1275-Pos
Characterization of Selectin-Mediated Cell Binding in Shear Flow Using Micro patterning Technology and Modeling
Konstantinos Konstantopolous, Ziqiu Tong, Luthuir Siu-Lun Cheung.
Johns Hopkins University, Baltimore, MD, USA.
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 micro patterning technology, the adhesion kinetics of the HL-60 leukocyte-like cells to immobilized P-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 0.25-2dyne/cm². Our data reveal the existence of a critical patch length, Lc, 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 \( L > L_c \), 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-associated rate ratio 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. 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
Bobby W. Laird, Gregory D. Reinhardt.
Texas A&M Univ. College Station, TX, USA.
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 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 and the oxygen atoms were placed 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. However, the Kd for PEP is more than 85 times that of wild type while the allosteric coupling is diminished in Km or Vmax compared to the wild type enzyme. However, the Km for PEP was determined for the first time. S58 was found to be in position for possible interactions with this bridging oxygen in PEP. The EcPFK with the mutation S58A was constructed and characterized. The modified enzyme exhibits no variation in Km or Vmax compared to the wild type. However, the Km for PEP is more than 85 times that of wild type while the allosteric coupling is diminished in Km or Vmax compared to the wild type enzyme. However, the Kd for PEP was determined for the first time. S58 was found to be in position for possible interactions with this bridging oxygen in PEP. The EcPFK with the mutation S58A was constructed and characterized. The modified enzyme exhibits no variation in Km or Vmax compared to the wild type. However, the Kd for PEP is more than 85 times that of wild type while the allosteric coupling is diminished in Km or Vmax compared to the wild type enzyme. However, the Kd for PEP was determined for the first time. S58 was found to be in position for possible interactions with this bridging oxygen in PEP.

1277-Pos
Superheating of Ice in the Presence of Ice Binding Proteins
Yeliz Celik, Laurie A. Graham, Yee-Foong Mok, Maya Bar, Peter L. Davies, Ido Bravslavsky.
1Ohio University, Athens, OH, USA, 2Queen’s University, Kingston, ON, Canada, 3Weizmann Institute of Science, Rehovot, Israel.
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 recrystallization. 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.

1278-Pos
Bacterial Porin Disrupts Mitochondrial Membrane Potential and Sensitizes Host Cells to Apoptosis
Anke Harsman, Michael Meinecke, Thomas Rudel, Joachim Rassow, Richard Wagner.
1University of Osnabrueck, Osnabrueck, Germany, 2MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, 3University of Wuerzburg, Wuerzburg, Germany, 4Ruhr-Universität Bochum, Bochum, Germany.
The bacterial outer membrane porin PorB of Neisseria gonorrhoeae is an essential lytic factor of these pathogens and a promising target for antibacterial agents. We show that PorB binds 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.

1279-Pos
Functional Reconstitution of Membrane Proteins Monitored by Isothermal Titration Calorimetry
Nadina Jahnke, Oxana O. Krylova, Sandro Keller.
1Leibniz Institute of Molecular Pharmacology (FMP), Berlin, Germany, 2Technical University Kaiserslautern, Kaiserslautern, Germany.
Membrane protein reconstitution denotes the transfer of a purified (but usually inactive) membrane protein to detergent micelles into lipid bilayers. The aim is to activate 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
Sarvenaz Sarabiipour, Kalina Hisrtova.
The Johns Hopkins University, Baltimore, MD, USA.
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
such as Crouzon syndrome and bladder cancer. This mutation has been shown to stabilize the isolated TM domain dimers in lipid membranes, but it is not known if it stabilizes the full length FGFR3 receptor dimers in the plasma membrane of mammalian cells.

To address the effect of the mutation in mammalian cells, we have determined free energies of dimerization for the wild type and mutant FGFR3 in mammalian (HEK293T and CHO) plasma membranes using the QI Förster resonance energy transfer (FRET) technique [Li et al., 2008]. The measured change in the dimerization free energy due to the Ala391Glu mutation is 1.2 kcal/mol, consistent with previous reports of hydrogen bond strength in proteins, as well as results for the isolated FGFR3 TM domains. Thus, we have shown that the mutation stabilizes the full length FGFR3 dimers in mammalian cells. We propose that this dimer stabilization is the major cause for FGFR3 overactivation and human pathologies.


