Interaction of Cationic Lipid Vesicles with Model Cell Membranes—As Determined by Neutron Reflectivity

P. Callow,[†,‡,§] G. Fragneto,[†] R. Cubitt,[†] D. J. Barlow,[*,‡] M. J. Lawrence,[‡] and P. Timmins[†]

Institut Laue-Langevin, 6 rue Jules Horowitz, BP 156, 38042 Grenoble Cedex 9, France, and Pharmacy Department, King's College London, The Franklin-Wilkins Building, Stamford Street, London, SE1 9NH, United Kingdom

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Transfection of cells by DNA (for the purposes of gene therapy) can be effectively engineered through the use of cationic lipid/DNA "lipoplexes", although the transfection efficiency of these lipoplexes is sensitive to the neutral "helper" lipid included. Here, neutron reflectivity has been used to investigate the role of the helper lipid present during the interaction of cationic lipid vesicles with model cell membranes. Dimethyldioctadecylammonium bromide (DDAB) vesicles were formed with two different helper lipids, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and cholesterol, and the interaction of these vesicles with a supported phospholipid bilayer was determined. DOPE-containing vesicles were found to interact faster with the membrane than those containing cholesterol, and vesicles containing either of the neutral helper lipids were found to interact faster than when DDAB alone was present. The interaction between the vesicles and the membrane was characterized by an exchange of lipid between the membrane and the lipid aggregates in solution; the deposition of vesicle bilayers on the surface of the membrane was not apparent.

Introduction

In the quest for safe and effective gene delivery vehicles, researchers have recently focused on nonviral vectors in order to circumvent the dangers inherent in using viral systems.[1,2] Cationic vesicles and polymers in particular have proved promising as they afford high gene transfection.[1] Cationic vesicles have intrinsic properties which make them attractive as vehicles for gene delivery. They are synthetic and so can be readily manufactured to drug standard; they are also potentially biodegradable, nonimmunogenic, and importantly are able to interact with DNA to promote the transfection of both replicating and nonreplicating cells.[3,4]

The vesicles manufactured solely from cationic lipids, however, have been shown to be far less effective than those made using mixtures of cationic and neutral "helper" lipids.[5] For example, if vesicles are prepared using the cationic lipid dimethyldioctadecylammonium bromide (DDAB), there is a marked improvement in transfection efficiency seen upon incorporating either 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or cholesterol (Chol). There are uncertainties as to the role(s) of the different "helper" lipids because their relative utility seems dependent on the nature of the test system employed. Although DDAB:DOPE vesicles show a high transfection efficiency in vitro, but not in vivo, DDAB:Chol vesicles show high transfection efficiency in vivo, but little activity in vitro.[6] Understanding the role of the "helper" lipids may allow for the rational design of future transfection systems.

Here we report on a study of the interactions of cationic lipid vesicles, composed of DDAB and the "helper" lipids DOPE and cholesterol, with a model cell membrane. Taking advantage of the different neutron scattering properties of hydrogen and deuterium, we have used neutron reflectivity to characterize a predominantly chain deuterated model cell membrane and then follow its interaction with hydrogenated lipid vesicles. This allows for the determination of the relative rates of interaction of the cationic vesicles containing the different "helper" lipids and the final destination of the lipid from the cationic lipid vesicles. The formation of different structures at the surface of the model membrane for the cationic lipid vesicles containing different "helper" lipids could potentially explain the different transfection efficiencies observed in vitro and in vivo for cholesterol and DOPE.

Materials and Methods

Neutron Reflectivity. In a neutron experiment, the specular reflection perpendicular to the reflecting surface, R, is measured as a function of the wave vector transfer, $Q = (4\pi/\lambda) \sin\theta$, where θ is the glancing angle of incidence and λ is the wavelength of the incident neutron beam. $R(Q)$ is related to the scattering length density (ρ) across the interface, $\rho(z)$,[7] by

$$R(Q) = (16\pi^2/Q^2)|\rho(Q)|^2$$

where ρ(Q) is the one-dimensional Fourier transform of ρ(z)

$$\rho(Q) = \int \exp(-i\kappa z)\rho(z)\, \mathrm{d}z$$

$\rho(z)$ being a function of the distance perpendicular to the interface (z).

* To whom correspondence should be addressed. Phone: +44 207 848 4827. Fax: 44 207 848 4800. E-mail: dave.barlow@kcl.ac.uk.

† Institut Laue-Langevin.

‡ King's College London.

§ Current address: Deuteration Laboratory, Institut Laue-Langevin, 6 rue Jules Horowitz, BP 156, 38042 Grenoble Cedex 9, France.

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The scattering length density $\rho(z)$ is related to the composition of the adsorbed species by

$$\rho(z) = \sum_j n_j(z) b_j$$

where $n_j(z)$ is the number of nuclei per unit volume and b_j is the scattering length of nucleus j.

In a typical analysis, the measured data are compared with reflectivity profiles calculated using the optical matrix method applied to different model density profiles.[8] Any model typically consists of a series of layers, each with a uniform scattering length density ρ and a thickness t. An additional parameter, σ, which measures the interfacial roughness between any two consecutive layers, may be incorporated if necessary. The calculated profile is compared with the measured profile and ρ and t for each layer varied until the optimum fit to the data is found. Although a given reflectivity profile may be satisfactorily accounted for by a variety of different structural models, the use of several profiles measured under different isotopic contrasts ensures that the fitted model provides a unique solution. This technique, known as contrast variation, relies on the fact that different nuclei scatter neutrons with quite different amplitude, and, in the case of protons and deuterons, with opposite phase. Therefore, using a combination of hydrogenated and deuterated materials, the reflectivity profile of a system can be substantially changed while keeping the same chemical structure at the interface. Moreover, it is possible (by adjusting the H/D ratio) to prepare solvents which are contrast matched to the silicon substrate used in the neutron reflectivity experiments, and the contrast between the surface and the solvent is then zero, giving a reflectivity profile arising only from the interfacial material.

The data described below were measured at the Institut Laue Langevin using the reflectometer D17. All measurements were made at 30 °C. Model fitting was performed using the programs AFIT[9] and WETDOC,[10] both allowing the simultaneous analysis of all data sets from the same sample in different H_2O:D_2O solvent contrasts. Errors on the parameters were calculated by varying each in turn, visually inspecting the quality of the fit and evaluating the change in the χ^2 value.

Materials. All chemicals used were obtained commercially and were used as received. Dimethyldioctadecylammonium bromide (DDAB) purity >98%, cholesterol (Chol) purity >99%, and dioleoyl-L-α-phosphatidylethanolamine (DOPE) purity ≥99% were supplied by The Sigma Chemical Co. Ltd., Dorset, U.K. Deuterated 1,2-dimyristoyl-*d*54-*sn*-glycerol-3-phosphocholine-1,1,2,2-*d*4,*N*,*N*,*N*-trimethyl-*d*9 (*d*-DMPC) >98% deuterium purity and hydrogenated 1,2-dipalmitoyl-sn-glycero-3-[phospho-L-serine] (*p*-DPPS) purity >99% were purchased from Avanti Polar-Lipids Inc., Alabaster, AL. D_2O was provided by the Institut Laue Langevin and was filtered through 0.22μm pore size filters (Millipore, Bedford, MA) before use.

