2230-Pos Board B200

Detection of Predominant Antiparallel Strand Registry in the Membrane-Associated HIV Fusion Peptide

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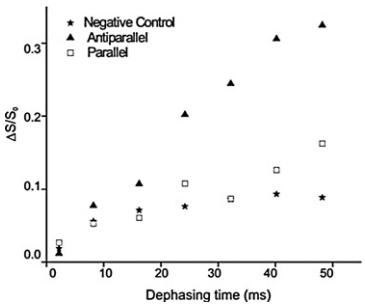
The HIV gp41 protein plays a significant role in the fusion of viral and host cell membranes. The ~16 N-terminal residues of gp41 form the apolar fusion peptide (HFP) that insert into the host cell membrane and play a critical role in fusion. A 30-residue peptide containing the HFP sequence was synthesized and was shown to induce fusion between vesicles. For HFP associated with membranes with physiological cholesterol content, solid-state nuclear magnetic resonance (SSNMR) measurements demonstrated a fully extended β strand conformation for the first 16-residues of HFP and that the HFPs were aggregated into a β sheet. In the present study, SSNMR was applied to probe for the relative population of parallel in-register strands and antiparallel strands in the β sheet region. Samples were prepared with a mixture of two HFPs, one of which contained a single ^{13}CO backbone label and one of which contained two sequential ^{15}N backbone labels. SSNMR measurements of ^{13}CO-^{15}N distances with different labeled HFPs showed that 50-60% of the strands in the β sheet were antiparallel and 5-15% were parallel. These data provide a clear structural model for the membrane-associated HFP.



2231-Pos Board B201

pH-Triggered Membrane Insertion Pathway of the Diphtheria Toxin T-Domain: 1. Insertion/Refolding Intermediate

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The T-domain of diphtheria toxin undergoes low pH-induced refolding that allows it to insert spontaneously into a lipid membrane. To gain insights into the general principles of non-constitutive membrane protein insertion we have examined pH-dependent membrane interactions of the T-domain using several spectroscopic techniques. Membrane topology of the NBD-labeled T-domain was studied using our fluorescence lifetime-based LysoUB quenching method. Our results reveal a well-defined translocation for NBD attached to a single cysteine in position 350 and no translocation for position 378C. We compared kinetics of membrane insertion and kinetics of binding of the T-domain. Energy transfer between donor-labeled T-domain and acceptor-labeled lipid vesicles demonstrates rapid membrane association for all pH values for which binding occurs. The insertion kinetics (measured with NBD probe attached at 369C) is significantly slower, and is also both pH- and lipid-dependent. This difference between FRET-based binding kinetics and NBD-based insertion kinetics indicates the presence of an interfacial intermediate on the insertion pathway of the T-domain. We examined the compactness of the fold of this intermediate using tryptophan quenching of bimane fluorescence. Bimane was attached at the 369C position, which places it in close proximity to the native W206, allowing one to monitor local unfolding of the protein. The pH-dependent reduction in quenching suggests that anionic lipids promote the unfolding of the intermediate on membrane interfaces. FCS measurements of the formation of the membrane-competent conformation of the T-domain indicate that the protonation of titratable residues occurs both in the bulk of the solution and near membrane interface. In an accompanying presentation (Rodnin et al., BPS Meeting 2009) we examine the role of several crucial histidine residues on the T-domain insertion pathway. Supported by NIH GM069783(-04S1).

2232-Pos Board B202

pH-Triggered Membrane Insertion Pathway of the Diphtheria Toxin T-Domain: 2. Role of Histidines

