

nanopore sequencing: results

I was awarded a Reed College Melon Foundation opportunity grant in November, 2008. This is a brief summary of my positive results, with an attached copy of the original grant.

Since I was only interested in bilayer formation, I constructed simple voltage divider to measure bilayer resistance. An approximately 9 V, 100 Hz sinusoidal signal was used to drive the system, and the potential across the divider resistor was measured with an oscilloscope. The apparatus was filled with either 1 M KCl or the ionic liquid, [bmim]⁺[BF₄]⁻. For both solvents, bubbles were a problem, but were removed via aggravated tapping or syringe. The conductivity of the ionic liquid actually seemed to be less than that of the 1 M KCl, contrary to what one would expect*. This is in agreement with new (to the author) experimental data reporting the conductivity of this liquid to be 0.4 S/m compared to that of 1 M KCl; 11 S/m. This is likely due to the much slower charge mobility, as the liquid is quite viscous.

Egg phosphocholines dissolved in decane were added to the cis side (the well containing the tapered end of the Teflon tube), and after some poking, the potential across the resistor was no longer detectable, presumably due to the very high resistance of tube blockage. In the room temperature ionic liquid, addition of small amounts of the lipid eliminated the potential across the sense resistor almost immediately. It remains to be seen whether these blockages are due to the formation of a single bilayer or a larger lipid structure.

These results suggest that some lipid structure is forming to block current in the ionic liquid. Because of the decreased (rather than increased, as was hoped) electrical conductivity of this particular ionic liquid compared to KCl solutions, however, it may not be very useful for nanopore sequencing.

I would like to thank MAGGIE GESELBRACHT for a loan of Pt wire to use as electrodes, GREG EIBEL for his extensive help in machining an apparatus, RANDIE DALZIEL for miscellaneous equipment & discussion, and LUCAS ILLING for discussion & electrical equipment.

* For 1 M KCl solution, only 1 in 55 atoms (1 M means one mole per litre, thus (1000 g/L)/(18.02 g/mol) = 55.49 mol/L) is charged, whereas for ionic liquids every molecule is charged, so naïvely we expect the conductivity to be about 50 times greater.

Nanopore sequencing refers to pulling a single strand of DNA or RNA through a hole on the order of a nanometer by a voltage. Most work in the field has used α -hemolysin, a protein that assembles across lipid bilayers to form a hole with inner diameter of 2.6 nm.

By putting a potential difference across the bilayer, both single stranded DNA and salt anions are pulled through the hole. As DNA moves through the hole, it blocks the flow of salt anions; this blockage is related to the size of the nucleotide bases, and thus in theory one could watch the current flow as DNA moves through the pore to sequence the polynucleotide.

The lipid bilayer self assembles across the tip of a Teflon cone made from heat-shrink tubing moulded around a sharp needle. Trimming the tip with a razor blade creates the conical aperture across which the bilayer is formed (David Deamer, personal communication);

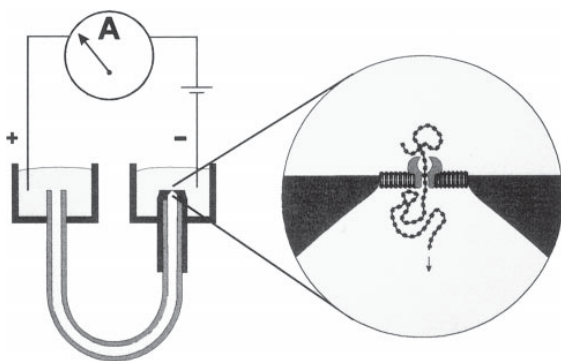


figure from Akeson *et al.*[1]. Note that we only need to hold a single strand of DNA, so both chambers and the connecting tube can fit in the palm of one's hand.

The ionic current through α -hemolysin is too small to detect with Reed's equipment (≈ 200 pA), but we can easily verify bilayer formation. I propose to replicate the first stage of these experiments—forming a bilayer across a Teflon aperture. Once this is done in aqueous buffer, I will attempt to form a bilayer in ionic liquid—a novel solvent that addresses the two primary hurdles of nanopore sequencing research.

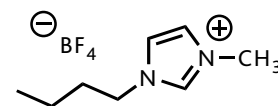
Ionic liquids are solvents with effectively every molecule carrying a full charge. The cation is typically organic with the charge delocalized to prevent crystallization. I suspect these solvents will be ideal for nanopore sequencing applications for two reasons. First, they increase the current blockage signal by virtue of consisting entirely of ions. Second, they are very viscous and remain liquid at low temperatures, both of which slow DNA translocation speed and thus provide more opportunity for the signal to reflect individual nucleotide bases.

The ionic liquid I propose to begin with, $[\text{bmim}]^+[\text{BF}_4]^-$, is miscible with water, and thus one would expect it to both solvate DNA and be polar enough to drive the formation of bilayers.

If the committee sees fit, I would like to initially request \$250 for device construction, the bilayer chemicals, and two ionic liquids (see attached cost sheet). If a bilayer can be reliably formed, in the spring I will need to purchase the α -hemolysin protein to begin the primary experiment. Unless the current signal is increased enormously, at this point work would move to OHSU to use a patch clamp amplifier. I will submit a second proposal at that point.

Thank you for your consideration.

[1] Mark Akeson, Daniel Branton, John J. Kasianowicz, Eric Brandin, and David W. Deamer. Microsecond Time-Scale Discrimination Among Polycytidylic Acid, Polyadenylic Acid, and Polyuridylic Acid as Homopolymers or as Segments Within Single RNA Molecules. *Biophys. J.*, 77(6):3227–3233, 1999



an ionic liquid:
1-butyl-3-methylimidazolium cation
with tetrafluoroborate anion