930-Pos Board B730
Functionalizing a Nanopore with Nano-Electrodes Towards the Control of the Translocation of DNA with Single Base Resolution
Hongbo Peng, Binquan Luan, Philip S. Waggoner, Stefan Harrer, Glenn J. Martyna, Stanislav Polonsky, Stephen M. Rossnagel, Gustavo Stolovitzky.
Recently, application of nanopores to low-cost DNA sequencing has attracted great interest as there is great need to reduce the cost of sequencing a whole human genome to $1000. A key issue in the field of nanopore DNA sequencing is to control the DNA translocation. Here we will report the development of what we call a ''DNA transistor'': a nanopore-based electrical device for controlling the translocation of DNA with single base resolution. The key part of this device is a free standing membrane, within which multiple layers of electrically addressable metal electrodes separated by dielectric layers are embedded. A 1-5 nanometer size pore is made through the membrane. We demonstrated that such a device is electrically viable for the electrode layer or the spacing dielectric layer as thin as 3 nm. Confirming the basic function of the device, induced electrical signals on the nano-electrodes by the translocating DNA, as well as the modulation of DNA translocation speed by the voltage bias applied on the nano-electrodes are also observed. Our ongoing experiments test if the modulated electrical field can trap or translocate DNA at a single base resolution.

931-Pos Board B731
Single Cell Immunoassay with Sting Sensors
Paolo Actis, Adam R. Seger, Boaz Vilozny, Olufisayo Jejelowo, Nader Pourmand.
Signal Transduction by Ion Nano Gating (STING) technology is a label-free biosensor capable of identifying DNA and proteins. Based on a functionalized quartz nanopipette, the STING sensor includes specific recognition elements for analyte discrimination based on size, shape and charge density. We demonstrated a detection limit toward environmental toxins as low as 100 fg/ml and linear range of 3 logs. STING paves the way for a new class of label-free, real time biosensors with unmatched sensitivity. The fully electrical read-out as well as the ease and low cost fabrication are unique features that give this technology enormous potential. Unlike other biosensing platforms, nanopipettes can be precisely manipulated with submicron accuracy and can be used to study single cell dynamics. We are currently investigating the application of STING sensors for the detection of oncoproteins inside a single living cell. We will be presenting preliminary results related to the detection of HPV proteins inside a single cancer cell.The STING platform paves the way for in vivo immunoassay down to the single cell level.
References:
1. P. Actis, O. Jejelowo and N. Pourmand, Biosensors and Bioelectronics, 2010, 26(2), 333-337.
2. P. Actis, A. C. Mak and N. Pourmand, Bioanalytical Reviews, 2010, 1, 177-185.

932-Pos Board B732
Surface Functionalization and Ion Selectivity in Sting Sensors
Boaz Vilozny, R. Adam Seger, Paolo Actis, Nader Pourmand.
Solid-state nanopores are a promising platform for sensitive detection of many analytes, but modifying the surface of pores to impart selectivity is a challenge. We are applying the principle of signal transduction by ion nano-gating (STING) to use modified quartz nanopipettes as selective electrical nanosensors. This mechanism uses modulations in ion current through a rectifying nanopore (roughly 50 nm in diameter) to detect analytes as they interact with surface-bound receptors. Because the current rectification is highly sensitive to changes in surface charge, receptors that reversibly bind metal cations can be used for label-free and continuous ion sensing. Using a combination of polyelectrolyte self-assembly on the pore surface and bioconjugation, we have modified the surface of quartz nanopipettes with calmodulin, a calcium-binding protein. At neutral pH, the sensor is selective for calcium over magnesium, with a limit of detection in the low micromolar range. Additional methods for detection of salts such as copper and zinc are also being pursued. These involve alternative recognition mechanisms include surface functionalization with chelating biopolymers such as chitosan, and voltage-directed nanoprecipitation of metal salts. The surface functionalization, sensor performance, and potential applications will be discussed. This electrical nanosensor may be applicable in many types of ion detection schemes, such as remote sensing applications or intracellular measurements for biological research.

