Posters

Protein Dynamics III

3277-Pos

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

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

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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

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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.

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Metric Scaling for Dimensionality Reduction of Disordered Protein Dynamics

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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

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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