3637-Pos Board B498
Observations of MHC Structural Changes Elicited from Antigenic Peptide using Diffracted X-Ray Tracking (DXT)
Yuji C. Sasaki[1,2], Kentaro Hoshisashi[1], Hiroshi Sekiguchi[1], Kouhei Ichiyanagi[1], Naoki Ogawa[3], Haruo Kozono[4], Osami Kanagawa[1].
[1]The University og Tokyo, Kashiwa-city, Japan, [2]JST/CREST Sasaki team, Osaka-fu, Japan, [3]Nihon University, Setagaya-ku, Japan, [4]Tokyo University of Science, Noda-shi, Japan.
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
Hisashi Kobayashi, Shinsaku Maruta, Keiko Tanaka, Shun Sugawara.
Soka Univ, Tokyo, Japan.
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
Paul Urayama, Jun Cheng, Michael Zatt.
Miami University, Oxford, OH, USA.
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
Maria B. Seabra[1], Rafael B. Lira[1], Ana Lívia Linard[1], Eneida de Paula[2], Renato Grillo[2], Vivaldo Moura[3], Adriana Fontes[1], Beate S. Santos[1].
[1]Federal University of Pernambuco, Recife, Brazil, [2]State University of Campinas, Campinas, Brazil, [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
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
Emrah Celik[1], Bijan Zakeri[2], Mark Howarth[2], Vincent T. Moy[1].
[1]University of Miami, Miami, FL, USA, [2]Oxford University, Oxford, United Kingdom.
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
Guillaume Tresset[1], Yves Lansac[2], Guillaume Romet-Lemonne[3].
[1]Université Paris-Sud, Orsay, France, [2]Université François-Rabelais, Tours, France, [3]CNRS, Gif-sur-Yvette, France.
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