can be reduced by modifying the electrode with graphene. Like in superconductors, electrons can flow across graphene nearly without scattering, which is crucial for constructing ultrasmall, high-speed devices. Therefore, understanding the interaction between the GOx and graphene is of prime importance. To this end, we have employed small angle neutron scattering technique to investigate the structural features of GOx covalently attached to the single layer graphene (SLG). Two different samples varying in density are examined and a 3-level Unified Fit is used to quantify the structural details. It is interesting to note that the radius of gyration of the primary protein reduced from ~30 Å in solution to about ~21-22 Å on the SLG, possibly indicating the disruption in the integrity of GOx’s quaternary structure. Further, though the increase in the GOX concentration resulted in similar structural topology, the clusters displayed a smoother surface with fracts of higher density.


**1646-Pos Board B597**

**Application of Strain and Calibration of FRET Emission for in vitro Live Cell Response to Cytoskeletal Deformation**

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Mandibular hypoplasia is a debilitating pediatric condition caused by underdevelopment of bone in young children. Current treatment options are expensive and morbid. The focus of our research is to elucidate the biophysical mechanisms by which mechanical strain can regulate bone growth via osteoblasts, and apply this knowledge to new therapies. As a first step, we have developed a novel non-invasive approach to quantify cellular strain in vivo by utilizing transfected tensile sensors in the form of Förster Resonance Electron Transfer (FRET) cassettes, and applied this tool to determine live cell responses to externally applied stretch. Stretching was controlled through a custom made device consisting of a micromanipulator and electric motor, mounted on the Nikon A1R multi-photon microscope stage. Adult mouse fibroblasts were transfected with FRET cassettes containing Venus (yellow) and Cerulean (blue) fluorescent proteins and an α-helix amino acid linker, followed by cell seeding onto a silicone membrane and stretching at 0.4% per minute. Cell images were recorded and analyzed to quantify stretch-induced change in FRET ratio (blue/yellow) and cell responses, including changes in cell morphology, dimensions and area. The results demonstrate a significant correlation between change in FRET emission ratio and cell stretch (R2=0.6). The trends show that as strain increases from 0% to 3% in a cell, the ratio of acceptor(yellow)/donor(blue) emission linearly decreases by ~50%, indicating a consistent loss in FRET efficiency and presenting a reliable non-invasive method for quantification of intracellular strain in live cells. Atomic force microscopy was utilized to confirm that the addition of a FRET cassette did not change the mechanical properties of the cell cytoskeleton. Overall, these results validate the potential of a FRET-based sensor for in vivo biomechanical cell studies.

**1647-Pos Board B598**

**Aptamer Sequence Deconvolution through Microarray Technology**

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Aptamer, which is generated by systematic evolution of ligands by exponential enrichment (SELEX), can recognize a specific target protein with high affinity. It is selected from a single-stranded DNA or RNA library, and the typical length is between 60 to 200 nucleotides, which may consist of some redundant components in cellular processes. Monitoring and/or inducing the interaction of the CRO maturation process. To this end, we are testing sequence tolerance of CRO-proximal residues using saturation mutagenesis, time lapse analysis of in vivo fluorescence and subsequent DNA sequence analysis.

**1649-Pos Board B600**

**High-Density, High Aspect Ratio Silicone Post Arrays for Magneto Optical Biosensing and Targeted Cell Capture**

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We have developed a dense microarray of tall, flexible silicone posts which has strong potential for use in magento-optical biosensing applications or as high-surface-area devices for cell capture. The posts in the array are 30 μm tall by 500 nm in diameter (AR ~50) with densities ranging from 269 to 27× POSTS/cm2 (2 to 7 microns average spacing). The posts are treated with a hydrophilic silicone surfactant which renders them stable against lateral collapse in fluid suspension. In addition, the posts can be fabricated using a magnetic-nanoparticle / silicone composite to produce a magnetically actutable array. The post array is fabricated by filling the pores of a polycarbonate track-etched membrane with liquid silicone or magnetic silicone composite. The filled membrane is placed on a glass substrate and the silicone is cured, after which the polycarbonate membrane is removed by dissolution. The surfactant treatment is applied during dissolution, allowing the completed array to be resuspended in a variety of solvents.

