene sulphonic acid, sodium lauryl ether sulphate, alpha olefin sulphonates, phosphate esters, sodium sulphosuccinates, hydrotropes, and the like, including mixtures thereof, can be used.

In another aspect, the bubbles disclosed herein can comprise a cationic surfactant. Any cationic surfactant can be used. Suitable cationic surfactants included, but are not limited to, quaternary ammonium compounds, imidazolines, betaines, etc. Such cationic surfactants can be obtained commercially or can be prepared by methods known in the art.

In still another aspect, the bubbles disclosed herein can comprise a nonionic surfactant. Any nonionic surfactant can be used. Suitable nonionic surfactants do not ionize in aqueous solution, because their hydrophilic group is of a non-dissociable type, such as alcohol, phenol, ether, ester, or amide. They can be classified as ethers (*e.g.*, polyhydric alcohols such as glycerin, solbitole, sucrose, etc.), fatty acid esters (*e.g.*, glycerin fatty acid ester, sobitan fatty acid ester, sucrose fatty acid ester, etc.), esters (*e.g.*, compounds made by applying, for example, ethylene oxide to a material having hydroxyl radicals such as high alcohol, alkyl-phenol, and the like), ether/esters (*e.g.*, compounds made by applying, for example, the ethylene oxide to the fatty acid or polyhydric alcohol fatty acid ester, having both ester bond and ether bond in the molecule), and other types (*e.g.*, the fatty acid alkanol-amide type or the alkylpolyglyceride type). Other suitable examples of nonionic surfactants can include, but are not limited to, alcohol ethoxylates and alkyl phenol ethyoxylates, fatty amine oxides, alkanolamides, ethylene oxide/propylene oxide block copolymers, alkyl amine ethoxylates, tigercol lubricants, etc.

In yet another aspect, the bubbles disclosed herein can comprise dipolar surfactants. Any dipolar surfactant can be used. Suitable dipolar surfactants (called amphoteric or zwitterionic) exhibit both anionic and cationic dissociation. Suitable examples of dipolar surfactants include, but are not limited to, products like betaines or sulfobetaines and natural substances such as amino acids and phospholipids. In one aspect, the betaines disclosed in U.S. Patent Nos. 6,852,816; 6,846,795; 6,846,352; and

6,849,426, which are incorporated by reference in their entireties, can be used herein.

Other examples of suitable surfactant include natural surfactants, which can have their source from plant or animal organs. In another example, a bolaform surfactant can be used. A bolaform surfactant is a surfactant that has two hydrophilic head groups at opposite ends of a hydrophobic tail.

Some specific examples of surfactants that can be used include, but are not limited to, sodium cholate, sodium deoxycholate, TRITON X-100 ™, TRITON X-114 ™, NONIDET P-40 ™, octylglucoside, TWEEN 20™, TWEEN 80™, BRIJ 35 ™, n-decyl-β-D-maltoside, n-dodecyl-N,N-dimethylamine-N-oxide, n-dodecyl-α-D-maltopyranoside, n-octyl-β-D-galactopyranoside, and n-undecyl-β-D-thiomaltopyranoside. Such surfactants are commercially available or can be prepared by synthetic methods known in the art.

Mixtures of these surfactants can also be used in the compositions and methods disclosed herein.

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 drug), 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_2NH—, —CH_2S—, —CH_2CH_2—, —CH=CH— (cis and trans), —$COCH_2$—, —$CH(OH)CH_2$—, and —CHH_2SO—. 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_2NH—, —CH_2CH_2—); Spatola *et al.*, *Life Sci* 1986, 38:1243-9 (—CH H_2—S); Hann, *J Chem 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): 97:39405 (—$CH(OH)CH_2$—); Holladay *et al.*, *Tetrahedron Lett* 1983, 24:4401-4 (—$C(OH)CH_2$—); and Hruby, *Life Sci* 1982, 31:189-99 (—CH_2S—) 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 may be 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-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) 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; Baggiolini *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 $F(ab')_2$, 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(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 oliognucleotides.

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.*, *Proc Natl Acad Sci 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.*, *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 1Plus 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.*, *Ann Rev Biochem* 1984, 53:323-56 (phosphotriester and phosphite-triester methods), and Narang *et al.*, *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.*, *Bioconjug 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, 6,011,020, 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 intramolecularly or intermolecularly. 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, VR1, CMR1, connexin,calreticulin, microtubule, S 100 proteins, heat shock proteins (hsps), 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 nanotubes), 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, polymethyl(meth)acrylate, 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 poly(ethylene 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.

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.

II. Methods or 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 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 5 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 5(a). While being blown with gas, the surfactant molecules (**71**) can self-assemble to form monolayers on the inside and outside surface of the water channel (**72**) (*see* Figure 5(b)). As a result, surfactant molecules can form two layers that sandwich a layer of water-containing secondary component (*e.g.*, in Figure 5, 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 6 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 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 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 $1x10^{-6}$ M, generally at least about $1x10^{-7}$ M, usually at least about $1x10^{-8}$ M, or at least about $1x10^{-9}$ M, or at least about $1x10^{-10}$ 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 prime, 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 R_f), 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 measures 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 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 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.

