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Hydrogel-Encapsulated Lipid Membranes

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Planar lipid bilayers provide an environment enabling single-molecule electrophysiological observations of membrane channel and pore proteins.[1] Such measurements are essential to understanding the proteins' biological function, as well as the basis of highly specific sensors capable of chemical detection[2] or potentially sequencing DNA at the single-molecule level.[3] Unfortunately, the physical properties of planar lipid bilayers limit their scientific and technological application: they are difficult to form, physically weak, subject to mechanical and acoustical perturbation, and short-lived. This paper describes a method for the creation of long-lived and physically robust membranes by encapsulating them in situ in a polymer hydrogel; further, it demonstrates their potential application for nanopore DNA sequencing.

A recent approach to address the fragility and short lifetime of lipid bilayers has been to tether them to solid surfaces.[4] Such systems combine the lipid bilayer membranes' fluidity and capacity for protein incorporation with the mechanical stability of a solid support. While electrical transport through ensembles of membrane protein channels has been measured in such systems,[5] no single channels have been detected to date. This is because tethered bilayer membranes have thus far proved incapable of producing the highly insulating seals necessary for single-molecule measurements,[6] although this is improving.[7] Studies with tethered bilayers have also shown an inability to quantitatively measure the magnitude of incorporated channel conductances, as a result of the high in-plane resistance of the electrolyte reservoir near the substrate.[8] Furthermore, the presence of the solid surface (typically a gold electrode) makes long-term DC measurements and analyte transport across the channel problematic.

Gels are appealing alternative materials for membrane supports. They can provide mechanical stability while allowing the membrane access to a bulk-like aqueous environment, enabling a low resistance path to the pore for ionic currents and diffusing analytes. Gels have been used previously to support membranes; in these cases, lipid solutions were deposited on top of pre-cast gels, but the resultant membranes were too leaky for single-channel measurement.[9] Ide and Yanagida formed high-resistance free-standing membranes self-assembled in aqueous solution and brought them into contact with a pre-cast gel on one side.[10] Although single channels were measured in that work, the membranes still suffered from short lifetimes. Peterson and co-workers physically sandwiched a lipid membrane between two preformed slabs of gel.[11] Their technique, however, has not been shown to achieve sufficiently high membrane resistances for single-molecule measurements. These techniques have all relied on pre-cast gels, and they have met with limited success. The approach discussed in this paper creates a stable long-lived platform for single-channel measurements by encapsulating a pre-existing free-standing high-resistance membrane within a gel polymerized around it in situ.

We create hydrogel-encapsulated membranes (HEMs) by first forming high resistance (>1 GΩ) lipid bilayer membranes on 200 μm diameter Teflon apertures from a solution of 3% (w/v) diphytanoylphosphatidylcholine (DPhPC) in *n*-decane using standard methods.[12] The aqueous solution surrounding these membranes contains 1 M KCl, 5 mM HEPES (pH 7.0), 7.5% (w/v) poly(ethylene glycol) dimethacrylate (PEG-DMA) monomers (1 kDa, Polysciences, Warrington, PA), and 1% (w/v) Irgacure 2959 UV photoinitiator (CIBA Specialty Chemicals, Tarrytown, NY). Following membrane formation, PEG-DMA polymerization was triggered by exposure to 400 W broad spectrum UV light for 5−6 min. The membranes before and after gelation were probed electrically using Ag/AgCl electrodes connected to an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Over 50 attempts, HEMs formed in this initial study remained intact for a mean duration of 48 h, with some lasting up to 5 days, as compared to a mean duration of 12 h (with a maximum of ∼24 h) without the presence of the gel.

The electrical characteristics of the HEMs were consistently stable over this period of time. Membrane thickness was determined by measuring the capacitance of the bilayer.[13] The approximate thickness of the HEM was determined (using a dielectric constant of 2.6 [14]) to be 4.7 ± 0.5 nm (n = 25), consistent with a molecular bilayer of DPhPC. HEM resistance was consistently greater than 10 GΩ over the period the membranes remained intact. In addition, HEMs showed unusual mechanical stability. While planar lipid membranes are typically quite susceptible to mechanical and acoustic perturbation, chambers containing HEMs could be handled roughly with no ill effect to the membrane. Following the completion of an experiment, the gel was physically removed and the chamber was disassembled. The chamber and Teflon partition could be reused following a simple cleaning procedure (wash with 5% (w/v) Triton X-100, rinse with water and 40% methanol in water).

