layered circuits. Biological nucleic acids such as microRNAs can serve as inputs, and circuits work reliably in a background of unrelated nucleic acids, suggesting applications to the analysis of complex biological samples.

Motions of Cell Surface Molecules

2891-Pos Membrane Diffusion Measured Using Fluorescence Correlation Spectroscopy in Polarized S. cerevisiae

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The cellular membrane not only serves as the interface and barrier between the extracellular environment and intracellular biochemical processes, but also a dynamic organelle where proteins are embedded, recruited and organized, in many cases free to diffuse around the cell surface creating a homogeneous protein concentration.

During polarization this uniformity is broken and localization of some proteins changes resulting in their localization becoming polarized. To explore the role of diffusion and how changes of a proteinâ™s diffusion rate may be involved in polarization, Fluorescence Correlation Spectroscopy (FCS) was utilized to measure membrane diffusion in the budding yeast, S. cerevisiae, during mating projection formation. Slow diffusion rates were determined for cytosolic and membrane bound (EGFPmem) EGFP which were consistent with previously reported values obtained using FRAP [1]. a-cells were exposed to varying concentrations of Î±-factor (0, 10, 100, 1000 nM) and localization of EGFPmem and the Î±-factor receptor (Ste2-EGFP) after the formation of a projection were observed. Localization of the Ste2-EGFP was highly polarized whereas localization of EGFPmem was only slightly weighted toward the projection. This may be the result of increased membrane order in the projection [2]. Diffusion rates of both Ste2-EGFP and EGFPmem were measured at the front and back of the polarized cell.

Future work will incorporate these measured diffusion rates as well as FCS derived molecule numbers into mathematical models of yeast cell polarization. EG&TH supported by NIH RR003155

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2892-Pos Cholesterol Mediates Heterogeneity in a Non-Equilibrium Lipid Membrane Model

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Cellular membranes show strong heterogeneity in their lateral organization, forming domains of various sizes. However, direct observations of the interactions between individual lipids and cholesterol have been challenging to obtain experimentally. Here, we simulate cellular membranes using a two-dimensional triangular lattice with the Monte Carlo method, including interactions between cholesterol, saturated and unsaturated lipids. In addition, simplified endo- and exocytosis events keep the system far from equilibrium. Results confirm that cholesterol plays the role of mediator between unsaturated and saturated lipids, consistent with experimental observations. Higher cholesterol concentrations lead to a weaker temperature dependence of the average energy per bond. At lower temperatures, under some conditions we also observe ordered cholesterol super-lattice structures within saturated domains. Finally, the endo- and exocytosis lead to a broader range of domain sizes, which we have quantified using Ripley's K-test. These non-equilibrium simulations of the direct interactions between cholesterol and lipids provide a more realistic picture of cellular membranes on the molecular scale, which may allow significant predictions to be made as a function of membrane composition.

2893-Pos Mobility Of Tsr, TatB And PhoR In The Inner Membrane Of Escherichia Coli

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Board B196

Many transmembrane and membrane associated proteins display non-random distributions, such as clustering into finite-sized domains or localization to specific regions, in both prokaryotic and eukaryotic cells. In many cases it is believed that these non-random distributions are essential for, or at least facilitate, function. Here we present observations of the distribution and mobility of three transmembrane proteins that show distinct localization patterns in the inner membrane of *Escherichia coli.*

1. The serine chemoreceptor, Tsr, labeled by the yellow fluorescent protein variant Venus, which is found primarily at the poles. Single molecule imaging of the mobility Tsr reveals a slow/immobile fraction (93% of the observed molecules) with an average diffusion coefficient D = 0.0125 ± 0.0024 $\mu m^2/s$

(n=402) restricted to movement in the polar regions and a small (7%) highly mobile fraction distributed throughout the cell (D = 0.243 ± 0.069 $\mu m^2/s$, n=30).
2. The twin arginine translocation protein, TatB, also labeled by Venus, which is found evenly distributed throughout the inner membrane. TatB molecules were found to be highly mobile throughout the membrane with an average diffusion coefficient D = 0.16 ± 0.02 $\mu m^2/s$ (n=394).
3. The transmembrane histidine sensor kinase, phoR, labeled by monomeric red fluorescent protein, mRFP, which is found in distinct domains that are evenly distributed throughout the inner membrane.

