several new algorithms and implementation techniques that enable the detection of significant structure-changing events in a molecular dynamics trajectory. These algorithms include a coarse graining of side chain contacts, a contact metric based on higher-order generalizations of the Delaunay tetrahedralization, and median filters for detecting significant shifts in the ensemble mean of the resulting time series. We have also developed numerical techniques for suppressing trivial re-crossing events and a new kernel-based estimator of the contact alteration activity. These methods will be disseminated in a newly developed package, "TimeScapes," which is compatible with molecular dynamics trajectories generated from any of a variety of popular simulation programs. Tests on microsecond time scale simulations suggest that the implementation is efficient and requires very little parameterization. The analysis provides a detailed listing of broken and formed contacts, and reliably detects allosteric and folding transitions, as well as stable intermediates, in the protein dynamics.

1874-Plat

Searching For the Hinge of E3 Ubiquitin Ligase Machinery with MD Simulations

Jin Liu, Ruth Nussinov.
National Cancer Institute, SAIC- Frederick, Frederick, MD, USA.

In Cullin-RING E3 ubiquitin ligases, substrate binding proteins, such as VHL-box, SOCS-box or the F-box proteins, recruit substrates for ubiquitination, accurately positioning and orienting the substrates for ubiquitin transfer. Yet, how the E3 machinery precisely positions the substrate is unclear. We performed molecular dynamics simulations for seven substrate binding proteins: Skp2, Fbw7, β-TrCP1, Cdc4, pVHL, SOCS2, and SOCS4, in the unbound form and bound to Skp1 or Elongin C. All seven proteins have two domains: one binds to the substrate; the other to E3 ligase modules Skp1/Elongin C. In all seven cases, the flexible inter-domain linker serves as a hinge rotating the substrate binding domain, optimally and accurately positioning it for ubiquitin transfer. A conserved proline is noticed in the linker of all seven proteins. The prolines pucker substantially and the pucker is associated with the backbone rotation toward the E2/ubiquitin. We further observed that the linker flexibility could be regulated allosterically by binding events associated with either domain. Thus searching for the allosteric sites to regulate the flexibility could provide a new strategy for drug discovery targeting the ubiquitin system. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number NO1-CO-12400.

1875-Plat

Identification Of Two Distinct Inactive Conformations Of The Beta-2 Adrenergic Receptor Reconciles Structural And Biochemical Observations

Ron O. Dror[1], Daniel H. Arlow[1], David W. Borhani[1], Mortenø Jensen[1], David E. Shaw[1,2].
[1]D. E. Shaw Research, New York, NY, USA, [2]Center for Computational Biology and Bioinformatics, Columbia University, New York, NY, USA.

Understanding the mechanisms of signaling proteins such as G-protein-coupled receptors (GPCRs) requires definition of their conformational states and the pathways connecting those states. The recent crystal structures of the beta-2 and beta-1 adrenergic receptors in a presumably inactive state constituted a major advance toward this goal, but also raised new questions. Although earlier biochemical observations had suggested that the beta adrenergic receptors possessed a set of contacts between helices 3 and 6, known as the ionic lock, which was believed to form a molecular switch for receptor activation, the crystal structures lacked these contacts. The unexpectedly broken ionic lock has provoked a great deal of speculation, raising questions about whether the structures accurately represent the inactive receptor state and whether the ionic lock plays a role in activation of these and other GPCRs. To address these questions, we performed microsecond-timescale molecular dynamics simulations of the beta-2 adrenergic receptor in multiple wild-type and mutant forms. Our observations of the behavior of the ionic lock, along with the formation of several novel structural elements in the extramembrane loops during our simulations, paint a more complete picture of the inactive state of the beta adrenergic receptors, reconciling the crystal structures with biochemical studies.

1876-Plat

Atomic Level Description of GPCR Activation Revealed by Microsecond Time Scale Molecular Dynamics

Diane Lynch[1], Dow Hurst[1], Patti Reggio[1], Alan Grossfield[2], Mike Pitman[3].
[1]University of North Carolina, Greensboro, Greensboro, NC, USA,
[2]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [3]IBM TJ Watson Research Center, Yorktown Heights, NY, USA.

