239-Wkshp
Ion Channel Simulation with Explicit Solvent and Lipid Membrane Based on the Drude Polarizable Force Field
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Accurate potential functions based on simple and computationally tractable functional forms are critical for meaningful MD simulation studies of ion channels based on atomic models. These systems are particularly challenging due to the large magnitude of the interactions involved and the contrasting features of the various molecular components. Water molecules provide a high dielectric bulk environment while the nonpolar hydrocarbon core of the lipid bilayer provides a low dielectric environment that surrounds the protein channel. The energetics of ion transport results from a delicate balance of very large ion-water and ion-protein interactions. These large energies are often in sharp contrast with the small activation energies, on the order of a few kBT, deduced from experimentally observed ion-fluxes. In the present work, a polarizable force field based on the classical Drude oscillator model was developed to enable all-atom MD simulations of biological ion channels. A systematic hierarchical strategy was used to optimize the parameters for water, ions, proteins, and lipids to best represent a collection of gas and liquid properties on the basis of small molecules representative of relevant functional groups. Ion models were optimized to reproduce both microscopic quantum mechanical and thermodynamic solvation data. Using MD simulations, a protein force field was tested for suite of peptides and proteins, and models of DPPC, DPPE, POPC and DOPC were tested against the experimental properties of bilayer membranes. First results from MD simulations of the KcsA channel embedded in a phospholipid bilayer with explicit solvent will be described.

240-Wkshp
Forcebalance: a Systematic, Reproducible, Statistically Driven Approach to More Accurate Molecular Dynamics Models
Vijay Pande.
Dept Chemistry, Stanford Univ, Stanford, CA, USA.
I will present our first application of the ForceBalance method for systematically parameterizing force fields: the iAMOEBA water model. Force balance uses a combination of experimental data and high-level theoretical calculations, combined with statistical methods to avoid overfitting and efficient schemes for optimizing in parameter space. We will describe our choice of reference data and provide a brief overview of the parameterization method. The accuracy of the direct polarization water model is demonstrated using a published, comprehensive benchmark of water properties across a wide range of phases and thermodynamic conditions. This suggests a new way to systemically create force fields, with both greater accuracy as well as reproducibility and transparency.

241-Wkshp
Atomistic and Coarse-Grained Models for Biomolecular Simulations
Teresa Head-Gordon.
University of California, Berkeley, Berkeley, CA, USA.
My talk will describe various theoretical approaches that allow for analysis and interpretation of hydration properties of peptides, bulk water structure properties, and biomolecular crowding. I will also introduce new theoretical models and methods that include direct and mutual polarization of aqueous solvent and how they can be used for computing relevant experimental observables for high quality interpretations of those experiments.

Workshop: Single Molecule Dynamics Using FRET/LRET

242-Wkshp
Theory of Single-Molecule Photon Sequences
Irina V. Gopich.
NIDDK, National Institutes of Health, Bethesda, MD, USA.
Single-molecule Förster resonance energy transfer (FRET) is a powerful tool to probe intramolecular distances and to study structure and dynamics of macromolecules. The distances fluctuate due to conformational dynamics on a wide range of time scales. Extracting information about the dynamics is particularly challenging when the fluctuations are as fast as the time between photons. Our study provides a general framework to describe the influence of fast and slow fluctuations of the energy transfer rate on the statistics of donor and acceptor photon counts, interphoton times and delay times (between a laser pulse and the arrival of the photon). I will discuss various methods to analyze photon sequences ranging from photon-by-photon analysis to FRET efficiency and lifetime distributions.

