sophisticated process and expensive instruments. Hence, We propose the method to use optical anisotropy and scattered light intensity as indices for accurate and robust discrimination between single AuNPs and dimers using single-particle polarization microscopy in aqueous solution. This method is called a direct light scattering(DLS) method. This is homogeneous, rapid low-cost, sensitive method. We tested several way to deduce distance between two AuNP in the step of fabricating AuNP dimers and evaluated these AuNPs to utilize DLS method in colloid solution. In this presentation, we report the result and conclusion about the influence of inter-particle distance in our experiment.

1640-Pos Board B591
Optically Tracing Electrical Synapses with Proton Channel-Based Voltage Sensing Protein
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The field of optogenetics has extensively improved over the last couple years allowing optical detection of single action potentials [CITE PAPERS]. In order to improve voltage sensors, a search strategy using the highly conserved S2 trans-membrane motif sequence identified 8 novel voltage sensing proteins. These proteins were fused to the fluorescent super eclipitc pHluorin A227D and transfected to HEK293 cells. The voltage sensing domain of a proton channel from liver fluke resulted in a sensor, Pado, which gave large optical signal and also responded to the induced pH change in the cell. This proton channel exhibits inhibition by extracellular Zn\(^{2+}\) and shifted response curves in differing pH environments. Higher buffering of the internal solution resulted in diminished pH dependent fluorescence change. With the ability to controllably manipulate pH of a cell, gap junctions can be traced through a network of HEK cells. Pado also provides a tool to screen fps for pH and voltage related activities. Ultimately Pado would make it possible to map electrical synapses and voltage activity simultaneously.

1641-Pos Board B592
Using Exploratory Data Analytics to Identify Deficiencies in mCherry Red Fluorescent Protein and Suggest Improvements
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Red fluorescent proteins are a valuable class of genetically encoded indicators for in vivo imaging. Even so, development of brighter variants has stagnated despite extensive efforts. To address this need, we have generated a library of over two hundred thousand simulated structures of mCherry fluorescent protein variants. The atomic positions of non-hydrogen atoms was then correlated with the planarity of the mCherry chromophore, a key determinant of molecular brightness. These studies revealed geometric instability of the mCherry chromophore. Further, we identified ten positional changes in mCherry side chains that strongly correlated with changes in the mCherry chromophore conformation. Site directed mutagenesis of key amino acids was then used to improve the mCherry quantum yield by approximately ten percent. These results suggest that a data analytics approach can be applied to structural engineering of fluorescent proteins for the first time.

1642-Pos Board B593
Design of a Theoretical Model to Identify Specific SSDNA Aptamers for Biosensing Applications
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Aptamers are single stranded DNA or RNA oligonucleotides which bind to proteins, small molecules, cells or organs with high affinity and specificity. Aptamers are designed to work in such a way that they only bind to their target proteins/cells, leaving other neighboring and non-targeting proteins/cells intact. This targeting efficiency makes them a promising solution to the problem associated with affinity and specificity of other bioactive agents, reducing harmful side-effects and costs. Target specific Aptamers can be produced by Systematic Enrichment of Ligands by Exponential Amplification, a method usually known as “SELEX Protocol”. However, this SELEX protocol needs a large pool of ssDNA/RNA library containing ~10^14 unique ssDNA/RNA sequences. Selection of only one aptamer for a target takes 15-18 rounds of selection and each round can take 2-3 days, which makes the process very time consuming and costly. In our model, we are trying to identify specific aptamer sequence for a target using a theoretical approach to experimental SELEX by a purely theoretical approach. This is done by rigorous theoretical studies on aptamer behavior in different biological environments. A molecular mean field theory is used which takes into account the size, shape, electrical properties and physical configurations of the aptamers along with the size, shape and physical properties of other chemical species associated with a particular biological environment. Coupling between different interactions, acid-base equilibrium and inhomogeneous distribution of different species along with the minimization of strong electrostatic repulsions gives us the most stable structure of aptamers specific to the target. So, this model can be a very efficient tool for designing aptamers to use in wide range of biosensing applications.

1643-Pos Board B594
Experimental Determination of Transition Dipole Moment Directions in Representatve Fluorescent Proteins
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Fluorescent proteins are the workhorses of biological molecular imaging. Important imaging modalities (such as polarization microscopy or FRET imaging) exploit anisotropic optical properties of fluorescent proteins. The anisotropy (directionality) of optical properties of fluorescent proteins is described by a vector, the transition dipole moment (TDM). Despite the importance of molecular TDM orientation for quantitative structural interpretation of many imaging experiments, experimental data on TDM direction in fluorescent proteins is very limited. Here we present the results of our optical measurements on crystals of representative fluorescent proteins, as well as mathematical interpretation of these results, yielding information on the orientation of TDMS within the investigated fluorescent protein molecules.

