

Single-Molecule Spectroscopy

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Membrane Deformation by HER2 Overexpression Disrupts Epithelial Integrity

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HER2 overexpression correlates with an increased metastatic potential in breast cancer (BC). While targeted therapies against HER2 effectively delay disease progression in this BC subtype, details of how HER2 overexpression drives these tumors to malignancy are still unclear. To gain a molecular understanding of this process on live cells, we employed quantum dot (QD) based single molecule imaging and analysis methods to monitor spatial arrangements of individual HER2s. Interestingly, overexpressed HER2s were not uniformly distributed on the cell membranes, but observed in clustered and elongated patterns. We found that these patterns resulted from deformed membrane morphologies, which appeared as irregularly shaped 'finger-like' structures (FLS) in electron micrograph images of HER2+ BC cells grown in vitro and taken from patients. Quantitative cluster analyses on cells that overexpress signaling-incompetent HER2 mutants showed that this membrane deformation was induced by high HER2 expression rather than by its signaling activities. The membrane deformation reduced cell adhesiveness by disrupting cell-substrate and cell-cell contacts, and perturbed 3D cell organization. These observations suggest that physical alteration of cell membranes by HER2 overexpression can increase the potential for cell dissemination in a non-canonical, signaling-independent manner, which may be involved in invasive progression of HER2+ BC.

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Blinking of Quantum Dot Probes in Single Membrane Molecule Rotation Measurements

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Asymmetric quantum dots (QD) provide non-bleaching probes of the rotation of individual cell surface proteins. We have conducted imaging measurements of individual 2H3 cell Type I Fcε receptor rotation on timescales down to 10 ms per frame. We have also used time-tagged single photon counting measurements of individual QD to examine μs timescales, though such rapid timescales are limited by QD emission rates. In both approaches we calculate the time-autocorrelation functions (TACF) for fluorescence polarization fluctuations. Decays of these fluctuations extend into the ms timescale, as implied by time-resolved phosphorescence anisotropy results. Effects of cell treatments such as receptor crosslinking suggest that this slow decay may be a property of the membrane itself, perhaps reflecting large-scale fluctuations of mesoscale membrane regions. However, depending on instrumental parameters used in data analysis, polarization fluctuation TACFs can contain a contribution from the intensity fluctuation TACF arising from QD blinking. Such QD blinking feed-through is extremely sensitive to these analysis parameters which effectively change slightly from one measurement to another. We discuss approaches based on the necessary statistical independence of polarization and intensity fluctuations to guarantee removal of a blinking-based component from rotation measurements. Supported by NSF grant MCB-1024668.

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Single-Molecule Fluorescence Microscopy and Tracking of Lipids in Mitochondrial-Like Supported Lipid Bilayers

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The mitochondrial membrane has a lipid and protein composition that results in unique physicochemical properties. These properties are important for many processes taking place at the mitochondrial membranes, such as the process of mitochondrial outer membrane permeabilization during apoptosis. In this study, we investigated the mobility of fluorescently-labeled lipids in supported lipid bilayers with mitochondrial-like lipid composition. We used and compared two different fluorescence methods: an ensemble method (image correlation spectroscopy) and a single particle method (single molecule tracking). Ensemble methods measure observables of the entire system. We use image correlation spectroscopy (ICS) on confocal microscopy images, in which the

autocorrelation function for scanned lines is calculated to obtain information on particle densities and dynamics of the entire system. Single-molecule fluorescence microscopy methods image proteins, enzymes or other molecules of interest as diffraction-limited spots, which can be individually located and tracked using suitable algorithms. Instead of averaging over the entire system, tracks are analyzed for each particle. We investigate the dynamics of supported lipid bilayers with a composition that aims to mimic that found in mitochondrial membranes. The bilayers were labeled with the lipophilic dye DiD and formed via vesicle fusion on a mica solid support. Diffusion coefficients are obtained by line correlation analysis on the confocal images and mean square displacement analysis as well as displacement distribution analysis at constant time steps on Total Internal Reflection Fluorescence Microscopy data. Both methods show the presence of mobile as well as immobile particles. The average diffusion coefficients of the mobile population obtained with either acquisition method are in agreement. This provides the foundation for further work concerning the interaction of membrane proteins with the lipid bilayer.

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The Role of Amino Acid Residues Located at the Catalytic Site in the Rotation of *Enterococcus hirae* V₁-ATPase

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V-ATPases are rotary molecular motors that generally function as proton pumps. We have recently solved the crystal structures of the V₁ moiety of *Enterococcus hirae* V-ATPase (EhV₁), providing the first high-resolution molecular structure of the V₁ moiety. Additionally, we have also verified the rotary catalysis of EhV₁ by using single-molecule high-speed imaging and have analyzed the properties of the rotary motion in detail. Here, to understand the role of amino acid residues located at the catalytic site on the ATP hydrolysis of EhV₁, we analyzed the rotation of the mutants of EhV₁. We observed the rotation of the mutants, A(F506E) (an amino acid residue which interacts with adenine ring of ATP) and B(R350K) (the arginine finger which interacts with β- and γ-phosphates of ATP) by using a 40 or 50 nm Au colloid as a low-load probe. These mutants rotate unidirectionally and the rotation rates obeyed the Michaelis-Menten kinetics. The maximal rotation rates for A(F506E) and B(R350K) were 86 rps and 0.3 rps respectively, and were 0.8 and 0.003 times that of the wild-type (107 rps). The second-order binding rate constant for ATP for A(F506E) and B(R350K) were $5.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ and $2.4 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ respectively, and were 0.02 times and equivalent to that of the wild-type ($2.3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$). These results strongly suggest that specific interaction of the catalytic site with the adenine ring of ATP is important to accelerate ATP binding, while interaction of arginine-finger with the β- and γ-phosphates of ATP accelerates the ATP hydrolysis.

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Investigating the Kinetics of HSF Binding Using High Throughput Single Molecule Imaging

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Transcriptional regulation in the heat shock response is vital for the proper function and survival of both prokaryotic and eukaryotic cells. The bulk of our current understanding of transcription has been obtained through biochemical assays such as pull-down, knockdown and genome wide assays. These have provided information on the identity of proteins involved in the regulation of various genes and the location at which they bind to DNA. However, the kinetics and architecture are not known at a single molecule level, in part due to the difficulty of obtaining enough data to provide statistics. A flow cell designed to extend DNA molecules under a hydrodynamic force, nicknamed DNA curtains, provides a high throughput method of imaging protein-DNA interactions at the single molecule level. Electron beam lithography is the