pro-survival (or in certain conditions, pro-apoptotic) signaling, playing a central role in healthy inflammatory response, but also in inflammatory disease and cancer. Binding has long been thought to be sufficient for receptor activation, however we have recently published evidence that TNFR1 undergoes a conformational change upon formation of an oligomeric network, suggesting that the ligand binds and arranges pre-formed receptor dimers into a highly organized lattice. Because lymphotoxin and TNF share the same receptors, their individual roles in signaling are not clearly delineated. We have discovered that oxidative stress abolishes bioactivity of lymphotoxin by causing sulfoxidation of several methionine residues that are absent in the homologous TNF sequence. We have shown in a recent publication that the lymphotoxin-TNFR1 binding is stabilized by Met120 of lymphotoxin through a sulfur-aromatic interaction and proposed that it may be disrupted by oxidation. However, immunoprecipitation shows that while ligand induced signaling is ablated after oxidative modification, ligand-receptor binding is not. This key distinction where the potency, but not the binding affinity of lymphotoxin is inhibited by oxidative stress, may play a key regulatory role in the highly complex lymphotoxin, TNF, TNFR1, and TNFR2 signaling system in which ligands and receptors cooperate and compete to dictate a specific signaling outcome, especially in the cases of inflamed or cancerous tissue, where the extracellular environment is strongly oxidative.

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Assessing Dynamic Features of NF-κB via Molecular Dynamics Simulations and Elastic Network Model

Arzu Uyar[1], Vineet Pande[2], Cameron Mura[3], J. Andrew McCammon[4], Pemra Doruker[1], Lennart Nilsson[2].
[1]Department of Chemical Engineering and Polymer Research Center, Bogazici University, Istanbul, Turkey, [2]Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden, [3]Department of Chemistry, University of Virginia, Charlottesville, VA, USA, [4]Chemistry and Biochemistry Department, University of California, San Diego, California, CA, USA.

Nuclear Factor kappa B (NF-κB) is an inducible transcription factor that regulates expression of numerous components of the immune system and binds to its target DNA sites in a dimeric butterfly-like conformation. Dysregulation of such factor can lead to a number of diseases, including cancer, acquired immunodeficiency syndrome and inflammation. Understanding the unbound form of this protein is crucial, since the determination of drug targets that inhibit free NF-κB has high potential in therapeutics. However, the form remains uncomprehended, despite the available experimental structures of several NF-κB dimers bound to DNA. In the present study, the dynamics and interactions of DNA-bound NF-κB dimer and its free form are investigated. We performed several independent molecular dynamics (MD) simulations using two different forcefields (CHARMM and AMBER), as well as the elastic network model (ENM) analysis. The results obtained from MD simulations and ENM reveal a consistent picture on global conformational dynamics of NF-κB. A common collective mode comprises an oscillatory opening-closing or *tong-like* motion in its N-terminal domains that bind to the DNA. Another mode represents *scissor-like* motion facilitating the movement of the recognition loop L1 towards the DNA, which is essential for the interactions between DNA and NF-κB. Moreover, occupancy analysis of MD trajectories supports that loop L1 makes the strongest hydrogen bonding network with bound DNA. Covariance maps based on the orientational cross-correlations between residues indicate significant differences in domain motions of free and bound forms of NF-κB. These findings provide insight on the unbound form of NF-κB.

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Conformational Properties of Kinesin's Neck Linker Across Species

Jason Doornenbal, Intisar Shaheed, Amanda Miguel, David Ando, Ajay Gopinathan.
Universtiy of California Merced, Merced, CA, USA.

Kinesin I, a homodimeric motor protein facilitates the movement of cellular cargo and vesicles toward the membrane, using microtubules as a bridge. Kinesin contains a short disordered neck linker region between the motor head and ordered stalk. The flexibility of the neck linker is a key parameter that governs the overall properties of the kinesin stepping mechanism. However, to date, our knowledge about the molecular structure and dynamics of this region remains incomplete. In order to explore its mechanical properties and propensity for preferred conformations, we conducted 1 microsecond implicit solvent molecular dynamic simulations using Gromacs with the AMBER force field. We analyzed the overall twist angle of the neck linker as a function of time and found that the twist angle distribution is bimodal indicating that the neck linker may have two preferred conformations. To determine the generality of this result, we ran simulations on disordered neck linker regions from multiple species including fruit fly, human and mouse and found that this appeared to be a generic feature of the kinesin I neck linker region.

