and promoter DNA containing a TATA-box, a mismatch bubble and a 7nt RNA transcript. Briefly, by using sm-FRET, we determine distances within double labelled FTC and the NPs computes 3-dimensional position probability densities of unknown sites on the TATA-box, TRF, TFIIIB and the non-template DNA. These results allow us to build a model of Pol II FTC, which is an important intermediate state of Pol II between initiation and elongation. Hence, our model helps understanding the transition mechanism of Pol II from its initiation to its elongation state.


2576-Pos Board B562 Detection of Mobile Single Fluorescent Particles by Confocal Microscopy: A Comparison with Fluorescence Correlation Techniques

Optidried Friaa, Meen Fi, Kostrewa, Radhika Voleti, Sanjeeva Shivakumar, Aisha Shamas-Din, Cecile Fradin.

To improve our understanding of proteins interactions with cellular membranes, we have developed a method based on confocal microscopy that enable us to correlate the number of proteins bound to a lipid vesicle with the vesicle properties (membrane integrity, membrane potential, vesicle size/s). This method consists in the simultaneous recording of confocal images in two different channels, and in the coincidental detection of single mobile particles in these channels. To demonstrate the usefulness of this method, we have used a model system consisting of small unilamellar liposomes (~ 100 nm in diameter) rendered fluorescent by the addition of a small percentage of fluorophore and interacting with fluorescently labeled proteins. To optimize image acquisition parameters for the detection of single diffusing liposomes, we systematically varied the fluorophore to vesicle ratio, the fluorescence excitation intensity, and the confocal pixel size and dwell time. We analyzed the images using different pixel binning values and different detection threshold. We then determined the range of parameters for which we were able to detect particles with independent positions. We also verified that the particle concentration and average particle fluorescence emission extracted from the images using this single particle detection method were consistent with results obtained by fluorescence correlation spectroscopy (FCS), rasterscanning image correlation spectroscopy (RICS) and fluorescence intensity distribution analysis (FIDA). Using vesicles labeled with two different fluorophores, we measured the probability that a vesicle detected in one channel would also be detected in the second channel, and compared our results with those obtained by fluorescence cross-correlation spectroscopy (XCS).

Our method should be useful for studies of protein or peptide insertion and pore formation into lipid membranes.

2577-Pos Board B563 Single-Image Molecular Analysis for Accelerated Fluorescence Imaging

Yan Mei Wang, Shengjie Zareh, Michael DeSantis, Shawn DeCenzo, Jonathan Kessler, Anthony Kovacs.

We have developed a single-molecule fluorescence imaging analysis method, SIMA (single image molecular analysis), to improve the temporal resolution of single-molecule localization and tracking studies to millisecond timescales without compromising the nanometer range spatial resolution [1]. This method, the width of the fluorescence intensity profile of a static or moving molecule, imaged using submillisecond to milliseconds exposure time, is used for localization and dynamics analysis. We apply this method to three single-molecule studies: (1) subdiffraction molecular separation measurements, (2) axial localization precision measurements, and (3) protein diffusion coefficient measurements in free solution. Applications of SIMA in flagella IFT particle analysis, localizations of U1BP in live cells, and diffusion coefficient measurements of LacI in E. coli will be discussed.


2578-Pos Board B564 Quantitative Analysis of the Lateral Organization of Plasma Membrane Proteins using Photoactivated Localization Microscopy (PALM)

Tijana Jovanovic-Talisman, Prabuddha Sengupta, Dunja Skoko, Jennifer Lippincott-Schwartz.

