Posters

Protein Dynamics III

3277-Pos

Green Fluorescent Proteins Aggregation Dynamics Explored with a Minimalist Coarse Grained Model

Fabio Trovato.
Nest at Scuola Normale Superiore, Pisa, Italy.

Green Fluorescent Proteins (GFP) are widely used as tags to monitor proteins motion in living cells[1]. GFP diffusional dynamics and optical properties, however, can be influenced by several hardly-controllable factors, such as self-aggregation and crowding in the cytoplasm. A quantitative evaluation of their impact can be of primary importance in the correct interpretation e.g. of FLIM and FRAP data, based on the measurement of diffusional dynamics[2]. A theoretical study of these aspects, however, requires the simulation of a statistical ensemble of GFPs embedded in a realistic cell environment, that is far beyond the capability of standard atomistic-based methods.

We shall present a simplified GFP model with the resolution of a single interacting center per amino-acid inspired to previous one-bead minimalist models for proteins and nucleic acids[3,4].The extreme reduction of the degrees of freedom allows us to simulate a large number of GFPs up to macroscopic time scales, and to collect sufficient statistical data for the comparison with experiments. A successful treatment of the internal dynamics is ensured by an accurate parameterization based on the Boltzmann inversion[5]. Particular attention will be devoted to the parameterization of protein-protein interactions in order to accurately simulate GFP aggregation. The cell environment is mimicked including crowder proteins in the system at lower resolution level. Results will be compared to experimental data on aggregation kinetic constants, diffusional constants and FRET experiments.

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3278-Pos

Asymmetric Dynamics of the Acyl-Carrier Protein Inside the Fungal Fatty-Acid Mega-Synthase

Claudio Anselmi, José D. Faraldo-Gómez.
Max Planck Institute for Biophysics, Frankfurt a.M., Germany.

Fatty-acid mega-synthases (FAS) are large multifunctional protein complexes responsible for the synthesis of fatty acids. In fungi, FAS features two distinct reaction chambers with three-fold symmetry, each of which includes all enzymes necessary to catalyze the iterative elongation of fatty acids, together with three acyl-carrier proteins (ACP), used for covalent substrate shuttling. Flexible linkers double-tether the ACPs to FAS scaffold to facilitate the delivery of chemical intermediates. However, the actual shuttling mechanism is still unknown; several contribution factors have been proposed, e.g. linker length and elasticity, electrostatics complementarity between the ACPs and the catalytic centers, etc.

To assess these and other factors, we have analyzed the dynamics of ACP within the FAS reaction chamber, using multi-scale molecular dynamics (MD) simulations. We have adopted a novel model, which comprises a coarse-grained (CG), semi-rigid-body representation of the ACPs; a CG, flexible representation of the ACP-chamber linkers; a grid-based representation of the FAS chamber; and an implicit description of solvent.

It was found that ACP dynamics is not hindered by the linker length nor its flexibility. Indeed, each ACP domain is able to visit all catalytic sites. Nonetheless, the probability of ACP encounters with the catalytic sites was found to differ between adjacent sub-chambers. It was found that this asymmetry arises from the steric hindrance imposed on the ACPs by the linkers. In conclusion, the dynamics of ACP within FAS appears to be essentially stochastic, and not limited by the native linker length; instead, it is modulated by volume-exclusion effects due to 'molecular crowding'and by electrostatic steering towards the chamber walls. It follows that residence times at each catalytic site will be primarily determined by their individual binding affinities for the ACP domain and its substrate.

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Interaction of CO/NO with the Apoptosis-Inducing Cytochrome C -Cardiolipin Complex

Ivan Husu[1,2], Sofia Kapetanaki[3], Gary Silkstone[4], Ursula Liebl[5], Michael T. Wilson[4], Marten Vos[5].
[1]Italian National Research Council (CNR), Rome, Italy, [2]Dept. of Chemistry, University of Rome ''La Sapienza'', Rome, Italy, [3]Dept. of Physics, University of Rome ''La Sapienza'', Rome, Italy, [4]Dept. of Biology, University of Essex, Colchester, United Kingdom, [5]Ecole Polytechnique, Palaiseau, Paris, France.

