ADVANCED MATERIALS

Supporting Information

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Supporting information for "Long-lived Planar Lipid Bilayer Membranes Anchored to an *In Situ* Polymerized Hydrogel"

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1. Single-channel insertion of alpha-hemolysin molecules.

To test membrane fluidity, α-hemolysin was inserted in the cgHEM. A representative conductance trace demonstrating single-channel insertion is shown in Figure 1.1 below.

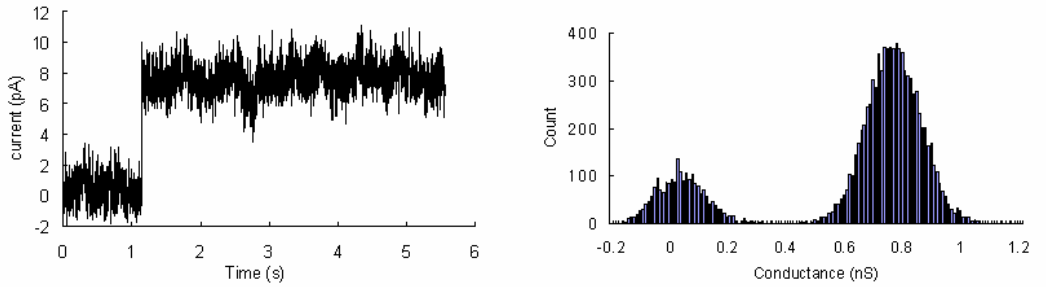

Figure 1.1: Conductance trace of an αHL channel inserted in a cgHEM and the corresponding conductance histogram

Alpha-hemolysin solution was deposited atop of the hydrogel; the protein diffused through the hydrogel and was successfully incorporated in the cgHEM, demonstrating that the cgHEM is sufficiently fluid to accommodate transmembrane proteins. The conductance level of the αHL on the cgHEM usually stayed within the ranges that have been reported in the literature. However, the current noise was high, likely due to the interaction of the αHL pore with the non-conjugated free PEG molecules (Bezrukov and Kasianowicz 1997). This noise was minimized by extending the UV illumination time to crosslink all of the free PEG molecules in the solution; however, some amount of noise was still present. This noise could potentially be reduced by using different types of crosslinkable monomers, such as acrylamide, to form the hydrogel, as well as different channel/pore proteins. For αHL insertion, 2 to 10 hours were required to have a first αHL channel inserted into cgHEM after the addition of the protein solution, as reported in our previous work (Jeon et al. 2006). In order to have a single protein insertion more efficiently, other researchers suggested in their recent work that buffer exchange can be done before the hydrogel is made (Kang et al. 2007; Shim and Gu 2007). The data shown in Figure 1.1 were acquired with the sampling frequency at 2 kHz and the current trace was filtered using Clampfit software (version 10, Axon Instruments, Foster City, CA) with a Gaussian low-pass filter at 200 Hz.

All references as listed in the communication, with the following additional reference:

Bezrukov, S. M., and J. J. Kasianowicz, *Eur. Biophys. J.* **1997**, *26*, 471.

2. Distinguishing the bilayer membrane from the solvent annulus in transmission microscopy images.

For experiments involving membranes with applied pressure gradients, the radius of the membrane was measured from microscopic images. A typical image is shown in Figure 2.1 below:

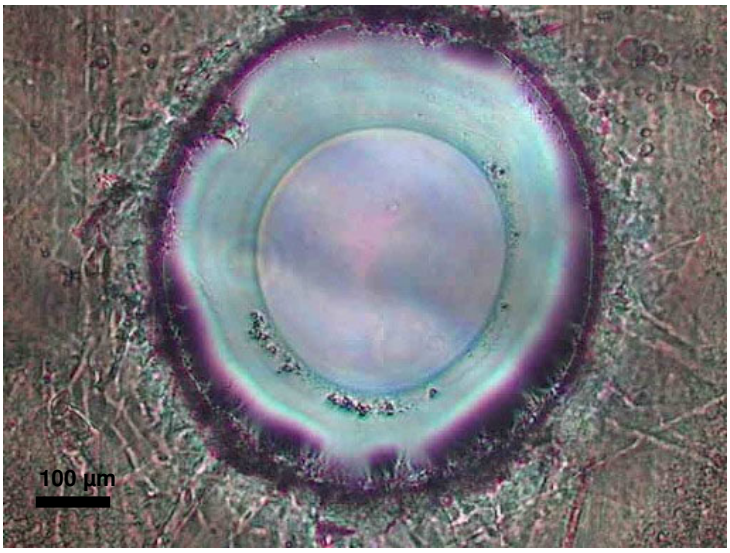

Figure 2.1: Transmission microscopy image of a freestanding planar lipid bilayer membrane

In Figure 2.2, this image is color-overlaid to distinguish the various visible features. The membrane can be seen at the center of the image, surrounded by the solvent annulus (Plateau-Gibbs border). The entire membrane/annulus system is supported on an orifice in a Teflon substrate.

