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(71) Applicant: **NANYANG TECHNOLOGICAL UNIVERSITY** [SG/SG]; 50 Nanyang Avenue, Singapore 639798 (SG).

(72) Inventors: **CHO, Nam-Joon**; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG).
JACKMAN, Joshua Alexander; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG).

(74) Agent: **VIERING, JENTSCHURA & PARTNER LLP**; P.O. Box 1088, Rochor Post Office, Rochor Road, Singapore 911833 (SG).

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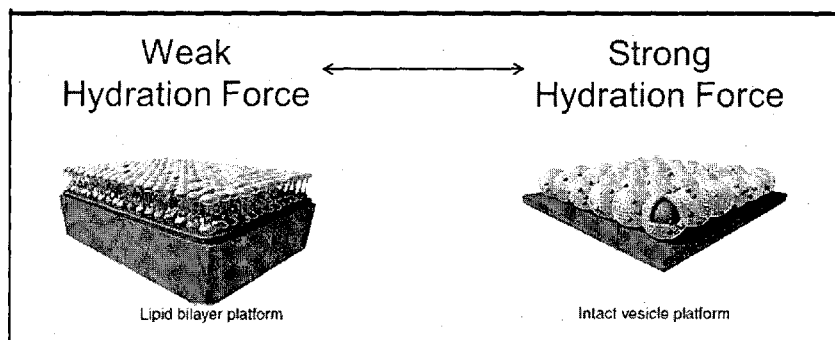
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(54) Title: METHODS FOR CONTROLLING ASSEMBLY OF LIPIDS ON A SOLID SUPPORT

FIGURE 1



(57) Abstract: The invention relates to a method of controlling adsorption of lipid molecules onto a solid support by tuning the steric-hydration force of the lipid vesicles and the surface of the solid support, such that the solid support either has a stabilized lipid bilayer adsorbed thereon or is resistant to adsorption of lipid molecules.

METHODS FOR CONTROLLING ASSEMBLY OF LIPIDS ON A SOLID SUPPORT

FIELD OF THE INVENTION

- 5 [001] The invention relates to methods of controlling adsorption of lipid molecules onto a solid support.

BACKGROUND OF THE INVENTION

- 10 [002] Lipid bilayer membranes on solid supports are a widely studied model system to understand the structure and function of biological membranes. There are many applications of lipid bilayer membranes on solid supports, including but not limited to biosensors, biocompatible surface coatings, and targeted drug delivery systems. Self-assembly fabrication of lipid bilayer membranes taking advantage of liposome
15 adsorption and spontaneous rupture on material substrates is popular, because the method does not require mechanical forces, occurs only at the solid-liquid interface, and is based on the diffusion-limited adsorption of liposomes onto the solid support. A wide range of experimental parameters influence the self-assembly process, including liposome properties (e.g., size, lipid composition, lamellarity and osmotic pressure),
20 solution conditions (e.g., ionic strength, temperature, solution pH) and material properties (e.g., crystallinity, topology).

- [003] Traditionally, adjustment of electrostatic forces has been the primary aim of interfacial science approaches to tune lipid-substrate interactions but this approach is
25 not always successful and the results are inconsistent. An example is the formation of a planar lipid bilayer on a solid support. On titanium oxide, formation of a planar bilayer by zwitterionic lipid vesicle adsorption and spontaneous rupture occurs at pH 4, which is below the isoelectric point (IEP) of titanium oxide (Tayebi, et al. Nature Materials 2012, Vol 11 pages 1074-1080). However, on aluminum oxide, formation of a planar
30 bilayer by zwitterionic lipid vesicle adsorption and spontaneous rupture does not occur under the same pH condition, although the condition is also below the isoelectric point of aluminum oxide (Keller, et al. Biophysical Journal 1998, 75, (3), 1397-1402). Considering that, in both cases, there is electrostatic attraction between the negatively charged zwitterionic lipid vesicles and the positively charged substrates, the evidence

supports that electrostatic attraction and methods to tune electrostatic forces are insufficient to form planar bilayers as a general means.

[004] It is generally understood that the overall adhesion process depends on the material properties of the solid support. Liposomes typically adsorb and either remain intact (e.g., gold and titanium oxide) or rupture to form a planar bilayer (e.g., silicon oxide and mica). Groves et al. (Langmuir 1998, 14, (12), 3347-3350) have reported that several oxide film substrates may serve as barriers that prevent the self-assembly of lipid bilayer membranes, including aluminum oxide, which can hinder liposome adsorption. As some substrates may serve as barriers that prevent the self-assembly of lipid bilayer membranes, the range of solid supports that can be used to support lipid bilayers is quite narrow. Groves et al. identified Type I barriers such as aluminum oxide that can prevent vesicle adsorption, and Type II barriers such as indium tin oxide and chrome that support vesicle adsorption but the resulting bilayers are effectively immobile.

[005] Several methods have been developed to form lipid bilayer membranes on solid supports which are intractable to liposome adsorption and spontaneous rupture, including covalently attaching auxiliary materials that facilitate adsorption and rupture of liposomes to the solid support. However, it has proven problematic to form a complete lipid bilayer membrane without adulterating the properties of the solid support or the lipid bilayer membrane through covalent modifications. These covalent modifications are time consuming, expensive and usually only work over small segments of the substrate.

[006] Therefore, there is need in the art for strategies that overcome the limitations of existing support materials while circumventing the laborious and costly surface modification approach.

[007] Furthermore, attempts to form lipid bilayer membranes on solid supports have only focused on the positive case, i.e., methods to form planar bilayers. There is also a need to develop methods to inhibit the formation of lipid bilayer membranes on solid supports tractable to vesicle adsorption and spontaneous rupture. As some materials adhere to cell membranes and lead to cell rupture, current nanoparticles and devices

used in animals including humans are limited to a narrow range of materials that are not harmful to cells. This limitation makes the range of materials suitable for nanoparticles and devices for targeted drug delivery very narrow.

- 5 [008] Accordingly, there is also need in the art for materials or methods that overcome the existing limitation in that the materials are imparted lipid rejecting properties.

SUMMARY OF THE INVENTION

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[009] In view of the above, it is an object of the invention to provide means to control adsorption of lipid molecules onto a solid support that are applicable to a wide range of solid supports.

- 15 [0010] The inventors have now surprisingly found that the adsorption of lipid molecules onto a solid support surface and the formation of lipid structures on said surface can be finely tuned by influencing the steric-hydration force of the lipids and the support surface.

- 20 [0011] In a first aspect, the invention therefore relates to a method of controlling adsorption of lipid vesicles onto a solid support comprising: tuning the steric-hydration force of the lipid vesicles and the surface of the solid support such that (i) the lipid vesicles rupture and form a bilayer membrane structure on the solid support; or (ii) the lipid vesicles are stably adsorbed to the solid support; or (iii) the adsorption of the lipid
25 vesicles onto the solid support is inhibited.

[0012] Another aspect of the invention relates to a solid support comprising a stabilized lipid bilayer adsorbed thereon obtainable according to the methods described herein.

- 30 [0013] Another aspect of the invention relates to a solid support resisting lipid vesicle adsorption thereon obtainable according to the methods described herein.

[0014] Other aspects of the invention will be apparent to a person skilled in the art with reference to the following drawings and description of various non-limiting

embodiments.

BRIEF DESCRIPTION OF THE FIGURES

[0015] The drawings are not necessarily drawn to scale, emphasis instead generally being placed upon illustrating the principles of various embodiments. In the following description, various embodiments of the invention are described with reference to the following drawings.

