A7 Structure-Based Design, Synthesis and Evaluation of Amyloid Fibril Inhibitors.
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A detailed understanding of the kinetics and thermodynamics of transthyretin (TTR) amyloid fibril formation has led to a small molecule strategy to prevent the protein-protein interactions that are enabled by TTR conformational changes. The dissociation of the normally folded and functional tetramer to an alternatively folded monomer is the rate-determining step in transthyretin amyloidogenesis. Small molecule inhibitors that block this step have been structurally characterized in complex to TTR at a resolution of 1.9 ˚ by X-ray crystallography. Several different inhibitor-TTR structures were used as a basis for structure-based designs of novel and optimized inhibitors. Several nM inhibitors have resulted from this effort. Most of the second-generation amyloidogenesis employing in vivo experiments.

A8 Molecular chaperones in protein folding in the cell
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Although the folded structure of a protein is determined by the information contained in its amino acid sequence, efficient realization of this information in vivo may require the assistance by molecular chaperones, at least for a significant fraction of newly-synthesized polypeptides. Dr native folding in the cytosol generally depends on chaperones of the Hsp70 family and on the cylindrical chaperonins. According to our current model, Hsp70 binds to nascent polypeptides on ribosomes, preventing misfolding until all the information required for productive folding is available. In E. coli, Hsp70 (DnaK) cooperates with the ribosome-bound chaperone and poly(U)-induced trigger factor (TF) in protecting nascent chains. The combined deletion of DnaK and TF is lethal, indicating that these components provide an essential function. Most proteins appear to fold rapidly upon release from this first set of chaperones, a subset of polypeptides must subsequently be transferred to a chaperonin for folding to the native state. The chaperonins are also essential for cell growth. They form large cylindrical complexes that bind unfolded polypeptides, up to ~60 KDa in size, in their central cavity. As shown for the E. coli chaperonin GroEL, the dome-shaped co-factor GroES caps the opening of the cylinder, resulting in the displacement of bound polypeptide into an enclosed folding cage, a mechanism that effectively prevents protein aggregation during folding. We have recently undertaken a proteomics approach to identify the E. coli proteins that utilize GroEL. Structural analysis of the identified proteins indicates the presence of preferred fold motifs and complex domain topologies, in support of the view that GroEL substrates fold slowly and tend to aggregate in the absence of chaperones.

A9 Interdomain communication in Hsp70 chaperones
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The ubiquitous Hsp70 molecular chaperones perform a wide range of cellular functions based on their ability to bind unfolded substrates. Hsp70s are two-domain allosterically-regulated proteins; binding of the C-terminal domain to exogenous hydrophobic residues on their protein substrates is regulated by ATP binding and hydrolysis in the N-terminal nucleotide-binding domain. We have biochemically and structurally characterized wild-type and mutant versions of the E. coli Hsp70, DnaK, to explore the mechanism of interdomain communication. The substrate-binding domain is made up of a distorted β-sandwich with a central polypeptide binding groove, covered by a long α-helical lid. All structures solved to date have the substrate-binding groove occupied. Critical to understanding the mechanism by which substrate is released in Hsp70s upon nucleotide binding is defining the structure of the substrate-free state. Recently, we have examined the nature of removal of the entire α-helical domain from the C-terminus of DnaK, creating DnaK(1-507) and a comparably truncated substrate-binding domain, DnaK(393-507) (Pellecchia et al., manuscript submitted). Surprisingly, DnaK(1-507) retained interdomain communication, arguing that the helix is not essential to the allosteric mechanism. NMR studies of DnaK(393-507) revealed significant dynamic and structural changes in the substrate-free β-domain upon removal of the C-terminal helix, which may result from lack of substrate or removal of the helical lid. We have now discovered that a longer construct with much of the helical lid intact, DnaK(387-507), is stable on a form with its binding site empty at pH 4.5. NMR studies at 500 and 800 MHz are enabling us to analyze in detail the structural differences between the free and bound forms of this construct, as well as one harboring a mutation, K414R, that abolishes allosteric interdomain communication (Montgomery et al., J. Mol. Biol. 286, 915 [1999]). Implications for the mechanism of nucleotide-mediated peptide release will be discussed.

A10 Chaperonin structure and function
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Chaperonins are a major family of molecular chaperones essential for the viability of all cells. The group I (GroE) chaperonins are found in eubacteria, mitochondria and chloroplasts, and group II (threonine or CCT) chaperonins occur in archaea and the eukaryotic cytosol. There is considerable detail known of their structure and mechanism of action, particularly for the GroE chaperonins.

In E. coli, chaperonin-assisted protein folding proceeds through cycles of ATPase action by the large chaperonin GroEL, and involves concerted, rigid body movements of the 3 domains in each subunit. Transient binding of the small chaperonin GroES forms an enclosed chamber about 70 Å across, in which folding of a protein substrate takes place. Access to the chamber is regulated by the nucleotide cycle, which controls the binding and release of GroES. In addition, the domain movements cause an alternation between low and high affinity binding for non-native protein substrates, by changing the accessibility of the hydrophobic binding sites. Binding, encapsulation and release states alternate between the two back to back 7-membered rings of GroEL, with the movements communicated by subtle alterations of the inter-ring interface. Using cryo EM we have obtained 3D reconstructions of chaperonin complexes in several different nucleotide states, revealing the very large hinge motions that accompany the functional cycle. We are now characterizing the folding-active states GroEL-ATP and GroEL-GroES-ATP at higher resolution, in order to observe the alterations in domain contacts. Combining the cryo EM map with atomic structures of the chaperonins provides a detailed structural basis for understanding the allosteric mechanism of chaperonins and control of their interactions with folding protein substrates.