

DEVELOPMENT OF BIOMIMETIC SURFACES BY VESICLE FUSION

Dimitrios Stroumpoulis*, Alejandro Parra, Jill Gliem and Matthew Tirrell
Department of Chemical Engineering, University of California Santa Barbara
Santa Barbara, CA, 93106

ABSTRACT

In this study fusion of lipid-peptide amphiphile vesicles is employed to form biomimetic coating materials that can modify cellular adhesion and growth on solid substrates. Ellipsometry has been used to monitor vesicle fusion at different concentrations on hydrophilic surfaces and to identify adsorption as its limiting step. Incorporation of small amounts of RGD containing peptide amphiphiles in cell adhesion resistant PC lipid membranes is shown to promote adhesion and growth only when a sufficiently long spacer is used to control the distance of the peptide ligand from the surface.

1. INTRODUCTION

Cellular events such as adhesion, migration, and proliferation are governed by interactions between molecules within the extracellular matrix (ECM) and receptors (integrins) on the cell surface. Biological cell membranes are lipid bilayers comprising phospholipids, glycolipids, proteins, glycoproteins and proteoglycans (Gutberlet, T. and Katsaras, J., 2001; Lodish, H. F., 1995). Supported planar bilayers (SPBs) represent a simplified model of the cell membrane and are increasingly used in bioengineering. The membrane consists of amphiphilic molecules, polymeric entities that possess both hydrophobic and hydrophilic moieties. In an effort to mimic the interactions of biological membranes peptide amphiphiles (Berndt, P., Fields, G. B., and Tirrell, M., 1995) can be synthesized encoded with the appropriate specific message via a peptide (Aota, S., Nomizu, M., and Yamada, K. M., 1994; Pierschbacher, M. D. and Ruoslahti, E., 1984) and equipped with hydrocarbon molecular regions to drive the assembly of the molecules into bilayers or vesicles. SPBs comprised of peptide amphiphiles have been shown to successfully and specifically engage receptor molecules. (Dillow, A. K., Ochsenhirt, S. E., McCarthy, J. B., Fields, G. B., and Tirrell, M., 2001).

SPBs and techniques to form them in a consistent and controlled fashion are becoming necessary as micro-scale level devices are developed for various technological applications (Tirrell, M., Kokkoli, E., and Biesalski, M., 2002). The lab-on-a-chip approach coupled to microfluidics and bio-MEMS devices will demand the development of new nanofabrication techniques (Texter,

J. and Tirrell, M., 2001). Promising alternatives include self-assembly, bottom-up processes such as the formation of SPBs from vesicle solutions, hence a basic understanding of such phenomena is essential to proceed to possible applications and to identify its limitations. SPB deposition from vesicle solutions has the potential of forming membrane mimics on flat and textured surfaces. This is an advantage with respect to Langmuir-Blodgett technique, which can only be effective on flat surfaces. A second advantage is the possibility of surface functionalization using composition arrays which would allow for the preparation of surface-composition gradients (Kam, L. and Boxer, S. G., 2000).

The kinetics of SPB formation from vesicle solutions has been studied in the past using quartz crystal microbalance (Keller, C. A. and Kasemo, B., 1998; Williams, L. M., Evans, S. D., Flynn, T. M., Marsh, A., Knowles, P. F., Bushby, R. J., and Boden, N., 1997) and surface plasmon resonance (Keller, C. A., Glasmaster, K., Zhdanov, V. P., and Kasemo, B., 2000). Other techniques that have been used to detect the formation of SPBs by this process are neutron reflectivity (Koenig, B. W., Gawrisch, K., Krueger, S., Orts, W., Majkrzak, C. F., Berk, N., and Silverton, J. V., 1996), atomic force microscopy (Egawa, H. and Furusawa, K., 1999; Reviakine, I. and Brisson, A., 2000), confocal fluorescence correlation spectroscopy and nulling ellipsometry (Benes, M., Billy, D., Hermens, W. T., and Hof, M., 2002). In these studies, rarely have there been any attempts to look at the kinetics of the process while making an effort to interpret the results in terms of mass transport models (Csucs, G. and Ramsden, J. J., 1998; Hubbard, J. B., Silin, V., and Plant, A. L., 1998). Such models, commonly used in chemical engineering applications, are important to the extent that they can give a quantitative and qualitative understanding of the processes under investigation. Computer simulations of the formation of SPBs from vesicle solutions can also give insight into the mechanics of the process (Zhdanov, V. P., Keller, C. A., Glasmaster, K., and Kasemo, B., 2000).