[0016] **Figure 1.** Conditions to control the interaction of lipid assemblies with solid supports. Conditions that induce weak steric-hydration force (weakly destabilizing) promote the formation of a two-dimensional, planar lipid bilayer. Conditions that induce strong steric-hydration force (strongly destabilizing) promote the formation of an intact vesicle layer because the total interaction energy is not strong enough to promote vesicle rupture.

[0017] **Figure 2** schematically shows a method to **A)** stabilize a lipid bilayer membrane on a solid support and **B)** inhibit formation of a lipid bilayer membrane on a solid support.

[0018] **Figure 3.** QCM-D monitoring of pH-dependent vesicle adsorption onto aluminum oxide. Graphs depict resonance frequency (Δf) and energy dissipation (ΔD) shifts as function of time as black and grey curves, respectively. **(A)** at pH 8, there was minimal vesicle adsorption. **(B)** at pH 6, vesicles adsorbed reversibly. **(C)** at pH 4, vesicles adsorbed irreversibly. **(D)** at pH 2, a planar lipid bilayer was formed.

[0019] **Figure 4.** Morphology Diagram of Model Membrane Platforms on Silicon Oxide. It is generally known in the art that lipid vesicles adsorb and rupture to form a planar bilayer on silicon oxide. However, the interaction between lipid vesicles and silicon oxide may be controlled. Using the prescribed methods to either promote or hinder this interaction, self-assembly of one of many different types of model membranes can occur on silicon oxide. Since the isoelectric point of silicon oxide is ~ 2 , the observed behavior demonstrates that the double-layer electrostatic force is insufficient by itself to control the interaction between vesicles and the substrate. Specifically, bilayer

formation may occur in the presence of electrostatic repulsion. Rather, the steric-hydration force can modulate this interaction, and strong steric-hydration force can inhibit bilayer formation even in cases of electrostatic attraction using, e.g., positively charged lipid vesicles. In turn, controlling the steric-hydration force interaction can be mediated by the prescribed method which tunes the steric-hydration force to be within an acceptable range.

[0020] **Figure 5.** QCM-D monitoring of vesicle adsorption onto titanium oxide pretreated with calcium ions. The graph depicts the resonance frequency (Δf) shift as a function of time. Before the measurement, the titanium oxide substrate was pretreated by incubation at room temperature in an aqueous buffer solution of CaCl_2 of the following molar concentration: 0, 10, 25, 100 or 250 mM, followed by rinsing with calcium-free aqueous solution.

DETAILED DESCRIPTION OF THE INVENTION

[0021] A method to control the interaction between lipid assemblies and inorganic materials was developed, which is based on an integrated framework to analyze the interaction of lipid assemblies on solid supports through three contributing interfacial forces, specifically the van der Waals force, double-layer electrostatic force and steric-hydration force. The van der Waals force is an attractive force between the planar lipid bilayer and the oxide film substrate. Hence, it always contributes positively to stabilization of solid-supported lipid assemblies. By contrast, the electrostatic double-layer interaction energy can be either repulsive or attractive, and hence its contribution to the total interaction energy is variable. The steric-hydration force is always repulsive, but the magnitude of the force varies and this is a key contributor to balance the other two forces to provide thermodynamic stabilization for the system.

[0022] Taken together, this framework provides a model which has three key contributions. First, it validates empirical findings that attractive electrostatic forces are important but not essential or sufficient for promoting vesicle rupture on solid supports. Second, it demonstrates that the steric-hydration force is also important to control the interaction of lipid assemblies with solid supports. Third, it demonstrates for the first time that the balance of these forces is the main contributor, primarily governed by the

steric-hydration force, to dictate how lipid assemblies interact with solid supports. Electrostatic forces, attractive, repulsive, or negligible depending on design scheme, are insufficient without requisite steric-hydration force conditions to control lipid adsorption. This knowledge provides design principles that can promote or inhibit the formation of planar bilayers and/or vesicle adsorption on a wide range of different solid supports.

[0023] Accordingly a first aspect of the invention relates to a method of controlling adsorption of lipid vesicles onto a solid support comprising: tuning the steric-hydration force of the lipid vesicles and the surface of the solid support such that (i) the lipid vesicles rupture and form a bilayer membrane structure on the solid support; or (ii) the lipid vesicles are stably adsorbed to the solid support; or (iii) the adsorption of the lipid vesicles onto the solid support is inhibited.

[0024] The term "steric-hydration force", as used in this connection, is a strong short range, typically repulsive, force between polar surfaces separated by a thin layer (<3nm) of water and/or ions and decays quasi-exponentially with the surfaces' spacing. It can be characterized by the decay length λ . The steric-hydration force may be tuned between a decay length of 0.1 and 0.6 nm by adjusting the spatial density and size of functional groups on the surface of the solid support. A weak steric-hydration force has a weakened magnitude of repulsion having less repulsion and is characterized by a shorter decay length towards 0.1 nm. A strong steric-hydration force has a strengthened magnitude of repulsion having greater repulsion and is characterized by a larger decay length towards 0.6 nm. The steric-hydration force is independent of the IEP.

[0025] In various embodiments, the method further comprises tuning the electrostatic forces between the surface of the lipid vesicles and the surface of the solid support. In various embodiments, both the electrostatic force, which is preferably attractive, and the steric-hydration force, which is typically repulsive, are finely tuned such that the desired form of lipid adsorption, including resisting the lipid adsorption, is achieved.

[0026] The term "electrostatic force", as used in this connection, refers to a force between electrical charges, for example on the surface of objects, which can be

attractive in case of opposite charges or repulsive in case of the same charges. Preferably, the solid support surfaces used in connection with the methods described herein have a positive charge, while the lipid vesicles are preferably negatively charged. This results in the electrostatic force between lipid vesicles and the solid support surface being attractive.

[0027] Conditions that induce attractive electrostatic force (strongly stabilizing) and weak steric-hydration force (weakly destabilizing) promote the formation of a two-dimensional, planar lipid bilayer. Conditions that induce repulsive electrostatic forces (strongly destabilizing) and strong steric-hydration force (strongly destabilizing) promote the formation of an intact vesicle layer (or complete inhibition of vesicle adsorption) because the total interaction energy is not strong enough to promote vesicle rupture (or vesicle adsorption).

[0028] The inventors have found that the magnitude and direction of the electrostatic force can be controlled by the solution pH and the ionic strength. The magnitude and direction of this force depend on the solution pH relative to the isoelectric point (IEP), the isoelectric point being an intrinsic material property corresponding to the pH value at which the surface has a net charge of about zero. Solution pH values below the IEP lead to positive net surface charges, and solution pH values above the IEP lead to negative net surface charges. Ionic strength controls the magnitude of the force insofar as that greater ionic strength attenuates the surface charge. Adjusting these conditions is useful for optimizing control over lipid adsorption onto solid supports.

[0029] In various embodiments the steric-hydration force of the solid support is tuned by controlling the pH. This adjustment influences the protonation state of functional groups on the surface and may influence bonding patterns between functional groups and between functional groups and water molecules. The pH control does not adjust the density of functional groups on the surface. Similarly, if desired, also the electrostatic forces may, in cases, be tuned by pH control depending on the IEP and the properties of the material. The pH control is typically done by changing the pH of the solution in which the lipid vesicles are provided and which is brought into contact with the solid support surface. The pH change can be done by any means known in the art and specifically includes the addition of an acid or a base. The pH change can be

monitored by known means, for example by means of a pH measuring electrode. The desired pH can for example be achieved by titrating the solution with an acid (for pH decrease) or a base (for pH increase) while monitoring the change via a pH electrode. In various embodiments, the tuning of the steric-hydration force and, optionally, the electrostatic forces is achieved by setting the pH to about 1.5 to 4.5, preferably pH 2 to 3.