Both the active (magnetic) and passive silicone arrays provide a very large surface area which may be well-suited for development of force-flying cells in a novel framework to identify the major protein-targeting motif of an aptamer. An aptamer microarray with 12,000 features was created to deconvolute the major protein-targeting motifs and the optimal length for the aptamers were accordingly determined through this microarray platform. Also, the structural and functional characteristics between the optimized aptamers and their parent sequences were compared in support of the microarray approach. In addition, we used the microarray and in silico docking approaches to investigate the targeting motif of an aptamer recognizing influenza hemagglutinin. The optimized motif obtained from the microarray approach correlated closely with the in silico model. This study indicates that the proposed microarray methodology can accelerate the aptamer sequence mining for post-SELEX optimization.
between proteins allows investigations of to specify their role of precise interactions in cellular mechanisms behaviors and to direct manipulation of these manipulable cellular responses. The conventional approaches to these measurements involve two tags conjugated to proteins of interest whereby the association of these two tags reveals the dimerization states of the targeted protein. Two major challenges for those probes include the reversibility of the tag interaction and the signal contrast for detection. The development of a dimerization inducer or reporter based on fluorogen activating proteins (FAP) takes the advantages of the high specificity of protein/fluorogenic dye interaction and the dynamic fluorescence enhancement upon complexation, allowing both the induction of dimerization and fluorescent detection of the protein interaction. We studied the tunability in binding properties of a malachite green (MG) binding homodimerizing FAP L5 by random and site-directed mutagenesis. One mutant L5_E52D showed low nanomolar binding affinity with large binding enthalpy, suggesting MG can be used as a strong chemical dimerization inducer. Saturation mutagenesis at the E52 site revealed another mutant, L5_E52K, which showed resulted in undetectable MG binding in monomeric form but displayed high fluorescence signal in tandem dimeric form, enabling the sense of dimerization. We present the characterization of the mutants from single-site mutation saturation of a Fluorogen Acti-vating Protein and the in vitro validations of two mutants being used for dimerization induction and dimerization detection.

1651-Pos Board B602 Engineering of Artificial pH Switch Proteins using Internal Ionizable Residues with Anomalous PKa Values Perégrine Bell-Upp, Jaime Sorensen1, Jamie L. Schlessman1, Bertrand E. Garcia-Moreno1.
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Many proteins act as pH sensors. They can respond to small changes in pH with a large conformational transition of functional significance. The thermodynamic nature of pH-driven conformational changes in proteins is well understood: a change in pH can drive a conformational transition when final and intermediate states have different affinity for H⁺. This requires that some ionizable groups titrate with different pKₐ values in the two states. Here we demonstrate how to engineer a pH switch protein with controllable, cooperative unfolding in response to small changes in pH in the physiological range. This study was enabled by our previous demonstration that most Lys and Gru residues buried in the interior of staphylococcal nuclease titrate with highly anomalous pKₐ values. We show that burying Lys or Ghu residues with highly anomalous pKₐ values can be used for rational engineering of artificial pH switch protein and that global cooperative unfolding in response to small changes in pH in the physiological range is possible. This is enabled by our previous demonstration that most Lys and Gru residues buried in the interior of staphylococcal nuclease titrate with highly anomalous pKₐ values as a consequence of being buried in the hydrophobic interior of the protein. The ionization of a single buried ionizable group is not sufficient to drive the unfolding of the protein, thus we engineered variants with two buried ionizable groups. The V66E/A109E and T62K/L125K variants of SNase were selected because, based on the pKₐ values measured for these buried residues, the protein was expected to act as a switch near pH 7. This was confirmed with fluorogenic dimerization experiments. The double Gru variant was stable at low pH and unfolded at high pH and the double Lys variant was stable at high pH and unfolded at low pH, as expected from the nature of the shifts in pKₐ values. Crystallography and NMR spectroscopy were used to characterize the folded and unfolded states. This strategy for the design of artificial pH sensing proteins is completely general and transferable to other proteins.

1652-Pos Board B603 The Design of an NAD⁺-BioSensor Based on Changes in Intermolecular HomofRET of Glucose-6-Phosphate Dehydrogenase William D. Cameron1, Cindy V. Bui1, Pamuditha N. Silva1, Jonathan V. Rocheleau1,2, 1Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, 2University Health Network, Toronto, ON, Canada.