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, 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 NaI (*see* Figure 7(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, 1.5 mL n-butyl lithium (Aldrich; 1.6 M in hexane) 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 benzyl chloride (Aldrich) 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 NaI (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 ^{1}H-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% $Na_2S_2O_3$ (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 $MgSO_4$, 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 ^{1}H-NMR spectrum. Figure 7(b) shows the ^{1}H NMR spectrum of the obtained PEtOz-PDMS-PEtOz measured in DMSO-d_6. It shows a sharp peak at δ=3.3 ppm (N-CH_2-CH_2-N) due to the PEtOz backbone and two broad peaks at δ=2.25 ppm (-C(O)CH_2-) and δ=0.93 ppm (CH_3-CH_2-), which represent the successful formation of PEtOz blocks. Gel permeation chromatography (GPC) analysis in THF revealed a molecular weight of M_n = 7800 g/mol and a polydispersity of M_w/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 KIMWIPES™, 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_0F_1-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_0F_1-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 µL of the PM (BR concentration: 4.8 mg/mL) with vigorous mixing for 1.5 hours. Then, 27.7 µL of F_0F_1-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 Na_2SO_4, 50 mM K_2SO_4, 2.5 mM $MgSO_4$, 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.

Example 4: 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: Proton pumping activity assays

The generation of a photo-induced electrochemical proton gradient was measured by trapping the fluorescent probe, 8-hydroxyprene-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.

Example 6: ATP synthesis activity assays

To measure ATP synthesis activity, a bioluminescence assay kit (FLAA Luciferin-Luciferase, Sigma) was used. The assay procedure was adapted from Hazard *et*

al., Arch Biochem Biophys 2002, 407:117-24. 50 μL ADP (0.2 M) and 25 μL Pi (1 M) were added to 500 μL of BR-ATP synthase-polymersome stock solution. ADP and Pi were incorporated into the polymersomes by vigorous mixing. Individual samples corresponding to each time point for measurements in the bubble architecture were prepared. Samples were illuminated by a 5.0 W green LED (λ = 570 nm) to generate a proton gradient. 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.

Example 7: Morphology and size distribution of polymersomes after BR-incorporation

The bright-field TEM images of BR-reconstituted polymersomes are shown in Figure 8(a). As seen in Figure 8(a), spherical polymersomes were observed distributed throughout the sample. Figure 8(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 9(a) shows ΔpH as a function of time together with a control. Intravesicular 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: 3.5×10-3 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 back-pressure 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 9(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 F_0F_1-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 surfactant; and
    b. a secondary component, wherein the secondary component is substantially 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 surfactant, wherein the inner wall and the outer wall comprises a gas between two layers of 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 present in the aqueous layer.

3. The bubble in claims 1 or 2, wherein the surfactant comprises a cationic surfactant.

4. The bubble in claims 1 or 2, wherein the surfactant comprises an anionic surfactant.

5. The bubble in claims 1 or 2, wherein the surfactant comprises a nonionic surfactant.

6. The bubble in claims 1 or 2, wherein the surfactant comprises a dipolar surfactant.

7. The bubble in claims 1 or 2, wherein the surfactant comprises a polymeric surfactant, oligomeric surfactant, and a natural surfactant.

8. The bubble in claims 1 or 2, wherein the bubble comprises two or more different secondary components.

9. The bubble in claims 1 or 2, wherein the secondary component comprises a biomolecule.

10. The bubble of claim 9, wherein the biomolecule comprises 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, or a mixture thereof.

11. The bubble in claims 1 or 2, wherein the secondary component comprises an indicator, a carbon based nanostructure, a dendrimer, a nanoscale device, microelectric machine (MEMs), a microbe, a non-water liquid, a gas, or a mixture thereof.

12. The bubble in claims 1 or 2, wherein the secondary component comprises a protein.

13. The bubble in claims 1 or 2, wherein the secondary component comprises a membrane protein.

14. The bubble in claims 1 or 2, wherein the secondary component comprises a receptor, a channel, a signal transducer, or an ion pump.

15. The bubble in claims 1 or 2, wherein the secondary component comprises bacteriorodopsin, cytochrome oxidase, aquaporin, or ATPase.

16. The bubble in claims 1 or 2, wherein the secondary component comprises bacteriorodopsin.

17. The bubble in claims 1 or 2, wherein the secondary component comprises a polymer matrix and a biomolecule, wherein the biomolecule is imbedded in the polymer matrix.

18. The bubble of claim 17, wherein the polymer matrix comprises a homopolymer.

19. The bubble of claim 17, wherein the polymer matrix comprises a copolymer.

20. The bubble of claim 17, wherein the polymer matrix comprises polyvinyl alcohol, polyacrylamide, a sol-gel, or mixture thereof.

21. The bubble of claim 17, wherein the polymer matrix comprises modified or unmodified polyethylene, polypropylene, polystyrene, polybutylene,

poly(meth)acrylate, polymethyl(meth)acrylate, polyacrylonitrile, ABS, polyethylene oxide, polypropylene oxide, polybutylene oxide, polyterephthalate, polyamide, nylon, or a mixture thereof.

22. The bubble of claim 17, wherein the polymer matrix comprises a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

23. The bubble in claims 1 or 2, wherein the secondary component comprises bacteriorodopsin imbedded in a polymer matrix comprising a polymer produced by the ring-opening cationic polymerization of ethyl oxazoline with bifunctional benzyl chloride-terminated PDMS.

24. A method for producing a bubble comprising admixing an aqueous solution comprising one or more secondary components and one or more surfactants to produce an admixture, and blowing a gas into the mixture.

25. A method for producing a bubble comprising
    a. admixing a first aqueous solution comprising one or more surfactants and blowing a gas into the mixture, thereby providing a first bubble mixture;
    b. admixing a second aqueous solution comprising one or more secondary components and blowing a gas into the mixture, thereby providing a second bubble mixture; and
    c. contacting the first bubble mixture to the second bubble mixture.