[0030] In various other embodiments that can be used in alternative to the pH change described above but also in combination therewith, the steric-hydration force and, optionally, also the electrostatic force of the solid support are tuned by pretreating the surface of the solid support. The pretreatment changes the properties of the surface with respect to the hydration properties. Typically, this change is caused by modification of surface exposed functional groups of the support material either by chemical (such as by reaction with another agent) or by physical (such as irradiation, heating, etc.) means. In some cases, pretreatment may change the protonation state of functional groups on the surface and may influence bonding patterns between functional groups and between functional groups and water molecules. Negatively charged functional groups may cause dipole alignment of water molecules leading to a strong repulsive steric-hydration force. Uncharged functional groups may cause hydrogen-bonded water molecules leading to a weak repulsive steric-hydration force. In other cases, pretreatment may change the spatial density of functional groups on the surface and affect the formation of hydration layers on the surface.

[0031] In various embodiments, the pretreatment of the support surface may include techniques such as, without limitation, thermal annealing, chemical titration, exposure to oxygen plasma, chemically modifying functional groups, treatment with a reducing or oxidizing agent, treatment with a catalyst, exposure to ultraviolet light, or the non-covalent attachment of ions to the solid support. The protonation state of the functional group, the spatial density of the functional group, and ions attached to the functional group are some of the factors involved in mediating the magnitude of the steric-hydration force.

[0032] As mentioned above, generally a solid support already has functional groups on its surface and modifying these functional groups by any of the pretreatments

mentioned herein can affect the steric-hydration force which in turn affects the interaction between the lipid vesicles and/or the surface of the solid support.

[0033] The type of functional groups that are present on the support surface depend on the material of the support and typically include, without being limited thereto, any one or more of hydroxyl (-OH), carboxyl (-COOH), carbonyl (-C(=O)-), amine (-NR₂), sulfhydryl (-SH) or phosphate (-PO₄²⁻) groups.

[0034] In various embodiments the pretreatment has the effect of reducing the spatial density of the functional groups on the surface. Such pretreatments may include exposure to oxygen plasma or exposure to ultraviolet light that may reduce the number of functional groups on the surface. Heat treatment such as thermal annealing may cause dissociation of the surface functional groups. Similarly chemical titration may actually remove surface functional groups.

[0035] Without wishing to be limited to any particular theory, it is however assumed that the surface functional groups create an energy barrier related to the repulsive steric-hydration force and reducing the spatial density of functional groups on the surface effectively decreases the strength of the repulsive steric-hydration force permitting the formation of a lipid bilayer on the solid support.

[0036] In various embodiments the pretreatment comprises protonating or deprotonating functional groups on the surface of the solid support. Where the pretreatment comprises protonating the surface functional groups a hydrogen bonding network is formed. The surface functional groups may be protonated by reducing agents or catalysts.

[0037] In various non-limiting embodiments, the ions non-covalently attached to the solid support surface are selected from the group consisting of magnesium, calcium and strontium.

[0038] Such ions may be non-covalently attached to the surface of the substrate by incubating the surface with an aqueous solution of the respective metal salts such as calcium chloride that will result in attachment of the calcium ions to the functional

groups on the surface of the solid support.

[0039] Without wishing to be limited to any particular theory, it is however assumed that this increases the decay length of the steric-hydration force preventing lipid vesicle absorption on the solid support.

[0040] In various embodiments the solid support comprises or consists of a material that is selected from metalloids, metalloid oxides, metals, metal oxides, and semiconductors. Alternatively, the solid support may have a surface or surface layer consisting of any one of these materials.

[0041] Metalloid oxides, as used herein, include silicon oxides and materials containing them, such as glass, silica and quartz.

[0042] Suitable metals for the solid support include, without limitation titanium, aluminum, and gold.

[0043] Metal oxides that can be used include, without limitation, aluminum oxide (alumina) and titanium oxide (titania).

[0044] Semiconducting materials that may be used for the solid support include, without limitation, silicon and indium tin oxide.

[0045] In preferred embodiments, the solid support material is a metal oxide, preferably aluminum oxide or titanium oxide.

[0046] In principle, the solid support may have any form or structure. It is however preferred that it includes a planar structure. Accordingly, the solid support may have the form of a plate or disk.

[0047] In various other embodiments the solid support comprises or consists of a particle, in particular a micro- or nanoparticle, preferably a nanoparticle. The nanoparticles may have a size between 2 - 400 nm, or 40 - 100 nm in diameter and are typically essentially spherical.

[0048] The nanoparticle may be suitable for use in an animal body such as a human body. In various embodiments the nanoparticle is suitable for drug delivery to an animal body.

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[0049] In various embodiments the lipid vesicles are provided in form of an emulsion in an aqueous solvent. It is to be understood that while throughout the present disclosure reference is made to lipid vesicles that the invention is not limited to such vesicles, but that other lipid assemblies can similarly be used. For example, other ordered lipid structures, like lamellar, cubic or tubular structures, or lipid aggregates may be equally suitable. It is thus understood that when reference is made herein to "lipid vesicles", this is by way of illustration and not limitation and that the term may include lipid assemblies in general.

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[0050] In various embodiments the lipid vesicles are liposomes. The liposomes are preferably artificially prepared spherical vesicles composed of lipids. The liposomes may have a mean diameter in the range of 10-100nm, preferably 50-80nm or more preferably 60-70nm. Preferably, the liposomes are unilamellar with a single lipid bilayer.

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[0051] The lipids forming the lipid vesicles, in particular the liposomes, may be selected from any suitable lipids and include well-known lipids, such as, for example, phospholipids, in particular glycerophospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, phosphatidylinositol and/or phosphatidylserine, or sphingolipids. While these are preferred lipids, any other lipid known to be suitable to form lipid vesicles/liposomes may similarly be used either individually or in combination, for example in combination with the aforementioned phospholipids.

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[0052] Examples for other lipids or lipid-like compounds that may be used include, without limitation, triglycerides, waxes, isoprenoids, in particular steroids, such as cholesterol, fatty acids, fatty alcohols and lipopolysaccharides.

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[0053] The lipids are typically characterized in that they comprise a polar or electrically charged, preferably (at neutral pH, i.e. about pH = 7) negatively charged head group

and an unpolar, uncharged tail group, the latter typically being a hydrocarbon, such as alkyl or alkenyl group.

[0054] The lipids used may be natural or synthetic in origin. While naturally occurring lipids, either isolated from natural sources or synthetically prepared, are preferred, it is also possible to use artificial lipids, for example lipids that have been modified compared to their natural counterparts.

[0055] In various embodiments, the lipid vesicles further comprise non-lipid components, such as proteins or peptides. Proteins or peptides that are to be used are preferably membrane-bound or membrane-associated proteins. Accordingly, the proteins and/or peptides may comprise lipid-like modifications, such as isoprenoid modifications, typically farnesyl or geranylgeranyl anchors, membrane-spanning domains or structures, or other domains or structures that facilitate membrane interaction by hydrophobic interactions.

