

Characterization of a Biomimetic Polymeric Lipid Bilayer by Phase Sensitive Neutron Reflectometry

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ABSTRACT:

Neutron reflectivity measurements were performed on a cell membrane mimic system consisting of a polyelectrolyte multilayer plus a synthetic terpolymer plus a phospholipid layer, at all of the intermediate steps in the assembly of the composite, to obtain neutron scattering length density depth profiles. The polyelectrolyte multilayer functions as a soft, water-containing cushion for the membrane mimic, formed by the synthetic terpolymer and the phospholipid layer. The systems were studied dry, in 92% humidity and wet, or fully hydrated, using a phase-sensitive neutron reflectometry technique. By using two water conditions (D_2O and H_2O mixtures) on the polyelectrolyte multilayer plus terpolymer plus phospholipid layer assembly, the distribution of water in the layers was obtained. It was found that the supported membrane-mimic has 40% water content in the “cushion” polyelectrolyte multilayer under humid conditions. Under the same conditions, a 10% water content was found in the terpolymer-phospholipid region. The overall thickness change due to the water uptake under these conditions was found to be 10 Å and was due to an increase in thickness of the polyelectrolyte multilayers. Because fusing phospholipid vesicles onto the polyelectrolyte multilayer plus synthetic terpolymer assembly shows the appearance of a 20 Å increase in thickness of the composite film system, it can be inferred that a phospholipid monolayer was formed. In addition, the terpolymer layer undergoes significant rearrangement upon the addition of the

phospholipid layer. Deuterated phospholipid was used to more accurately assess the location of the phospholipid layer. Even though the measurements under wet, or fully hydrated, conditions were limited by lower spatial resolution and reduced intensity, the scattering length density profiles obtained qualitatively agreed with the structures observed under dry and humid conditions.

INTRODUCTION

Lipid membranes, the boundaries for cellular and intracellular structures, regulate many crucial biological processes. Planar supported mimics of cell membranes are of great interest as model systems for the study of membrane structure/function phenomena in fundamental biophysics research.¹ Supported mimics of cell membranes are also of practical interest. They enable functionalization of inorganic materials (semiconductors, gold-covered surfaces, and polymeric materials) through the creation of biocompatible surfaces such as biofunctional coatings for artificial organs and other implanted devices.² They allow the preparation of ultra thin, high-electrical-resistance layers on conductors and since they provide a natural environment for the immobilization of proteins they lend themselves for the design of biosensors.³⁻⁹

Several approaches can be used for the assembly of biomembranes onto various solid supports and have been recently reviewed.^{1,10-12} Incorporation of transmembrane proteins into solid-supported membranes as well as membrane stability for a broad range of applications continue to be major obstacles. Several strategies have been developed to decouple the biomembranes from the underlying solid support surface to better accommodate transmembrane proteins.^{3,7-9,12-16} One such method is to have the

biomembrane rest on a soft “cushion” composed of multilayers of water-swellaable hydrophilic polyelectrolytes.¹⁵ The polymer layer acts as a support for the biomembrane, not unlike the cytoskeletal support found in actual mammalian cell membranes.¹⁷ The "cushion" polymers are fixed to the hard surface through electrostatic interactions.

Anchoring the biomembrane to the "cushion" has important applications for systems under external force fields, like shear flow for cardiovascular devices. Orban et al. use a synthetic terpolymer that interacts strongly with the polyelectrolyte layers through its charged styrene sulfonate component and at the same time mimics the lower leaflet of a membrane with its N,N dioctadecylcarbonyl propionic acid component.¹⁸ Acrylate phospholipid vesicles are then able to fuse to it and, for substantial increase in stability, the planar phospholipid assembly is polymerized *in situ*. While membrane fluidity is critical for many of the functional responses in biological membranes, certain applications lend themselves to compromise, albeit with a reduction in lateral mobility.

