quantify the $BSCaM_{IQ}$ sensor for live cells at single-cell resolution, based on the competition between CaM and the channel (Liu *et al.*, *Nature* 2010) and also the *in vitro* measurement of $BSCaM_{IQ}$ sensors (Black *et al.*, *Biochemistry* 2006). Next, we move up to multiple cells in phantom of FMT (Hu, *et al.*, *Progress in Natural Science*, 2008). Cell lyses are loaded into phantom in FMT system to perform FRET, with different titration of CaM concentrations upon lyses with $BSCaM_{IQ}$. Finally, cells expressing $BSCaM_{IQ}$ are suspended as solutions in phantom. Suspensions of cells with distinct expression levels of CaM, as scalable mimics of bulky tissue, are imaged in full-angle FMT to examine spatial resolution and other key issues in FRET sensor-based FMT. Here, we present an innovative approach to systematically quantify $BSCaM_{IQ}$ sensors at single-cell, multi-cell and tissue levels for FMT, which is readily applicable for other FRET sensors.

2636-Pos Board B622

Amino Acid-Specific Attachment of a Protein to the Termini of Carbon Nanotube Without Affecting its Enzymatic Function

Shahbaz J. Khan.

In single-molecule measurement and manipulation of biological materials, as well as constructing biological devices by utilizing nano-materials, the interaction between biomolecules and nano-material largely affect the quality of the device and accuracy of the measurement. Especially, when a small number of the molecules are targeted, the way of attaching protein to the nano-material largely affects the result. We describe a strategy for site-specific conjugation of target proteins to carbon nanotubes. Non-natural amino acid, azidotyrosine, was incorporated in a protein of interest at a desired position by utilizing amber mutation method. This azide group was then reacted with carboxyl group at the end(s) of nanotube through a set of cross linkers. We successfully demonstrated that calmodulin can be attached to the end of nanotube without losing its enzymatic functions. This approach allows the covalent conjugation of any nanostructure and/or nano-device to any proteins without affecting their biological functions.

2637-Pos Board B623

Kinetic Study of De Novo Chromophore Maturation of Fluorescent Proteins

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Green fluorescent protein (GFP) has a chromophore that forms autocatalytically within the folded protein. Although many studies have focused on the precise mechanism of chromophore maturation, little is known about the kinetics of de novo chromophore maturation. Here, we present a simple and efficient method for examining the de novo kinetics. GFP with an immature chromophore was synthesized in a reconstituted cell-free protein synthesis system under anaerobic conditions. Chromophore maturation was initiated by rapid dilution in an air-saturated maturation buffer and the time course of fluorescence development was monitored. Comparison of the de novo maturation rates in various GFP variants revealed that some folding mutations near the chromophore promoted rapid chromophore maturation and that the accumulation of mutations could reduce the maturation rate. Our method will contribute to the design of rapidly-maturing fluorescent proteins with improved characteristics for real-time monitoring of cellular events.

2638-Pos Board B624

Photothermal Lens Spectoscopy in Highly Scattering Media

Omar Ziane, **Aristides Marcano**, Hacene Boukari, Noureddine Melikechi.

Photothermal lens spectrometry is a highly-sensitive technique used to measure simultaneously absorption and thermal parameters of various scatter free samples. However, recently researchers have extended the use of this technique to for measuring the absorption of light in complex samples, including highly scattering media such as biological samples, . It is commonly applied in either scattering-free samples or without a systematic study of assumed that the effects of scattering on the photothermal signals. measurements are negligible. In this work, we report the results of a pump-probe experiment in which In the present study we measured both the photothermal signal and the scattering intensity at 90^{o} angle from samples of water solutions prepared with a mixture of absorptive gold nanoparticles (concentration 150 ppb and 2-nm diameter) and scattering latex microspheres (0.2-25 microm diameter and concentrations 1.3-50 microg/ml). The data show that the photothermal signal slowly decreases when the concentration of the latex scatterers is increased. At latex concentration 50 microg/ml, the scattered signal increases more than 2 orders of magnitude for 0.2-microm latex microspheres while the photothermal signal reduces by less than 50%. This result suggests that the signal reduction may be caused by the distortion of the excitation beam profile due to multiple scattering, inducing hence reduction of the local temperature gradient necessary for thermal lensing. In the case of Latex, we show that the photothermal lens method can be considered scattering free for turbidity values smaller than ?? (1/cm).

