Integrated Massively Parallel Arrays of Stochastic Sensors (IMPASS)

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Our contract started 1 July. We immediately started to place orders for supplies and equipment. We obtained two Axopatch 200B patch clamp amplifiers, and air tables to eliminate vibrations. We placed an order for a KSV Langmuir trough for the creation of large areas of polymer monolayers. We placed an order for an incubating shaker table from Brunswick. We placed an order for an FPLC to facilitate protein purification.

We obtained a custom synthesis of polymer from Polymer Source Inc. (Quebec Canada). We also obtained glassware necessary for the in-house custom synthesis of polymer.

Our work proceeded along several fronts: 1) Synthesis and manipulation of the polymer 2) Synthesis and manipulation of the protein component 3) Construction and testing of the membrane apparatus. 4) Incorporation of protein into polymer membranes and measurement of transmembrane conductance 5) Creation of polymer membranes supported by micromachined substrates

1) Synthesis and manipulation of the polymer

While constructing the synthetic chemistry apparatus for the creation of the polymer, we worked with the commercially obtained polymer. One of the important properties of the polymer is that it is able to crosslink. We performed NMR experiments on polymer unexposed to UV and with a 30 minute exposure and found that the degree of crosslinking can be easily observed (see figure). The top figure shows the spectrum of unexposed polymer, with the peaks corresponding to the unmodified methacrylate groups circled in red. The bottom figure shows the result of crosslinking, as the peaks have disappeared. We performed other experiments in which the time and intensity
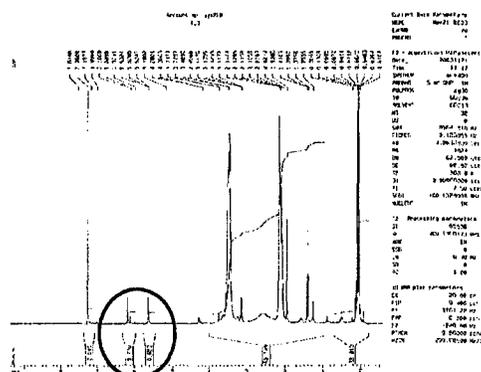
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14. ABSTRACT
We report the successful formation of biomimetic polymer membranes approximately 5-6nm in thickness made from the biomimetic amphiphilic triblock copolymer poly(methyloxazoline)-poly(dimethylsiloxane)-poly(methyloxazoline). We have also successfully inserted a number of membrane proteins (OmpG, MscL, alpha-hemolysin, and alamethicin) into these membranes and demonstrated that these proteins retain their natural function and capabilities. We have found that these membranes exhibit extended lifetimes compared to lipid membranes and on micromachined substrates the lifetime is extended even further (~3 days). As a result of this work, we have found that biomimetic polymer membranes are suitable subjects for further research in membrane protein-based stochastic sensing.

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of illumination was varied, and we found that the integrated area under these peaks was directly indicative of the amount of uncrosslinked chemical groups.

After 4 months, the polymer synthetic apparatus was complete and over the next 14 months a number of amphipathic biomimetic polymers were constructed. There were two main categories of synthesized polymer. **1) symmetric tri-block copolymers** (reactions shown below for poly(methyloxazoline)-poly(dimethylsiloxane)-poly(methyloxazoline) (PMOXA-PDMS-PMOXA)

ABA (A: PMOXA, B: PDMS, A: PMOXA)
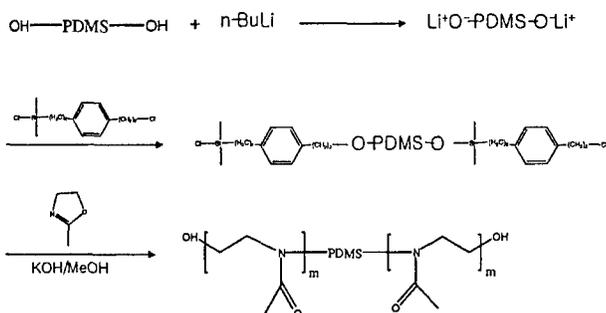
2) asymmetric tri-block copolymers (reactions shown below for poly(ethyleneglycol)-poly(dimethylsiloxane)-poly(ethyleneglycol) (PEG-PDMS-PEG'), where the PEG chains are of different lengths.