These domains are mobile with a diffusion coefficient of 0.025 ± 0.0014 $\mu m^2/s$ (n=180). Observation of individual PhoR molecules reveals a diffusion coefficient of 0.031 ± 0.0046 $\mu m^2/s$ (n=23) implying that most PhoR proteins in the membrane are contained within these domains. After longer observation, PhoR is found to be localized primarily in large domains that form at each pole implying a slow coalescence of the mobile PhoR containing domains to the polar regions.

2894-Pos A Hidden Markov Model of Single Particle Tracks to Estimate Kinetic Parameters

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Board B197

Single particle tracks of labeled plasma membrane-associated proteins frequently show deviations from random Brownian motion and exhibit confinement. Such confinement is usually transient, and is variously attributed to membrane heterogeneities, such as lipid-raft domains, the presence of rigid obstacles, or interaction with other membrane-bound or cytosolic proteins. We examine the dynamics of a diffusing membrane-anchored protein, that interacts with a homogeneously distributed binding partner, such as a cytoskeletal tether. The system is described by the diffusion coefficient in the free and bound states, and the transition probabilities between the two states. We model the resulting single particle tracks as the outcome of a hidden Markov model, whose underlying state sequence (free or bound) is hidden, while the particle position at each observation time is recorded. In this formulation, the likelihood of the observed sequence of displacements in a track is obtained as a function of the four model parameters. We then optimize the model parameters to maximize this likelihood function and reliably estimate the diffusion coefficient for each state and the equilibrium constant for transitions between the two states. We assign statistical error bounds on these estimates. Further, if the two diffusion coefficients are sufficiently well-separated, we also obtain first order reaction rates for the interstate transitions, and classify the free and bound segments of a single track. Thus, our model estimates important biophysical parameters in a statistically rigorous fashion from single particle tracks.

2895-Pos Large Scale Organization In Crowded Membranes

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Board B198

Over the past years, the classical fluid mosaic model - in which membrane proteins have ample space to explore the entire membrane - has undergone some serious revision. In actuality, the membrane environment is highly crowded and heterogeneous. Crowding has profound implications for the dynamical behavior of the proteins, and is therefore a determining factor for the mechanisms of cell signaling. Only very recently the importance of membrane mediated interactions in such processes was recognized. Here we use two-phase GUVs (giant unilamellar vesicles) with multiple budded, liquid ordered domains to model this class of interactions. Such budded domains repel as our recent analytical model of completely phase separated GUVs (Semrau, Idema et al.) suggests. Here we measure the strength of the repulsion by analysis of domain diffusion and find that it gives rise to a preferred domain size. Furthermore, we observe that the interaction strength has peaks at distinct domain sizes. These sizes correspond to the addition of a domain to a shell of domains surrounding a central, pinned domain. In a crowded system governed by membrane mediated interactions this "shell model" has two important biological implications:

1. clustering of proteins of similar size or interaction strength is promoted
2. a highly ordered state with mostly 5, 6 or 7 neighbors is favored.

In conclusion, membrane mediated interactions, which have a much longer range than electrostatic, van der Waals or hydrophobic forces, give rise to large scale organization in crowded cell membranes.

2896-Pos MHC Class II Molecules on B cells: Biophysical Studies of Raft Localization

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Board B199

MHC class II molecules have been reported to associate with membrane rafts on various cell types. We have examined the extent and nature of raft association of various truncated and mutated I-A^k molecules expressed on M12C3 and K46J B cells and having various capacities to present antigen to T cells. Quantitative analysis of Western blots of class II from sucrose density gradient ultracentrifugation shows that the amount of low buoyant density I-A^k is reduced or eliminated by methyl β-cyclodextrin (MBCD) treatment and is often increased by antibody crosslinking. For nine cell lines examined, the degree of raft localization appears significantly correlated with the level of surface expression as evaluated by flow

cytometry. Similarly, time-resolved phosphorescence anisotropy measurements show that cholera toxin B (CTB) treatment typically increases the average anisotropy of class II molecules and that cells pretreated with MBCD exhibit reduced effects of CTB. However, measurements of FRET between CTB-labeled GM1 and mAb-labeled class II have not shown proximity between these molecules, presumably because specific association is lacking. Polarization homo-FRET imaging suggests that, at least for wild-type I-A^k, some class II molecules are constitutively aggregated into dimers or larger structures. Such "dimers of dimers" or "superdimers" have been observed for other class II molecules and their existence for I-A^k has been inferred from mutational analysis. However, the question of whether such aggregated class II molecules are those localized in rafts has not been resolved. The relation of these data to a role for raft association of class II species in signal transduction in antigen-presenting cells and the possible linkage of class II molecule homotropic association to such signal transduction are discussed further.

