

TriC to restore unfolded  $\gamma$  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.

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#### 2235-Pos Board B205

##### H $\alpha$ B-Crystallin Suppresses The Aggregation Upon Refolding Of Its Physiological Substrates H $\gamma$ D-, H $\gamma$ C- And H $\gamma$ S-Crystallin

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The passive chaperone  $\alpha$ -crystallin, a small heat shock protein, is one of the ubiquitous crystallins in vertebrate lenses, along with the  $\beta\gamma$ -crystallins. It is composed of two subunits ( $\sim 20$  kDa)  $\alpha$ A- and  $\alpha$ B-crystallin ( $\alpha$ A- and  $\alpha$ B-crys), which form an hetero-oligomeric, polydisperse complex of  $\sim 800$  kDa in the lens. Aggregates isolated from mature-onset cataracts, the major cause of sight loss worldwide, contain damaged and misfolded forms of  $\beta\gamma$ -crystallins, as well as  $\alpha$ -crystallins. We have studied the chaperone function of Human  $\alpha$ B-crystallin interacting with its physiological Human  $\gamma$ -crystallin substrates. Human  $\gamma$ D-crystallin (H $\gamma$ D-crys) and  $\gamma$ C-crystallin (H $\gamma$ C-crys) are stable and long-lived mammalian  $\gamma$ -crystallins localized in the lens nucleus. Human  $\gamma$ S-crystallin (H $\gamma$ S-crys) is abundant in the lens outer cortex. All three  $\gamma$ -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 H $\gamma$ C- and H $\gamma$ D-Crys intermediates competes with productive refolding. Diluting unfolded H $\gamma$ C-, H $\gamma$ D-, or H $\gamma$ S-crys to low GdnHCl concentrations (100  $\mu$ g/ml, 37°C) resulted in the protein population partitioning between productive refolding and aggregation pathways. H $\gamma$ D-, H $\gamma$ C- or H $\gamma$ S-Crys protein was allowed to refold and aggregate in the presence of H $\alpha$ B-Crys homo-oligomers at different monomer-to-monomer ratios of  $\gamma$ -Crys to  $\alpha$ B-Crys. H $\gamma$ D- and H $\gamma$ C-Crys aggregation was suppressed to similar levels, whereas H $\gamma$ S-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 1 $\gamma$ C:5 $\alpha$ B and 1 $\gamma$ D:5 $\alpha$ B chains. This complex was still present 4 days after the suppression reaction was initiated. These results provide a model for how  $\alpha$ -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 $\alpha$ -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  $\rightarrow$  R and R'  $\rightarrow$  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 develop a yeast system to identify clients that rely on Hsp90 ATPase activity. Using this approach,