ABA´(A: PEG, B: PDMS, A´: PEG´)
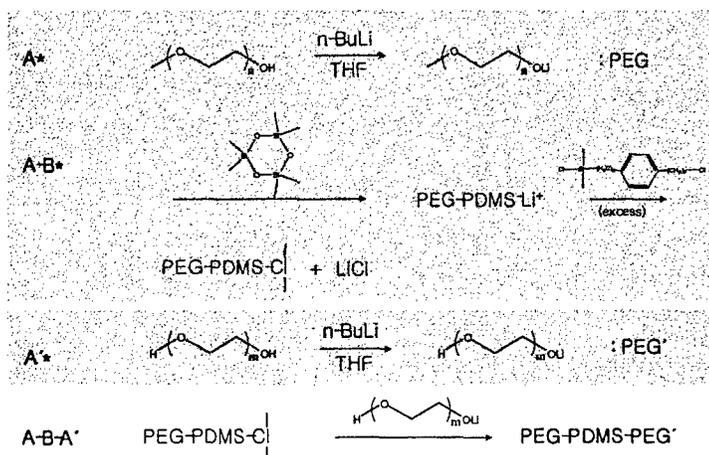
## 2) Synthesis and manipulation of the protein component

Following published data (Conlan et al., Biochemistry 2000, 39, 11845-11854) we ordered forward and reverse primers for OmpG and cloned the gene from E. coli using PCR. We then inserted this gene into a plasmid and attached a 6xHis tag onto the N-terminus of the protein. There was considerable difficulty in inserting this plasmid into porin deficient bacteria for overexpression and protein production.

On the protein OmpF, we identified a candidate residue for mutagenesis to Cysteine, residue 183. Initial energy minimization calculations were made to determine whether the putative mutagenesis would have any deleterious effects on the protein structure. We did not determine this to be the case and went ahead with the modifications. We verified the successful changes by sequencing the mutant OmpF gene.

Initial GROMOS96 Energy/Conformation Calculations
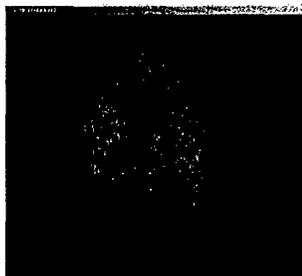

SPDB E-field Analysis of Protein
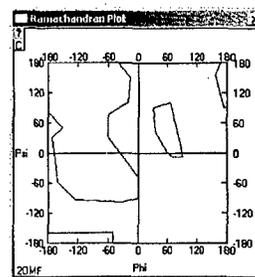
Ramachandran plot analyzing phi/psi angles of conformation- Determines integrity of β-barrel structure.

We aimed to test as many membrane proteins as possible in the polymer membranes to test the extent of their biomimicry.

We chose OmpF, OmpG, α-hemolysin, MscL, and alamethicin. Having already produced, purified and modified OmpF, we cloned and purified OmpG and MscL from bacterial sources, purchased alamethicin from Sigma Chemicals, and obtained αHL as a gift from Stephen Cheley at Texas A&M. We show SDS-PAGE gels below to demonstrate that the protein for experimentation was pure and that transports results arise solely from the intended protein.

Insertion of protein into polymer: MscL, OmpG, αHL, Alamethicin
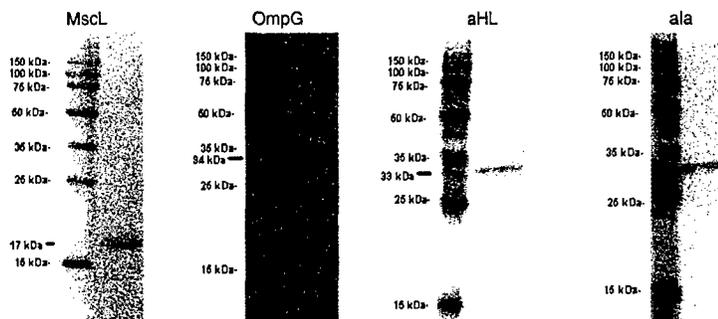
αHL was a gift from Stephen Cheley; Alamethicin obtained commercially; MscL and OmpG produced in house

3) Construction and testing of the membrane apparatus

We machined teflon BLM chambers in-house as well as obtaining them commercially. We formed lipid and polymer membranes over 100 μm and 200 μm holes. To measure these membranes we used both commercial and homebuilt electronics.