Neutron reflectometry is a very powerful technique for determining the compositional depth profile of multilayer structures, with a resolution that can be of the order of angstroms.¹⁹ For a given chemical composition profile, there exists a corresponding scattering length density (SLD) profile determined by the atomic and isotope specific interactions of the film's constituents with neutrons. To analyze neutron reflectivity data, SLD profiles are modeled using knowledge of the constituents of the films. It is known, however, that a single neutron reflectivity measurement on a system is consistent with more than one SLD profile. On the other hand, phase-sensitive neutron reflectometry, involving multiple measurements of the reflectivity for various references, makes possible direct inversion to obtain the unique SLD profile of the system without *a priori*

knowledge of the multilayer composition. By using the surround variation method, which consists of preparing the same sample on two different substrates or by having the sample in contact with two other different environments that will not change its SLD profile, two reflectivity measurements with the same sample are made, and from them, the complex reflection amplitude of the sample is obtained. Using the Gel'fand-Levitan-Marchenko integral equation, or a differential formulation alternative, the real part of the complex reflection amplitude is inverted to obtain the unique SLD profile of the sample, independent of the substrate or surround environment with which it was in contact.²⁰⁻²³ This method is especially powerful for complex systems such as membrane mimics.

The present work reports the compositional depth profile of a multilayer system consisting of a polyelectrolyte multilayer “cushion” onto which is tethered a membrane mimic consisting of a synthetic terpolymer and a phospholipid leaflet. The same sample was prepared on two substrates: silicon (Si) and sapphire (Al_2O_3) single crystal wafers, to be able to use the direct inversion technique. The compositional depth profile was obtained for every step in process of assembling the soft supported membrane mimic. The assembly process consisted of three steps: a) polyelectrolyte multilayer, b) polyelectrolyte multilayer plus terpolymer and c) polyelectrolyte multilayer plus terpolymer plus phospholipid layer. The measurements were conducted under dry conditions, in 92% humid conditions using different D_2O and H_2O mixtures and in wet conditions (bulk water of 50% D_2O and 50% H_2O).

MATERIALS AND METHODS*

SAMPLE PREPARATION

Reagents:

Poly-L-lysine (21-479kD) and Alginate (ca. 41% guluronic acid) were purchased from Sigma and ISP respectively. The terpolymer: styrene sulfonate-2 hydroxyethyl Acrylate-N,N dioctadecylcarbamoyl propionic acid (SSS-HEA-DOD)1:6:3, and the acrylate PC: 2 were hydroxyethyl Acrylate 1-palmitoyl-2-(12-(acryloyloxy)dodecanoyl)-sn-gycero-3-phosphorylcholine, were synthesized as previously described.¹⁸ 1,2-Dipalmitoyl-D62-sn-Glycero-3-Phosphocholine (d-DPPC) was purchased from Avanti Polar Lipids, Inc.

Sample substrates:

The substrates for the samples were 4.76mm thick Si and 3.175mm thick Al₂O₃ wafers, 76.2mm in diameter. The surface where the sample was assembled was polished (0.3nm RMS roughness). The Si wafers were purchased from Polishing Corporation of America and the Al₂O₃ wafers were purchased from Meller Optics Inc.

The wafers were all coated with a thin film of chromium (Cr) followed by a thin film of gold (Au) using a high vacuum system (Edwards 306) equipped with two magnetron sputtering sources (one for Cr and the other for the Au). The power for gold deposition was 750W and for chromium was 440W. The basic vacuum level was at least 1×10^{-5} Pa. During the sputtering process argon (Ar) gas is introduced in the chamber at a pressure of 6×10^{-1} Pa. The wafers rotate over the Au/Cr targets (Superior Electric SLO-SXN stepper motor, Warner Electronic). A specially shaped mask, placed in between the targets and the wafers, ensures the deposition of homogeneously thick films on the wafers. The thickness of the Cr sub layer was 20 ± 0.4 Å and the Au film was 80 ± 10 Å thick. Both Cr/Au films were the same thickness on the Si and Al₂O₃ substrates to within 1Å. Previous atomic force microscope measurements on equivalent samples indicated that the gold films prepared in this way had a RMS surface roughness of approximately 3Å.

