

## Employing an Amphipathic Viral Peptide to Create a Lipid Bilayer on Au and TiO<sub>2</sub>

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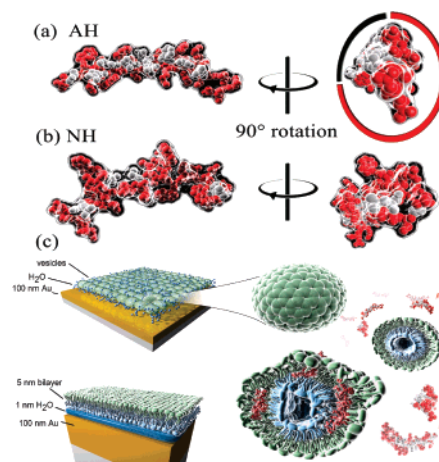
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Supported lipid bilayers formed by the fusion of small unilamellar vesicles onto silicon oxide<sup>1,2</sup> or organic film-modified surfaces<sup>3</sup> enable the biofunctionalization of inorganic solids,<sup>3</sup> such as semiconductors, gold-covered surfaces,<sup>4</sup> and optoelectronic lab-on-a-chip devices.<sup>5</sup> They have proven valuable in the study of the characteristics and behavior of membrane-bound proteins, membrane-mediated cellular processes, protein–lipid interactions, and biological signal transduction.<sup>1,2,6</sup> Because of the complexity of biomembranes, there is a clear need to develop model membrane systems, where one or a few membrane components can be isolated and studied. In addition, a wide range of available surface-sensitive techniques can be used to study natural biological systems effectively by supporting model membranes on a solid surface. Applications of supported membranes on solid surfaces potentially include biosensors,<sup>5</sup> programmed drug delivery devices,<sup>7</sup> surface modification of medical implants,<sup>6</sup> and the production of catalytic interfaces.<sup>7</sup>

In order to mimic natural biological systems, researchers have employed the vesicle fusion method to form supported bilayers on substrates, such as glass, mica, self-assembled monolayers, and quartz. However, it has proven problematic to create planar bilayers on certain preferred solid substrates, such as gold and TiO<sub>2</sub>. For example, scientists have attempted to modify gold surfaces using self-assembled monolayers (SAMs),<sup>5</sup> which may require special synthesis, but the structure of the SAMs that are formed may not be well-defined.<sup>8</sup>

Here, we have utilized an amphipathic  $\alpha$ -helix (AH) peptide derived from the N-terminus of the hepatitis C virus NS5A protein<sup>19</sup> (Figure 1) to create planar bilayers on gold and TiO<sub>2</sub> solid supports. In this work, we successfully demonstrate that an AH peptide destabilizes and ruptures the leaflets of intact lipid vesicles, allowing the ruptured vesicles to fuse and form planar bilayers on preferred solid substrates, such as gold and TiO<sub>2</sub>.

In order to systematically investigate the ability of AH peptides to rupture vesicles, we initially tested vesicles extruded through 30 nm polycarbonate etch-tracked (PEC) membranes on a gold surface in the absence of the AH peptide, then introduced the peptide to induce bilayer formation. It has been well-documented that the high polarizability of the gold surface maximizes the attractive potential, which is the driving force that enables vesicles to remain intact and stable on a gold support.<sup>1,2,9,10</sup> When vesicles adsorb, a large amount of trapped water exists within the intact vesicles as well as between vesicles adsorbed on the surface. This trapped water is able to dissipate a large amount of energy (Figure 2a), unlike the water that rests on top of a bilayer. Due to the viscoelastic nature of the films, the large energy dissipation



**Figure 1.** The proposed schematic of vesicle rupture and fusion in forming supported bilayers on gold or TiO<sub>2</sub> solid surfaces. Space-filling models of (a) AH peptide (sequence: SGSWLRDWDWICTVLTDFKTWLQSKLDYKD) and (b) NH peptide (sequence: SGSWLRDDW DWECTVLTDDK-TWLQSKLDYKD). (c) Speculative model of rupturing process by AH peptide.

