recordings from neurons in the brains of living flies reveal changes in macroscopic outward current in Slob null and Slob over-expression flies. These changes are consistent with the effects of Slob described previously in a heterologous expression system. Furthermore, in vivo single channel recordings demonstrate large changes in Slowpoke channel activity in Slob null and Slob over-expression flies. Our results provide evidence that an ion channel regulatory protein complex can modulate neuronal physiology, and ultimately behavior, in an intact organism.

975-Symp MPS-1 is a serine/threonine kinase

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MPS-1 is an accessory subunit of K^+ channels which belongs to the evolutionarily conserved family of KCNE proteins. MPS-1 is expressed in the metazoan *Caenorhabditis elegans* where it co-assembles with voltage-gated K^+ channel KVS-1 to form a complex that is necessary for the normal function of the sensory apparatus of the worm. Of particular interest is that MPS-1 possesses kinase activity. Thus, MPS-1 can phosphorylate KVS-1 and other substrates. Electrophysiological analysis in CHO cells, shows that MPS-1 activity acts to specifically regulate the magnitude of the macroscopic potassium current by controlling the open probability of the channel. We found that disruption of MPS-1 kinase activity leads to learning-disability in *C. elegans*. Worms bearing a dead kinase variant, obtained by mutating a D to N in the catalytic site retain normal nervous function but cannot habituate to repeated sensory stimulation. Taken together these data indicate that the enzymatic activity of an ancillary subunit can produce plasticity of K^+ channel function to specifically control neuronal excitability and habituation behavior in the nematode.

976-Symp Modulation Of Kv1 Channel Activity By Structural Changes Of An Associated Oxidoreductase

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Beta subunit (Kvβ) of the *Shaker* family voltage-dependent potassium channels (Kv1) assembles with the channel early in biogenesis to form a permanent macromolecule complex. We have shown that Kvβ is a functional aldo-keto reductase that utilizes NADPH as a cofactor. Furthermore, structural changes on Kvβ, for example, oxidation of the Kvβ-bound NADPH, changes Kv1 channel activities. These results suggest that Kvβ could transduce changes in cellular metabolic redox state into altered cell excitabilities, and imply a new way of regulating Kv1 channels. Small-molecule compounds that interact with Kvβ at low-micromolar concentrations were identified using an automated high-throughput screen in addition to a manual screen. Positive hits from the screens were examined for their effects on channel activities and then were co-crystallized with Kvβ for structural analysis. Atomic resolution structures of the complexes revealed different binding sites on Kvβ, likely representing different mechanisms of channel modulation. These small-molecule compounds are used as a tool to probe both the physiological functions of Kvβ, as well as the molecular mechanisms of channel modulation.

Platform AA: Coarse-Grained and Enchanced Sampling Biophysical Simulation Methods

977-Plat Internal Coordinate Molecular Dynamics Using Efficient Multibody Dynamics Algorithms

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In this work a methodology for reduced order modeling of biomolecular systems as sub-structured multi-rigid body articulated systems is presented and the integration of molecular dynamics software with multibody dynamics software to facilitate this modeling effort is discussed. Holonomic constraints are used to selectively curtail the frequency content of the system, thereby allowing for large temporal integration steps. These constraints are used to either freeze out select degrees of freedom or make rigid clusters of sets of interaction sites. An efficient, recursive method based on Kane's method is used for generating and solving the internal coordinate dynamics equations of motion of the coarse-grained bio-molecular and bulk materials. The methodology is verified by simulating several systems including explicit water, alkane chains, Alanine dipeptide and carboxyl terminal fragments of Calmodulin, Ribosomal, Rhodopsin L7/L12 and RuBisCO proteins. The stability and validity of the simulations are studied through thermodynamics properties and conformational analysis of nano-second long simulations of these systems. In these simulations, a speed up of an order of magnitude (or more) is realized as compared to classical molecular dynamics while preserving the essential dynamics of the system (within conservative error bounds). As a part of this work, a freely available, open-source computational tool was developed by combining a classical molecular dynamics software LAMMPS and a multibody dynamics research code called POEMS. This tool gains on the complementary nature of the two codes by coupling the efficient force calculation algorithms in LAMMPS with the efficient algorithm in POEMS for generating and solving the internal coordinate dynamics equations of motion.

