3637-Pos Board B498
Observations of MHC Structural Changes Elicited from Antigenic Peptide using Diffracted X-Ray Tracking (DXT)
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The conformation changes of functional proteins are important process in biological reactions. The major histocompatibility complex (MHC) molecules play an important role in the immune systems. MHC molecules are transmembrane heterodimers with extracellular domains that bind antigen peptide for presentation to T-cells. It is considered that the affinity of peptides for the MHC molecule is crucial factor to determine the antigenicity of peptide to elicit T cell responses. This TCR/peptide/MHC tri-molecular interaction has been studied extensively. However, what determines immunogenicity of the peptide as well as strength of the immunological interaction is not fully understood. Here, we show correlations between the MHC stability and active motions of the peptide/MHC molecule using the diffracted X-ray tracking (DXT) that monitors real-time molecular motions of individual I-A^k MHC molecule in aqueous solution at single-molecule level. DXT can observe the dynamical different motions of many peptide/MHC with high accurate two rotational views (2θ and χ). We obtained the relationship between diffusion coefficients from DXT measurementsand the stability of I-A^k MHC complex from macro-SDS-polyacrylamide gels.This relationship (van't Hoff plot) was divided into two types of molecule stable states. Entropy evaluation from our van't Hoff plot was carried out by each conformational type in many peptides/MHC complexes, and it was shown clearly that the stability and dynamical intramolecular motions or elicited structural changes of peptides/MHC complex are related strongly. Additionally, we tried to measure SDS stability of peptides/MHC complex using DXT in order to get van't Hoff plot from only single-molecule technique.

3638-Pos Board B499
Photo-Control of the Small G Protein Ras using Photochromic Molecule
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Small GTPase Ras is one of the G proteins. It was shown that the crystal structure of the GTPase domain strikingly resemble to that of ATP driven motor proteins, myosin and kinesin. We have previously incorporated photochromic molecules into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon ultraviolet and visible light irradiation. In this study, we performed basic study to control the Ras function reversibly by light using photochromic molecules. First, in order to monitor GTPase of Ras, we synthesized a new fluorescent GTP analogue, NBD-GTP that changes its fluorescent intensity along the formation of Ras-GTP, Ras-GDP-Pi and Ras -GDP state. Quantitative analysis of cysteine residue on the surface of Ras using DTNB revealed that one cysteine locates on the surface of wild type Ras. However, the cysteine reside was not modified with thiol reactive photochromic molecule, 4-phenylazophenyl maleimide (PAM). Therefore, We prepared the mutant T59C which has an additional cysteine residue at T59 where is believed as a functional region. However, T59C mutant did not show GTP binding and GTP hydrolysis. And ultraviolet (UV) and visible (VIS) light photo irradiation did not alter the GTPase activity of the PAM-modified T59C. Subsequently, we designed and prepared further mutants K5C, I36C, Y64C, which have single cysteine in the site near the switch2 region or GAP binding site. The Ras mutants I36C and Y64C bound to NBD-GTP and hydrolyzed NBD-GTP. The mutants were modified with PAM and reversible alterations of GTPase activity by the VIS and UV light irradiation were studied.

3639-Pos Board B500
Monitoring Metabolic Responses in Saccharomyces Cerevisiae using Fluorescence-Based Detection of NADH Conformation
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Metabolic co-factors such as reduced nicotinamide adenine dinucleotide (NADH) are fluorescent and can be used to monitor cellular metabolism related to respiration and mitochondrial function. While fluorometric monitoring typically focuses on total NADH concentration, it is the free, as opposed to protein-bound, NADH pool that couples the various biochemical reactions (e.g., involving dehydrogenases) and that is the determinant of reaction velocities. Thus the level of free versus protein-bound NADH is an important physiologic parameter for monitoring metabolic responses. This work explores changes in intracellular NADH conformation when S. cerevisiae (baker's yeast) is subject to various metabolic modifiers (including cyanide, FCCP, glucose, and ethanol) and various oxygenation levels. NADH tends to exist in a folded conformation when free and an unfolded conformation when protein bound, with the excited-state emission shifting to shorter wavelengths upon unfolding. Thus we excite intracellular NADH at 337-nm wavelength using a nitrogen laser, recording the emission spectrum in real time using a spectrograph coupled to an intensified CCD detector. Results indicate that when NADH concentration increases or decreases, the proportion of free NADH increases or decreases as well, although there are differences in the time course of the two measures.