Using the method of Canal and Peppas,[15] we determined the hydrogel polymer mesh size to be approximately 7 nm. To establish that this gel allows the diffusion of molecules of interest to the encapsulated membrane, the pore protein α-hemolysin (αHL) was introduced to the HEM. αHL is a 34 kD water-soluble polypeptide from *Staphylococcus aureus* that combines with other αHL monomers to form a 1.5−2.5 nm diameter heptameric pore in lipid membranes;[16] 1.7 ng of heptameric αHL in an aqueous solution of 200 mM NaCl and buffered with 100 mM Tris·HCl (pH 8.2) was deposited atop the gel, about 0.5 cm from the membrane. αHL insertion into the membrane was observed as a discrete 0.8 nS jump in membrane conductance within 2−10 h of this deposition. Without the presence of the gel, addition of the same amount of protein resulted in the first incorporation about 0.5−2 h later in an unstirred solution. The increase in time is consistent with a 70% decrease of the effective diffusion constant of the protein caused by the gel, as predicted by the theory of Lustig and Peppas[17] for the gel mesh size and protein molecular weight in question. We have also observed a similar stabilization effect for membranes supported by gel on only one side. In preliminary experiments, we formed a gel on one side of a membrane, added protein (which incorporated

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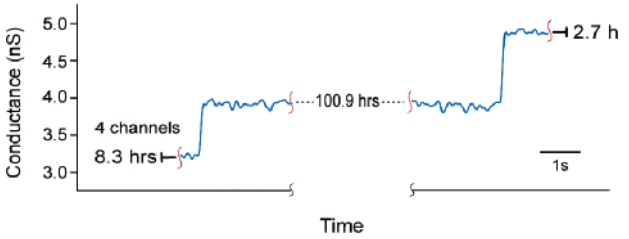

Figure 1. The stable conductance of five αHL channels incorporated into a HEM was observed over 100 h.

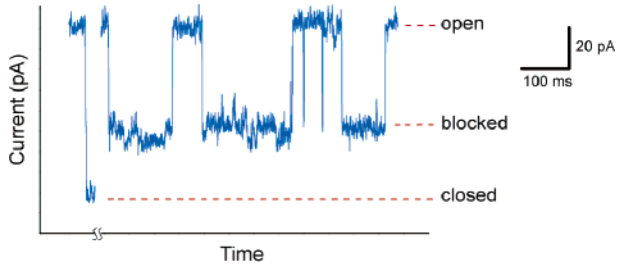

Figure 2. Current trace of DNA translocation through αHL incorporated into a HEM at 80 mV potential. Three blockages of the open channel current are shown with durations of 125, 180, and 86 ms.

quickly) on the free solution side, and changed solution to halt further incorporation. The solution could then be replaced with gel precursor solution and UV polymerized for full encapsulation. No changes to the transport properties of αHL were observed as a result of the solution exchange or UV illumination.

Hydrogel encapsulation greatly stabilized membranes both with and without protein incorporated into them. Stable single-channel currents of α-hemolysin in a HEM were measured for several days (Figure 1). The voltage gating property and characteristic conductance of αHL in DPhPC were measured. Upon application of a 100 mV potential, spontaneous closure of active αHL was observed, a well-known characteristic of α-toxin pores.[18]

To test the delivery of analyte molecules to a channel protein embedded in a HEM, we added 150 base-long single strands of DNA consisting only of the bases A and C to HEMs containing incorporated αHL. Recent work examining the electrophoretic transport of DNA through nanopores has observed that the DNA travels through the pore so quickly that the passage of single bases cannot be resolved at standard electronic measurement bandwidths.[3,19] However, in our system, 40 min after the addition of DNA, current blockages were observed 3−700 ms in length, over 100 times longer than those reported previously (Figure 2). The slowest translocation times indicate that each base traverses the pore in milliseconds, well within the reach of conventional electronics. This DNA is not expected to have any secondary structure, so the delayed transit time is presumed to be due solely to the presence of the gel. Since the free coil radius of the DNA is expected to be >10 nm and larger than the mesh size of the gel,[20] it should reptate rather than freely diffuse through the gel,[21] decreasing its effective diffusivity by over 50-fold.[22] Meller and co-workers found that the DNA translocation was significantly slowed by its interaction with or confinement by the pore, and that there appeared to be an energy barrier impeding DNA entry into the pore as well,[23] all which could be increased by the presence of the gel. Work with DNA translocation through nanopores in HEMs is ongoing and can be expected to contribute to the understanding of the fundamental physics of DNA nanopore translocation.

In summary, in situ hydrogel encapsulation imparts to lipid bilayer membranes the durability and longevity necessary for extended single-molecule biophysical studies and engineered device applications of integral membrane proteins. These include field applications of protein-based sensors and investigations of interactions between membrane proteins and small molecules for drug discovery. Of particular interest is the potential of the gel to slow the transit of single-stranded DNA driven electrophoretically through the pore for the purposes of electrical characterization.

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