[0056] In various embodiments the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by pretreating the solid support to reduce the magnitude of the repulsive steric-hydration force and, optionally, promote electrostatic attraction, preferably both. This provides a support surface that promotes lipid vesicle adhesion and subsequent rupture, such that the lipid vesicles form a stabilized bilayer membrane structure on the solid support. Generally, the formation of such a bilayer structure may include some deformation of the vesicles upon adsorption that leads to fusing of the vesicles and subsequent self-assembly of a lamellar bilayer structure on the support surface.

[0057] To achieve such an bilayer formation-promoting support surface, which is also referred herein as a surface which stabilizes bilayer membrane structures, the pretreatment may be selected from the group consisting of thermal annealing, chemical titration, exposure to oxygen plasma, exposure to ultraviolet light, protonating functional groups on the surface of the solid support, treatment with a reducing agent, or treatment with a catalyst. The pretreatment is believed to have the effect of stabilizing the formation of a lipid bilayer on the solid support (Figure 2A).

[0058] In various embodiments, the presently described methods aim at providing surfaces that resist bilayer formation or lipid vesicle adsorption in general. In these cases, it is desirable that the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by pretreating the solid support in order to increase the magnitude of the steric-hydration force and, optionally, increase electrostatic repulsion such that adsorption of the lipid vesicles onto the solid support is inhibited.

[0059] The treatment necessary to achieve such effect may aim at increasing the repulsive steric-hydration force that blocks interaction between the lipids/lipid vesicles and the surface of the solid support.

[0060] To achieve this, the pretreatment may be selected from the group consisting of chemical titration, exposure to oxygen plasma, exposure to ultraviolet light, deprotonating functional groups on the surface of the solid support, chemical titration, an oxidizing agent, or treatment with a catalyst. The pretreatment aims at increasing the spatial density of the functional groups and/or inducing ionization of the functional groups, and thus increasing the magnitude of the steric-hydration force, which in turn inhibits formation of a lipid bilayer on the solid support (Figure 2B).

[0061] In various embodiments of the invention, the control of lipid vesicle adsorption is done by control of the solution pH. Such pH control may be used for the pretreatment of the solid support but may also be used to directly tune the interaction between lipid vesicles and solid support. Where the solid support is an oxide film with hydroxyl functional groups, the steric-hydration force is weakened by decreasing the pH to a range of about pH 2-3, as at this pH the hydroxyl groups of the oxide induce the formation of a hydration layer that embodies a weaker steric-hydration force. This allows the formation of a lipid bilayer on the solid support. In contrast known methods only call for decreasing the pH slightly below the isoelectric point of the support material. This typically leads to protonation of the support surface, thus increasing its positive charge, which in turn results in increased electrostatic attraction forces. However, it has been found that these methods lead only on some substrates to the formation of a lipid bilayer on the solid support. Surprisingly, the inventors have now found that even the previously unsuited solid support materials can accommodate lipid

bilayer formation, if the pH is not only lowered slightly below the IEP, but rather to a value where the steric-hydration force can be tuned. This range is typically between about pH 2 and 3 for oxide films with hydroxyl functional groups.

5 [0062] The present invention also encompasses the structures obtainable by the methods described herein. In various embodiments, these structures are the solid supports comprising a stabilized lipid bilayer adsorbed thereon that are obtainable according to the methods described above. In other embodiments, the structures are the solid supports resisting lipid bilayer formation and/or lipid vesicle adsorption
10 thereon that are obtainable according to the methods described above.

[0063] As described above the present invention focuses on the formation thermodynamics of model membranes on solid surfaces, such as oxide substrates, to either promote or prevent the formation of the planar lipid bilayers. The insight gained
15 from the experiments allowed the formation of a lipid bilayer on aluminum oxide, which was previously not considered possible due to the properties of aluminum oxide surfaces.

[0064] In addition, the finding that surfaces that were previously assumed to strongly
20 promote lipid adsorption and bilayer formation can be treated such that they become resistant to lipid adsorption and bilayer formation, advantageously lead to a possibility for the reduction of cytotoxicity of nanoparticles, such as those intended for therapeutic uses. This reduced cytotoxicity can be attributed to the fact that the nanoparticles resist adsorption to lipids, as they occur in the cell membranes.

25 [0065] Generally, the terms used herein have, unless explicitly stated otherwise, the accepted meaning in the art.

[0066] By "comprising", as used herein, it is meant including, but not limited to,
30 whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

[0067] By "consisting of" is meant including, and limited to, whatever follows the

phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present.

[0068] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0069] The invention has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0070] The following examples are illustrative of the invention and its preferred embodiments, and are not to be taken as limiting the disclosure or claims in any way.

EXAMPLES

Example 1: Formation of a Lipid Bilayer Membrane on Aluminum Oxide.

5 **Surface Treatment.**

[0071] For all experiments, AT-cut crystals (Q-Sense) with 14 mm diameter and a 50 nm thermally evaporated aluminum oxide coating were used. Prior to experiment, each sensor crystal was treated with oxygen plasma at 80 W for 5 min (March Plasmod Plasma Etcher, March Instruments, California).

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Vesicle Preparation.

[0072] Small unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids, AL, USA) were prepared according to the extrusion method, as previously described. Extrusion was performed through a series
15 of track-etched polycarbonate membranes with decreasing 100, 50 and 30 nm pore sizes. Vesicles were prepared using a Tris buffer solution (10 mM Tris and 150 mM NaCl, pH 7.4) at a nominal lipid concentration of 5 mg•mL⁻¹. Before the experiment, the resulting small unilamellar vesicles were diluted in the appropriate Tris buffer solution to 0.125 mg•mL⁻¹ concentration, and were used within 24 hours of preparation. All
20 buffer solutions were prepared in 18.2 MΩ•cm MilliQ water (MilliPore, Billerica, MA). The average diameter of the vesicles was 60-nm, as measured by dynamic light scattering

Dynamic Light Scattering.

25 [0073] The size distribution of extruded vesicles was determined by using a 90Plus particle size analyzer (Brookhaven Instruments, Holtsville, NY, USA) that was equipped with a 658.0 nm monochromatic laser. In order to minimize the reflection effect, the scattering angle was set at 90°. Experimental data was collected by digital autocorrelator software, and all autocorrelation functions were analyzed by CONTIN
30 and nonnegatively constrained least-squares algorithms to identify multimodal distributions, if any. For experiments reported in this work, the average diameter of vesicles was 60.3 nm with a polydispersity of 0.054, which is in agreement with literature values

Quartz Crystal Microbalance-Dissipation (QCM-D) Method.

[0074] The adsorption kinetics of POPC lipid vesicles onto aluminum oxide were monitored by a Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden), as previously described (Richter & Brisson, 2005 Biophysical Journal 88 (5), 3422-3433). For all experiments, AT-cut crystals (Q-Sense) with 14 mm diameter and a 50 nm thermally evaporated aluminum oxide coating were used. Prior to experiment, each sensor crystal was treated with oxygen plasma at 80 W for 5 min (March Plasmod Plasma Etcher, March Instruments, California). Experimental data was recorded at the third (15 MHz), fifth (25 MHz) and seventh (35 MHz) overtones, and the data presented in the main text was recorded at the third overtone (15 MHz). The temperature of the measurement cell was 25.0 °C with fluctuations no greater than ± 0.5 °C.

Controlling formation of lipid bilayers.

[0075] Chemical titration strategies were employed in order to control adsorption kinetics of POPC lipid vesicles onto an aluminum oxide surface, as monitored by using the quartz crystal microbalance with dissipation (QCM-D) monitoring technique (Figure 3). Before experiment, surfaces were incubated in solutions of varying pH in order to change the protonation state of hydroxyl functional groups on the aluminum oxide surface.

