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Measurement of Temperature Change in Single Hela Cells with Thermosensitive Polymers

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Temperature is one of the most important parameters for living cells. Relationship between temperature and the processes in cells, i.e. effects of temperature on the cellular activities, has been studied for many years. However, there have been few studies focusing on the intracellular temperature of single living cells, because there have been few appropriate methods. In this study, we have developed a new method to measure a local temperature change inside single cells with molecular thermometers. We used thermosensitive fluorescent polymer, poly(DBD-AE-co-NNPAM-co-NIPAM) as a molecular thermometer. The fluorescence intensity of the polymer in cytosol sharply increases more than 10-fold by 6-7 °C increase around the transition temperature. We first tried to detect the thermogenesis in single HeLa cells induced by ionomycin, which was previously studied with using a micro thermometer made of a glass capillary (Suzuki, M. et al., Biophys. J. 92, L46-L48, 2007). When ionomycin was added to extracellular solution, the fluorescence intensity of polymers inside HeLa cells increased. However, the polymers also responded to DMSO, in which ionomycin was dissolved, so that this property made it difficult to accurately determine the temperature change. Therefore, we have developed another method composed of a thermosensitive sheet, which complements the measurement with polymers. As a result, we found that the temperature increase occurred due to ionomycin treatment.

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Mapping Cell Adhesion Patterns with a Modified DNA Hairpin

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New contrast methods and sample preparation techniques for microscopy have made it possible to collect quantitative data from images while offering a less invasive and more direct experimental approach. Fluorescence, for instance, takes advantage of the versatility of labeling chemistry and spectral properties of probes to provide spatial and temporal resolution in multiple channels of information. Alternatively, a higher power light source can be introduced to a sample to manipulate molecules mechanically through an anchor. However, while fluorescence can be readily adapted to a system of interest, mechanical measurements often require specialized instrumentation and thus lack the versatility offered by fluorescence microscopy. To bring mechanical detection to a more common microscope platform, we need a contrast scheme that combines mechanical sensitivity with fluorescence imaging. Taking advantage of the well characterized chemical, mechanical, and physical properties of DNA, we previously presented the force-fluorescence relationship for a DNA hairpin. Given the low force of unzipping, 18pN, we expect that the hairpin molecule will be sensitive to molecular-scale mechanical changes. We modified this DNA hairpin by adding a cell adhesion peptide, RGD, to its terminus. Accordingly, we hypothesize that the hairpin can be used to detect cell adhesions to a surface coated with the hairpin molecule. Here, we present steps towards a molecular contrast scheme for imaging patterns of cell-surface adhesions as changes in fluorescence intensity.

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Improving the Photostability of Membrane Probes through Fluorination

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High photostability is a desired property for fluorescent probes, especially when long recording time is necessary. It becomes more crucial for long wavelength fluorescent dyes because they generally have lower fluorescence quantum yields and are more vulnerable to chemical and photochemical attacks. We have developed a series of optical probes with high membrane affinities that are able to report on their local membrane environment and act as sensors of membrane voltage. These dyes have hemicyanine chromophores with donor-bridge-acceptor structures. Following our recent success in developing near-infrared fluorescent membrane probes, we further tried to improve the performance of these dyes through fluorination. Synthetic pathways to hemicyanines with fluorine substitution at various positions have been developed and the performances of these fluorinated dyes have been evaluated. It was found that the effect of fluorination depends critically on the site of fluorination: donor, acceptor, or bridge. Fluorination on the vinylene bridge appears to give optimal outcomes: improvement in photostability, red-shifts in absorption and emission spectra, and increase in absorption cross section. On the other hand, fluorination on the pyridinium acceptor results in an undesirable outcome: the dye is much less stable than the nonfluorinated counterpart in PBS buffer. Fluorinated membrane dyes generally show response kinetics and voltage sensitivities similar to their nonfluorinated counterparts when tested in a voltage-clamped hemispherical lipid bilayer (HLB) apparatus and in nonlinear optical imaging. (Supported by NIH grants EB001963 and U54RR022232)

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Fluorogen Activating Peptide Based Energy Transfer Donors for FRET in Living Cells

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Ensemble FRET based biosensors have been demonstrated by a number of groups that allow for direct interrogation of signaling pathways in living cells, with a range of activities including protein modification, protein conformational changes, and protein interactions. The response strength of these probes is limited by FRET efficiency and ensemble averaging, as the responses typically come from a heterogeneous population of responders in the dynamic cellular situation. We present a novel approach to FRET in living cells that allows single molecule FRET determinations based on stochastic activation of an expressible donor moiety. This approach allows low-density single molecule FRET measurements to be determined in living cells, and correlated to cellular location.

We have isolated Fluorogen Activating Peptides (FAPs) based on single chain antibody fragments that noncovalently bind to dyes and enhance their fluorescence by factors of 2000-20,000. In the apo-form, the FAP has no chromophore or detectable fluorescence, while diffusion controlled binding of dye results in ''switching'' of the module with a very high contrast ratio. Use of FAP-based FRET donors, in low concentrations of fluorogen with respect to the FAP-Fluorogen Kd results in a low occupancy of FRET donors when paired with other expressible FRET acceptors, such as RFP or ReAsH domains. This stochastic occupancy allows single molecule determinations of FRET efficiency, at a rate equal to the object localization rate, governed by binding of the fluorogen to the FAP (see posters by Qi Yan et al. and Keith Lidke et al.), and breaking from the ensemble averaged FRET measurements. We present data and formalisms for analysis of the FRET efficiency of incompletely occupied ensembles of molecules, and feasibility demonstrations for single-molecule FRET determination using sparse single molecule measurements on living cells.

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Visualizing Receptor Internalization using a New Fluorescence Labeling Method

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The specific labeling of proteins in living cells using a genetically encodable tag and a small synthetic probe targeted to the tag has several advantages over widely used larger fluorescent proteins. We recently developed a quick labeling method using a high-affinity heterodimeric coiled-coil formation between the E3 tag (EIAALEK)3 attached to the target protein and the Kn probes (KIAALKE)n (n = 3 or 4) labeled with a fluorophore [1]. The size of the heterodimer (5-6 kDa) is significantly smaller than that of fluorescent proteins (~27 kDa), minimizing perturbation to receptor function. The labeling is cell-surface specific therefore suitable to detect receptor internalization. The agonist-induced internalization of β2-adrenergic receptor (β2AR) and EGF receptor transiently expressed in Chinese hamster ovary (CHO) cells can be clearly visualized using the K4 probe. Taking the advantage of reversible labeling of the K3 probe, cell-surface and internalized β2ARs can be labeled with different fluorophores. In this study, fluorescence ratiometric detection of receptor internalization for a high throughput screening was examined using β2AR stably expressed in CHO cells. The receptors were doubly labeled with pH-sensitive fluorescein (FL) (pKa~6.5) and pH-insensitive tetramethylrhodamine (TMR). FL fluorescence was attenuated following internalization after agonist stimulation (isoproterenol, 30 min) because of a lower pH in endosomes (pH 5-6), whereas TMR fluorescence was unchanged. An increase in the intensity ratio TMR/FL from 1 to ~3 was observed in endosomes. Thus, the fluorescence ratio-imaging method was found to be useful for evaluation of receptor internalization.

[1] Yano, Y., Yano, A., Oishi, S., Sugimoto, Y., Tsujimoto, G., Fujii, N., Matsuzaki, K. (2008). ACS. Chem. Biol. 3, 341-345.