Vesicle Preparation. Vesicle samples were prepared using the "thin film method" as described by Ma et al.[11] The required mass of lipid was placed in a round-bottom flask and dissolved in excess chloroform. The chloroform was then removed by rotary evaporation at 50 °C and the resulting lipid film stored overnight at ~5 °C. An aliquot of D_2O (at 25 °C) was then added to give a final concentration of 1 mg/mL lipid and the solvent allowed to hydrate the lipid under atmospheric pressure at 50 °C. When the lipid was fully hydrated, the multilamellar vesicles so formed were size-reduced by probe sonication (Soniprobe, Lucas Dawe Ultrasonics) for 30 min over ice. Vesicle suspensions of lower concentration were prepared by dilution of these 1 mg/mL preparations. Throughout the following, the concentrations of lipid within the vesicle samples are given as the final concentration of DDAB present. Where there is a mixture of lipid present the molar ratio of DDAB to "helper" lipid is given. All vesicle preparations used in this study had mean vesicle radii of less than 40 nm (determined by dynamic light scattering using a Malvern 4700c light scattering instrument: Malvern Instruments Ltd., Malvern, Worcester, U.K.) and can therefore be considered to be unilamellar.

Preparation of the Model Cell Membrane. Lipid bilayers were deposited on the (111) surface of silicon single crystals by using a combination of the Langmuir−Blodgett (first layer) and Langmuir-Schaeffer (second layer) techniques.[12] Briefly, after spreading a phospholipid monolayer on the surface of water, at a pressure <0.1 mN/m, and allowing for the evaporation of the solvent, the monolayer was compressed to a surface pressure of 30 mN/m and the highly hydrophilic block (a silicon crystal previously cleaned with organic solvents and UV/ozone treatment[13]) was immersed in the subphase. By withdrawing the block from water, at a speed of 5 mm/min, a monolayer of lipid was deposited on its surface. The solid was then rotated by 90° and slowly reimmersed with the large face parallel to the water surface for the formation of the bilayer. The pressure was kept constant all the time. Stable and reproducible bilayers were obtained.

Solvents. Four D_2O:H_2O mixtures were prepared for use in characterizing the layer found at the surface of the silicon block. Before use all the solvents were filtered through 0.22 μm pore size membranes to remove any large contaminants. The four solvents each had different scattering length densities, thus contrasting or contrast matching different regions of the sample. D_2O ($\rho = 6.35 \times 10^{-6}$ Å^{-2}) offered maximum contrast with the hydrogenated lipid, $CMSiO_2$ solvent ($\rho = 3.41 \times 10^{-6}$ Å^{-2}) contrast matched the silicon oxide layer at the surface of the silicon substrate, CMSi solvent ($\rho = 2.07 \times 10^{-6}$ Å^{-2}) contrast matched the silicon substrate, and H_2O ($\rho = -0.56 \times 10^{-6}$ Å^{-2}) resulted in the maximum contrast with the deuterated lipid present at the silicon surface. To change the solvent contrast a 3-fold excess of the required solvent was pumped into the sample environment[14] (15 mL volume) at a rate of 150 mL/h.

Results and Discussion

Silicon Block Exposed to DDAB Vesicles. The surface of a silicon block was first characterized using one solvent contrast, provided by a D_2O subphase. Using the optical matrix method the data collected were simulated employing a single layer to model the presence of silicon oxide at the surface of the silicon block. This layer was assigned a fixed scattering length density (ρ) of 3.41×10^{-6} Å^{-2}, which is the calculated scattering length density of SiO_2. The resulting model required a layer of 13 ± 2 Å thick with an interfacial roughness of 2 ± 1 Å to achieve the quality of fit shown in Figure 1. These model parameters are reasonable for a silicon oxide layer at the surface of a silicon block and are in good agreement with the data reported by other workers using more than one solvent contrast.[15]

After characterization of the silicon block the D_2O subphase was replaced by a 0.1 mg/mL DDAB vesicle suspension in D_2O and the reflectivity data then recorded as a function of time. On the addition of the hydrogenated 0.1 mg/mL DDAB vesicles the reflectivity curve is modified within 90 min (Figure 1), indicating that the characteristics of the surface of the silicon block have been altered by the presence of the cationic vesicles. Little further variation in the reflectivity curve is observed over a timescale of 3 days, but after 10 days exposure an additional change is seen (in the region of $Q > 0.1$ Å^{-1}: Figure 1). Goloub and Koopal[16] describe a two-step process occurring during the interaction of the silicon oxide surface and

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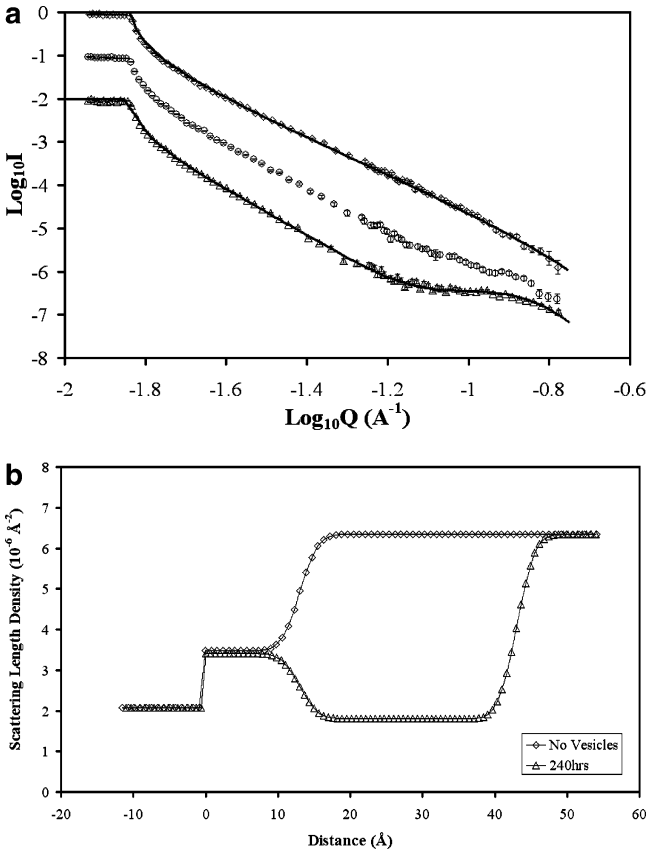

Figure 1. (a) Silicon surface characterized by neutron reflectivity, D_2O as the subphase (◇). Silicon surface 90 min after injection of 0.1 mg/mL DDAB vesicles (suspended in D_2O) into the subphase (○). Silicon surface after 10 days exposure to 0.1 mg/mL DDAB in D_2O (△). Solid lines represent model fits to the experimental data. Data are offset for reasons of clarity. (b) Scattering length density profiles for the model fits.

cationic vesicles within the subphase; the first being the adsorption of individual molecules to the silicon oxide layer and the subsequent change resulting from the formation of a lipid bilayer at the surface. The initial change in the reflectivity curve followed by its slower modification observed here supports this two step model.

