visited. We have thus established that there is a difference in the movement of membrane proteins in living and dead cells. The active component of the motion of the λ-receptor in living cells has been modeled as an artificial temperature, which estimates the energy necessary for this active motion. The influence of antimicrobial peptides (AMPs) on the outer membrane of bacteria was investigated using the mobility of the λ-receptor as a membrane marker. With the growing resistance to antibiotics AMPs are gaining increased interested. Using the AMPs polymyxin B (PMB), and the non toxic derivate polymyxin B nonapeptide (PMBN), we have investigated the influence of AMPs on the outer bacterial membrane. Cells exposed to PMB showed a decrease in the spread of position visited by the λ-receptor upon poisoning. PMBN is known to increase the permeability of the outer membrane with out killing the cells. Exposure to PMBN did, however, not influence the mobility of the λ-receptor.

1430-Pos Board B274

Investigating Axonal Outgrowth and Orientation of Neuroblasts through an Alternating Stiffness Substrate

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At the interface of cell-substrate interactions, substrate elasticities strongly influence the morphology and function of cellular responses. This is important in diverse areas including neural function, metastasis, and heart disease. Cells are subject to mechanical signals in addition to biochemical signals; therefore, understanding the cellular interactions to substrate stiffness and with extracellular matrix is an important step to define how cultured cells respond when grown on materials that have similar characteristics to physiological conditions. To address the effects of localized elasticity, we developed a new method to control the microenvironment through generating a substrate with localized alternating stiffnesses interacting with cells to affect their structural response. This technique was accomplished through first fabricating polymeric microchannels using conventional soft lithography. We made channels either 30 μm or 100 μm wide, and 50 μm deep with poly(dimethylsiloxane) (PDMS) with a 5:1 ratio of base/curing agent. We then poured PDMS with a 30:1 ratio of base/curing agent into the channels and then removed the extra PDMS to produce a level surface. This produced alternating surfaces with elastic modulus of 800 kPa and 200 kPa adjacent to one another. We coated the surfaces with extracellular matrix and seeded neuroblasts onto the systems. We then differentiated them using retinoic acid (20 μM). We found that the neuroblasts had distinct patterns that emerged as they extended processes to and across these alternating stiffness substrates. Depending on the location of the cell body and the direction of the outgrowth when compared to the alternating stiffness interface, the processes would extend forward in different paths. We believe that this approach will enable greater understanding of axon outgrowth as well as provide insight into a variety of diseases linked to cell-ECM-material interactions.

1431-Pos Board B275

FRAP and Photoconversion in Multiple Arbitrary Regions of Interest Using a Programmable Array Microscope (PAM)

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Photomanipulation (photobleaching, photoactivation, or photoconversion) is an essential tool in fluorescence microscopy. Fluorescence recovery after photobleaching (FRAP) is commonly used for the determination of lateral diffusion constants of membrane proteins, and can be conveniently implemented in confocal laser scanning microscopy (CLSM). Such determinations provide important information on molecular dynamics in live cells. However, the CLSM platform is inherently limited for FRAP because of its inflexible raster (spot) scanning format. We have implemented FRAP and photoconversion protocols using a programmable array microscope (PAM). The bleaching or photoconversion patterns are arbitrary in number and shape, dynamic, and adjustable to and by the sample characteristics. We have used multi-spot PAM-FRAP to measure the lateral diffusion of the erbB3 (HER3) receptor tyrosine kinase labeled by fusion with mCitrine on untreated cells and after treatment with reagents that perturb the cytoskeleton or plasma membrane or activate coexpressed erbB1 (HER1, the EGF receptor EGFR). We also explored the versatility of the PAM for photoconversion in arbitrary regions of interest, in cells expressing erbB3 fused with the fluorescent protein dronpa.

1432-Pos Board B276

Glycosphingolipids and Non-Raft Phospholipids Exhibit Very Similar Dynamics in Single-Molecule Observations

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The diffusion of typical raft-associated molecules, glycosphingolipids, GM_1, GM_3, GD_{1a}, was observed using high-speed single-particle tracking (HS-SPT) at a temporal resolution of ~6 μs. Each glycosphingolipid molecule was tagged with a 40-nm-gold particle conjugated by the respective Fab antibody or a Cy3-conjugated Fab antibody. At video rate, both probes exhibited the same diffusion behavior, undergoing apparent simple Brownian diffusion at the same effective diffusion coefficient (about 0.2 $\mu m^2/s$). In HS-SPT observations, each glycosphingolipid molecule underwent actin-dependent hop diffusion over the membrane compartment of an average of 110 nm in diameter at an average frequency of once every 25 ms. Surprisingly, this behavior is the same as that of a typical non-raft phospholipid DOPE, qualitatively and quantitatively. These results are at variance with the previous FCS/FRAP studies. In addition, previous investigations concluded that GM_1, labeled either with cholera toxin (CTX) in its headgroup or with Bodipy-FL on the alkyl chain, diffused much more slowly than non-raft phospholipid probes. Here, we found that CTX bound to GM_1 in the plasma membrane diffused as fast as DOPE during the initial 0.5 s after its binding to the membrane, but slowed by a factor of 4 within 10 min after its binding. These results suggest that the slowed diffusion of GM_1 found in previous observations would probably be due to the influences of CTX-induced GM_1 crosslinking or of perturbation by Bodipy-FL on the alkyl chain. These results indicate that in the plasma membrane of non-stimulated cells, glycosphingolipids movements are not slowed by the possible presence of raft, further suggesting that the rafts in the steady-state cells are much smaller than the compartment size (110 nm) and/or short-lived ($<<$25 ms).

