Protein-Ligand Interactions II

3062-Pos Board B109

Mechanism of Interaction between the General Anesthetic Halothane and a Model Ion Channel Protein: Structural Investigations via X-ray Reflectivity from Langmuir Monolayers

Joseph Strzalka, Jing Liu, Andrey Tronin, Inna Y. Churbanova, Jonas S. Johansson, J. Kent Blasie.
University of Pennsylvania, Philadelphia, PA, USA.

De novo designed model membrane proteins provide an alternate platform for investigating the interaction of general anesthetics and proteins, particularly for obtaining structural information, while avoiding the complexity of natural membranes. We previously reported the synthesis and structural characterization of a model membrane protein comprised of an amphiphilic 4-helix bundle peptide with a hydrophobic domain based on a synthetic ion channel and a hydrophilic domain with designed cavities for binding the general anesthetic halothane (Ye *et al,* Biophys. J. **87:** 4065 (2004)). In the present work we synthesized an improved version of this halothane-binding amphiphilic peptide with only a single cavity and an otherwise identical control peptide with no such cavity, and applied x-ray reflectivity to monolayers of these peptides in order to probe the distribution of halothane along the length of the core of the 4-helix bundle as a function of the concentration of halothane. At the lower concentrations achieved in this study, about three molecules of halothane were found to be localized within a broad symmetric unimodal distribution centered about the designed cavity. At higher concentrations, about six-seven molecules were found to be uniformly distributed along the length of the bundle, corresponding to approximately one molecule per heptad. Monolayers of the control peptide showed only the latter behavior, namely a uniform distribution along the length of the bundle irrespective of the halothane concentration. The results provide insight into the nature of such weak binding when the dissociation constant is in the mM regime, relevant for clinical applications of anesthesia. They also demonstrate the suitability of both the model system and the experimental technique for additional work on the mechanism of general anesthesia. Supported by NIH GM55876.

3063-Pos Board B110

Probing Peptide Material Interactions At The Single Molecule Level

David C. Appleyard, Jacquelyn A. Kunkel, Matthew J. Lang.
Massachusetts Institute of Technology, Cambridge, MA, USA.

Optical tweezers are well suited to probe the characteristics of molecular scale interactions at the interface between biology and materials. The high resolution force and position measurements can offer unique insights into the mechanism and kinetics of adhesion between short peptide sequences, aptamers, and a variety of material surfaces used in microfluidics, medicine, and semiconductor technologies. We have developed a flexible assay to characterize the binding distribution and off-rate for a collection of peptides. The peptide sequences of interest are attached to a polystyrene bead via a 3500 bp DNA spacer using a cysteine on the peptide and a primary amine on the DNA. The modularity of the assay allows any peptide sequence containing a N-terminal cysteine to be linked to the DNA and evaluated. Casein and on certain surfaces, the DNA, act as blocking molecules to reduce non-specific adhesion. Two previously elucidated peptide sequences selected for sapphire affinity were examined and exhibited dissimilar binding distributions, mean adhesion force, and rate constants. To explore the broad applicability of the assay, the adhesion of a universal binder on slide glass was inspected.

3064-Pos Board B111

Effects of Load and Contact Time on the Stability of Bimolecular Integrin-Fibrinogen Bonds Under a Constant Tensile Force

Rustem I. Litvinov, Joel S. Bennett, John W. Weisel, Henry Shuman.
University of Pennsylvania, Philadelphia, PA, USA.

The regulated ability of integrin alphaIIbbeta3 to bind fibrinogen plays a crucial role in platelet function, thrombosis, and hemostasis. Previously, we found that the rupture force distribution of individual integrin alphaIIbbeta3-fibrinogen interactions under ramp loads display at least two components that differ in kinetics, loading rate dependence, and susceptibility to activation/inhibition of the integrin, which suggests that specific binding and unbinding of the integrin alphaIIbbeta3 with fibrinogen is a complex multi-step process (Litvinov et al., Biophys J. 2005; 89:2824). Employing a new laser tweezers-based electronic force clamp (Litvinov et al., Biophys J. 2008, 94, Suppl., Abstract, 1724-Pos.), we now study interactions of purified integrin alphaIIbbeta3 and fibrinogen under constant tensile force, mimicking the effect of hydrodynamic blood flow on an adherent platelet. Under a constant tensile force of 50 pN, the distribution of durations of the alphaIIbbeta3 and fibrinogen bonds is bimodal, with specific integrin-fibrinogen interactions mostly lasting more than 2 sec. At the same constant unbinding force, the bond lifetimes increase as the duration of contact between alphaIIbbeta3- and fibrinogen-coated interacting surfaces is increased from 0.1s to 2s, again suggesting that the initial interaction of fibrinogen with alphaIIbbeta3 is followed by reorganization of the binding interface, enhancing the strength and stability of binding. In further experiments, the average bond lifetimes exponentially decrease as tensile force is increased from 0 to 30 pN, suggesting that in this force range the alphaIIbbeta3-fibrinogen interactions represent classical ''slip bonds''. Taken together, these data provide important quantitative characteristics of the alphaIIbbeta3-fibrinogen binding and unbinding, which underlie the dynamics of platelet adhesion and aggregation in the blood flow.