Fitting the reflectivity curve of the silicon block after 10 days exposure to 0.1 mg/mL DDAB vesicles, with D_2O as the subphase, can be achieved with a simple two-layer model: the first layer, representing the silicon oxide surface of the block, being allocated a thickness of 13 ± 2 Å, a scattering length density (ρ) of 3.41×10^{-6} Å^{-2} and an interfacial roughness of 2 ± 1 Å; the second layer with a thickness of 30 ± 3 Å with a ρ value of 1.8 (±0.2) $\times 10^{-6}$ Å^{-2} and an interfacial roughness of 2 ± 1 Å. Complete coverage of the silicon surface with a DDAB bilayer would result in a ρ value of approximately -0.4×10^{-6} Å^{-2}, therefore we can determine that 70% of the surface is coated by lipid. It should be noted that as only one solvent contrast was used here (D_2O) these parameters may not be a unique solution to fitting the experimental data but, while this model may be an oversimplification of the structure at the surface, it does provide evidence that a cationic lipid bilayer, and not a monolayer, is forming on the silicon oxide surface.

9:1 *d*-DMPC:*p*-DPPS Bilayers. During this study, many 9:1 *d*-DMPC:*p*-DPPS bilayers, adsorbed to the surface of a silicon block, were prepared and characterized. All of the neutron reflectivity data collected for these separately prepared 9:1 *d*-DMPC:*p*-DPPS bilayers could be fitted using a common five layer model. The mean values

Table 1. Parameters Used to Fit the Neutron Reflectivity Curves for the 9:1 *d*-DMPC:*p*-DPPS Bilayers[a]

model layer description	thickness (Å)	scattering length density ($\times 10^{-6}$ Å^{-2})	interfacial roughness (Å)
SiO_2 layer	13 ± 2	3.4 ± 0.2	2 ± 1
solvent layer	3 ± 1		2 ± 1
headgroup layer	8 ± 1	5.4 ± 0.2	3 ± 1
hydrocarbon layer	27 ± 2	6.8 ± 0.2	3 ± 1
headgroup layer	8 ± 1	5.4 ± 0.2	3 ± 1

[a] The solvent layer has a scattering length density determined by the H:D contrast used and, as more than one solvent contrast was used, a single value cannot be quoted.

for layer thicknesses, scattering length densities, and interfacial roughnesses were calculated from the modeling of each individually characterized 9:1 *d*-DMPC:*p*-DPPS bilayer and then kept constant within the five layer model. By varying the volume of solvent present within the layers of the model which represent the hydrophilic "headgroups" and hydrophobic "tails" of the lipid bilayer fits of comparable quality were obtained for each deposited bilayer. The volume of solvent within each layer was calculated based on the simultaneous fitting of several sets of reflectivity data, each for an individual 9:1 *d*-DMPC:*p*-DPPS bilayer, collected with different H:D solvent contrasts present in the subphase (specifically D_2O, $CMSiO_2$, CMSi, and H_2O).

Table 1 shows the parameters for layer thickness, scattering length density, and interfacial roughness, which did not vary beween separately prepared samples, that were used to fit the neutron reflectivity curves of the 9:1*d*-DMPC:*p*-DPPS bilayers. A fourth parameter (discussed below) was required to describe the level of hydration of each layer, which varied from sample to sample depending on the surface coverage of each 9:1 *d*-DMPC:*p*-DPPS bilayer. This model can be interpreted as a lipid bilayer (of total thickness, 8 + 27 + 8 = 43 Å) separated by a 3 Å thick layer of solvent from the surface of the silicon block, with the phospholipid "headgroup" layers of 8 Å thickness bordering a hydrocarbon layer of 27 Å thickness. The scattering length densities of the layers represent the weighted sums of the densities of both the hydrogenated and deuterated lipid present in the bilayer. A 9:1 mixture of deuterated and hydrogenated hydrocarbon chains has a calculated mean scattering length density of 6.8×10^{-6} Å^{-2} and a 9:1 mixture of deuterated DMPC "headgroups" and hydrogenated DPPS "headgroups" has a mean density of 5.4×10^{-6} Å^{-2}.[17] The experimental values, derived from the model fitting, and the theoretical values are in good agreement indicating that the hydrogenated DPPS molecules are incorporated into the deuterated DMPC bilayer at the surface of the silicon in the expected molar ratio of 9:1 *d*-DMPC:*p*-DPPS.

Although the thickness, scattering length density, and interfacial roughness of the layers used to model the reflectivity curves of the 9:1*d*-DMPC:*p*-DPPS bilayers can be kept constant for all samples without altering the quality of fit achieved, this is not the case for the volume of solvent present within each layer. A typical volume of solvent present within the hydrocarbon core of the model is approximately 25 ± 5% of the total volume of the layer, with "headgroup" layers modeled as 35 ± 5% solvent. This is equivalent to describing a mean coverage of the silicon surface for these 9:1 *d*-DMPC:*p*-DPPS lipid bilayers of 75%, a reasonable surface coverage from the deposition

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Table 2. Model Used to Fit the Measured Q Range ($Q = 0.01-0.2$ Å^{-1}) of the Reflectivity Curve of a 9:1 *d*-DMPC:*p*-DPPS Bilayer before and after 6 Hours Exposure to 0.1 mg/mL DDAB Vesicles Suspended in D_2O[a]

layer description	thickness (Å)	roughness (Å)	ρ ($\times 10^{-6}$ Å^{-2}) 9:1 *d*-DMPC:*p*-DPPS bilayer	ρ ($\times 10^{-6}$ Å^{-2}) 9:1 *d*-DMPC:*p*-DPPS bilayer after 6 h exposure to DDAB vesicles
SiO_2	13 ± 2	2 ± 1	3.4 ± 0.2	3.4 ± 0.2
solvent	3 ± 1	2 ± 1	6.4 ± 0.2	6.4 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	6.1 ± 0.2
hydrocarbon	27 ± 2	3 ± 1	6.7 ± 0.2	6.7 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	6.1 ± 0.2

[a] Data and fit shown in Figure 3. ρ = scattering length density of the modeled layer. SiO_2 = silicon oxide layer.

of the lipid layer. Fragneto et al.[17] describe surface coverages of 70% or greater for lipid bilayers deposited on silicon blocks using a similar deposition technique.

The model summarized in Table 1, which was used to fit the neutron reflectivity data collected for all the 9:1*d*-DMPC:*p*-DPPS lipid bilayers discussed in this study, was the simplest model tested that could successfully fit the neutron reflectivity data. Models using one layer to simulate the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer, adsorbed directly to the silicon oxide surface of the silicon substrate or separated by a trapped solvent layer, could not fit the data regardless of the values assigned for scattering length density, interfacial roughness or solvent presence. Using a two layer model, to simulate the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer, resulted in a successful fit of the neutron reflectivity data; however, the parameters derived from the model did not correlate with the known properties of the *d*-DMPC and the *p*-DPPS molecules deposited at the surface. Only when the three layer model described in Table 1 was used could the data be successfully fitted with a reasonable physical model.

