this study are generally applicable for studying diffusion and exchange of membrane associated fluorophores using FRAP on widely available commercial confocal laser scanning microscopes.

166-Pos Board B45

Dynamic Spatial Distribution of RNA Polymerase in Live E. coli

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Escherichia coli is one of the few model systems that has been extensively studied in biology. Because of their small size, bacterial cells have always been difficult to study with light microscopy. Electron microscopy can give spectacular images of the static structure of fixed bacterial cells, but fluorescence microscopy provides quantitative information about subcellular structure and dynamics in living cells.

One of these quantitative methods is fluorescence recovery after photobleaching (FRAP). FRAP can be used to study dynamic redistribution of fluorescent tracer particles in cells. Of interest to our lab is *E. coli* RNA polymerase, the enzyme responsible for transcribing DNA into RNA. FRAP provides information on the diffusion of polymerase on the ~1 μm length scale. We have measured the fluorescence distribution recovery in live *E. coli* held at 30°C in flowing aerated growth media. Preliminary results indicate that there is a population of fluorescently tagged RNAP that recovers on the time scale of seconds. This leads to a rough estimate of $D_{apparent}$ ~0.2 μm^2-s^{-1}. As a comparison, the mean *in vitro* 1D sliding diffusion constant of T7 RNAP as reported by Kim and Larson in 2007 was 0.12 μm^2-s^{-1}. Somewhat surprisingly, it appears as if RNAP can diffuse as quickly inside the cell with its hop and slide method as it can outside the cell with 1D sliding. Actively transcribing polymerases should appear stationary over this time scale. This allows us to estimate the fraction of actively transcribing RNA polymerase, or otherwise immobile, to be about 0.6.

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Morphogen Gradient Formation Unraveled Using In Vivo Three-dimensional Single Molecule Microscopy

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Positional information is essential for the cell's fate in tissue. In the wing imaginal disk of Drosophila melanogaster positional information is provided by a concentration gradient of the morphogens Decapentaplegic (Dpp) and Wingless. We use a 3D-epifluorescence setup to unravel the spatio-temporal distribution of YFP-labeled Dpp after secretion by specialized producing cells. With our approach we are able to characterize the Dpp distribution in the wing disk in all three dimensions in vivo. Most Dpp is located apically in a layer of ~5 μm. To elucidate how the gradient is maintained individual endosomes containing Dpp are followed. We found that endosomes contain up to 100 Dpp molecules allowing us to follow endosomes for hundreds of frames with high spatio-temporal accuracy in three dimensions.

The Dpp concentration in each endosome was directly determined from the fluorescence intensity. We find a constant Dpp fraction of 60% in endosomes, agreeing with the fixed fraction found in FRAP experiments. Sudden changes in Dpp content of up to 15 Dpp molecules are observed, indicating that Dpp is endocytosed in clusters into vesicles before vesicle fusion with endosomes occurs. Surprisingly multiple preferred Dpp cluster sizes are found. Measuring Dpp in- and outflow results in rates on the order of minutes. Labeling different types of endosomes allows us to calculate Dpp degradation and recycling rates. Endosome mobility plays an important role in maintaining the Dpp gradient. We find that Dpp-containing endosomes close to the Dpp source are transported during 15% of the time. Further away this percentage drops, indicating that transport via endosomes is playing a less important role in maintaining the morphogen gradient. Our study leads to a mechanistic model for gradient formation on the level of the mobility of individual Dpp endosomes and molecules.

168-Pos Board B47

$G\alpha_q$ Binds Two Effectors Independently in Cells: Evidence for Predetermined Pathways

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G proteins transduce signals along diverse pathways, but the factors involved in pathway selection are largely unknown. Here, we have studied the ability of $G\alpha_q$ to select between two effectors, phospholipase Cβ (PLCβ) and phosphoinositide-3-kinase (PI3K). Specifically, we expressed eCFP and eYFP tagged proteins in HEK293 cells and monitor their interactions throughout stimulation using Forster resonance energy transfer (FRET). We find separate and stable pools of $G\alpha_q$-PLCβ and $G\alpha_q$-PI3K complexes existing in both the baal and stimulated states. These separate complexes exist despite the ability of $G\alpha_q$ to simultaneously bind both effectors as determined by *in vitro* measurements using purified proteins. Pre-formed G protein-effector complexes will limit the number of pathways a given signal will take and may simplify predictive models.

