molecular crowding impacts biomolecule behavior as expected inside the cell. Fluorescence emission from a single photoactivated probe captured in an oil immersion, high numerical aperture objective, produces a spatial pattern on the detector that is a linear combination of 4 independent and distinct spatial basis patterns with weighting coefficients specifying emission dipole orientation. Basis patterns are tabulated for single photoactivated probes labeling myosin cross-bridges in a permeabilized muscle fiber undergoing total internal reflection (TIR) illumination. Emitter proximity to the glass/water interface at the coverslip implies the dipole near field and dipole power normalization are significant effectors of the basis patterns. Other characteristics of the basis patterns are contributed by field polarization rotation with transmission through the microscope optics. Pattern recognition utilizes the generalized linear model (GLM), maximum likelihood fitting, for Poisson distributed uncertainties. This fitting method is more appropriate for treating low signal level photon counting data than χ² minimization. Results indicate that emission dipole orientation is measurable from the intensity image except for the ambiguity under dipole inversion. The advantage over an alternative method comparing two measured polarized emission intensities using an analyzing polarizer is that information in the intensity spatial distribution provides more constraints on fitted parameters and a single image provides all the information needed. Also, axial distance dependence in the emission pattern is exploited to measure relative probe position near focus. Supported by NIH NIAIMS and NHLBI grants R01AR049277 and R01HL095572.

1728-Pos Board B638
Fast Topology Changes During SNARE-Mediated Vesicle Fusion Observed in Supported Membranes by Polarized TIRFM
Kiessling Volker, Marta K. Domanska, Lukas K. Tamam.
In vitro reconstitution experiments have played an essential role in a large body of research on SNARE-mediated membrane fusion. Recently, new single vesicle assays have been developed to gain more detailed insight into the kinetics of vesicle docking and fusion. Previously, we used supported membranes in combination with total internal reflection microscopy (TIRFM) to record the docking and fusion of synaptobrevin containing vesicles with 4 ms time resolution. Depending on the lipid conditions, we found that between 3 and 8 SNARE complexes are needed for fast fusion in this system. Here we utilize polarized TIRFM to investigate topology changes that the docked vesicles undergo after the onset of fusion. The theory that describes the fluorescence intensity during the transformation of a single vesicle from a spherical particle to a flat membrane patch is developed and confirmed by experiments with the three fluorescent probes Rh-DOP, NBD-DPPE and BODIPY-PC. Our results show that, on average, the fusing vesicles flatten and merge into the planar membrane within 8 ms after fusion starts.

1729-Pos Board B639
Assessing the Metabolic State of Cultured Cells and the Intact Cochlea by Two-Photon Fluorescence Intensity and Lifetime Imaging
The ability to match energy production with demand is a fundamental determinant of the health of cells, tissues, and organs of the body. Metabolism and mitochondrial dysfunction have been broadly implicated in many disease states, including hearing disorders and noise-induced hearing loss. We have employed two-photon fluorescence imaging of intrinsic mitochondrial reduced nicotinamide adenine dinucleotide (NADH) and flavoprotein (Fp) to quantify the metabolic state of several cultured cell lines, multicell tumor spheroids, and the intact mouse organ of Corti. Historically, the fluorescence intensity has commonly been used as an indicator of the reduced NADH and/or oxidized Fp concentration in cells and tissue. More recently, fluorescence lifetime imaging has revealed that changes in metabolism produce not only changes in fluorescence intensity, but also significant changes in the lifetimes and concentrations of free and enzyme-bound pools of NADH. This presents a new opportunity to track the cellular metabolic state as NADH binding changes with metabolism. To better understand what is revealed by variations in the fluorescence intensity and lifetime distributions, we have used mitochondrial uncouplers and inhibitors in conjunction with manipulation of substrate and oxygen concentrations to systematically adjust the metabolic state. We will compare the similarities and differences of the intensity- and FLIM-based assessment of metabolic rate in model systems of increasing complexity, ranging from single cells to intact tissue, highlighting the advantages and limitations of each technique.
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1730-Pos Board B640
Genetically Targetable Cell Surface Selected pH Sensor
Anmol Grover, Brigitte S. Schmidt, Laura Kremer, Qi Yan, Marcel P. Bruechle.
The specific noncovalent interactions between a monomethine dye and a single chain variable fragment antibody can be used to target and activate dye molecules that are otherwise nonfluorescent in solution at a specific protein partner in living cells. This interaction/activation process allows detection of bound dye in the presence of a large excess of unbound dye, a useful property for dynamic imaging. To apply this approach to environmental sensing, a series of tandem dye molecules were prepared consisting of a fluorogen and a pH sensitive cyanine dye linked at distances where intramolecular energy transfer was dominant. The binding of these molecules to their respective fluorogen activating peptides provided a series of genetically targeted and activated probes that function as single excitation ratiometric emitter probes, or as ratiometric excitation probes, depending on the arrangement of the energy transfer pair, and displayed pH responses with pKa values from 6.0 to 8.0. These probes allowed tracking and repeated pH measurements of 2-adrenergic receptor internalization and sorting at a single vesicle level, in 4-d confocal microscopy.

Bioinformatics

1731-Pos Board B641
The Structural Biology Knowledgebase - search Online for Protein Sequences, Structures, Functions, Methods and More
The Structural Biology Knowledgebase (SBKB, URL: http://sbkb.org) is a free online resource designed to combine all protocols and results of the structural genomics and structural biology efforts with information from the biological community in order to have a better understanding of living systems and disease. We will present examples of how to navigate the SBKB and how to use it to enable biological research. For example, a protein sequence or PDB ID search will provide a list of protein structures from the Protein Data Bank, associated biological descriptions (annotations), homology models, structural genomics protein target information, experimental protocols, and the ability to order available DNA clones. A text search will find technology reports and publications that were created by the Protein Structure Initiative’s high-throughput research efforts. Web tools that aid in bench top research, such as the Sequence Comparison and Analysis tool for protein construct design, will also be demonstrated. Created in collaboration with the Nature Publishing Group, the Structural Biology Knowledgebase Gateway provides a research library, editorials about new research advances, news, and an events calendar also present a broader view of structural genomics and structural biology.
The SBKB is funded by the Protein Structure Initiative/NIGMS.

1732-Pos Board B642
Moonlighting Proteins Database
Mathew Mani, Constance Jeffery.
Moonlighting proteins comprise an interesting subset of multifunctional proteins in which the two functions are found in a single polypeptide chain. They do not include proteins that are multifunctional due to gene fusions, families of homologous proteins, splice variants, or promiscuous enzyme activities. Moonlighting proteins include several different kinds of enzymes and other proteins and different combinations of functions. Recent crystal structures of some moonlighting proteins have provided clues to the molecular mechanisms of one or both functions, and in some cases how a protein can switch between functions. We are preparing a curated, searchable database that contains information about the sequences, structures, and functions for known moonlighting proteins.

1733-Pos Board B643
TMBB-DB: A Proteomic Database of Transmembrane β-Barrier Predictions
Thomas C. Freeman Jr., William C. Wimley.
The transmembrane β-barrel (TMBB) is one of the two major structural motifs found in membrane-spanning proteins. TMBBs are found exclusively in the outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts. Because TMBBs perform a vast array of functions (e.g. signal transduction and cellular adhesion) and are surface-exposed, they present an exploitable vulnerability in drug-resistant pathogenic Gram-negative bacteria. Technical deficiencies have impeded progress in the structural study of TMBBs, which is