proteins with a battery of ligands or a plethora of buffer conditions. In all cases, an environmentally sensitive fluorescent dye (SYPRO orange) is used to monitor melting temperature shifts under various conditions. HTTS has also been used to characterize protein-protein interactions, but its general applicability to protein complexes remains to be tested. Here, we use the complex formed by the N-terminal fragments of Protocadherin-15 (pcdh15) and Cadherin-23 (cdh23), essential for inner-ear mechanotransduction, to test modified HTTS assays that evaluate protein-protein binding affinities. By mixing cdh23 and pcdh15 at different ratios, we observed increased thermal stability of pcdh15 caused by shifts in chemical equilibrium towards the complex. In addition, protein fragments carrying missense mutations known to cause deafness and reduce binding affinity lack these shifts. These results suggest that HTTS can be used to qualitatively evaluate cadherin protein-protein interactions. We further validated our HTTS results using surface plasmon resonance-based affinity measurements. Surprisingly, we found that in some cases thermal stability shifts correlate well with koff rather than Kd. Our modified protocols might be applicable to any heterodimeric protein complex with affinities ranging from ~ 1 nanoM to 100 microM.

1920-Pos Board B64

An iLBP Family Member Domain Swapped Dimer is Evidence for a Highly Ordered Folding Intermediate

Zahra Assar[1], Zahra Nossoni[1], Wenjing Wang[2], Babak Borhan[1], James H. Geiger[1].
[1]Chemistry, Michigan State University, East Lansing, MI, USA, [2]Massachusetts Institute of Technology, Cambridge, MA, USA.

Human Cellular Retinol Binding Protein II (hCRBP II), a member of the intracellular Lipid binding protein (iLBP) family, is a monomeric protein responsible for the intracellular transport of retinol and retinal. HCRBP II and other members of this family have been shown to be remarkably stable to mutations. Previous studies on other proteins of this family have shown that they fold into monomeric species. However we report, for the first time, that wild-type (WT) hCRBP II gives rise to an extensive domain swapped dimer (involving almost 50% of the protein sequence) during bacterial expression. In addition, there is no evidence of interconversion between monomer and dimer at room temperature, even after weeks of incubation, suggesting that two non-interconverting folds can result from the same amino acid sequence. Though wild-type hCRBP II forms the dimer, the propensity for dimerization can be substantially increased via mutation at Tyr60. Structural studies of wild-type and several mutant dimers suggest that an ''open monomer'' folding intermediate gives rise to both monomer and dimer, and their ratio depends on the relative orientation of the two halves of the protein in the open monomer intermediate. Therefore, Nature may build in an interaction (a Tyr60-Glu72 hydrogen bond) in a folding intermediate to prevent dimerization to yield the physiologically relevant monomeric protein, begging the question of whether other members of the iLBP family form physiologically relevant dimer species.

1921-Pos Board B65

Increase in Domain Swapping of the DNA-Binding Domain of Human FoxP1 is Related to a Decrease in Monomer Folding Stability

Exequiel Medina, Cristóbal Córdova, Javiera Reyes, César A. Ramírez-Sarmiento, Jorge Babul.
Universidad de Chile, Santiago, Chile.

Domain swapping is a folding-upon-binding phenomenon that allows proteins to form dimers by exchanging segments of their structure with another equivalent subunit. Recently, solved structures of the DNA-binding domain of the P subfamily of forkhead box transcription factors, FoxP, showed that these proteins can also form swapped dimers. Biologically, mutations of the DNA-binding domain of these proteins are linked to diverse diseases in humans. Moreover, FoxP1 and FoxP2 can reach monomer-dimer equilibrium in solution after hours of incubation, suggesting a low kinetic barrier separating both species in contrast to other domain-swapping proteins where this process takes days. Using wild type FoxP1 (WT) as a model of domain swapping, we analyzed the temperature and protein concentration effects on dimer dissociation, obtaining the free energy change and enthalpy of the process, indicating that the dimerization is an enthalpy-driven process. To understand how FoxP1 could swap, we performed equilibrium unfolding experiments with the dimer, showing that it follows a three-state mechanism, where the first transition corresponds to dimer dissociation and occurs with little loss of secondary structure. To further corroborate the relevance of hinge region and helix H3 in the domain swapping process of FoxP1, we engineered a previously described hinge monomeric mutant of FoxP1 (A539P) and we constructed a mutant in helix H3 (R553H) related to human health diseases. The dimerization propensity of R553H is higher than WT, suggesting that helix 3 and hinge region are relevant in the dimerization. Folding stability comparison of A539P and R553H monomers with WT show that the order of stability is A539P>WT>R553H, concluding that the ability of FoxP1 to domain swap rapidly can be partially explained by its low monomer stability. Funding: FONDECYT 1130510, 11140601.