[0076] The average diameter of the vesicles was 60-nm. After establishing a baseline signal, vesicles were added and changes in resonance frequency (Δf) and energy dissipation (ΔD) were monitored as functions of time. At pH 8, vesicle addition led to changes in resonance frequency and energy dissipation of -32 Hz and 2.2×10^{-6} , respectively (Fig. 3A). The $\Delta D/\Delta f$ ratio is indicative of a viscoelastic adsorbate, suggesting the adsorption of a few vesicles rather than a planar bilayer. The same experiment was also performed at pH 8.5, no vesicle adsorption was observed. This treatment prevents vesicle adsorption on the aluminum oxide support.

[0077] At pH 6, vesicle addition led to the formation of an intact vesicle adlayer (=adsorbed layer) (Fig. 3B). The maximum changes in resonance frequency and energy dissipation were -126 Hz and 8.7×10^{-6} , respectively, which are consistent with an intact vesicle adlayer. However, there was a change in the physical properties of the adlayer as flow continued. Within 15 min, the final changes in resonance frequency and

energy dissipation had reached -116 Hz and 6.3×10^{-6} , respectively. Reversible vesicle adsorption suggests relatively weak vesicle-substrate interactions. At pH 4, vesicle addition led to changes in resonance frequency and energy dissipation of only -121 Hz and 6.3×10^{-6} , respectively (Fig. 3C). The vesicle adlayer was irreversibly adsorbed, suggesting stronger vesicle-substrate interaction as compared to pH 6.

[0078] Vesicle adsorption was next investigated at pH 2 (Fig. 3D). Two-step adsorption kinetics were observed, which corresponded to planar bilayer formation via vesicle rupture after reaching a critical coverage. Upon adsorption, the critical vesicle coverage corresponded to changes in resonance frequency and energy dissipation of -40 Hz and 3.0×10^{-6} , respectively. Afterwards, vesicles began to rupture and the final changes in resonance frequency and energy dissipation were -24 Hz and 0.6×10^{-6} , respectively. Taken together, the QCM-D measurement results indicate that chemical titration influences the interaction between vesicles and aluminum oxide.

Equation Validation Data

[0079] Experimental data for controlling lipid adsorption onto silicon oxide and aluminum oxide as representative materials. Mathematical equations describe how to control the interaction via modulating the steric-hydration force on titanium oxide as a representative material. A general equation is described below:

W_1 = van der Waals force

W_2 = Double-layer electrostatic force

W_3 = Steric-hydration Force

W_{Total} = Total Interaction Energy

W_0 = Threshold Energy

$$W_{\text{Total}} = W_1 + W_2 + W_3$$

[0080] The methods described herein may control W_{Total} to be greater or less than W_0 in order to promote attractive or repulsive interactions between lipid membranes and oxide materials. W_1 and W_2 are fixed parameters under physiological conditions and W_3 may be freely tuned within a certain range (decay length, λ_0 , between 0.1 and 0.6 nm).

[0081] The isoelectric point of aluminum oxide is reported to be approximately 8.8. Below this pH, the substrate has a positive charge while the vesicles are negatively charged. Nevertheless, the vesicles only weakly adsorb and do not form a planar bilayer until pH 2. Changes in the double-layer electrostatic force are insufficient to explain the experimental findings on aluminum oxide. It is likely that the steric-hydration force is the primary factor controlling adsorption of lipid vesicles onto aluminum oxide, and hence this finding enables additional strategies to adjust the steric-hydration force.

[0082] Since the electrostatic force is not the main factor, the pH effect is likely due to either the van der Waals force or steric-hydration force. The van der Waals force is always attractive and its strength is independent of pH so the steric-hydration force is likely the main factor to explain the pH effect on aluminum oxide.

[0083] Vesicles interact with aluminum oxide, going from strong steric hydration force to a weak steric-hydration force in the following self-assembly pathway: (i) barrier; (ii) reversibly adsorbed vesicle adlayer; (iii) irreversibly adsorbed vesicle layer; and (iv) planar bilayer.

Example 2: Lipid Membrane Platforms on Silicon Oxide.

[0084] Using the methods as described in Example 1 to either promote or hinder lipid self-assembly of many different type of model membranes on silicon oxide—a prototypical surface known in the art to promote bilayer formation under standard conditions—a scale was formed. Since the isoelectric point of silicon oxide is ~2, the observed behavior demonstrates that the double-layer electrostatic force is insufficient by itself to control the interaction between vesicles and the substrate (figure 4). In every case the pH that forms a lipid bilayer on the solid support is above the IEP. Rather, the steric-hydration force can modulate this interaction. In turn, controlling this interaction can be mediated by the prescribed method which tunes the steric-hydration force to be within an acceptable range.

Example 3: Resistance of Treated Titanium Oxide to Liposome Adsorption.

Surface Treatment.

[0085] For all experiments, AT-cut crystals (Q-Sense) with 14 mm diameter and a 50 nm thermally evaporated titanium oxide coating were used. Prior to experiment, each sensor crystal was treated with oxygen plasma at 80 W for 5 min (March Plasmod Plasma Etcher, March Instruments, California).

Vesicle Preparation.

[0086] Small unilamellar vesicles composed of 70 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 30 mol% 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (POPS) (Avanti Polar Lipids, AL, USA) were prepared according to the extrusion method, as previously described. Extrusion was performed through a series of track-etched polycarbonate membranes with decreasing 100, 50 and 30 nm pore sizes. Vesicles were prepared using a Tris buffer solution (10 mM Tris and 150 mM NaCl, pH 7.4) at a nominal lipid concentration of 5 mg•mL⁻¹. Before experiment, the resulting small unilamellar vesicles were diluted in the appropriate Tris buffer solution to 0.125 mg•mL⁻¹ concentration, and were used within 24 hours of preparation. All buffer solutions were prepared in 18.2 MΩ•cm MilliQ water (MilliPore, Billerica, MA).

QCM-D Method.

[0087] The adsorption kinetics of POPC lipid vesicles onto titanium oxide were monitored by a Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden), as previously described above. For all experiments, AT-cut crystals (Q-Sense) with 14 mm diameter and a 50 nm thermally evaporated titanium oxide coating were used. Prior to experiment, each sensor crystal was treated with oxygen plasma at 80 W for 5 min (March Plasmod Plasma Etcher, March Instruments, California). Experimental data was recorded at the third (15 MHz), fifth (25 MHz) and seventh (35 MHz) overtones, and the data was recorded at the third overtone (15 MHz). The temperature of the measurement cell was 25.0 °C with fluctuations no greater than ±0.5 °C.

Controlling lipid bilayers.

[0088] Titanium oxide surfaces were pre-incubated with calcium chloride solutions in order to deposit divalent calcium ions on the substrate. Depending on the molar concentration of calcium chloride in solution, the layer coating could be adjusted in

order to impart a steric-hydration force. After the incubation step, 70/30 mol% POPC/POPS lipid vesicles were added to the substrate and the vesicle adsorption process was tracked by QCM-D monitoring as shown in Figure 5. The calcium layer imparts a positive charge so it was initially hypothesized that the attached calcium ions would facilitate rupture of the negatively charged vesicles.