26. The bubble produced by the method of claim 24 or 25.

27. A method of assaying an interaction between a first compound and a second compound, comprising:
    a. providing the bubble according to claims 1-23, or 26, wherein the secondary component comprises the second compound;
    b. contacting the bubble with the first compound; and
    c. detecting an interaction between the first compound and the second compound.

28. The method of claim 27, wherein the second compound comprises a protein and the first compound comprises a potential therapeutic agent, a kinase, a phosphatase, a protease, a methylating agent, an antibody, or fragment thereof.

29. The method of claim 27, wherein the second compound comprises a potential therapeutic agent, a kinase, a protease, a methylating agent, an antibody, or fragment thereof and the first compound comprises a protein.

30. The method of claim 27, wherein the second compound comprises a nucleic acid and the first compound comprises a primer, a probe, a ligase, an endonuclease, a transcriptase, a ribozyme, or fragment thereof.

31. The method of claim 27, wherein the second compound comprises a primer, a probe, a ligase, an endonuclease, a transcriptase, a ribozyme, or fragment thereof and the first compound comprises a nucleic acid.

32. The method of claim 27, wherein the second compound comprises a protein and the first compound comprises a potential therapeutic or the second compound comprises a potential therapeutic agent and the first compound comprises a protein, and wherein the detectable interaction indicates potential therapeutic activity.

33. The method of claim 27, wherein the second compound comprises a protein and the first compound comprises a kinase, a phosphatase, a protease, a methylating agent, or a fragment thereof, or the second compound comprises a kinase, a phosphatase, a protease, a methylating agent, or fragment thereof, and the first compound comprises a protein, and wherein the detectable interaction indicates enzymatic activity.

34. The method of claim 27, wherein the second compound comprises a protein and the first compound comprises an antibody or fragment thereof, or the second compound comprises an antibody or fragment thereof, and the first compound comprises a protein, and wherein the detectable interaction indicates binding activity.

35. The method of claim 27, wherein the second compound comprises a nucleic acid and the first compound comprises a ligase, an endonuclease, a transcriptase, a ribozyme, or a fragment thereof, or the second compound comprises a ligase, an endonuclease, a transcriptase, a ribozyme, or a fragment thereof, and the first compound comprises a nucleic acid, and wherein the interaction indicates enzymatic activity.

36. The method of claim 27, wherein the second compound comprises a nucleic acid and the first compound comprises a primer, probe, or aptamer, or the second compound comprises a primer, probe, aptamer, and the first compound comprises a nucleic acid, and wherein the interaction indicates hybridization.

37. The method of claims 27-36, further comprising contacting the bubble with a third compound.

38. The method of claim 37, wherein the third compound comprises 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.

39. A method of assaying a condition, comprising subjecting a bubble according to claims 1-23, or 26, wherein the secondary component comprises an indicator to a condition to be assayed; and detecting the indicator.