In the present work we observe SPB formation from vesicle solutions on hydrophilic silicon substrates with the optical technique of phase modulated ellipsometry, and the results are further interpreted in terms of a previously published mass transfer model (Hubbard, J. B., Silin, V., and Plant, A. L., 1998). Conclusions about the kinetics are

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extracted from fitting the model equations to the experimental data. Our understanding of the vesicle fusion process is then applied to develop biomimetic interfaces between solid substrates and cells. Peptide amphiphiles containing the arginine-glycine-aspartate (RGD) cell adhesion promoting amino acid sequence are used to control cell binding and growth on supported membranes.

2. KINETICS OF VESICLE FUSION

2.1. Vesicle Fusion

Vesicle fusion is a flexible method for depositing supported lipid bilayers on both flat and textured topologies. Important theoretical (Lipowsky, R. and Seifert, U., 1991; Seifert, U., 1997) and experimental (Benes, M., Billy, D., Hermens, W. T., and Hof, M., 2002; Csucs, G. and Ramsden, J. J., 1998; Egawa, H. and Furusawa, K., 1999; Hubbard, J. B., Silin, V., and Plant, A. L., 1998; Johnson, J. M., Ha, T., Chu, S., and Boxer, S. G., 2002; Keller, C. A. and Kasemo, B., 1998; Keller, C. A., Glasmaster, K., Zhdanov, V. P., and Kasemo, B., 2000; Koenig, B. W., Gawrisch, K., Krueger, S., Orts, W., Majkrzak, C. F., Berk, N., and Silverton, J. V., 1996; Lingler, S., Rubinstein, I., Knoll, W., and Offenhausser, A., 1997; Reimhult, E., Hook, F., and Kasemo, B., 2003; Reviakine, I. and Brisson, A., 2000; Williams, L. M., Evans, S. D., Flynn, T. M., Marsh, A., Knowles, P. F., Bushby, R. J., and Boden, N., 1997) work has been conducted in an effort to understand this useful process.

The formation of a SPB from a vesicle solution is believed to proceed through the following mechanism (Seifert, U., 1997): Vesicles adsorb on the surface and subsequently rupture or fuse with each other before rupturing. In either case single bilayer disks are formed, which will then grow and coalesce to form a continuous SPB. Vesicles adsorb on the surface and subsequently rupture to form single bilayer disks, which will then grow and coalesce to a continuous SPB.

There has been extensive experimental work on vesicle fusion by the quartz crystal microbalance-dissipation technique (Reimhult, E., Hook, F., and Kasemo, B., 2003). It was concluded that vesicles adsorb irreversibly on SiO₂, Si₃N₄, TiO₂, oxidized Pt and oxidized Au, but vesicle to bilayer transformation occurs only for the first two after a critical vesicle coverage is reached, while in the other cases the vesicles remain intact but deformed. Deformation of vesicles on SiO₂ is found to be larger than on TiO₂ something which is interpreted as stronger attraction to the surface. Larger vesicles are more deformed by the surface interaction than smaller ones in both cases. The different polarizabilities and isoelectric points of the surfaces mentioned above are considered

important parameters governing their distinct behavior as far as vesicle surface interaction is concerned.

Same type of experiments (Reimhult, E., Hook, F., and Kasemo, B., 2003) have also demonstrated that the presence of a critical vesicle coverage threshold required for rupture is independent of the vesicle size. This is inconsistent with a mechanism requiring vesicles to fuse with each other on the surface until a critical radius is reached for rupture to take place. Instead it suggests that the critical vesicle coverage required for rupture is essentially a steric condition (packing of the vesicles), which enhances vesicle deformation.

The rupture point on the vesicle is probably where the radius of curvature is smallest, most likely located where the membrane curves away from the surface. At that point, adhesion to the surface and deformation due to steric interactions between vesicles increases the lateral tension Σ , within the membrane. In principle (Lipowsky, R. and Seifert, U., 1991) the formation of a pore can reduce the vesicle's energy at sufficiently large values of Σ since a pore of radius L within a planar membrane has energy $F_{\text{pore}} = 2\pi L \Sigma_e - \pi L^2 \Sigma$, where Σ_e is the line tension due to hydrophobic effects. Once a pore is formed L will tend to increase until Σ decreases enough (lateral tension is relieved) and equilibrium is reached ($dF_{\text{pore}}/dL = 0$).