[0089] To test this hypothesis, we first performed a control experiment in which the vesicles were added to a titanium oxide substrate that was not treated with calcium chloride before experiment. The average diameter of the vesicles was 70-nm. In this case, vesicle adsorption led to formation of an adsorbed vesicle layer with a final frequency shift of -104 Hz. By contrast, there was no frequency shift associated with vesicle adsorption to surfaces already containing attached calcium ions from deposited solutions of 10, 25, 100, or 250 mM calcium chloride despite an attractive electrostatic interaction. This indicates that attached calcium ions inhibit vesicle adsorption.

CLAIMS

1. A method of controlling adsorption of lipid vesicles onto a solid support comprising: tuning the steric-hydration force of the lipid vesicles and the surface of the solid support such that
- 5 (i) the lipid vesicles rupture and form a bilayer membrane structure on the solid support; or
- (ii) the lipid vesicles are stably adsorbed to the solid support; or
- 10 (iii) the adsorption of the lipid vesicles onto the solid support is inhibited.
2. Method according to claim 1, further comprising tuning the electrostatic force between the surface of the lipid vesicles and the surface of the solid support.
- 15 3. Method according to claim 1 or 2, wherein the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by controlling the pH.
4. Method according to claim 1, wherein the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by
- 20 pretreating the surface of the solid support.
5. Method according to claim 4, wherein the pretreatment comprises thermal annealing, chemical titration, exposure to oxygen plasma, chemically
- 25 modifying functional groups, treatment with a reducing or oxidizing agent, treatment with a catalyst, exposure to ultraviolet light, or the non-covalent attachment of ions to the solid support.
6. Method according to claim 5, wherein the pretreatment comprises
- 30 protonating or deprotonating functional groups on the surface of the solid support.
7. Method according to claim 5 or 6, wherein the functional groups that are chemically modified comprise hydroxyl, carboxyl, carbonyl, amine, sulfhydryl

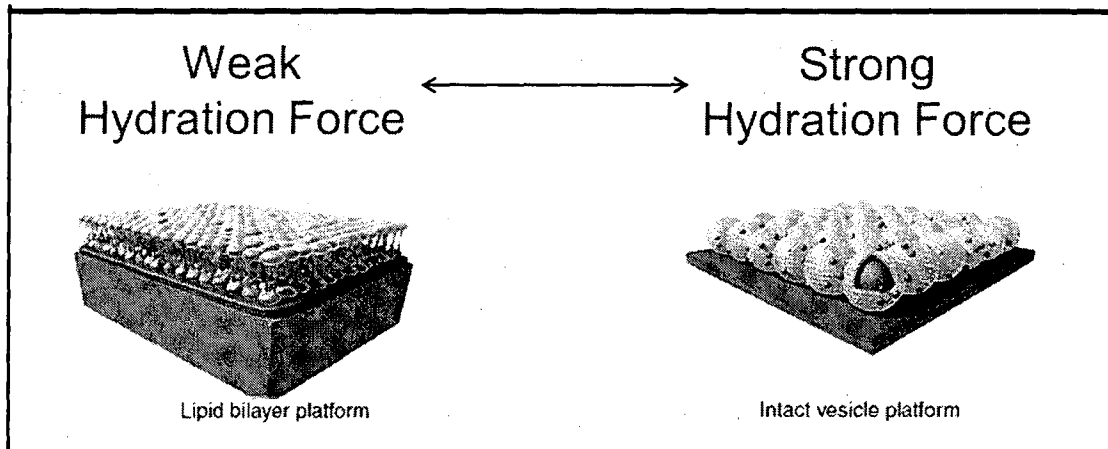
or phosphate groups.

- 5 8. Method according to claim 5, wherein the ions non-covalently attached to the solid support surface are selected from the group consisting of magnesium, calcium and strontium.
- 10 9. Method according to any one of claims 1 to 8, wherein the solid support comprises or consists of a metalloid or oxide thereof, a metal, a metal oxide, or a semiconductor.
- 15 10. Method according to claim 9, wherein the metal oxide is selected from the group consisting of aluminum oxide and titanium oxide.
11. Method according to claim 9, wherein the metalloid oxide is selected from the group consisting of glass, silica and quartz.
12. Method according to claim 9, wherein the metal is selected from the group consisting of titanium, aluminum and gold.
- 20 13. Method according to claim 9, wherein the semiconductor is silicon or indium tin oxide.
14. Method according to any one of claims 1 to 13, wherein the solid support comprises a planar structure.
- 25 15. Method according to any one of claims 1 to 13, wherein the solid support comprises a nanoparticle.
16. Method according to any one of claims 1 to 15, wherein the lipid vesicles are liposomes.
- 30 17. Method according to any one of claims 1 to 16, wherein the lipid vesicles are provided in form of an emulsion in an aqueous solvent.

18. Method according to any one of claims 1 to 17, wherein the lipid vesicles comprise or are composed of phospholipids.
- 5 19. Method according to any one of claims 1 to 18, wherein the lipid vesicles further comprise proteins or peptides.
- 10 20. Method according to any one of claims 1-19, wherein the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by pretreating the surface of the solid support said pretreatment reducing the steric-hydration force such that the lipid vesicles rupture and form a stabilized bilayer membrane structure on the solid support, wherein the pretreatment is selected from the group consisting of thermal annealing, chemical titration, exposure to oxygen plasma, exposure to ultraviolet light, protonating functional groups on the surface of the solid support, a reducing agent, or treatment with a catalyst.
- 15 21. Method according to any one of claims 1-19, wherein the steric-hydration force and, optionally, the electrostatic force of the solid support are tuned by pretreating the surface of the solid support said pretreatment increasing the steric-hydration force such that adsorption of the lipid vesicles onto the solid support is inhibited, wherein the pretreatment is selected from the group consisting of chemical titration, exposure to oxygen plasma, exposure to ultraviolet light, deprotonating functional groups on the surface of the solid support, chemical titration, an oxidizing agent, or treatment with a catalyst.
- 20 22. Solid support comprising a stabilized lipid bilayer adsorbed thereon obtainable according to the method of any one of claims 1 to 20.
- 25 23. Solid support resisting lipid vesicle adsorption thereon obtainable according to the method of any one of claims 1 to 19 and 21.
- 30

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FIGURE 1



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FIGURE 2 A

i)

Solid Support

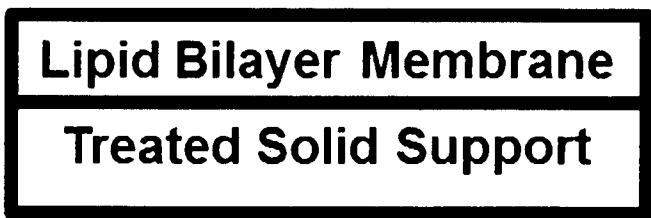
Surface Treatment

ii)

Treated Solid Support

Add Lipid Solution

iii)