978-Plat Accelerating the Convergence of Slow Diffusive Conformational Transitions with Molecular Dynamics

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Slow diffusive conformational transitions play key functional roles in biomolecular systems. Our ability to sample these motions with

molecular dynamics simulation in explicit solvent is limited by the slow diffusion of the solvent molecules around the biomolecules. Previously, we proposed an accelerated molecular dynamics method that has been shown to efficiently sample the torsional degrees of freedom of biomolecules beyond the millisecond timescale. However, in our previous approach, large-amplitude displacements of biomolecules are still slowed by the diffusion of the solvent. Here we present a unified approach of efficiently sampling both the torsional degrees of freedom and the diffusive motions concurrently in explicit water molecules. We show that this approach samples the configuration space more efficiently than normal molecular dynamics, and that ensemble averages converge faster to the correct values.

979-Plat Finding Dynamical Pathways for Large Conformational Changes of Proteins with Swarms of Trajectories

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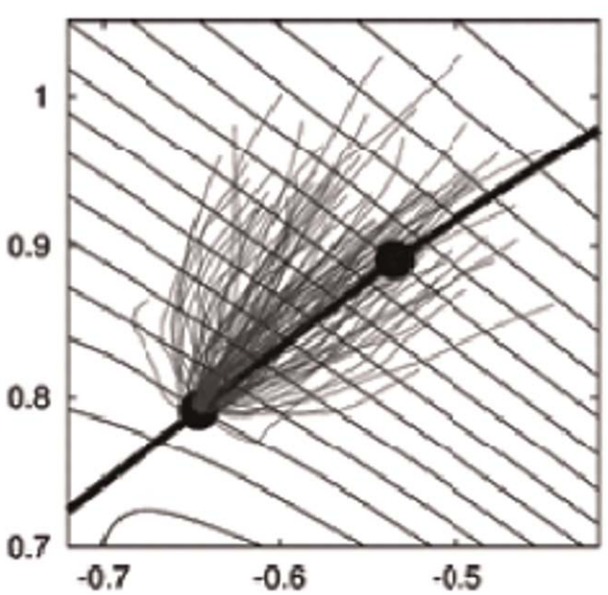
We have developed a simple and efficient molecular dynamics computer simulation method to find transition pathways between two stable protein conformations. The method refines a putative transition pathway using the average drift of a set of collective variables describing the path. The drift is estimated on-the-fly via swarms of short trajectories. Successive iterations of this algorithm, which can be naturally distributed over many computer nodes with negligible inter-processor communication, refine an initial trial path toward the most probable transition path between two states. Once the path is found, quantities of interest like free energies and rates can also be calculated. Applications of this method to signal transduction proteins and ion channels will be presented and discussed.



980-Plat Progress Toward a General Peptide Force Field Based on Systematic Multi-scale Coarse-graining

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Coarse-grained (CG) modeling of proteins has garnered significant attention in recent years because it offers one route by which the length and time scales accessible to simulation studies of these systems can be increased. Recently our group described a systematic multi-scale approach for generating peptide CG models. These models reproduce the structural properties of peptides representing basic secondary structural motifs of protein structure. Due to the averaging inherent in coarse-graining, one might expect the resulting effective interactions to be highly dependent on the chemical composition of the peptides studied as well as the region of configuration space explored by each. As expected, the peptides did display different sets of effective interactions. However, from the perspective of protein folding studies, it would be quite advantageous to obtain a set of CG interactions that could be used to represent peptides of disparate amino acid composition or occupying different regions of configuration space. We describe efforts toward using the multi-scale approach to represent peptides of dissimilar amino acid composition and secondary structure using a single set of CG interactions. Our observations suggest that it may be possible to identify sets of CG effective interactions that can be used to represent quite different peptide systems. This would be of great utility in the field of protein folding, as a single set of CG interactions could be used to model the entire folding process. This finding is also of relevance to protein structure prediction studies, where the final peptide configuration is unknown and the amino acid sequences of interest can be diverse. In both cases the capacity of CG models to correctly represent native configurations while facilitating exploration of the underlying configuration space would be quite beneficial.