Vesicle to bilayer transition is shown to be thermally activated (Reimhult, E., Hook, F., and Kasemo, B., 2003) in that the critical vesicle coverage required for rupture is inversely proportional to temperature. Bilayer formation can be prevented by adsorption at sufficiently low temperature (e.g. below the melting point of vesicles). Outward osmotic gradient also favors rupture by causing compressive stress on the vesicles. An inverse gradient on the other hand has a smaller effect on rupture.

An important conclusion that was drawn out of fusion experiments with proteoliposomes is that the inner surface of the vesicle becomes primarily the leaflet of the bilayer facing the surface (Salafsky, J., Groves, J. T., and Boxer, S. G., 1996).

Given all the aforementioned experimental observations the possible steps of the vesicle fusion mechanism are summarized in Figure 1. Initially vesicles diffuse from the bulk close to the surface. The diffusion coefficient can be approximated by the Stokes-Einstein model for diffusion of hard spheres. Once close to the surface adsorption begins and proceeds until a critical concentration of adsorbed vesicle mass is reached. Essentially this requirement reflects the deformation of vesicles due to adsorption and steric interactions between them. The small radius of curvature at the point that the vesicles curve away from the surface initiates a mechanism of pore formation that is propagated by the

“catalytic” action of hydrophobic edges, based on the same principle that pre-rupture vesicles are believed to catalyze vesicle fusion (Johnson, J. M., Ha, T., Chu, S., and Boxer, S. G., 2002).

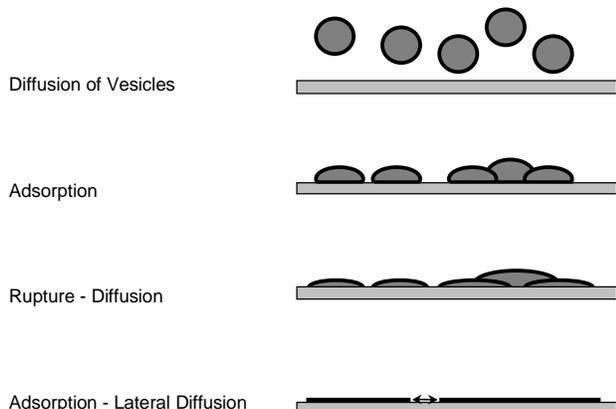


Figure 1: Possible mechanism of vesicle fusion.

The ruptured vesicles begin to diffuse close to the surface. The diffusion coefficient can no longer be approximated by the Stokes-Einstein model but its value is affected by the dominant close range attractive van der Waals forces. Finally adsorption of ruptured vesicles (or bilayer islands) takes place and diffusion of bilayer fragments on the surface ensures minimization of the hydrophobic edge energy.

2.2. Results

The observed values of the experimental quantity Δy (see Data Analysis section) were always in the range of 0.032 to 0.034 for fusion of DMPC vesicles. These are sufficiently small to make equation (5) a valid approximation. Substitution of the experimental values of Δy along with the refractive indices for silicon (3.88) and water (1.33) leave two values in equation (6) undefined, namely, the film thickness Δz and its refractive index n .

The refractive index of DMPC lipid molecules arranged in a bilayer is not known, but it might be different from that in a bulk solution (Petrov, J. G., Pfohl, T., and Mohwald, H., 1999). As a first approximation it is reasonable to expect it to be in the same range as the refractive indices of alkanes and fatty acids of similar chain lengths in film state. Substitution in (6) of values for n ranging from 1.44 to 1.50 gives values of 56 to 39 Å for Δz . This is close to the expected thickness of a single lipid bilayer (40-50 Å).

On this basis and for later convenience when performing the kinetic analysis it was assumed that the final surface coverage in all experiments was 100 percent.

Since the headgroup area for DMPC is known (Koenig, B. W., Strey, H. H., and Gawrisch, K., 1997) to be 59 \AA^2 , the maximum surface coverage then corresponds to 3.8 mg m^{-2} and this value was used to scale all the experimental data. Again we emphasize that the exact value of surface coverage can only be obtained if the refractive index of a DMPC bilayer is known. Given the uncertainty behind this number we can only get an estimate of the final surface coverage but this does not interfere in any way with the kinetic study performed here.