Lipid Bilayer Membrane**Treated Solid Support**

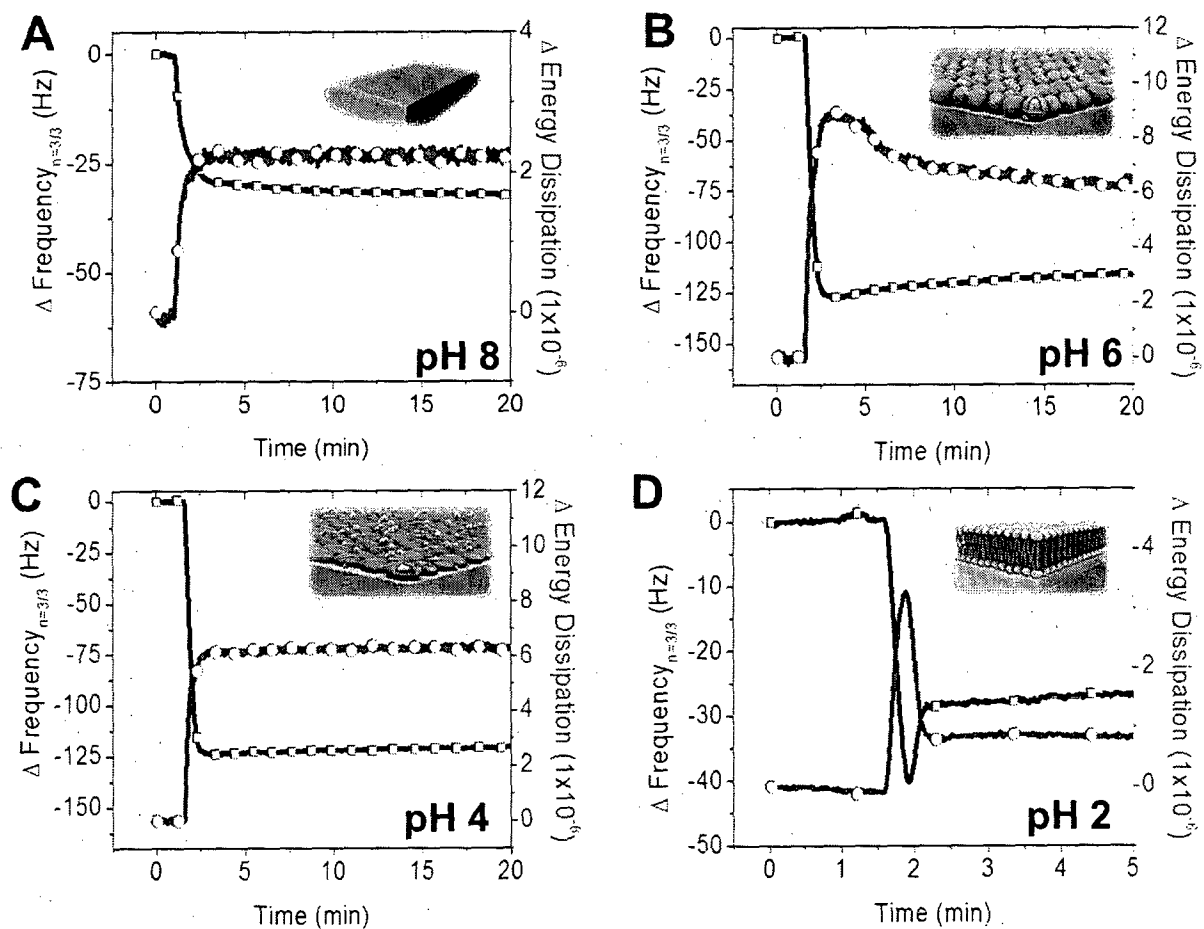
3/6

FIGURE 2 B

i)**Solid Support****Surface Treatment****ii)****Treated Solid Support****Add Lipid Solution****iii)****Treated Solid Support**