40. The method of claim 39, wherein the condition is pH, light intensity, electrochemical potential, or ionic strength.

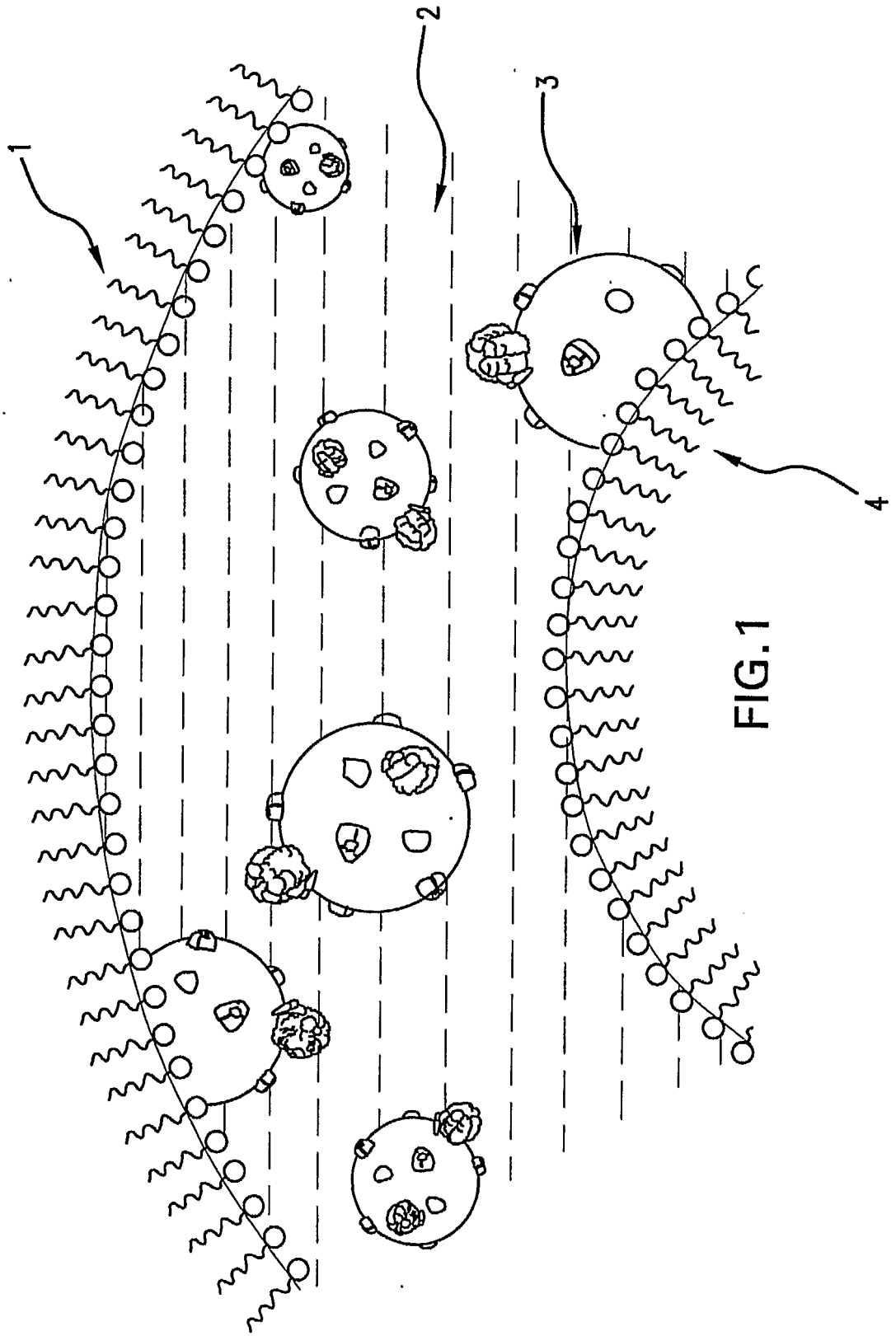

FIG.1

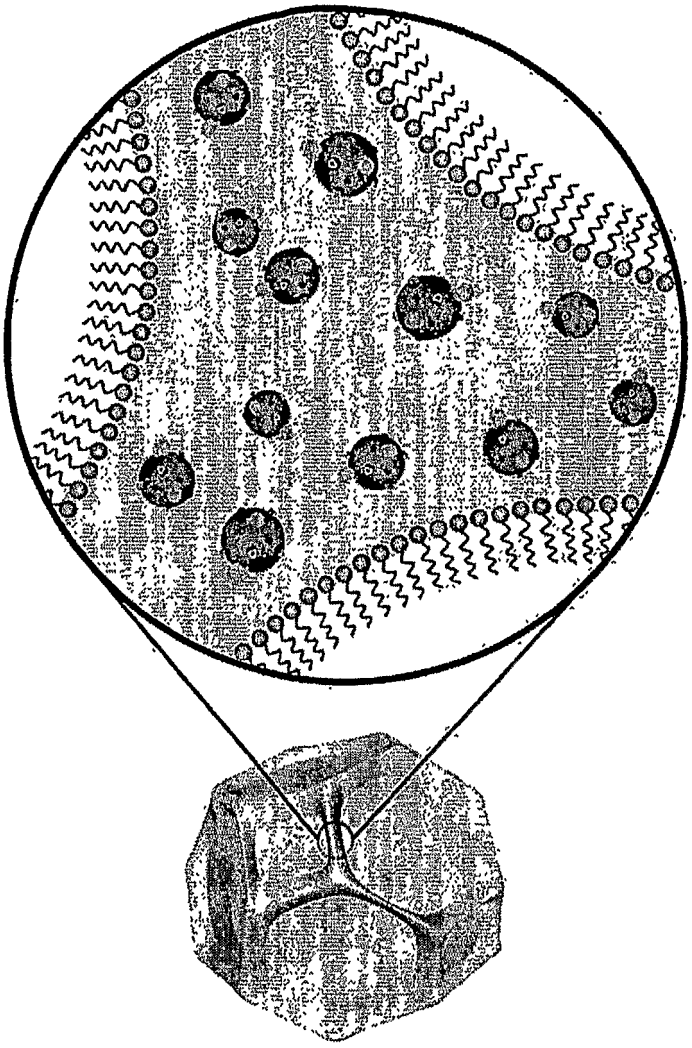

FIG.2b

