1219-Pos

Discrete and Continuous Three Dimensional Simulations for Fluorescence Recovery In Bacteria

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Recent evidence indicates that components of functional molecular complexes in living cells may turnover relatively rapidly over timescales of seconds. Experimental methods such as fluorescence recovery after photobleaching (FRAP) may be used to probe the kinetics of the dynamic turnover process. Several models exist to model the FRAP process within a cell, however the majority of these require assumptions specific to the size and topology of eukaryotic cells. In the present work we present a robust discrete stochastic simulation of the FRAP process in three dimensional space for application to turnover processes in the bacterial cytoplasm. This is compared with a complementary continuous numerical simulation based on the finite element method (FEM). The effect of the dimensions and shape of the bacterial cell are analysed to elucidate the rôle of boundary effects, and low copy number régimes are simulated to study the effect of intrinsic noise in such systems. We show how these simulations may be used to optimise kinetic parameters based on experimental data.

1220-Pos

pH Induced Dynamics Enables the Peptide Exchange of MHCII Molecules

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MHC II acquires antigenic peptide at acidic endosomes, which contain proteases and DM for peptide generation and peptide exchange reactions. The mechanism of peptide exchange reaction catalyzed by DM is still an enigma. We noticed that peptide exchange can be completed by just lowering pH for most MHC II isotypes. Thermal denaturation studies by differential scanning colorimeter have shown that the MHC II are more stable at pH5 than pH7, and the effect was entropy driven which imply that molecular flexibility at low pH has some role for the reaction. Thus, we carried out the diffracted X-ray tracking (DXT) analysis of peptide/MHC II, which should detect even a slight movement of the peptide/MHC II complex at the single molecule level. The molecular movement of peptide/MHC II complexes appeared to be larger at pH5 than pH7 when low affinity peptide was bound. This sustains results of the thermodynamic studies. In order to test the effect of the increased flexibility, we artificially restrained the movement of MHC molecule. When MHC II was produced as leucine-zipper rigidified form at the C-terminus, the movement was heavily restricted on DXT measurement. Furthermore, the rigidified MHC II molecule show reduced peptide exchange capability. These results entail that the flexibility at lower pH has a role for the peptide exchange reaction of MHC II molecules.

1221-Pos

Molecular Mechanisms for Phosphorylation Driven Dissociation of Rb-E2F Complexes

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The Retinoblastoma Protein (Rb) functions as a negative regulator of cell growth in part by physically sequestering and repressing the transactivation activity of E2F. It is well established that phosphorylation of Rb by Cyclin Dependent Kinases disrupts binding between Rb and E2F, however it is unknown which of the 15 CDK consensus phosphorylation sites on Rb are required to disrupt the interaction between the pocket domain of Rb and the transactivation domain of E2F (E2FTD). In this work, we use calorimetric assays to reveal that phosphorylation at S608/S612 and T356/T373 are together sufficient to reduce the affinity of E2FTD for Rb pocket 250-fold, the same as fully-phosphorylated Rb. Nuclear Magnetic Resonance is used to identify the phosphorylation-dependent conformational changes that directly inhibit E2FTD binding. Specifically, we have found that phosphorylation of S608/S612 promotes intramolecular binding between the flexible pocket linker and the pocket domain of Rb, while phosphorylation at T356/T373 enhances binding between the N-terminal domain and pocket domain of Rb. Taken together, our results reveal two novel mechanisms for how phosphorylation of Rb modulates binding between E2FTD and Rb pocket, and we describe for the first time a function for the N-terminal domain in the inactivation of Rb.

1222-Pos

Expanding the Range of Redox Potentials of the 2Fe-2S Clusters of the Outer Mitochondrial Membrane Protein MitoNEET

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MitoNEET is a recently discovered outer mitochondrial membrane protein that harbors a 2Fe-2S cluster bound to a unique 3Cys-1His coordination (1). We measured a pH-dependent redox potential with a value near 0 mV at pH 7.0 ($E_{m,7}$). This value lies intermediate between most low potential 4Cys-coordinated ferredoxin-like (Fd) centers (~-300 mV) and most high potential 2Cys-2His-coordinated Rieske centers (~+300 mV) (2). Upon replacing the single His87 ligand with Cys, we obtained an $E_{m,7}$ near 300 mV closer to that of Fd clusters (Figure). Upon replacing Lys55 located near His87 with Met, $E_{m,7}$ increases to near +250 mV, closer to that of high potential Rieske clusters (Figure). This shows that there is a large interaction between Lys55 and His87 and that mitoNEET is robust to large changes in the $E_{m,7}$ of its 2Fe-2S clusters. Thus, we have engineered stable mutant mitoNEET with $E_{m,7}$ values over a range of almost 600 mV.