Previously, we had reported the results of microsecond molecular dynamics simulations for the interaction of 2-arachidonoylglycerol (2AG), the endogenous ligand of the Class A cannabinoid CB2 receptor, with the CB2 receptor in an explicit POPC lipid bilayer[1]. These results show the initial stages of agonist binding to and activation of the CB2 receptor. Analysis of these trajectories reveals that upon the binding of 2AG, which occurs via lipid between transmembrane helix 6 (TMH6) and TMH7, the intracellular portions of TMH3 and 6 separate with a concurrent breaking of an intracellular salt bridge. The latter event has been probed by an Essential Dynamics analysis of the trajectory during the binding event. This analysis indicates that a single eigenvector captures the motion of the breaking of this salt bridge and the opening of the intracellular surface of the receptor, events that are believed to be associated with activation. These results will be presented and discussed, particularly in light of recent experimental results of spin label measurements by Altenbach et al. [2] and the crystal structures of opsin [3,4] which both show an intracellular separation of between 6-7 Å between the ends of TMH3/TMH6 of rhodopsin upon light activation.

[1] Reggio, P. et al. Biophys. Supplement 94, 2676 (2008).
[2] Altenbach, C. et al. PNAS, 105, 7439-7444 (2008).
[3] Park, J.H. et al. Nature 454, 183-187 (2008).
[4] Scheerer, P. et al. Nature 455, 497-502 (2008).

1877-Plat

Potential of Mean Force Calculations of Ion Permeation in Gramicidin A Channel

Yuhui Li[1], Toby W. Allen[2], Olaf S. Andersen[3], Benoit Roux[1].
[1]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA, [2]Department of Chemistry, University of California at Davis, Davis, CA, USA, [3]Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY, USA.

The potential of mean force (PMF) for K^+ ion permeation through the gramicidin A (gA) channel were calculated from the molecular dynamics (MD) simulations with four different force fields (FF): CHARMM27, CHARMM27 with dihedral-based correction map (CHARMM27+CMAP), CHARMM27 with a improved FF parameters for tryptophan indole ring (CHARMM27+Trp), and CHARMM27 with the CMAP and the improved FF parameters for Trp (CHARMM27+CMAP+Trp). When comparing the PMFs obtained with these four different FF, we find that both CHARMM27 and CHARMM27+Trp predict free energy profiles that are in semi-quantitative agreement with measurements of the conductance and dissociation constant. The combination CHARMM27+Trp gives the best agreement. However, the CHARMM27+CMAP yields a larger barrier in the PMF and CHARMM27+CMAP+Trp generates a deeper binding potential well. These calculations illustrate the sensitivity of the PMF controlling ion permeation to subtle changes in the FF. We also compute a 2-ion PMF for a doubly occupied gA channel. The effect of the number of water molecules in the channel on the effective ion-ion interactions is also studied. Elucidating the properties of the doubly occupied channel is important because experiments are often carried out at fairly high concentration where double ion occupancy is predominant.

1878-Plat

A Solvent-Free Coarse-Grained Model for Quantitative POPC Bilayer Simulations

Zun-Jing Wang, Markus Deserno.
Carnegie Mellon University, Pittsburgh, PA, USA.

We presented an implicit solvent CG model in a bottom-up scheme for simulations of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer membranes. The usage of implicit solvent enables membrane simulations on large length- and time-scale at modest computational expense. Despite an improved computational efficiency, the model preserves chemical specificity and quantitative accuracy in comparison with top-down solvent-free CG bilayer models. In the CG model, each of the CG sites was associated with the center-of-mass of a specific group of atoms in the all-atom representation of POPC. The bonded and non-bonded interaction parameters together with the effective cohesive interactions mimicking the hydrophobic effect were systematically derived by matching radial distribution functions, density and pressure profiles of the bilayer, and self-assembly of lipids in all-atom simulations of POPC phospholipids. The CG model is especially useful for studies of large-scale phenomena in membranes which require a detailed description of chemical specificity, e.g. membrane patches interacting with movable and transformable membrane proteins/peptides.