9:1 *d*-DMPC:*p*-DPPS Bilayer Exposed to 0.1 mg/mL DDAB Vesicles. DDAB vesicles (0.1 mg/mL) were injected into the subphase of a deposited 9:1 *d*-DMPC:*p*-DPPS bilayer. It was expected that there would be a rapid interaction between the positively charged DDAB vesicles and the negatively charged 9:1 *d*-DMPC:*p*-DPPS bilayer, in a manner similar to that observed for a silicon surface exposed to DDAB vesicles. Therefore data were only collected for the "low Q" region of the reflectivity curve ($Q < 0.08$ Å^{-1}) to allow greater time resolution, as the higher intensity of reflection in this region allows faster data acquisition.

Given the difference in charge between the lipid present within the vesicles and the lipid at the surface of the silicon, it was thought that an interaction may occur that would result in the deposition of an additional lipid bilayer, composed of hydrogenated DDAB molecules, on the surface of the 9:1 *d*-DMPC:*p*-DPPS bilayer. The deposition of such a layer would result in changes to the reflectivity curve in the $Q < 0.08$ Å^{-1} region. However, during the first 6 h of exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to DDAB vesicles no change was observed in this "low Q" region. This is in direct contrast to the observations made for a silicon block exposed to DDAB vesicles (Figure 1). Since no such changes were observed it is concluded that a new layer is not deposited onto the supported bilayer over this (6 h) time period.

The full reflectivity curve for the 9:1 *d*-DMPC:*p*-DPPS bilayer before and after 6 h exposure to 0.1 mg/mL DDAB vesicles shows a slight change in the higher Q range ($Q = 0.075-0.2$ Å^{-1}) suggesting that the DDAB vesicles were indeed interacting with the supported bilayer. Only the D_2O contrast was measured after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to the vesicles, and so the modeling used to compare the two data sets does not contain the "percentage solvent" parameter, as it is not possible to determine the level of solvent present within the layers in the absence of data for varying H:D solvent contrasts (Table 2).

Fitting of the data collected for the 9:1 *d*-DMPC:*p*-DPPS bilayer after 6 h exposure to 0.1 mg/mL DDAB vesicles can be achieved by varying the scattering length density parameter of the modeled layers. Varying the thickness of the layers of the model results in significant changes in the simulated reflectivity curve, which decreases the quality of the fit, even if the scattering length densities of the layers are also allowed to vary. Certainly, the addition of a layer of hydrogenated material, simulating the deposition of a DDAB bilayer at the surface of the 9:1 *d*-DMPC:*p*-DPPS bilayer cannot be used to fit the data.

As can be seen in Table 2, the change in the reflectivity curve can be attributed to an increase in the scattering length density of the "headgroup" regions (model layer 3 and 5) of the 9:1 *d*-DMPC:*p*-DPPS bilayer. This must be the result of an increase in the presence of deuterated material in this region. The most plausible explanation for this is that more D_2O is now present in these layers. It would be expected that any interaction between the vesicles and the supported bilayer would initially result in a change in the "headgroup" region and this increased presence of D_2O may be the precursor to more significant changes over a longer period of time.

9:1 *d*-DMPC:*p*-DPPS Bilayer after 15 Days Exposure to 0.1 mg/mL DDAB Vesicles. As exposure of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to DDAB vesicles for 6 h showed modification of the supported lipid bilayer, a 9:1 *d*-DMPC:*p*-DPPS bilayer was prepared and 0.1 mg/mL DDAB vesicles injected into the subphase 15 days prior to characterization by neutron reflectivity. It was hoped that this prolonged period of exposure before characterization would allow sufficient time for the lipid present at the surface of the silicon block and the lipid present in the vesicles to interact and reach equilibrium, possibly forming new structures at the surface. The vesicles were washed out of the subphase, by the injection of pure D_2O, before the neutron reflectivity data were collected. This was to ensure that the structure formed at the surface remained constant during data collection. Reflectivity data were also collected in the presence and absence of vesicles in the subphase to confirm that the removal of the vesicles did not modify the structure of the adsorbed layer. The curves collected in the presence and absence of the DDAB vesicles were identical, showing that the removal of the vesicles did not result in the destruction of the lipid structures formed at the silicon surface.

After the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to 0.1 mg/mL DDAB vesicles for 15 days there appears to be exchange of lipid between the hydrogenated lipid of the vesicles and the predominantly deuterated lipid of the deposited 9:1 *d*-DMPC:*p*-DPPS bilayer (Table 3). This deduction is based on the lowering of the scattering length density (ρ) of the "hydrocarbon" layer of the 9:1 *d*-DMPC:*p*-DPPS bilayer from 6.8×10^{-6} Å^{-2} to the ρ value of 0.8×10^{-6} Å^{-2} for the newly formed layer. It is clear, if one compares the neutron reflectivity data collected for the

Table 3. Model Parameters Required to Fit the Neutron Reflectivity Curves of a 9:1 *d*-DMPC:*p*-DPPS Bilayer after 15 Days Exposure to 0.1 mg/mL DDAB Vesicles (Figure 4)[a]

layer description	thickness (Å)	ρ ($\times 10^{-6}$ Å^{-2})	roughness (Å)	% solvent
SiO_2	13 ± 2	3.4 ± 0.2	2 ± 1	0
DDAB	37 ± 3	0.8 ± 0.2	2 ± 1	49 ± 5

[a] ρ = scattering length density of the modeled layer, SiO_2 = silicon oxide layer.

9:1 *d*-DMPC:*p*-DPPS bilayer after prolonged exposure to the DDAB vesicles with simulated curves for the deposition of a hydrogenated lipid bilayer at the surface of the 9:1 *d*-DMPC:*p*-DPPS bilayer, that exchange of lipid has occurred rather than the formation of an additional lipid layer at the silicon surface (data not shown).

In contrast to the 9:1 *d*-DMPC:*p*-DPPS bilayer, the new lipid layer, after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to DDAB vesicles, can be modeled as a single 37 Å thick layer. This is reasonable as, if exchange of lipid is occurring resulting in the formation of a DDAB bilayer at the silicon surface, the "headgroups" of a DDAB molecule consists only of a nitrogen atom with two bound methyl groups and is, therefore, too small to require modeling as discrete layers. Modeling of the small angle neutron scattering data collected for DDAB vesicles made this assumption and resulted in allocating a thickness of 38 Å ± 1 to the lipid bilayers of DDAB vesicles.[18] The similarity between the DDAB vesicle bilayer thickness (38 Å) and the thickness of the layer modeled at the surface of the silicon block as a result of the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to DDAB vesicles (37 Å) is clearly apparent and supports the proposed process of lipid exchange between the vesicles and the supported lipid bilayer.