1922-Pos Board B66

Coiled-Coil Probes Identified the Unfolding Pathway of Yeast Phosphoglycerate Kinase

Qing Li[1], Zackary N. Scholl[2], Piotr E. Marszalek[1].
[1]MEMS, Duke University, Durham, NC, USA, [2]Duke University, Durham, NC, USA.

Large multi-domain proteins which have complicated reacting interfaces and dominate the proteomes of life remain mysterious regarding to their folding behaviors and mechanisms. Comprehensive studies towards the understanding of the fundamentals of protein folding mechanisms tend to bias small single-domain monomeric proteins, possibly due to their simplicity and reversible refolding capabilities. Yet the understanding of protein folding behaviors acquired from studying small single-domain proteins are not adequate to generate an accurate extrapolation for predicting the folding mechanisms of proteins that contain more than one domain. Here we present the characterization and interpretation of the unfolding pathway of a model large multi-domain protein yeast phosphoglycerate kinase (PGK) using single-molecule force spectroscopy (SMFS) and insertion of the antiparallel coiled-coil (CC) polypeptide probe we developed previously. When placed into different loop regions inside of yeast PGK, the CC probe affected different portion of the unfolding profile. Statistical comparison between the force-extension (FE) curves of multiple CC inserted yeast PGK's and the wildtype yeast PGK provided insights into figuring out the effects of conformational changes in domains and across the interfaces between domains on the unfolding pathway of the entire yeast PGK protein. Together with coarse-grain simulation modeling of the unfolding pathway, the sequence of the unfolding events occurred along the unfolding pathway of yeast PGK is precisely determined.

1923-Pos Board B67

Folding Analyses of the Major Folding Intermediate of Prouroguanylin using Deletion Mutants

Kenta Hattori[1], Masaki Okumura[2], Shigeru Shimamoto[1], Yuji Hidaka[1].
[1]Kinki University, Higashi-osaka, Japan, [2]Tohoku University, Sendai, Japan.

Uroguanylin serves as an endogenous ligand for guanylyl cyclase C, and consists of 16 amino acid residues and two disulfide bonds. Uroguanylin is secreted in the form of the precursor protein prouroguanylin, in vivo, and the propeptide region of prouroguanylin plays the role of an intra-molecular chaperone in the formation of the native conformation of the mature peptide, uroguanylin [1,2]. Our previous studies dealing with the disulfide-coupled folding pathway of prouroguanylin suggested that a folding intermediate (isomer 2) with one and two mis-bridged disulfide bonds in the pro-peptide and mature region is formed and is then was converted to the native form by a disulfide exchange reaction. Structural analyses of isomer 2 and the native form of prouroguanylin indicated that isomer 2 possesses a slightly larger of α-helical structure content than the native form. These results indicate that isomer 2 is a major folding intermediate in the formation of prouroguanylin and the disulfide conversion, which may be induced by β-sheet formation between the N-terminal and the C-terminal region of the pro-peptide and the mature region, respectively, from isomer 2 to the native form results in a decrease in the α-helical structure content of the molecule. Therefore, a structural analysis of isomer 2 is important in terms of understanding the disulfide-coupled folding of prouroguanylin. In this study, to investigate the folding mechanism of prouroguanylin, a series of C-terminal deletion mutants of isomer 2 was prepared and their secondary structures estimated by means of CD spectroscopy. The results indicated that the C-terminal Cys77-Ala81 region is required for the kinetic or thermodynamic stability of isomer 2. The folding mechanism of prouroguanylin via isomer 2 will be discussed in this presentation.

1924-Pos Board B68

Interdomain Contacts and RNA Polymerase Control Native State Interconversion of the Transformer Protein RfaH on a Dual-Funneled Landscape

César A. Ramírez-Sarmiento[1], Jeffrey K. Noel[2], Sandro L. Valenzuela[1], Irina Artsimovitch[3].
[1]Departamento de Biologia, Universidad de Chile, Santiago, Chile, [2]Center for Theoretical Biological Physics, Rice University, Houston, TX, USA, [3]Department of Microbiology and The Center for RNA Biology, Ohio State University, Columbus, OH, USA.