The interaction of mitochondrial cytochrome (cyt) *c* with cardiolipin (CL) is involved in the initial stages of apoptosis, leading to dissociation of the heme-Met80 bond and to a cyt *c* ''second life'' as a peroxidase.

We show that in the presence of CL both carbon monoxide (CO) or nitric oxide (NO) bind to the cyt *c* heme with high affinity. Specifically, in cyt *c*-CL complexes the NO binding leads to a pentacoordinate heme In contrast, native cyt *c* involved in the respiratory chain does not bind CO. Nitrosyl adducts of ferrocyt *c* arepossible instead, though always hexacoordinate.

Photodissociated CO from cyt *c*-CL-CO complexes gives rise to predominant bimolecular rebinding, while ~20% is geminate in a ps timescale. This contrasts with Met80X mutant cyt *c*, where geminate recombination dominates due to the protein rigidity. These and other results are consistent with a CL anchorage model showing an acyl chain impaled in the protein: CL leads to changes in the protein conformation and flexibility, allowing ligands access to the heme. The CO affinity for cyt *c*-CL is high enough to envisage an antiapoptotic effect of nanomolar CO concentrations via inhibition of the cyt *c* peroxidase activity. Furthermore an unusually complex set of kinetic steps follows the initial NO binding to the cyt *c*-CL complex heme. NO binds via a scheme comparable to that described for cyt c' and guanylate cyclase, the final product being NO attached to the proximal side of the heme. Features such as high yield of NO escape after dissociation, rapid dissociation of proximal histidine upon NO binding and its very fast binding after NO dissociation, the formation of hexacoordinate nitrosyl intermediates then indicate a remarkable mobility induced by CL on the proximal heme environment.

3280-Pos

Metric Scaling for Dimensionality Reduction of Disordered Protein Dynamics

Joshua L. Phillips[1], Edmond Y. Lau[2], V.V. Krishnan[3], Michael Rexach[4], Shawn Newsam[1], Michael E. Colvin[1].
[1]University of California, Merced, Merced, CA, USA, [2]Lawrence Livermore National Laboratory, Livermore, CA, USA, [3]University of California, Davis, Davis, CA, USA, [4]University of California, Santa Cruz, Santa Cruz, CA, USA.

One of the central tenets of molecular biology is the ''protein structure-function'' paradigm, which states that proteins adopt rigid 3-dimensional structures that are responsible for their function. There is now growing evidence that some proteins and protein domains exist as intrinsically disordered forms. Since traditional tools of biomolecular modeling focus on the fluctuation of the protein around a reference or canonical structure, new approaches are needed that do not use a single reference structure to define a metric for the dynamics of disordered biomolecules. Seemingly similar dynamics are observed for globular proteins during the folding process, so such techniques would also be beneficial to the study of non-equilibrium processes involving globular proteins. We show how classical metric scaling applied to molecular dynamics (MD) simulations of a class of entirely disordered proteins (outside of a small anchoring domain) involved in nucleocytoplasmic transport, the FG-nucleoporins (FG-nups), develops several key insights into the dynamics of the FG-nups, the adequacy of our simulation protocols, and also provides low-dimensional, detailed maps of the conformation space explored by the FG-nups. We then compare our results to those obtained from simulations of several unfolded globular proteins to see if the dynamics of folding proteins differ even at the earliest stages of the folding process.

3281-Pos

Detection of Oxidation and Palmitoylation in Snare Proteins

April Adams, Liz C. Flores, Dixon J. Woodbury.
Brigham Young University, Provo, UT, USA.

Cysteine residues play an important role for protein localization, function, and structure. Therefore, it is useful to determine the extent of cysteine modification. We have developed a sensitive assay that is able to determine the number of free (reduced) cysteines in proteins. GST-linked proteins are bound to

glutathione coated wells. The free cysteines are biotinylated and detected via streptavidin-HRP. We used this assay to study the amount of oxidation and palmitoylation in SNARE proteins (SNAP-25, Syntaxin 1A, and a no cysteine mutant of SNAP-25). Each protein contains 0-4 cysteine residues and results were compared to the background signal obtained from just GST. Reducing agents (Cu^{2+}, Fe^{2+}, Cystine) and oxidizing agents (cysteine and Zn^{2+}), were used to alter the extent of oxidation/reduction of cysteine residues. Alternatively, reduced cysteines were blocked by reactions with NEM or Palmitoyl-CoA. Except for palmitoylation, all reactions could be driven to near completion during a 10 minute incubation on ice. Palmitoylation (without enzymes) required incubation for 1 hour at RT and high doses of Palmitoyl-CoA to palmitoylate >50% of the cysteine residues. This assay is simple, inexpensive, and relative fast, and should allow greater elucidation of the chemistry of cysteine residues in proteins due to its high resolution.

