5-bisphosphate carboxylase/oxygenase (RuBisCO). The challenge associated with structure and functional investigation of Rca can be attributed to its exceptionally low thermo-stability, high degree of size polydispersity and tendency toward subunit aggregation.
In this work we have successfully employed fluorescence fluctuation methods to study the nucleotide-dependent stoichiometry of fluorescently tagged Rca for a wide range of concentrations. Our results show a stepwise assembly pathway of Rca under different assay conditions. In presence of Mg+2 -ADP, the oligomerization state of Rca is largely dominated by monomers at concentrations below 0.5 μM. The state of oligomerization gradually changes in steps of two subunits. The most probable model for this assembly supports the dissociation coefficients of ~4, 1, 1 μM for the monomer-dimer, dimer-tetramer and tetramer-hexamer equlibria respectively. Continued assembly at even higher concentrations suggests self association through the formation of spiral arrangements that grow along the helical axis.

2706-Pos Board B725
Inferring Subunit Stoichiometry from Single Molecule Photobleaching
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Single molecule photobleaching is a powerful tool for determining the stoichiometry of protein complexes. By attaching fluorophores to proteins of interest, the number of associated subunits in a complex can be deduced by imaging single molecules and counting fluorophore photobleaching steps. Since some bleaching steps might be unobserved, the ensemble of bleaching steps will be binomially distributed and it has been commonly assumed that the highest number of observed bleaching steps is indicative of the stoichiometry of the complex. However, we point out that inferring the true composition of a complex from such data is non-trivial because binomial processes are ill-posed. As a result, there may be a significant probability that the true complex is larger than the data indicate simply due to finite sample size and the variance of binomial processes. Because of this possibility, calculating likelihoods to establish parameter confidence can be misleading. What is needed is a reliable method to quantify one's conclusions about stoichiometry. We present a Monte Carlo method which does not rely on likelihood calculation and provides a reliable estimate of confidence. The formalization and methods presented here provide a rigorous analytical basis to this pervasive experimental tool.

Biosensors

2707-Pos Board B726
Simulations and Modelling of Biomimetic Nanopores
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Nanopores are fast becoming a major scientific tool in molecular analysis and detection due to their ability to detect polynucleotides, proteins, and small molecules. Previous work has included the manipulation of α-hemolysin into a DNA sequencing pore however further development is needed for the use of a simple, monomeric pore for this purpose.
Biomimetic modelling of nanopores allows for a specific function to be built into the model based on the replication and analysis of existing selectivity present in proteins.
An initial analysis of known porin structures using computational techniques (pore radius profiles and electrostatic calculations) has identified potential structures for manipulation. Steered Molecular Dynamics simulations on a select number of membrane proteins have given insight into possible residue motifs of interest. With this, ''simple protein'' pores have been made with varying number of beta strands based on models of existing barrel proteins. Motifs have been modelled for exploration via MD simulations.

2708-Pos Board B727
Polymers Pushing Polymers: Polymer Mixtures in Thermodynamic Equilibrium with a Pore
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Studies on the ejection of viral genomes from capsids use osmotic stress to push a DNA molecule into the viral capsid by the action of PEG in solution. Identities of the polymer forced into the nano-cavity (DNA) and the polymer pushing it (PEG) differ. We analyze a variation on the problem with a simple model of a binary polymer mixture and find that polymer size dependent partitioning into a pore also has a complex dependence on the composition of the polymer mixture and the pore-penetration penalty. We analyze a polydisperse polymer solution of big and small polymer chains (bPEG and sPEG, respectively) of which only the sPEG is allowed to pay an energy penalty and enter the pore. We introduce an ansatz for the free energy of the polymer mixture that is consistent with our previous phenomenological fit to the equation of state of bulk uncharged polymers. We then analyze the osmotic pressure of this solution in equilibrium with a pore for various amounts of sPEG. Calculating the corresponding partition coefficient allows us to assess the pushing forces exerted by the external polymer solution via its osmotic pressure and ascertain that some polymers can push others to enter the pore in a kind of osmotic tug-of-war. We find that pore penetration is governed by two different penetration free energy scales. One scale represents the osmotic pushing strength of the external polymer mixture. The other scale comprises the interplay between the pore energy penalty and the translational mixing entropy (van't Hoft) and configurational (des Cloizeaux) fluctuations of the external solution. This principle should have applications in many areas of nano-science dealing with partitioning of polymer chains into small enclosures.

2709-Pos Board B728
Sequence-Specific Nucleic Acid Detection from Binary Pore Conductance Measurement
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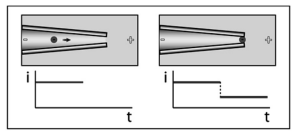
We describe a platform for sequence-specific nucleic acid (NA) detection utilizing a micropipette tapered to a 2 μm diameter pore and 3 μm diameter polystyrene beads to which uncharged peptide nucleic acid (PNA) probe molecules have been conjugated. As the target NAs hybridize to the complementary PNA-beads, the beads acquire negative charge and become electrophoretically mobile. An applied electric field guides these NA-PNA-beads toward the pipette tip (Figure left), which they obstruct, leading to an indefinite, electrically detectable, partial blockade of the pore (Figure right). In the presence of non-complementary NA, even to the level of single base mismatch, permanent pore blockade is not seen. We show application of this platform to detection of the anthrax lethal factor sequence. We are currently working to reduce the concentration limit of detection through the use of longer target ssDNA oligomers.



L. Esfandiari et al., *J. Am. Chem. Soc.*, vol. 134, no. 38, pp. 15880–15886, 2012.

2710-Pos Board B729
DNA Sequence Detection using the Nanopore MspA
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Nanopore DNA sequencing is a single molecule sequencing method in which DNA is pulled through a nanometer-sized pore, while the nucleotides modulate an ionic current. We use a protein pore based on Mycobacterium smegmatis porin A (MspA) that we developed to have sensitivity to single nucleotides in DNA. When using the phi29 DNA polymerase to move DNA in single-nucleotide steps through MspA, we observe a succession of well resolved ionic current levels. Each current level is primarily determined by the two nucleotides centered in MspA's narrowest constriction and is partially influenced by the flanking nucleotides. We present progress towards decoding algorithms that translate the observed ionic current patterns to DNA sequence.

2711-Pos Board B730
Biomolecular Detection with Engineered Robust Nanopores
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Nanopores have become indispensable, powerful tools for bimolecular detections and molecular manipulations at single-molecule level, and they have been realized as an alternative to current analytical devices in biomedical molecular diagnosis. With a limited number of protein nanopores available, designing robust, functionally-intact protein scaffolds under various detection conditions is a daunting challenge in developing protein nanopore-based technologies. We used ferric hydroxamate uptake component A (FhuA), a bacterial β-barrel membrane protein as a template for protein engineering to produce an exceptionally-functional nanopore for sampling biomolecular events in harsh environments.