A number of genetically targetable biosensors are based on the allosteric binding of a ligand to a protein, inducing changes in the molecular distance between two spectrally overlapping fluorescent proteins (e.g. Cerulean and Venus). Ligand concentration is reflected in FRET efficiency, which is typically determined by emission wavelength or fluorescent lifetime. Due to the number of correction factors required for this technique, biosensor design has been largely limited to intramolecular FRET. In nature, however, protein homodimerization is a fairly common event. Therefore, the ability to reliably detect intermolecular FRET would open a much larger design space. To that end, we are investigating FRET between identical fluorophores (homofRET) as a method of detecting intermolecular homodimerization through changes in fluorescence anisotropy. To address an ongoing need for measuring cellular NADPH in the field of diabetes, we have developed an NADP⁺-sensor based on the dimerization of Glucose-6-Phosphate Dehydrogenase. We describe the various stages of sensor development including: linker optimization, enzymatic inactivation and a confirmation of dimerization using Number and Brightness analysis. We further demonstrate other advantages of homofRET sensor design, such as the ability to swap between fluorescent protein colours and an improved capacity for multiparametric imaging. Functionally, we show that the sensor responds consistently with two-photon excitation NAD(P)H autofluorescence (which cannot distinguish between NADPH and NADH), but could also capture previously inaccessible events unique to NADPH. Finally, the sensor was applied to study nutrient-stimulated changes in NADP⁺ in beta-cells, where we found significant NADPH depletion in the mitochondria, but not in the cytoplasm, after fatty acid treatment. Overall, this project demonstrates the utility of intermolecular biosensors using homoFRET thus revealing a new paradigm for biosensor design.

Micro- and Nanotechnology II

1653-Pos Board B604 Pores with Undulating Opening Diameter can Determine Particles by Size and Shape Crystal Yang1, Preston Hinkle1, Dmitry Melnikov1, Henriette E. Bakker1, Arnout Imhof1, Eugenia Toomil-Molares2, Maria Gracheva1, Zuzanna Siwy1.
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The resistive-pulse technique has been applied to detect and size particles as they pass through a pore. The pulse, defined as a change in current with respect to the baseline, carries information about the particle’s volume. Pores characterized with an undulating opening diameter could be used to identify the shape of a passing object using the resistive-pulse technique. The experiments were performed with 12 micrometer long pores in polyethylene terephthalate (PET) prepared by the track-etching technique. Due to the laminar structure of PET films, the subsequent wet chemical etch formed longitudinal irregularities along the pore wall. The topography of each pore is examined nondestructively using polystyrene particles and observed as a series of peaks within a pulse. We studied the effects of the pore undulations on differently shaped particles by comparing pulses of polystyrene spherical beads with pulses created by silica rod-shaped particles with lengths of 590 and 1950 nm and diameter of ~200 nm. To understand the undulating diameter’s role, we also passed both particles through a smooth, 30 μm long polycarbonate (PC) pore. We found that the rods’ passage induced averaging of the pulses so that a smaller number of sub-peaks was observed compared to the pulses of the spheres. The degree of the pulse averaging could be correlated with the rod length. Pulses of beads and rods were virtually indistinguishable in the smooth PC pores, emphasizing the importance of the pore undulations’ ability to distinguish shapes. In studying non-spherical particles, we discovered that the pore’s pulse amplitude was higher than expected from volume exclusion. This suggests that the pulse amplitude of non-spherical particles depends not only on particle volume, but also on its angular orientation and rotational motion as it travels across the pore.

1654-Pos Board B605 Solvent Free Bilayer Recordings using a Novel All-In-One Miniaturized Amplifier Federico Thei1, Michele Rossi1, Marco Bennati1, Alessandro Marabelli1, Matthias Becker1, Niels Fertig2.
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We present a new miniaturized all-in-one Amplifier suitable to perform high quality electrophysiology measurements. The instrument has been evaluated on a platform where ion channel proteins are reconstituted in solvent-free bilayers and multiple corresponding experimental data sets are shown. Characterization of reconstituted ion channels is still challenging as low noise current measurements in the pA range have to be combined with a large bandwidth of tens of kilohertz. Traditional transimpedance amplifiers use resistive or capacitive feedback to generate an analog output which needs to be digitized for further processing and display, resulting in complex, bulky and expensive systems. The amplifier shown here uses a switched capacitor gain stage combined with a sigma-delta digitizer which directly produces a digital signal. The actual layout is completely done in one silicon die (ASIC). This novel approach allows the integration of a multiple gain stage, filters, signal conditioning circuitry, digitizer and USB interface into the size of a pack of cigarettes while noise performance (~100fA rms @ 1KHz) and bandwidth (up to 100KHz) are comparable to state-of-the-art patch clamp amplifiers.