3282-Pos

Application of Gaussian Network Model to Elucidate Functional Modes of Motion in a Glutamate Transporter

Indira Shrivastava, Jie Jiang, Susan G. Amara, Ivet Bahar.
University of Pittsburgh, Pittsburgh, PA, USA.

Glutamate transporters located on the plasma membrane of glia and neurons are responsible for clearing glutamate from the extracellular space and maintaining its concentration below toxic levels. Disruption in glutamate uptake is associated with acute brain injury (ischemia, stroke, seizures) and chronic pathological conditions, including amyotrophic lateral sclerosis, Hungtington's disease and Alzheimer's disease. Moreover, glutamate transporters possess a substrate-activated chloride current which may regulate neuronal activity. Understanding the mechanism of transport is therefore, of great physiological and medical importance. High resolution structures of a glutamate transporter homolog (GltPh) from an archaeal species were determined recently (Boduker et al, 2007) allowing for establishing a basis for the structural mechanism of binding and transport at the molecular level. We used the Gaussian Network Model (GNM) for protein dynamics to elucidate the most cooperative modes of motions intrinsically accessible to the homotrimer. A concerted opening and closing of the extracellular vestibule is found to be the most dominant mode of motion near the equilibrium structure. This mode of motion is expected to aid in the substrate binding process of the transport machinery. The movement of the extracellular portions of the protein, towards the center of the aqueous cavity is supported by cross-linking experiments with mutants where residues mutated to cysteine are observed to form disulfide bridges with their counterparts in the neighboring subunit. In the X-ray structure, these residues are more than 40 Å apart. The formation of these disulfide bridges also results in reduced transport of glutamate, suggesting a functional role for the motion induced by the most cooperative GNM mode.

3283-Pos

Simulation of Membrane Sculpting by EFC F-BAR Domain Lattices

Hang Yu[1], Ying Yin[1], Anton Arkhipov[1], Klaus Schulten[1,2].
[1]Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Physics, University of Illinois Urbana Champaign, Urbana, IL, USA.

Cells, during cellular morphogenesis, are dynamically sculpted into different compartments by membranes with the help of proteins. The BAR domain is one of the conserved protein domains that is involved in shaping cellular membranes in vivo, and is observed to induce tubule formation from liposomes in vitro. Previous simulations showed that certain lattice arrangements of N-BAR domains shape membranes into tubules (Yin, Arkhipov, and Schulten, 2009). Here we show, by means of several microsecond coarse-grained simulations of F-BAR domains in different lattice configurations on POPC/POPS membranes, that extended-FCH (EFC) F-BAR domains shape membrane in a fashion similar to what has been seen in N-BAR simulations. The membrane bending property of several F-BAR domain lattice arrangements is characterized, showing that different lattice configurations induce a range of membrane curvatures. A highly detailed view of the dynamic membrane sculpting process by F-BAR domain lattices on a microsecond timescale is obtained.

Yin, Y., Arkhipov, A., and Schulten, K. (2009). Simulations of membrane tubulation by lattices of amphiphysin N-BAR domains. *Structure* **17**(6)**,** 882-92.

3284-Pos

A Dynamical Model for Heat Shock Protein (HSP) Transcription and its Correlation with HSP70 in vitro Experiments