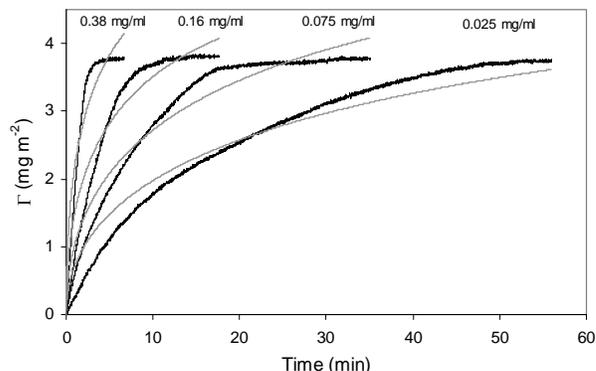


Figure 2: Supported lipid bilayer formation kinetics. Experimental data (dark lines) and fitting of the diffusion-limited case of solution to the mass transport model, (equation 14, light lines).

The experimental data after processing as well as the fitting of the diffusion limited mass transport model (equation 15) and the adsorption limited one (equation 16) are presented in Figure 2 and 3 in the form of surface coverage (mg m^{-2}) as a function of time (min). It can be readily seen that the rate of coverage increases with the lipid concentration in the bulk and that good fitting is achieved for the adsorption limited case.

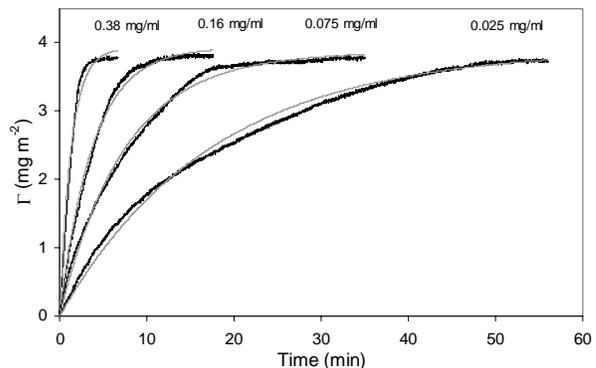


Figure 3: Supported lipid bilayer formation kinetics. Experimental data (dark lines) and fitting of the adsorption-limited case of the mass transport model, (equation 16, light lines).

Table 1 and 2 show the corresponding parameters for the fitting, namely the maximum surface coverage Γ_m , the

adsorption rate constant K and the mass diffusion coefficient D . Not surprisingly, the values for Γ_m are close to the maximum coverage used to scale the curves. Also we would expect K to be independent of concentration and that is the case, indicating that a simple model can be used to describe this process.

Table 1: Fitting parameters for the diffusion-limited case of the mass transport model (equation 15).

Lipid concentration C_0 , mg ml ⁻¹	Maximum surface coverage Γ_m , mg cm ⁻²	Mass diffusion coefficient D , cm ² s ⁻¹
0.4	6.0×10^{-4}	6.6×10^{-9}
0.2	5.5×10^{-4}	1.6×10^{-8}
0.08	6.1×10^{-4}	3.0×10^{-8}
0.02	5.7×10^{-4}	1.2×10^{-7}

Based on the fittings of the two limiting cases the process seems to be adsorption limited or that the rate of diffusion of vesicles close to the surface is faster than the rate they get adsorbed as ruptured vesicles.

Table 2: Fitting parameters for the adsorption-limited case of the mass transport model (equation 16).

Lipid concentration C_0 , mg ml ⁻¹	Maximum surface coverage Γ_m , mg cm ⁻²	Adsorption rate constant K , cm s ⁻¹
0.4	3.9×10^{-4}	1.3×10^{-5}
0.2	3.9×10^{-4}	1.1×10^{-5}
0.08	3.9×10^{-4}	1.2×10^{-5}
0.02	3.9×10^{-4}	1.5×10^{-5}

