Measuring Height Mismatch and Miscibility Temperatures of Model Cell Membranes

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Introduction:

Hundreds of different lipids exist in the cell membrane, and little is understood regarding their physical and organizational properties [1]. Model lipid bilayers serve as a simplified means of studying many of the inter-molecular interactions that occur within cell membranes. For example, interactions between two or more lipids and cholesterol can result in demixing of lipid bilayers into regions with different lipid compositions. This membrane phase separation can be observed in model lipid bilayers composed of a high-melting temperature (T_m) lipid, a low-T_m lipid, and cholesterol. At temperatures above the phase separation temperature (miscibility temperature) of a composition, lipids mix together into one uniform liquid phase. At lower temperatures, two distinct phases coexist: a liquid ordered (L_o) and liquid disordered (L_d) phase. The L_o phase is primarily composed of high T_m lipids, whereas the L_d phase is composed mainly of low T_m lipids. After phase separation occurs, like phases coalesce, forming larger single-phase domains [2].

Currently, the factors determining the miscibility temperature of a lipid composition are unknown. Recent research proposes that miscibility temperature increases with the height mismatch of the L_o and L_d phases [3, 4, 5]. When the membrane separates into macroscopic phases of different heights, the total area of the hydrophobic lipid tail group exposed to the solvent (water) is thought to be reduced, making this configuration energetically favorable [3] (Figure 1).

The goal of our research was to elucidate the contribution of height mismatch vs. other lipid structural parameters in determining miscibility temperature. The purpose of this project was to discover whether two bilayers with similar miscibility temperatures could have different height mismatches between the demixed phases. In order to accomplish this, we had to perfect our methods of measuring miscibility temperatures and height mismatch in lipid bilayers. In this study, miscibility temperatures were determined by observing phase separation of giant unilamellar vesicles (GUVs) using fluorescence microscopy, and height mismatch was measured by atomic force microscopy (AFM) of supported lipid bilayers (Figure 2).

Methods:

Giant Unilamellar Vesicles (GUVs). Dipalmitoyl-sn-glycero-3-phosphotidylcholine (DPPC, 16:0) and dioleoyl-sn-glycero-3-phosphotidylcholine (DOPC, 18:1) were purchased from Avanti Polar Lipids, Inc. Cholesterol

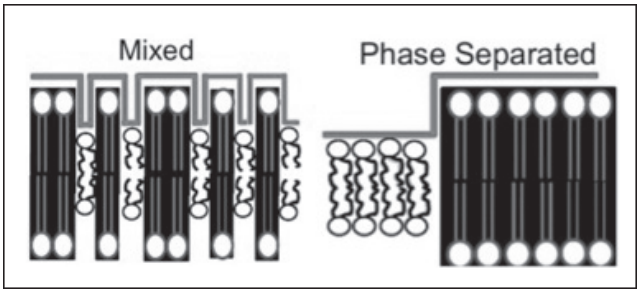

Figure 1: Schematic of height mismatch; the top line represents the surface exposed to water, thecircles are the lipid head groups, and the straight and bent lines are the lipid tails.

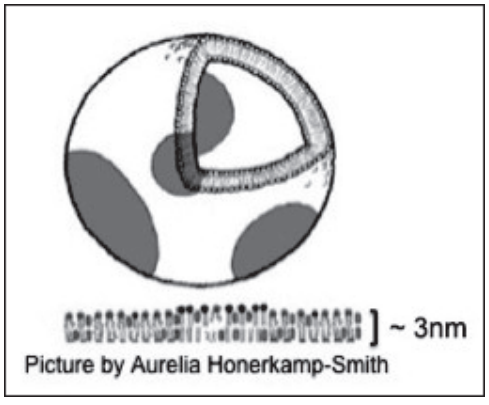

Figure 2: The top part of the figure shows a micron-scale, phase separated vesicle. The bottompicture shows a supported bilayer.

was purchased from Sigma, and Texas Red DHPE, a fluorescently labeled lipid, was purchased from Invitrogen®. All were purchased dissolved in chloroform.

Solutions of DOPC, DPPC, cholesterol (2:2:1), and 0.8% Texas Red were vortexed, spread on the conductive surface of indium tin oxide coated glass slides, and vacuum dried for 1/2 h. GUVs were formed by electroformation [6] at 60°C, 1.5 V, and10 Hz for 1 h. Vesicles were imaged using fluorescence microscopy. The transition temperature was determined to be the temperature at which 50% of vesicles showed phase separation.