1281-Pos
The Physical Basis Behind Achondroplasia, the Most Common Form of Human Dwarfism
Lijuan He, Kalina Hristova,
Johns Hopkins University, Baltimore, MD, USA.
Fibroblast growth factor receptor 3 (FGFR3) is a receptor tyrosine kinase which plays an important role in long bone development. The Gly380Arg mutation in FGFR3 transmembrane domain has been linked to achondroplasia, the most common form of human dwarfism. However, the exact mechanism underlying the pathology is not well understood. One hypothesis is that the mutation stabilizes the active FGFR3 dimer in the plasma membrane. To test this hypothesis, here we measure the activation of wild type and mutant FGFR3 in mammalian cells, and analyze the activation using a physical-chemical model accounting for dimerization, ligand binding and phosphorylation probabilities. Our results demonstrate that the achondroplasia mutation does not increase the dimerization propensity of FGFR3. Instead, the data suggest that the mutation induces a structural change in the unliganded dimer. We propose that this structural change is a cause for pathogenesis in achondroplasia.

1282-Pos
Spectroscopic Design of Phospholamban Mutants to Treat Heart Failure
Simon J. Gruber1, Suzanne Hayden1, Kim N. Ha1, Roger J. Hajjar2, Gianluigi Veglia1, David D. Thomas1.
1University of Minnesota, Minneapolis, MN, USA, 2Mt. Sinai Medical School, New York, NY, USA.
Ca2+ cycling through the SR in muscle cells is largely controlled by the Ca-pump (SERCA). SERCA transports Ca2+ into the SR and is inhibited by phosphorylamban (PLB) at submicromolar [Ca2+]i, and this inhibition can be relieved by adrenergic stimulation. Contraction takes place when the Ca-release channel opens and the intracellular [Ca2+]i is high. One of the most common symptoms of heart failure (HF) is impaired calcium handling, frequently resulting from decreased SERCA activity. We are using EPR and NMR to study the relationships among structure, dynamics, and function of PLB, with the goal of designing LOF-PLB mutants (PLBM) that can compete with WT-PLB and thus relieve SERCA inhibition. Several studies have shown that a pseudophosphorylated PLB (S16E-PLB) is effective for gene therapy in rodents and sheep, and we are using spectroscopic methods to refine this approach. We have developed a system for examining the function and interactions of SERCA and PLB in HEK cells. Active SERCA is expressed at a level high above basal ATPase activity, and cells are co-transfected with WT-PLB and/or PLBM to measure SERCA inhibition in living cells. Unlike S16E-PLB, these mutants are able to respond to adrenergic stimulation. In addition to quantifying SERCA activity in the presence of PLBM, we measure the ability of each mutant to compete with WT-PLB for binding to SERCA. This is done by measuring fluorescence resonance energy transfer (FRET) between labeled SERCA and WT-PLB. If PLBM displaces WT-PLB, less energy is transferred between fluorophores and a decrease in FRET is observed. Based on these results, rAAV is used to test PLBM in rodent and porcine models of HF for efficacy in vivo and ability to respond to adrenergic stimulation, with the goal of developing a novel, gene therapy based treatment for HF.