The mean scattering length density of the 37 Å thick layer, used to model the reflectivity curves collected for the 9:1 *d*-DMPC:*p*-DPPS bilayer after its exposure to DDAB vesicles for 15 days, is 0.8×10^{-6} Å^{-2}. As the scattering length density (ρ) of the hydrocarbon region of the 9:1 *d*-DMPC:*p*-DPPS bilayer has been shown to be 6.8×10^{-6} Å^{-2} and a 100% DDAB lipid bilayer has been shown to have a ρ value of approximately -0.5×10^{-6} Å^{-2} [18] one can calculate the level of exchange of lipid between the predominantly deuterated lipid of the 9:1 *d*-DMPC:*p*-DPPS bilayer adsorbed to the silicon surface and the hydrogenated lipid of the DDAB vesicles. It would appear that approximately 20% of the lipid bilayer at the surface of the silicon block, after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to DDAB vesicles, is composed of a 9:1 mixture of *d*-DMPC:*p*-DPPS, whereas approximately 80% of the lipid bilayer at the surface of the silicon block is composed of DDAB molecules. It is not possible to determine from these neutron reflectivity measurements whether the DDAB, DMPC, and DPPS molecules are dispersed uniformly throughout this lipid bilayer or whether domains of *d*-DMPC:*p*-DPPS are present within a DDAB matrix.

It was thought possible that the lowering of the scattering length density of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer from 6.8×10^{-6} to 0.8×10^{-6} Å^{-2}, on exposure to hydrogenated DDAB vesicles, may have been the result of DDAB molecules adsorbing to the silicon surface only in regions not covered by the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer. From the solvent presence modeled within the 9:1 *d*-DMPC:*p*-DPPS lipid bilayers, we know that typically 75% of the silicon surface is coated with an adsorbed lipid bilayer after the deposition process. Therefore, in order for 80% of the surface to be coated with the hydrogenated DDAB molecules, after the exposure of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to DDAB vesicles for 15 days, exchange of lipid between 9:1 *d*-DMPC:*p*-DPPS bilayer and the DDAB molecules of the vesicles must be occurring.

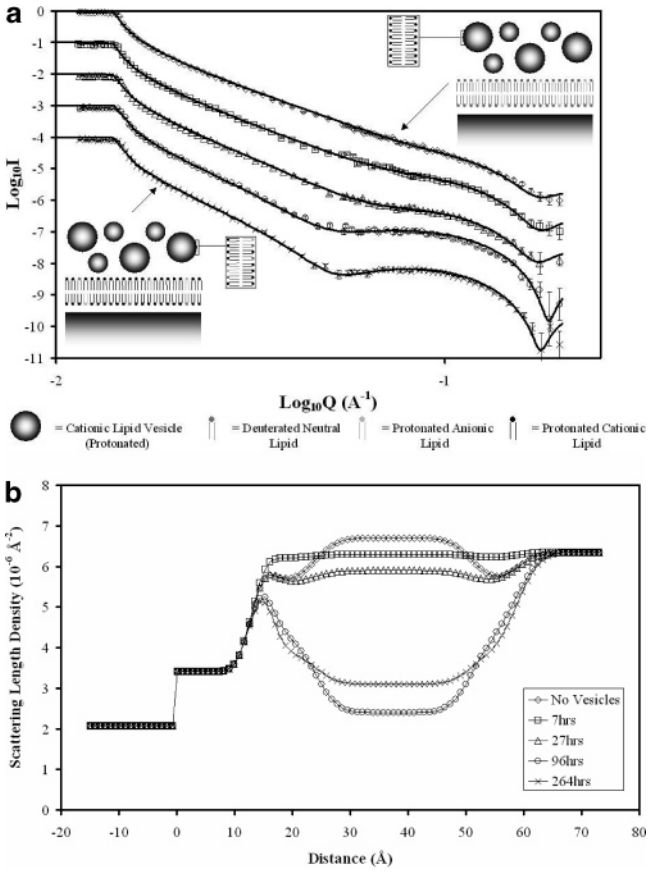

Figure 2. (a) Neutron reflectivity curves for a 9:1 *d*-DMPC:*p*-DPPS bilayer in the presence of 0.1 mg/mL 1:1 DDAB:Chol vesicles, suspended in D_2O, as a function of time. 9:1 *d*-DMPC:*p*-DPPS bilayer characterized with D_2O in the subphase (◇), 7 h after exposure to 0.1 mg/mL 1:1 DDAB:Chol (□), 27 h after exposure (△), 96 h after exposure (○) and 264 h after exposure (×). Solid lines represent model fits to the experimental data. Model parameters as per Table 4. Data are offset for reasons of clarity. (b) Scattering length density profiles for the model fits.

The results discussed here suggest that the exposure of a 9:1 *d*-DMPC:*p*-DPPS bilayer, which is adsorbed to the surface of a silicon block, to 0.1 mg/mL DDAB vesicles results in a slow process of lipid exchange between the supported bilayer and the vesicles in the subphase.

9:1 *d*-DMPC:*p*-DPPS Bilayer Exposed to 0.1 mg/mL 1:1 DDAB:Chol Vesicles. Once a 9:1 *d*-DMPC:*p*-DPPS lipid bilayer had been characterized by neutron reflectivity measurements, 0.1 mg/mL 1:1 DDAB:Chol vesicles, suspended in D_2O, were injected into the subphase. The effect of the 1:1 DDAB:Chol vesicles on the 9:1 *d*-DMPC:*p*-DPPS supported lipid bilayer was recorded as a function of time.

Figure 2 shows the modification of the neutron reflectivity curve of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer resulting from its exposure to 0.1 mg/mL 1:1 DDAB:Chol vesicles. A clear comparison can be drawn between the effect of the 1:1 DDAB:Chol vesicles and the effect of 100% DDAB vesicles on the 9:1 *d*-DMPC:*p*-DPPS bilayer after

(18) Callow, P.; Fragneto, G.; Barlow, D. J.; Lawrence, M. J.; Heenan, R. K.; Timmins, P. Manuscript in preparation.

Table 4. Model Parameters Required to Fit the Neutron Reflectivity Curves of a 9:1 *d*-DMPC:*p*-DPPS Bilayer as a Function of Time of Exposure to 0.1 mg/mL 1:1 DDAB:Chol Vesicles (Figure 5)[a]

layer description	thickness (Å)	roughness (Å)	ρ ($\times 10^{-6}$ Å^{-2}) no vesicles	ρ ($\times 10^{-6}$ Å^{-2}) 7 h	ρ ($\times 10^{-6}$ Å^{-2}) 27 h	ρ ($\times 10^{-6}$ Å^{-2}) 96 h	ρ ($\times 10^{-6}$ Å^{-2}) 264 h
SiO_2	13 ± 2	2 ± 1	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
solvent	3 ± 1	2 ± 1	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.2
headgroup	8 ± 1	3 ± 1	5.6 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	4.3 ± 0.2	3.9 ± 0.2
hydrocarbon	27 ± 2	3 ± 1	6.7 ± 0.2	6.3 ± 0.2	5.9 ± 0.2	2.4 ± 0.2	3.1 ± 0.2
headgroup	8 ± 1	3 ± 1	5.6 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	4.3 ± 0.2	3.9 ± 0.2

[a] SiO_2 = silicon oxide layer. ρ = scattering length density.

6 h, for example both reflectivity curves exhibit an increase in intensity at $Q \approx 0.1$ Å^{-1}. As with the 100% DDAB vesicles, the 9:1 *d*-DMPC:*p*-DPPS bilayer was kept in contact with the 1:1 DDAB:Chol vesicles for a prolonged period of time and neutron reflectivity data were collected up until 11 days after the initial exposure of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to the 1:1 DDAB:Chol vesicles.