The super-resolution method of photoactivated localization microscopy (PALM) can be used to analyze the distribution and dynamics of single molecules within bigger structures, making it an ideal tool for mechanistic investigation of biological processes. This technique is particularly useful for the investigation of protein organization on the cell surface due to spatial and temporal resolution advantages over conventional fluorescence microscopy. However, because of photophysical properties of fluorescent molecules and the uncertainty of their localization, quantitative determination of oligomeric structures is challenging. To address this, we developed a new method to analyze the distribution of single molecules obtained with PALM by separating contributions from stochastic clustering (corresponding to multiple appearances of a single protein) and protein clustering (corresponding to homo- and hetero-oligomers) using autocorrelation analysis. We probed six different plasma membrane proteins that have distinct anchoring mechanisms and lipid affinities. We showed that our approach provides insight into the organization of proteins with ~25 nm resolution and identifies conditions that significantly change their distribution. In particular, we found that glycosylphosphatidylinositol anchored protein (GPI) did not form significant clusters in the steady state. However, we observed large clusters upon antibody crosslinking of GPI with concomitant rearrangement of the actin cytoskeleton. Formation of actin rings around large protein platforms could have important implications in cell signaling. Vesicular stomatitis virus glycoprotein and transferrin receptor showed moderate and significant clustering, respectively, whereas LAT, Lyn, and ABCB11 did not exhibit appreciable clusters. Our analysis method, which provides insight into membrane organization on a single molecule level, is an excellent tool for quantitatively probing numerous biological mechanisms because the segregation of plasma membrane molecules is thought to be critical for various physiological processes.

2579-Pos Board B565 Superresolution Localization Microscopy Using Fluorescent Activating Proteins

Qi Yan, Samantha L. Schwartz, Suvarjit Maji, Diane S. Lidke, Keith A. Lidke, Marcel P. Bruchez.

Fluorogen Activating Proteins (FAPs) are single chain antibodies that were selected to specifically activate the fluorogenic dyes thiazole orange (TO) and malachite green (MG) with nanomolar affinities. The fluorogens are not fluorescent except when bound to the cognate FAPs, resulting in thousands of fold increase in fluorescence intensity. The fact that FAPs are small in size (12 to 25 kDa) and genetically expressible makes them a useful tool for live cell fluorescence imaging. We have shown the feasibility using MG-FAPs in Stimulated Emission Depletion microscopy to achieve a 3-fold resolution improvement compared to a confocal image. Here we demonstrate the utility of MG-FAPs for superresolution imaging using a non-photoactivated single molecule localization approach. At equilibrium the labeling density of FAP sites is controlled by the concentration of fluorogenic dye. By using dye concentrations lower than the Kd of the fluorogen and FAP, we obtained sparse labeling where individual FAPs could be resolved with a localization accuracy of < 10 nm on both live and fixed/permeabilized cells.

2580-Pos Board B566 Single Molecule Immunoprecipitation

Ankur Jain, Ruijie Liu, Biswa Ramani, Yang Xiang, Taekjip Ha.

Protein-protein interactions form the cornerstone for most biological pathways. Governed by numerous factors, same protein can associate with a host of different proteins and exhibit diverse functionality in a specific physiological setting. This heterogeneity in protein interactions is difficult to probe by using bulk assays like Western blots. We present a single molecule assay for direct visualization of in vivo biomolecular complexes. Protein complexes are immunoprecipitated directly from cell and tissue extracts. The proposed interacting partners are labeled with fluorophores. Using multicolor fluorescence colocalization with single fluorophore sensitivity, we are able to determine the complex composition under physiological stimulations. Discrete photo-blinking events provide information on the complex stoichiometry. The method is amenable for pull down of endogenous proteins. Pulled down complexes can be used for single molecule functional analysis. Single molecule immunoprecipitation provides a rapid, sensitive and robust platform for analyzing multimeric protein assemblies in different biological pathways.

2581-Pos Board B567 Generating Global Protein Dynamics with Structure-From-FRET

Andraj Savol, Arvind Ramani, Chang Chen, Xiaofang Li, Keith A. Lidke, Marcel P. Bruchez.

The remarkable specificity and diversity of function of protein function are consequences of native conformational fluctuations, and more specifically transitions between conformational substates. Experimentally, these dynamics are increasingly resolvable (with high temporal detail) using single molecule Forster Resonance Energy Transfer (smFRET) methods, which can capture rare structural extensions not discernable with bulk methods. Still, even with multiple fluorophore pairs, dye-separation trajectories lack the atomic detail of crystallographic or NMR-based methods, and, at present, merging smFRET data with high resolution structural data remains problematic. Moreover, many functionally relevant transitions proceed within timescales far beyond those accessible to current molecular dynamics approaches. We address this common tradeoff between detail and sampling by posing the following inference problem: given a single dynamic distance constraint, what is the most likely protein structure?