3640-Pos Board B501
Liposomes Encapsulating Quantum Dots as Luminescent Probes
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Liposomes are being studied as drug delivery systems, membrane models, as well as encapsulating systems of nanostructured materials for cellular imaging and diagnostics applications. Taking advantage of the use of Quantum Dots (QDs) as fluorescent probes over many conventional fluorescent dyes, several research groups are developing methods to encapsulate lypophilic and hydrophilic QDs into liposomes either in the aqueous compartment or in their bilayer in order to have a multifunctional system and provide therapy and diagnostics at the same time [1].
In this work, Cadmium Telluride/Cadmium Sulfide (CdTe/CdS) quantum dots functionalized with mercaptopropionic acid (MPA) was synthesized in aqueous medium and characterized by optical spectroscopy and X-Ray diffractometry. Synthesis was carried out by adding Te^{-2} to a Cd^{+2}/MPA refluxed for 7 h at ~ 95°C. Cationic multifluorescent quantum dot liposomes (QD-Ls) formed by phosphatidylcholine (PC) and 1,2-dioleoyl-3-trimethylammonium-propane(DOTAP) (80:20) were prepared by lipid film hydration with pure water. QDs were encapsulated by a new method using freeze-and-thaw cycle. Liposomes (100 or 800 nm in diameter) encapsulating QDs were characterized by zeta potential, size distribution, fluorescence and transmission electron microscopy and show great potential for diagnostic purposes.
[1] G. D. Bothun, A. E. Rabideau, and M. A. Stoner, *J. Phys. Chem B Lett* **2009** 113, 7725-7728.
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3641-Pos Board B502
An Irreversible Lock to Proteins for Dynamic Force Spectroscopy at the Mammalian Cell Surface
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Exploring the role of force in biology is often limited by breakage of protein interactions. We have developed a protein interaction that will not break, because a covalent bond forms spontaneously between a genetically-encoded protein (termed SpyCatcher) and a peptide (SpyTag). The covalent bond was formed between engineered fragments of a protein from Streptococcus pyogenes, which naturally forms an intramolecular isopeptide bond. SpyTag could be inserted at various locations in proteins and reacted rapidly and specifically, both in bacterial and mammalian systems. Single molecule dynamic force spectroscopy uncovered the intermediate complex before covalent reaction and the covalent complex which resisted more than 1 nN. SpyTag was applied to provide an irreversible lock to a specific protein on the mammalian cell surface. Combining AFM and confocal microscopy, SpyTag enabled us to visualize how high forces change membrane structure, cytoskeletal organization, and signaling in mammalian cells.

3642-Pos Board B503
Supramolecular Assemblies of Lipid-Coated Polyelectrolytes
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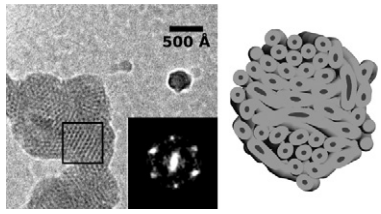
We reveal the existence of a general class of supramolecular assemblies made up of lipid-coated polyelectrolytes including the celebrated lipid-nucleic acid complexes. With the aid of high-resolution cryo-electron microscopy, we unveil the nanoscale internal organization of assemblies generated with a wide range of synthetic and biological polyelectrolytes, from short stiff up to long flexible ones. A coarse-grained model of semi-flexible self-assembling tubes allows us to construct a full phase diagram of the three-dimensional morphologies in good agreement with experimental observations, and to better understand the relationship between the varying degree of order and the physical properties of lipid-coated polyelectrolytes. We find out that assemblies can

exhibit a local hexagonal packing and evolve from disordered agregates to spooled then entangled straight bundles as the rigidity of lipid-coated polyelectrolytes increases. These assemblies may constitute a generic route for interfacing polyelectrolytes to living cells like in gene delivery.