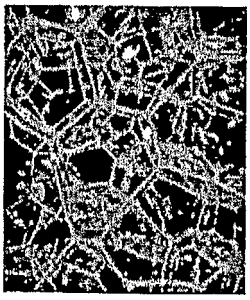

FIG.2a

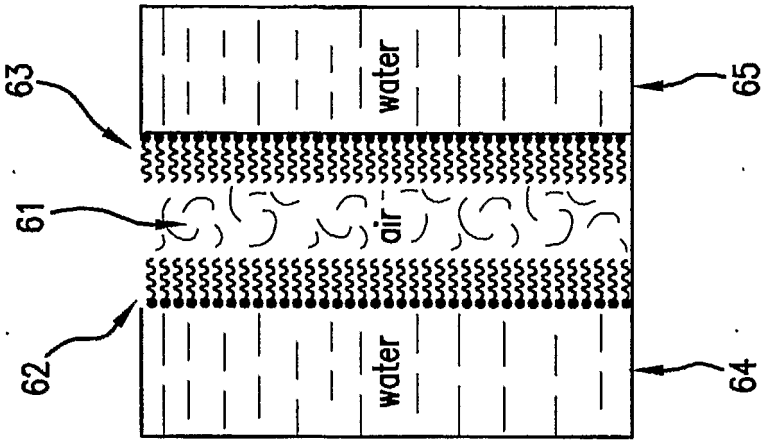

FIG.3b

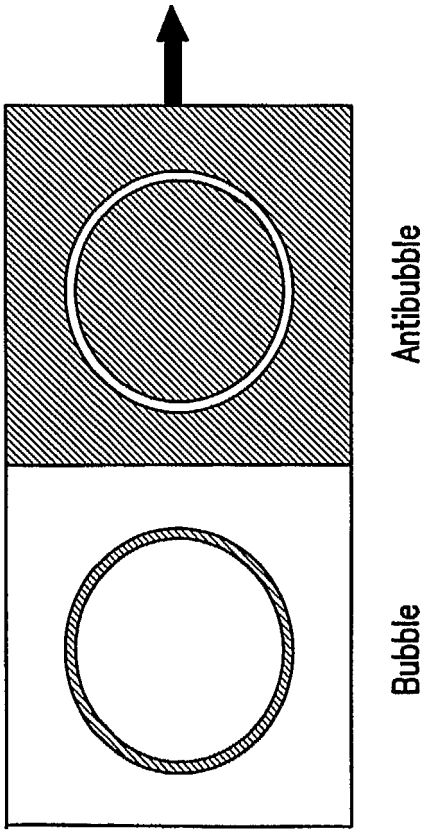

FIG.3a

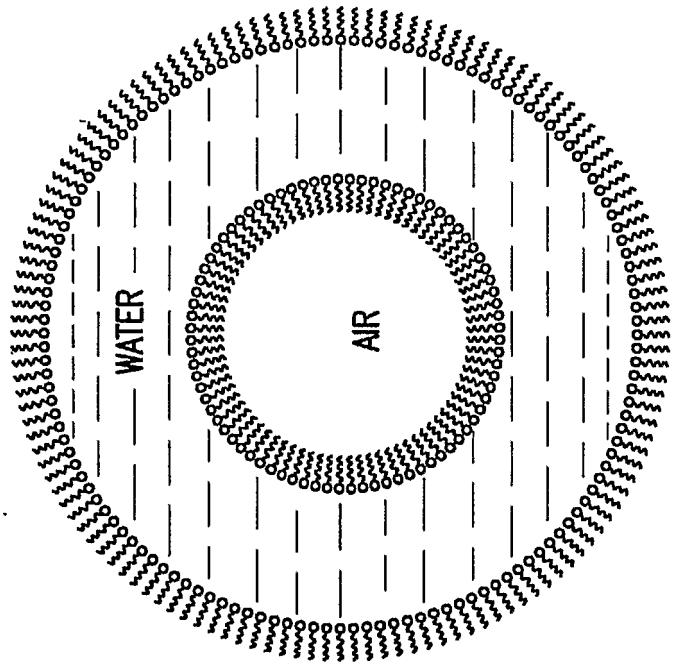

FIG.4b

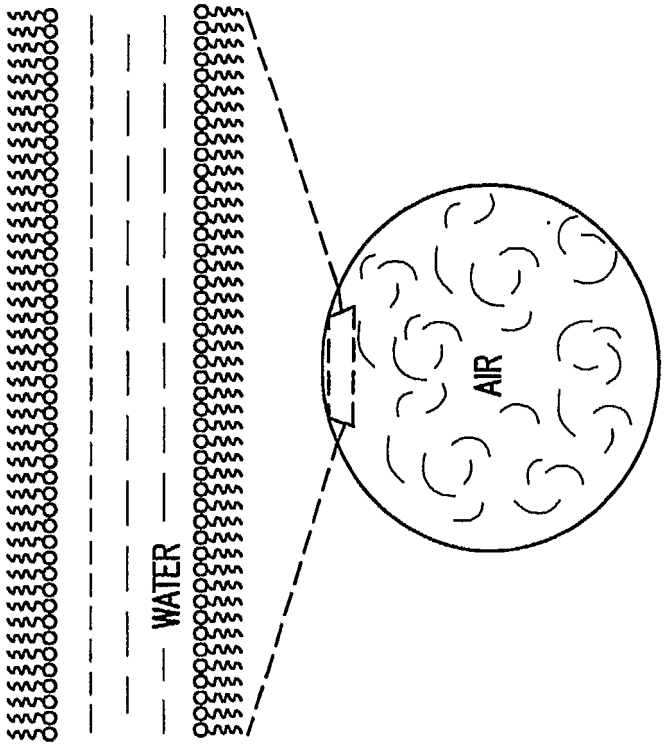

FIG.4a