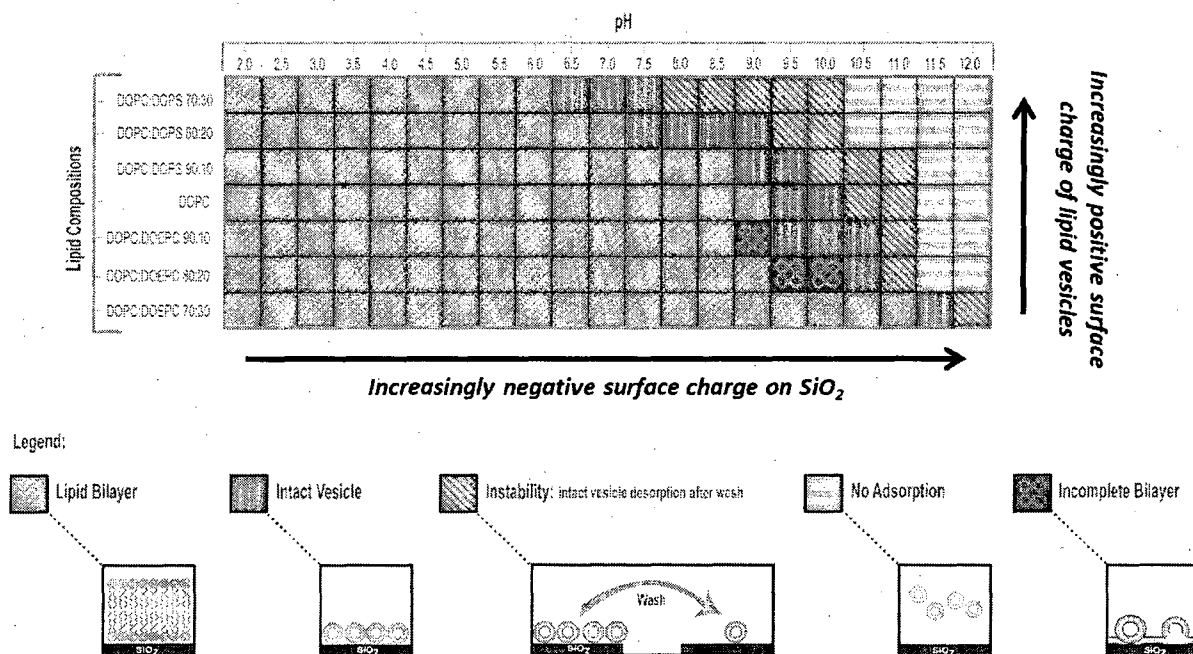
4/6

FIGURE 3



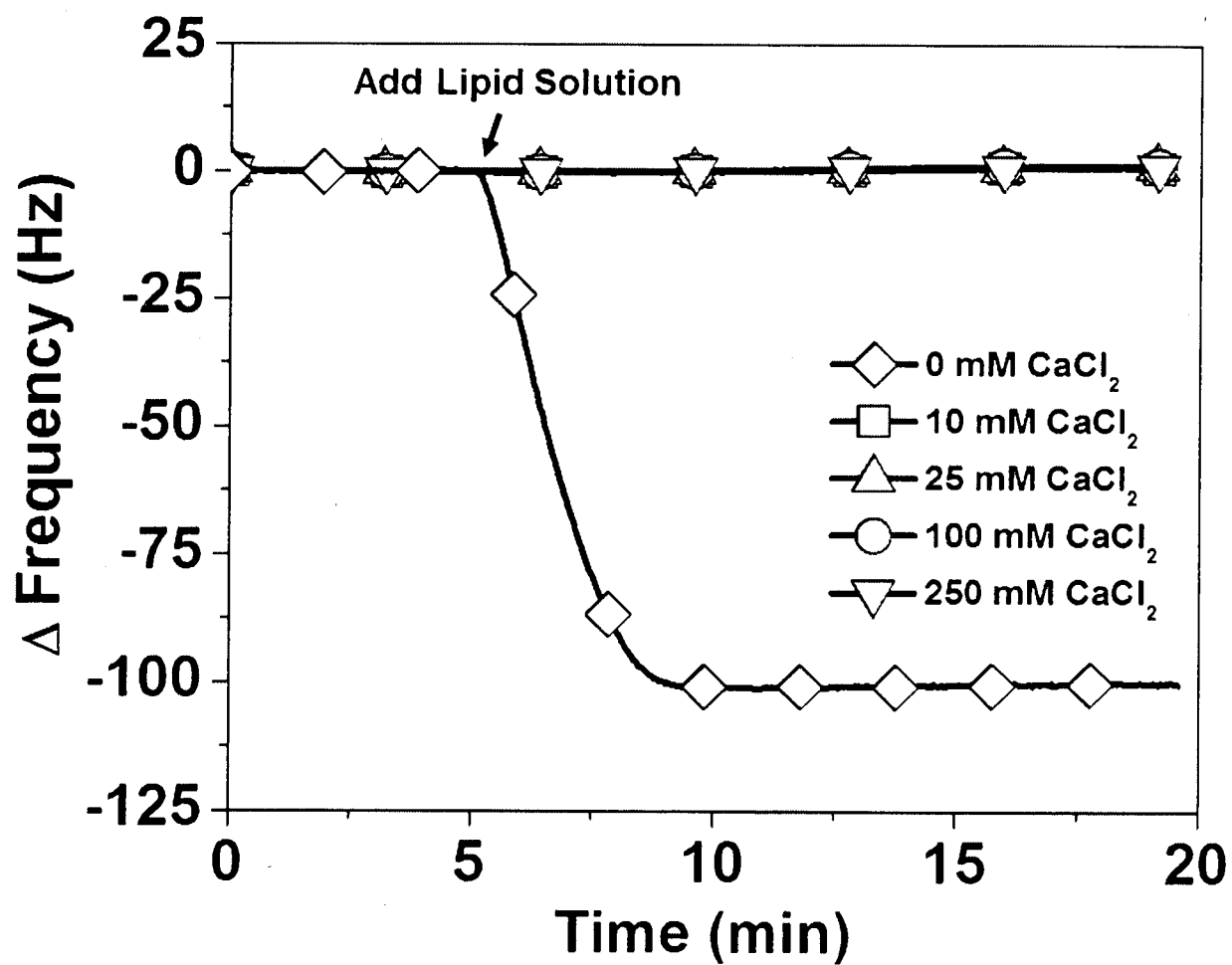
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FIGURE 4



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FIGURE 5



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2014/000449

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/127 (2006.01) A61K 47/44 (2006.01) G01N 33/543 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPODOC and WPI/IPC/CPC A61K47 or A61K9 and Keywords (lipid, hydration, tuning, pH, bi_layer, adsorption, prepar+, support, membrane+) & like terms.

Cluster - SPATEN-English full text databases TXTAU1 TXTCA1 TXTEP1 TXTGB1 TXTSG1 TXTUS0 TXTUS1 TXTUS2 TXTUS3 TXTUS4 TXTUS5 TXTWO1 WPI EPODOC and Keywords (lipid, hydration, tuning, pH, bi_layer, adsorption, prepar+, support, membrane+) & like terms.

EPODOC and WPI: (membrane+ or bi_layer or BLM or monolayer) and Keywords ((steric 8d (dehydration or hydration or water)), vesicle, tuning, adsorption) & like terms.

Transferred results to cluster: Cluster - SPATEN-English full text databases TXTAU1 TXTCA1 TXTEP1 TXTGB1 TXTSG1 TXTUS0 TXTUS1 TXTUS2 TXTUS3 TXTUS4 TXTUS5 TXTWO1 WPI EPODOC and Keywords ((steric 8d (dehydration or hydration or water)), vesicle, tuning, adsorption) & like terms. GoogleScholar search and keywords (liposome adsorption tuning bilayer "steric hydration"). GooglePatent search and keywords (dehydration adsorption "solid support" "lipid membrane" pH tuning). AusPat ("cho, nam-joon" IN INV).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 22 October 2014		Date of mailing of the international search report 22 October 2014	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer John Deuis AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832146	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/SG2014/000449
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8211712 B2 (Cho et al.) 03 July 2012 See figure 1A; and col. 2 lines 37 to 43.	22, 23
A	WO 2002/071944 A1 (SAAVEDRA, Scott, S. [US/US]) 19 September 2002 See figure 7; and page 11 lines 18 to 24.	1
A	WO 2001/026800 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]) 19 April 2001 See page 8 line 30 to page 9 line 12.	1
A	WO 1996/010178 A1 (PHARMACIA BIOSENSOR AB) 04 April 1996 See the abstract.	1
A	"Contributions of hydration and steric (entropic) pressures to the interactions phosphatidylcholine bilayers: experiments with the subgel phase."; McIntosh, T. J., and S. A. Simon; Biochemistry. 1993, 32:8374-8384. See whole document.	1

Form PCT/ISA/210 (fifth sheet) (July 2009)

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

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

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

See Supplemental Box for Details

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

Remark on Protest

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

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

☐ Claims 1-21 are directed to a method of controlling adsorption of lipid vesicles onto a solid support and a solid support thereof comprising tuning the steric-hydration force of the lipid vesicles and the surface of the solid support. The feature of controlling adsorption of lipid vesicles by tuning the steric-hydration force of the lipid vesicles and the surface, is specific to this group of claims.

☐ Claim 22 is directed to a solid support comprising a stabilized lipid bilayer adsorbed thereon. The feature of a solid support comprising a stabilized lipid bilayer adsorbed thereon, is specific to this group of claims.

☐ Claim 23 is directed to a solid support resisting lipid vesicle adsorption thereon. The feature of a solid support resisting lipid vesicle adsorption thereon, is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is a solid support comprising lipid vesicles adsorbed thereon. However this feature does not make a contribution over the prior art because it is disclosed in:

US 8211712 B2 (Cho et al.) 3 July 2012

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature present in the claims and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/SG2014/000449	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 8211712 B2	03 July 2012	US 8211712 B2	03 Jul 2012
		JP 2008538280 A	23 Oct 2008
		WO 2006110350 A2	19 Oct 2006
WO 2002/071944 A1	19 September 2002	US 2006014013 A1	19 Jan 2006
WO 2001/026800 A1	19 April 2001	AU 5462498 A	22 Jun 1998
		AU 6468501 A	26 Nov 2001
		AU 8099901 A	18 Feb 2002
		AU 2003256703 A1	09 Feb 2004
		CA 2388173 A1	19 Apr 2001
		CA 2408351 A1	22 Nov 2001
		CA 2417569 A1	14 Feb 2002
		CA 2492899 A1	29 Jan 2004
		EP 0941474 A1	15 Sep 1999
		EP 0941474 B1	29 Mar 2006
		EP 1218097 A1	03 Jul 2002
		EP 1287351 A2	05 Mar 2003
		EP 1305614 A2	02 May 2003
		EP 1543317 A2	22 Jun 2005
		JP 2005534010 A	10 Nov 2005
		JP 4493496 B2	30 Jun 2010
		JP 2003511679 A	25 Mar 2003
		JP 2003533211 A	11 Nov 2003
		JP 2004506208 A	26 Feb 2004
		US 6228326 B1	08 May 2001
		US 6503452 B1	07 Jan 2003
		US 2003096418 A1	22 May 2003
		US 6699719 B2	02 Mar 2004
		US 2002009807 A1	24 Jan 2002
		WO 0188182 A2	22 Nov 2001
		WO 0212546 A2	14 Feb 2002
		WO 9823948 A1	04 Jun 1998
		WO 2004010074 A2	29 Jan 2004
		WO 2004010074 B1	01 Jul 2004
WO 1996/010178 A1	04 April 1996	EP 0784793 A1	23 Jul 1997
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.
Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/SG2014/000449	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		EP 0784793 B1	18 Dec 2002
		JP H10507126 A	14 Jul 1998
		US 5922594 A	13 Jul 1999
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			