981-Plat Development of a Transmembrane Protein Force Field for Coarse Grained Molecular Dynamics

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G-Protein Coupled Receptors (GPCRs) are transmembrane proteins that are important in the signal transduction process. They are implicated in the pathology of many diseases. Therefore, it is critical to understand the molecular mechanism underlying the signal transduction process. The fundamental necessary step for signal transduction via GPCRs, is the activation of GPCRs by agonists and G-proteins, that leads to subsequent secondary events in the signal transduction pathway. Molecular dynamics (MD) simulations can be used to investigate these processes. Since the time scales accessible by conventional MD simulations are still below the time scale of the activation of GPCRs, we have developed a coarse

grained model for transmembrane proteins reducing the simulation time considerably, and allowing focus on the overall behavior of transmembrane proteins, such as aggregation and internalization. Based on the coarse grained lipid model by Markvoort et al. [1], a two particle per amino acid model for the protein has been developed, one for the backbone and one for the side chain [2]. To account for the different nature of the amino acids, the side chain particles are furthermore split into four categories [3]. The intramolecular interactions in the coarse grained protein are dominated by bonds and angles only. The secondary structure arises mainly from the nonbonded interactions alone. Using this new coarse grained model, simulations have been performed on the coarse grained representation of rhodopsin embedded in a lipid bilayer. Both visual and numerical analysis of the trajectories have shown that the coarse grained force field produces stable transmembrane proteins.

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982-Plat A Systematic Methodology for Capturing Essential Dynamics in Coarse-grained Models

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Coarse-grained (CG) models of biomolecules have recently attracted considerable interest because they enable the simulation of complex biological systems on length-scales and time-scales that are inaccessible for atomistic molecular dynamics (MD) simulation. Associated with any CG model is a map that transforms an atomically detailed configuration into a CG configuration. For CG models of relatively small biomolecules or in cases that the CG and all-atom models have similar resolution, the construction of this map is relatively straightforward and can be guided by chemical intuition. However, it is more challenging to construct a CG map when relatively large and complex biomolecules have to be represented by relatively few CG sites. The present work introduces a new and systematic methodology for constructing a CG mapping with a particular resolution for an arbitrarily complex biomolecule. In particular, the resulting CG map optimally captures biologically-relevant motions within the "essential dynamics subspace" characterized by principal component analysis (PCA) of an atomistic MD trajectory. Numerical calculations illustrate the methodology for several important complex proteins, such as the HIV-1 CA protein and G-actin. These illustrations demonstrate that the methodology determines a CG mapping that is optimized for representing large-scale and functionally-relevant fluctuations in biomolecules.

983-Plat From Discoidal to Spheroidal HDL particles through Coarse Grained and All Atom Molecular Dynamics Simulations

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In vivo high density lipoproteins (HDL) originate as discoidal complexes of apolipoprotein (apo) A-I, phospholipids (PL) and cholesterol. These nascent HDL complexes are remodeled by the enzyme Lecithin Cholesterol Acyl Transferase (LCAT), that catalyses the transition from discoidal (PL-rich) to spherical HDL (the form of circulating HDL) by generating cholesteryl esters (CE) and lyso-PC. The phase separation of neutral lipids, CE and triglycerides (TG), creates a hydrophobic core encapsulated by the protein and amphipathic lipid molecules. To investigate the conformational change of apoA-I in the transition from PL-rich to CE-rich HDL particles, we initially performed all atom (AA) and coarse grained (CG) molecular dynamics (MD) simulations at 310 K on two starting model discoidal HDL particles containing palmitoyloleoyl-phosphatidylcholine (POPC), cholesterol (UC) and full length apoA-I molecules with molar ratios of 160:24:2 and 160:64:2, respectively. In the 100 ns coarse grained structures a fraction of the cholesterol molecules was mutated to cholesteryl oleate (CO) molecules and an equivalent number of POPC molecules were removed. The main goal was to mimic the LCAT activity in silico by simulating model CE-rich HDL particles representing small HDL_2 particles with a cholesterol concentration similar to that of circulating HDL. Then, the two mutated structures containing POPC:CO:UC:apoA-I molar ratios of 142:18:6:2 and 105:55:9:2, respectively, were subjected to a 100 ns CG MD simulation at 310K. In both CG MD simulations CO molecules form a hydrophobic core in the 100 ns time scale, indicating that hydrophobic interactions play an active role in the stabilization of spheroidal HDL particles. It is also interesting to note the separation of UC molecules, as observed experimentally, into two distinct environments: free and bound to the protein.