933-Pos Board B733
Stochastic Sensing of His-Tagged Proteins with a NTA-Functionalized Solid-State Nanopore
Ruoshan Wei, Christian Grunwald, Robert Tampé, Ulrich Rant.
Artificial nanopores in solid-state membranes have emerged as a versatile tool to study single biomolecules. Employing nanopores as stochastic sensors offers the attractive prospect of detecting and quantifying molecular interactions and bindings on a single-molecule level. Individual interactions between analyte molecules and receptor sites within the pore can be observed as transient modulations of the trans-pore current.
Here we demonstrate the stochastic sensing of single proteins in a solid-state nanopore functionalized with nitrilotriacetic acid (NTA) chelator groups as specific binding sites for histidine-tagged proteins. The non-covalent yet selective His-tag/NTA interaction is well suited as a model system, since the binding strength depends on NTA valency and presence of competitive binders.
NTA affinity groups are tethered within the nanopores by first coating the walls of pores in SiN membranes with gold, and subsequently adsorbing NTA-functionalized alkane-thiol self-assembled monolayers.
Individual binding events of His-tagged proteins were successfully detected in real-time. Analysis of the binding times provide kinetic information and corroborate the occurrence of bimolecular interactions. The binding is shown to be reversible and specific, as the kinetics can be significantly altered by the addition of imidazole as a competitive binding agent and is completely inhibited by removal of the chelated metal ions. In concentration dependent studies we elucidate the influence of the competitive binder on the single-molecule binding times.
We systematically studied the influence of the NTA valency on the binding strength, comparing mono-, bis-, and tris-NTA receptors and present a ranking of single-molecule binding times. Our results indicate that multivalent interactions are strong enough to stably immobilize His-tagged proteins within the pore, so their interactions with third-party proteins can be studied. This paves the way for the use of NTA functionalized metal nanopores as generic tools to study protein-protein interactions one-on-one.

934-Pos Board B734
Nanopores with Fluid Walls
Erik C. Yusko, Jay M. Johnson, Sheereen Majd, Panchika Prangkio, Ryan C. Rollings, Jiali Li, Jerry Yang, Michael Mayer.
This work introduces the concept of synthetic nanopores with fluid lipid walls and describes the benefit of these pores for single protein translocation experiments. The inspiration for this design comes from lipid-coated nanopores in olfactory sensilla of insect antennae. Multifunctional coatings of fluid lipids enable characterization of single proteins with unprecedented information content and address currently unmet challenges of single protein investigations with nanopores. For instance, translocation events of lipid-anchored proteins reveal the charge, ligand affinity, volume, and molecular shape of single proteins in high throughput. Moreover, translocation of amyloidogenic peptides through a pore with fluid walls makes it possible to characterize a large number of individual Alzheimer's disease-related amyloid-beta fibrils in situ; these fibrils clog conventional nanopores. The choice of lipids in the coating makes it possible to fine-tune the thickness, surface chemistry, functionality, and viscosity of the coating in the pore. These characteristics enable increasing the signal to noise ratio, eliminating non-specific binding, capturing and concentrating specific analytes, increasing translocation times, and time-resolving translocation events completely. Finally, these coatings provide the exquisite sensitivity to monitor thermally-induced conformational changes in bimolecular sheets of lipids and these phase transitions make it possible to actuate the diameters of synthetic nanopore dynamically in situ.

935-Pos Board B735
Microfluidic Fabrication of Asymmetric Giant Lipid Vesicles
Peichi C. Hu, Noah Malmstadt.
We have developed a microfluidic layer-by-layer fabrication technology for assembling asymmetric giant unilamellar vesicles (GUVs). The vesicles formation was a two-step process. In each step, a lipid monolayer is formed at a water-oil interface. The inner monolayer is formed inside of microfluidic device based on a droplet flow configuration consisting of a continuous oil flow stream in which water droplets are formed. The outer monolayer is formed by transferring the water droplets from oil to water. By dissolving different lipid compositions in the different oil phases, the composition of each leaflet of the resulting lipid bilayer can be controlled. Membrane asymmetric is confirmed by showing successful targeting of fluorescently labeled lipids to each leaflet and by demonstrating that asymmetric GUVs will bind an avidin-coated surface only when biotinylated lipids are targeted to the outer leaflet. In addition, asymmetry is confirmed by binding of labeled annexin V to membranes formed with an asymmetric composition of the phosphatidylserine lipids. Unilamellarity is confirmed by membrane permeabilization with alpha-hemolysin. Aside from the capacity to form asymmetric bilayers, this technique has an advantage over the standard electroformation method in being capable of forming GUVs at high salt concentration.