binding. The dynamics and kinetics of the observed opening transitions allow for the estimation of free energy differences for opening. We obtained values for wild-type Cys283 bound TnC (~4 kcal/mol), V44Q Cys283-bound TnC (3.2 kcal/mol), E40A Ca2+-bound TnC (~12 kcal/mol) and wildtype apo TnC (~20 kcal/mol). These results suggest that the mutations have profound impact on the frequency of presenting the hydrophobic patch to TnI. These simulations also corroborate that cardiac wildtype TnC does not open on timescales relevant to contraction without calcium being bound. Additionally, Brownian dynamics simulations are used to investigate TnI association with TnC. Simulations of full length tropomyosin model elucidate the dynamical interplay between the TnC, TnI and TnT subunits.

1699-Pos Board B591
Molecular Dynamics Simulations of Vibrational Labels in Rabbit Muscle Creatine Kinase
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Molecular Dynamic Simulations of Vibrational Labels in Rabbit Muscle Creatine Kinase Creatine kinase functions as a dimer with two subunits that crystallize symmetrically in the apo form but asymmetrically in a transition state analog complex (TSAC). Previous spectroscopic results from our group on the active-site cysteines in the dual active sites of the creatine kinase dimer indicate that both active sites can adopt a range of structures, but the conclusions of data from both S-H stretching bands of free cysteine and CN stretching bands of cyanated cysteine are ambiguous as to whether the protein rests in an asymmetric configuration with one active “closed” and one “open” to reactivity with its substrate. With the goal of analyzing local cysteine orientations in creatine kinase to interpret spectroscopic results, Gromacs (force field) was used to generate trajectories starting from both the apo and substrate-induced crystal structures. Multiple explicit water models were used including TIP3P and SPC/E. The goals were to simulate unmodified and cyanated CYS 283 and analyze the movement of the thiol or thio cyanate vs the local protein backbone to determine whether there are specific H-bond partners or dominant geometries for either residue that might be reflected in results from vibrational spectroscopy of those residues. The results showed that unmodified Cys283 in creatine kinase adopts a single, long-lived orientation about 40% of the time, due to a specific H-bond between the thiol and a specific water molecule. Similar results suggesting a locally preferred conformation were seen for cyanlated Cys283. The re-interpretation of spectroscopic results in light of these simulations will be discussed.

1700-Pos Board B592
Exploring an Intermediate State of F1-ATPase by Atomistic Molecular Dynamics Simulation
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F1-ATPase, the catalytic domain of FoF1-ATP synthase, is a rotary molecular motor that reversibly interconverts ATP hydrolysis free energy and mechanical work associated with the rotation of the central stalk. In the structure, α- and β-subunits alternatingly arrange to form a hexameric ring, with the rod-like γ-subunit located at its center. Single-molecule experiments showed that the γ-subunit rotates in 120° steps, and that this step is further divided into 80° and 40° substeps. We thus expect two metastable conformations of F1; one before the 80° substep (binding dwell state) and the other before the 40° substep (catalytic dwell state). X-ray structures of F1-ATPase in the catalytic dwell state have provided tremendous functional insight. However, to complete our understanding of the mechanochemical coupling of the F1 motor, it is important to explore also the binding dwell state, and the transitions between the substates. In this work, we use molecular dynamics simulations to study the structure, motions, and ligand dissociation energetics of F1-ATPase as a function of the γ-subunit rotation angle. The γ-subunit is rotated with the help of a newly developed torque simulation method. In our all-atom/explicit solvent molecular dynamics simulations we use enhanced sampling techniques to study phosphate (Pi) release in the transition from the catalytic to the binding dwell state, and to characterize the conformational responses of the β-subunits. From the simulations, we obtain a detailed picture of the conformational changes in F1-ATPase induced by γ-subunit rotation, their energetics, and of their relevance to function.

1701-Pos Board B593
Molecular Dynamics Simulations of Yeast F1-ATPase Before and after 16-Degree Rotation of the Gamma Subunit
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We performed molecular dynamics simulations for crystal structures of yeast F1-ATPase whose crystallographic unit contains two different states. One complex binds Pi in the β subunit, and the other liberated it. In the latter structure, the position of central stalk is rotated +16° in the hydrolysis direction. Because single molecule experiments have proven that Pi release occurs at the β subunit, and the γ subunit is rotated after ATP hydrolysis and Pi release, these structures are supposed to represent snapshots before and after Pi releases for the 40° substep, respectively.
We describe how the F1-ATPase complex changes the structure after Pi release, based on the results of the MD simulations for γF1,II and γF1,III. Then, we propose a possible mechanism of the 40° rotation of the γ subunit.

1702-Pos Board B594
Mapping the Structural and Dynamical Features of Kinesin Proteins
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Kinesin motor proteins drive intracellular transport by coupling ATP hydrolysis to conformational changes and directed movement along microtubules. Characterizing distinctive conformations and their interconversion mechanisms is thus essential to determining an atomic-level model of kinesin action. Here we report a comprehensive principal component analysis of 114 experimental structures along with the results of accelerated molecular dynamics simulations that together map the structural and dynamical features of the kinesin motor domain. All experimental structures were found to reside in one of eight distinct conformational clusters comprising two major groups. These groups differ in the orientation of key functional elements, including the microtubule binding alpha4-alpha5 subdomain. Group membership was found not to correlate with the nature of the bound nucleotide in a given structure. Accelerated molecular dynamics simulations of ATP, ADP and nucleotide free Eg5 indicated that all three nucleotide states could sample the major crystallographically observed conformations. Differences in the dynamic coupling of distal sites were evident in the simulations. In the ATP and ADP simulations the neck-linker, loop8 and the alpha4-alpha5 subdomain display correlated motions that are absent in ADP simulations. Additional simulations predict that mutations G325A and G326A reduce the flexibility of these regions and disrupt their couplings. Furthermore, only in ADP simulations was the neck-linker region observed to undock. Additional APO simulations, commenced with an undocked neck-linker, formed coordinations reminiscent of the docked state. These interactions were absent in simulations of I539A mutants. Our combined results indicate that the reported ATP and ADP-like conformations of kinesin are intrinsically accessible regardless of nucleotide state. Furthermore, simulations highlight sites critical for large-scale conformational changes. We expect that further application of these methods will provide a framework for understanding the complete sequence of conformational changes and their relation to kinesin’s ATPase cycle.