separate different species, all parameters necessary for performing an accurate spFRET experiment can be obtained in a single measurement.

1894-Plat
Single-Molecule Counting with Palm
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Photoactivated Localization Microscopy (PALM) is a single-molecule based imaging technique. By repeated activation and sampling of sparse subsets, densely expressed fluorescent proteins can be resolved in time. Here, we describe how to exploit this property for single-molecule counting in cells. A strategy is developed for differentially activating and optimized imaging of PA-GFP and PA-Cherry. Coupled PA-FPs are used as calibration probes for standardization of single-molecule counting and determination of photoactivation efficiency. With these tools, we demonstrate how single-molecule counting enables to assess stoichiometries and to relate such counts to absolute molecule numbers in a biological specimen.

1895-Plat
Microsecond Single Molecule Tracking: Probing Protein Diffusion at High Spatial and Temporal Resolution
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In recent years advancement in light microscopy have made possible to break the resolution limit and to achieve super- resolution positional accuracy. However, these techniques are limited in their temporal resolution to tens of millisecond, and thus cannot access the faster dynamics relevant for most biological processes. Here we present a versatile technique which allows to track a molecule with nanometer spatial and microsecond temporal accuracy. The method is based on partially overlapping excitation of two spectrally separated fluorophores. A wedge is introduced in the setup so the fluorescent signals from the two emitting species are recorded independently onto different area of the CCD. In this way we record the position of a single molecule with nanometer accuracy at two different points in time. A cross-correlation algorithm is subsequently applied and information on the mean square displacement of the molecule is retrieved. The temporal accuracy is ultimately limited by the diffusion coefficient of the molecule under study, and the accuracy by which the position can be determined. This method was proved using short DNA oligonucleotides and here applied to live cells. We followed the diffusion of GPI anchor protein dually labeled with a refolded split GPF and an Alexa 647 dye attached to the complementary peptide. This approach guarantees the 1:1 stochiometry needed and we were able to track GPI down to microseconds timeresolution, gathering information on the diffusion properties of the anchor in the membrane.
Our results did not exhibit hop-diffusion behavior at short time-scale as previously predicted.

1896-Plat
Nano-Scale Spatial Organization of Plasma Membrane Revealed by Pair-Correlation Analysis
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TIRF-based photoactivated localization microscopy (PALM) offers a powerful tool to interrogate the spatial distribution of proteins in the plasma membrane (PM) at scales inaccessible by conventional light microscopy. We employed PALM to map out the nanoscopic organization of PM and construct a mechanistic perspective of PM organization. Cluster analysis of PALM data is complicated by the multiple appearances of a single protein with associated stochastic uncertainty in position, and reversible blinking of individual fluorophores. We developed a statistical algorithm to rigorously analyze PALM-data by performing spatial pair auto- and cross-correlation on the entire ensemble of detected molecular peaks, and subsequently separating out the contribution resulting from multiple appearances of the same molecule from that arising due to the actual lateral organization of the protein molecules. Using this approach, we could describe protein organization in an accurate and quantitative way, and extract reliable physical parameters like cluster-radius and number of molecules in a cluster. We examined the distribution of a diverse set of PM-proteins carefully chosen based on their different membrane-anchors and differential partitioning in phase-separated PM vesicles. Correlation analysis of PALM data revealed distinct steady state organization of these proteins: Transferrin Receptor-PAGFP and VSVG-PAGFP were organized into discrete clusters of ~150nm and 70nm radius, respectively. PAGFP-GL-GPI, Lyn-PAGFP and Lat-PAGFP, in contrast, exhibited random distribution. Interestingly, cross-correlation of 2-color PALM

images showed dramatic reorganization of actin into ring-like structures around antibody-crosslinked PAGFP-GPI clusters (~100-200nm); similar actin-structures can modulate protein-platforms during cell-signaling. The combination of PALM and correlation analysis provides a robust and quantitative approach to study PM reorganization during various physiological processes.

Platform AK: Membrane Active Peptides

1897-Plat
Probing Structural Features of Alzheimer's β-Amyloid Ion Channels in Membranes Using Aβ Mutants
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A current hypothesis for the pathology of Alzheimer's disease (AD) proposes that amyloid-beta (Aβ) peptides induce uncontrolled, neurotoxic ion flux across cellular membranes. The resulting inability of neurons to regulate their intracellular concentration of ions, in particular calcium ions, has been associated with cell death and may thus contribute to cognitive impairment typical for AD. The mechanism of the ion flux is not fully understood since no experimentally based Aβ channel structures at atomic resolution are currently available, and few polymorphisms have been predicted by computational models. Structural models and experimental evidence suggest that Aβ channel is an assembly of loosely-associated mobile β-sheet subunits. Histidines were proven to be on or near the mouth of the Aβ pore, but no other amino acids have been tested. Using planar lipid bilayers, we present a study showing that amino acidic substitutions can be used to infer which residues line the pore and are water accessible. For example, the substitution of F19P is capable of undermining the amyloid structure such that bilayer membranes exposed to it do not support ion channel formations for prolonged periods of time. This and other structural information on or in membrane are needed to aid drug design aiming to control unregulated Aβ ion fluxes.

1898-Plat
Evolution of Membrane Leakage by Pre-Amyloidogenic Oligomers of Islet Amyloid Polypeptide
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Islet amyloid polypeptide (IAPP) is the primary amyloid component found in patients suffering from type II diabetes. IAPP has been implicated in the death of pancreatic beta-cells, resulting in a greater strain on the remaining insulin producing cells and contributing to further progression of the disease. While fibers are the most obvious characteristic of any amyloid disease, it has been found that smaller soluble oligomers are the most likely source of cytotoxicity. Previous research has shown that IAPP oligomers are capable of permeabilizing lipid bilayers under physiological conditions. Permeabilization of cellular membranes resulting in ionic imbalance and subsequent apoptosis is a leading theory for the cause of beta-cell death in type II diabetes. In order to better understand the mechanism by which IAPP causes cell death we have examined the evolution of membrane-bound leakage states over time. Using a combination of bulk and single molecule techniques we have uncovered a multi-phasic leakage mechanism in which we observe two clear time regimes of leakage behavior induced by soluble pre-amyloidogenic oligomers. Our results give us further insight into the mechanism by which IAPP is capable of inducing membrane leakage.

1899-Plat
In Situ Mapping of Membranolytic Peptide-Membrane Interactions by Coupled Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy-Atomic Force Microscopy (ATR-FTIR-AFM)
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The mechanisms by which membranolytic peptides may exert their action are highly complex and specific. They may involve peptide self-association, membrane-induced refolding, or targeting of specific lipid domains or components. Insights into these behaviours are critical for the *de novo* design of peptide-based antimicrobial agents. To better understand these mechanisms, we have applied coupled attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR)-atomic force microscopy (AFM) to directly