

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 ( $\text{Ca}^{2+}/\text{Fe}^{2+}$ ) 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 transit 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 sta-

bility 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 characteristics of the porins MspA,  $\alpha$ -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 *Mycobacterium 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  $\sim 3$  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  $\beta$ -barrel channel

of alpha-hemolysin. It is argued that channel confinement plays a critical role in dynamics of these complex processes. Our results shed light on the way inter-molecular processes affect nucleic acids' kinetics.

#### 2184-Plat

##### Measuring Direct Forces on dsRNA in Solid State Nanopores

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In recent years, far-reaching discoveries about the functionality of RNA in biology have been made. Especially double stranded RNA (dsRNA) is found to play a key role in the process of RNA interference. We employ solid state nanopores (nanometer sized holes in a thin SiN membrane) to study single RNA molecules. By applying an electrical field over the nanopore, RNA molecules can be threaded into the nanopore, causing a change in the ionic current. This change can provide insight into some of their structural properties, such as charge density, diameter, and possibly also their local structure. We have integrated our nanopore setup with optical tweezers, which allows us to also measure and apply forces to the molecule inside the nanopore.

Here, we present the first application of this new technique to the study of RNA molecules, in this case long dsRNA. We show that the force experienced on these molecules is very similar to that on DNA molecules, as one would expect from the very similar structure of these molecules. In addition, we show that the measured force is independent on the distance of the optical trap to the nanopore, even at very close range ( $< 500$  nm). Measuring forces at such close distances may be required for the application of this technique to more complicated molecules, such as single stranded RNA molecules or RNA-protein complexes. Finally, we have further extended the use of this technique to very small nanopores (down to  $\sim 3$  nm in diameter), also an important future requirement to study more complex molecules. Combined, these measurements represent important steps towards the detection of local structure along RNA molecules.

#### 2185-Plat

##### A Pore-Cavity-Pore Nanodevice to TRAP and Electro-Optically Investigate Single Molecules

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Single engineered nanopores in solid state membranes have attracted broad attention in recent years as a tool to study single biological molecules like DNA or proteins. Here we introduce a novel solid-state device which comprises two stacked nanopores defining the in- and outlet of a pico liter cavity. This pore-cavity-pore (PCP) architecture allows for the electrical as well as optical examination of single molecules.

The PCP device is fabricated by structuring nanopores into a sandwich SiN/Si/SiN wafer using e-beam lithography, wet chemical etching, and feedback controlled electrochemical etching steps. The in- and outlet nanopores of the fabricated PCP-devices are characterized by transmission electron microscopy, evidencing that the pore diameters may be controlled independently down to 10 nm.

We demonstrate that the double pore geometry enables a novel measurement mode for nanopore devices, namely, time-of-flight experiments. In DNA translocation experiments we find time-correlated pulses in the measured ionic trans-doublepore current, which arise from single DNA molecules translocating one pore after the other. From correlation analysis we are able to deduce molecular mobilities for DNA molecules of different lengths. Moreover, we present fluorescence experiments of single DNA molecules and nm-sized polystyrene beads inside the PCP device. Through electric potential control we are able to inject and eject nano-objects into and out of the PCP device. We utilize fluorescence to monitor hybridization of DNA molecules trapped in the cavity in order to demonstrate how the PCP device may be used as a pico liter reaction chamber.

## Platform AJ: Protein Aggregates

#### 2186-Plat

##### Low Resolution Structure of a Membrane-Permeabilizing Oligomer of $\alpha$ -Synuclein: the Basis for a High-Throughput Screening of Compounds against $\alpha$ -Synuclein Aggregation

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$\alpha$ -synuclein is a 140-residue natively unfolded protein, whose aggregation is implicated in the development of Parkinson's Disease. It is thought that the cytotoxic species is not the mature fibril, but rather a prefibrillar aggregate which has membrane-permeabilizing properties. We have used Small Angle X-ray Scattering (SAXS) to determine the low-resolution structures of the different species formed during  $\alpha$ -synuclein fibrillation in a non-invasive fashion. In addition to the starting monomer-dimer equilibrium and two *bona fide* fibril types accumulating towards the end of the aggregation process, we have identified a wreath-shaped oligomeric state which has a very distinct central hole. Both its structure and the kinetics of its formation are consistent with an on-pathway role, while its membrane-permeabilizing properties identify it as a putative cytotoxic species. We have also used SAXS to monitor the fibrillation of  $\alpha$ -synuclein in the presence of the surfactant SDS and find that the fibrillar aggregates grow in a continuous fashion, forming beads on a string where the individual beads are stabilized by intermolecular  $\alpha$ -synuclein contacts. The high reproducibility of this aggregative behaviour has formed the basis for a high-throughput screening assay involving 746,000 compounds that has allowed us to identify a significant number of compounds with the ability to inhibit early-stage aggregation of  $\alpha$ -synuclein. This distinguishes the assay from previous assays that have focused mainly on the ability to prevent formation of  $\alpha$ -synuclein fibrils. The hits from our assay may form the basis for a therapeutic intervention against Parkinson's Disease.

#### 2187-Plat

##### Molecular Insights into the Role of Serum Amyloid-P Component in the Stabilization of Fibrillar Beta-2 Microglobulin

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Serum amyloid-P component is found ubiquitously in amyloid deposits and has been shown to stabilize fibrillar structures and prevent clearance by the host's defences. Here we report on solid-state NMR studies on fibrillar deposits formed from  $\beta$ 2-microglobulin, typically found in patients with dialysis related amyloidosis, and their interactions with serum amyloid-P component.

We have successfully undertaken the expression, purification and refolding of the 99 residue  $\beta$ 2-microglobulin and established conditions for optimal binding of serum amyloid-P component. High resolution solid-state magic-angle spinning (MAS)-NMR spectra obtained from the fibrils indicate that within the fibrils the  $\beta$ 2-microglobulin adopts a homogeneous structure. Using two-dimensional homo- and hetero-nuclear correlation spectroscopy we have been able to assign several of the sites within the protein. Currently we are using a range of labelling schemes and acquiring three-dimensional data-sets to complete this assignment. Comparison of the assignment with that obtained from monomeric  $\beta$ 2-microglobulin in solution is beginning to provide valuable insights into the structural changes occurring upon fibrilization. Similar comparisons with fibres decorated with serum amyloid-P component should provide valuable insights into how this molecule interacts and stabilizes amyloid fibrils at a molecular level.

During the course of these experiments we have also obtained 2D correlation data on inclusion bodies of  $\beta$ 2-microglobulin. The resolution attained is not as high as that observed in the fibrillar spectra, however they permit the assignment of resonances to amino acids with the  $\beta$ 2-microglobulin. This suggests that within the inclusion bodies the  $\beta$ 2-microglobulin adopts a well defined conformation with the lower spectral resolution arising from reduction in dynamics in these highly packed structures. Detailed comparisons of the data with that obtained from the soluble and fibrillar  $\beta$ 2-microglobulin should provide insights into the nature of this structure.

#### 2188-Plat

##### The Role of Small Oligomers on an Amyloidogenic Free Energy Landscape

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We combine atomic force microscopy particle size distribution measurements with earlier measurements on 1-anilino-8-naphthalene sulfonate, thioflavin T and dynamic light scattering to develop a quantitative kinetic model for the aggregation of beta-lactoglobulin into amyloid. We directly compare our simulations to the population distributions provided by dynamic light scattering and atomic force microscopy. We combine species in the simulation according to structural type to compare with the fluorescence fingerprint results. The kinetic model of