( $\Delta D \sim 8 \times 10^{-6}$ ) leads to an underestimation of the layer thickness using the Sauerbrey equation.<sup>11</sup> This underestimate can be corrected by applying the viscoelastic model described by Voinova et al.<sup>11–13</sup> After introducing 0.05 mg·mL<sup>-1</sup> of the AH peptide to the vesicle layer on the gold surface (Figure 2-1b), a large frequency decrease ( $\sim 40$  Hz) and a dissipation increase ( $\sim 14 \times 10^{-6}$ ) occur within 2 min. This result, although unexpected, provides a clue in our effort to understand the molecular interactions that occur when the AH peptide and lipid bilayer interact.

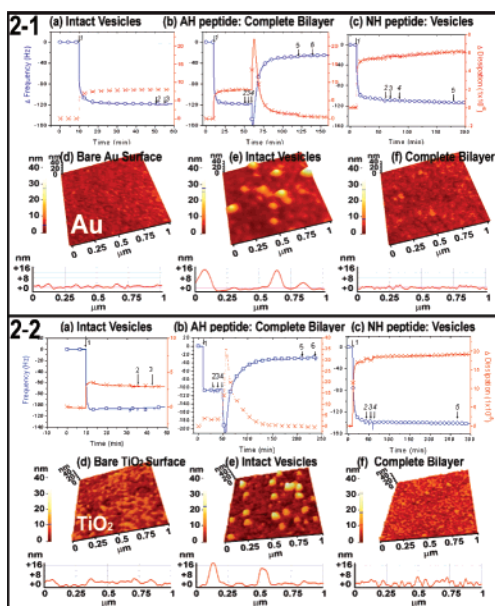
One possible explanation for this phenomenon is that the AH peptide first creates an instability on the vesicle surface by electrostatic interaction. This may lead to expansion of the vesicles<sup>14,15</sup> as well as to the creation of microvilli<sup>16,17</sup> (finger-like structures) on the outer leaflet of the vesicles.<sup>18</sup> This could explain both the large changes in frequency due to the presumed expansion of vesicles as well as the increase in dissipation ascribable to both expansion and microvilli structure. The most exciting results are that the final frequency shift relative to the initial state was  $25.5 \pm 0.5$  Hz, and the final dissipation value was only  $0.08 \times 10^{-6}$  (Figure 2-1b), both values corresponding to a complete bilayer. According to the Sauerbrey equation, from which the bilayer thickness can be calculated, these QCM-D parameters indicate a transition of the vesicles to a thin and rigid bilayer film as a result of the action of the AH peptide.

To further verify that the AH peptide, with its amphipathic  $\alpha$ -helical structure, destabilized the vesicles, we repeated the experiments under the same conditions using a modified peptide in which three charged amino acids were introduced into the

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**Figure 2.** Analysis of rupturing and fusion process of bilayer formation from vesicles on Au (2-1) and  $\text{TiO}_2$  (2-2) by QCM-D and AFM. (2-1a–c) Change in QCM resonant frequency and dissipation as a function of time for novel bilayer formation process on gold surface. (a)  $\Delta f(t)$  (blue curve) and  $\Delta D(t)$  (red curve) show vesicle adsorption on oxidized gold surface. After 10 min (arrow 1) of stabilizing frequency signal, POPC vesicle solution was injected into the liquid cell. After 50 and 55 min (arrows 2 and 3), the same buffer was used for two washes. (b) At 60 min (arrow 4), the amphipathic  $\alpha$ -helix peptide (AH peptide) solution was added (0.05 mg/mL) to the intact vesicles on the gold surface. After 120 and 140 min (arrows 5 and 6), the same buffer was used for two washes, and the stability of the bilayers on the gold surface was observed. (c) The effect of the NH peptide was studied in an analogous manner to the AH peptide as described for Figure 2-1b. (2-1d–f) AFM images demonstrating intact vesicles rupturing and spreading after addition of AH peptide on Au surface. The images are presented in the Height mode: (d) bare Au surface; (e) vesicles (0.1 mg/mL) deposited on Au surface; (f) after treatment with the AH peptide (0.05 mg/mL). (2-2) Analogous experiments to 2-1 except performed on a  $\text{TiO}_2$  surface (see Supporting Information for details).