Sample assembly:

Separate samples were prepared representing the three consecutive steps in the assembling process of the biomimetic membrane. For each of these steps two equivalent samples were prepared, one on Si and one on Al₂O₃ single crystal wafers, with Cr and Au thin films deposited as described above.

polyelectrolyte multilayer (PE):

The polyelectrolytes used were poly-L-lysine (PLL), which has a positive charge, and Alginate, which has an equivalent negative charge. A polyelectrolyte layer is formed by self-assembly. At room temperature, the polished side of the single crystal wafer was coated with a 0.1% w/v solution poly-L-lysine (PLL) solution, then incubated for 60 seconds and finally rinsed thoroughly three times with deionized H₂O. After removing the excess water the process was repeated with a 0.15% w/v Alginate solution. Once the excess water on the Alginate layer was removed the process was repeated with the 0.1% w/v solution PLL solution, for a total of three layers of polyelectrolytes: PLL-Alginate-PLL.¹⁸

polyelectrolyte multilayer plus terpolymer (PE+TER):

After forming the polyelectrolyte multilayer as described in a) the wafer was coated with a terpolymer poly(HEA₆:DOD₃:SSS₁) solution, (0.1 mM SSS in 1% Dimethyl Sulfoxide, also purchased from Sigma) for 90 seconds and subsequently rinsed seven times with deionized H₂O.

polyelectrolyte multilayer plus terpolymer plus phospholipid layer (PE+TER+PC):

After forming the polyelectrolyte multilayer, followed by the addition of the terpolymer as described above, the wafer was incubated in a 1.2 mM acrylate PC vesicle solution in

20 mM NaH₂PO₄ 750 mM NaCl pH = 7.4 buffer for 12-14 hours at 40°C. The vesicles were formed by three cycles of liquid nitrogen freezing followed by a 65°C thawing and vortexing of the lipid in 20 mM NaH₂PO₄ pH = 7.4 buffer and then followed by the extrusion (using an extruder made at Emory University) of the solution through 2.0 μm and 0.6 μm Millipore polycarbonate filters (Fisher Scientific) 21 times. The vesicles were 424±150nm in size as measured by (@ NIST, Coulter Submicron Particle Analyzer Model N4MD). At the end of the incubation period, 10μL of a photo initiator solution was added. The photo initiator solution was a 10 mM xanthine 225mM triethanolamine 37 mM 1-vinyl-2-pyrrolidinone (all purchased from Aldrich) in deionized H₂O solution. Once the photo initiator solution was added to the sample, it was immediately irradiated with visible light (50mW/cm²) for 30 minutes at room temperature in a nitrogen atmosphere in a glove box. This forms crosslinks between acyl chains in the Acrylate PC layer as well as in the terpolymer. The surface then is rinsed thoroughly with deionized water.

polyelectrolyte multilayer plus terpolymer plus a deuterated DPPC layer (PE+TER+d-DPPC):

After all neutron reflectivity measurements were done on the polyelectrolyte multilayer plus terpolymer samples described above, the wafers were put in a cell (described in the next section) and incubated in a 1.2 mM d-DPPC vesicle solution in a 50%/50% D₂O/H₂O 16 mM NaH₂PO₄ 150mM NaCl pH = 7.4 buffer for 12-14 hours at 40°C. The vesicles were formed as describe above for the PE+TER+PC sample. Neutron reflectivity measurements on these samples were performed after the cell, which holds 500μL of liquid, was flushed with 10mL deionized 50%/50% D₂O/H₂O. Unlike the

PE+TER+PC sample preparation, there was no *in situ* polymerization step for the d-DPPC layer.

NEUTRON REFLECTIVITY MEASUREMENTS

Measurements were performed at the NG1 vertical sample plane reflectometer at the National Institute of Standards and Technology Center for Neutron Research (NCNR). In specular reflection the incident angle, θ , of the neutron beam with respect to the surface of interest, is equal to the angle of the reflected beam and the reflected intensity is measured as a function of θ . The SLD is a function of distance, z , along the surface normal, whereas the reflectivity and complex reflection amplitude are functions of the wavevector transfer, Q . For specular reflection, z and Q lie perpendicular to the surface of interest. Q is related to θ as follows: $|Q| = (4\pi/\lambda) \sin \theta$, where λ is the wavelength of the neutron. λ was fixed at 4.75 Å with $\Delta Q/Q \sim 0.025$ over the entire range of wavevector transfer for which the reflectivity was measured. Thus, the “footprint” of the beam on the sample was kept approximately constant by varying the apertures which defined the incident beam angular divergence. The reflectivity data from a sample is obtained by subtracting from the specularly reflected intensity the “noise” coming from the incoherently scattered neutrons, as well as stray neutrons from other sources, and then dividing by the incident intensity. The details have been described elsewhere.²⁴ The spatial resolution, d_{min} , for a SLD profile obtained from a reflectivity measurement is given, to first order:²⁵