1879-Plat

Interaction of Fullerene with Model Cell Membranes: a Computer Simulation Study

Luca Monticelli[1,2], Emppu Salonen[1], Pu C. Ke[3], Ilpo Vattulainen[2,1].
[1]Helsinki University of Technology, Espoo, Finland, [2]Tampere University of Technology, Tampere, Finland, [3]Clemson University, Clemson, SC, USA.

Nano-sized fullerene aggregates can enter cells and alter their functions, but the mechanisms of cell damage is unclear. In our previous work [1] we used coarse-grained molecular dynamics simulations to characterize the thermodynamics and kinetics of permeation of fullerene clusters through a model membrane. We also showed that high fullerene concentrations induce changes in the structural and elastic properties of the lipid bilayer, but these are not sufficient to cause a direct mechanical damage to the membrane. Now we explore the effect of fullerene on model membranes including an ion channel protein, Kv1.2, using computer simulations with both a coarse-grained and an atomistic representation. We also investigate the effects of a naturally abundant organic compound, gallic acid, on fullerene-membrane interactions. Recent work [2] has shown that gallic acid-coated fullerenes cause cell contraction. We use computer simulations to describe possible mechanisms of cell damage.

[1] Wong-ekkabut et al., Nature Nanotech (2008), 3, 363.

[2] Salonen et al., Small, in press.

1880-Plat

Force Calculations for DNA-PAMAM Dendrimer Interactions from Molecular Dynamics Simulations

Kathleen M. Mills[1], Brad Orr[1], Mark Banaszak Holl[1], Ioan Andricioaei[2].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California Irvine, Irvine, CA, USA.

Polyamidoamine (PAMAM) dendrimers are known to bind and condense plasmid DNA (1,2,3). However, the nature of the interaction between dendrimers and DNA and the mechanism of compaction is not fully understood. Potentials of mean force and forces of interaction were calculated from all-atom umbrella sampling simulations of amine-terminated G3 dendrimers and a 24bp strand of double-stranded DNA. Our simulations show that dendrimers and DNA interact with each other over large distances. Simulations also reveal that even low-generation dendrimers can induce significant bending in DNA and that the dendrimer also deforms considerably upon interaction with the DNA. We compare forces calculated from these simulations with optical tweezer experiments on DNA condensation by dendrimers (3) and propose an explanation for the compaction of DNA by dendrimers observed at forces over 60pN.

1. A.U. Bielinska, J.F. Kukowska-Latallo, J.R. Baker, Gene Structure and Expression, 1353, 180 (1997).
2. W. Chen, N.J. Turro, D.A. Tomalia, Langmuir, 16, 15 (2000).
3. F. Ritort, S. Mihardja. S.B. Smith, C. Bustamante, Phys. Rev. Letters, 96, 118301 (2006).

Platform AC: Protein-Nucleic Acid Interactions

1881-Plat

The Impact of Bending and Twisting Rigidity of DNA on Protein Induced Looping Dynamics

Niels Laurens[1], Christian Pernstich[2], Lucy E. Catto[2], August F. Harms[1], Yana S. Kovacheva[2], Stuart R.W. Bellamy[2], Stephen E. Halford[2], Gijs J.L. Wuite[1].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]University of Bristol, Bristol, United Kingdom.

Protein induced DNA looping is a key regulatory mechanism involved in important processes such as gene regulation, DNA-transcription and -replication. The relation between the induced loop topology and DNA-protein dynamics is essential for understanding these processes. Bending and twisting rigidities of DNA are shown to have a profound influence on the formation and stability of these loops. We used FokI, a restriction enzyme that binds two asymmetrical recognition sites enhancing its specificity, as our model system. Controlling the orientation of both binding sites enabled us to explore the impact of the physical properties of DNA by inducing different loop topologies and measuring the resulting changes in DNA-protein dynamics.

The looping behavior is quantified using a tethered particle assay. With this assay we obtained the kinetics of protein induced loop formation with a single measurement by tracking up to 50 DNA tethers in parallel. The dwell times are extracted and compared using both a running average method and a hidden Markov analysis.

We used DNA substrates with a range of different spacing's between the two asymmetric recognition sequences. In addition we varied the orientation of these recognition sites and sampled how binding and loop formation is influenced by these different topologies. We show that both, the separation and orientation of the two recognition sites have a profound influence on the formation and stability of these looped DNA-protein structures. The results are understood and modeled in terms of the helical pitch and the bending energy involved in protein induced loop formation.

