Class a Beta-Lactamase Dynamics from Molecular Dynamics Simulations

ARTICLE *in* BIOPHYSICAL JOURNAL · FEBRUARY 2011

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2010.12.3111

3 AUTHORS, INCLUDING:

Olivier Fisette

Ruhr-Universität Bochum

12 PUBLICATIONS **44** CITATIONS

SEE PROFILE

Patrick Lagüe

Laval University

34 PUBLICATIONS **396** CITATIONS

SEE PROFILE

Protein Dynamics - Simulations

2896-Pos Board B1

Identifying Binding Cooperativity in Protein Kinase a through Community Analysis

Mikolai Fajer, J. Andrew McCammon.

The binding of ATP and PKI to the catalytic sub-unit of protein kinase A is highly cooperative. A point mutation distal from both binding sites abolishes the cooperativity, and appears to be part of an allosteric network. To further investigate the allosteric network the apo, bound and mutated forms of the protein are simulated with replica exchange accelerated molecular dynamics (REXAMD). The REXAMD ensembles are validated by comparing computed NMR chemical shift perturbations against those derived from experiment.

Network models of the residue level correlation are constructed and separated into coarse grained communities that capture the allosteric network. The generality of these network models is tested by comparing mutations made directly to the network models against the mutated simulation. A broader mutational analysis of the network models is then performed in order to map out the allosteric network and identify key residues including the catalytic and regulatory spines.

2897-Pos Board B2

Molecular Dynamics Investigation on Conformational Dynamics of G Proteins

Jackson Chief Elk, J.B. Alexander Ross, Stephen R. Sprang.

We have carried out Principle Component Analysis (PCA) on a set of Molecular Dynamics (MD) trajectories computed from a set of G Protein X-ray crystal structures. G Proteins are comprised of three subunits and are important signal amplifying molecules that initiate secondary messenger cascades. Messenger cascades are responsible for initiating many biological processes and cataloging the behavior of the individual steps provides important data on cellular signaling that has potential pharmaceutical applications. In our studies the α subunit was studied exclusively because it contains the binding pocket for GDP/GTP and is the first in a series of molecules activated in signaling cascades. The specific regions Switch I-III, and P-loop on these proteins compose the binding pocket for GDP/GTP, and Switch III serves as an antenna for effector recognition. The Cα atoms on the polypeptide backbone were used in the PCA to examine the dynamics of the protein's conformation. These conformational dynamics are proposed to play an integral role in the enzyme's natural ability to shift from the resting to the active state where further rearrangements promote catalysis.

2898-Pos Board B3

Microscopic Picture of the Mechanism of Energy Transmission in F1-ATPase as Revealed by Molecular Dynamics Simulations

Jacek Czub, Helmut Grubmueller.

FoF1-ATPase is a rotary motor protein that synthesize ATP using the proton gradient across a membrane as a free energy source. The proton flow through the membrane-embedded Fo generates the rotary torque that drives the rotation of the F1 asymmetric shaft. Mechanical energy of the rotating shaft is used by the catalytic subunit of F1 to synthesize ATP against thermodynamic potential gradient. Here, we used fully atomistic molecular dynamics simulation to study the distribution of torsional elasticity in the F1 motor. Structural analysis of the rotational fluctuations revealed that the elasticity of the F1 shaft, as sensed by Fo or observed experimentally, arises from two distinct contributions: its intrinsic elasticity and an effective potential imposed by the catalytic subunit. We proposed also a simple model of the F1 energetics along the rotary degrees of freedom in the proximity of the resting state observed in the crystal structures. As opposed to the usually employed models where the motor mechanical progression is described by a single angular variable our multidimensional treatment emphasizes the spatially non-homogeneous nature of the central shaft and its interactions with the stator. We used it to predict the distribution of elastic energy stored within F1 when it is driven away from the resting state by the Fo power stroke. To directly investigate the mechanism of energy transmission between the rotor and stator subunits of F1 we employed a non-equilibrium MD approach where the central shaft is driven to rotate by externally applied torque. These simulations allowed for the elucidation of the mechanism by which the rotating shaft induces a sequence of conformational changes at the active sites.

