Molecular Chaperones

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Rocking Motion of a Protein-Folding Nano-Machine Revealed By Single-Particle Cryo-Em

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The protein folding machine Methanococcus maripaludis chaperonin (Mm-cpn) is a type II archael chaperonin that has a built-in lid. It is a 16-subunit homo-oligomer of ~1 MDa arranged in a two back-to-back rings that is structurally very similar to the mammalian chaperonin such as TRiC. The substrate folding is accompanied by a conformational change triggered by nucleotide binding and hydrolysis.

Using single particle cryo-EM and image reconstruction, we solve both the wild type and lidless mutant Mm-cpn in open and closed states respectively at resolutions between 10 and 4.3 Å. The open state is a nucleotide-free state while the closed state corresponds to the transition state of ATP hydrolysis. Cα backbone models of these four 3-D reconstructions have been hand traced or flexibly fitted depending on their resolutions. The models show clearly the subunits' equatorial domain rotation between the open and closed states, which is unique and different from the well-studied type I chaperonin (GroE) found in E.Coli.

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The Group II Chaperonin Mm-Cpn Binds and Refolds Human Gamma D Crystallin

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The aggregation of damaged or misfolded proteins is associated with a number of human diseases, including Alzheimer's disease, Huntington's disease, and cataract. In this study, we investigate the ability of the Group II chaperonin from Methanococcus marapaludis, Mm-Cpn, a homolog of the eukaryotic chaperonin TRiC, to bind and refold human γD 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. Mm-Cpn interactions with both wild type HγD-Crys, and damage and disease model mutant HγD-Crys were evaluated. Solution turbidity studies indicate that Mm-Cpn suppresses aggregation of both wild type and disease model mutant HγD-Crys, and exhibits a greater affinity for the destabilized mutant HγD-Crys. In addition, size exclusion chromatography and fluorescence spectroscopy show that Mm-Cpn can refold HγD to a native like state, as well as form a long-lived Mm-Cpn/HγD complex with both the wild type and mutant HγD crystallins. This long-lived complex may be ideal for imaging of the chaperonin/substrate complex by cryo-EM or x-ray crystallography. These data suggest that the Mm-Cpn/HγD interaction may reveal aspects of the mechanism of binding and refolding of beta-sheet domains by the Group II chaperonins.

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Chaperone Interactions of the Small Heat Shock Protein Human αb-Crystallin With Its Physiological Substrate γd-Crystallin and Its Isolated Domains

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α-crystallin, a small heat shock protein chaperone, is one of the ubiquitous crystallins in the vertebrate lens, along with the βγ-crystallins. It is a polydisperse complex of ~800 kD consisting of two subunits (~20 kD) αA- and αB-crystallin (αA- and αB-Crys). Its chaperone activity involves suppressing aggregation by binding aggregation-prone species. Aggregates isolated from mature-onset cataracts, the major cause of blindness worldwide, contain damaged and misfolded forms of βγ-crystallins. The γ-crystallins are structural, monomeric proteins that consist of four Greek-key motifs organized into two domains. Human γD-crystallin (HγD-Crys) is a stable and long-lived mammalian γ-crystallin localized in the lens nucleus. It can refold *in vitro* to its native state after unfolding in high concentrations of GdnHCl. However, at very low denaturant concentrations (< 1 M GdnHCl) aggregation of refolding HγD-Crys intermediates competes with productive refolding. We have previously determined that the conformation of the bound HγD-Crys substrate in γD-αB complexes resembles the partially folded intermediate populated during refolding/unfolding equilibrium experiments, which has its N-terminal domain unfolded and its C-terminal domain folded. We have utilized single domain constructs to further characterize the binding interactions of HαB-Crys to different regions of HγD-Crys. The HγD-Crys C-terminal domain construct (γD-Ctd) aggregates upon refolding, while the N-terminal domain construct (γD-Ntd) does not aggregate under similar conditions. However, when γD-Ctd and γD-Ntd are unfolded and refolded together, γD-Ctd recruits γD-Ntd into the aggregate. HαB-Crys can suppress the aggregation of the γD-Ctd and forms γD-Ctd-αB complexes. Using a no-Trp mutant of HαB-Crys (W9F/W60F), we have determined, through the fluorescence emission of γD-Ctd tryptophans, that the γD-Ctd in the γD-Ctd-αB complexes is partially folded. These results provide further insight into how α-crystallin interacts with aggregation-prone substrates *in vivo*.

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Identification of a Consensus Motif in Substrates Bound by a Type I Hsp40

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Protein aggregation is a hallmark of a large and diverse number of conformational diseases. Molecular chaperones of the Hsp40 family (Escherichia coli DnaJ homologs) recognize misfolded disease proteins and suppress the accumulation of toxic protein species. Type I Hsp40s are very potent at suppressing protein aggregation and facilitating the refolding of damaged proteins. Yet, the molecular mechanism for the recognition of nonnative polypeptides by Type I Hsp40s such as yeast Ydj1 is not clear. Here we computationally identify a unique motif that is selectively recognized by Ydj1p. The motif is characterized by the consensus sequence GX[LMQ]{P}X{P}{CIMPVW}, where [XY] denotes either X or Y and {XY} denotes neither X nor Y. We further verify the validity of the motif by site-directed mutagenesis and show that substrate binding by Ydj1 requires recognition of this motif. A yeast proteome screen revealed that many proteins contain more than one stretch of residues that contain the motif and are separated by varying numbers of amino acids. In light of our results, we propose a 2-site peptide-binding model and a plausible mechanism of peptide presentation by Ydj1p to the chaperones of the Hsp70 family. Based on our results, and given that Ydj1p and its human ortholog Hdj2 are functionally interchangeable, we hypothesize that our results can be extended to understanding human diseases.

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Investigating Interactions Between the Hsp90 Molecular Chaperone and Unfolded Protein Substrates

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The ubiquitous protein chaperone Hsp90 plays an integral role in cellular homeostasis and protein folding by interacting with substrate proteins. Only recently have three-dimensional structures of the full-length Hsp90 been determined and a reaction cycle been proposed. Despite this achievement, remarkably little is known about the molecular basis for substrate interactions. To investigate this issue, I am utilizing a well-studied model of an unfolded protein, Δ131Δ, a fragment of staphylococcal nuclease. Using small angle x-ray scattering and structure-based fitting we have found that Hsp90 undergoes an open/closed conformational change in the presence of Δ131Δ. NMR measurements of Δ131Δ and domain fragments of Hsp90 have indicated the interaction location on both the chaperone and the substrate. We use this information to build a mechanistic model of how Hsp90 interacts with unfolded protein substrates and how different nucleotide states influence these interactions.

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Insertion of Hsp70 Into Membranes Correlates With the Flipping of Phosphatidlyserine Across the Lipid Bilayer

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Expression of heat shock proteins is the primary event in the cellular response to stress. Indeed, these proteins are crucial in preventing cell death and recovery after different physiological and environmental stresses. Hsp70 (Hsp72), which is the major stress-inducible member of the heat shock protein family, is primarily located in the cytosol. However, recent evidence has shown that this protein can be detected on the cell surface of transformed cells inserted into the plasma membrane. Hsp70 does not contain any hydrophobic domains that could predict its insertion into membranes. Consequently, the possible mechanism for translocation into membranes is likely a non-classical process. Pure recombinant Hsp70 was incubated with phosphatidylserine (PS) liposomes, and a concentration-dependent incorporation of the protein into the bilayer was observed. On the contrary, Hsp70 did not get incorporated into phosphatidylcholine (PC) liposomes. Liposomes made of a PS:PC mixture