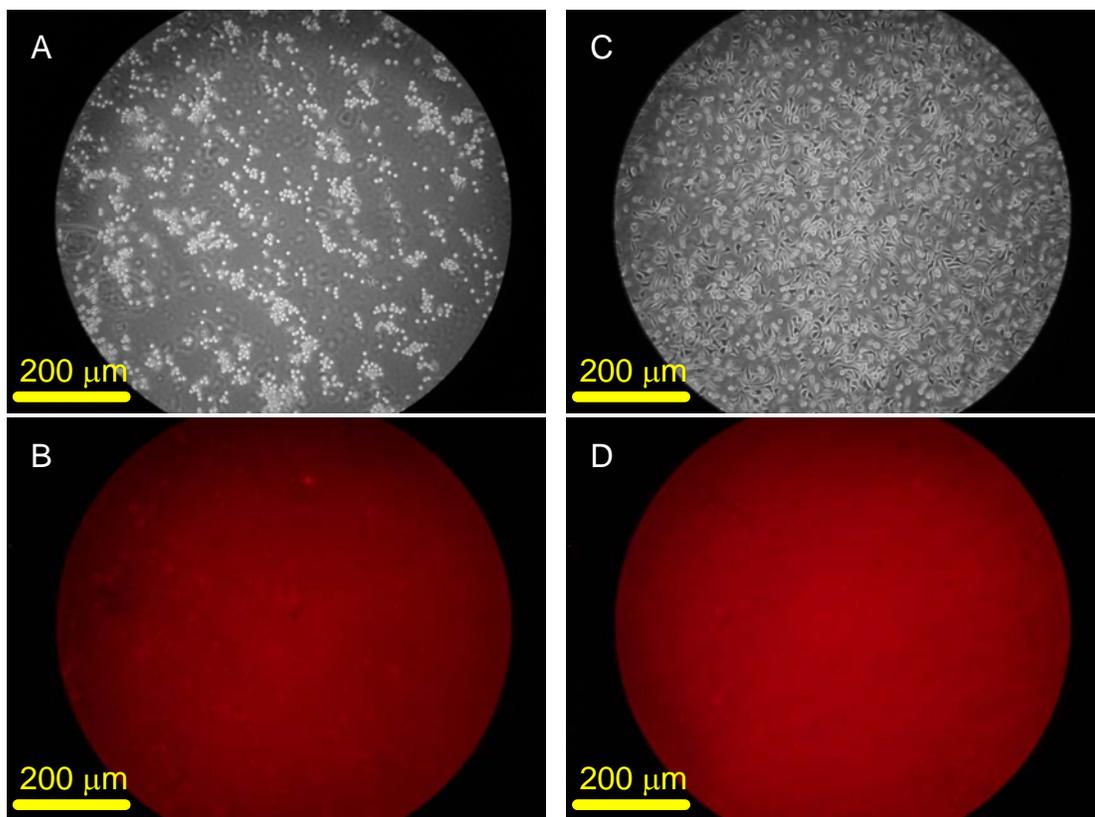


Figure 4: NIH3T3 cells cultured on supported membranes after 4 hours. Membranes in (A) & (B) are 99% EggPC and 1% Texas Red DHPE while in (C) & (D) 94% EggPC, 5% (C₁₆)₂-Glu-PEO-GRGDSP and 1% Texas Red DHPE. Pictures (A) and (C) are in optical mode, while (B) and (D) in fluorescent mode on the same surface spot respectively.

3. CELL ADHESION ON PA MEMBRANES

The ability of RGD containing peptide amphiphiles to modify the cell adhesion resistant behavior of phosphatidylcholine (PC) lipid supported membranes deposited on borosilicate coverslips was investigated. Mouse fibroblast cells were incubated on three types of

surfaces: egg-phosphatidylcholine (egg-PC) membranes and egg-PC membranes containing 5% mol of either (C₁₆)₂-Glu-C₂-GRGDSP or (C₁₆)₂-Glu-PEO-GRGDSP. In all cases the presence of 1% mole of Texas Red labeled lipids allowed for bilayer visualization. Only membranes containing the (C₁₆)₂-Glu-PEO-GRGDSP amphiphile promoted cell adhesion while the two other types of surfaces effectively blocked cell adhesion. The results

obtained from a typical adhesion experiment are presented in Figure 4. Focal adhesion sites were formed after 4 hours when supported membranes contained $(C_{16})_2$ -Glu-PEO-GRGDSP. On the other hand cells seeded on Egg-PC membranes or $(C_{16})_2$ -Glu- C_2 -GRGDSP containing membranes did not adhere, as inertial motion under mild shaking revealed, but tended to clump together.

In all cases a membrane was present between the cells and the substrate as it's evident from the pictures taken in fluorescent mode. However, the stretched morphology of the cells on the $(C_{16})_2$ -Glu-PEO-GRGDSP containing membranes can only be explained by a scenario involving cells partially penetrating the membrane to form focal adhesion sites with the glass substrate, since a fluid bilayer could not possibly support the tensile forces exerted by the cells (Groves, J. T., Mahal, L. K., and Bertozzi, C. R., 2001). The RGD motif likely initiates the cascade of events that enables the cell to spread by specifically interacting with the integrins,

located on the cell surface. The presence of the RGD amino acid sequence in the membrane does not guarantee cell adhesion as proven by the inability of the shorter $(C_{16})_2$ -Glu- C_2 -GRGDSP amphiphile to alter the adhesion resistant nature of the Egg-PC bilayer. It appears that the accessibility of the ligand is equally important. Effective control over the vertical distance of the integrin specific peptide from the ambient lipid molecules in the membrane environment can actuate the receptor engagement.