RfaH is a bacterial virulence factor that belongs to the universally conserved NusG family of transcription factors and is commonly referred as the first example of a transformer protein. In contrast to other NusG proteins, the C-terminal domain (CTD) of RfaH folds into an α-helical hairpin that is stabilized by interactions with the N-terminal domain (NTD), which in turn masks an RNA polymerase (RNAp) binding site and thus constitutes an autoinhibited state.
This autoinhibition is relieved when binding to a specific DNA element in the transcription elongation complex triggers domain dissociation, allowing the NTD to interact with RNAp to facilitate transcription while the CTD dramatically refolds into a NusG-like β-barrel that interacts with the ribosome to activate translation, thus changing both the structure and function of RfaH.
Since this phenomenon is experimentally challenging, their structural details remain to be elucidated. Here, we explore the mechanism of the conformational switching of RfaH in the full-length protein using a dual-basin structure-based model. Our simulations capture several features described experimentally, such as the requirement of disruption of interdomain contacts to trigger the dramatic α-to-β transformation of RfaH, confirms the roles of previously indicated residues E48 and R138, and suggests a new important role for F130, in the stability of the interdomain interaction. These native basins are connected through an intermediate state that builds up upon binding to the NTD and shares features from both folds. We also examine the competitive binding between RfaH-CTD and RNAp for the NTD, showing that RNAp binding favors the β fold.
Our study shows that native-biased models are appropriate for interrogating detailed mechanisms of the structural rearrangements during the native state interconversion of RfaH. FUNDING: Fondecyt 11140601.

1925-Pos Board B69
Fast Closuer of Long Loops at the Initiation of a Protein Folding Pathway
Elisha Haas[1], Dan Amir[1], Tomer Orevi[1], Gil Rahamim[1], Sagar Kathuria[2], Robert C. Matthews[3], Osman Bilsel[3].
[1]Bar Ilan University, Ramat Gan, Israel, [2]Biochemistry, UMass school of Medicine, Worcester, MA, USA, [3]UMass school of Medicine, Worcester, MA, USA.
What are the initial steps in the folding of globular proteins? In order to test the hypothesis that long loop closure transitions are part of the initiation of the folding pathways of globular proteins, our groups are studying the initiation of the folding transition of a model protein by time resolved excitation energy transfer (trFRET) detected fast kinetics experiments. Site specific double labeling is used to study the timing of conformational transitions of individual loop forming chain segments at the μs time regime. Previously, it was shown that at least three long loops in the Escherichia coli adenylate kinase (AK) molecule close within the first 5 ms of the 6 seconds folding kinetics of AK. We applied a continuous flow based double kinetics experiment. These measurements enabled us to obtain μs series of transient time dependent distributions of distances between the ends of the labeled loops. Analysis of the trFRET experiments shows that the N terminal loop (loop I) is closed within less than 60 μs after the initiation of refolding. Loop II is also mostly closed within that time step but shows an additional small reduction of the mean end to end distance in a second phase at a rate of $0.005\mu s^{-1}$. This second phase can either reflect tightening of a loosely closed loop in the ensemble of conformers or may reflect two subpopulations in the ensemble, which differ in the rate of closure of loop II, but not in the rate of closure of loop I. This study shows the very fast closure of long loops in the otherwise disordered backbone and fine details of the very early hidden pretransition state stepsthat are essential for the fast and efficient folding of the protein molecule.

1926-Pos Board B70
Examining the Vectorial Folding Pathway of the β-Helical Peptide, Pertactin, using Molecular Dynamics Simulations
Anthony Hazel, James C. Gumbart.
Georgia Institute of Technology, Atlanta, GA, USA.
Gram-negative bacteria, lacking an energy source at the outer membrane (OM), have evolved a number of unique systems in order to carry out necessary exchange of molecules across the OM. An example is the export of so-called ''virulence factors'', the passenger domain of autotransporters (AT), >97% of which are predicted to adopt right-handed β-helical structures. Given that these passenger domains have a stable C-terminal core, and cross the OM C-terminus to N-terminus, a vectorial folding pathway could direct secretion of the passenger domain across the OM without the need for an external energy source. To better understand the proposed vectoral folding pathway, we calculate the free energy landscape for the first turn of the β-helical protein, pertactin (PDB: 1DAB). Using the radius of gyration, RG, and the number of native hydrogen bonds, QH, we performed 2D umbrella sampling with replica exchange (REMD-US) on residues 438 to 481, while restraining residues 482 to 539 to their crystal structure. We examine how kinetic traps may affect the folding rate in the unconstrained pathway, whereas the vectorial pathway avoids these traps, leading to faster folding rates for the latter, which has been observed experimentally.