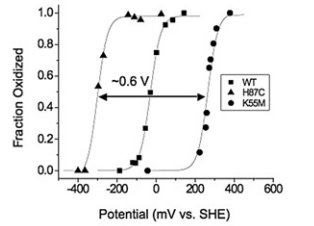

Figure. Spectroelectrochemical titrations of WT MitoNEET (squares) with H87C (triangles) and K55M (circles) mutants. The fraction oxidized versus solution potential was fit to a Nernst equation to obtain the redox potential. Measurements were done in 50 mM Tris 100 mM NaCl pH 8.0. All potential values were corrected to Standard Hydrogen Electrode (SHE).

(1) Paddock et al. (2007) Proc Natl. Acad. Sci USA 104, 14342-14347
(2) Meyer (2008) J Biol Inorg Chem 13, 157-170

Supported by NIH (GM41637, GM54038 and DK54441).

1223-Pos

"A Comparative Thermal Kinetic Investigation of the Fresh and Stale-Expired Protein Denaturing Transition"

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Protein, in eggs, has a biological quality greater than any other natural food. The quality of the protein in eggs means that all egg protein can be used for synthesis and limits the amount burned as fuel or stored as fat. Hence, the quality of egg protein can be an important factor in weight loss and essential to understand more facts about egg proteins. In this study, a comparative thermal kinetic investigation of the fresh and stale-expired proteins was made using calorimetric technique. Three different scans as temperature scans, heating rate scans and time scans were performed using differential scanning calorimetry varying temperature range from 0 °C to 200 °C and heating ramp rates from 1 °C/min to 20 °C/min. Two protein samples were used; one obtained from a fresh egg and other from the stale-expired egg from the same batch. The denaturing transition for the stale egg protein occurred at the higher temperature with smaller enthalpy and needed a larger activation than the fresh protein. This indicates that stale-expired egg provides less energy and need more energy to be activated or burnt than the fresh egg protein. Hence, the stale protein would increase more fat and need more energy to burn if eaten in the food.

Keywords: Calorimetry, fresh and stale Protein, Kinetics, Activation Energy, Denaturing transition, thermodynamics

1224-Pos

High-Order Correlations in Internal Protein Motions and Energetics

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Background: Despite originating as a linear sequence of amino acids, folded proteins display a remarkable diversity and specificity of motions, or dynamics, which constitute the building blocks of cellular metabolism. A growing body of evidence also suggests that these motions are hierarchical, involving a multitude of spatial and temporal scales. A key task in biology is thus to elucidate the relationship between hierarchical nature of protein dynamics and function. Characterizing these spatial/ temporal fluctuations in molecular dynamics (MD) simulations is critical to understanding enzyme catalysis, ligand binding, and allosteric signaling - all therapeutically exploitable processes.
Results: Using 0.5us simulation for ubiquitin, a 76 residue protein that labels other proteins for degradation, we show that positional deviations exhibit non-Gaussian behavior (kurtosis $>>$ 3), at functionally important regions of the protein. To analyze the spatial deviations we propose a general and statistically rigorous method Quasi Anharmonic Analysis (QAA) that meaningfully captures non-Gaussian behaviors overlooked with established methods. QAA,

which is an extension of independent component analysis (ICA) techniques, is a more realistic encoding of protein fluctuations and atomic coupling since its basis vectors capture, in addition to variance, higher-order spatial statistics. QAA benefits from relaxing the constraint of orthogonality in basis vectors (e.g., PCA) or assumptions of Gaussian deviations. This coupling between the basis vectors from QAA allows one to elucidate how 'fast' and 'slow' motions in ubiquitin allow it to bind to different substrates with high specificity. Conclusions: QAA is a novel approach to organize and visualize conformational landscape spanned by a protein. QAA naturally characterizes long-tailed distributions and separates conformational clusters with exceptional clarity when projected onto the novel representation space. The transitions we observe in ubiquitin signal biologically important structural shifts and highlight meaningful energetic barriers in the underlying energy landscape.

1225-Pos

An Electrohydrodynamic Model and Extensive MD Simulations Agree on the Positional and Intra-Residual Relaxations Up to Sub-Microsecond Dynamics

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Our recent simulations have indicated that the number of water molecules within a cutoff distance of each residue scales linearly with protein depth. At physiological temperatures, while the translational memory of water molecules around a residue is proportional to its depth, the orientational memory is independent of the residues position[1]. These corroborate the recently reported result that water density fluctuations around hydrophobic surfaces are considerably larger than those near hydrophilic surfaces[2].

We develop an efficient, simple model that characterizes protein dynamics both at picosecond and sub-microsecond timescales[3], which are coupled through conformational motions and catalysis[4]. Our approach is based on our earlier two-degree-of-freedom model, coupling the protein's fluctuations to the vicinal layer[5]. It proves to be efficient in estimating dynamic transitions in different solvents. The model emanates from geometric Brownian motion, similar in spirit to those including hydrodynamic interactions[6]. In our formulation, however, the traditional intra-molecular interactions are coupled to an electrostatic field that increases the flexibility of the regions in contact with hydrophobic residues, and almost exhibits a decoupled dynamics from hydrophilic regions where the solvent friction term dominates. We derive analytically the decay of the positional fluctuations and the relaxation of the distances between the residues which are not in direct contact.

