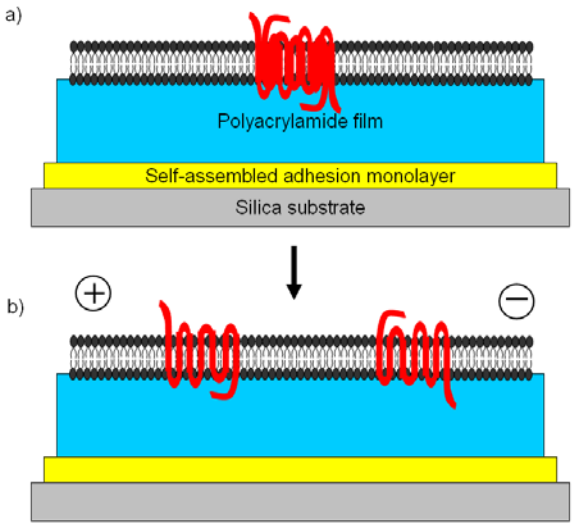
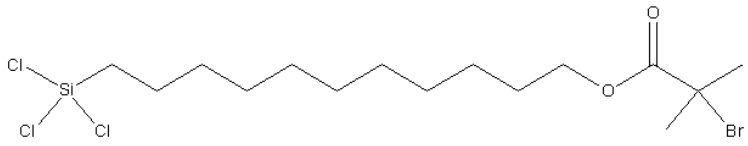
Final progress report, February 2011. PI: Mary J. Wirth; DE-FG02-04ER15596
Linear and Nonlinear Spectroscopic Probing of Solute Interactions with Chemically Modified Silica Surfaces

Perspective. Solar energy conversion through biology would provide a renewable and nonpolluting abundance of energy. The bacterium *Halobacterium salinarum* converts solar to electrical energy by virtue of a transmembrane protein, bacteriorhodopsin. This transmembrane protein pumps protons across a nonconducting bilayer upon irradiation with green light. The bacterium evolved to perform this function inefficiently. If we were able to understand this process to engineer this protein for efficiency, then inexpensive energy production could be achieved. There are tens of thousands of different types of halobacteria, giving the opportunity to study different efficiencies and relating these to the protein structures. Technology does not yet exist to perform such screening. The goal of this research is to generate new separation technology that can ultimately enable such screening. This involves creating a method for separating oriented and functional transmembrane proteins that remain in an electrically insulating lipid bilayer, with aqueous solutions on either side of the bilayer. A pH change across the lipid bilayer upon irradiation of a known concentration of proteins would probe function. Differences in proton pumping efficiency for different proteins variants would provide structure-function information for engineering the proteins.

Introduction. A schematic diagram from the original proposal is shown here. The idea is that a) a lipid bilayer supported on a hydrophilic polymer film will make the bilayer fluid, and b) applying an electric field will cause electrophoretic migration of the transmembrane proteins. We demonstrated this concept experimentally in a paper that was published just after this new grant period started (Lipid Bilayers on Polyacrylamide Brushes for Inclusion of Membrane Proteins, Emily A. Smith, Jason W. Coym, Scott M. Cowell, Victor J. Hruby, Henry I. Yamamura, Mary J. Wirth, *Langmuir*, *21*, 9644-9650, **2005**). The electrophoretic mobility was slow (10^{-8} cm^2/Vs), and we project that a two order of magnitude increase would make this a practical tool. We are investigating two ways of improving electrophoretic mobility: better polymer supports, and a novel nanoporous medium that suspends the bilayer over free solution.



Polymer supports. In a previous grant period we had shown that termination of polymerization was significant, and this causes polydispersity. The work at the end of the last grant period showed that polydispersity slows the electrophoretic mobility. To this end, we implemented a new initiator, shown below, that has its radical stability better matched to that of acrylamide than does the benzyl chloride that we had used previously. We are presently characterizing its reaction kinetics with different catalysts, and it does give thicker polyacrylamide films. We have found that this slows the lateral diffusion of lipid probes, as we had anticipated in the original proposal. The next step is to put spacers in between the initiators to lower the

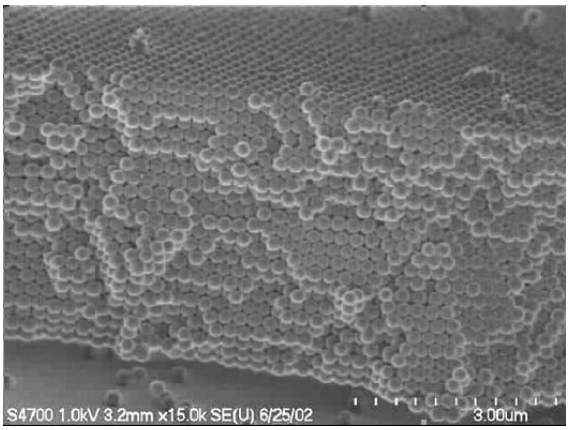


$C_{15}H_{28}BrCl_3O_2Si$
Exact Mass: 452.01
Mol. Wt.: 454.73
C, 39.62; H, 6.21; Br, 17.57; Cl, 23.39; O, 7.04; Si, 6.18

effective viscosity of the polymer film. This laborious work is underway ernestly, requiring many person-hours for the polymerizations, the depositions of bilayers and the studies of fluorescence recovery after photobleaching.

Nanoporous supports.

We are investigating colloidal crystals for various applications in our other sponsored projects, including as HPLC stationary phases and as porous coatings on coverslips for optically imaging live cells. A scanning electron micrograph (SEM) image is shown in the figure to the right, illustrating a crystal of 200-nm colloids. It struck us that these could possibly support lipid bilayers, suspending regions of the bilayer over the pores of the colloidal crystal. Both sides of the bilayer would be in contact with water, which would give high mobility. We extruded vesicles larger than the pore sizes to form lipid bilayers on the colloids.



We confirm the bilayer is across the top of the colloids by monitoring mobility of a fluorescence-labeled lipid. The fluorescence recovery curve after photobleaching is shown in the figure below. This gives two results that are exciting and unprecedented. 1) The recovery fits to a single exponential (red curve). Supported lipid bilayers normally fit to a double exponential because the side of the bilayer facing the support has a higher friction coefficient than the one facing the solution. This backs the conclusion that the bilayer is truly suspended over the pores. The bilayer is presumed to attach at the tops of the colloids, and the results suggest that the labeled lipids do not significantly exchange with the attachment sites. 2) A 100% recovery is observed. This is unheard of in studies of supported lipid bilayers, because the support side normally has sites for irreversible adsorption, thus it further confirms that the bilayer is suspended over aqueous pores. We found that the conditions of deposition are critical in forming the bilayer on top of, rather than inside of, the crystals. No recovery was observed when the bilayer formed in the crystal. We introduced labeled bacteriorhodopsin, but it was mobile. Perhaps it exchanges with the attachment points, or perhaps the high concentrations of protein pulled the bilayer into the crystal.