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Neuronal mRNA trajectories exhibit a diverse range of behaviors along tracks including cytosolic diffusion and retrograde/anterograde transport. From our analysis, we obtain detailed distributions of transport rates and diffusivities along individual mRNA trajectories, as well as the lifetimes of each state of motion. We find that the rate of anterograde transport is greater than that of retrograde transport, indicating a possible role of forwards-backwards transport of the mRNA particles in distributing these molecules throughout the cell. To illustrate the broad applicability of our approach, we also use it to classify the heterogeneous oscillatory motion of sister kinetochores during HeLa cell division. While our results are consistent with sliding window averages commonly employed to analyze kinetochore dynamics, the Bayesian HMM infers local switching and pausing dynamics that are uniquely resolved by single-particle-based analysis.

825-Pos Board B605

Single Molecule Studies of Tau Protein in the Abel Trap

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Tau protein is an intrinsically disordered protein that stabilizes microtubules of neurons mainly in the central nervous system. Abnormalities in Tau are thought to be involved in Alzheimer's disease. The aggregation process of monomeric Tau into an oligomeric species and the intermediate conformational states that may facilitate aggregation are of particular interest. To investigate the disordered nature and aggregation of Tau protein, single, solution-phase Tau proteins and oligomers were isolated in a microfluidic trap, called the Anti-Brownian Electrokinetic (ABEL) Trap. The ABEL trap cancels Brownian motion of a molecule of interest via electrokinetic and/or electroosmotic flow, allowing prolonged examination of a single, fluorescently-labeled analyte in solution. Combining this technique with fluorescence anisotropy enabled studies into how monomer structure and aggregation changes with cellular conditions known to accelerate aggregation, including concentration effects, solution viscosity, heparin, and cellular crowding. Characteristics of disordered Tau were compared with those of a globular protein of similar size, microbial transglutaminase (MTG). The ultimate goal is to provide the missing link between non-aggregated, monomer Tau protein and the toxic species that result in devastating disease, offering insight into how this pathway may be upset.

826-Pos Board B606

Epidermal Growth Factor Receptor (EGFR) Membrane Organization and Dynamics Investigated by Sw-Fccs and Imaging Fcs

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The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase involved in various cellular processes including cell differentiation, proliferation, and migration. Despite many years of intensive research its mode of activation is still not fully understood, partly because its dimerization and its membrane localization are still under discussion. Here we use Single Wavelength excitation Fluorescence Cross-Correlation Spectroscopy (SW-FCCS) and Imaging Fluorescence Correlation Spectroscopy (Imaging FCS), to investigate receptor dimerization and receptor localization in membranes.
The fraction of EGFR preformed dimers, i.e. dimerization in the absence of ligands, has been determined under various conditions with very different results, ranging from exclusive monomeric states to distributions between monomers dimers and possibly higher oligomers. The reason for these discrepancies is unclear. We therefore measure the dimerization on different cell lines (CHO-K1, Cos7, HEK293) at different membrane locations (basal and apical membrane) and at room and physiological temperature to determine whether dimerization is sensitive to these different conditions. We observe a cell line specific sensitivity of dimerization to temperature and membrane localization that can change the detected dimer fraction by more than a factor of 2, hinting at an explanation why different experiments provide different answers to the question of preformed dimers. Furthermore, we use Imaging FCS to investigate the localization of the EGFR within the membrane and its interaction with the cytoskeleton. Our study shows that the EGFR is interacting with cholesterol dependent and independent domains and its motion is influenced by the cytoskeleton. Overall these measurements provide a complex picture of EGFR organization and dynamics, which might play essential roles in receptor function and signaling.

827-Pos Board B607

Rapid Measurement of Molecular Transport and Interaction Inside Living Cells with Single Plane Illumination Microscopy

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Fundamental physiological processes within cells and tissues including cell adhesion, signaling, movement, division or metabolism are based on the transport and interaction of biomolecules. Biomolecular dynamics is usually measured by applying single particle tracking (SPT) analysis to camera images or by using fluorescence fluctuation spectroscopy (FFS) methods based on single point detection. We show that image mean square displacement (*i*MSD) analysis, applied to single plane illumination microscopy (SPIM) data, is a faster and more efficient way of unravelling rapid, three-dimensional molecular transport and interaction within solutions and in living cells. From a stack of camera images recorded in a few seconds only, the type of dynamics such as free diffusion, flow or binding can be identified and quantified without suffering from the limitation of current camera frame rates. Also, the SPIM-*i*MSD method does not require calibration of the microscope point spread function (PSF) and light exposure levels are very low. We quantified the dynamics of several different proteins in the cyto- and nucleoplasm of living cells to demonstrate the advantages of our approach. As an example, from a single measurement, we were able to determine the diffusion coefficient of free clathrin molecules as well as the transport velocity of clathrin-coated vesicles involved in endocytosis. Used in conjunction with dual view detection, we further show how protein-protein interactions can be quantified.
Work supported in part by NIH grants P50 GM076516 and P41 GM103540.

828-Pos Board B608

Characterization of Fluorescent 3DNA Dendrimers with Fcs and Single Molecule Imaging

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3DNA dendrimers are uniform three-dimensional DNA structures commercially available from Genisphere, Hatfield, PA. The base unit is made by two strands of DNA that are complementary only in the central region, while the four end regions can be annealed with other base units of different sequences. Thus, dendrimers can grow in layers with finite control. At two layers, the dendrimer has 36 free arms which are available for attaching multiple labels of interest. In our study, eight arm extensions were incorporated into the dendrimer. Each of these arms is capable of annealing to three independent short oligomers labeled with a fluorophore. Using fluorescence correlation spectroscopy, we monitor changes of the dendrimer brightness and calculate the number of the labeled oligonucleotides incorporated into the dendrimers in a titration experiment. We confirmed that the incorporation of the labeled oligomers into the dendrimer is close to the expected value and the fluorescence of the labeled nucleotides is not quenched. Single molecule images of the dendrimers show high homogeneity and multiple bleaching steps. Therefore, 3DNA dendrimers can serve as good scaffolds for advanced fluorescence imaging.

829-Pos Board B609

Concentration Estimates from Counting Individual Molecules

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The transmission of information in cells usually involves changes in concentration that need to be read by different cell components. Very often small molecule numbers are involved so that fluctuations play a relevant role. In this work we study molecule number fluctuations when molecules diffuse and react. Building upon our previous works on the analysis of Fluorescence Correlation Spectroscopy experiments we derive a formula for the observation time that is needed to estimate concentrations with a given precision out of counting individual molecules. In this way we determine that fluctuations in the number of bound molecules can be averaged out on a relatively fast timescale due to correlations and that they are described by either one or two characteristic timescales depending on the concentration of free molecules. We show that how our results can be used to understand the transformation from a highly fluctuating instantaneous transcriptional activity into the expected output concentration relatively fast and to analyze the dynamics of enzymes at the single molecule level.