We have performed 200ns MD simulations both above and below the dynamic transitions of lysozyme and myoglobin to validate our analytical results. We obtain a remarkable agreement between simulations and our model for the decay time of both positional fluctuations and intra-residual distances.

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1226-Pos

Trade-Off Between Localization and Expression Levels in Flagellar Pole Development of the Bacterium Caulobacter Crescentus

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The bacterium Caulobacter crescentus is a model organism for cell cycle regulation and development. Upon division Caulobacter differentiates into two phenotypically distinct cells, a sessile stalked cell and a motile swarmer cell. Throughout the Caulobacter cell cycle, the localization of several key proteins is highly regulated. In this study, we address the importance of spatial localization in signal transduction and development using synthetic redesign of protein localization coupled with mathematical modeling. Development at the flagellar pole is controlled by the response regulator DivK, whose phosphorylation state is controlled by the histidine kinase DivJ and the phosphatase PleC. PleC localizes to the swarmer cell pole, while DivJ localizes at the stalked pole. To address the importance of localization, To address this question, we have constructed strains with a variety of PleC and DivJ localization patterns, including delocalization and mislocalization to the opposite pole. To determine whether phenotypic changes can be explained purely on the basis of altered localization, we have developed a mathematical model that suggests that flagellar pole development does not rely critically on precise localization of DivJ and PleC, and that developmental defects due to complete mislocalization of PleC can be compensated for by overexpression. Our results indicate that localization is not absolutely necessary for some cellular functions, but that localized proteins enhance the robustness of the system to fluctuations.

1227-Pos

Free Energy Profiles of Large Scale Protein Conformational Changes

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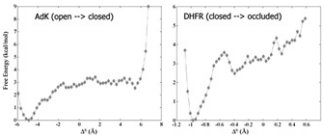
The function of many enzymes requires a transformation between widely different conformational states. Often simulations of all-atom models are not capable of determining the transformation between experimentally known end states because of the large system size and wide range of these conformational changes. However, the characterization of such transition pathways using coarse-grained models can identify significant features such as free energy barriers. In this study we employed a Monte Carlo simulation framework where bond lengths and bond angles are preserved in order to generate an initial pathway along the change in RMSD connecting these end point conformations. Within this framework, rotatable dihedral bonds along the main chain and side chain serve as the effective degrees of freedom. We then sampled conformations for each of the intermediates along this pathway without bias. The resulting conformations were combined in order to calculate the free energy profiles for these conformational changes in different proteins. These profiles yield realistic free energy barriers and indicate the degree to which conformational change is coupled to ligand binding in these enzymes.



1228-Pos

A20 Negative Feedback Regulates Period of NF-KB Oscillations

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The nuclear-cytoplasmic shuttling of NF-κB is characterized by damped oscillations of the nuclear concentration with a time period of around 1.5 hours. The NF-κB network contains several feedback loops modulating the overall response of NF-κB activity. While IκBα is known to drive and IκBϵ is know to dampen the oscillations the precise role of A20 negative feedback remains to be elucidated. Here we propose a reduced model of the NF-κB system focusing on three negative feedback loops (IκBα, IκBϵ and A20) which capture the experimentally observed responses in wild-type and knockout cells. We find that A20, like IκBϵ, efficiently dampens the oscillations although through a distinct mechanism. In addition however we have discovered a new functional role of A20 by which it controls the oscillation period of nuclear NF-κB. The design based on three nested feedback loops allows an exploration of different oscillatory responses where both period and amplitude decay can be modified. Based on these results we predict that adjusting the expression level of A20, by e.g. siRNA, the period can be changed from 1h to 3h.

1229-Pos

Systematically Defining Coarse-Grained Representations of Large Biomolecular Complexes

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Various biomolecular complexes are involved in many important biological processes. For example, the ribosome is a very large RNA-protein assembly that plays a central role in protein biosynthesis. Microtubules serve as a structural component of the cytoskeleton. It would be difficult to use large-scale atomistic molecular dynamics (MD) simulations to study the functional motions of these systems because of computational expense, and furthermore, high resolution atomic structures for such complexes may not even be available. Therefore various coarse-grained (CG) approaches have attracted rapidly growing interest, which enable simulations of large biomolecular complexes over longer effective timescales than MD simulations. We have developed a novel and systematic method for constructing CG representations of arbitrarily complex biomolecules, in order to preserve the large-scale and functionally relevant essential dynamics (ED) at the CG level. In the ED-CG scheme, the essential dynamics can be captured from principal component analysis (PCA) of a MD trajectory, elastic network model (ENM) of a single atomic structure, or a low-resolution cryo-electron microscopy density map. The method has been applied to the E. coli. ribosome and a microtubule to characterize CG models with different resolutions. The results illustrate that functionally important essential dynamics can still be captured even with aggressive coarse-graining.