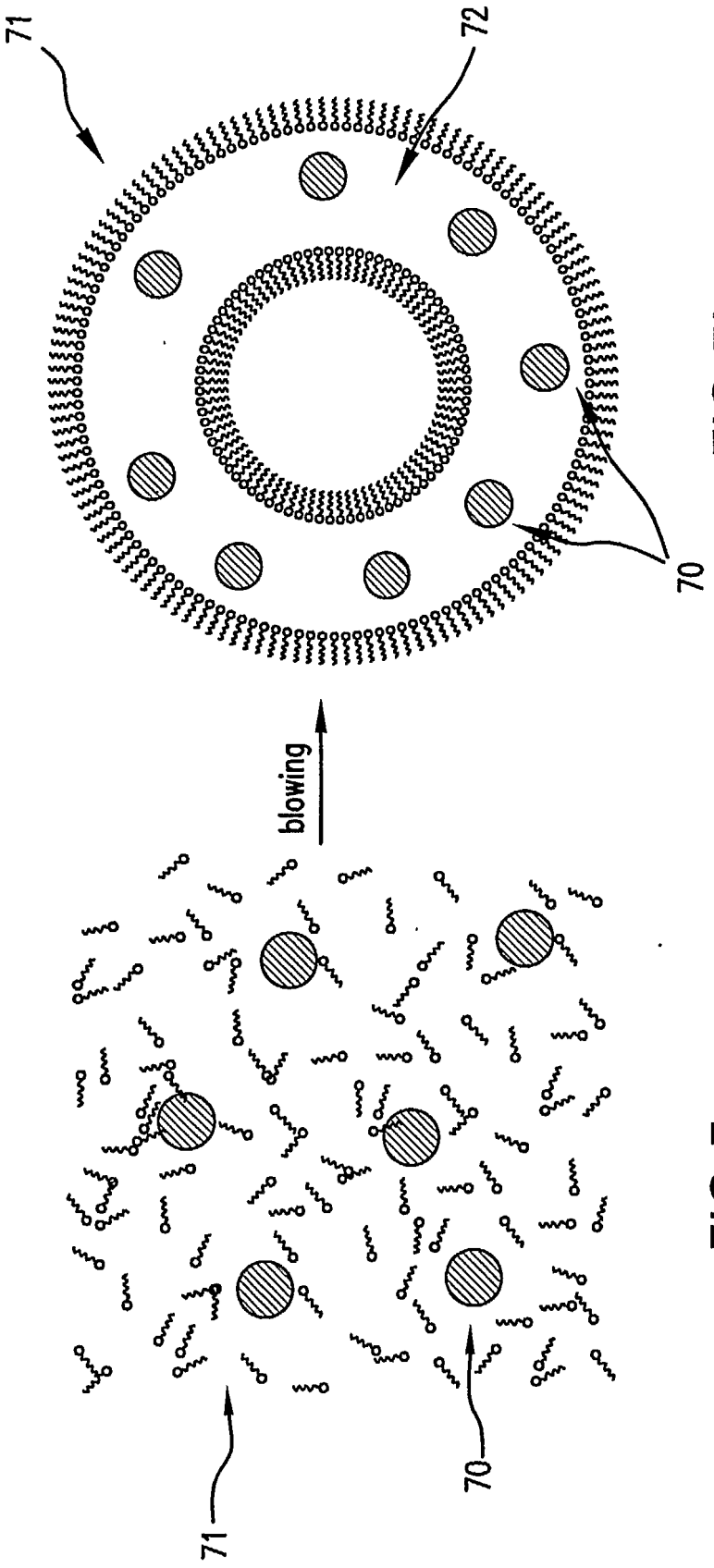

FIG.5b

FIG.5a

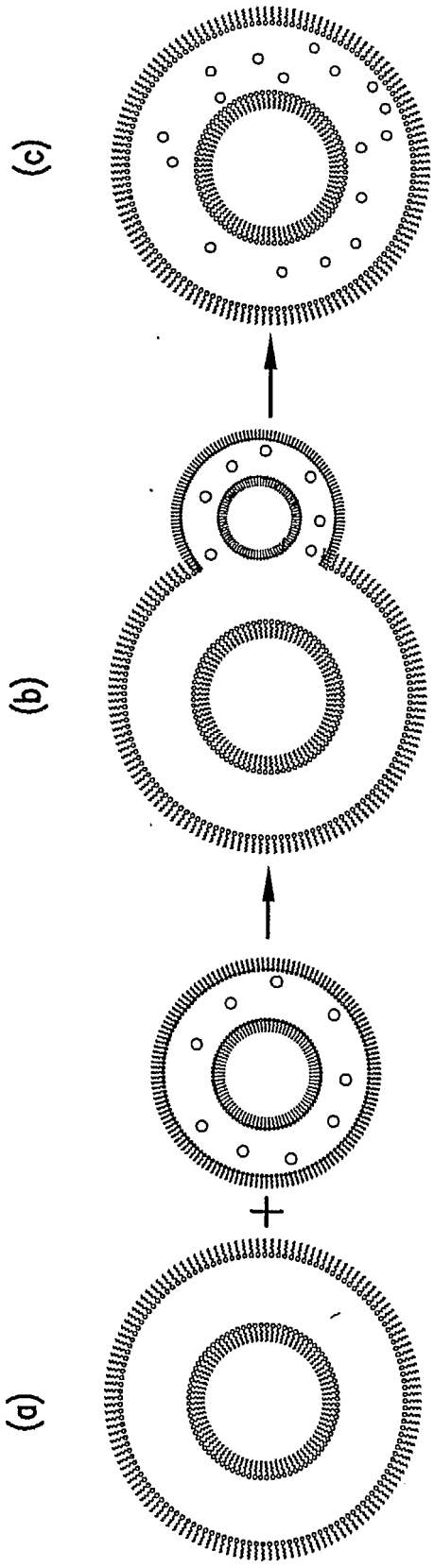

FIG.6

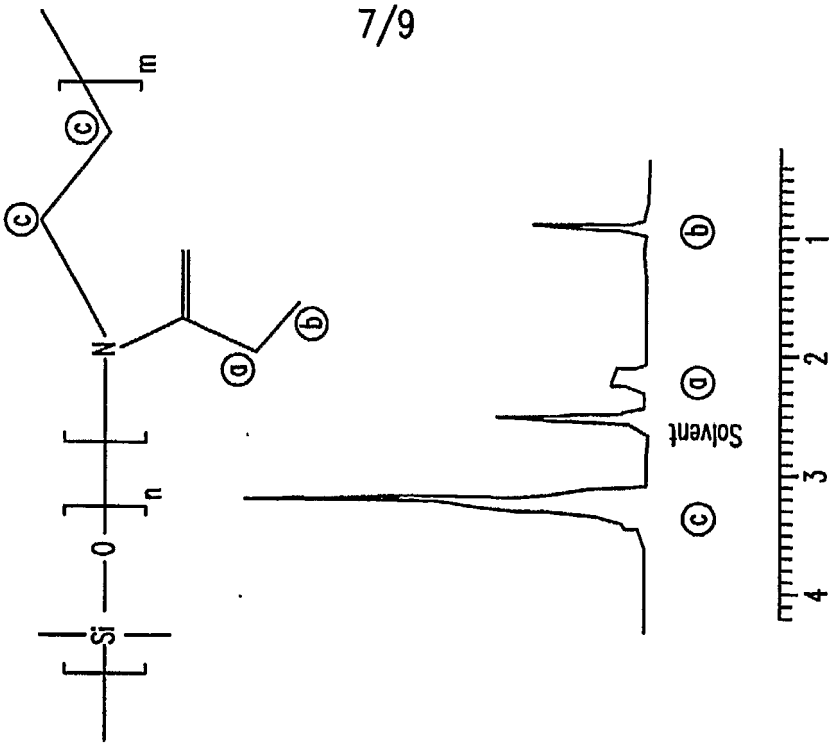
Solvent
a
b
c

FIG.7b

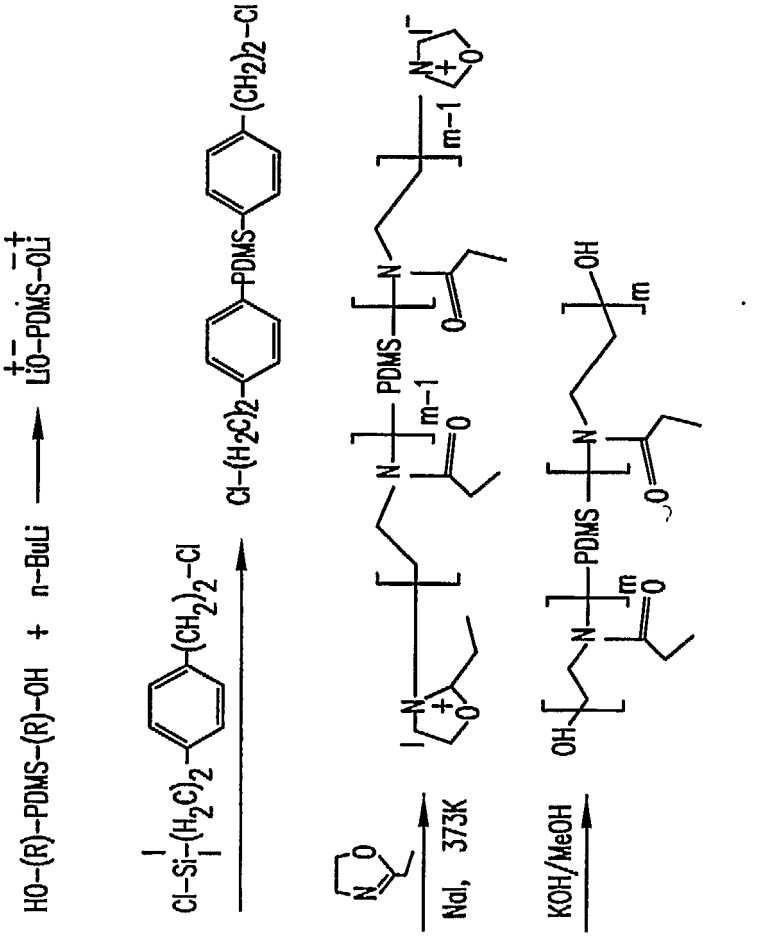
HO-(R)-PDMS-(R)-OH + n-BuLi