We installed and tested the Axopatch 200B. Below is a sample data trace taken of the β-amyloid peptide at 1kHz and filtered with a 100 Hz Bessel filter (Major vertical ticks are 5 pA, and the major horizontal ticks are 2000 ms). The noise is sub-pA, more than suitable for the experiments we plan to do.
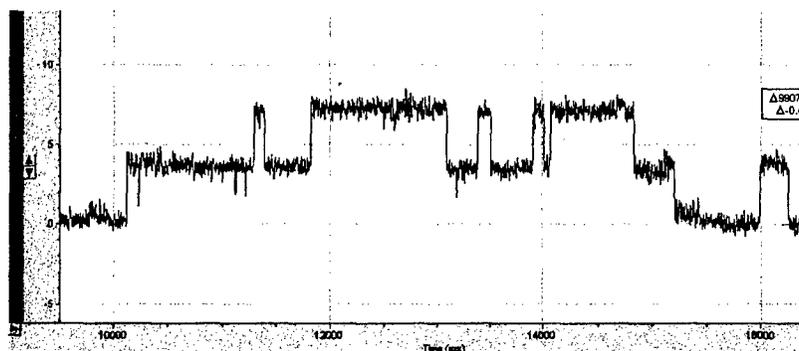
A measurement of the polymer membrane with the homebuilt electronics and software verified that the hardware and software were working properly and that the membrane monolayers were able to be made successfully and their thickness measured (see figure below).
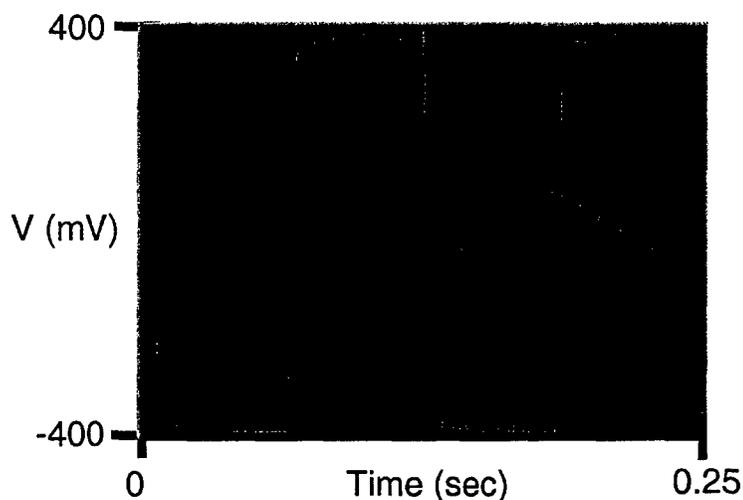

In this figure, an alternating triangle wave voltage is applied across the membrane, which acts like a parallel plate capacitor. This produces a current which has a waveform which is the derivative of the applied voltage, and the amplitude of which is proportional to the capacitance, which itself is inversely proportional to the thickness of the membrane. The waveform above was a measurement of a polymer membrane monolayer with a thickness of 8.5 nm. We have made and measured polymer membranes 5 nm thick as well.

4) Incorporation of protein into polymer membranes and measurement of transmembrane conductance

We incorporated the proteins from 2) above into membranes made in step 3) from the polymer synthesized in 1). The incorporation of all proteins was successful and easily detected using our homebuilt and commercial electronics. The incorporation of a protein appeared as a stepwise, quantized increase in the transmembrane conductance, shown below for αHL.

# α-hemolysin Incorporated into Polymer Membrane
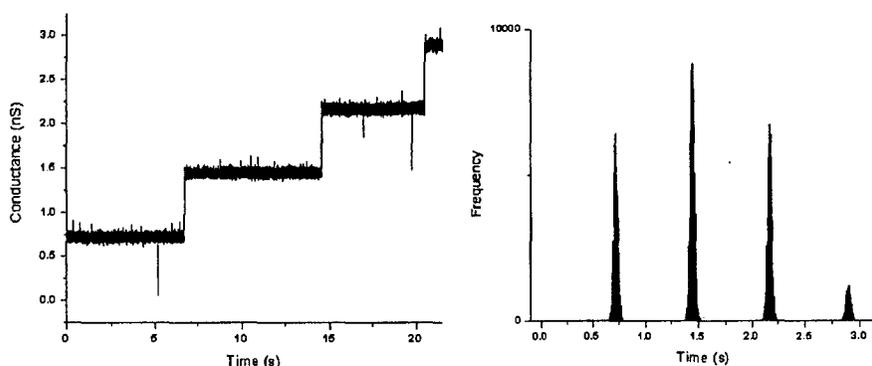
aHL incorporates readily (~.72 nS in 1M KCl)