1283-Pos
The Activity of a Low-Affinity L-Arginine Transporter Quenches Peroxy-nitrite-Induced Fluorescence in Ventricular Cardiomyocytes
Jayalakshmi Ramachandran, R. Daniel Peluffo.
UMDNJ-New Jersey Medical School, Newark, NJ, USA.
We discovered a low-affinity, high-capacity L-arginine (L-Arg) transport process in rat cardiomyocytes consistent with the activity of the CAT-2A member of the y+ family of cationic amino acid transporters (Peluffo, J Physiol, 580:925-936, 2007), set to function in parallel with the previously described high-affinity, low-capacity CAT-1 (Lu et al., Biosci Rep, 29:271-281, 2009). In assessing the role of a low-affinity transporter in this setting, we propose that CAT-2A protects cardiac muscle cells by ensuring the availability of proper L-Arg levels for the synthesis of nitric oxide (NO) via NO synthase (NOS). To test this hypothesis, acutely-isolated cardiomyocytes were loaded with the dye coelenterazine that greatly increases its fluorescence quantum yield in the presence of peroxynitrite (ONOO·) and superoxide radicals. Cells were then exposed to 20 or 100 μM ONOO· and changes in fluorescence were followed with a spectrofluorometer. Addition of extracellular L-Arg reduced ONOO-induced fluorescence in a concentration-dependent manner, an effect that was not mimicked by D-arginine or L-lysine and was fully blocked by the NOS inhibitor L-NAME. L-Arg reduced fluorescence with Kᵢ values of 0.84 ± 0.12 and 1.26 ± 0.16 mM at 20 and 100 μM ONOO·, respectively. L-Arg “zero effect” on ONOO-induced fluorescence was also dependent on ONOO concentration, with values of 145 and 363 μM for 20 and 100 μM ONOO·, respectively. Below these values, decreasing concentrations of L-Arg progressively increased ONOO-induced fluorescence, an effect that was also blocked by L-NAME. All these effects can be explained by NOS-mediated NO synthesis, which may turn to ONOO production at limiting L-Arg. Since ONOO has detrimental effects on cardiac contractility, these results suggest a cardioprotective role for the low-affinity L-Arg transporter, ensuring proper supply of NOS substrate under a variety of physiological and pathological conditions.

1284-Pos
Structure and Function of the Intracellular Region of the Plexin-B1 Transmembrane Receptor
Prasanta K. Hota1, Yufeng Tong2, Junia Y. Penachioni1, Luca Tamagnone1, Hee-Won Park2, Matthias Buck1.
1School of Medicine, Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA, 2Structural Genomics Consortium, Department of Pharmacology, University of Toronto, Toronto, ON, Canada, 3Institute for Cancer Research and Treatment, University of Torino, Candilo, Italy.
Plexin family are unique transmembrane receptors protein known to regulate several cellular processes including axonal guidance in the developing nervous system. Upon activation, plexin initiates signaling processes, which involve several small GTPases of the Ras and Rho families (R-Ras, Rac1, Rnd1, and RhoD) that regulates cytoskeletal dynamics and cell adhesion. Plexins are unique amongst transmembrane receptors because its several cytoplasmic regions interact directly with small GTPases. Specifically, plexins possess a domain with homology to GTPase activating proteins (GAPs). As part of their activation, plexin family shows GAP activity toward R-Ras. However, the mechanism of activation is not known till date because of lack of information about the structure and function of these receptor proteins. In this context, we have studied the structure, function of intracellular region of PlexinB1 and their binding interaction with small GTPase. The structure is monomeric and binds to Rac1, Rnd1 as well as Rras, but not H-Ras. These findings suggest that the monomeric form of the intracellular region is primed for GAP activity and extend a model for plexin activation.


Protein Structure I

1285-Pos
Structural and Functional Studies of Bacterial Toxin-Antitoxin Systems
Gregory Verdun1, Lauren DeStefano2, Chi Wang3, Gregory Boel1, Guy Montelone1, Nancy Woychik3, John H. Hunt1.
1Weill Medical College of Cornell University, New York, NY, USA, 2UMDNJ-RWJMS Department of Molecular Genetics and Microbiology & Immunology, Piscataway, NJ, USA, 3Columbia University, Dept. of Biological Sciences, New York, NY, USA, 4Rutgers - State University of New Jersey, New Brunswick, NY, USA, 5UMDNJ-RWJMS Department of Molecular Genetics and Microbiology & Immunology, Piscataway, NJ, USA.
TA systems have recently been linked to medically important processes such as biofilm formation, bacterial persistence after exposure to antibiotics, and bacterial pathogenesis. Toxin-Antitoxin (TA) systems are stable protein complexes consisting of a toxin, whose action is mechanistically distinct from exotoxins (e.g. botulinum, anthrax or cholera toxins), in complex with an antitoxin, its specific inhibitor. The release of TA toxin occurs under specific...