LiO-PDMS-OLi
Cl-Si-(H2C)2-(CH2)2-Cl
Cl-(H2C)2-PDMS-(CH2)2-Cl
NaI, 373K
KOH/MeOH

FIG.7a

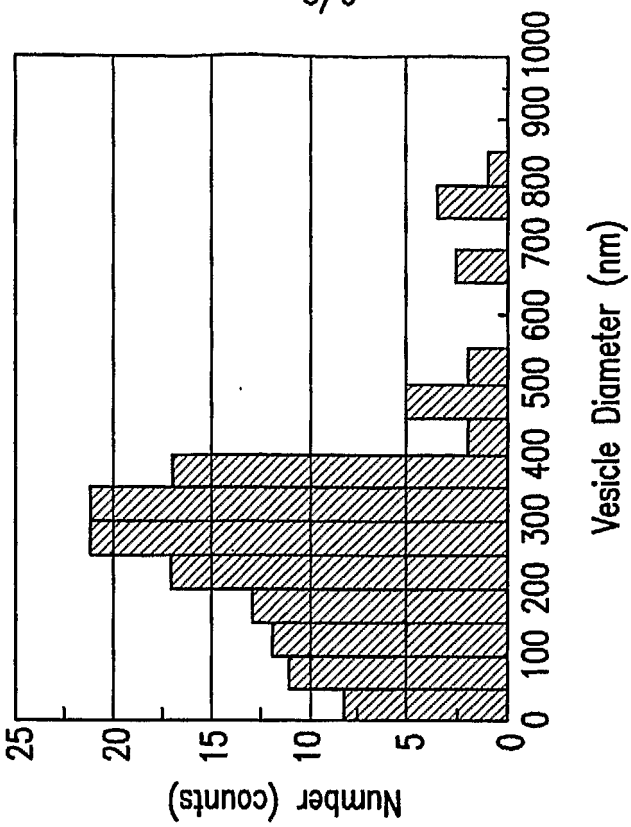
Number (counts)
25
20
15
10
5
0
0 100 200 300 400 500 600 700 800 900 1000
Vesicle Diameter (nm)

