mouse sperm by a cAMP/PKA-mediated pathway with a pharmacological profile suggestive of a Slc29a (ENT) equilibrative nucleoside transporter. Using qRT-PCR we find in adult mouse testis 160- and 32-fold greater expression of Slc29a1 than of the other surface-membrane ENT transporters Slc29a4 and Slc29a2. However, Slc29a1 protein was not found on mature sperm using immunocytochemistry. Moreover, wildtype and Slc29a1-null mice accelerate at similar rates (1.5-2.0 Hz min^{-1}) in response to Cl-dAdo indicating that Slc29a1 is not required for Cl-dAdo action. Consistent with this observation, the accelerating action of Cl-dAdo resists the Slc29a-selective inhibitor nitrobenzylthioinosine (NBTI; 10 μM). The accelerating action of Cl-dAdo additionally resists replacement of external Na^+ with $NMDG^+$ indicating that Slc28a concentrative nucleoside transporters (CNTs) also are not required. Interestingly, the Adenosine A3 receptor-selective agonist Cl-IB-MECA (25 μM) is nearly as effective as Cl-dAdo in accelerating sperm beat frequency, suggesting a possible role for cell surface A3 receptors in Cl-dAdo-mediated increases in sperm motility. Two A3 isoforms are expressed in the mouse Adora3i1 and Adora3i2; Adora3i2 expression is testis specific. Adora3i1 null sperm increase beat frequency in response to both Cl-IB-MECA (25 μM) and Cl-dAdo (25 μM), so this isoform is not needed for sperm response to adenosine. Sperm response to Cl-dAdo and Cl-IB-MECA is diminished after pertussis toxin treatment of cells suggesting the receptor is $G_{\alpha i/o}$ coupled. We are currently testing functionality of the testis-specific novel Adora3i2 isoform in a heterologous system. *Support from U54-HD12629 of the SCCPRR program of NICHD. L.A.B. supported in part by 5-T32-HD007453.*

2576-Pos

Novel Receptor-Mediated Endothelial Cell Chemotaxis

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The directed migration of endothelial cells away from existing blood vessels is a critical step during cancer progression. Specific receptor-ligand interactions initiate intracellular signal transduction pathways and asymmetric cytoskeletal reorganization, leading to migration towards ligand sources. Disruption of these receptor-ligand interactions is a common goal in cancer drug development, and a key hurdle is the discovery of new receptor-ligand partners that are suitable targets. The G-protein-coupled receptor 124 (GPR124) is enriched in the endothelium of the developing brain and regulates embryonic CNS angiogenesis; however, its ligand and its potential role in endothelial cell chemotaxis are unknown. We examined the migration of mouse brain-derived endothelial cells (bEnd3) within custom microfluidic devices capable of generating stable concentration gradients of chemotactic ligands. The bEnd3 were genetically modified either to overexpress (GPR124+) or knock down (GPR124-) receptor expression. GPR124+ cells were observed to chemotax in response to a 0.025% gradient/micron of embryonic brain cortical cell conditioned medium (CM), while GPR124- cells followed random walk statistics in identical gradients. Mathematical analysis of the cell migration pathways (n~100 for each condition) revealed that net migration displacement, migration persistence time, and migration speed were increased for GPR124+ cells over GPR124- cells in both CM gradients as well as uniform CM concentrations. This suggests that in addition to being able to initiate asymmetric cytoskeletal reorganization, GPR124 signaling also triggers a general enhancement in cell motility. GPR124+ cells were also found to migrate towards VEGF-depleted CM, demonstrating that GPR124-mediated chemotaxis is independent from VEGF-mediated chemotaxis, a widely studied ligand of endothelial cells and a common cancer drug target. GPR124+ cells did not chemotax towards CM of 193T cells, a negative control kidney cell line. These results demonstrate utility of a new quantitative microfluidic platform to identify novel receptor-ligand partners for potential cancer drug development.