Yusheng Feng[1], Carolina Livi[2].
[1]The University of Texas at San Antonio, San Antonio, TX, USA, [2]The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Heat-shock proteins (HSPs) are well know for their protective mechanism when cells undergo stress conditions such as heat by enhancing protein folding and mitigating apoptosis Heat-shock causes the denaturing of proteins to increase, which leads to the aggregation of denatured and functional proteins. On the other hand, cells develop thermotolerance to protect themselves from later exposure to heat shock or other stresses. Once a cell has been exposed to the heat-shock, the fate of the cell depends on the amount of protein denaturation and aggregation. The cell's ability to become tolerant of heat after the heat-shock is one of the most widely studied activating responses experimentally. In the paper, we develop three mechanistic models in a hierarchical fashion that follow a biological pathway from the initial heat-shock to the HSP70:HSF complex that dissociates to HSP70 and HSF, and aim to predict the HSP concentration in a cell. These models allow investigators to simulate the major/important mechanisms in the upregulation of HSP70. This study was performed to create a depiction of the cellular and nuclear events of the HSP70 response to the heat-shock. The data generated from the model were correlated to favorably experimental data under the assumption of time delay.

3285-Pos

Tuning Protein Dynamics by Modulating Solvent Motions through Glassy Matrices, Sol-Gels and Reverse Micelles

David Dantsker, Camille Roche, Mahantesh Navati, Joel M. Friedman.
Albert Einstein College of Medicine, Bronx, NY, USA.

Many proteins can be viewed as nanodevices whose functions are controlled by a complex array of molecular motions. Functionally important protein dynamics pose a difficult challenge for study in that they span orders of magnitude with respect to amplitudes and time scales. One approach to organizing and systematically studying protein dynamics is based on the degree to which the different protein motions are slaved to different categories of solvent motion. This approach is the basis for the solvent slaving model of Frauenfelder and coworkers and our protein dynamic state model. In the present work, we describe how sol-gels, trehalose-derived glasses and reverse micelles can be used as matrices to systematically tune these different categories of protein dynamics through modulation of solvent dynamics and confinement volume. Through this approach we can dissect out the factors that control the activation energies and onset of activation for dynamics in hemeproteins such as hemoglobin and myoglobin that control substrate/ligand diffusion within the protein as well as escape from or entry into the protein from the solvent.

3286-Pos

Structural and Dynamical Behavior of the HIV-1 Nucleocapsid Protein by Molecular Dynamics and Quantum Mechanical Simulations

Jooyeon Hong, Sihyun Ham.
Sookmyung Women's University, Seoul, Korea, Republic of.

The human immunodeficiency virus-type 1 (HIV-1) nucleocapsid (NC) protein is critical for the recognition and packaging of the viral genome and has been a potential target for the development of antiviral drugs. All retroviral NC proteins contain one or two copies of an invariant array of CCHC ($Cys\text{-}X_2\text{-}Cys\text{-}X_4\text{-}His\text{-}X_5\text{-}Cys$) that are crucial for RNA genome recognition, packaging, and infectivity and have been proposed to function as zinc-binding domains. Although the solution structures of the NC protein with Zinc binding have been determined by experimental studies, the physiological relevance of zinc coordination has not been revealed. Here, we present the structural characteristics of the NCp7 protein with Zn and without Zn ions to investigate the coordination effect in the structural transitions upon inhibitory process by potential drugs by using molecular dynamics simulations with explicit water. For the elucidation of the structural and dynamic properties of the Zn-ligand binding domain in NCp7 protein, quantum mechanical calculations were executed to properly develop the force field for Zn. Upon ligand binding to NCp7 protein, correlation between experimental and computed dissociation constants will be discussed with the mechanistic variations for the ligand-binding and inhibition of NCp7 protein.

3287-Pos

Bio-Mechanical and Binding Effects in Cleavage of Von Willebrand Factor A2 Domain by Adamts13: Molecular Dynamics, Quantum Mechanics and Force Distribution

Agnieszka K. Bronowska, Carsten Baldauf, Frauke Graeter.
EML Research gGmbH, Heidelberg, Germany.

ADAMTS13 is a plasma zinc metalloproteinase crucial for primary haemostasis, the enzymatic activity of which is biomechanically controlled. Shear stress-induced partial unfolding of its substrate, the von Willebrand factor (vWF) A2 domain, is essential for efficient cleavage of the target 1605Y-1606M peptide bond. However, the mechanisms of stress-activated vWF A2 recognition and cleavage by ADAMTS13 have yet to be fully resolved.

From our newly developed Molecular Dynamics (MD) based force distribution analysis, we find force to propagate in the cleavage-ready vWF A2 unfolding intermediate such that the target peptide bond is selectively pre-stressed and