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984-Plat Time Evolution of Multicellular Systems Studied by Cellular Particle Dynamics Simulations

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Theoretical and computer modeling of the time evolution of multicellular systems provides valuable insight and guidance to in vitro experiments relevant to embryogenesis, tumor growth, angiogenesis, cell sorting and self-assembly of artificial cell aggregates that are used in bioprinting. The cellular particle dynamics (CPD) computer simulation method is designed to describe and predict the time evolution of multicellular systems that behave like viscoelastic liquids. In CPD each cell is modeled as an ensemble of interacting cellular particles. The time evolution of the multicellular system is determined by recording the 3D trajectories of the cellular particles by integrating their equations of motion. To test the CPD method we applied it to

(i) cell sorting in spherical aggregates composed of cells with different adhesion strengths and
(ii) the fusion of two identical spherical cellular aggregates.

For both cases the results of the CPD simulations agree well with the experimental data. In particular, we compared the time evolution of the interfacial area between the two fusing aggregates obtained from experiment, a CPD simulation, and an analytical calculation based on a continuum theory for viscous fluids. We used the comparison to relate the simulation time and the experimental time, and we demonstrate how the comparison can be used to relate cellular level CPD parameters to tissue level biophysical quantities such as surface tension and viscosity. As an application, we used the obtained model parameters in large scale CPD simulations to predict the formation of complex 3D cellular structures as a results of the fusion of bioprinted cell aggregates.

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Platform AB: Single Molecule Biophysics - I

985-Plat Single-molecule Fret Observations And Large-scale Simulations Of Ribosome Dynamics

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High-spatial and -time resolution single-molecule fluorescence resonance energy transfer measurements have been used to probe the structural and kinetic parameters of transfer RNA (tRNA) movements within the ribosome. Our investigation of tRNA motions, quantified on wild-type, mutant, and L1-depleted ribosome complexes, reveals a dynamic exchange between multiple metastable tRNA configurations. This new dynamic information, together with supporting evidence, will be presented in the context of a framework in which the formation of intermediate states in the translocation process is achieved through global conformational rearrangements of the ribosome particle. All-atom explicit solvent simulations performed on the 70S ribosome have been used to investigate the metastable states visited during the translocation reaction coordinate. These simulations shed light on the stereochemical feasibility of various models, including independent vs. simultaneous movement of the aminoacyl- and peptidyl-tRNAs. The relationship between the movement of tRNA, the small subunit, and the L1 stalk will be explored in detail.

986-Plat Single-Molecule Tracking of mRNA Exiting from RNA Polymerase II

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Single-pair fluorescence resonance energy transfer was used to track RNA exiting from RNA polymerase (Pol II) in elongation complexes. Measuring the distance between the RNA 5'-end and three known locations within the elongation complex allows us to determine its position by means of triangulation.

RNA leaves the polymerase active center cleft via the previously proposed exit tunnel. When the RNA reaches lengths of 26 and 29 nucleotides, its 5'-end associates with Pol II at the base of the dock domain.

TFIIB changes the position of the nascent RNA presumably due to a competition for the binding site on the dock domain. Thus exiting RNA may contribute to TFIIB displacement during the transition from initiation to elongation and may prevent TFIIB reassociation during elongation.

A more general implication is that the applied single-molecule triangulation technique combining multiple FRET measurements with high resolution crystallographic data provides an accurate tool for determining the positions of flexible domains in large multiprotein complexes.

987-Plat Assembly And Activity Of Transcription Complexes Detected At The Single-molecule Level

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Initiation of messenger RNA (mRNA) transcription in eukaryotes involves assembly of RNA polymerase II (Pol II), general transcription factors, activators, and co-activators on a promoter, unwinding of the promoter, initiation of mRNA synthesis, and promoter escape by Pol II. The mechanism of assembly of the components of the preinitiation complex on promoter remains poorly understood. We have built an instrument that combines total-internal-reflection microscopy with magnetic nanomanipulation of single DNA molecules. The instrument is designed to directly detect assembly of Pol II transcription complexes from fluorescently labeled components, and to detect promoter unwinding activity of Pol II, all in the same experiment. As a proof of principle, using our instrument we have detected binding by fluorescently labeled *E coli* RNA polymerase (RNAP) to a single, immobilized DNA molecule, and, simultaneously, detected promoter unwinding by RNAP. Thus, the rate of isomerisation of "closed" RNAP-promoter complex into "open" RNAP-promoter complex can be measured at the single-molecule level. To demonstrate that our instrument can detect