1433-Pos Board B277

Role of Membrane Domains in Interferon Receptor Signaling: a Single-molecule Study

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Signaling in living cells is largely mediated through multi-protein complexes, and is triggered by recognition of a chemical ligand by membrane receptors at the extracellular side, leading to activation of cytoplasmatic effectors. Thus, understanding the dynamical behavior of receptors in the cell membrane is fundamental to understand the processes of cell signaling. The system we are studying, type I interferon (IFN) receptor, is a member of the cytokine family, which plays a key role in early innate and adaptive immune responses upon infection by pathogens. Different members of the type-1 IFN family elicit differential responses although binding to the same receptor. The latter comprises two subunits ifnar1 and ifnar2. Upon ligand binding a ternary complex is formed and signaling pathways activated. Using single-molecule wide-field fluorescence microscopy we follow receptor diffusion in the plasma membrane of living HeLa cells. Receptor subunits are labeled through post-translational labeling with synthetic dyes (e.g. Cy5) coupled to coenzyme-A. Each of the receptor subunit is transfected and expressed, both separately and simultaneously, in HeLa cells, allowing measurements on single component as well as on the ternary complex formed upon IFN binding. Using correlation analysis we obtained information on receptor diffusion constants. Switching between fast and slow motility and vice versa was observed, and interpreted as association/dissociation of ternary complex. Mutants ligand with different affinity toward ifnar1 and ifnar2 are also tested to probe the effect on ternary complex dynamics. Finally we show that membrane nanostructure is possibly involved in the dynamic behavior of the complex. Our data lead to a kinetic model for receptor assembly which may help to obtain a better understanding of transmembrane signaling.

1434-Pos Board B278

Cortical Cytoskeletal Structures Constrain CD36 Receptor Motion at the Cell Surface to Enhance Aggregation and Signaling

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CD36 is a key receptor in human macrophages, binding to multivalent ligands such as oxidized LDL and malaria-infected red blood cells. To study the dynamics and aggregation kinetics of CD36 receptors in the plasma membrane, we used single-molecule imaging combined with single-particle tracking and mathematical modeling of individual receptor behavior. We immuno-labeled

CD36 receptors with a primary Fab fragment and a Cy3-conjugated secondary Fab fragment or with quantum dots, and imaged the dorsal surface of macrophages using live-cell epifluorescence microscopy. To track the imaged CD36 receptors, we developed a single-particle tracking algorithm that detects particles with sub-pixel localization and follows dense particle fields, capturing particle association and dissociation events and recovering trajectory interruptions resulting from temporary particle disappearance (Jaqaman Nat. Methods 2008). We found that, at rest, surface-bound CD36 receptors existed as discrete multimers that contained up to six monomers. A subpopulation of the detected CD36 multimers (~30%) moved along linear tracks that radiated from the perinuclear region. The movement required both the acto-myosin network and microtubules. Temporal multi-scale analysis of receptor frame-to-frame displacement and run-time toward and away from the perinuclear region using sampling frequencies of 10-125 Hz revealed that the linear motion of CD36 multimers was due to constrained diffusion within cytoskeleton-mediated linear corridors in the membrane. Importantly, the dimensionality reduction resulting from motion within corridors increased the probability of CD36 aggregation two-fold. Cytoskeleton perturbations that inhibited the linear motion of CD36 inhibited the uptake of its ligands and the phosphorylation resulting from CD36 cross-linking. These data provide the first direct demonstration of the functional requirements of cortical cytoskeletal structures in the regulation of receptor activation via the spatial organization of receptor motion and aggregation at the level of the plasma membrane.

1435-Pos Board B279

Plasma Membrane Topology and Membrane Models

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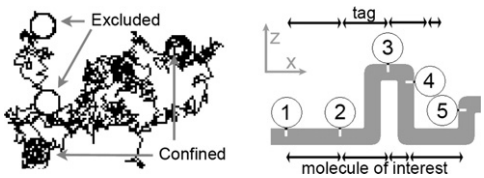
Single particle tracks (SPTs) can be followed over the plasma membrane with high spatial and temporal resolution. The interpretation of SPTs, combined with Monte Carlo simulations, form the basis of complex membrane models. Most interpretations of SPTs rest on the convenient assumption that cell membranes are flat. However simulations of simple diffusion over surfaces that include pillars, when the locations are known in X and Y but movement is possible in Z, substantially reduce the apparent rate of diffusion and produce tracks that could be interpreted as transient anchorage and confinement (Figure, left).
In addition SPTs usually follow a tag attached to the molecule of interest, not the molecule itself. When the molecule is confined to the plasma membrane, with the tag in the extracellular fluid and the surface is flat, they may be coincident. However gradients produce a variable offset between the molecule and its tag, misreporting the actual movement (Figure, right).
Plasma membranes are not flat and projections or depressions produce the appearance of anomalous subdiffusion. We suggest that complex explanations, transient anchorage or barriers, should only be considered once more obvious ones have been disproved.



