reflection-fluorescence correlation spectroscopy (ITIR-FCS) enabled direct determination of the local diffusion coefficients of CEACAM1 in different regions of the cell membrane while number and brightness analysis (N&B) and spatial intensity distribution analysis (SpIDA) provided insights into the oligomeric state of CEACAM1. These strategies for characterizing the dynamics of transmembrane receptors in live cells show clear promise for probing glycoprotein function in normal and disease states.

3397-Pos Board B258
Multimerization Studies of Membrane Receptors via μ-Patterned Surfaces
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Studying dimerization or multimerization processes of plasma-membrane localized receptor proteins is important for a better understanding of various cellular processes. While the physiological role of receptor multimerization remains a matter of debate for many proteins, it has been proved that such events are crucial for the function of different classes of membrane receptors (e.g. EGF or Insulin receptor). Several biochemical tools including cross-linking or co-IP assays are used to unravel protein multimerization. However, these techniques suffer from being indirect and hardly quantitative and give high number of false-positives and/or negatives. Indeed, there are biophysical assays to address this question, but the evaluation of the recorded data remains complicated and laborious. Here we describe how a combination of Total Internal Reflection Fluorescence (TIRF) microscopy and micro-structured surfaces (''micropatterning assay'') can be used to easily prove multimerization of membrane receptors. This technique was developed to detect protein-protein interactions (Schwarzenbacher et al., 2008; Weghuber et al., 2010) and offers the possibility to measure and quantify also weak or short-lived interactions in vivo. In a proof of concept experiment we studied the multimerization of YFP and/or CFP labeled ß1 and ß2 adrenergic receptors. The question of homo- and especially hetero-association of these G-protein coupled receptors (GPCR) is far from being completely understood. Receptor maturation, G-protein coupling, downstream signaling and regulatory processes such as internalization might be influenced by dimerized adrenergic receptors. We unequivocally show that ß1 as well as ß2 receptors form homo- and hetero multimers in living CHO-K1 cells. Our findings contribute to the field of adrenergic receptors. In general, our system might be of great interest for a fast and straightforward analysis of membrane-protein multimerization.

3398-Pos Board B259
Mobility of MenC and PhoU in Live E. Coli: A Single Molecule Tracking Study
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Innumerable signal transduction systems have evolved in which an environmental signal is transferred across the cell membrane to the cell interior via a series of protein movements and protein-protein interactions. Knowledge of the diffusion of signalling proteins in these networks and the effects of the presence of their interacting partners is required to understand the dynamics of the signalling response of such networks. Here, we will discuss our recent research using single molecule imaging techniques to determine the mobility of several proteins labelled by the fast folding variant of yellow fluorescent protein, Venus, in live E. coli. Specifically, we have determined the mobility of PhoU, a member of the PhoR-PhoB two-component signalling system and MenC, the o-succinylbenzoyl-CoA synthase, which has been found to interact with PhoU (Y.-J. Hsieh, Y. Yang, and B. L. Wanner, unpublished data). The effect of interactions of these proteins on their mobilities was investigated through monitoring protein diffusion in cells having deletions of the interacting partner genes. Results will be discussed in terms of their implications on the dynamics of signal transduction systems and in comparison to the known mobilities of other proteins and lipids in E. coli and eukaryotic cells.