2577-Pos

Mobility of G Proteins is Heterogeneous and Polarized During Chemotaxis

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The interaction of G-protein-coupled receptors with G proteins is a key event in transmembrane signal transduction leading to vital decision-taking of the cell. Here we applied single-molecule epifluorescence microscopy to study the mobility of both the Gβγ and the Gα2 subunits of the G protein heterotrimer in comparison to the cAMP-receptor responsible for chemotactic signaling in Dictyostelium discoideum. Our experimental results suggest that ~30% of the G protein heterotrimers exist in receptor pre-coupled complexes. Upon stimulation in a chemotactic gradient this complex dissociates, subsequently leading to a linear diffusion/collision amplification of the external signal. The further observation of partial immobilization and confinement of Gβγ in an agonist, F-actin and Gα2-dependent fashion led to the hypothesis of functional nanometric domains in the plasma membrane that locally restrict the activation signal and in turn lead to faithful and efficient chemotactic signaling.

2578-Pos

Physical Properties of Fibrinogen Substrates Control Integrin Mediated Cell Adhesion

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The physical properties of substrates are known to control cell adhesion via integrin-mediated signaling. Recently, we have shown that binding of fibrinogen to the surface of fibrin gel prevents integrin $\alpha_M\beta_2$-mediated leukocyte adhesion by creating an anti-adhesive layer. Furthermore, fibrinogen immobilized on various surfaces at high density supports weak cell adhesion whereas at low density it is highly adhesive.

To gain an understanding of the mechanism underlying differential cell adhesion, we extended the cell adhesion assays to platelets carrying integrin $\alpha_{IIb}\beta_3$. The results showed a similar behavior indicating that the process is independent of the type of integrins. In order to quantify the adhesion forces, we applied single cell force spectroscopy (SCFS). In this assay, a single cell is attached to a tipless cantilever of an atomic force microscope (AFM) and force-distance curves for different surfaces are acquired. For cells carrying $\alpha_M\beta_2$-integrins we found significant lower adhesion forces for high- compared to low-density fibrinogen substrates.

Furthermore, we analyzed the adhesive behavior of fibrinogen surfaces using force spectroscopy with a silicon nitride AFM tip. These experiments, unrelated to the cells and integrins, show similar behaviors as the cell adhesion assays. AFM images of the different substrates indicate that fibrinogen deposition at high density results in an aggregated multilayered material characterized by low adhesion forces. However, low-density fibrinogen produces a single layer in which molecules are directly attached to the solid surface resulting in higher adhesion forces.

The data suggest that deposition of a multilayered fibrinogen matrix prevents stable cell adhesion by modifying the physical properties of surfaces resulting in reduced force generation with implications for hemostasis and biomaterial applications.

2579-Pos

Mechanochemical Signaling in Glomerular Podocytes

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Kidney glomeruli function as filters, allowing the passage of small solutes and waste products into the urinary space, while retaining essential proteins and macromolecules in the blood stream. They achieve this through a filtration apparatus comprised of three layers, a fenestrated glomerular endothelium, a basement membrane (GBM) and an epithelial cell layer. Glomerular epithelial cells (podocytes) culminate into interdigitating foot processes (FPs) between adjacent cells. Specialized structures known as slit diaphragms (SDs) function as modified adherens junctions connecting podocyte foot processes. These structures are under constant mechanical stress due to fluid pressure, driving filtration across the barrier. Disruption of the SDs or podocyte damage can lead to progressive loss of protein into the urine (proteinuria) and ultimately to kidney failure. Dysregulation of the podocyte actin cytoskeleton has been implicated in mechanisms of proteinuria.

Podocyte adhesion on collagen activates the FAK/ERK pathway, a known mediator of F-actin assembly. Here, we mechanically stimulated adherent podocytes using a cell stretcher. A 20% strain at 1 Hz for 20 min increased ERK phosphorylation compared to control. We observed a peak after 2 min and thereafter an exponential decrease. This suggests that podocytes are responsive to early external strain, which might be transduced into a chemical signal capable of modulating the actin cytoskeleton. We hypothesize that adhesion via integrin receptors triggers mechanochemical signals which may in turn affect cell-cell and cell-GBM connections. Currently, we are studying the effects of cytoskeletal organization/regulation in wildtype and mutated podocytes, with a focus on determining cell mechanical properties using magnetic tweezers and atomic force microscopy.