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Conformational Changes in the β Subunits of F_1-Atpase Revealed by Fret Measurements During the Rotation of the γ Subunit

Mitsuhiro Sugawa[1], Masaru Kobayashi[1], Takashi Matsui[1], Tomoko Masaike[2], Takayuki Nishizaka[1].
[1]Gakushuin University, Tokyo, Japan, [2]Tokyo University of Science, Tokyo, Japan.

To uncover conformational changes in biomolecules accompanied with biochemical events remains challenging, even though the atomic structures and the kinetics of the biomolecules are revealed. F_1-ATPase is a rotary molecular motor in which a central γ subunit rotates against hexagonally arranged subunits $\alpha_3\beta_3$, hydrolyzing ATP sequentially in three β subunits. Previous study using a single-molecule fluorescence polarization method has proposed sets of the β subunit conformations during the rotation (Masaike et al., Nat. Struct. Mol. Biol. 2008). However, further information is indispensable for identifying the ATP-waiting form of which the atomic structure has not been revealed. Here we performed single-pair FRET measurement to detect distance changes between two β subunits. Time trajectories of FRET efficiency showed two-state transitions between high (0.8) and low (0.5). Given the crystal structures, low FRET efficiency indicates one β subunit in the open form but another in the closed form. On the other hand, high FRET efficiency indicates both of the β subunits in the closed form. Next, we performed a simultaneous measurement of FRET between two fluorescently labeled β subunits and the rotation of the γ subunit. High FRET efficiency was occurred in one of the three catalytic dwells. In the remaining five dwells, other two catalytic dwells and three ATP-waiting dwells, FRET efficiency was lower. These results suggest that in the ATP-waiting dwell two of three β subunits would not take the closed form as in the catalytic dwell. We are performing further experiments with a magnetic tweezers.

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Effects of Phosphomimetic Mutations on the Persistence Length and Thin Filament Binding Properties of α and β-Tropomyosin

Greg Medlock[1], Worawit Suphamungmee[2], An-Yue Tu[1], Xiaochuan (Edward) Li[2], Michael Regnier[1], Michael A. Geeves[3], William Lehman[2].
[1]University of Washington, Seattle, WA, USA, [2]Boston University School of Medicine, Boston, MA, USA, [3]University of Kent, Canterbury, United Kingdom.

The azimuthal movement of tropomyosin (Tm) over actin filaments to expose myosin binding sites is a fundamental step in the regulation of muscle contraction. Tm monomers link end to end over actin, so shifting of one Tm monomer to expose myosin binding sites may cause strain in neighboring monomers via end-to-end interactions, resulting in cooperative behavior. In fact, such cooperativity may extend even further when Tm is phosphorylated at Ser-283 (Rao et al., 2009), which might reinforce the overlap region and increase the effective Tm stiffness, thereby allowing more effective tropomyosin movement along the length of the thin filament. However, effects on the binding affinity of Tm to actin and troponin T due to phosphorylation of Ser-283 must also be taken into account. To test these possibilities, we produced phosphomimetic mouse α-Tm using the point mutation S283D. Samples of rotary shadowed and EM images were assessed for stiffness. The persistence length of single molecules was obtained using manual protein skeletonization, which will be compared to the results from a novel automated skeletonization algorithm. Additional mouse ß-Tm mutants with and without the phosphomimetic mutation that also contained a R133W mutation, implicated in familial distal arthrogryposis and found to increase contractility (Robinson et al., 2007), are also being produced and analyzed. Preliminary results show an increase in persistence length with S283D α-Tm, indicating increased stiffness, and a modest decrease in cTnT1 binding to actin/α-Tm with the phosphomimetic mutant. In contrast, there is a modest increase in cTnT1 binding with the non-phosphomimetic S283A α-Tm. Supported by HL65497, HL11197, and HL36153.

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Dynamics in the Transmembrane Segment of the Influenza a M2 Proton Channel

Joana Paulino[1,2], Ivan Hung[2], Tymothy A. Cross[2,3].
[1]Institute of Molecular Biophysics, FSU, Tallahassee, FL, USA, [2]NHMFL, Tallahassee, FL, USA, [3]Department of Chemistry and Biochemistry, FSU, Tallahassee, FL, USA.

Influenza A virus, responsible for the flu, infects respiratory tract epithelial cells. M2 is a tetrameric proton channel essential for the virus life cycle. The antiviral drug amantadine (Amt) used to block M2 channel prior to the recent S31N mutation. With the known backbone structure of the M2 transmembrane (TM) domain, polypeptide backbone and side chains dynamics can be characterized.