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The function of diphtheria toxin T-domain is to insert into the lipid bilayer and translocate the catalytic domain across the membrane in response to acidification of the endosome. In the accompanying presentation (Kyrychenko et al., BPS Meeting 2009) we have characterized the membrane insertion pathway of the T-domain WT. Here we examine the role of several crucial histidine residues in pH-dependent insertion by comparing WT to various mutants with one or more H-to-Q or H-to-R substitution(s). Thermal denaturation measured by CD indicates that all tested substitutions lead to lower stability of the protein. Kinetic measurements of guanidinum denaturation reveal that the folding branch of the chevron plot for the WT is independent of pH, while the unfolding branch becomes more prominent with acidification (a feature lost in some of the mutants). We have examined the insertion of the TH8-9 helical hairpin by following the topology of the NBD probe attached to C350, which is translocated across the bilayer in the WT. Interestingly, a single mutation of a remote histidine residue (H257R or H257Q) abolishes the insertion, while triple mutations of the H322, H323, H372, located on the top of the TH8-9 hairpin, do not affect the topology. This result is confirmed by emission measurements of the bimane probe placed in the middle of TH-9 helix: (1) WT-like blue shift for triple-R or triple-Q mutants and no shift for H257R or H257Q. The kinetics of membrane insertion, however, is affected in triple mutants: abolishing the charge (triple-Q mutant) results in slower insertion kinetics, while placing constant charges (triple-R mutant) in the same positions accelerates the insertion at intermediate pH. These results are consistent with the proposed role of His protonation in triggering membrane refolding. Supported by NIH GM069783(-04S1).

Molecular Chaperones

2233-Pos Board B203

Characterization of Substrate Binding to the Group II Archael Chaperonin from Methanococcus maripaludis (Mm-Cpn)

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Molecular chaperones are important for the proper folding and function of many proteins, and malfunction of chaperone-assisted folding has been implicated in several protein aggregation diseases. For this reason, chaperones have been identified as a potential area for drug development. An important step in developing specific therapeutic targets is the identification of the recognition site on the substrate as well as the binding site on the chaperone. In this study, we use a number of biophysical and biochemical methods to elucidate the binding site on a Human γ-Crystallin substrate and the recognition site on the Methanococcus maripaludis chaperonin (Mm-Cpn). A homologue of human chaperonins, Mm-Cpn has been shown to bind and inhibit the aggregation of several closely related members of the Human γ-Crystallin protein family including HγD- and HγC-crystallins. Although members of this protein family share a high degree of homology, Mm-Cpn suppression of HγD aggregation is twice as efficient as the suppression of HγC aggregation. It remains unclear whether this difference in suppression efficiency is linked to sequence-determined substrate binding kinetics or to differences in substrate aggregation kinetics. To better understand how sequence difference may affect aggregation and chaperone binding, we studied the chaperone-induced aggregation suppression for the isolated N-terminal and C-terminal domains of both HγD and HγC. Supported by an NIH Roadmap Award to the Center for Protein Folding Machinery (http://ncmi.bcm.tmc.edu/nanomedicine).

2234-Pos Board B204

Characterization of the Group II Chaperonin TriC derived from Human Cervical Adenocarcinoma (HeLa) Cells

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Molecular chaperones are key elements in protein folding pathways, and there are numerous proteins which are unable to fold in the absence of one or more molecular chaperones. Eukaryotic chaperones can be broadly separated into two families: chaperones, which sequester and protect unfolded proteins, but do not facilitate folding, and chaperonins, which require ATP, and actively fold nacent or misfolded proteins. The mammalian chaperonin TRiC, in addition to being required for actin and tubulin folding, binds and refold several disease causing proteins *in vitro*, including those associated with Alzheimer's disease, Von Hippel Landau tumor, and Huntington's disease. This refolding activity has revealed TRiC as a possible therapeutic agent for the treatment and prevention of aggregation diseases. We have purified TriC from cervical adenocarcinoma cells. To assess the properties of Human TriC, we are characterizing its interactions of with human γ Crystallins. Crystallins are a family of structural proteins found in the lens of the human eye, and aggregation of these proteins is thought to be the cause of cataract. *Methanococcus marapaludis* chaperonin Mm-Cpn, a homolog of human TriC, has been shown to both suppress aggregation of Human γ Crystallins, and refold the Crystallins to a native-like conformation. Suppression of γ Crystallin aggregation is being investigated using UV/Vis spectroscopy, and the ability of

TriC to restore unfolded γ Crystallin to a native fold investigated using size exclusion chromatography. In collaboration with fellow members of the Center for Protein Folding Machinery, we are investigating the possibility of visualizing the crystallin substrate in the chaperonin/substrate complex by Cryo-EM.