Supported Bilayers. Solutions of DOPC, DPPC, and cholesterol (2:2:1) were dried under nitrogen and vortexed with purified water, making the final concentration of lipids 1 mM. In cases where phase separation was confirmed by fluorescence microscopy, the lipid solution contained 0.8% Texas Red DHPE. The solution was bath-sonicated for 1 h to form small vesicles and centrifuged for 1/2 h. 20 μL of solution was added to 180 μL of 5 mM $CaCl_2$ and immediately deposited as a thick droplet on a 4.91 cm^2 freshly cleaved mica substrate. An additional 5 ml of $CaCl_2$ was added for 1/2 h to allow a bilayer to form on the mica.

The preceding steps were carried out above the transition temperature of the composition (~34°C). The mica-supported bilayer was thoroughly rinsed with purified water. Supported bilayers containing dye were imaged by fluorescence microscopy. Supported bilayers without dye were imaged by AFM in constant-amplitude tapping mode in a liquid cell. The liquid cell kept bilayers submerged in purified water. We used a SNL-10 tip (spring constant: 0.32 N/m), a scan area of 100 μm^2, and a scan frequency of 1 Hz. The amplitude set point was varied to reduce artifacts.

Results:

Using fluorescence microscopy, we determined the miscibility temperature of a vesicle containing 2:2:1 DOPC, DPPC, and cholesterol to be 34.6 ± 0.6°C (Figure 3). The uncertainty represents the full range of miscibility temperatures. Additionally, we observed phase separation in supported bilayers with fluorescence microscopy. Using AFM, we observed nanometer-scale height mismatch in supported lipid bilayers.

Discussion:

In our research we observed phase separation on both supported bilayers and GUVs. Because the miscibility temperature we measured for DOPC, DPPC, and cholesterol (2:2:1) agrees with previous measurements by our lab [7], we have confidence in our lipid composition. In the future, our lab will study compositions of lipids with greater height mismatches and similar miscibility temperatures to continue this project.

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References:

[1] Mouritsen, O. Life- As a Matter of Fat. Springer, 2005.

[2] Veatch, S.L., Keller, S.L. Biophys. J. 2006, 90, 4428-4436.

[3] Garcia-Saez, A.J., Chiantia, S., Schwille, P. J Biol.Chem. 2007, 282, 33537-33544.

[4] Perlmutter, J.D., Sachs, J.N. J. Am. Chem. Soc. 2011, 133, 6563-6577.

[5] Kuzmin, P.I., Akimov, S.A., Chizmadhev, Y.A., Zimmerberg, C., Frederic, S. Biophys. J. 2005, 88, 1120-1133.

[6] Angelova, M. I., Soleu, S., Meleand, P., Faucon, J.F., Bothorel, P. Prog. Colloid. Polym. Sci. 1992, 89, 127-131.

[7] Veatch, S.L., Keller, S.L. Biophys. J. 2003, 85, 3074-3083.

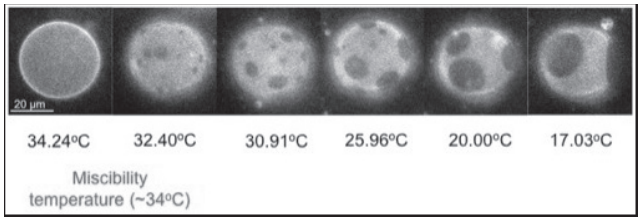

Figure 3: These pictures show the same GUV at different temperatures. Its miscibility temperature is ~34°C.

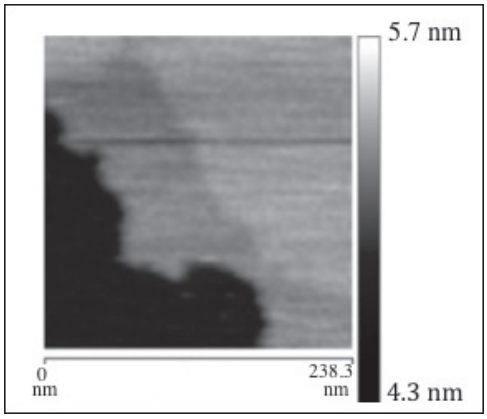

Figure 4: AFM image; the black is the mica substrate, and the two lighter shades show a height difference in the sample.