amyloidogenesis progresses much slower, on the days and weeks timescale. As conditions are changed from folding to misfolding, formation of the native structure slows down indicating the increase of the barrier separating the molten globule and native states. In the meantime, the native state becomes more unstable as well. Amyloid formation is only observed among solvent conditions where folding is absent.

Probing Aggrecaan Interactions by Atomic Force Microscopy

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Aggrecaan, the major extracellular matrix proteoglycan in cartilage, is a highly charged bottlebrush shaped macromolecule. It consists of negatively charged glycosaminoglycan (GAG) chains attached to a protein backbone. The bottlebrush structure enables aggrecaan to maintain an extended conformation responsible for the high osmotic pressure sustaining compressive loads in cartilage. Alterations in aggrecaan bottlebrush structure with age and disease lead to bone deformities, dwarfism, arthritis, and other pathological conditions. In solution, aggrecaan bottlebrushes show distinct osmotic pressure versus concentration regimes. They self-assemble and form large clusters. Using Atomic Force Microscopy, we measured Static Light Scattering Second Virial Coefficient. The interaction mechanism does not appear to be dominated by an electrostatic effect of the charge at the capsid surface, and a dependence on the solvent ionic strength is observed. Keywords: Dicistroviridae; Triatoma virus; electrostatic energy; virus aggregation; Second Viral Coefficient. Figure: TriV surface charge at pH 4.0

Protein Folding & Stability III

High Pressure FTIR Studies on Model a-Helical Peptides

Teraya Donaldson, Alice Smith-Gickhorn, Sean M. Decatur.

High-pressure conditions force water into internal cavities of proteins; the presence of water in a hydrophobic interior can destabilize the tertiary structure resulting in protein unfolding. This effect has been predicted in molecular dynamics simulations for model alpha-helical peptides at high pressures, where water is forced into closer contact with backbone carbonyls (as measured by an increased in backbone hydration). According to simulations, this increased hydration depends on local sequence and the impact that specific side chains have on backbone conformation. We have investigated the effects of pressure on a series of 20- residue peptides. Model peptides based on the alanine and lysine repeats form water soluble, stable alpha helices (1). The sequence (AAAAK)3-AAAAAY is a well-characterized, synthetic polypeptide, ideal for the study of helix propensity (2). Using the model peptides, we experimentally confirm that the helical content is conserved under pressure. Perturbations were monitored using a probe of secondary structure, the amide I’ band, with infrared (IR) spectroscopy and a diamond anvil cell. Amide I’ mode shifts to a lower frequency with the increase of pressure. Local information is obtained by measuring amide I bands in 13C labeled peptides, where 13C alanines are placed at different positions relative to the lysines within the peptide. We examined the shielding effects of lysine at the various positions and confirm that shielding of backbone carbonyls occurs.


SAXS Study of Cytochrome-C Cold Denaturation

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We present a study of the cold denaturation of proteins using Small Angle X-Ray Scattering. The size and shape of equine cytochrome-c is determined at varying salt and pH conditions from –25 to 60 °C and compared to the two-steps of Thermal Protein Denaturation (Dill (PNAS 2009)). The incorporation of a temperature-dependent pH and solvent dielectric constant is critical to model electrostatic interactions over this broad temperature range and properly predict the observed protein stability from sequence. Under suitable conditions, the protein can be made to increase in size by nearly 9 Angstroms (over 60% of its native radius of gyration) when dropped in temperature from 0 to –25 °C. Cold denaturation under these conditions is also verified by monitoring fluorescence from the native tryptophan in this protein. This allows us to compare denaturation monitored at one location inside the protein with global structural changes observed by SAXS.

Apoprotein B Reconstruction at Single Molecular Level


Low density lipoprotein-cholesterol (LDL-C) is a clinical significant marker of cardiovascular disease risk. Each particle of LDL contains only one protein, apolipoprotein B (apoB). In human, the liver secretes full-length apoB (apoB-100) which also serves as a ligand for receptor-mediated uptake of LDL by a variety of cell types, such as monocyte and A549. Many diseases’ progression may cause by the deficiency of the LDL assembly. Therefore, it is desired to reveal the folding/assembly process of apoB. However, it is challenge to refold the apoB, because of its high insolubility in solution. In this study, the native apoB had been purified from LDL by ice shear method, and truncated mutants of various lengths of apoB were recombinant expressed from E. coli system. The apoB-100 and its truncated proteins were then dissolved in the denature buffer which contained additional detergents and were refolded via an over-critical refolding process. The folding intermediates of apoB-100 and its truncated mutants could be observed by immunofluorescence microscopy at the single molecular level and the lipidation processes and secondary structures of apoB can be also analyzed by SRCD. Moreover, the con-focal microscopy showed that the refolded and native LDL could be absorbed by THP-1 and A549 cell. According to our observation the LDL assembly process can be proposed. This is the first study to refold the structural and function of apoB in vitro. Meanwhile, this refolded lipoprotein can be used as carrier for hydrophobic particles delivery.

Folding Studies of Beta-Strand-Containing Repeat Proteins Through Naturally-Occurring and Consensus-Designed Sequences

Thuy P. Dao, Ananya Majumdar, Doug Barrick.

Repeat proteins, devoid of sequence-distant contacts observed in globular proteins, are ideal candidates for the dissection of local stability, nearest-neighbor contact parameters, cooperativity, and determination of folding energy landscapes. Recent studies on HEAT, TPR, Ankyrin, and Armadillo have contributed to the understanding of the folding of helical repeat proteins. Moreover, constructs composed of simplified “consensus” sequence...