broad-spectrum phenotypes of both ML4 and *Va* appear to result from certain aspects of endosomal/lysosomal dysfunction. Lysosomes, traditionally believed to be the terminal ''recycle center'' for biological ''garbage'', are now known to play indispensable roles in membrane traffic and multiple intracellular signaling pathways. The putative lysosomal function(s) of TRPML proteins, however, has been unclear until recently. Studies on animal models and cell lines in which TRPML genes have been disrupted or genetically depleted have discovered roles of TRPMLs in a variety of cellular functions including membrane traffic, signal transduction, and organellar homeostasis. Physiological assays on cells in which TRPMLs are heterologously over-expressed revealed the channel properties of TRPMLs, suggesting that TRPMLs mediate cation (Ca^{2+}/Fe^{2+}) efflux from endosomes and lysosomes in response to unidentified cellular cues. Using our recently developed lysosome patch-clamp technique, we screened a variety of cytosolic and luminal factors that are known to affect endolysosomal functions and have identified an endogenous agonist for TRPML channels. We are currently investigating the activation mechanism in detail.

Platform AI: Micro & Nanotechnology, Nanopores

2178-Plat
Quantized Ionic Conductance in Nanopores
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Ionic transport in nanopores is a fundamentally and technologically important problem in view of its occurrence in biological processes and its impact on novel DNA sequencing applications. Using molecular dynamics simulations we show that ion transport may exhibit strong nonlinearities as a function of the pore radius reminiscent of the conductance quantization steps as a function of the transverse cross section of quantum point contacts. In the present case, however, conductance steps originate from the break up of the hydration layers that form around ions in aqueous solution. We discuss this phenomenon and the conditions under which it should be experimentally observable.
M. Zwolak, J. Lagerqvist, and M. Di Ventra, Phys. Rev. Lett. 103, 128102 (2009)

2179-Plat
Base-By-Base Ratcheting of Single Stranded DNA through a Solid-State Nanopore
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The benefits of low-cost, high-throughput human genome sequencing to medical science has inspired recent experimental work focused on DNA translocation through solid-state nanopores. Given that microelectronic fabrication methods permit the integration of nano-electronics devices to sense each DNA base, the genetic code (DNA sequence) could be read out during translocation by measurement of transverse electrical current, voltage signal, ionic current or hydrogen-bond mediated tunneling signal generated by each base in turn. However, DNA translocation inside a solid nanopore remains poorly controlled and DNA moves too rapidly to be detected at the desired single-base resolution. Here we show using realistic atomistic modeling that the recently proposed DNA transistor can achieve single-base control. These simulation results and a simple theoretical model inspired by the numerical studies demonstrate that when pulled by an optical tweezer as in a single molecule experiment or driven by a biasing electric field as in a high-throughput screening mode, the DNA transistor allows single stranded DNA to transits a nanopore in a stick-slip or thermal ratchet-like fashion, i.e. DNA alternatively stops and advances quickly one nucleotide spacing. During a stick state, a DNA base could be positioned before a sensor for an accurate read-out. We expect that the DNA transistor could be utilized in conjunction with a nanopore-based DNA sensing technology to achieve the goal of fast and cheap DNA sequencing.

2180-Plat
Synthetic Mycolic Acid Bilayers with Applications in Nanopore Sequencing
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To date, work in lipid bilayers has been primarily limited to a handful of small-chain lipid chemistries. We demonstrate and characterize lipid bilayer formation using pure mycolic acid, a long-chain saccharolipid, in circular apertures less than 50 microns in size. The resultant bilayers exhibit high mechanical stability over 12-hour timescales, breakdown voltages exceeding 1 V, as well as electric seals exceeding 400 GOhm, making them particularly useful for nanopore sequencing. We find these bilayers permeable to transmembrane porins and have analysed the insertion charachteristics of the porins MspA, α-Hemolysin and gramicidin, as well as demonstrating ssDNA translocation. Moreover, this result yields further understanding of the outer membrane structure of mycobacteria.

2181-Plat
Novel Nanoscale Tunneling Architectures for DNA Analysis
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Rapid, label-free analysis of individual biopolymers, specifically of individual DNA fragments is of great importance to many areas of biology and medicine. In recent years, translocation experiments within solid-state pores and protein channels combined with ionic current blockade measurements have become the technology of choice when detection is needed at the single molecule level. For linear biopolymers such as DNA and RNA however, detection based on ionic current blockade seem to lack the signal sensitivity necessary to obtain structural information with single base resolution. Transverse (perpendicular to the helix axis) conductance measurements of DNA in nanometer-sized tunneling junctions promise current detection limits within single nucleotide resolution. Yet, the exact alignment of nanoscale electrodes in tunneling regime to a solid-state nanopore has proven to be a significant challenge. We address this shortcoming by developing a novel method for aligning nanopore and tunneling junction in a nanoscale tunneling architecture by electrochemical metal deposition. As a result, tunnelling electrodes can be fabricated with atomic sharpness and precisely aligned to the nanopore. DNA can be driven electrophoretically through the tunneling architecture and it may be possible to detect modulations in the tunneling current specific to each base in the DNA.

2182-Plat
A Novel DNA Sensing Technique using the Nanopore MspA
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Nanopores of both protein and solid-state composition provide an excellent tool for single molecule sensing, particularly for DNA. Nanopores are nanometer-sized holes that provide the only pathway between two ionic baths. DNA is sensed by electrophoretically driving it into the nanopore which temporarily causes a reduction in ionic current as the DNA translocates. The protein nanopore Mycobacteria smegmatis porin A (MspA) has a geometry enabling the discrimination of the four nucleotides using ssDNA. This discrimination is easily observed when ssDNA translocation is briefly interrupted by complimentary oligonucleotides which must dissociate before ssDNA translocation can occur. We show that such duplex-interrupted translocation yields the ability to sense nucleotide composition on various strands of DNA. Such sensing could be useful in next-generation sequencing techniques with nanopores.

2183-Plat
Single Molecule Studies of Polyadenylic Acid Helix-Coil Kinetics using Nanopore
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Polyadenylic acid (poly(A)) forms helical configuration in aqueous solution at neutral and alkaline pH. The transition between its helical and random coil structures has been studied using bulk spectroscopic or calorimetric methods, revealing its thermodynamic properties. Recently, optical tweezers pulling experiments provided further support for the stacked helix structure of poly(A). While the bulk and the single-molecule experiments used 'bare' ssRNA molecules, the biological function of poly(A) entails the interactions with multiple proteins, such as poly(A)-binding proteins (PABP). In this study, we explore the helix-coil dynamics of poly(A) inside a small protein channel (alpha-hemolysin) at the single molecule level. The fluctuations between stacked and unstacked states are directly observed and quantified using statistical averaging over multiple individual events. An extensive temperature-dependent study of the process provides us with activation energies of the helix to coil (and vice versa) transitions, which are found to obey first order kinetics and results agree with bulk measurements. Surprisingly, time scales extracted from the single-molecule measurements are ~3 orders of magnitude longer than temperature-jump kinetics using 'bare' RNA. We provide a model that explains these results based on the protein-nucleic acid interactions inside the β-barrel channel