CONCLUSION

Ellipsometry was used to study the kinetics of vesicle fusion on hydrophilic surfaces and adsorption was determined to be the limiting step of the process. Biomimetic membranes deposited by vesicle fusion on glass coverslips are shown to promote cell adhesion and growth only when the binding specific ligands are effectively presented to cells.

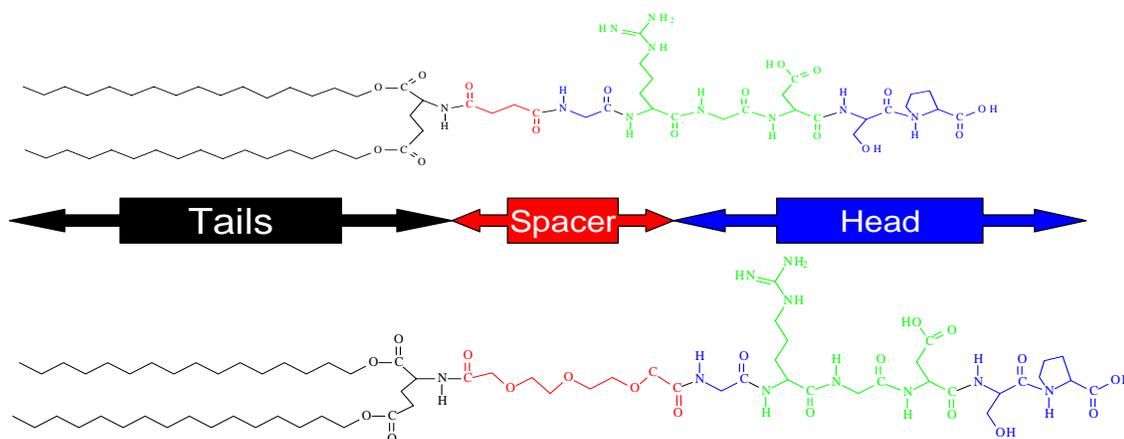


Figure 5: Chemical structure of the peptide amphiphiles used in this study: $(C_{16})_2$ -Glu- C_2 -GRGDSP and $(C_{16})_2$ -Glu-PEO-GRGDSP.

EXPERIMENTAL PROTOCOL

Peptides were purchased from SynPep (Dublin, CA). Dialkyl ester lipid tails (C_{16}) were connected to the peptide headgroup by a L-glutamic acid linker and a succinic anhydride (Berndt, P., Fields, G. B., and Tirrell, M., 1995; Dillow, A. K., Ochsenhirt, S. E., McCarthy, J. B., Fields, G. B., and Tirrell, M., 2001) or a 3,6,9-trioxaundecanedioic acid (polyethylene oxide) spacer. The general molecular structure of the peptide amphiphiles is shown in Fig. 5.

Small unilamellar vesicle (SUV) solutions were prepared by mixing one or more of the following: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or 1- α -Phosphatidylcholine from egg (egg-PC), purchased from Avanti Polar Lipids (Alabaster, AL), 1,2-dihexadecanoyl-

sn-glycero-3- phosphoethanolamine, triethylammonium salt (Texas Red® DHPE) purchased from Molecular Probes (Eugene, OR) and $(C_{16})_2$ -Glu- C_2 -GRGDSP or $(C_{16})_2$ -Glu-PEO-GRGDSP. 10 mg total amount of amphiphiles were dissolved in chloroform and a thin layer was formed on the walls of a test tube by rotation and nitrogen flow induced evaporation. The sample was left overnight in a vacuum chamber to ensure complete removal of the solvent. The film was then reconstituted in 6 ml of Millipore water (18 M Ω -cm), briefly vortexed and left to hydrate in a 37 °C water bath for one hour before being passed five times through a temperature controlled extruder (at 40 °C) containing a polycarbonate membrane filter with 100 nm size pores. Dynamic light scattering was used to confirm the formation of 100 nm diameter vesicles. The extruded solutions were kept at 5 °C and used within 2 weeks.

Silicon dioxide substrates were treated in piranha (70% H₂SO₄, 30% H₂O₂ v/v) for thirty minutes to oxidize organic contaminants, briefly rinsed with Millipore water and quickly placed in a 20 ml quartz cylindrical cell filled with Millipore water to minimize exposure to the ambient air. The cell was subsequently fastened on a phase modulated ellipsometer (Beaglehole Instruments) and rapidly fed with 10 ml of the desired vesicle solution through the cell inlet in an effort to minimize any possible sample loss through the cell outlet, while enhancing the mixing of the injected volume with the water in the cell. After injection ellipsometric measurements were taken at room temperature (25°C) for one hour with a time interval of one second at the Brewster angle of incidence for the silicon-water interface (71°).