3643-Pos Board B504

Structure, Assembly and Mechanical Properties of DNA Nanoparticles Condensed with Peim

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DNA is an anionic polyelectrolyte, which occupies a large volume in salt free solution due to the coulomb repulsion between the charged groups. In the presence of polymer cations, DNA condenses into nanoparticles. DNA nanoparticles have generated a lot interest as a preferred vehicle for delivering therapeutic DNA in gene therapy. The efficiency of gene delivery is determined by stability and compactness of the particles. However, not much is known about the organization of DNA within the nanoparticles. Large polymer cations condense DNA rapidly, with no distinct intermediate stages that could give insight into the arrangement of DNA. In our work, we modulate the DNA length to slow down nanoparticle formation; and, by imaging with Atomic Force Microscopy, reconstruct stages in the particle assembly. The polymer cation used was polyethyleneimine modified with sugar residues (PEIm). The cation:base pair ratio was ~60. The DNA is found to be arranged within the nanoparticle as an inter-weaving network of long fiber condensates. The fiber condensates form from DNA folding along its length, and appear to be the unit of DNA organization within the particle. The fiber-condensate network is highly deformable, having as much as 95% water content. Nano-indentation experiments suggest that the nanoparticles have a hollow sphere architecture.

3644-Pos Board B505

Extracellular Matrix Proteins are Necessary for Mouse Embryonic Stem Cell Differentiation and May Guide Stem Cell Fate

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While in vitro differentiation protocols often rely exclusively on soluble growth factors to direct mouse embryonic stem cell (mESC) fate, the ESC niche contains both soluble factors and fibrillar extracellular matrix (ECM) proteins, including fibronectin (FN). Moreover, some of the soluble factors used to guide differentiation (e.g. Activin A) are known to increase the expression of ECM proteins, though the functional importance of this change is not well understood. We examined whether ECM proteins were necessary for promoting and directing mESC differentiation. mESCs, grown as embryoid bodies under differentiating conditions in the absence of FN, maintained expression of the pluripotency marker, Nanog. The embryoid bodies also showed a spatiotemporal correlation between expression of FN and GATA4, a marker for differentiation. When differentiated on a gelatin substrate, mESCs create a fibrillar ECM containing fibronectin and laminin components, while the presence of leukemia inhibitory factor (LIF), a maintainer of mESC pluripotency, inhibits the production of this fibrillar matrix. Ongoing work is investigating how the composition of this cell-derived ECM changes when soluble growth factors are used to guide ESC fate and whether these changes are necessary to efficiently direct differentiation. Together these data imply that FN is necessary for mESC differentiation and that the extracellular matrix may be an important director of stem cell fate.

3645-Pos Board B506

Investigating the Effects of Dynamic External Stimuli on Single Cell Fitness and Gene Expression in Escherichia Coli

