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Platform: Imaging & Optical Microscopy: New Fluorescent Methods & Probes

2127-Plat
Combined Single Molecule Fluorescence and Force Microscopy to Study Lipid Transfer from Lipoproteins to Biomembranes

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The High Density Lipoprotein (HDL) is one of the smallest and densest lipoproteins and carries besides their protein parts various lipids. Hitherto, the mechanisms how lipids flow from lipoproteins into the cellular plasma-membrane are far from being understood: it remains elusive whether the receptor directly influences lipid efflux or keeps the lipoprotein particle attached to the plasma-membrane, thereby enhancing the probability for lipid transfer.
To get a molecular understanding of these key processes, we have developed a combined and fully synchronized single molecule fluorescence and force microscope. Here we use two complimentary approaches, either by anchoring a single cell on a tipless cantilever or by utilizing the AFM tip to deliver HDL particles to the cell. With the first approach we found out that transfer of lipids indeed happens on living cells. For this, single cells were moved towards a surface covalently functionalized with HDL-particles. As soon as a cell got in contact with the surface, particles fused with the cell membrane and transferred their cargo. With the second approach, a single bioparticle could be delivered in a controlled way to membranes. Upon specific delivery of one particle attached to the AFM tip, the flux of single fluorescently labelled molecules out of the particle into a supported lipid bilayer (SLB) was measured. In particular, we compared the transfer of the fluorescently labelled lipids DiI, Bodipy-labelled cholesterol and cholesteryl-ester.

2128-Plat
Intracellular Pressure Measurement by using Pressure Sensitive Yellow Fluorescent Protein

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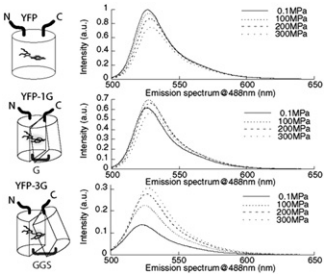
Fluorescent protein-based indicators for intracellular environment conditions such as pH and ion concentrations are commonly used to study the status and dynamics of living cells. Despite being an important factor in many biological processes, pressure, however, is an exception. Development of the pressure sensitive fluorescent protein would blaze a new path to the future biology. Recently, we enhanced the pressure sensitivity of yellow fluorescent protein (YFP) by inserting several glycines into it. Our mutation enhanced the interaction between the chromophore and the solvent that is responsible for pressure changing. In response to changes in pressure, a spectrum shift and an intensity change of the fluorescence was observed, indicating applicability of the present YFP for measuring the intracellular pressure on a live specimen. By measuring the fluorescence of the mutant YFP, we succeeded in measuring the intracellular pressure changes that follow actin filament disruption in living cells. This study shows that our mutation enhanced unknown properties of YFP that may allow us to measure the intracellular pressure.



2129-Plat
Binding-Activated Localization Microscopy of DNA Structures

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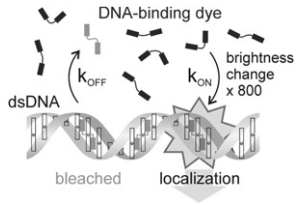
Localization microscopy relies on the separation of the emission of individual fluorophores within a diffraction limited spot. In popular techniques like PALM, STORM, or blinking the structure of interest is first labeled and lasers/chemicals are then adjusted such that only a sparse subset of all labels is fluorescent at the same time. We propose an alternative strategy, namely to use fluorophores that are ''switched on'' when bound to the target structure and to localize them while they are binding in the presence of free dye. This approach is best described by the term Binding-Activated Localization Microscopy (BALM). Here we demonstrate BALM with DNA-binding dyes that show a strong fluorescence enhancement when bound to dsDNA. Surface-immobilized DNA molecules were imaged with a resolution of ~14 nm (full-width at half-maximum), reaching a spatial sampling of nearly 1/nm. We further show measurements of the bacterial chromosome in fixed E. coli cells and of artificial DNA structures. In general, the extension of BALM to other fluorophores will complement the experimental toolbox for superresolution imaging.



Reference:
Schoen et al., Nano Letters 11: 4008-4011 (2011).

2130-Plat
A Simple, Versatile Method for GFP-Based Single Molecule Localization Microscopy

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Single-molecule localization-based superresolution microscopy methods such as PALM or STORM, have been breakthrough techniques of the last years. Until now however, they require special fluorescent proteins to be cloned or high-affinity antibodies to be generated for specific labeling. On the other hand, many laboratories will have most of their constructs in GFP form and entire genomes are available as functional GFP-fusion proteins.
Here, we report a method that makes all these constructs available for superresolution microscopy by targeting GFP with tiny, high-affinity antibodies coupled to blinking dyes. It thus combines the molecular specificity of genetic tagging with the high photon yield of organic dyes and minimal linkage error. Direct STORM on microtubules labeled with our novel antibodies showed that indeed the linkage error was minimal, whereas the large size of standard antibodies resulted in an additional error of >10 nm in immunolabeling. The high brightness of our labels enabled us to perform rapid time-lapse dSTORM and sptPALM on living neurons expressing the outer membrane protein GPI-GFP. Three-dimensional dSTORM on microtubules using the bi-plane approach allowed us to distinguish overlapping microtubules with an axial separation of ~100 nm. Using a budding yeast GFP-tag genomic library we could readily image several GFP-tagged proteins targeted to specific intracellular locations.
In summary, targeting of GFP-labeled constructs with tiny antibodies provides fast and simple access to superresolution microscopy of virtually any known protein in cells. Since for several organisms the entire genome is available as GFP-tagged constructs, all these proteins are immediately accessible without the requirement for cloning or the generation of antibodies. Finally, due to a simple one-step labeling protocol, our technique opens the door to high-throughput localization analysis of entire genomes at the nanoscopic level in cells.

2131-Plat
Biological Structure from Precise and Accurate Estimation of Fluorophore Orientations and Distances: Proof-of-Principle using Internally Labeled dsDNA

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Structure and structural transitions are at the heart of the molecular basis of biological function. In super-resolution microscopy and single-molecule biophysics, such information is probed using fluorophores. The emitted light gives rise to diffraction-limited spots whose centers are routinely localized with nanometer precision when spots are isolated. Thus, distances shorter than the diffraction limit may be assessed by filtered imaging of differently colored fluorophores. Popular approaches assume rotational freedom of the fluorophore emission dipole moment and hence fit a 2D Gaussian intensity distribution to the image of a fluorophore. This is done using least-squares method. However, when the dipole moment is resolved in time or deliberately