molecular extension of single oligomers having controlled composition varied with the force applied by an optical trap. Whereas monomeric α-synuclein is mostly unstructured, α-synuclein dimers and tetramers formed many different structures with a surprisingly broad range of sizes and stabilities. Many were highly stable, while others were only marginally so and interconverted rapidly; most stable structures formed quickly, within tens of seconds. These results provide a new window into the complex folding landscape of protein oligomerization, highlighting the structural heterogeneity of even the smallest oligomers.

3196-Pos Board B57

α-Synuclein Monomer Conformations Studied by in Silico and in Vitro FRET

Reinhard Klement[1], Helmut Grubmüller[1], Elisha Haas[2].
[1]MPI for Biophysical Chemistry, Goettingen, Germany, [2]Bar Ilan University, Ramat Gan, Israel.

The pathological aggregation of α-synuclein (AS), a 140-aa presynaptic protein, is cytotoxic to motor neurons in the brain and thus constitutes a major factor in the etiology of Parkinson's disease (PD). AS is intrinsically unstructured and adopts no preferred fold in solution. Since low molecular weight oligomers of AS are considered to be the most toxic species in PD it is important to study the conformers of monomeric AS. In our current investigation we combine classical molecular dynamics (MD) simulations with experimental FRET measurements to determine distance relations in the N-terminus and the NAC region of AS. With extended MD simulations (~ 90μs) we first generated ensemble structures of monomeric AS molecules. From these trajectories snapshots were extracted and the appropriate amino acids mutated at eight different FRET label positions respectively. The FRET pair consists of a tryptophan as the donor and a coumarine dye attached to a cysteine as the acceptor. QM calculations were carried out to parameterize the coumarine dye for the AMBER 99SB force field and to determine transition dipole moments. From the AS MD simulations with the attached FRET labels instantaneous donor-acceptor distances r_{DA} and values of the orientation factor κ^2 can be extracted. Applying Förster's theory energy transfer efficiencies are calculated and compared to the experimental findings. With the directly available values r_{DA} and κ^2 we get an improved structural characterization of the disordered AS ensemble.

M. Hoefling, N.Lima, D.Haenni, C.A. M.Seidel, B.Schuler, H.Grubmüller, PLoSONE 6(5): e19791. doi:10.1371/journal.pone.0019791.
A.Grupi, E.Haas, JMB, 405:1267-1283 (2011).
M.J.Roberti, C.W.Bertoncini, R.Klement, E.A.Jares-Erijman, T.M.Jovin, *Nature Methods*, 4(4):345–351 (2007).

3197-Pos Board B58

Critical Assessment of Paramagnetic Relaxation Enhancement for Ensemble Calculation of Disordered States

Jordi Silvestre-Ryan[1], Santi Esteban-Martín[1], Xavier Salvatella[1,2].
[1]Joint BSC-IRB Research Programme in Computational Biology, Institute for Research in Biomedicine, Barcelona, Spain, [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

In NMR spectroscopy, paramagnetic relaxation enhancement (PRE) can be used to probe transiently formed long-range contacts in macromolecular structures. This is particularly powerful for the structural characterization of intrinsically disordered and unfolded proteins. Here we assess the structural information encoded in PRE data, and its use in describing ensemble representations of disordered states. We seek to determine both the optimal number of PRE labels for structural determination as well as their capabilities in reproducing contact regions and contact populations. We first constructed several ensembles of a model protein with a variety of imposed tertiary contacts, and then attempted to reproduce the ensembles through simulations restrained by theoretical PRE intensities. Almost universally, the PRE data is able to recapture the contacts of the synthetic ensembles provided that one PRE label is placed every 10-20 residues. However, the degeneracy inherent in ensemble-averaged PRE data, compounded by the radius to the minus sixth dependency, makes it difficult to distinguish between contact distances and populations. As a consequence, while the contacts themselves remain visible, their populations are largely determined by the size of the simulated ensemble.

3198-Pos Board B59

Conformational Ensembles of Intrinsically Disordered Proteins are Determined by Charge Patterning

Rahul K. Das, Rohit V. Pappu.
Washington University in St. Louis, Saint Louis, MO, USA.

