1073-Plat
Dynamics of Conformational Transitions in DsRed as Detected by Polarization-Modulated MFICS
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Non-invasive spectroscopic approaches that are sensitive to fluctuations on the single molecule scale are desirable as methods to characterize the behaviour of proteins in living cells. Here we present molecular Fourier imaging correlation spectroscopy (MFICS) as a novel, high signal-to-noise approach to detect the time-varying translational movement and conformational transitions of the fluorescent protein DsRed. Thermally induced conformational transitions of the DsRed tetramer lead to changes in the optical dipolar coupling between adjacent chromophore sites, and our implementation of polarization- and intensity-modulated photo-excitation generates a phase sensitive signal that is separable into a component that comes from internal, thermally induced conformational transitions and a component which stems from translational diffusion. Based upon joint probability distributions and two-dimensional spectral densities of these measurements in conjunction with existing structural information, we interpret our measurement in terms of transitions between distinct energy-transfer-coupled conformations of DsRed.

1074-Plat
Nucleotide Movement within SERCA Detected by FRET Resolved Simultaneously on the Nanosecond and Millisecond Time Scales
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We are using time-resolved fluorescence resonance energy transfer (TR-FRET) to investigate nucleotide binding of the Ca-ATPase (SERCA). Based on biochemistry and crystallography, it has been proposed that SERCA has two distinct modes of nucleotide binding. To extend this observation from the crystal to the functional sarcoplasmic reticulum membrane, we have performed TR-FRET to measure the distance between C674 (labeled with the dye IAEDANS) and the fluorescent nucleotide TNP-ADP, in the presence and absence of inhibitors. In TR-FRET, a pulsed laser excites the AEDANS donor, and the emission is detected with sub-nanosecond time resolution, yielding a detailed description of the distance distribution between donor and acceptor. To determine whether these modes of nucleotide binding occur in solution during the Ca-ATPase reaction mechanism, we performed transient TR-FRET ([TR]^{2}FRET) experiments, in which a complete subnanosecond TR-FRET decay (from 0 to 100 ns after the pulse) was recorded every 0.1 ms after rapid mixing of AEDANS-SERCA and TNP-ADP in a stopped-flow instrument. We observed clearly a biphasic reaction with a fast component (rate constant proportional to nucleotide concentration, presumably representing the nucleotide binding reaction) and a slower component ($14 s^{-1}$) representing a conformational change within SERCA, apparently corresponding to the predicted nucleotide rearrangement. TR-FRET is a powerful technique for connecting structural dynamics of SERCA with its static crystal structures.

1075-Plat
Modular Scanning FCS quantifies ligand-receptor interactions in live multicellular organisms
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Analysis of receptor-ligand interactions in situ belongs to the most relevant tasks in life sciences, but still poses considerable challenges to quantitative microscopy. Here we show that by extending the concept of fluorescence correlation spectroscopy to a modular beam-scanning scheme featuring steady volume, two-focus and dual-color scanning in one setup, this task can be solved even for complex biological environments.
We were able to quantify the mobility of fibroblast growth factor receptors in cell membranes of living zebrafish embryos and show that Fgfr1 and Fgfr4 have similar binding affinities to the ligand Fgf8. This finding is in contrast to previous in vitro data, underlying the importance of in vivo data acquisition.
Modular scanning FCS can be easily implemented with modern laser scanning microscopes and permits quantitative measurements of dynamic parameters in the membranes of living multicellular organisms, systems previously inaccessible by FCS.

1076-Plat
Multicolor Fluctuation Spectroscopy in Cells: Obtaining the Stoichiometry of Molecular Complexes
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We have developed a method that is capable of measuring the stoichiometry of these molecular complexes and mapping dynamic processes in living cells such as molecular interactions and binding to specific locations. The method is based on measuring simultaneously fluctuations of the fluorescence intensity at two image channels, each detecting a different kind of protein. The statistical analysis of correlated fluctuations is performed by measuring the duration of correlated fluctuations and the covariance of the amplitude of the fluctuations giving the relative intensity of the complex in two separate channels. We apply the cross-variance method to determine the stoichiometry of complexes containing paxillin and vinculin or FAK (focal adhesion kinase) in disassembling adhesions in mouse embryo fibroblasts expressing FAK, vinculin and paxillin tagged with EGFP and mCherry. One important finding of our studies is that we were unable to detect complexes of these proteins in the cytoplasm far from the adhesions. At the disassembling edge, large molecular aggregates leave the bright adhesions. This sudden and correlated change of intensity shows cross-correlation between FAK and paxillin and vinculin and paxillin. From the amplitude of the correlated fluctuations we determine the composition of the aggregates leaving the adhesions. These aggregates rapidly break down in the cytoplasm since large complexes are found only in very close proximity to the adhesions or at their borders. This methodology is applicable to the study of all kinds of molecular interactions in which two (or more) of the partners can be labeled with probes of different colors. Work supported in part by U54 GM064346 Cell Migration Consortium (MD,RH,PW and EG), NIH-P41 P41-RRO3155 (EG) and P50-GM076516 (EG).

1077-Plat
Molecular Crowding Effects On Multiscale Diffusion As A Function Of The Hydrodynamic Volume Of Both The Solute And Solvent Molecules
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Molecular crowding in living cells influences passive intracellular transport, which regulates a number of cellular processes such as signal transduction, macromolecular assembly, and reaction kinetics. Recent studies indicate that crowding molecules influence both the effective viscosity and free-volume exclusion available for the diffusing solutes. In this contribution, we investigate the size effect of both the crowding as well as solute molecules on multiscale diffusion. Ficoll-70 and -400 are used as crowding agents as compared with a buffer with different glycerol content as a continuum. The rotational (ps - ns) and translational (μs-s) diffusion kinetics of rhodamine green, EGFP, and DsRed, in different crowding environments, are characterized using time-resolved fluorescence anisotropy and fluorescence correlation spectroscopy. Our single-molecule and ensemble studies suggest that molecular crowding effects on diffusion depend on the size of crowding agent, the hydrodynamic volume of the solute, and the temporal resolution of the technique used.

1078-Plat
Diffusional Characteristics as a Readout for the Folding Status of Transmembrane Proteins
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A multitude of transmembrane and secretory proteins enter the endoplasmic reticulum (ER) as unfolded polypeptide chains. In the ER, chaperones supervise the folding process and prevent unfolded proteins from entering anterograde transport intermediates, hence ensuring that only properly folded cargo is transported along the secretory pathway. It has been shown, however, that also unfolded proteins are very mobile in the ER thus questioning the model of a chaperone-mediated retention mechanism. To revisit this problem, we have used fluorescence correlation spectroscopy (FCS) on the temperature-sensitive tsO-45-G mutant of the vesicular stomatitis virus glycoprotein. Probing the short-range diffusional characteristics via FCS, we observed a strongly anomalous diffusion of tsO-45-G the in the ER in the unfolded state (at 39.5°C). The subdiffusion subsided when shifting to the permissive temperature (32°C) at which the protein is folded. Blocking the interaction of chaperones with the unfolded protein lead to a diffusion behavior similar to the folded protein. Based on our experimental results, we propose a model for the complex interplay between the chaperone machinery and unfolded membrane proteins.