3065-Pos Board B112

Solvent Exposure- and Distance-Dependent Dielectric Function for Ligand-Protein Interactions

Daniel P. Garden, Boris S. Zhorov.
McMaster University, Hamilton, ON, Canada.

Physics-based force fields for high throughput ligand docking usually determine electrostatic energy with distance-dependent dielectric (DDD) functions, which do not fully account for the dielectric permittivity variance between ~ 2 in the protein core and ~ 80 at the protein surface. We propose here a new dielectric function that depends on the distance between a pair of interacting atoms and the degree of their exposure to the aqueous environment. This solvent exposure- and distance-depend dielectric (SEDDD) function accounts for both electrostatic and dehydration energy components. Using a training set of ten x-ray structures, we first optimized the global-minimization protocol in the ZMM program with the AMBER force field, implicit solvation, and the DDD function $\epsilon = 2r$. A search was considered a success if the root mean square deviation (RMSD) of the ligand's atoms in the apparent global minimum from the x-ray structure was less than 2 Å. For each complex, the apparent global minimum was found by sampling hundreds of thousands of the rigid-ligand binding poses and refining low-energy poses by Monte Carlo-minimizing energy with flexible ligand and flexible protein. For an examining set of 60 structures, the global-minimization protocol with implicit solvation and various DDD functions ($\epsilon = r$, $\epsilon = 2r$, and $\epsilon = 4r$) yielded success rates of 66.7%, 73.3% and 75.0% respectively. In most outliers, the ligand-binding sites were located at the protein surface. Using a second training set of 16 ligand-protein complexes, we parameterized the SEDDD function to minimize the average RMSD between the apparent global minima and x-ray structures. Application of the parametrized SEDDD function to the examining set yielded a success rate of 91.7%, a substantial improvement versus the best-performing DDD function with implicit solvation.

3066-Pos Board B113

IRRAS Studies of the Host Defense Effect of Pulmonary Surfactants SP-A and SP-D

Guangru Mao[1], Lin Wang[1], Joseph W. Brauner[1], Francis X. McCormack[2], Erika Crouch[3], Barbara Seaton[4], James Head[4], Kelly Smith[3], Carol R. Flach[1], Richard Mendelsohn[1].
[1]Rutgers University, Newark, NJ, USA, [2]University of Cincinnati, Cinninati, OH, USA, [3]Washington University School of Medicine, St. Louis, MO, USA, [4]Boston University, Boston, MA, USA.

The pulmonary surfactant proteins SP-A and SP-D have been proven to play a role in host defense. SP-A binds phosphatidylcholine, galactosylceramide, and the lipid A portion of lipopolysaccharide (LPS), which is found in Gram-negative bacterial cell walls. SP-D binds phosphatidylinositol, glucosylceramide and LPS. Although X-ray crystallography and NMR spectroscopy have provided atomic level information about SP-A and SP-D structures, molecular level information about their binding to biological ligands is lacking. IR reflection-absorption spectroscopy (IRRAS) is used in the current work to study the properties of SP-A and SP-D at air/water interfaces and their interaction with biological ligands. The monolayer properties of recombinant rat neck + carbohydrate recognition domain (NCRD) SP-A and its point mutant D215A SP-A are compared. Both NCRD SP-A and D215A SP-A adsorb to the air/water interface, DPPC monolayers, and Lipid A monolayers, but D215A/PL SP-A displays higher affinity and greater stability. Measurements of surface pressure, Amide 1 intensity, and lipid acyl chain conformational ordering can help to elucidate the mechanism of interaction of SP-A and its ligands. Similar experiments have been carried out with SP-D.

3067-Pos Board B114

Communication Via Structural Water: Changes In The Thrombin Water Channel And Active Site Due To Sodium Binding

Rachel L. Rice, **Nathan A. Baker**.
Washington University in St. Louis, St. Louis, MO, USA.

Thrombin plays a pivotal role in the blood coagulation cascade, functioning as both a procoagulant and anticoagulant. Thrombin exists in the blood in equilibrium between these two catalytic forms, and the transition from the slow to the