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2897-Pos Imaging Membrane Dynamics Participating in Immunoreceptor Signaling

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Board B200

The molecular ordering of the lipids within a biomembrane can profoundly change the local biophysical properties, such as lateral and rotational diffusion, permeability, and fluidity, and these properties, in turn, may affect protein or enzyme activity, transient compartmentalization, and trafficking of vesicular compartments. Specialized nanodomains (or "rafts") within the plasma membranes of mast cells (immune cells) have been implicated in facilitating the allergic response by compartmentalizing (or excluding) transiently relevant signaling proteins; however they have not yet been observed directly in living cells. We have developed a quantitative optical approach toward the elusive goal of visualizing the spatial and temporal dynamics of these nanodomains with specific signaling molecules. By exploiting the nanoscale sensitivity of fluorescence lifetime and fluorescence polarization anisotropy, we can probe membrane nanodomain dynamics below optical resolution under physiological conditions. We find that the membrane nanostructure changes where antigen-crosslinked high affinity IgE receptor localizes. Importantly these changes correlate well with the functional response, as assessed by stimulated tyrosine phosphorylation. Our biophotonics experimental approach should be directly applicable for investigating any signaling pathway that may be dependent upon localize membrane ordering.

2898-Pos Lateral Mobility Enhances E-cadherin Signaling

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Board B201

Cell-cell communication is crucial for proper tissue development and function. Membrane-associated adhesion proteins that mediate these interactions exhibit complex patterns of motion along the cell surface, a property that is difficult to capture in model systems but is often posed to modulate cell signaling. Here, we examine the impact of lateral mobility on E-cadherin signaling using a supported lipid bilayer (SLB) model. Glass beads (5 micrometer diameter) were modified with a dimeric protein consisting of the extracellular domain of human E-cadherin fused with an Fc region (IgG constant region) and appended with a poly-His tail (hEF6H). Immobilized hEF6H was prepared by adsorption onto these beads, yielding controlled surface concentrations of up to 1200 molecules / micrometer^2. Laterally mobile hEF6H was prepared by forming SLBs of DOPC + 6% DOGS-NTA-Ni on beads, which were then loaded with this protein. Tethered hEF6H on glass exhibited a lateral diffusion coefficient of ~0.4 micrometer^2/sec, and a concentration of 300 molecules/micrometer^2. MDCK cells expressing both GFP-labeled Rac1 and DsRed-labeled E-cadherin were seeded onto glass substrates coated with collagen I and allowed to spread for 1hr. Response to hEF6H beads seeded directly onto individual cells was examined by live microscopy.

Laterally mobile hEF6H was more effective in generating a cellular response than its immobile counterpart. Beads containing 300 molecules/micrometer^2 of mobile hEF6H induced clustering of both Rac1 and cellular E-cadherin. Similar clustering of these proteins was also observed on beads containing 1200 molecules/micrometer^2 of immobilized hEF6H but not at 300 molecules/micrometer^2. Interestingly, we did not observe significant recruitment of bilayer-tethered hEF6H to the sites of cell-bead contact, suggesting that this effect is not simply the result of a local increase in concentration on the bead. The effect of protein mobility on specific pathways involved in E-cadherin signaling is currently under investigation.

2899-Pos Single Molecule Fluorescence Studies of Protein Domain Docking and Diffusion on Model Membrane Surfaces

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Board B202

Proteins containing membrane-binding PH and C2 domains are involved in cellular signaling in many pathways. The thermodynamics and kinetics of membrane docking control the physiological function of these targeting domains *in vivo*. Here, we measure the equilibrium binding and dissociation kinetics of individual domains

and full-length proteins to lipid bilayers using total internal reflection fluorescence (TIRF) microscopy. In addition, we use TIRF to measure lateral diffusion of individual molecules in order to directly determine diffusion constants and gain information on specific protein-lipid interactions. Single-molecule techniques enable kinetic measurement under conditions in which equilibrium is not perturbed, as well as allowing resolution of multiple populations with distinct lateral diffusion properties. To our knowledge, this is the first single-molecule study using isolated proteins and supported lipid bilayers to probe molecular mechanisms of protein membrane docking. Both Ca^{2+}-regulated docking of C2 domains, and PIP_3-targeted docking of PH domains are being studied and the latest findings will be reported. Overall, we find that single molecule measurements complement and extend traditional measurements for the elucidation of protein-membrane docking mechanisms.