3399-Pos Board B260
Cross Regulation in Signaling Networks: A FRET Study of the PhoB-PhoR Two-Component System in E-Coli
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The signal for the PhoR-PhoB two-component regulatory system of E. coli is environmental inorganic phosphate (Pi). A signal for environmental Pi insufficiency is propagated across the membrane to the cytosolic domain of the signalling histidine kinase PhoR via the ABC (ATP-binding cassette) transporter, the Pst (Pi-specific transport) system, in the absence of transport by protein-protein interactions, both in the membrane and the cytoplasm of the cell. Cross regulation also exists between PhoR-PhoB two-component system and key regulatory systems in E. coli, including the ArcB-ArcA two-component system for redox signalling. Such cross regulation among key signalling systems is thought to be important for integration of diverse cellular pathways in the control of cell growth and for survival under conditions of stress. Knowledge of how these interactions is brought about necessary to understand fully the dynamics of the response of the Pi signalling network to environmental Pi insufficiency and to understand interactions of the PhoR-PhoB and other signalling systems. Here we have employed Förster resonance energy transfer (FRET) techniques (acceptor photobleaching and sensitized emission) coupled with specific knock-out mutants to investigate in live E. coli cells interactions between ECFP-labelled members of the PhoR-PhoB system and protein targets with which they are thought to interact such as the o-succinylbenzoyl-CoA synthase, MenC. We will show a strong enhancement of the FRET signal through the use of protein fusions and knock-out mutants. We will discuss results of this study in terms of their implication on the dynamics of the PhoR-PhoB two-component signal transduction system.

3400-Pos Board B261
A Receptor Guanylyl Cyclase Reveals Auto-Phosphatase Activity
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In sea urchin sperm of Arbacia punctulata, a guanylyl cyclase (GC) serves as chemotaxis receptor that enables sperm to respond to a single molecule of the chemoattractant, resact. The efficiency of resact capture is high, because GC covers about 50% of the flagellar surface and binds resact with picomolar affinity. Furthermore, the binding affinity is controlled by the level of occupancy of the receptor. At high occupancy the resact affinity is lowered through negative cooperativity among subunits of the trimeric GC complex. The lifetime of active GC is controlled by its phosphorylation state. At rest GC is phosphorylated at six serine residues. After activation by resact, the GC becomes dephosphorylated with a biphasic time course, whereas dephosphorylation strongly coincides with the decrease of cGMP synthesis. During the initial fast phase the amplitude of GC dephosphorylation increases with the occupancy level. However, the time constant of this phase is independent of receptor occupancy. Moreover, dephosphorylation is superstoichiometric: even if only 5% of the GCs are occupied by resact, approximately 70% become dephosphorylated. We conclude from these results, that the occupied GC inactivates by auto-dephosporylation (fast phase) and additionally can dephosphorylate adjacent non-occupied GCs (slow phase). We could show for the first time that a receptor GC is regulated by auto-dephosphorylation.

Synaptic Transmission

3401-Pos Board B262
Conical Tomography of Ribbon Synapses of Light and Dark-Adapted Rod and Cones Photoreceptors
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Rod and cone photoreceptors undergo tonic transmitter release following changes in illumination. In both rods and cones, the fusion competent (i.e., docked and primed) synaptic vesicles are thought to be associated preferentially to a distinctive electron-dense structure referred as the ''ribbon.'' The aim of this study was to evaluate the location and number of docked (i.e., ''hemi-fused'') and fully fused (i.e., ''omega'' figures) vesicles in the axon terminal of rods and cones in light and dark-adapted mice. Using conical electron tomography, we reconstructed ribbon synapses of mice exposed to steady bright light or dark-adapted for 3 to 180 minutes. The conical tomograms were analyzed using density segmentation methods based on the watershed algorithm. We found that in the light, in both rods and cones the docked vesicular pool extended alongside the entire area of contact between the axon terminals and the horizontal cell endings. In rod axon terminals, the docked pool was comprised of 460-480 hemi-fused vesicles and remained essentially unchanged during dark-adaptation. In the dark, the terminals underwent rapid but reversible changes, including an increase in plasma membrane area and the number of ''omega'' figures (from ~190 to ~1300 in rods terminals). After ~30 minutes in the dark, the axon terminals returned to the conditions observed in mice exposed to steady light conditions. We thus conclude that in mouse rod and cone photoreceptors both ribbon-associated and non-ribbon regions of the

plasma membrane of the axon terminals contribute to the shaping of signaling to post-synaptic neurons.

