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.

[1] Lidke et al J Cell Biol. 170:619 (2005)
[2] Hagen et al "Single Molecule Dynamics" 117 (2008)
[3] Bharde et al submitted (2009)
[4] Arndt-Jovin et al IEEE Trans NanoBios 8:65 (2009)

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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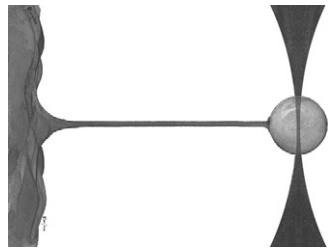
Intracellular cargo trafficking involves dramatic changes in membrane shape, the mechanics of which remain poorly understood. We focus on Sar1, the key regulator of the coat protein complex II (COPII) family that ferries newly synthesized proteins from the endoplasmic reticulum (ER), and the only member 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.