Further we created nanometer-scale transmembrane channels in lipid bilayers using self-assembled DNA-based nanostructures [2]. Scaffolded DNA origami was used to create a stem that penetrates and spans a lipid membrane, and a barrel-shaped cap that adheres to the membrane in part via 26 cholesterol moieties. In single-channel electrophysiological measurements, we find similarities to the response of natural ion channels, such as conductances on the order of 1 nS and channel gating. In single-molecule translocation experiments, we highlight one of many potential applications of the synthetic channels, namely as single DNA molecule sensing devices.

References
[1] Wei*, R., Martin*, T.G., Rant, U. & Dietz, H.: DNA origami gatekeepers for solid-state nanopores. Angew. Chem. Int. Ed. Engl. 51, 4864-4867 (2012).
[2] Langecker*, M., Arnaut*, V., Martin* T.G., List, J., Renner, S., Mayer M., Dietz H. & Simmel F.C.: Synthetic lipid membrane channels formed by designed DNA nanostructures. Science, in press.
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2656-Pos Board B675
Detection of Sequence-Specific Proteins Bound to DNA using Solid-State Nanopores
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Nanopore analysis holds great promise for performing DNA footprinting, wherein the locations of binding entities can be mapped along single molecules to reveal substructure. Towards this goal, we describe progress in the use of solid-state nanopores for the detection of sequence-specific proteins bound to double-strand DNA. We detect multiple specific binding entities during translocation using a common current sensing technique. We further investigate custom molecular constructs that offer single protein binding sites as a model system. Successful implementation of this measurement technique has implications for future use in rapid genetic and epigenetic diagnostic systems.

2657-Pos Board B676
Engineering a Biomimetic Biological Nanopore to Selectively Capture Folded Target Proteins
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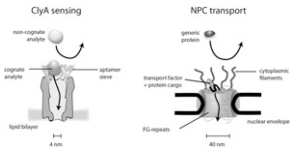
Nanopores have been used in label-free single-molecule studies, including investigations of chemical reactions, nucleic acid analysis and applications in sensing. Biological nanopores generally perform better than artificial nanopores as sensors, but they have disadvantages including a fixed diameter. Here we introduce a biological nanopore ClyA that is wide enough to sample and distinguish large analytes proteins, which enter the pore lumen. Remarkably, human and bovine thrombins, despite 86% sequence identity, elicit characteristic ionic current blockades, which at −50 mV differ in their main current levels by 26 ± 1 pA. The use of DNA aptamers or hirudin as ligands further distinguished the protein analytes. Finally, we constructed ClyA nanopores decorated with aptamers covalently attached to the nanopore exterior. Like nuclear-pore complexes (NPC), these nanopores selectively captured and translocated cognate protein analytes into their interiors, but excluded non-cognate analytes.



2658-Pos Board B677
Sit, Roll Over, Play Dead: Investigating the Effects of pH on the Translocation of Double-Strand DNA through Solid-State Nanopores
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Nanopore translocation experiments have revealed many interesting single molecule characteristics, including molecular size and local structure. In this work, we investigate the effects of pH on Lambda (Δ) DNA translocations through solid-state nanopores. We measure dwell time and conductance blockage of unfolded Δ DNA while varying the pH systematically over a range from 3 to 10. Our measurements indicate that electrophoresis (EP), electroosmosis (EM), and diffusion (DF) all play important roles during the transport process. We find that Δ DNA mean dwell times increase as the electrolyte pH approaches the DNA isoelectric point and translocation direction changes polarity at low pH. The relative contributions of EP, EM and DF change at different rates with pH to produce the observed net effects. Our results emphasize the importance of DNA and nanopore zeta potential considerations when analyzing single molecule translocation dynamics and offer a possible route towards reduced translocation speed while retaining high signal-to-noise ratio.

2659-Pos Board B678
Force Spectroscopy of Single Stranded DNA with Biological Nanopore MspA
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Biological nanopores are single molecule sensors able to probe individual DNA molecules. In this method, a nanometer scale pore is inserted into a lipid bilayer in an electrolyte solution. Voltage is applied across the bilayer and the ionic current through the pore is measured. DNA nucleotides residing in the pore constriction modulate the resistance of the DNA-pore system. Our group previously introduced Mycobacterium smegmatis porin A (MspA) as a biological nanopore sensor with high signal to noise resolution of DNA nucleotides. In this work, we perform force spectroscopy on individual single-stranded DNA molecules. DNA molecules are attached to NeutrAvidin and driven into MspA until the NeutrAvidin comes to rest on MspA's entrance. Approximately 14 nucleotides span the distance to MspA's constriction. The nucleotides within the pore constriction experience a force proportional to the applied voltage. By varying the voltage applied across the pore, we detect stretching of the DNA with angstrom precision. These experiments validate MspA as a single molecule tool to study mechanical properties of DNA.

2660-Pos Board B679
Single Enzyme Activity Detected with a Nanoelectronic Sensor
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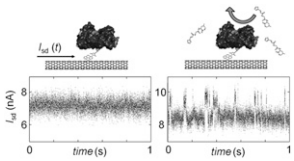
Today's single molecule studies rely almost exclusively on force-based detection and/or optical detection. A third, fundamentally new, detection technology utilizes the electrostatic signals generated by single molecules. using the protease thermolysin we show that enzymatic activity can be detected with single turnover resolution using a carbon nanotube field-effect transistor sensor. A single thermolysin molecule is site-specificially immobilized to the carbon nanotube via a cysteine residue. In the presence of a thermolysin substrate the sensor shows discrete switching between two conductivity levels. The switching signal depends on the substrate concentration, and does not occur in the absence of substrate. We envision that this approach can be generalized to a large number of enzymes, allowing for single enzyme studies of many enzyme-substrate systems.



Micro- and Nanotechnology I

2661-Pos Board B680
Lipid Bilayers with Integrated Microelectronics for High-Resolution Ion Channel Recordings
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Applications in drug discovery and nanopore DNA sequencing demand highly integrated and scalable electrophysiology platforms for ion channel recordings. Both the physical size and high-frequency signal-to-noise ratio of such systems are commonly constrained by the interconnects and fluidics accompanying a measurement, rather than by fundamental properties of the channel proteins or electronics involved. Here we present a platform for high-resolution ion channel recordings which physically integrates custom low-noise electronic voltage-clamp preamplifiers with ion channel proteins in reconstituted lipid membranes. We demonstrate scenarios in which single-channel resolution is maintained to very high signal bandwidths, and provide a window into the high data rates and channel densities which can be achieved by leveraging existing complementary metal-oxide-semiconductor (CMOS) microelectronics for advanced electrophysiology platforms.

2662-Pos Board B681
Estimating the Geometry of Scanning Ion Conductance Microscope Pipettes from Resistance Variation with Breakage
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