3402-Pos Board B263

Acute Damage to Synaptic Ribbons Differentially Affects Evoked and Spontaneous Neurotransmitter Release from Rod Bipolar Cells

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Ribbon synapses are features of non-spiking tonic releasing cells of the retina and inner ear. These synapses are named for their electron dense synaptic ribbons, which tether an abundance of synaptic vesicles near release sites. Here, we used illumination of fluorescein-tagged ribbon-binding peptides to acutely damage synaptic ribbon function in mouse rod bipolar cells using fluorophore assisted light inactivation (FALI), while monitoring neurotransmitter release from a post-synaptic AII amacrine cell. Illumination of ribbon-targeted peptides, but not scrambled controls, revealed an immediate drop in the frequency and amplitude of mEPSCs at −60 mV without effect on the event kinetics, whereas the amplitude of the first EPSC evoked by a step to −10 mV for 100 ms was unaltered. These results suggest that two independent ribbon-associated pools of vesicles contribute to release at −60 mV and in response to steps to −10 mV. Our results are also consistent with a role for the ribbon in coordinating multivesicular release.

3403-Pos Board B264

A Catalytic Slot Model for Exocytosis with a Single Release Sensor

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Regulated secretion occurs on different timescales: fast (synchronous) release is mediated by high-release probability vesicles, whereas vesicles with low release probability contribute to slow (asynchronous) release. Current models of exocytosis incorporate parallel calcium-sensors, to account for the slow release remaining after deletion of the fast sensor. However, the identity of the slow calcium sensor remains elusive, and no molecular manipulation has been identified, which eliminates slow, but not fast release.

Using mathematical modeling we found that a simpler, sequential model with only a single release sensor suffices to describe previously obtained data in chromaffin cells and neurons: we suggest that during maturation vesicles associate with a catalyst at the release site. This catalyst facilitates priming by a calcium-dependent increase of the interconversion rate between un-primed and primed vesicles without changing the population of those states in equilibrium. We suggest that the calcium sensor for exocytosis (usually assumed to be synaptotagmin-1 or −2) regulates release by two distinct actions. It facilitates priming while clamping release in a calcium-independent manner. Calcium-binding to synaptotagmin relieves the clamp. In the absence of synaptotagmin spontaneous fusion depletes the primed vesicle state, thereby uncovering the upstream calcium-dependent catalysis step as the slow calcium sensor described in synaptotagmin nulls.

The model can explain salient observations, including calcium-dependence of pool sizes in chromaffin cells, fusion and recovery kinetics, and it accounts for a number of observations not easily explained in earlier models, including sub-maximal release (decreased fraction of fast/slow release at low calcium concentrations) and the phenotype of synaptotagmin knockouts when stimulated by high-frequency trains.

We suggest that slow release is not mediated by a parallel-acting, competing sensor, but by an upstream calcium-dependent catalysis step, making slow release a fundamental property of fast release itself.

3404-Pos Board B265

Observation of Two-Step Unzipping of a Single SNARE Complex by using Nano-Mechanical Measurement

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Soluble NSF Attachment Protein Receptors (SNARE) complex is known as the minimal machinery for synaptic vesicle exocytosis in neuronal communication. The conformational transition from ''trans'' to ''cis'' form of SNARE complex is an essential step of a Ca2+ triggered vesicle fusion to release neurotransmitters. Until recently, it has been debated whether the trans-SNARE complex is fully-zipped or partially-zipped before transition toward cis-form. We observe from nano-mechanical measurement by magnetic tweezer that a single soluble SNARE complex shows a sequential two-step unzipping, about 10nm each. It implies that trans-SNARE complex could be partially-zipped when force is applied like the hydration force between lipid bilayers. Other neuronal proteins might act on the unzipped domain to clamp trans-SNARE complex before conformational transition to cis-SNARE complex.