$$d_{min} = \frac{\pi}{Q_{max}}$$

The data in these experiments were generally taken up to a Q_{max} of 0.3 \AA^{-1} , which meant that the spatial resolution was approximately 10 \AA , or about half the thickness of a

membrane leaflet. Under these experimental conditions it is possible to distinguish the two leaflets of a membrane, but not the detailed structure of a single leaflet. However, neutron reflectivity is sensitive with angstrom resolution to overall changes in thickness for a layer at least d_{min} in thickness.

SAMPLE ENVIROMENTS FOR NEUTRON REFLECTIVITY EXPERIMENTS

The samples were measured dry and in approximately 92% humidity (at room temperature under Ar atmosphere). The humidity was controlled using a Li-Cor Li-610 dew point generator (LI-COR, Inc.) that delivered a moist Ar flow to the chamber containing the sample. Relative humidity and temperature were measured just above the surface of the sample with a Vaisala humidity and temperature sensor (VAISALA, Inc.) model HMD70Y. Temperature was controlled by a Neslab model RTE-111 chiller-heater (Thermo Neslab). 92% humidity conditions were used both in 100% D₂O and 50%/50% D₂O/H₂O. The humidity sensor calculates relative humidity (RH) at a particular temperature, T , from the actual vapor pressure (P) using the following relation:

$$RH_{\text{SENSOR}}(T) = \frac{P(T)}{\text{saturation vapor pressure } (P_s) \text{ for H}_2\text{O at } T} \times 100\%$$

To obtain the same RH conditions for the D₂O/H₂O combinations used, the above equation was modified as follows:

$$RH_{\text{TRUE}}(T) = \frac{RH_{\text{SENSOR}}(T) \times (P_s \text{ for H}_2\text{O at } T)}{P_s \text{ for the D}_2\text{O/H}_2\text{O combination at } T}$$

The saturation pressure, P_s , for any isotope of H₂O at a given T is given by:²⁶

$$P_s(T) = P_c \exp[(T_c / T)(B_1\tau + B_2\tau^{1.5} + B_3\tau^3 + B_4\tau^{3.5} + B_5\tau^4 + B_6\tau^{7.5})]$$

where $\tau = T - T_c$, and P_c and T_c are the critical pressure and temperature, respectively.

The values of the constants P_c , T_c , and B_i ($i=1-6$) are isotope specific and are given in table IV of reference (24). The corresponding $P_s(T)$ for a mixture of H₂O and D₂O is obtained by linear combinations (weighted by the molar fractions) of the corresponding isotope specific $P_s(T)$.

The polyelectrolyte multilayer plus terpolymer sample pair (on Si and Al₂O₃) was measured under wet conditions in bulk 50%/50% D₂O/H₂O. To achieve this, a Si wafer with two 1mm in diameter machined holes diametrically opposite from each other and at 5mm from the edge was pressed against the sample by a set of springs that delivered uniform pressure on the crystals to avoid bending them. In between the two crystals was a foam gasket providing the space (approximately 110 μ m) for the water reservoir. The cell was loaded with water through the holes in the Si wafer. By making the thickness of the water reservoir in the cell 110 μ m, the incoherent scattering from the water is significantly reduced and the specularly reflected intensity can be measured above the background up to a Q value of approximately 0.2 \AA^{-1} .

RESULTS AND DISCUSSION

GOLD AND CHROMIUM LAYERS

The Au layer in all of the substrates was the main contributor to the reflected intensity, both by its thickness and by its SLD contrast to the medium surrounding the samples. The Au layer was reproducibly obtained from the direct inversion of the real part of the complex reflection for all the assemblies studied under all conditions. The Cr layer appeared under all conditions for the PE+TER assembly. For these samples the Cr layer was found to be 20 Å thick and the Au layer was found to be 80 Å thick. However, for the PE and PE+TER+PC assemblies only the Au layer was apparent and was found to be 100 Å thick. The SLD for the Au layer in the dry and humid conditions was found to be on average $4.1 \pm 0.2 \times 10^{-6} \text{Å}^{-2}$, which is near the SLD expected for bulk Au.