169-Pos Board B48

Trafficking Of Glutamatergic And Peptidergic Vesicles In Astrocytes

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In neurodegenerative disorders and in trauma of the central nervous system (CNS) excitotoxic stress is developed due to highly increased extracellular concentrations of neurotransmitters. Astrocytes are, in addition to neurons, sensitive to excitotoxic stress, leading to an increase in the intracellular free calcium concentration ([Ca2+]i). This elicits a discharge of several gliotransmitters from membrane-bound vesicles and probably also affects the pattern of vesicle trafficking in astrocytes. Several aspects of the trafficking of membrane-bound vesicles in astrocytes have been studied, but their recycling is poorly defined. We labeled recycling vesicles containing either the vesicular glutamate transporter 1 (VGLUT1) either vesicles containing atrial natriuretic peptide (ANP). We examined their number, fluorescence intensity and mobility by confocal microscopy. A rise in [Ca2+]i elicited an increase in the number and fluorescence intensity of the puncta. In contrast to non-stimulated cells, where VGLUT1 vesicles cycle slowly between the plasma membrane and the cytoplasm, in stimulated cells many vesicles exhibited higher, directional mobility. The opposite effect of stimulation was measured for ANP-vesicles. In CNS pathologies astrocytes change the expression of many genes, including genes encoding intermediate filament proteins. Since cytoskeleton-severing agents abolished vesicle mobility, this indicates a cytoskeleton dependent vesicle recycling. Our findings importantly contribute to the understanding of how vesicle mobility is regulated.

170-Pos Board B49

Developing Statistical Diagnostic Tools For Discriminating Between Different Diffusive Modes Of Fluorescently Tagged Protein Complexes In Living Cells For Short Duration Trajectories

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Several protein molecular complexes in living cells are known to diffuse in many different modes. These include normal Brownian diffusion, anomalous or sub-diffusion, confined/picketed diffusion and facilitated diffusion. Such a variety of diffusive modes belies the heterogeneity in the cellular environment, both in terms of effective viscosity of the intracellular medium and marked differences in packing densities and spatial organisation of molecular substructures, both in the cell membrane and the cytoplasm. Discriminating diffusive modes is relatively easy for long duration trajectories, however obtaining such trajectories typically requires a relatively cumbersome tag, such as using colloidal gold beads of several 10s of nm is diameter, or functionalised quantum dots again in excess of 10nm in effective diameter, which inevitably affects normal physiological function and diffusion and may lead to misinterpretation of the underlying biology. This has applications in topical questions such as the degree to how freely mixed the membrane is or whether interacting proteins are confined through the membrane micro-structure. A far better approach is the use of smaller fluorescent tags under more physiological conditions, such as genomically-encoded fluorescent protein fusions such as GFP. However, such fluorescent proteins have photophysical properties that typically only allow optical tracking over short time windows for tracked protein complexes. Here we present new statistical approaches which allow discrimination of different diffusive modes on such short duration trajectories.

171-Pos Board B50

Probing the Intracellular Reaction Dynamics of Low Density Lipoprotein Using Single Particle Tracking Fluorescence Microscopy

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Interactions between substrate-containing late endosomes and enzyme-containing lysosomes mediate intracellular reactions that lead to the degradation of the substrate. To monitor the intracellular degradation of low density lipoprotein (LDL) in live cells, individual LDL particles were labeled with approximately 200 lipophilic, fluorescent dye molecules. Due to the close proximity of individual fluorophores, the emission of photons by the fluorophore is quenched. Upon enzymatic degradation, the LDL particle exhibits a 3-fold fluorescence