243-Wkshp
Transition-Path Times in Protein Folding from Single-Molecule FRET
Hoi Sung Chung.
NIDDK/NIH, Bethesda, MD, USA.
The transition-path is the tiny fraction of an equilibrium, single-molecule trajectory when the free-energy barrier is actually crossed. For a two-state protein-folder, the transition-path contains all the mechanistic information on how a protein folds. However, because it is so fast a transition-path has never been observed experimentally for any molecular system in the condensed phase. As a first step toward observing transition-paths, we are using single molecule FRET to measure average transition-path times (t_{TP}). Using the Gopich/Szabo maximum-likelihood method to analyze photon trajectories, we have now determined t_{TP}'s for all-beta (FBP28-WW domain, t_{TP}=2μs) and all-alpha proteins (α_3D, t_{TP}=12 μs), and an upper bound for an alpha/beta protein (protein G, t_{TP} <10 μs) (Chung *et al*., *Science* 2012; Chung, Eaton, *Nature*, 2013). The surprising result is that the folding times for the WW domain and protein G differ by 10,000-fold, yet the t_{TP}'s differ by less than 5-fold, so a fast and slow-folding protein take almost the same time when they actually fold. Szabo's theory for barrier crossing of a Brownian particle explains this result by showing that t_{TP} is insensitive to the barrier height, i.e. t_{TP} μ $\ln(3\beta\Delta G^*)/D^*$. Studies of the viscosity- and temperature-dependence of t_{TP} for α_3D suggest that the extremely low viscosity-dependence ($\sim\eta^{0.3}$) arises from a lower D^* due to increased internal friction (''rougher'' energy landscape). Lowering the pH to neutralize 12 carboxylates eliminates potential salt bridges and reduces the viscosity dependence to that previously observed ($\sim\eta^{0.6}$) for other all-alpha proteins. These results provide the first glimpse of the structural origin for internal friction in protein folding, suggesting that the lower D^* for α_3D arises from making and breaking non-native salt-bridges during the transition path, as observed in MD simulations by the Shaw group (Best, Hummer, Eaton, *PNAS* 2013).

244-Wkshp
New FRET Methods for Studying Processing of Nucleic Acids by Protein Machines
Achillefs Kapanidis, PhD.
Physics, University of Oxford, Oxford, United Kingdom.
Single-molecule FRET microscopy is a versatile fluorescence technique that serves as a molecular ruler reporting on nanoscale distances and distances changes within biomolecules. During the past 15 years, studies based on single-molecule FRET have uncovered substantial information about the dynamics of nucleic acids (DNA and RNA), as well as the mechanisms of nucleic acid processing by a large variety of protein machines. Such studies are often based on direct, real-time observation of the dynamics of surface-immobilized individual molecules of nucleic acids or their complexes with proteins, and offer first views of intermediates undetectable by more conventional structural and biochemical methods.
However, many single-molecule FRET techniques are often limited to a single observable, namely the FRET efficiency between the donor and acceptor probes; as a result, one may need considerable information about a system to interpret the FRET signal appropriately. In my talk, I will describe how the coupling of the FRET signal with additional fluorescence observables at the single-molecule level can be used to explore transient reaction intermediates and reaction paths. Specifically, I will discuss combinations of single-molecule FRET with alternating-laser excitation (ALEX), tethered fluorophore motion (TFM) and protein-induced fluorescence enhancement (PIFE). I will also describe examples of applications of such FRET-based techniques in mechanistic studies of gene transcription and site-specific DNA recombination. Prospects of extending FRET and its combinations in vivo will also be discussed.

245-Wkshp
Single Molecule Four Color FRET Reveals the Mechanism of an ATP Driven Multicomponent Motor
Thorsten Hugel.
Technical University of Munich, Garching, Germany.
During the last years single molecule methods have allowed for unprecedented insights into the dynamics of proteins. Usually, the relative movement of two fixed positions (fluorophores) in the protein or in between two proteins is monitored over time. Here we present first results on the simultaneous monitoring of FRET between four fluorophores. Two fluorophores describe conformational changes in the molecular chaperone and heat shock protein Hsp90, the third the binding kinetics of the co-chaperone p23 and the fourth the binding kinetics of ATP. We show that binding of p23 is able to transform the thermal fluctuation controlled yeast Hsp90 into a directed nucleotide dependent multicomponent motor.