HUMIDITY EXPERIMENTS

To be able to use the direct inversion technique two samples of the same type were made onto two substrates, Si and Al₂O₃ as mentioned above. However, because of time constraints, not all conditions studied were measured for both equivalent samples. The 50%/50% D₂O/H₂O humidity condition was measured on only one substrate (Si). Also, for the study of the PE, only the sample on Si was measured. In these cases, the “model-independent” fitting program, PBS²⁷, developed at the NCNR, was used to fit the reflectivity data and obtain a SLD profile. This method uses parametric B-splines to describe the SLD as a function of z . Input parameters are the SLD values of the Si substrate and D₂O/H₂O solution, and the total thickness of the sample, including the Cr and Au layers. Information about the SLD profiles obtained using the direct inversion method on the samples measured using other D₂O/H₂O conditions was used in constructing starting SLD profiles for the data that were fit with PBS.

The real part of the complex reflectivity amplitude times Q^2 is shown in Figure 1a as a function of Q for the PE+TER assembly and the PE+TER+PC assembly in D₂O humidity. Figure 1b shows the corresponding curves for the imaginary part of the complex reflectivity amplitude. The quality of the oscillations (smooth and continuous, rather than discontinuous) along the Q -axis, indicates that the sample pair was not only homogeneous but also essentially identical. Figure 2 shows the reflectivity of the PE assembly on Si under D₂O humidity and the solid curve through the data corresponds to the calculated reflectivity from the SLD profile for PE shown in figure 3a. Also shown in figure 3a are the corresponding SLD profiles for the PE+TER and the PE+TER+PC assemblies under D₂O humidity. The calculated real part of the reflectivity amplitudes corresponding to these SLD profiles are shown as the solid lines in Figure 1a.

Figure 3b shows the SLD profiles for the PE+TER and the PE+TER+PC assemblies dry. The observed SLD for the polyelectrolyte multilayer in these profiles is consistent with the calculated SLDs for Alginate and PL, which are $1.7 \pm 0.5 \times 10^{-6} \text{ \AA}^{-2}$ and $0.7 \pm 0.2 \times 10^{-6} \text{ \AA}^{-2}$ respectively (assuming the density to be $1 \pm 0.3 \text{ gr/cm}^3$).

As can be seen from comparing figures 3a and 3b, the polyelectrolyte multilayer region is significantly hydrated in a humid atmosphere. The terpolymer layer in the PE+TER assembly, which appears as a region of slightly negative SLD, consistent with the calculated SLD of the DOD component of the terpolymer, or $-0.024 \pm 0.003 \times 10^{-6} \text{ \AA}^{-2}$ (assuming a density of $0.9 \pm 0.1 \text{ gr/cm}^3$), does not seem to incorporate water in a humid atmosphere. The terpolymer layer is also observed to have no strong effect on the hydration of the polyelectrolyte multilayer. The exact thickness of the terpolymer region in the PE+TER assembly only becomes apparent after the addition of the phospholipid

layer. Without the phospholipid layer, the small difference in SLD between the terpolymer and air makes it impossible to distinguish the edge of the film. The PE + TER + PC assembly in figure 3b indicates a non-negligible, 20Å phospholipid region with an average SLD of $0.35 \times 10^{-6} \text{Å}^{-2}$. The overall thickness of the PE+TER+PC assembly is observed to increase in thickness from 110Å when dry to 120Å when the system is exposed to a humid atmosphere. The thickness difference of 10Å between the dry and the humid conditions for the PE+TER+PC assembly is due to the water swelling of the polyelectrolyte multilayers. In contrast to the PE+TER assembly in humid conditions, both the terpolymer and the phospholipid layers are significantly hydrated in the PE+TER+PC assembly (figure 3a). This strongly suggests that a rearrangement of the terpolymer layer occurs upon addition of the phospholipid layer. This result is in agreement with those obtained from recent polarized infrared spectroscopy experiments²⁸. At the same time, the polyelectrolyte multilayer in the PE+TER+PC assembly seems to be less hydrated than in the PE and the PE+TER assemblies. Figure 4a shows the SLD profile for the PE+TER+PC assembly under humid conditions in D₂O and in 50%/50% D₂O/H₂O. Figure 4b shows the water fraction in the assembly under humid conditions. This is obtained by assuming that the distribution of each component in the layers is unaffected by having either D₂O or 50%/50% D₂O/H₂O so that the water fraction, x , at any given point in the profile is given by:

$$x(z) = \frac{\rho(z)_{D_2O} - \rho(z)_{50\%/50\%D_2O/H_2O}}{\rho_{D_2O} - \rho_{H_2O}}$$

From figure 4b it can be seen that the polyelectrolyte multilayer has a maximum uptake of 40% of water. This is a significant amount of water, which suggests that a polyelectrolyte multilayer can work as a “cushion” for membrane mimetic systems. The

terpolymer and the phospholipid layers contain an average of 10% water, which is also significant, suggesting that these layers are not a tightly packed.. A water depletion layer in the phospholipid region in figure 4b suggests a very specific location for the acyl chains of the phospholipid. The calculated SLD value from acyl chains is approximately $-0.055 \pm 0.005 \times 10^{-6} \text{ \AA}^{-2}$ (assuming a $0.8 \pm 0.1 \text{ gr/cm}^3$ density). It is not possible to discern such a structure from figure 4a. The average SLD of a phospholipid layer is calculated to be $0.4 \pm 0.05 \times 10^{-6} \text{ \AA}^{-2}$ (assuming a $0.9 \pm 0.1 \text{ gr/cm}^3$ density), consistent with figure 3b. If this layer is 10% hydrated, the SLD for the layer changes to $0.99 \times 10^{-6} \text{ \AA}^{-2}$ in D_2O (consistent with figure 3a) and to $0.65 \times 10^{-6} \text{ \AA}^{-2}$ in 50%/50% $\text{D}_2\text{O}/\text{H}_2\text{O}$. The $0.34 \times 10^{-6} \text{ \AA}^{-2}$ difference in the SLD profile for the PE+TER+PC assembly in 50%/50% $\text{D}_2\text{O}/\text{H}_2\text{O}$ is too small to be detected.

After fusing d-DPPC vesicles to the PE+TER assembly and letting the samples dry at 45°C for 48 hours the surfaces were again measured under a dry Ar flow at room temperature. Figure 5 shows the SLD profiles for the PE+TER+PC assembly and the PE+TER+d-DPPC assembly. A rearrangement in the terpolymer layer upon addition of the phospholipid layer is again observed upon comparison of the SLD profile of the PE+TER+d-DPPC assembly to that of the PE+TER assembly. The PE+TER+PC assembly shows an outermost layer with a SLD of $0.34 \times 10^{-6} \text{ \AA}^{-2}$, consistent with a complete phospholipid layer. In contrast, the PE+TER+d-DPPC assembly shows a SLD of $1.7 \times 10^{-6} \text{ \AA}^{-2}$ for the phospholipid layer, compared with an expected average SLD of $4.5 \pm 0.5 \times 10^{-6} \text{ \AA}^{-2}$ (assuming a $0.9 \pm 0.1 \text{ gr/cm}^3$ density) for a complete d-DPPC layer. This result suggests that, although the d-DPPC layer is located at the surface of the film, it only covers about 40% of the surface. Another possibility is that the interface between

the terpolymer and d-DPPC is very rough. Since the d-DPPC is not polymerized to the PE+TER layers, the surface coverage of the d-DPPC layer is not necessarily correlated to that of the Acrylate PC. Future measurements on a partially deuterated terpolymer layer and a crosslinkable deuterated phospholipid to be studied under dry and humid conditions are expected to provide further insights into the structure of the PE+TER+PC assembly.