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In this work, we study the bacterial cellular response to time-dependent external stimuli in single living cells. We developed a microfluidic platform for single cell analysis that allows for dynamic control of well-defined environmental growth conditions and culture media. Using this platform, we studied the effect of small molecule inducers on gene expression in the *lac* operon using fluorescent reporter proteins and cell growth rates as a proxy of cellular fitness. We applied temporally varying inducer concentrations by translating single cells between two adjacent fluid streams containing either growth medium or growth medium and inducer. We observed that single cell gene expression depends on growth rate and frequency of exposure to inducer concentrations. Single cell induction experiments are compared to control experiments with and without continuous fluid flow in microfluidic channels. For these experiments, single cell analysis is facilitated by a microfluidic-based hydrodynamic trap recently developed in our lab. The hydrodynamic trap enables confinement and manipulation of single cells in free solution using the sole action of fluid flow. Automated feedback control is integrated into the device using an ''on-chip'' valve, which allows for precise confinement of cells in free solution. Using this device, cells are confined at a fluid stagnation point generated in a cross-slot microfluidic geometry, thereby enabling non-perturbative trapping of cells for long time scales. Using optical microscopy, we observe single *Escherichia coli* cells growing and dividing both at room temperature and at 37^0C, and cell division rates in the microfluidic trap compare favorably to the growth rates of *E. coli* measured in bulk studies. We anticipate that the microfluidic-based trap is an ideal platform to study cellular regulation and gene network dynamics of single living cells in free solution.

3646-Pos Board B507

Infrared Light Excites Cells via Transient Changes in Membrane Electrical Capacitance

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Control of excitable cells using optical technologies such as optogenetics has enabled important advances in neuroscience and the development of clinical applications. Most existing methods of optical control require the use of genetic or chemical sensitizers that enable light to alter the ionic conductance of cell membranes. By contrast, infrared (IR) light of wavelengths $> 1.5\mu m$ has been shown in vivo to excite neural and muscle tissue without any pretreatment. Unfortunately, the mechanism of IR stimulation is unknown. Here, we describe how IR light excites cells by transiently altering their membrane electrical capacitance. Our data from voltage clamped Xenopus laevis oocytes, mammalian cells and artificial lipid bilayers shows that IR energy absorbed by water produces a rapid local increase in temperature at the cell membrane, transiently increasing its electrical capacitance, and generating depolarizing currents. Correspondingly, under current clamp conditions, IR pulses produce rapid changes in membrane potential. This unexpected mechanism is fully reversible and requires only the most basic properties of cell membranes. Changes in capacitance were verified by direct measurement in mammalian cells and artificial bilayers, and are consistent with a classical theoretical description of cell membranes as coupled double-layer capacitors. In shedding light on the mechanism of IR stimulation, our findings point to this technology's unique generality as a means to control excitable cells, and raise questions about other thermal phenomena that may meaningfully affect membrane electrostatics. Supported by the NIH: GM030376 and DC011481-01A1.

3647-Pos Board B508

Mechanical Derivation of Functional Myotubes from Adipose-Derived Stem Cells

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In recent years, ECM stiffness and resulting cell contractility have been identified as potent stem cell differentiation regulators. Successful stem cell-based therapies will require acclimating cells to the abnormally stiff ECM of muscular dystrophy while inducing and/or maintaining myogenesis, fusion, and dystrophin delivery. Here we directly compare ASC to BMSC stiffness responsiveness and show myotube formation derived from ASCs on matrices that mimic skeletal muscle. ASCs are shown here to not just simply reflect the qualitative stiffness sensitivity of bone-marrow-derived stem cells (BMSCs) but to exceed BMSC myogenic capacity (40-fold higher myogenic marker expression on myogenic stiffness), expressing the appropriate temporal sequence of muscle transcriptional regulators on muscle-mimicking extracellular matrix in a focal adhesion- and contractility-dependent manner. 2% of ASCs formed multi-nucleated myotubes with a continuous cytoskeleton (10-fold higher than chemical induction) that was not due to misdirected cell division; microtubule depolymerization severed myotubes, but after washout, ASCs refused at a rate similar to pretreated values. BMSCs never underwent stiffness-mediated fusion. ASC-derived myotubes, when replated onto non-permissive stiff matrix, maintain their fused state. Fusion frequency was increased by a contractile agonist, lysophosphatidic acid and decreased by a myosin inhibitor, blebbistatin. ASCs generated higher tangential force than BMSCs and