Prolonged exposure of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to 1:1 DDAB:Chol vesicles resulted in a significant change in the neutron reflection data collected. Clearly the 9:1 *d*-DMPC:*p*-DPPS bilayer at the surface of the silicon block had been significantly modified by the presence of the 1:1 DDAB:Chol vesicles. However, whether exchange of lipid had occurred between the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer and the 1:1 DDAB:Chol vesicles in the subphase, as was the case for 100% DDAB vesicles, or whether there had been the deposition of a new hydrogenated lipid bilayer at the surface of the 9:1 *d*-DMPC:*p*-DPPS bilayer, driven by the presence of the cholesterol "helper" lipid, was not immediately apparent.

The good quality fits, shown for the neutron reflectivity data in Figure 2, were achieved by fixing the model layer thicknesses and allowing their scattering length densities to vary (Table 4). As the data were collected with only D_2O as solvent within the subphase, only the mean scattering length density of each of the model layers could be calculated, and the volume of solvent within each layer could not be ascertained.

As can be seen in the models described in Table 4, the changes in the neutron reflection curves of the 9:1 *d*-DMPC:*p*-DPPS bilayer, as it is exposed to 1:1 DDAB:Chol vesicles, can be simulated by the lowering of the scattering length densities of the model layers as a function of exposure time. The only explanation for the lowering of these ρ values (to the extent seen) is that there is an increase in hydrogenated material at the surface of the silicon block. This must be the result of exchange of lipid between the predominantly deuterated material of the 9:1 *d*-DMPC:*p*-DPPS bilayer adsorbed to the surface of the silicon and the hydrogenated DDAB and cholesterol of the vesicles present within the subphase.

Although modeling the neutron reflection data collected for the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer during its exposure to 1:1 DDAB:Chol vesicles in D_2O, attempts were made to model the changes in the reflectivity curves as the deposition of a hydrogenated lipid bilayer at the surface of the 9:1 *d*-DMPC:*p*-DPPS bilayer. It was thought that this might be the result of the inclusion of the 1:1 molar ratio of cholesterol within the DDAB vesicles modifying the behavior of the 100% DDAB vesicles described above. However, the experimental data could not be successfully modeled as a hydrogenated 1:1 DDAB:Chol bilayer being deposited on the 9:1 *d*-DMPC:*p*-DPPS supported bilayer.

With only one solvent contrast used to collect the data it is always possible that more than one model may be produced to account for the neutron reflectivity curves. Therefore, an additional experiment was performed to confidently determine the structures formed at the surface of the silicon block after the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer had been exposed to 1:1 DDAB:Chol vesicles.

Table 5. Model Parameters Required to Fit the Neutron Reflectivity Curves of a 9:1 *d*-DMPC:*p*-DPPS Bilayer after 8 Days Exposure to 0.1 mg/mL 1:1 DDAB:Chol Vesicles (Figure 6)[a]

layer description	thickness (Å)	ρ ($\times 10^{-6}$ Å^{-2})	roughness (Å)	% solvent
SiO_2	13 ± 2	3.4 ± 0.2	2 ± 1	0
solvent	3 ± 1		2 ± 1	100
headgroup	8 ± 1	$-2.5 \times 10^{-2} \pm 0.2$	3 ± 1	10 ± 5
hydrocarbon	27 ± 2	−0.29 ± 0.2	3 ± 1	5 ± 5
headgroup	8 ± 1	$-2.5 \times 10^{-2} \pm 0.2$	3 ± 1	10 ± 5

[a] SiO_2 = silicon oxide layer, ρ = scattering length density. The solvent layer has a scattering length density determined by the H:D contrast used and, as more than one solvent contrast was used, a single value cannot be quoted.

9:1 *d*-DMPC:*p*-DPPS Bilayer Exposed for 8 Days to 0.1 mg/mL 1:1 DDAB:Chol Vesicles. A 9:1 *d*-DMPC:*p*-DPPS lipid bilayer was prepared and 0.1 mg/mL 1:1 DDAB:Chol vesicles injected into the subphase 8 days prior to characterization by neutron reflection. The vesicles were then removed from the subphase and the structures resulting from the 8-day exposure to the vesicles characterized using four H:D solvent contrasts.

The neutron reflectivity data collected for the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer exposed for 8 days to 0.1 mg/mL DDAB:Chol vesicles supports the deductions made above. Good quality model fits of the reflectivity curves, collected using four H:D solvent contrasts, can be achieved by varying the scattering length density of the "headgroup" and "hydrocarbon" layers of the model used to simulate the data collected for the 9:1 *d*-DMPC:*p*-DPPS, and the volume of solvent present within each of these layers (Table 5). Although it is conceivable that more than one model could be used to fit the reflectivity data collected with only one H:D solvent contrast present in the subphase, it is unlikely that more than one model would be capable of successfully fitting all four curves collected with different H:D solvent contrasts present in the subphase.

The model described in Table 5 supports the proposal that exchange of lipid has occurred between the predominantly deuterated bilayer adsorbed to the surface of the silicon block and the hydrogenated material of the 1:1 DDAB:Chol vesicles. This can be the only explanation for the lowering of the scattering length densities of the "headgroup" and "hydrocarbon" layers, when compared to the values used to fit the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer (5.4×10^{-6} Å^{-2} and 6.8×10^{-6} Å^{-2} respectively).

Hydrogenated hydrocarbon chains have been shown to have scattering length densities (ρ values) of approximately -0.4×10^{-6} Å^{-2},[15] therefore, in order for the "hydrocarbon" layer of the model to have a ρ value of -0.29×10^{-6} Å^{-2}, the layer adsorbed to the surface of the silicon block, after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to hydrogenated 1:1 DDAB:Chol vesicles, must be

composed of approximately 99% hydrogenated hydrocarbon chains. It is, therefore, reasonable to propose that the exposure of the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to 1:1 DDAB:Chol vesicles for 8 days results in the complete exchange of lipid between the adsorbed (deuterated) layer at the surface of the silicon block and the hydrogenated lipid of the 1:1 DDAB:Chol vesicles.

The effect of exposing a 9:1 *d*-DMPC:*p*-DPPS lipid bilayer to 100% DDAB vesicles for 15 days has been described above. In that case the exchange of lipid between the adsorbed, deuterated layer and the hydrogenated DDAB molecules of the vesicles in the subphase led to 80% of the modeled layer being composed of hydrogenated lipid, compared to ~99% for the 1:1 DDAB:Chol vesicles after 8 days. It would appear, therefore, that the inclusion of a 1:1 molar ratio of the cholesterol "helper" lipid into the DDAB vesicles increases the rate of lipid exchange between the two lipid structures present within the sample. However, the rate of exchange is still slow, requiring days rather than hours to see significant changes in the reflectivity curves of the 9:1 *d*-DMPC:*p*-DPPS bilayer, possibly suggesting that these vesicles do not fuse with biological membranes.