2580-Pos

A Model of Atherosclerosis Plaque Formation and Development

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Atherosclerosis is a progressive disease characterized in particular by the accumulation of lipids and fibrous elements in arteries. Over the past decade,

scientists come to appreciate a prominent role for inflammation in atherosclerosis. This work is devoted to the construction of a model of formation of lesions of earliest type arising in atherosclerosis. We consider that the inflammatory process starts with the penetration of Low Density. Lipoproteins cholesterol in the intima. This phenomenon is related to the local blood flow dynamics. The blood flow is simulated by the Navier-Stokes equations, together with the continuity equation. LDL transport in lumen of the vessel is modelled with convective-diffusion equation, and inflammatory process is solved with three additional reaction-diffusion partial differential equations. The plaque growing is modeled by Stokes equation [1], [2] and [3]. The input data for the flow waveforms are taken from MR phase contrast flow measurements of the patient. The computed results show velocity profiles, shear stress distribution and LDL distribution in blood lumen [2] and [3]. Computed concentration oxidized LDL, macrophages and cytokines indicate that there is a newly formed matter in the intima, especially in the flow separation region of LAD during most of the diastole. In summary, full model of plaque formation and development, coupled with blood flow and LDL concentration in blood, is created.

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2581-Pos

Determination of Single Molecule erbB1 Homodimer Lifetimes Using Single Quantum Dot Tracking and a Diffusive Hidden Markov Model

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Signaling by the erbB family of transmembrane tyrosine kinase receptors controls many cellular pathways, including growth and differentiation, while dysregulation of erbB signaling is a hallmark of many cancers. To understand how receptor interactions regulate signaling, we have developed new multi-color single molecule tracking and analysis techniques. Diffusion and dimerization of erbB1 receptors on live cells were monitored with single particle tracking by using antibody fragments or ligand probes coupled to quantum dots (QDs). As a probe for resting receptors, we used a non-activating, non-competing, monovalent fragment of an anti-erbB1 heavy-chain only antibody (VhH). To track activated receptors, QDs conjugated to EGF ligand were employed. Dimer lifetimes were determined from two-color single particle trajectories using a modified Diffusive Hidden Markov Model. This analytic two-state (bound and free) model uses the two-color QD trajectories to find bound states and quantify the dimerization lifetime using a global estimation over many trajectories. We capture the behavior of erbB1 receptors diffusing on the surface of live A431 cells, including the formation of long-lived dimers between two EGF-QD-erbB1 complexes. Treatment with PD153035, a specific inhibitor of erbB1 tyrosine kinase activity, increases receptor diffusion rate and reduces the lifetime of erbB1 homodimers, suggesting a role for the active kinase domain in formation of stable dimers. These results demonstrate the capabilities of innovative imaging and analysis approaches to measure protein-protein interaction kinetics in real time.

2582-Pos

Quantum-Dot, Magnetic Particle and Expression-Probe Based Sensing of erbB Protein Dynamics and Development of Tumor Diagnostics

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The bright fluorescence emission and resistance to photobleaching make quantum dots (QDs) ideal for single-particle detection and permit imaging over prolonged time periods. Because of these advantages, we have used QDs in combination with expression probes and advanced microscopy techniques to investigate the protein dynamics of tyrosine kinase receptors in vivo and in vitro [1,2]. A new class of nanoparticle actuators has been devised that we denote magnetic switches [MS]. These switches are superparamagnetic iron oxide nanoparticles (SPION) specifically targeted to cell surface receptors that serve as magnetic actuators, uncoupling oligomerization and ligand binding [3].

Activation of the erbB receptor tyrosine kinases (RTKs erbB1-4) induced by the extracellular binding of peptide ligands triggers signaling cascades responsible for cellular motility, cell division, and differentiation. We have genetically tagged the ErbB proteins with the acyl carrier protein (ACP) sequence. QDs have been targeted to receptors on the external cell surface via the growth factor receptor, EGF, or by covalently linking to the ACP tag allowing the visualization in living cells of individual receptors, the diffusion of which has been determined on different cell types. We have also used them to detect dimerization, receptor recycling and endosomal trafficking.

