Intrinsically Disordered Proteins I

305-Pos Board B105

A Coarse-Grained Model for Unfolded Proteins

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There is increasing evidence that natively-unfolded proteins play a key role in many important biological processes, including nucleocytoplasmic transport. In this work we propose an implicit solvent one-bead per amino-acid coarse-grained molecular dynamics model to study the characteristics of unfolded proteins. Experimentally-obtained Ramachandran plots for the coil regions of proteins are converted into distributions of pseudo-bond and pseudo-dihedral angles between neighboring alpha-carbons in the polypeptide chain. These are then used to derive bending and torsion potentials, which are residue- and sequence-specific. We show that the radius of gyration of denatured proteins can be well predicted by the developed potentials.

Figure 1 shows the radius of gyration (Rg) as a function of the number of residues (N) for a range of denatured proteins. Both our simulation results and experimental data can be well fitted to a power-law relation with a similar exponent. The experimental data are slightly overestimated, which could be explained by the presence of hydrophobic clusters and residual structures for the used experimental conditions. In future work our model will be extended to include hydrophobic and electrostatic effects as well.

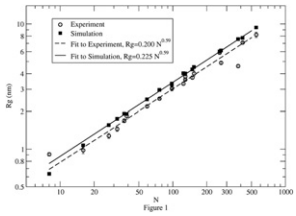

Figure 1

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Impact of Covalent Modifications on Binding and Conformational Propensities of Histone Tails

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Histone tails are highly flexible N terminal protrusions of histone proteins which help to fold DNA into dense superstructures known as chromatin. On a molecular scale histone tails are polyelectrolites with high degree of conformational disorder, allowing them to function as bio-molecular ''switches'', regulating various genetic regulatory processes via diverse types of covalent modifications. Because of being intrinsically disordered, the structural and dynamical aspects of histone tails are still poorly understood. In this work we have investigated the impact of experimentally well studied covalent modifications on conformational and DNA-binding propensities of H4/H3 histone tails(methylation/acetylation respectively). We carried out long time REMD simulations on wild-type and covalently modified forms of H4/H3 tails in presence of explicit water and ions. Our results demonstrated how covalent perturbations impact the stability of secondary structural elements that are present in the wild-type forms. We reconciled some of our previous results with in vivo experiments by probing DNA-binding energy landscapes of H4 and H3 tails showing that in presence of DNA intrinsic conformational preferences are well preserved, which is manifested in formation of fairly similar secondary structural elements upon binding. Our results also showed that binding free energy gain is sufficiently small(~3kT) to set dynamic equilibrium between DNA-bound and unbound states. Additionally we have computed the free energy profiles of DNA binding for wild and covalently modified forms finding a correlation between binding strength and change in stability of the chromatin fiber that takes place upon covalent modification of H3/H4 histone tails.

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Broad Ensemble Generator (BEG): Software to Generate Ensembles for Intrinsically Disordered Proteins

Stepan Kashtanov, F. Marty Ytreberg.

n this presentation the broad ensemble generator (BEG) software is introduced, designed to efficiently construct diverse all-atom protein structures from sequence information alone. The BEG software utilizes a build-up approach where the protein chain is grown one amino acid at a time. All generated structures follow random spatial patterns by varying phi-psi angles along the peptide bonds. There is also an option present to allow changes of amino-acid side-chain torsions. Only steric interactions are currently included, but the method is flexible enough to allow for other interaction types. The application of BEG to generating structural ensembles for intrinsically disordered proteins is also presented.

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Structural and Functional Characterization of a RecQ-Like Helicase in S. cerevisiae with a 647 Residue Disordered Region

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Sgs1 is a RecQ-like helicase in budding yeast that functions in the maintenance of genomic stability. In concert with the Top3 protein, Sgs1 has been implicated in the repair of double strand DNA breaks, the restart of stalled replication forks, and as a resolver of DNA structural intermediates, including double Holliday junctions. Loss of proper function in 3 of the 5 human homologs (BLM, WRN and RecQL4) results in Bloom's, Werner's and Rothman-Thomson syndrome respectively, diseases characterized by a high incidence of cancer and/or accelerated aging. To date, genetic and structural studies have focused primarily on the C-terminal region (residues 647-1447), which contains a conserved, catalytic helicase region and associated domains. While the N-terminal region has been implicated in several important events, including Top3 binding and ssDNA annealing, little information has been gathered about the structure and overall function of the first 640 amino acids. Here, we show via computer models (IUPRED) that the first 647 amino acids of Sgs1 are disordered. While little significant sequence homology exists for fungal Sgs1 homologs, IUPRED models reflect a similar pattern of predicted disorder correlating to evolutionary distance from S. cerevisiae. The region corresponding to vital Top3/Rmi1 binding (residues 1-125) in S. cerevisiae has been purified and exhibits an NMR spectrum that is characteristic of a disordered protein, lending support to the computer models. Future studies will focus on developing structural models for this fragment using chemical shifts and residual dipolar couplings and determining how the structure changes when bound to Top3. Data from these structural studies will be used to guide the design of mutants that will be used in genetic studies of DNA damage.

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Expression of Soluble Recombinant Proteins via Translational Fusion to Novel IDPS

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The heterologous production of proteins in Escherichia coli cells is a common practice in basic research laboratories. However, overexpressed proteins frequently aggregate as folding intermediates in insoluble inclusion bodies thereby complicating downstream applications. While strategies have been developed to recover the aggregated proteins from inclusion bodies they are not universally effective. An alternative approach is to express heterologous proteins in the soluble form through the use of gene fusions. A variety of fusion proteins have been described for this purpose including thioredoxin (Trx), maltose binding protein (MBP), glutathione S-transferase (GST), and NusA. In this work, we present a new set of intrinsically disordered polypeptide (IDP) fusion partners that promote the soluble production of recalcitrant protein targets from E. coli. A portfolio of recalcitrant proteins was expressed in E. coli as translational fusions to N-terminal 6xHis, 6xHis-MBP, or a set of 6xHis-IDP fusions. Under a standard set of growth conditions the novel IDP-fusion proteins outperformed both the 6xHis and 6xHis-MBP fusion partners in promoting soluble expression. Furthermore, the N-terminal fusions did not inhibit affinity-purification, biological activity where tested, or fusion-tag removal by enterokinase cleavage. The higher percentage of soluble protein recovered coupled to the purification and cleavage efficiencies make these novel intrinsically disordered fusion tags attractive alternatives for protein scientists.

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Structural Basis for the Dynamic Behavior of a Family of Disordered Proteins

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Intrinsically disordered proteins (IDPs) are characterized by a lack of stable tertiary structure and sample a dynamic range of conformations. When binding, IDPs often transition from a disordered to ordered state, with helices being the most common conformation adopted (1). The p53 tumor suppressor protein is an IDP that transitions from an unfolded (disordered) to folded (ordered) state upon binding. The p53 transactivation domain (TAD) forms an alpha helical structure when it binds to either the 70 kDa subunit of replication protein a (RPA70) or the murine double minute 2 protein (MDM2). We analyzed chemical shifts of RPA70 after titrating with either human p53 TAD, or the canine p53 TAD homologue using ^{15}N-^{1}H HSQC NMR experiments. Human p53 TAD appears to bind to RPA70 with a higher affinity than canine p53 TAD. Furthermore, canine p53 TAD is more dynamic than human p53 TAD, which is consistent with the titration results. There are two mutations in the canine p53