An effect of the formation of a bilayer at the surface of the silicon block predominantly composed of DDAB and cholesterol, as a result of the exchange of lipid between the 9:1 *d*-DMPC:*p*-DPPS bilayer and the lipid of the vesicles, is the increased exclusion of solvent from this region of the sample. For the deposited 9:1 *d*-DMPC:*p*-DPPS bilayer, "headgroup" layers of the model commonly have 35% of their volume occupied by solvent, with the "hydrocarbon" layer having 25% of its volume occupied by solvent. In the case of the model described in Table 5, the "headgroup" layers have only 10% of their volume occupied by solvent and the "hydrocarbon" layer has only 5% of its volume occupied by solvent. It would appear that the packing of the DDAB and cholesterol molecules that form the bilayer at the surface of the silicon block, after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to 1:1 DDAB:Chol vesicles, is more ordered, and of higher density, than that of the initial 9:1 *d*-DMPC:*p*-DPPS lipid bilayer.

9:1 *d*-DMPC:*p*-DPPS Bilayer Exposed to 0.1 mg/mL 1:1 DDAB:DOPE Vesicles. Although 1:1 DDAB:Chol vesicles have been shown to have high transfection efficiencies in vivo, 1:1 DDAB:DOPE vesicles have been shown to have high transfection efficiencies in vitro.[6] It was hoped, therefore, that a comparison of the behavior of these two compositions of vesicle in the absence of DNA, when a 9:1 *d*-DMPC:*p*-DPPS lipid bilayer was exposed to them, would give an insight into these differences in transfection efficiency.

If one compares the change in the neutron reflectivity curve of the 9:1 *d*-DMPC:*p*-DPPS bilayer caused by its exposure to 0.1 mg/mL 1:1 DDAB:DOPE vesicles for 9 h (Figure 3) with that seen for 1:1 DDAB:Chol vesicles after 7 h (Figure 2) one can see that there is a significant change in the reflectivity curve, whereas this was not the case for the data of 1:1 DDAB:Chol vesicles over a comparable period of time. DOPE has been described as a "fusogenic" lipid, which promotes fusion of lipid:DNA complexes with endosomal membranes.[5,19] One can clearly see by the comparison of the data for 100% DDAB, 1:1 DDAB:Chol, and 1:1 DDAB:DOPE vesicles, in the absence of DNA, that DOPE increases the rate of interaction of the vesicles with the supported 9:1 *d*-DMPC:*p*-DPPS lipid bilayer.

Modeling of the neutron reflectivity data collected for the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer exposed to 1:1 DDAB:DOPE vesicles suspended in D_2O (Figure 3) was performed assuming that exchange of lipid was occurring between the predominantly deuterated bilayer at the surface of the silicon block and the hydrogenated lipid of the vesicles in the subphase, in much the same as described for 100% DDAB and 1:1 DDAB:Chol vesicles (Table 6).

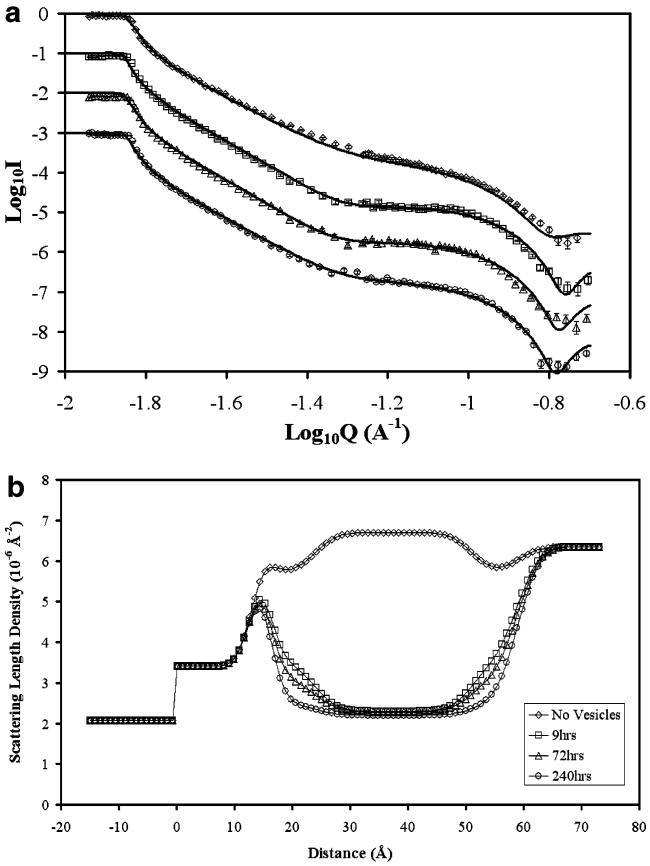

Figure 3. (a) Neutron reflectivity curves for a 9:1 *d*-DMPC:*p*-DPPS bilayer in the presence of 0.1 mg/mL 1:1 DDAB:DOPE vesicles, suspended in D_2O, as a function of time. 9:1 *d*-DMPC:*p*-DPPS bilayer characterized with D_2O in the subphase (◇), 9 h after exposure to 0.1 mg/mL 1:1 DDAB:DOPE (□), 72 h after exposure (△) and 240 h after exposure (○). Solid lines represent model fits to the experimental data. Model parameters as per Table 6. Data are offset for reasons of clarity. (b) Scattering length density profiles for the model fits.

One can see in Table 6 that, after 9 h exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to 1:1 DDAB:DOPE vesicles, the "hydrocarbon" layer of the model has a scattering length density (ρ) of 2.3×10^{-6} Å^{-2}. Considering that this layer has a ρ value of 6.8×10^{-6} Å^{-2}, before the addition of the 1:1 DDAB:DOPE vesicles, one can calculate that approximately 60% of the lipid bilayer adsorbed to the surface of the silicon block is composed of a mixture of DDAB and DOPE after 9 h. This level of lipid exchange between the 9:1 *d*-DMPC:*p*-DPPS bilayer and the vesicles in the subphase required an exposure time of approximately 4 days for the 1:1 DDAB:Chol vesicles (Table 4). Clearly the inclusion of DOPE as the 'helper' lipid increases the rate of interaction of the vesicles in the subphase with the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer when compared to cholesterol.

9:1 *d*-DMPC:*p*-DPPS Bilayer Exposed for 12 Days to 0.1 mg/mL 1:1 DDAB:DOPE. To confirm that a similar process of lipid exchange had occurred for the 1:1 DDAB:DOPE vesicles compared to that described for 100% DDAB and 1:1 DDAB:Chol vesicles, instead of the formation of novel structures at the surface, a 9:1 *d*-DMPC:*p*-DPPS

(19) Farhood, H.; Serbina, N.; Huang, L. *Biochim. Biophys. Acta* **1995**, *1235*, 289.

Table 6. Model Parameters Required to Fit the Neutron Reflectivity Curves of a 9:1 *d*-DMPC:*p*-DPPS Bilayer as a Function of Time of Exposure to 0.1 mg/mL 1:1 DDAB:DOPE Vesicles (Figure 7)[a]

layer description	thickness (Å)	roughness (Å)	ρ ($\times 10^{-6}$ Å^{-2}) no vesicles	ρ ($\times 10^{-6}$ Å^{-2}) 9 h	ρ ($\times 10^{-6}$ Å^{-2}) 72 h	ρ ($\times 10^{-6}$ Å^{-2}) 240 h
SiO_2	13 ± 2	2 ± 1	3.4	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
solvent	3 ± 1	2 ± 1	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	3.5 ± 0.2	3.1 ± 0.2	2.5 ± 0.2
hydrocarbon	27 ± 2	3 ± 1	6.7 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.2 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	3.5 ± 0.2	3.1 ± 0.2	2.5 ± 0.2

[a] ρ = scattering length density, SiO_2 = silicon oxide layer.