hydrophobic face of the AH peptide,<sup>19</sup> disrupting its amphipathic nature and destroying its helical structure. As shown in Figure 2-1c, this nonhelical peptide (termed NH peptide) does not destabilize intact vesicles since there are no changes in  $\Delta f$  and  $\Delta D$  after injection of the NH peptide. This indicates that the amphipathic helical structure is one of the essential characteristics in vesicle destabilization.

While the QCM-D results demonstrate the process of kinetic changes from adsorption of vesicles to the formation of bilayers, we utilized atomic force microscopy (AFM) in order to confirm and directly display rupture of vesicles and bilayer formation by the destabilizing agent, the AH peptide. AFM examined the bare Au surface as a control with  $R_q$  of  $1.13 \pm 0.21$  nm ( $\pm$ SE,  $n = 8$ ). Vesicles (0.1 mg/mL) were carefully added through the injection system, incubated for 30 min, and thoroughly rinsed three times with Tris buffer. Intact vesicles were clearly identified by AFM and the average  $R_q$  increased to  $2.49 \pm 0.32$  nm ( $\pm$ SE,  $n = 8$ ), as shown in Figure 2-1e.

The AFM images in Figure 2-1f show the effect of the AH peptide on the vesicles as a destabilizing agent, which we examined by injecting the peptide (0.05 mg/mL) and incubating the solution for 2 h prior to scanning the images. These images clearly confirm the QCM-D data, indicating that vesicles ruptured as a result of the treatment with AH peptide. The average  $R_q$  of  $1.67 \pm 0.12$  nm ( $\pm$ SE,  $n = 15$ ) indicated that the roughness became similar to the bare Au surface (average  $R_q$  of  $1.32 \pm 0.25$  nm ( $\pm$ SE,  $n = 8$ )), as

expected for a bilayer. Grain analysis identified no vesicle-like structures ( $P \leq 0.001$ ), indicating that the AH peptides ruptured vesicles to form bilayers. These results correlate with the QCM-D kinetic data shown in Figure 2-1b. An identical experiment has been done on  $\text{TiO}_2$  (Figure 2-2) and has shown similar results.

In conclusion, we describe a novel method wherein an amphipathic  $\alpha$ -helical peptide can be used to destabilize a collection of intact vesicles, transforming them into a planar bilayer structure on various substrates, such as gold and titanium oxide. Both the frequency and dissipation values from QCM-D analysis indicate the formation of complete synthetic biomembranes in the configuration of two-dimensional complex fluids. The superior properties of gold and titanium oxide substrates can now be utilized in various applications, such as biosensor and lab-on-a-chip devices utilizing the benefits of phospholipid membranes. Using this new approach to form lipid bilayers may eliminate the need to use less desirable substrates and allow researchers to take advantage of the electrical properties of gold and the biocompatibility of titanium oxide surfaces. This novel process to form bilayers on gold and  $\text{TiO}_2$  shifts the focus to a material-based solution and away from surface-dependent constraints that had limited scientists to inferior substrates, such as silicon oxide solids, on which to fabricate supported lipid bilayers.

**Acknowledgment.** This work was supported in part by NASA 1025-679-1-REDAL and the Center for Polymer Interfaces and Macromolecular Assemblies (CPIMA), an NSF-MRSEC. This work was also supported by a Burroughs Wellcome Fund Career Award (to J.S.G.) and 1RO1-DK064223 and KRF-2004-015-c00426 to S.-J.C.

**Supporting Information Available:** Material and methods, and characterization and investigation of different size vesicles that rupture on Au and  $\text{TiO}_2$  by QCM-D and AFM. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0701412