FIG.8b

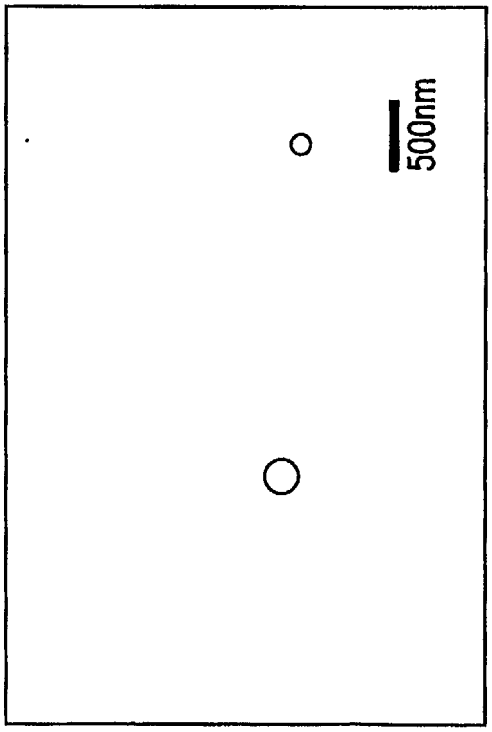
500nm

FIG.8a

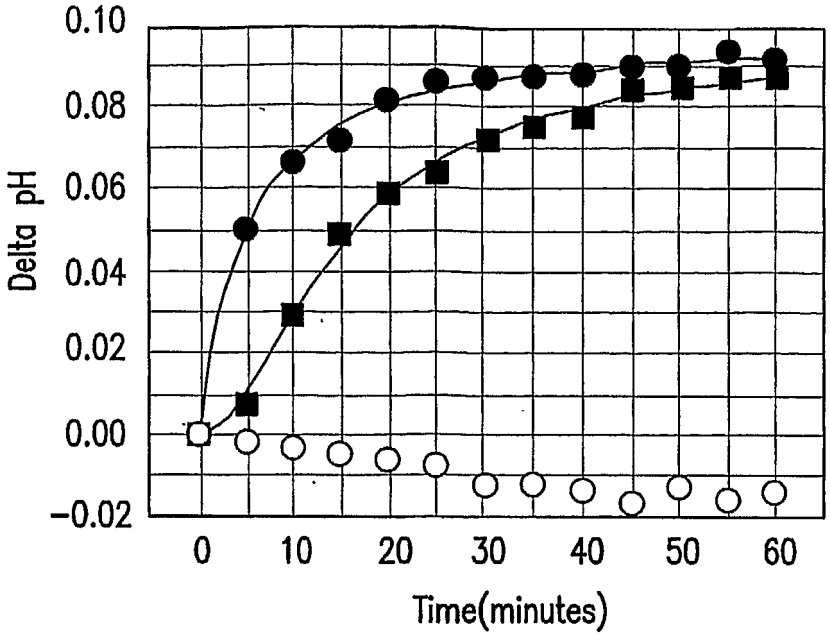
0.10
0.08
0.06
0.04
0.02
0.00
−0.02
Delta pH
0
10
20
30
40
50
60
Time(minutes)

FIG.9a

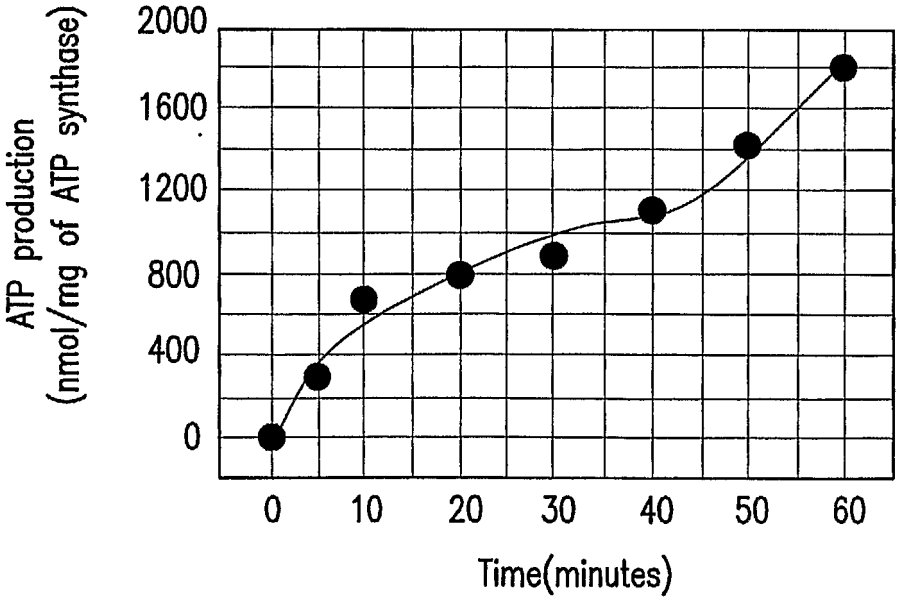
2000
1600
1200
800
400
0
ATP production
(nmol/mg of ATP synthase)
0
10
20
30
40
50
60
Time(minutes)

FIG.9b

专利名称(译)	气泡结构及其制造和使用方法		
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[标]申请(专利权)人(译)	加利福尼亚大学董事会		
申请(专利权)人(译)	加利福尼亚大学董事会		
当前申请(专利权)人(译)	加利福尼亚大学董事会		
[标]发明人	MONTEMAGNO CARLO D CHOI HYO JICK		
发明人	MONTEMAGNO, CARLO, D. CHOI, HYO-JICK		
IPC分类号	G01N33/53 C07K1/00 A61K9/12 A61K9/127		
CPC分类号	B01J13/02		
优先权	60/653354 2005-02-16 US		
其他公开文献	EP1853916A4		
外部链接	Espacenet		

摘要(译)

公开了与气泡有关的组合物和方法，其可用于化学和生物化学分析和合成。