Intrinsically disordered proteins (IDP) or regions (IDR) fail to adopt stable secondary or tertiary structures under physiological conditions. 30% of eukaryotic proteins are IDPs while 50% of eukaryotic proteins and 70% of signaling proteins contain IDRs. IDPs sample heterogeneous conformations and characterizing their conformational ensembles is important to understand the role of disorder in IDP function. Recent work has shown that the phase behavior of IDPs with up to 50 residues can be predicted from their net charge per residue. To test the validity of this phase diagram for longer IDPs, we have carried out systematic and comparative computational studies on different IDPs/IDRs with more than 100 residues and low net charge per residue (~0.1). Our results show that in contrast to the phase diagram predictions of disordered globules, these longer IDPs can adopt semi-extended conformations that appear to have the characteristics of fractal globules or other polymeric mesophases with large conformational heterogeneity. The sequence patterning of positively and negatively charged "blobs" appears to be the primary determinant of deviations from predictions based on net charge per residue. Although the latter is valid within locally compact domains, the charge patterning acts as the primary modulator of stochastic inter-blob and inter-domain electrostatic interactions. The encoding between sequence patterning and the probabilities of through-space inter-blob and inter-domain contacts can be inferred and our ongoing efforts are focused on formalizing a theoretical framework to predict the effects of variations in charge patterning at the sequence level on the conformational ensembles of longer, functionally relevant IDPs and IDRs.

3199-Pos Board B60

Simulations of Denatured Protein Ensembles Reproduce and Rationalize Experimental Observations of Persistent Contacts Between Residues Distal in Protein Sequence

Nicholas J. Lyle[1], Wenli Meng[2], Daniel P. Raleigh[2], Rohit V. Pappu[1].
[1]Washington University, St. Louis, MO, USA, [2]State University of New York at Stony Brook, Stony Brook, NY, USA.

Interactions in protein denatured state ensembles (DSE) influence protein stability and folding kinetics. Characterization of DSE is difficult because of the underlying conformational heterogeneity. Recent paramagnetic relaxation enhancement (PRE) measurements show evidence for persistent non-local interactions in the N-terminal domain of ribosomal protein L9 (NTL9) under strongly denaturing conditions. Surprisingly, these contacts between residues distal in sequence prevail despite the lack of detectable secondary structure and evidence that the protein samples significantly expanded conformations. We present results from computational thermal unfolding studies on the NTL9 protein to provide an explanation for the observed long-range contacts. The simulations are based on the ABSINTH implicit solvation model and utilize efficient Monte Carlo methods for conformational sampling. We identify a temperature range that yields ensembles consistent with the experimental data as inferred by direct, quantitative comparison of experimental observables from simulation results to experimental data. We refer to these ensembles as TD-ensembles which were generated without any a priori knowledge of the experimental data and analyze these ensembles to provide a quantitative interpretation of experimental results. Persistent non-local contacts are made throughout the NTL9 DSE without appreciable secondary structure; the most significant being relatively low-likelihood hydrophobic clusters and contacts between the N-terminal and central portion of NTL9. Our results explain how the average dimensions of denatured proteins conform to the statistics of self-avoiding random walks despite sampling conformations with low likelihoods of long-range contacts.

3200-Pos Board B61

Assessing the Structural Ensemble and Folding Propensity of Intrinsically Disordered Proteins

Christopher B. Stanley[1], Amanda Debuhr[2], Laura Grese[1,2], Erica Rowe[1,2], Hugh O'Neill[1], Valerie Berthelier[2].
[1]Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN, USA.

The coupled folding and binding mechanism of intrinsically disordered proteins (IDPs) creates energetic consequences that yield strong, specific interactions yet with the ability to adapt toward multiple different binding targets. To understand the role of IDP structural flexibility, characterization of the conformational ensemble of the unbound molecule along with determination of its trajectory toward the bound state is required. We used small-angle neutron scattering (SANS) to investigate the structural ensemble of an intrinsically disordered region of activator for thyroid hormone and retinoid receptor (ACTR), a binding partner of the transcription coactivator CREB binding protein (CBP). The structures generated computationally using the ensemble optimization method (EOM) to fit the SANS data displayed size distributions that indicate unbound ACTR samples compact states that are comparable to the bound