FhuA has a luminal cross-section of ~3.1×4.4 nm and is plugged by a globular N-terminal cork domain (C). Single-channel electrical recordings with extensive protein redesign of FhuA resulted in identifying four long extracellular loops (4L) that partially block the lumen upon the removal of the cork. The newly engineered protein, FhuA ΔC/Δ4L, was the result of a removal of almost 33% of the total number of amino acids of the wild-type FhuA (WT-FhuA) protein. The crown achievement in this work was combining direct genetic engineering with a refolding approach to produce this unusually-stable protein nanopore. Critical to its future nanotechnological applications, FhuA ΔC/Δ4L was functional under structure-altering conditions, including low ion concentration and highly acidic aqueous phase.

To tailor the FhuAΔC/Δ4L protein to the use in nanopore-based detection devices, we show that the FhuA-based nanopores function as stochastic biosensing elements. For example, we monitored the proteolytic activity of an enzyme at highly acidic pH and we were able to determine the kinetics of protein-DNA aptamer interactions at physiological salt concentration. These two assays have not been demonstrated with the existing nanopores.

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Insights into the Size and Geometry of a Robust Engineered Membrane Protein Nanopore

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The maximum utility of protein nanopores in biotechnological applications is generally dictated by the size and internal geometry of the protein. The knowledge of these two parameters is imperative, particularly when extensive protein redesign is employed to produce task-specific proteins.

Recently, we used ferric hydroxamate uptake component A (FhuA), a β-barrel bacterial outermembrane protein, as a protein-engineering template to produce an unusually-rigid, open nanopore (FhuA ΔC/Δ4L). This new nanopore maintains its stability under harsh experimental conditions. It was essential to couple comprehensive protein redesign with protein refolding protocols to obtain such a nanopore. With the radical revamp of the FhuA template, it was necessary to shed light on the overall structure of the new FhuA ΔC/Δ4L protein. Thus, we employed water-soluble, flexible poly(ethylene glycols) and dextran polymers to inspect the interior of FhuA ΔC/Δ4L during single-channel recordings.

The addition of poly(ethylene glycols) to solution produced alterations in the single-channel conductance, allowing for the calculation of the nanopore diameter. We report that FhuA ΔC/Δ4L features an approximate conical internal geometry with the *cis* entrance (extracellular) smaller than the *trans* entrance (periplasmic). This finding is in accord with the asymmetric nature of the crystal structure of the wild-type FhuA protein. Additionally, experiments with impermeable dextran indicated an average internal diameter of ~2.4 nm, an estimate based on the polymer-induced alteration of the access resistance contribution to the total resistance of the nanopore. The insights into size and geometry of FhuA ΔC/Δ4L deduced from these polymers experiments will aid in future protein engineering of FhuA, opening new engineering avenues for specific tasks in biotechnological applications.

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Fabrication of Surface-Attached Magnetic Post Arrays for Biosensing Applications

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We present a new biosensing device which consists of an array of flexible high-aspect ratio magnetic structures cantilevered to a solid substrate. The structures are 25 x 2.5 micron cylindrical silicone rods which are encased with a shell of nickel; the silicone structure lends the rod flexibility while the nickel enables magnetic control. Fabrication begins in a 25-micron-thick porous polycarbonate track-etched membrane in which pore diameter is tuned by etching in a sodium hydroxide solution. A gold and aluminum working electrode is sputter-coated onto the back of the membrane, followed by electrodeposition of 100-200 nm of nickel onto the pore wall. This deposition forms a hollow nickel cylinder which is then filled with a poly(dimethyl siloxane) core. After curing, dichloromethane is used to dissolve the polycarbonate template, resulting in a magnetically active microrod array containing two million rods per square centimeter. Furthermore, we demonstrate that changes in the range of motion of the oscillation of the microrod array can be measured by monitoring optical transmission through the sample. This allows us to monitor the aggregation kinetics of the rod array or coagulation of an intervening fluid, leading to a generalized platform for biosensing applications.

2714-Pos Board B733

Non-Invasive Real-Time Study of Cell Adhesion using Dissipation Monitoring of the QCM-D

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Cell adhesion is an essential biological process for cell survival, differentiation, and migration. There are two types of cell adhesion to the extracellular matrix (ECM) and adhesion to adjacent cells. Cell-substrate adhesion is mediated through a class of receptors known as integrins. There receptors simultaneously connect the ECM on the outside to the actin filaments on the inside of the cells. Another important cell adhesion is cell-cell adhesion; it is an essential component of epithelial morphology, cellular communication and function. Epithelial cells adhere tightly to their neighbors through adhesive structures and these structures are connected to intermediate filaments microfilaments. This association with the cytoskeletal network is necessary for stable cell-cell adhesion and for the communication of the changes in neighboring cells. There are many methods that can be used to measure the changes in cell adhesion, but each has its disadvantages. Many of these methods are based on end-point detection which don't allow real-time detection or are based on the introduction of foreign objects which can affect the response of cells. We have developed a non-invasive real time method using the quartz crystal microbalance with dissipation monitoring (QCM-D) to quantitatively monitor such cellular processes using the dissipation factor ΔD. Previously, we've successfully tracked real time changes in cellular adhesion due to induction of the EGFR pathway using the QCM-D. The QCM-D technique can be a useful application in studying other cellular process such as cell signaling and trafficking and can potentially be a useful in vitro method for drug and biomarker screenings. Here we will take a look at pathways that contribute to changes in cell adhesion through cell-ECM adhesion and cell-cell adhesion.

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Frequency Locked Microtoroid Optical Resonators as a Non-Invasive Tumor Biopsy Alternative

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Whispering gallery mode optical resonators offer an unusual coupling of rapid response time and ultra-sensitive biological and chemical detection. We have improved the signal to noise ratio of microtoroid optical resonators ~1000-fold over standard techniques by using laser frequency locking and have applied this to assay tumor progression in mice by sensing the low concentrations of exosomes, shed by tumor cells, in serum samples collected from the animals. Serum samples from normal or experimental mice cause no shift in the resonance wavelength of the microtoroids; however, after using antibodies toward specific tumor markers to sensitize the toroid surface, we detected changes in the resonance frequency of the microtoroid when exposed to the serum of tumor-implanted mice. Serum from control (tumor-free) mice caused no shift. The wavelength shifts observed were 600 times the noise and drift of the sensor, even for a million fold dilution of the serum sample. Analysis of the shifts showed unitary steps of ~ 0.5 fm, suggesting that the assay may be sensitive enough to detect individual binding events, offering a means to analyze the size of the biomolecules that are binding to the resonator. If validated, this approach offers a non-invasive tumor ''biopsy,'' exploiting the circulation of blood to collect a sample of tumor surfaces without the need to find or access the tumors.

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Cell-Free Electrophysiological Functional Measurements with Native Membranes of *Torpedo Californica* Electric Tissue

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