Data in 50%/50% H₂O/D₂O bulk solution could only be obtained to a Q_{\max} value of 0.2 Å⁻¹, due to the higher background resulting from the incoherent scattering from hydrogen (or, to a lesser extent, deuterium) in the solution. Thus, the spatial resolution is only about 15Å, making it difficult to compare the resultant SLD profiles quantitatively to those obtained under humid or dry conditions, where the spatial resolution is 10Å. However, the SLD profiles under wet and humid conditions were qualitatively the same. Since the data obtained in solution did not provide further insight into the structure of the PE, PE+TER or PE+TER+PC samples, it will not be discussed further here. However, when the PE+TER+d-DPPC assembly was measured under wet conditions, the d-DPPC layer was not discernable. Yet, when the sample was dried, the d-DPPC layer was clearly visible (see Figure 5). This supports the conclusion that the layer is not complete as it is highly hydrated under wet conditions.

CONCLUSIONS

Phase-sensitive neutron reflectivity measurements were successfully performed on a cell membrane mimic system consisting of a polyelectrolyte (PE) multilayer plus synthetic terpolymer (TER) plus a phospholipid (PC) layer assembly, at all of the intermediate steps in the assembly of the composite, to directly obtain neutron scattering length density depth profiles. The PE+TER+PC supported membrane-mimetic was

found to contain 40% water in the “cushion” PE multilayer under humid conditions. The TER-PC region was found to contain 10% water under the same conditions. The overall thickness change due to the incorporation of water under humid conditions was found to be 10Å and was due to a change in thickness in the PE multilayers. Upon fusion of phospholipid vesicles, the composite PE+TER+PC assembly shows an increase in thickness of 20Å, consistent with the formation of a phospholipid monolayer at the surface. This was further confirmed by fusing d-DPPC vesicles to the PE+TER assembly, which resulted in a much larger increase in the SLD of the assembly at the surface. Upon comparing the SLD profiles for the PE+TER and PE+TER+PC assemblies, it is evident that the terpolymer layer undergoes significant rearrangement upon the addition of the phospholipid layer.

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FIGURE CAPTIONS

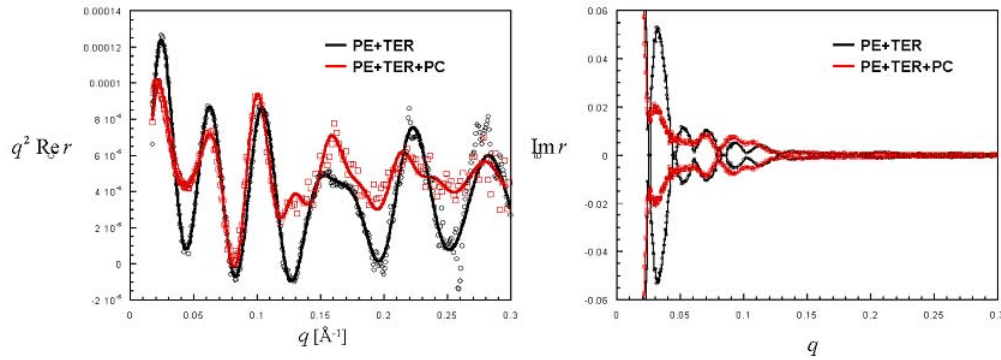


Figure 1. (a) Real part of the complex reflectivity amplitude times Q^2 as a function of Q for the (o) PE+TER assembly and the (\square) PE+TER+PC assembly in 92% D_2O humidity. The solid curves through the data are the calculated real part of the complex reflectivity amplitude from the corresponding SLD profiles shown in figure 3. (b) Corresponding imaginary part of the complex reflectivity amplitude.

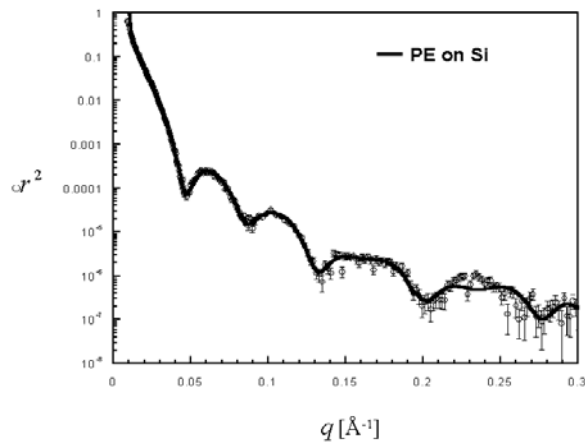


Figure 2. Reflectivity of the PE assembly on Si in 92% D_2O humidity. The solid curve through the data corresponds to the calculated reflectivity from the SLD profile shown in figure 3.