3405-Pos Board B266

Effects of Presynaptic Calcium Stores on Short-Term Synaptic Plasticity

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In reconstructions of hippocampal neuropil, Smooth Endoplasmic Reticulum (SER) appears in a majority of the presynaptic terminals. In the presence of ongoing electrical activity, Inositol Triphosphate Receptors (IP3Rs) on the SER initiate a positive feedback loop that can lead to release of calcium from the SER via an IP3-mediated pathway. We investigated how the presence of this additional source of calcium in addition to the Voltage Dependent Calcium Channels (VDCCs) can regulate synaptic transmission. We carried out 3D Monte Carlo simulations of the molecular interactions that govern transmitter release in a 1) Canonical CA3-CA1 synapse 2) Synapse reconstructed from serial section Transmission Electron Microscope images. The relatively simple geometry of CA3-CA1 synapses allows activity-dependent local calcium at the active zone and the related transmitter release profiles to be quantitatively analyzed. In paired-pulse stimulation, the presence of molecular pathways that regulate the calcium stores increased the calcium buffering capacity of the synapse, which decreased the initial release probability and enhanced paired-pulse facilitation. In contrast, a high-frequency stimulus could trigger the activation of presynaptic Metabotropic Glutamate Receptors (mGluRs) leading to IP3 production and ultimately to release of calcium from the SER. IP3Rs operated at a much slower time scale, on the order of seconds compared to the millisecond timescale of the VDCCs. This led to an increase in the basal level of intracellular calcium and enhanced transmitter release rates. We further explored the functional implications of the range of SER geometries observed in the synaptic ultrastructure and the effect of different arrangements between IP3Rs and VDCCs on synaptic plasticity. The synaptic ultrastructure precisely orchestrated the degree of facilitation and depression and the existence of presynaptic calcium stores provided the synapse with an additional intrinsic time scale that could be regulated by activity.

3406-Pos Board B267

Three Distinct Mechanisms of Neuro-Muscular Junction Excitation by Infrared Pulses

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Post-synaptic currents (PSCs) were recorded using whole cell voltage clamp to examine excitatory responses of the neuro-muscular junction to infrared pulses (1862nm, 200μs/pulse, 5-30 pulses/s, 20-1000 μJ/pulse over 125 nm^2). In wild type Caenorhabditis elegans, optical stimuli excited the post-synaptic cell by 1) immediate opto-mechanical triggering of pulse-by-pulse miniature excitatory currents (mLEC) with 0.7ms latency to peak and 2) relatively slow (τ~1.3s onset) thermodynamically driven reduction in a tonic outward rectified K^+ current (LTC). The same optical stimuli acted on the pre-synaptic neuron to 3) rapidly increase the rate of synaptic vesicle release and the rate of miniature PSCs (mPSCs). In addition, mPSC kinetics were increased with infrared stimulation resulting in a decrease in average charge per event from 52 to 32fC. The pulse-by-pulse mLECs were enhanced in muscle degenerin gain of function mutant (unc-105) suggesting the fast response was due to opto-mechanical activation of the degenerin stretch receptor. The slow tonic current (LTC) reversed at the K^+ equilibrium potential, exhibited a highly rectified outward conductance, and a thermal-dependent closure analogous to shaker related channels including $K_v1.1$. In the pre-synaptic neuron, the spontaneous rate of synaptic vesicle release and the laser-evoked increase was nearly eliminated in a loss of function mutation of the voltage insensitive cation leak channel (unc-77, nca-1). The increased mPSC rate (presynaptic action) and reduction in the tonic outward K^+ current (post-synaptic action) contributed in nearly equal proportions at −60mV holding potential and accounted for over 90% of the total laser-evoked PSC. [Supported by NIH R01 DC006685 & R01DC011481]

3407-Pos Board B268

Optical Activation or Silencing of Neural Activaty in Neurons that Regulate Energy Balance in the Hypothalamus

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