Optical borosilicate cover glasses (Fisherbrand) for membrane deposition were cleaned via plasma oxidation (Harrick, NY). A 200 µl drop of a 5% w/w sodium chloride solution followed by a 100µl drop of the desired vesicle solution (at a concentration of 1.66 mg/ml) was placed in the center of the coverslip that was affixed to the bottom of a sterile petri dish with vacuum grease. A second coverslip was placed on top in a sandwich arrangement and the substrate was allowed to interact with the vesicles for 30 minutes. The petri dish was then filled with Millipore water being careful not to expose the membrane to air and the top coverslip was removed. The petri dish was flushed several times with Millipore water to remove excess vesicles and then flushed with Dulbecco's modified Eagles medium (DMEM) (1X) at a final volume of 7 ml. NIH3T3 (mouse fibroblast) cells were cultured in DMEM containing 5% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. Cells were grown in a 37 °C incubator with 5% CO₂ atmosphere. Cells were washed with a solution of 10% phosphate buffer saline (PBS) 7.2 (10X) in water, trypsinized, and resuspended in DMEM with 5% FBS at a concentration of 1,000,000 cells/ml. An average of 200,000 cells was added per sample (approx. 20,000 cells/cm²).

Imaging of the membranes and cells was performed on a Nikon Eclipse TE-200 microscope using a 10x Phase 1 DL objective. Fluorescence images were taken with the same objective in fluorescence mode using a 100 W mercury arc lamp. The supported membranes were fluorescently labeled with 1 mol % of Texas Red lipids. Images were recorded with a digital color CCD camera (Cannon model Coolpix 995).

DATA ANALYSIS

The Fresnel reflection coefficients of an interface are given by:

$$r_p = \frac{|E_p^r|}{|E_p^i|} e^{i\Delta\varepsilon_p} \quad (1)$$

$$r_s = \frac{|E_s^r|}{|E_s^i|} e^{i\Delta\varepsilon_s} \quad (2)$$

where $E_{p,s}^{r,i}$ and $\Delta\varepsilon_{p,s} = \varepsilon_{p,s}^r - \varepsilon_{p,s}^i$ correspond respectively to the electric field amplitudes and phase differences before and after reflection in the p (parallel to the plane of incident) or s (perpendicular to the plane of incident) polarizations (subindices) for the incident beam (superindices). See Figure 6.

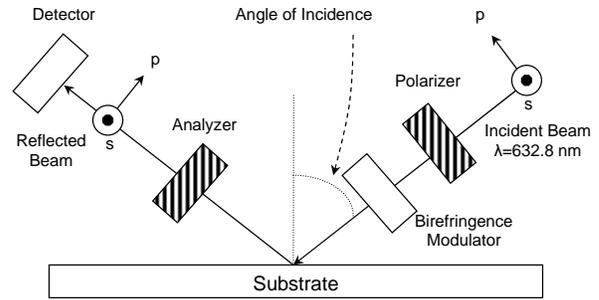


Figure 6: Phase modulated ellipsometer experimental setup.

The ratio of the complex Fresnel reflection coefficients is the most fundamental ellipsometric quantity:

$$r = \frac{r_p}{r_s} = \text{Re}(r) + i \text{Im}(r) \quad (3)$$

The measured quantity y is given by the following equation:

$$y = \text{Im}(r) \frac{2}{1 + \text{Re}(r)^2 + \text{Im}(r)^2} \quad (4)$$

This expression can be simplified due to the following facts: (1) the measurements are performed at the pseudo-Brewster angle of the system, which for nonabsorbing films implies that $\text{Re}(r) = 0$, and (2) the measured values for $\Delta y = y_{\text{final}} - y_{\text{initial}}$ are positive and small, which implies that Δy grows linearly with $\Delta \text{Im}(r)$. From these simplifications we get:

$$\Delta y = 2\Delta \text{Im}(r) \quad (5)$$

Finally, Δy can in turn be related to the thickness of the film (Δz) through (Petrov, J. G., Pfohl, T., and Mohwald, H., 1999):

$$\Delta y = \frac{2\pi\sqrt{n_1^2 + n_2^2}}{\lambda(n_1^2 - n_2^2)} \cdot \frac{(n^2 - n_1^2)(n^2 - n_2^2)}{n^2} \cdot \Delta z \quad (6)$$

where n_1 , n_2 and n are the refractive indices of the solvent, the substrate and the film, respectively, assuming an isotropic refractive index for the film.

