1275-Pos
Characterization of Selectin-Mediated Cell Binding in Shear Flow Using Micropatterning Technology and Modeling
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Cellular interactions play an essential role in diverse (patho)physiological processes such as leukocytes extravasation and cancer metastasis. Selectins initiate the tethering and rolling of free-flowing cells on activated endothelium before the integrin-dependent firm adhesion under flow. Utilizing microfluidics devices and protein micropatterning technology, the adhesion kinetics of the HL-60 leukocyte-like cells to immobilized P-/L-selectin was investigated. Multiple selectin patches of varying lengths (6-160μm; in the direction of flow) and constant width (10μm) were patterned on a glass substrate to provide specific adhesion. A PDMS layer, which had been cured and peeled off from a 25μm height microchannel mold, was bonded on the patterned glass substrate to form the microfluidics device. Cell suspensions were perfused through the device under prescribed shear stresses varying from 0.25-2dyn/cm^2. Our data reveal the existence of a critical patch length, L_c, which represents the cell rolling distance to form an initial binding and is a function of the wall shear stress and the selectin density on the substrate. At $L<L_c$ no cell binding is detected on the patch. At $L \geq L_c$, the number of tethered cells increased with the patch length. A theoretical model was developed to analyze our data, and accounted for the selectin-mediated reversible binding kinetics, the shear-controlled association rate and two-pathway dissociation rate. Our model successfully fits to the experimental results and, in particular, the transition of the ''shear threshold phenomenon'' was correctly captured. Our model also shows that the ''shear threshold phenomenon'' is not observed if the selectin site density is >3000sites/μm^2. In summary, our analysis has identified the minimum cell rolling distance required for selectin-dependent binding to occur in a shear flow. Moreover, we have developed the first analytical equation to model cell binding with the shear threshold phenomenon.

1276-Pos
Distinguishing Binding from Allosteric Action in Escherichia Coli Phosphofructokinase
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Thermodynamic linkage analysis involves the determination of binding parameters for substrate and allosteric ligand individually to free enzyme plus a coupling parameter that quantifies the nature and magnitude of allosteric interaction. Thus allosteric action can be differentiated from allosteric ligand and substrate binding affinities. Previously thermodynamic linkage analysis of the allosteric inhibition of E. coli PFK (EcPFK) by either phosphoenolpyruvate (PEP) or its analogs implied that different functional groups within the allosteric molecules play different roles in each of these two attributes. The data suggested in particular that the oxygen that bridges the phosphate group to the rest of the molecule in PEP plays a larger role in ligand binding than in allosteric signal propagation. In an effort to continue to elucidate the role of the PEP bridging oxygen the crystal structure of wild type EcPFK bound to PEP was determined for the first time. S58 was found to be in position for possible interactions with this bridging oxygen of PEP. EcPFK with the mutation S58A was constructed and characterized. The modified enzyme exhibits no variation in Km or Vmax compared to the wild type enzyme. However, the Kd for PEP is more than 85 times that of wild type while the allosteric coupling is diminished by 27%. The analysis of the S58A mutant provides additional evidence that the bridging oxygen in PEP primarily contributes to the binding free energy of PEP and makes a relatively minor contribution to the allosteric effect per se. Supported by NIH Grant GM33216 and Welch Foundation Grant A1548.

1277-Pos
Superheating of Ice in the Presence of Ice Binding Proteins
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Antifreeze proteins (AFPs) are a class of ice binding proteins that are found in many cold-adapted organisms, where they serve as inhibitors of ice crystal growth and recrystalization. Adsorption-inhibition theory suggests that AFPs adsorb to ice surface and surface-adsorbed AFPs should prevent ice from melting as well as from freezing and evidence for such activity was shown experimentally (Knight and Devries, Science 1989). However, so far there has been no measurement of the melting inhibition of ice in AFP solutions. We examined a series of hyperactive and moderate AFPs and measured the melting hysteresis activity, which is defined as the elevation of the actual melting temperature above the equilibrium melting point. We observed that superheated ice crystals can be held stable for hours in AFP solutions. The measured superheating values were much more appreciable for hyperactive AFPs in comparison to moderate ones. The amount of this elevation was only a fraction of depression the freezing. Still, in these temperatures, we measured remarkably fast melting velocities of the superheated ice crystals which were proportional to the superheating values. Furthermore, we visualized fluorescently labeled AFPs on superheated ice crystals. The observation of superheating of by AFPs strongly suggest that AFPs adhere to ice surface as a part of their mechanism of action and the binding to ice surface is irreversible.

Membrane Protein Function II

1278-Pos
Bacterial Porin Disrupts Mitochondrial Membrane Potential and Sensitizes Host Cells to Apoptosis
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The bacterial outer membrane porin PorB of Neisseria gonorrhoeae is an essential virulence factor of these pathogenic gram-negative bacteria which finally lead to the apoptotic degradation of host cells. In course of the infection process PorB is known to be transferred to the mitochondrial membranes of infected cells where it induces the dissipation of mitochondrial membrane potential by a so far unknown mechanism. Using single channel electrophysiology and spectroscopic techniques we were able to reveal the molecular mechanisms underlying this regulated process.
Based on a detailed study of the single channel characteristics of wildtype PorB and the mutant PorBK98Q lacking the putative ATP-binding site, we could identify the prerequisites for the formation of open pores in the mitochondrial inner membrane at physiological membrane potentials. Spontaneous incorporation of the β-barrel protein into membranes was followed by using CD-spectroscopy revealing large structural rearrangements during membrane insertion. We are now able to explain the molecular mechanisms by which targeting of bacterial toxin PorB to mitochondria of infected cells is setting up host cells to apoptosis.

1279-Pos
Functional Reconstitution of Membrane Proteins Monitored by Isothermal Titration Calorimetry
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Membrane protein reconstitution denotes the transfer of a purified (but usually inactive) membrane protein from detergent micelles into lipid bilayers. The aim is to restore the native protein fold and function in a well-defined membrane environment. The reconstitution yield critically depends on a wide range of parameters, including temperature, pH, ionic strength, as well as the type and concentration of detergent, lipid, protein, and additives. Moreover, it is of paramount importance to initiate the reconstitution process from a suitable lipid-to-detergent ratio. Unfortunately, however, assessing the success of a reconstitution experiment has thus far been limited to a trial-and-error approach, which has substantially slowed progress in the field.
To address this problem, we have established high-sensitivity isothermal titration calorimetry (ITC) as a powerful tool for monitoring the reconstitution of membrane proteins into lipid vesicles. Using ITC, the complex changes in the physical state of a protein/lipid/detergent mixture during reconstitution can be followed in a non-invasive and fully automated manner. Here we exemplify this approach for the prokaryotic potassium channel KcsA, which we first purified in detergent micelles and then reconstituted into stable proteoliposomes at very high protein densities. Electrophysiological experiments performed in planar lipid membranes confirmed that KcsA regained its functional activity upon reconstitution.

1280-Pos
Quantitative Measurements of Receptor Interactions in Mammalian Cells: Implications for Human Pathologies
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Receptor Tyrosine kinases (RTKs) are family of single-pass cell membrane receptors with extracellular ligand-binding domains and intracellular kinase domains, which conduct biochemical signals via lateral dimerization in the plasma membrane. Mutations in the transmembrane (TM) domains of these receptors are known to promote unregulated signaling. An example is the Ala391Glu mutation in the TM domain of FGFR3, which leads to pathologies