1644-Pos Board B595
Glycine Protects Hepatocytes through a Chloride Independent Mechanism
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Cytoprotection by the amino acid glycine was first described more than two decades ago. Accumulated evidence shows that glycine protects against hypoxia, ischemia-reperfusion and several toxins in hepatocytes, renal tubular cells and other cell types. The exact mechanism of the protection is still under investigation and multiple models have been proposed. One working model is that the protection exerted by glycine is mediated by inhibition of ligand-gated chloride channels. In our study, we used a genetically encoded chloride biosensor to test this working model. Isolated rat hepatocytes infected with adenovirus for expression of the chloride biosensor were treated with the mitochondrial uncoupler, carbonyl cyanide m-chlorophenyl hydradozone (CCCP), in the presence and absence of 3 mM glycine. With and without glycine, intracellular chloride concentration increased upon addition of CCCP. Without glycine, CCCP led to swelling, membrane bursting and death of hepatocytes in Krebs-Ringer-Hepes buffer. By contrast in the presence of glycine, cell death was markedly delayed. Moreover instead of swelling, hepatocytes underwent cell shrinkage after CCCP. These findings suggest that glycine alters cell volume regulation after CCCP and prevents cellular swelling leading to plasma membrane rupture and consequent loss of cell viability. DK073336, DK037034 and 14.250.31.0028.

1645-Pos Board B596
Small Angle Neutron Scattering Studies of Glucose Oxidase Immobilized on Single Layer Graphene: Relevant to Protein Microfluidic Chip
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Chronic diseases like diabetes has become more pervasive. Blood glucose monitoring is a valuable tool in the health management and consequently point-of-care devices like glucometers has now become indispensable. Since, obtaining and reliably accurate measurements are very essential, a promising solution is to incorporate enzymes into the biosensor [1]. Enzymes like glucose oxidase (GOx) are ideal for consistent measurements because of their specificity and the ability to electrochemically transduce from the enzymatic reaction. Consequently, the presence of glucose in blood plasma can be detected by the change in the potential at the electrode adjacent to GOx during catalysis of glucose [2]. Further, the oxidation overpotential of the electrode
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 sensors. 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. Furthermore, though the increase in the GOX concentration resulted in similar structural topology, the clusters displayed a smoother surface with fructals 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 of how 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 strain. 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 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.

1648-Pos Board B599
Developing Leave One Out GFP Based Biosensors
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The green fluorescent protein (GFP) has gained its utility expand far beyond that of a fluorophore in Aequoria victoria. Some of the main drivers of this have been work done to increase the spectroscopic range of the protein as well as to develop GFP as a biosensor. In our study we have generated leave-one-out variants of GFP (LOO-GFPs) that have been circularly permuted with a secondary structural element omitted. Co-expression of the truncated GFP (sensor) with wt peptides (target) reconstitutes fluorescence to varying degrees depending upon which strand has been omitted. We have observed that the efficiency in chimera formation (CRO) matures with the addition of mutations throughout the CD signal and the thermodynamic stability of the sensor-target complex.

Additionally, we envision that these insights can prove useful for computationally designing LOO-GFP biosensors with the ability to detect exogenous targets. Given the structure of a desired target protein, the primary sequence of the target protein is scanned to find fragments whose chemical composition are similar to that of one of the late folding β strands of GFP. These fragments can replace the corresponding β strand of GFP and the region around the new strand can be redesigned to find a set of biosensor primary sequences that best accommodates the exogenous target sequence. Initial screening of a library of biosensor sequences, even with covalent linkage of the LOO-GFP to the target, failed to produce a functional protein; suggesting that additional considerations are necessary. In particular, success depends on understanding the sequence determinants 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 magneto-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 260 to 2700 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 docking approaches to investigate cell mechanics, such as magnetic-head-based sandwich assays. In addition, the chaotic flow produced within a magnetically-actuated array dramatically increases opportunities for contact with targeted materials such as circulating tumor cells in a cell-capture device.

1650-Pos Board B601
Dimerization Induction and Measurement using Fluorogen Activating Proteins Guided by Saturation Mutagenesis
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Protein-protein interactions in living cells are one of the most fundamental opportunities for contact with targeted materials such as circulating tumor cells in a cell-capture device.