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.

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Plasma Membrane Topology and Membrane Models

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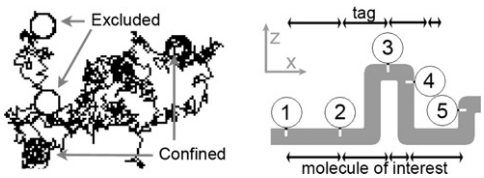
Single particle tracks (SPTs) can be followed over the plasma membrane with high spatial and temporal resolution. The interpretation of SPTs, combined with Monte Carlo simulations, form the basis of complex membrane models. Most interpretations of SPTs rest on the convenient assumption that cell membranes 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.

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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.

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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.

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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