Molecular Dynamics II

1673-Pos Board B583
Evidence for Pre- and Post-Power Stroke of Cross-Bridges of Contracting Skeletal Muscle
Krishna K. Midde.
We examined orientational fluctuations of a few molecules of myosin in working *ex-vivo* skeletal myofibril. Light Chain 1 (LC1) of myosin was labeled with fluorescent dye and exchanged with native LC1 of skeletal myofibril. A small volume within the A-band (~10^{-16}L), containing on average 3-4 fluorescent myosin molecules, was observed by confocal microscopy. The myofibrils were cross-linked with EDC [1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide] to prevent shortening. During muscle contraction myosin tail (containing LC1) undergoes cyclic fluctuations of orientation. We measured these fluctuations by recording the parallel and perpendicular components of fluorescent light emitted by myosin LC1-bound fluorophore. The histograms of fluctuations of fluorescent molecules in rigor were represented by a single Gaussian distribution. In contrast, histograms of contracting muscles could be fitted by at least two Gaussians. This provides evidence that cross-bridges in working skeletal muscle assume two distinct conformations, presumably corresponding to the pre- and post-power stroke.

1674-Pos Board B584
Drift-Oscillatory Steering with the Forward-Reverse Method for Calculating the Potential of Mean Force
Bryan W. Holland, Mostafa NategholEslam, Bruno Tomberli,
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We present a method that enables the use of the forward-reverse (FR) method on a broader range of problems in soft matter physics. Our method, which we call the OFR method, adds an oscillatory steering potential to the constant velocity steering potential of the FR method. This enables the calculation of the potential of mean force (PMF) in a single unidirectional oscillatory drift, rather than multiple drifts in both directions as required by the FR method. By following small forward perturbations with small reverse perturbations, the OFR method is able to generate a piecewise reverse path that follows the piecewise forward path much more closely than any practical set of paths used in the FR method. We calculate the PMF for four different systems: a dragged Brownian oscillator, a pair of atoms in a Lennard-Jones liquid, a Na^+-Cl- ion pair in an aqueous solution, and a deca-alanine molecule being stretched in an implicit solvent. In all cases, the PMF results are in good agreement with those published previously using various other methods, and to our knowledge we give for the first time PMFs calculated by nonequilibrium methods for the Lennard-Jones and Na^+-Cl- systems.

1675-Pos Board B585
Dynamics of the Drosophila Circadian Clock: Theoretical Anti-Jitter Network and Controlled Chaos
Hassan M. Fathallah-Shaykh.
Electronic clocks exhibit undesirable jitter or time variations in periodic signals. The circadian clocks of humans, some animals, and plants consist of oscillating molecular networks with peak-to-peak time of approximately 24 hours. Clockwork orange (CWO) is a transcriptional repressor of Drosophila direct target genes.
Theory and data from a model of the Drosophila circadian clock support the idea that CWO controls anti-jitter negative circuits that stabilize peak-to-peak time in light-dark cycles (LD). The orbit is confined to chaotic attractors in both LD and dark cycles and is almost periodic in LD; furthermore, CWO diminishes the Euclidean dimension of the chaotic attractor in LD. Light resets the clock each day by restricting each molecular peak to the proximity of a prescribed time.
The theoretical results suggest that chaos plays a central role in the dynamics of the Drosophila circadian clock and that a single molecule, CWO, may sense jitter and repress it by its negative loops.