2639-Pos Board B625

Synthesis of Photochromic ATP Analogue and its Interaction with Motor Protein

Kazuya Aritomi, Taro Kimura, Takeshi Itaba, Shinsaku Maruta.

Azobenzene is one of the photochromic molecules, which undergoes rapid and reversible transitions between the cis isomer and trans isomer by visible and ultra-violet light irradiation. We have been trying to control the activities of motor proteins using the photochromic molecules as photo-regulatory devices. We have recently demonstrated that microtubules dependent ATPase activity of the kinesin modified by azobenzene derivative was regulated by UV-VIS light irradiation. However, it was not so easy to incorporate the photochromic molecules into the functional site of motor proteins without altering the native enzymatic properties.

In the present study, we have designed the novel ATP analogues consist of photochromic molecules in order to photo-regulate the motor protein kinesin without their chemical modification. It is expected that the ATP analogues induce the reversible conformational change in the active site by alternate UV-VIS light irradiation. We have synthesized non-nucleotide ATP analogue composed of azobenzene derivative, Phenylazobenzoic-aminoethyl -triphosphate (PABATP). PABATP showed UV/VIS light absorption spectral change accompanied by transition between cis and trans in a similar manner observed in azobenzene. PABATP was hydrolyzed by conventional kinesin and the hydrolysis rate was activated by microtubules. It has been demonstrated that the cis isomer and trans isomer perform differently on a microtubule gliding motility assay. We have also examined the formation of kinesin-PABADP-Pi analogues (BeFn, AlF4-, Vi) which may mimic the transient states in ATPase cycle.

2640-Pos Board B626

Photocontrol of the Small G Protein Ras Using Photochromic Molecule

Hisashi Kobayashi, Shinsaku Maruta.

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 demonstrated that incorporation of photochromic molecules in the functional sites of kinesin enable the photo-regulation of their ATPase activities. Therefore, it is expected that Ras may be also regulated using photochromic molecules. In this study, basic experiments were performed in order to photoregulate GTPase activity of Ras. A new fluorescent GTP analogue, NBD-GTP was synthesized in order to monitor the GTPase Ras.

NBD-GTP showed initial burst enhancement of the fluorescent intensity by 120% when GDP free Ras was added and subsequently slow increase of fluorescence. The results may suggest that the initial burst of fluorescence reflects the binding of GTP to the free GTPase site of Ras, and the slow phase corresponds to the formation of Ras-GDP-Pi state. Quantitative analysis of cysteine residue on the surface of Ras using DTNB revealed that one of the intrinsic three cysteine locates on the surface. However, SH group reactive photochromic molecule, 4-phenylazophenyl maleimide (PAM) was not incorporated into the cysteine residues of wild type Ras. We designed the cDNA encoding the ras, which has an additional reactive cysteine residue at near 12, 59 and 61, which are suggested as an essential for GTPase activity. We prepared Ras mutant T59C that have a reactive cysteine residue in the functional key region for the signal transduction. Incorporation of PAM into T59C abolished GTP binding and GTP hydrolysis of the mutant. UV and Visible light photo irradiation did not alter the GTPase activity of the mutant modified with PAM.

2641-Pos Board B627

Conformational Change of Myosin Head Induced by Photo-Isomerization of Photochromic ATP Analogue

Takeshi Itaba, Hideki Shishido, Yasunobu Sugimoto, Katsuzo Wakabayashi, Shinsaku Maruta.

Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the cis- and trans-forms in response to ultraviolet (UV) and visible (VIS) light irradiation, respectively. Previously, we have cross-linked reactive cysteine SH1 and SH2 of skeletal muscle myosin with the sulfhydryl-reactive bifunctional azobenzene derivative, azobenzene-dimaleimide (ABDM) and succeeded to induce lever arm swinging reversibly by photo-irradiation. However, incorporation of the photochromic molecules into the functional regions abolished native enzymatic properties of myosin.

In the present study, we have employed photochromic ATP analogues that change its structure reversibly by light irradiation in order to photo-regulate function of myosin. Phenylazobenzoyl-iminoethyl-Tri-Phosphate (PABITP) have been designed and synthesized. In the preliminary experiment cis isomer of PABITP was hydrolyzed by skeletal and smooth muscle myosin in the presence of Mg2+ much faster than trans isomer. The results suggested that the cis isomer and trans isomer perform differently as a substrate of myosin. PABITP induced dissociation of acto-myosin. Myosin-PABIDP-Pi analogues (BeFn) ternary complex that may mimic transient state in ATPase was formed. X-ray solution