Table 7. Model Parameters Required to Fit the Neutron Reflectivity Curves of a 9:1 *d*-DMPC:*p*-DPPS Bilayer after 12 Days Exposure to 0.1 mg/mL 1:1 DDAB:DOPE Vesicles (Figure 8)[a]

layer description	thickness (Å)	ρ ($\times 10^{-6}$ Å^{-2})	roughness (Å)	% solvent
SiO_2	13 ± 2	3.4 ± 0.2	2 ± 1	0
solvent	3 ± 1		2 ± 1	100
headgroup	6 ± 1	1.3 ± 0.2	5 ± 1	36 ± 5
hydrocarbon	23 ± 2	1.1 ± 0.2	5 ± 1	26 ± 5
headgroup	6 ± 1	1.3 ± 0.2	5 ± 1	36 ± 5

[a] SiO_2 = silicon oxide layer. ρ = scattering length density. The solvent layer has a scattering length density determined by the H:D contrast used and, as more than one solvent contrast was used, a single value cannot be quoted.

bilayer was prepared and 0.1 mg/mL 1:1 DDAB:DOPE vesicles injected into the subphase. The vesicles were allowed to interact with the supported lipid bilayer for 12 days before they were removed by the injection of pure D_2O. The resulting structures at the surface of the silicon block were then characterized by neutron reflection using four H:D solvent contrasts in the subphase.

After 12 days exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to 0.1 mg/mL 1:1 DDAB:DOPE vesicles, the model used to fit the neutron reflectivity curves is shown in Table 7. The thicknesses of the layers of the model are smaller than those used to model the 9:1 *d*-DMPC:*p*-DPPS bilayer (reducing from 8 ± 1 Å to 6 ± 1 Å for the "headgroup" layers and 27 ± 2 to 23 ± 2 Å for the "hydrocarbon" layer), with the interfacial roughness of these layers increasing (from 3 ± 1 to 5 ± 1 Å). However, the change in layer thickness is within the experimental error and coupled with the increase in interfacial roughness can be considered to be comparable with the models described previously.

As observed with only the D_2O solvent data, the scattering length densities of "headgroup" and "hydrocarbon" layers of the model are reduced by the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to the hydrogenated 1:1 DDAB:DOPE vesicles. With the additional solvent contrast data, it can be seen that the percentage of the volume of each modeled layer occupied by solvent is comparable between that of the 9:1 *d*-DMPC:*p*-DPPS bilayer before and after exposure to the 1:1 DDAB:DOPE vesicles (both values being approximately 35% for the "headgroup" and 25% for the "hydrocarbon" layers). This confirms that the exchange of lipid between the predominantly deuterated 9:1 *d*-DMPC:*p*-DPPS bilayer and the hydrogenated vesicles must be occurring, rather than the inclusion of hydrogenated material into regions at the silicon surface that were not covered by the 9:1 *d*-DMPC:*p*-DPPS lipid bilayer.

If no deuterated lipid was being removed from the surface, hydrogenated lipid would have to be incorporated into the 9:1 *d*-DMPC:*p*-DPPS bilayer to effect the lowering of scattering length density. This, in turn, would result in a reduction in the volume of solvent present within each modeled layer, as the lipids would be becoming more densely packed excluding solvent, and reduce the volume of the modeled layer occupied by solvent. For the volume of solvent present within the modeled layers to be similar before and after the exposure of the 9:1 *d*-DMPC:*p*-DPPS bilayer to 1:1 DDAB:DOPE vesicles the surface coverage of lipid at the surface of the silicon block must be unchanged by the exchange of lipid. A scattering length density of 1.1×10^{-6} Å^{-2} for the 'hydrocarbon' layer of the model (Table 7) corresponds to 15% of the layer being composed of the initial 9:1 *d*-DMPC:*p*-DPPS layer and 85% being composed of a mixture of DDAB and DOPE molecules.

Conclusions

During this study many 9:1 *d*-DMPC:*p*-DPPS bilayers have been prepared and characterized by neutron reflectivity. The data collected for these layers, used as model cell membranes, can be simulated using a five layer model of constant thickness, scattering length density, and interfacial roughness. The volume of solvent present within the layers representing the lipid at the surface varies as a result of differences in coverage of the silicon surface.

The exposure of DDAB vesicles to a bare silicon surface leads to the formation of a lipid layer 30 ± 3 Å thick at the surface. However, this layer has a thickness less than that observed for a DDAB lipid bilayer in vesicular form (38 Å). This is due to a tilting of the axes of the DDAB molecules as a result of incomplete coverage of the surface. Further prolonged exposure of the silicon surface to the vesicles would increase the surface coverage, forcing the DDAB molecules to orient with their axes perpendicular to the silicon surface and most likely resulting in a layer thickness more consistent with a DDAB lipid bilayer. Indeed, when a 9:1 *d*-DMPC:*p*-DPPS bilayer is exposed to DDAB vesicles for the period of 15 days lipid exchange occurs between the deposited lipid and the lipid present in the vesicles, resulting in a layer of predominantly DDAB molecules at the surface with a layer thickness of 37 ± 3 Å, which is in good agreement with the value we have determined for a DDAB lipid bilayer using small angle neutron scattering.[18]

The data described in this study demonstrate that the interaction between a model lipid membrane and cationic lipid vesicles is one of lipid exchange, rather than the deposition of new structures at the surface for the three vesicle compositions we have used. For 100% cationic lipid (DDAB) vesicles the rate of exchange is slow. The presence of "helper" lipid increases the rate of exchange with 1:1 DDAB:Chol being faster than 100% DDAB and 1:1 DDAB:DOPE being faster than 1:1 DDAB:Chol (i.e., 1:1 DDAB:DOPE > 1:1 DDAB:Chol > 100% DDAB), with the models reasonably describing the layers' new composition at the surface.

If one assumes that the behavior of these systems is similar in the presence of DNA, then it would appear that

there are different rates of exchange that are optimum for in vivo and in vitro transfection. Fast rates of lipid exchange appear to be appropriate in vitro with slower rates being more efficient for transfection in vivo, as 1:1 DDAB:DOPE has a higher transfection efficiency in vitro than 1:1 DDAB:Chol and vice versa. This may be a result of the increased circulation time that will be observed for 1:1 DDAB:Chol vesicles in vivo due to the slower rate of interation of the vesicle with cell membranes compared to 1:1 DDAB:DOPE vesicles.

Further neutron reflectivity experiments investigating the interactions of this model cell membrane with these cationic lipid:"helper" lipid vesicles complexed with DNA will confirm whether the exchange of lipid observed here is an appropriate model for the initial interactions that occur during transfection.

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Supporting Information Available: Figures depicting neutron reflectivity curves of a 9:1 *d*-DMPC:*p*-DPPS bilayer (Figures i−v). This material is available free of charge via the Internet at http://pubs.acs.org.

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