1882-Plat

Target Site Search Strategy Of Gene Regulatory Proteins

Mario Diaz de la Rosa, **Andrew J. Spakowitz**.
Stanford University, Stanford, CA, USA.

Gene expression is orchestrated by a host of regulatory proteins that coordinate the transcription of DNA to RNA. Regulatory proteins function by locating specific binding sequences of DNA and binding to these sequences to form the transcription initiation complex. In many instances, these regulatory proteins only have several hundred copies that must efficiently locate target sequences on the genome-length DNA strand. The non-specific binding of regulatory proteins to random sequences of DNA is believed to permit the protein to slide along the DNA in a stochastic manner. Periodically, a thermal kick or an interaction with another bound protein will disengage the regulatory protein from the DNA surface, leading to three-dimensional diffusion. Eventually, the protein will reattach to the DNA at some new location that is dictated by both the diffusivity of the protein and the DNA configuration. Cycling through these random events constitutes a search strategy for the target site. We build a reaction-diffusion theory of this search process in order to predict the optimal strategy for target site localization. The statistical behavior of the DNA strand acts as a necessary input into the theory, and we consider several governing behaviors for the DNA strand. We explore the impact of DNA configuration on target site localization in order to predict how protein expression will vary under different experimental conditions.

1883-Plat

Structural and Thermodynamic Means for Adaptable 3′ Splice Site Recognition

Clara L. Kielkopf.
University of Rochester School of Medicine, Rochester, NY, USA.

The essential splicing factor U2 Auxiliary Factor (U2AF^{65}) identifies the 3′ splice sites of pre-mRNAs during the initial stages of spliceosome assembly. Short, poorly conserved pre-mRNA sequences mark human 3′ splice sites, including polypyrimidine (Py) tracts that are recognized by two consecutive RNA recognition motifs (RRMs) of U2AF^{65}. To understand how U2AF^{65} adapts to divergent pre-mRNA splice sites, high resolution structures were determined of U2AF^{65} complexes with a series of Py tracts. In parallel, the affinities, enthalpy and entropy changes associated with Py tract binding were analyzed using fluorescence anisotropy assays and isothermal titration calorimetry. The different Py tracts bind with optimized registers across the U2AF^{65} surface, placing cytidines and uridines in preferred binding pockets. Small angle X-ray scattering (SAXS) analysis of wild-type and variant U2AF^{65} proteins further demonstrated that the tandem RRMs adopt an extended, bilobal arrangement in solution (Fig. 1). The preferences for binding specific nucleotides at a subset of U2AF^{65} sites, combined with the loose arrangement of RRM domains, altogether supports adjustable binding registers as a means for universal recognition of diverse 3′ splice sites.

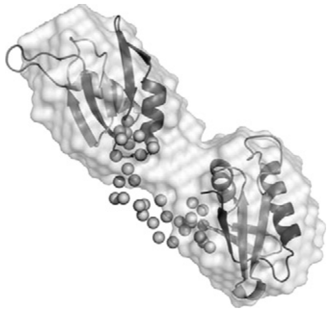

Fig. 1. Shape reconstruction of the U2AF^{65} RNA binding domain.

1884-Plat

Single Molecule FRET Studies of Binding and Conformational Dynamics of HMGB -DNA Systems

Yuyen Lin[1], Phil Hardwidge[2], Emily Bystry[2], L. James Maher III[2], **Ivan Rasnik**[1].
[1]Department of Physics Emory University, Atlanta, GA, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

HMGB proteins are abundant non-histone proteins in eukaryotic chromatin. HMGB proteins are thought to act as architectural factors that enhance DNA bending and flexibility. The dynamic aspects of DNA bending by these proteins remain elusive. It has been proposed that these proteins associate and dissociate from DNA at fast rates. This highly dynamic behavior makes it difficult to study binding and conformational dynamics by traditional biochemical techniques. In this work we use single molecule fluorescence resonant energy transfer (smFRET) to study the binding dynamics of human HMGB2A and *S. cerevisiae* Nhp6A sequence non-specific single box proteins to DNA substrates. Studies were done using both total internal