different orientations can be significantly narrowed by rotating a linear polarized excitation beam together with a wide-field de-excitation beam of perpendicular polarization (excitation polarization angle narrowing, Ex-PAN) resulting in short periodical flashes from different standard markers after each other. We present images of membrane-labelled neuron samples and surface labelled nanospheres in which different molecules cause periodic fluorescence modulation of different phases in different image pixels that are directly connected to the nanometric sample structure. This modulation and phase information can be used to deduce fluorescence images at higher resolution (SPoD, super-resolution by polarization demodulation). We demonstrate that in membrane-labelled neurons structures such as the spine-apparatus in individual spine heads can be resolved that cannot be resolved using conventional fluorescence microscopy. In addition, we present latest developments such as the extension to a double modulation approach for full 3D-determination of the molecular orientation.

[1] N. Hafi, M. Grunwald, L. S. van den Heuvel, T. Aspelmeier, J.-H. Chen, M. Zagrebelsky, O. M. Schütte, C. Steinem, M. Korte, A. Munk and P. J. Walla, ***Nature Methods***, 11,5, 579-584 (**2014**).

38-Subg

Understanding Gene Expression Heterogeneity in Living Cells with Single-Molecule Fluorescence Microscopy

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Gene expression refers to the sum of processes that enable cells to control their complement of RNA, and the study of gene expression has been spurred by genome-wide techniques such as microarrays and chromatin immunoprecipitation. Placing these data within a cellular context to reveal the underlying mechanisms of gene regulation has been a central challenge in the field of systems biology. In recent years, through parallel advances in microscopy, fluorescent probe development, and computational modeling, it has become possible to describe gene expression in a fundamentally different way: one can now directly observe single molecules of RNA in living and fixed cells using the fluorescence microscope. In this talk, I will describe the use of this single-molecule approach to study transcription kinetics of the GAL10 locus in budding yeast, which is regulated by sugar availability. Transcription is observed to occur in bursts of high activity followed by periods of inactivity, each lasting several minutes. This stochastic, punctate behavior results in 'noise' in gene expression and is not visible in population studies, which instead give the impression of a gradual response to sugar availability. I will describe recent results on the role of non-coding RNA in transcriptional regulation. Genomic data indicates that eukaryotic genomes are ubiquitously transcribed, but the function of these RNAs is largely unknown. Our results indicate that noncoding RNA displays different behavior during repression and activation, suggesting multiple roles, even at the same locus.

39-Subg

Metabolic Imaging of Living Tissues by Fluorescence Lifetime Microscopy (FLIM) and Endogenous Biomarkers

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Cellular metabolism plays a crucial role in several physiological processes such as cancer progression, cell migration, stem cell differentiation, embryo morphogenesis and neurodegenerative diseases. A non-invasive and high resolution mapping of cell metabolism in vivo is fundamental for the understanding of tissue development and for testing effective treatments. We established a sensitive method for metabolic imaging by combining nonlinear optical microscopy and the use of endogenous fluorophores that are naturally present in cells and tissues. We provide functional images of tissues autofluorescence by using two-photon-excitation microscopy, fluorescence lifetime microscopy (FLIM) and the metabolic coenzyme nicotinamide adenine dinucleotide (NADH). NADH is the principal electron acceptor in glycolysis and electron donor in oxidative phosphorylation and is a very informative intrinsic biomarker for metabolism, mitochondrial function, oxidative stress and apoptosis. NADH ubiquity renders metabolic imaging straightforward and label-free in any type of biological tissue. Fluorescence lifetime measurements of free and protein-bound NADH sense small changes in cellular redox state and quantifies different rates of oxidative phosphorylation and glycolysis in single cells within the tissue microenvironment. FLIM of NADH has the capability to discriminate different metabolic states of undifferentiated progenitor stem cells and differentiated cells, to predict stem cell fate to different lineages, to identify proliferating cancer cells and delineate tumor borders. In conclusion, optical metabolic imaging by NADH FLIM represents a promising experimental technique for measuring single-cell metabolic phenotype in living intact tissues with minimal phototoxicity and can be widely applied non-invasively for longitudinally studies in vivo.

Subgroup: Membrane Biophysics

40-Subg

Allosteric Mutant Phenotypes Investigated on an α1 Glycine Receptor Transmembrane Structure

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The glycine receptor (GlyR) is a pentameric ligand-gated ion channel (pLGIC) mediating inhibitory transmission in the nervous system. Its transmembrane domain (TMD) is the target of allosteric modulators such as general anesthetics and ethanol, and is a major locus for hyperekplexic congenital mutations altering the allosteric transitions of activation or desensitization. We previously showed that the TMD of the human α1GlyR could be fused to the extracellular domain of GLIC, a bacterial pLGIC, to form a functional chimera called Lily. Here, we overexpress Lily in S2 insect cells and solve its structure by X-ray crystallography at 3.5 Å resolution. The TMD of the α1GlyR adopts a closed-channel conformation with an unusual gate made up by a single ring of hydrophobic residues at the center of the pore. Electrophysiological recordings show that the phenotypes of key hyperekplexic mutations of the α1GlyR and scattered all along the pore are preserved in this chimera, including those that confer decreased sensitivity to agonists, constitutive activity, decreased activation kinetics or increased desensitization kinetics. Combined structural and functional data suggest a pore opening mechanism for the α1GlyR providing a structural explanation for the effect of some key hyperekplexic mutations. The first X-ray structure of the TMD of the α1GlyR solved here using GLIC as a scaffold paves the way for mechanistic investigation and design of allosteric modulators of a human receptor.

41-Subg

Thermodynamics of AChR Activation

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Energy changes in muscle acetylcholine receptor (AChR) gating were estimated from single-channel currents by using a cyclic activation scheme. Mutations mainly cause local and independent free energy changes that can be mapped onto structures and used to engineer rate and equilibrium constants. Residue-by-residue maps of reaction progress (phi) show that two separated regions, the agonist binding sites and linkers in the membrane domain, are the first to reach the gating transition state, followed by the extracellular domain, most of the membrane domain and a gate that appears to unlock in three steps. Maps of ground state free energies show big changes at the agonist sites, domain and subunit interfaces, a transmembrane helix and the gate. The local character of side chain energy changes and the similar phi values of the agonist sites and distant linkers suggest that the gating allosteric transition is not a purely mechanical process. Each AChR has 2 agonist sites in the extracellular domain, at αδ and either αε (adult) or αγ (fetal) subunit interfaces. Here, an increase in affinity for the ligand increases Po much as the movement of S4 does in voltage-gated channels. We measured the effects of mutations at each site with regard to their contribution to the binding free energy, active vs. resting (ΔGB). In both adult and fetal AChRs the two agonist sites behave independently. For 4 different agonists ΔGB is more favorable at αγ compared to the others. Only 3 aromatics contribute to ΔGB at the adult sites but 5 do so at αγ. ΔGB values calculated from MD simulations are consistent with the experimental ones and suggest that the αγ site is more compact, better organized and less dynamic than the others.

42-Subg

Structural Determinants of TRPV Channel Activation and Modulation

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My lab is broadly interested in the mechanisms of signaling and transport across cellular membranes. Much of our research centers on TRP channels