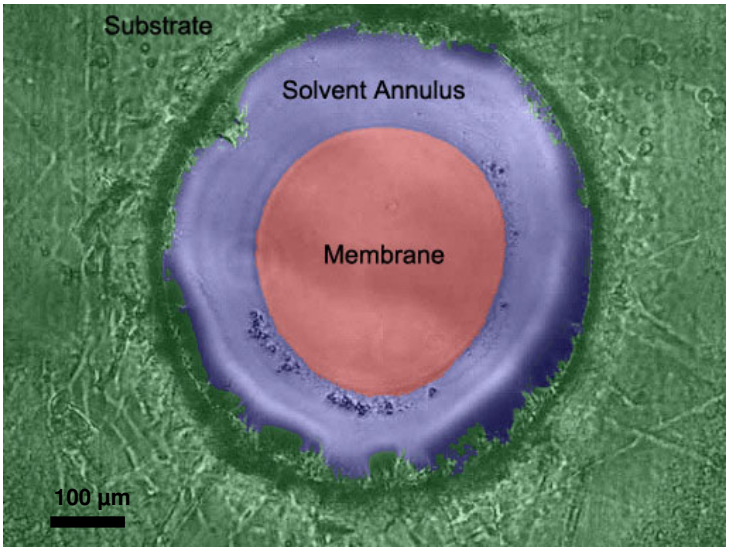

Figure 2.2: Membrane image color-coded to identify features

3. Membrane bulging out from the substrate.
The file entitled '`http://schmidtlab.seas.ucla.edu/membrane_bulge.avi`' is a stop-motion video of an unencapsulated membrane subjected to increasing pressure. At each frame, the pressure is increased by .36 Pa and the membrane is allowed to equilibrate for two minutes before the image is captured. The solvent annulus can be seen to shrink, and the interface between the membrane and the substrate can be seen to eventually leave the orifice completely. At this point, it has taken on a hemispherical shape, and is bulging away from the camera. Finally, the membrane bursts.

4. Change in annulus size with applied pressure for a membrane supported on one side by a hydrogel.
Figure 4.1 shows a series of images of a membrane being subjected to an increasing pressure gradient. A hydrogel has been formed on the side of the membrane opposite to the one shown here. The trace of

membrane area (measured using ImageJ, http://rsb.info.nih.gov/ij/) versus pressure shows the linear relationship expected for a uniformly compressed membrane. The pressure at which this curve plateaus corresponds both to the complete filling of the orifice with the membrane and with the kink in the HEM curve in Figure 2b in the main paper.

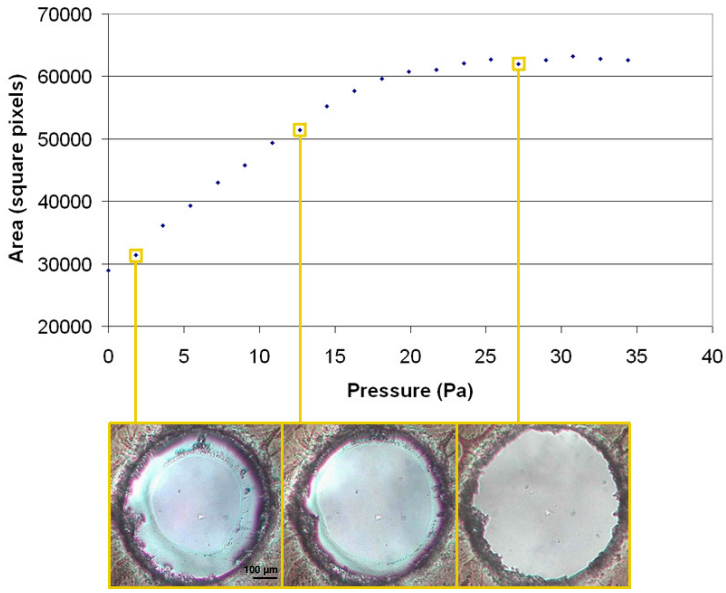

Figure 4.1: Change in solvent annulus size and membrane area of a singly-supported HEM with applied pressure

5. Solvent in the annulus of a cgHEM subsequent to membrane failure

The video file entitled '`http://schmidtlab.seas.ucla.edu/solvent_adhesion.avi`' shows a cgHEM system in which the membrane has just failed under the application of a ~50 Pa pressure gradient. The solvent from the Plateau-Gibbs border can be seen wetting the hydrogel on the left of the image, flowing freely rather than contracting into a minimum surface area configuration. This indicates that the surface of the hydrogel has been made hydrophobic, most likely by conjugation to acrylate-modified lipids.

6. The mobility of solvent islands in unencapsulated membranes and HEMs.

The video file entitled '`http://schmidtlab.seas.ucla.edu/diffusing_solvent_island.avi`' shows the formation of a solvent-rich island in a BLM during the process of membrane thinning. This island can be seen to quickly diffuse in the plane of the membrane until it fuses with the solvent annulus at the membrane-substrate boundary. The video file entitled '`http://schmidtlab.seas.ucla.edu/immobile_solvent_islands.avi`' shows a HEM with similar solvent-enriched islands. These islands, however, are immobile, held in place by the hydrogel.