Supported by an NIH Roadmap Award to the Center for Protein Folding Machinery (http://ncmi.bcm.tmc.edu/nanomedicine).

2235-Pos Board B205

HαB-Crystallin Suppresses The Aggregation Upon Refolding Of Its Physiological Substrates HγD-, HγC- And HγS-Crystallin

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The passive chaperone α-crystallin, a small heat shock protein, is one of the ubiquitous crystallins in vertebrate lenses, along with the βγ-crystallins. It is composed of two subunits (~ 20 kDa) αA- and αB-crystallin (αA- and αB-crys), which form an hetero-oligomeric, polydisperse complex of ~ 800 kDa in the lens. Aggregates isolated from mature-onset cataracts, the major cause of sight loss worldwide, contain damaged and misfolded forms of βγ-crystallins, as well as α-crystallins. We have studied the chaperone function of Human αB-crystallin interacting with its physiological Human γ-crystallin substrates. Human γD-crystallin (HγD-crys) and γC-crystallin (HγC-crys) are stable and long-lived mammalian γ-crystallins localized in the lens nucleus. Human γS-crystallin (HγS-crys) is abundant in the lens outer cortex. All three γ-crystallins can refold in vitro to their native state after unfolding in high concentrations of GdnHCl. However, at very low denaturant concentrations (< 1 M GdnHCl) aggregation of refolding HγC- and HγD-Crys intermediates competes with productive refolding. Diluting unfolded HγC-, HγD-, or HγS-crys to low GdnHCl concentrations (100 μg/ml, 37°C) resulted in the protein population partitioning between productive refolding and aggregation pathways. HγD-, HγC- or HγS-Crys protein was allowed to refold and aggregate in the presence of HαB-Crys homo-oligomers at different monomer-to-monomer ratios of γ-Crys to αB-Crys. HγD- and HγC-Crys aggregation was suppressed to similar levels, whereas HγS-Crys aggregation was not suppressed as strongly in assays measuring solution turbidity at 350 nm. SEC chromatograms of the products of suppression reactions showed the presence of a high molecular weight complex containing the chaperone-substrate complex in ratios of 1γC:5αB and 1γD:5αB chains. This complex was still present 4 days after the suppression reaction was initiated. These results provide a model for how α-crystallin interacts with aggregation-prone substrates in vivo.

2236-Pos Board B206

Interaction between Molecular Chaperone Prefoldin with Group II Chaperonin in the Presence of Nucleotides: Implication for Substrate Transfer Mechanism from Prefoldin to Chaperonin

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Prefoldin (PFD) is a molecular chaperone that captures a protein-folding intermediate and transfers it to a group II chaperonin (CPN) for correct folding. However, mechanism of substrate transfer from PFD to CPN remains to be elucidated. Previous studies showed that CPN has a helical protrusion as a built-in-lid, and uses ATPase cycling to promote the conformational change necessary to open and close the lid. In this study, we have examined interaction between archaeal PFD and CPN in the presence of various nucleotide analogs. Affinities between fluorescein-labeled Pyrococcus PFD (PhPFD) and Thermococcus CPN (ThCPN) in the absence or presence of ADP and AMPPNP were examined by fluorescence anisotropy measurement. In the presence of ADP and AMPPNP, ThCPN was shown to take open and closed conformation, respectively.

The affinity of PhPFD to ThCPN was weakest in the presence of AMPPNP, which suggests that PFD does not bind to CPN in closed-state. In contrast, PhPFD bound more tightly to ThCPN (nucleotide free or ADP) in open-state. Interestingly, affinity of PhPFD to ADP-ThCPN was higher than nucleotide free-ThCPN, even though both take open conformations. This result also implies that these open conformations are different, which is supported by other experiments indicating that ADP-ThCPN can suppress thermal aggregation of citrate synthase more efficiently than nucleotide free-ThCPN. Our data implicates that substrate protein is delivered from PFD to CPN of the open conformation selectively in ADP bound-state rather than nucleotide free-state.