The mass transport model used to interpret the experimental results was published by Hubbard *et al* (Hubbard, J. B., Silin, V., and Plant, A. L., 1998). and was applied for interpretation of the formation of a lipid monolayer on top of a supported hydrophobic alkanethiol monolayer already in place on the substrate.

The model is based on Fick's second law of diffusion (z is the direction normal to the surface):

$$\frac{\partial c(z,t)}{\partial t} = D \frac{\partial^2 c(z,t)}{\partial z^2} \quad (7)$$

where $c(z,t)$ is the concentration of lipids at a distance z from the surface at time t and D is the diffusion coefficient of mass.

An initial and a boundary condition are required to obtain the flux of mass on the surface:

$$c(z,t)|_{t=0} = C_0 \quad (8)$$

$$D \frac{\partial c(z,t)}{\partial z} \Big|_{z=0} = K c(z,t) \Big|_{z=0} \quad (9)$$

The first condition states that the initial concentration along the normal direction is equal to the concentration in the bulk at time zero. The second is a mixed boundary condition, which states that mass arriving on the surface is being adsorbed. The constant K represents the mass adsorption rate constant and typically it's the same constant found in the original paper by Hubbard *et al* (Hubbard, J. B., Silin, V., and Plant, A. L., 1998) under the name reorganization rate constant.

Combining equations (7), (8) and (9), the diffusive flux on the surface is obtained as a function of time:

$$J_{surf} = -D \frac{\partial c(z,t)}{\partial z} \Big|_{z=0} = K C_0 \exp\left(\frac{K^2 t}{D}\right) \operatorname{erfc}\left(\frac{K t^{1/2}}{D^{1/2}}\right) \quad (10)$$

The most important assumption in the development of this model is the existence of an imperfectly adsorbing surface. In other words mass already adsorbed on the surface prevents additional mass from being adsorbed. This can be mathematically formulated by stating that the mass accumulation on the surface per area is equal to the mass flux on the surface multiplied by the free surface area fraction:

$$\frac{d\Gamma}{dt} = J_{surf} \cdot \beta \quad (11)$$

where Γ is the surface coverage and β is the free surface area fraction, given by:

$$\beta = 1 - \frac{\Gamma(t)}{\Gamma_m} \quad (12)$$

where Γ_m is the maximum surface coverage.

An interesting characteristic of this model is that an analytical solution of equation (11) can be found (Carslaw, H. S. and Jaeger, J. C., 1986):

$$\Gamma(t) = \Gamma_m \left\{ 1 - \exp\left[\frac{-KC_0}{\Gamma_m} \left[\frac{D}{K^2} \left(\exp\left[\frac{K^2 t}{D} \right] \cdot \operatorname{erfc}\left[\left(\frac{K^2 t}{D} \right)^{1/2} \right] - 1 \right) + \frac{2}{K} \left(\frac{Dt}{\pi} \right)^{1/2} \right] \right] \right\} \quad (13)$$

Defining the characteristic time for adsorption as:

$$\tau = \frac{D}{K^2} \quad (14)$$

two limiting cases of equation (13) are possible:

For $t / \tau \gg 1$ (diffusion-limited case):

$$\Gamma(t) = \Gamma_m \cdot \left(1 - e^{-\frac{C_0}{\Gamma_m} \left(2\sqrt{\frac{Dt}{\pi}} \frac{D}{K} \right)} \right) \quad (15)$$

For $t / \tau \ll 1$ (adsorption-limited case):

$$\Gamma(t) = \Gamma_m \left\{ 1 - \exp\left(\frac{-KC_0 t}{\Gamma_m} \right) \right\} \quad (16)$$

At this point it is important to elucidate the significance of the constant K . The adsorption rate constant represents all the parameters that affect the rate of mass adsorption on the surface i.e. temperature, mass-surface attraction, osmotic pressure, etc. excluding concentration. Mass can be adsorbed in different organization schemes. For example it is possible to have adsorption of vesicles or adsorption of ruptured vesicles (bilayer fragments). On top of that and taking into account the complexity of the vesicle fusion mechanism it is likely to have partial desorption of vesicles as a intermediate step in the reorganization of adsorbed vesicles to adsorbed bilayers. Therefore it is quite fundamental to identify that K symbolizes the net adsorption of mass on the surface.

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