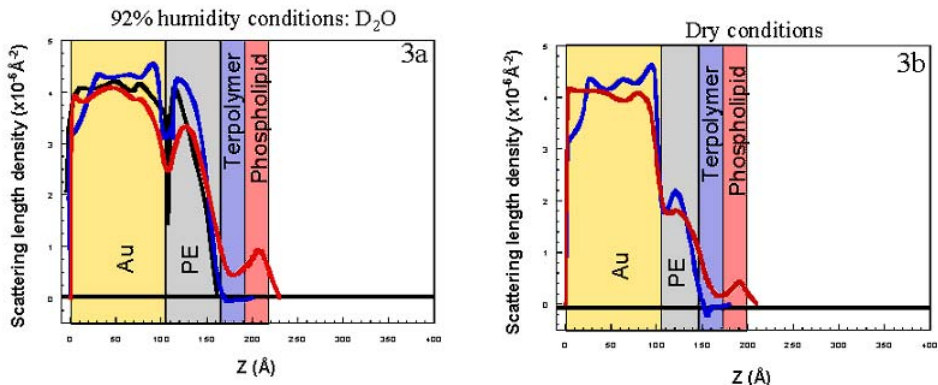


Figure 3. (a) SLD profiles for the (—) PE, the (—) PE+TER and the (—) PE+TER+PC assemblies in 92% D₂O humidity. (b) SLD profiles for the PE+TER and the PE+TER+PC assemblies dry.

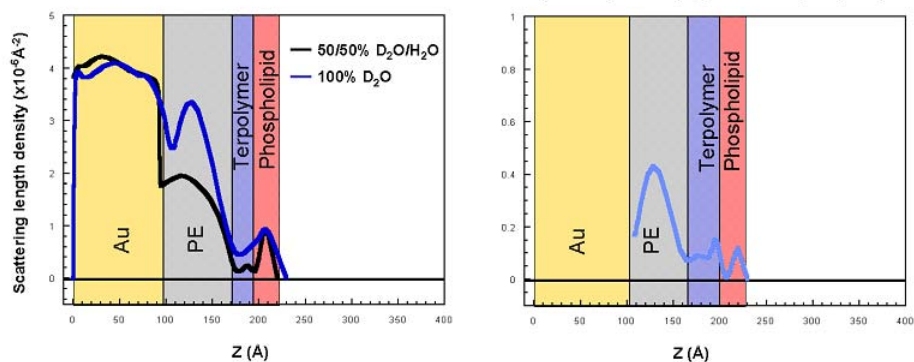


Figure 4. (a) SLD profile for the PE+TER+PC assembly under (—) 92% humid conditions in D₂O and (—) 50%/50% D₂O/H₂O. (b) Water fraction in the assembly under humid conditions.

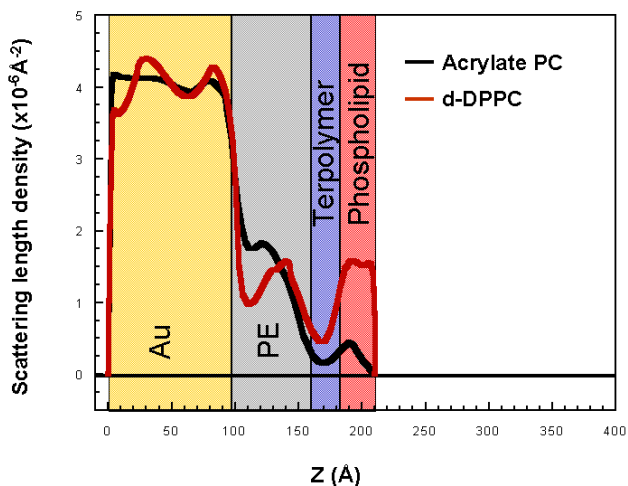


Figure 5. SLD profiles for the (—) PE+TER+PC assembly and the (—) PE+TER+d-DPPC assembly dry.

*Certain commercial materials, instruments, and equipment are identified in this manuscript in order to specify the experimental procedure as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials, instruments, or equipment identified is necessarily the best available for the purpose.

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