The conductance for aHL was approximately 10% less than measured in lipid membranes, but this is within the variability of protein conductances within membranes of different lipid consitutents.

We also determined that the polymer membrane does not alter any function of incorporated protein, for example voltage gating of OmpG (below). OmpG naturally closes in transmembrane voltages around 80mV. We were able to demonstrate this effect also in OmpG incorporated into polymer membranes.
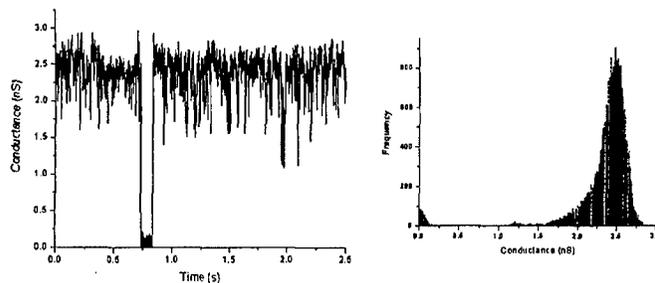
Gating of OmpG
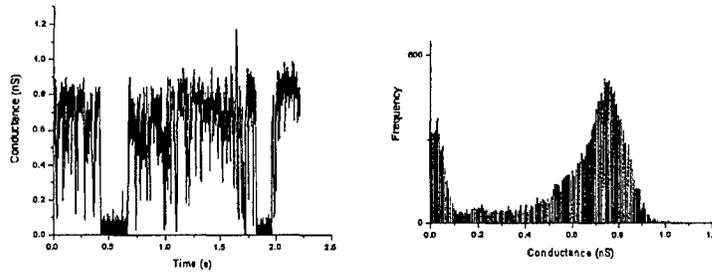
Voltage gating occurs as in lipid;

80 mV applied

Likewise, MscL's conductance is modulated by the membrane tension (pressure gated), and we were able to reproduce this behavior in polymer membranes:

Pressure gating of MscL in polmyer

16 mmHg applied

Finally, the fluidity of the membrane can also affect protein function and interactions. We probed this property through the incorporation of the oligomerizing transmembrane peptide alamethicin. We found that we were able to incorporate this protein and that it has similar conductance characteristics and behavior as seen in lipid membranes (shown below).

Alamethicin in Polymer
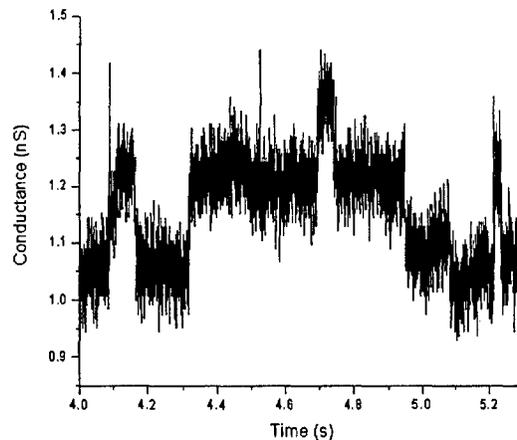
5) Creation of polymer membranes supported by micromachined substrates

Finally, we also experimented with replacing the membrane support substrates traditionally made out of teflon with micromachined substrates made from silicon. We coated these substrates with a hydrophobic SAM (shown below), which enabled the lipid and polymer membranes to be successfully formed on the Si holes. We found that we were able to create membranes on these substrates with longevity ten times longer than the same membranes. Although the cause of this significant improvement in lifetime is not yet fully understood, this result causes us to be very optimistic going forward about the prospects of the polymer membranes as long-lived, robust substitutes for lipid membranes in membrane protein-based stochastic sensing applications.