1676-Pos Board B586
A novel Method for Coarse Graining of Atomistic Simulations Using Boltzmann Inversion
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Molecular dynamics (MD) simulations play an important role in many physical, chemical and biological applications. To allow MD methods to be applied to sufficiently large systems and sufficiently long timescales, coarse grained (CG) molecular dynamics methods have been developed in which groups of atoms are represented by a single pseudo-atom, or coarse grained bead. In general, two groups of coarse-grained force-fields for molecular simulation exist, one in which standard form potentials are tuned to reproduce certain thermodynamic properties of a system of interest, and a second group in which potentials are chosen to reproduce simulation results from an atomistic MD simulation. This second procedure to develop a CG force field depends on the ability to map coarse grained particles on atoms in an atomistic simulation. An example of this is the Boltzmann inversion method, in which radial distribution functions are calculated between the mapped CG particles. For (macro)molecules that are mapped onto multiple CG centers this mapping is relatively straightforward. However, for small molecules, like water which is the basic component of most biophysically relevant systems, one must map a group of molecules onto a single CG particle. Finding the optimal division of molecules into groups for each frame of the atomistic trajectory is far from trivial. Here, a novel method is introduced that allows for this mapping of CG particles on a pre-fixed amount of molecules. Our coarse graining algorithm involves the optimization of the division of the molecules, using simulated annealing. The feasibility of the method is demonstrated on various systems containing either pure water, a water-octanol mixture or a solution of sodium chloride in water and may be very useful for large biological systems such as membranes in the future.

1677-Pos Board B587
Diffusion and Binding of RNase A in Dextran Polymeric Solutions Studied by Fluorescence Correlation Spectroscopy
Silviya Zustiak, Ralph Nossal, Dan Sackett.
Diffusion of molecules in the cytoplasm of the cells has long been of interest in the area of targeted drug delivery as well as for describing basic cellular processes. Diffusion of molecules through the cytoplasm or especially the nucleoplasm involves movement of relatively small, typically charged molecules through a sea of much larger, charged, molecules and supramolecular assemblies and is mainly affected by molecular crowding and charge-mediated binding. For small molecules such as metabolites and nucleic acids, an important parameter that describes the cytoplasmic rhelogy is the translational diffusion coefficient. In this work, we have developed a model system in which both molecular crowding and charge-mediated binding were addressed independently in a controlled manner. In particular, we obtained the translational diffusion coefficient of the positively charged protein, RNase A, in polymeric solutions of dextrans of various charges (which affects binding) and differing dextran concentrations (which affects crowding), as well as combinations of both. Using Fluorescence Correlation Spectroscopy (FCS), we observed that the diffusion of RNase A was unaffected by the presence of the positively charged or the neutral dextrans up to 20 μM dextran, above which concentration the diffusion was hindered by crowding. On the other hand, the presence of negatively charged dextrans slowed the protein diffusion significantly even at 0.2 μM dextran. The compound translational diffusion of RNase A decreased with increase in negative dextran concentration. The % bound RNase A also increased until it reached equilibrium binding of ~90% bound RNase A at 14 μM dextran. Binding of RNase to the negatively charged dextrans was further confirmed by ultrafiltration. In addition, exact equilibrium dissociation constants were determined.

1678-Pos Board B588
Conformational Relaxation and Water Penetration Triggered by the Ionization of Internal Groups in Proteins
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Bernard R. Brooks.
Internal ionizable groups in proteins play essential functional roles in biochemical processes related to bioenergetics, including catalysis, proton transport, and electron transfer reactions. Many proteins harness the coupling between the ionization of internal groups and structural reorganization for functional purposes. To elucidate the mechanisms and the structural basis of function in these proteins, it is necessary to understand how the ionization of internal groups is coupled to structural reorganization of the protein and how the protein environment influences the pK_a values of internal groups. Through molecular dynamics simulations and Self-guided Langevin dynamics simulations we are studying the types of structural responses that can be triggered by ionization of internal groups. Water penetration, side-chain rotation and backbone relaxation are among the structural changes that have been detected with molecular dynamics simulations. A large family of variants of staphylococcal nuclease with internal ionizable groups (Lys, Arg, Asp and Glu) are being used to examine these issues systematically. Some of the ionizable groups exhibit pK_a values that are shifted significantly compared to the normal values in bulk water. Most computational methods for pK_a calculations cannot reproduce accurately the pK_a shifts observed in such variants, primarily because they cannot reproduce correctly the structural response to the ionization of internal groups. The physical and structural insight into structural changes promoted by internal charges will be useful to guide the development of more accurate methods for structure-based pK_a calculations.