1927-Pos Board B71
Resolving the Heterogeneity of the Ensemble of Unfolded States by a Combination of Fluorescence Spectroscopic Methods
Katherina Hemmen[1], Dmitro Rodnin[1], Igor Markovic[1], Suren Felekyan[1], Ralf Kuehnemuth[1], Hugo Sanabria[2], Claus A. Seidel[1].
[1]Institute of Molecular Physical Chemistry, Heinrich-Heine-University, Düsseldorf, Germany, [2]Department of Physics and Astronomy, Clemson University, Clemson, SC, USA.
Intense debate exists on the possibility that unfolded proteins show, to certain extent, residual secondary structure. Transient structure formation might facilitate folding (nucleation site) and/ or enhance binding to ligands. Some proteins such as those categorized as intrinsically disordered proteins take advantage of these features. Here, we used the model enzyme lysozyme from the phage T4 (T4L), a simple two subdomain protein, which already exists in an equilibrium of at least three conformations under native conditions. We created a set of 24 double mutants of the cysteine-free pseudo-wild type by inserting an unnatural amino acid and a cysteine mutation and site-specifically labeled them using orthogonal chemistry with a Förster resonance energy transfer (FRET) dye pair. Our set of variants allows us to build up a network of distances spanning the enzyme in order to monitor the ensemble of conformations. The behavior of the protein under highly denaturing conditions was observed by a combination of ensemble (ensemble time-resolved fluorescence lifetime and anisotropy) and single-molecule spectroscopic (multiparameter fluorescence detection, photon distribution analysis, (filtered) fluorescence correlation spectroscopy) methods. Our network covered all possible directions over the whole protein allowing us to map specifically the local motions (dye mobility) and global changes. We identified regions with residual structure which exist even under highly denaturing conditions. Additionally, the combination of ensemble and single-molecule methods allows us to resolve the full heterogeneity of the proteins' denatured conformations.

1928-Pos Board B72
Key Roles of Translocating Loops in the Mechanochemical Coupling and Power Production of a AAA^+ Protease Machine
Piere Rodriguez-Aliaga[1], Luis Ramirez[2], Frank Kim[2], Carlos Bustamante[3], Andreas Martin[4].
[1]Biophysics Graduate Group, University of California at Berkeley, Berkeley, CA, USA, [2]University of California at Berkeley, Berkeley, CA, USA, [3]Chemistry, Physics, and Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA, USA, [4]Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA, USA.
ATP-dependent proteases are crucial to maintain cellular protein homeostasis. Here we used single-molecule assays to characterize the mechanism by which the AAA^+ protease ClpXP from E. coli transforms energy from ATP hydrolysis into mechanical work to drive protein translocation and unfolding. We establish that ATP hydrolysis and phosphate release occur in the burst phase during conformational changes of the motor, while ADP release and ATP binding happen in the dwell between bursts. We find that the residues of the highly-conserved translocating pore loops in the central ClpX pore determine the efficiency of substrate unfolding and translocation, and are crucial for the mechanochemical coupling and the power generated by ClpXP. Interestingly, we observe that the conformational resetting of the pore loops between consecutive power strokes appears to time both the dwell duration and the release of ADP. Together, our results indicate that: i) the mechanochemical coupling of the motor and its unfolding capability are mediated by the size of the residues in the translocating loops, ii) the unfolding capability of the motor does not depend on the burst size or the grip as previously proposed, but instead it depends on the power -work produced per unit time- generated by the motor, iii) provide insights into why evolution has selected a conserved sequence-motif for the translocating loops of prokaryotic and eukaryotic AAA^+ proteases, and iv) indicate that ClpXP's mechanism deviates from other well studied molecular motors (such as the Phi29 DNA packaging motor).