Single-Molecule Spectroscopy

**810-Pos Board B590**

**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 Fce 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 single 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 fluorescence anisotropy measurements. 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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**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 investigate 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 diffusion-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.