2237-Pos Board B207

Networks of Functional Residues in GroEL and GroES

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The chaperonin GroEL and its cofactor GroES make up a molecular machine that rescues aggregation-prone misfolded proteins. The GroEL functional cycle consists of a series of large-scale allosteric transitions between the T, R, R′ and R″ states. The corresponding large structural rearrangements facilitate substrate protein capture, refolding, and release, and are thus essential for the proper operation of the chaperonin. Using a C_α-sidechain elastic network model-based structural perturbation method, that probes the response of a local perturbation at all residue sites, we have studied the molecular details of the T -> R and R″ ->T transitions and determined the key mechanical residues that support the allosteric cycle - the allostery wiring diagram. We provide a molecular level interpretation for the intraring positive cooperativity and interring negative cooperativity as well as the role of GroES in the GroEL allosteric cycle.

2238-Pos Board B208

ClpXP Degradation of the DNA-Protection Protein Dps Requires Auto-Tethering to the Enzyme

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Dps is a dodecameric bacterial protein that acts to prevent the formation of hydroxyl radicals and condenses cellular DNA to form ''biocrystals'' under stressful conditions, protecting the chromosome from damage. During exponential growth, Dps is continually synthesized but rapidly degraded by the AAA+ protease ClpXP, resulting in a low cellular concentration. Dps degradation is rapidly turned off when cells respond to nutritional or oxidative stresses, allowing Dps to accumulate swiftly and counteract the damaging effects of the stressors. This environmental regulation of degradation is highly specific; stressors such as hydrogen peroxide result in the stabilization of Dps, while the degradation of other ClpXP substrates is not affected by this treatment. Maintenance of genomic integrity then crucially depends upon selective proteolysis of Dps by ClpXP only during non-stress conditions. The molecular mechanism of Dps recognition and degradation by ClpXP was probed through a combination of *in vivo* and *in vitro* techniques. Dps degradation exhibits an absolute requirement for the N-terminal domain of ClpX, a region that mediates interaction with substrate-delivery proteins called adaptors. The characterized ClpX adaptor SspB as well as a peptide representing only the ClpX-binding region of SspB are each able to compete efficiently with ClpXP for Dps degradation. The N-terminus of Dps seems to interact with ClpX, primarily on its N-domain. An extended region or multiple regions within the N-terminus of Dps are required for efficient competition of Dps degradation by ClpXP. Thus, Dps functions analogously to an adaptor protein by using its unstructured N-terminus to tether itself to ClpX during the degradation process. This mechanism may increase the affinity of Dps for ClpX by allowing the two proteins to engage in multiple contacts simultaneously.

2239-Pos Board B209

Controlling oligomerization through protein engineering: in vivo analysis of Hsp90

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Many homo-oligomeric proteins are vital for biology including ion-channels, the p53 tumor suppressor, and the essential kinase-associated chaperone Hsp90. Mutational analyses of these homo-oligomeric systems in vivo is complicated by cross-oligomerization between wild-type and mutant subunits. We have devised a generalizable thermodynamic strategy to prevent cross-dimerization. Appending an oligomerization domain to the mutant subunits reduces the free energy of homocomplexes relative to wild-type/mutant heterocomplexes. We have used this strategy to engineer super-stabilized Hsp90 dimers that do not cross-oligomerize with wild-type Hsp90. Super-stabilized Hsp90 supports yeast viability and is fully active in the maturation of v-src kinase. Thus, our stabilization strategy does not disturb the biochemical function of Hsp90.

We have used superstabilized Hsp90 to address a fundamental and long-unanswered question regarding Hsp90: what clients or substrate proteins depend on Hsp90 ATPase activity in vivo. The identification of ATP dependent Hsp90 substrates has been a major challenge both in vitro and in vivo. In vitro studies are complicated by the large number of co-chaperones required for Hsp90 to function efficiently. In vivo studies are complicated both because ATPase deficient Hsp90 mutants do not support viability and because when different Hsp90 variants are co-expressed they form a mixture of different dimer species. We have used our engineered super-stabilized Hsp90 to developed a yeast system to identify clients that rely on Hsp90 ATPase activity. Using this approach,