2899-Pos Board B4

Class a Beta-Lactamase Dynamics from Molecular Dynamics Simulations

Olivier Fisette, Patrick Lagüe, Stéphane Gagné.

Two model class A beta-lactamases were studied using molecular dynamics. TEM-1, responsible for ampicillin resistance in E. coli, is the canonical beta-lactam-hydrolying enzyme. PSE-4 confers carbenicillin resistance to P. aeruginosa, an opportunistic pathogen. A total simulation time of 1.0 us for each enzyme in the free form allowed a precise determination of protein dynamics on the ~ 100 ns timescale. Comparison with NMR relaxation experiments and their model-free analysis shows quantative agreement between the two techniques. We also demonstrate how simulations can be used to interpret relaxation results and identify instances of over- and under-fitting in model-free analysis. Benzylpenicillin and carbenicillin were parameterised within the context of CGenFF, and an additional 1.0 us simulation time was acquired for both the TEM-1-BZP and PSE-4-CBC enzyme-substrate pairs. The dynamics of these complexes will be discussed, along with the effects of substrate binding and the role of the omega-loop.

2900-Pos Board B5

"DFG-flip" in the Insulin Receptor Kinase is Facilitated by a Helical Intermediate State of the Activation Loop

Harish Vashisth, Cameron F. Abrams.

The insulin receptor (IR) is a ligand-activated tyrosine kinase, whose ligand-stimulated catalytic activity and biological function depends upon trans-autophosphorylation of three activation loop (A-loop) tyrosines located in each of its cytoplasmic kinase domain (IRKD). Excised crystal structures of the inactive and active IRKD reveal that the A-loop is displaced by ~20A on activation. The highly conserved residues Asp1150, Phe1151, and Gly1152 at the N-terminus of the A-loop (the "DFG" motif) collectively "flip" to bury the Phe1151 underneath alpha-C-helix, and simultaneously present Asp1150 for ATP binding. However, the exact mechanism of the DFG-flip in the IRKD remains elusive, chiefly due to the unavailability of structural data on intermediate conformations of the A-loop. In this work, we have studied the inactive to active structural transition of the A-loop using temperature accelerated molecular dynamics (TAMD). Starting with the inactive A-loop conformation, a 50-ns of TAMD generated a target A-loop conformation within ~8A (RMSD) of the known active A-loop conformation. A further 20-ns MD-equilibration of this structure in the presence of ATP and phosphotyrosines stabilizes the A-loop conformation to an RMSD of ~4A with respect to its active state. Significantly, we also capture the DFG-flip during TAMD, and observe that this flip is facilitated by a transient three-turn helical conformation of the A-loop, the folding of which draws the placement of the side-chains of Phe1151 and Asp1152. Such transient helical conformations of the A-loop can potentially be exploited for the design of novel inhibitors that target a specific DFG conformation.

2901-Pos Board B6

Heirarchical Constrained Molecular Dynamics Simulations for Proteins

Nagarajan Vaidehi, Gouthaman Balaraman, In-Hee Park, Jeff Wagner, Abhinandan Jain.

Here we report a constrained molecular dynamics method that allows molecular models ranging from all-torsion to freezing parts of the protein as rigid body connected by flexible hinges, and studying the dynamics of proteins. This method is known as GNEIMO (Generalized Newton-Euler inverse mass operator method). We have derived new algorithms to assign initial velocities in the torsional space that obey the Boltzmann distribution. The effect of assigning initial velocities in the dihedral space (as opposed to transforming the Cartesian velocities to dihedral velocities), on the time taken for equilibrating the molecular system is analyzed.

We have demonstrated the capability of the GNEIMO method in handling a range of constrained dynamics simulations - from all torsion dynamics to freezing secondary structures as rigid bodies while modeling the rest of the protein connecting the rigid bodies with torsional degrees of freedom. The simulations where secondary structures are kept rigid, allowing torsions connecting these rigid bodies, are termed here as ''hierarchical'' simulations. We have applied the the hierarchical constrained dynamics for folding of small proteins, and refinement of low resolution protein models to high resolution structures. We find that the conformational sampling in hierarchical simulations is not only wider in range, but samples ''native like'' conformations more often than all-torsion or all-atom molecular dynamics simulations. We demonstrate the application of GNEIMO method for protein structure refinement.