Lipids and Signaling on Membrane Surfaces

2900-Pos Lipid Raft Disruption Alters Both Local And Global IP_3-mediated Calcium Release In Human Neuroblastoma Cells

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Board B203

Localized calcium signals such as calcium 'puffs' regulate physiological processes as varied as synaptic transmission and gene transcription, and arise because of the clustered distribution of inositol trisphosphate receptor (IP_3R) channels in the endoplasmic reticulum (ER) membrane. In light of growing evidence for the role of lipid rafts in spatially organizing membrane proteins, we sought to examine the possible role for lipid rafts in regulation of IP_3-mediated calcium signaling. Two-photon microscopy of laurdan fluorescence confirmed that the membrane order of SHSY-5Y cells increased when treated with methyl-beta-cyclodextrin (mβCD) for 10 minutes to remove membrane cholesterol, presumably disrupting lipid rafts, and subsequently decreased with replacement of cholesterol. Furthermore, we found that mβCD treatment reduced the amplitudes (but not wave velocity) of global intracellular $[Ca^{2+}]_i$ transients evoked by carbachol stimulation and by UV-flash photolysis of caged-IP_3, whereas these responses recovered following subsequent re- addition of cholesterol. The reduction in amplitude appears not to arise *via* calcium inhibition of IP_3R, because basal calcium levels remained unchanged after mβCD treatment. To then explore the effects of lipid environment on local calcium signals, we used short UV-flash photolysis durations to dissociate global rises of calcium into discrete puffs. We observed no changes in puff amplitudes or numbers of responding puff sites per cell following mβCD treatment, but the frequency of puffs was significantly reduced.

2901-Pos Endogenous Phosphatidylinositol 4, 5-bisphosphate Directly Regulates Cerebral Artery BK Channel Function

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Board B204

We have shown that exogenous application of phosphatidylinositol 4, 5- bisphosphate (PIP_2) increases BK channel activity (NPo) in cerebral artery myocytes and modifies cerebral artery myogenic tone (Liu et al., 2006; Vaithianathan et al., 2007). Here, we conducted a series of experiments to determine whether endogenous PIP_2 controls cerebral artery myocyte BK NPo and arterial tone. First, under physiological $Ca^{2+}{}_i$ (3 μM; Pérez et al., 1999), BK NPo ran down almost immediately after patch excision from the myocyte, becoming ~50% of initial values 30 min after. In the presence of 0.1 μ M okadaic acid, bath application of 0.5 mM Mg-ATP (Lin et al., 2005) totally rescued the rundown, which likely reflects channel activation by PIP_2 being regenerated *via* PI4KIIα (Yaradanakul et al., 2007). Moreover, PIP_2 monoclonal antibodies (1:1000) applied on top of Mg-ATP to the cytosolic side of the plasma membrane dropped NPo to <35% of control Second, co-transfection of HEK293 cells with BK-channel forming (cbv1)+smooth muscle-abundant (β_1) subunits and PI4kinaseIIα resulted in robust potentiation of NPo when compared with cbv1+ β_1 alone. This result is consistent with the idea that transfection of PI4KIIα leads to increased PIP_2 levels (Yaradanakul et al., 2007) and, thus, increased NPo. Third, perforated-patch recordings in freshly isolated myocytes under physiological conditions of voltage and Ca^{2+} demonstrated that inhibition of PLC under block of PKC and SR Ca^{2+}-ATPase (IP3 and DAG final targets) resulted in a dramatic increase in both activation slope and amplitude of total BK current, peak amplitude reaching 6,608.5±1,983.1% of control. Finally, PLC inhibition on top of block of PKC and SR Ca^{2+}-ATPase caused a robust increase in arterial diameter (+15.1±0.1%). We conclude that endogenous PIP_2 directly activates cerebrovascular myocyte BK channels and, thus, decreases vascular tone.

2902-Pos Conformational Manifold And Bilayer Insertion Of A Membrane-anchored Ganglioside, GM1

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Board B205

Specific recognition of membrane-bound gangliosides by proteins is critical for many biological processes including cell growth and maturation; however, little is known about the conformation and orientation of gangliosides in their biological environment. Here we report molecular dynamics (MD) simulations and NMR experi-