1436-Pos Board B280

Lateral Organization in Simulated Four-Component Non-equilibrium Model Membranes

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Lateral organization in biomembranes plays a major role in membrane topology, and is thus implicated in many basic functions of biomembranes such as endocytosis and signal transduction. In this study, non-equilibrium Monte Carlo simulations are used to investigate two related scenarios: 1. the effect of a rigid distribution of proteins on the lateral organization of lipids in a biomembrane, and 2. the degree to which lipid interactions influence the lateral organization of membrane-associated proteins that are free to translate laterally. Our model includes generic saturated and unsaturated lipids, proteins, and cholesterol, and is driven out of equilibrium through simulated endo- and exo-cytosis events. By varying the temperature, the protein mole fraction, and the interaction strengths, we examine the conditions under which various types of lateral organization occur. Simulation results are analyzed with pair-correlation functions and the Ripley K-test. We compare results from simulations of the two scenarios above and from simulations of biomembranes lacking protein.

1437-Pos Board B281

Growth Cones As Sensing, Amplifying And Filtering Modules

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Sensitivity to weak directional signals is a striking feature of chemotactic systems. In eukaryotic cells, it is often attributed to spatial amplification in the detection of the gradient of guidance cues due to an asymmetric distribution of signaling molecules of the signaling pathway. By combining single-quantum dot imaging with a guidance assay, we probed the dynamics of GABAA receptors (GABAARs) in nerve growth cones. In presence of a GABA gradient, we observed a lateral redistribution of the receptors towards the GABA source. This effect was both reversible and specific of GABA signals. Its functional implication was established by calcium imaging which showed that the redistribution was accompanied by an enhanced asymmetry of the calcium response. Furthermore, single quantum-dot tracking of GABAARs revealed a ''conveyor-belt'' type of motion in which receptors randomly alternated between periods of free diffusion and of microtubule-dependent directed movement.
We therefore propose a model in which an asymmetric activation by the signaling gradient leads to oriented growth of the MTs which, in turn, contributes to an asymmetric distribution of the receptors and amplification in gradient sensing. This simple model for the formation of polarity at the cell membrane is supported by numerical simulations that describe with minimal hypothesis the results of our experiments. These simulations also provide predictions on the dependence of the formation of polarity as a function of gradient parameters. We will finally show our current effort to place neurons in microfluidic devices, to generate controlled gradients and to characterize the growth cone as a sensing, amplifying and filtering module.

1438-Pos Board B282

Imaging Of The Diffusion Of Individual Band 3 Molecules On Whole Erythrocytes From Patients With Hereditary Hemolytic Disorders

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The plasma membrane of the human erythrocyte is a composite structure that consists of a fluid lipid bilayer (including membrane-embedded and membrane-associated proteins) and a sub-surface scaffolding consisting of a six-fold network of the tetrameric flexible structural protein spectrin. The spectrin network is pinned to the fluid bilayer through a series of transmembrane and membrane associated proteins both at the six-fold junctions and near the mid-points along the spectrin tetramers between junctions. Common to both of these pinning points is the presence of the transmembrane anion exchange protein band 3 (AE1). Band 3 ties the six-fold junction to the bilayer through interaction with the protein adducin and ties the midpoint to the bilayer through interaction with ankyrin. Much of the remarkable mechanical characteristics of the red cell have been attributed to this membrane architecture. Further, red cells become fragile in pathologies known to disrupt the spectrin network or its pinning points to the membrane. To assess changes in the structure of the plasma membrane of pathologic red cells at the single molecule level, the mobility of individual band 3 molecules was observed on normal red cells as well as those from patients with several types of hereditary diseases including spherocytosis, elliptocytosis, and pyropokilocytosis. Specifically, individual band 3 molecules on whole red cells were labeled by quantum dots through the band 3 inhibitor 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS). We will present data on the mobility of band 3 in each of these cell types recorded at video imaging rates of 120 fps. The observation that membrane pathologies can be distinguished by the mobility of individual membrane molecules suggests that single particle tracking might constitute a useful tool for characterizing the ''health'' of a membrane.

1439-Pos Board B283

Dynamics Of Folate/Folate Receptor Complexes In KB Cells Observed Through Single Molecule Fluorescence Imaging

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Folate (vitamin B_9) is essential in the synthesis of nucleotide bases and amino acids. Cellular uptake (endocytosis) of folate is mediated by the membrane bound folate receptor (FR), a glycosylphosphatidylinositol-anchored protein. FR has been found to be up-regulated and/or redistributed in the plasma