The basic research performed on erbB RTKs has led to the application of targeted QDs and magnetic NPs to distinguish glioblastoma tumors from healthy brain tissue [4]. These probes can distinguish both high grade and low grade tumors, the latter of which show no contrast by MRI nor take up probes such as 4-ALA. These targeted nanoparticles have great promise for facilitating the accurate resection of the tumor (including small cellular foci) with minimal loss of normal brain tissue.

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Endoplasmic Reticulum & Protein Trafficking

2583-Pos

Geometric Curvature Sensing of Alpha-Helical Proteins

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Understanding how specific proteins are recruited to membranes is crucial to understanding key biological functions. Membrane curvature sensing is increasingly recognized as a powerful means for guiding protein localization, coupling protein binding to the local membrane geometry. The mechanisms underlying curvature sensing remain poorly quantified, however. We focus on the two curvature-associated proteins that share an amphipathic alpha-helical structure. The first, SpoVM, is a small (26 residue) protein from Bacillus subtillus that has recently been shown to preferentially bind to convex surfaces [K.S. Ramamurthy et al., Science 323: 1354 (2009)]. The second is the 23 amino acid N-terminal helical domain of Sar1, which initiates the assembly of COPII coated vesicles at the endoplasmic reticulum and which recent work [Parthasarthy Lab, unpublished] has shown to dramatically alter membrane rigidity. Using both microfabricated surfaces that present controlled curvatures to the proteins and optical-trap based assays involving dynamic membrane deformation, we quantify the curvature dependence of the membrane binding affinity of these structurally similar yet functionally disparate proteins.

2584-Pos

Modulation of Membrane Rigidity by Sar1, a Vesicle Trafficking Protein

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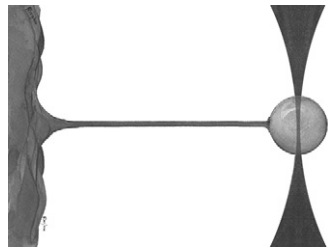
Intracellular cargo trafficking involves dramatic changes in membrane shape, the mechanics of which remain poorly understood. We focus on Sar1, the key regulator of the coat protein complex II (COPII) family that ferries newly synthesized proteins from the endoplasmic reticulum (ER), and the only member of the COPII coat that interacts directly with the ER lipid bilayer membrane. To investigate whether Sar1 has a role beyond merely localizing the other COPII proteins, we directly measure the force involved in membrane deformation as a function of its concentration, using optically trapped microspheres to pull tethers from in vitro lipid membranes whose composition and large surface area mimic the composition and geometry of the ER. We find that Sar1 lowers the rigidity (bending modulus) of lipid membranes to nearly zero in a concentration-dependent manner. Moreover, Sar1 lacking its N-terminal amphipathic helix induces negative (concave) spontaneous membrane curvature. These results reveal a paradigm-altering insight into COPII trafficking: Sar1 actively alters the material properties of the membranes it binds to, lowering the energetic cost of curvature generation.



2585-Pos

Tail-Anchored Membrane Protein Recognition by Get3

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Targeting of newly synthesized membrane proteins to the endoplasmic reticulum is an essential cellular process. Most membrane proteins are recognized and targeted co-translationally by the signal recognition particle. However, nearly 5% of membrane proteins are 'tail-anchored' (TA) by a single C-terminal transmembrane domain that cannot access the co-translational pathway. Instead, TA proteins are targeted post-translationally by a conserved ATPase termed Get3. The mechanistic basis for TA protein recognition or targeting by Get3 is not known. Here we present crystal structures of Get3 in 'open' (nucleotide-free) and 'closed' (ADP•AlF4–bound) dimer states. In the closed state, the dimer interface of Get3 contains an enormous hydrophobic groove implicated by mutational analyses in TA protein binding. In the open state, Get3 undergoes a dramatic rearrangement that disrupts the groove and shields its hydrophobic surfaces. These data provide a molecular mechanism for nucleotide-regulated binding and release of TA proteins during their membrane targeting by Get3.