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).
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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.