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A Putative Network of Interactions Controls the Opening and Closing Dynamics of the ATPase Domain of E. Coli Hsp70, DNAk

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Hsp70 chaperones play important roles in cells including protein folding, trafficking, degradation and enabling survival under stress conditions. DnaK is an *E. coli* Hsp70 homolog comprising an ATPase domain and a substrate-binding domain. DnaK has two substrate-affinity states: ATP binding lowers the affinity of the substrate, whereas its hydrolysis leads to a higher affinity of substrate for binding. ATP-dependent communication between the two domains is essential for chaperone function and mediated via a conserved hydrophobic linker (^{384}GDVKDVLLL^{392}). Previous studies showed that when the linker interacts with the ATPase domain, which was studied by the construct containing the entire linker, DnaK(1-392), an enhanced ATPase rate is observed compared to the construct lacking the conserved ^{389}VLLL^{392} linker region, DnaK(1-388). This observation suggests that structural rearrangements caused by linker docking adopt the ATPase domain in a closed conformation, leading to an enhanced, pH-dependent ATPase activity. Here, our aim is to delineate the residues that are responsible for the linker induced conformational rearrangements. In that line, using molecular dynamic simulations we identified a putative network of interactions through Arg71-Glu81-Asp85-Thr225-His226 at the lobe interface of the ATPase domain that might be critical in stabilization of the domain in the so called ''open'' and ''closed'' conformational equilibrium. We made point mutations for these sites on the two ATPase domain constructs, and studied the structural and functional effects of these residues on the ATPase domain as a function of pH using various biophysical and biochemical methods. Mutations' effects studied by equilibrium thermodynamic measurements showed variations for the constructs, but dramatic changes were observed in the dynamics of the constructs. Our results suggest the linker as a controller for ATPase domain dynamics changes partly through the identified network.

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Divalent Recognition of the E. Coli Cytoskeletal Protein FtsZ by the ATP-dependent Protease ClpXP

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Cell division in bacteria requires the coordinated interactions of cytoskeletal motor proteins, their regulators and proteins that remodel the outer cell layers. In Escherichia coli, the tubulin homolog FtsZ is essential for cell division. FtsZ forms a large ring-like structure, called the Z-ring, inside the cell at the site of constriction. In vitro FtsZ hydrolyzes GTP and assembles into dynamic polymers when GTP is present. One modulator of FtsZ assembly is the ATP-dependent protease ClpXP. ClpX, a AAA+ ATPase, forms a hexameric ring that unfolds proteins with specific recognition signals. ClpX associates with ClpP, a barrel-shaped compartmentalized protease, to degrade proteins in a regulated manner. ClpXP degrades FtsZ in vivo and in vitro and promotes the disassembly of FtsZ polymers. Site-directed mutagenesis of FtsZ indicated that two discrete regions of FtsZ near the C-terminus are important for degradation by ClpXP. One region is within the C-terminal four amino acids of FtsZ and overlaps with the recognition site for several other FtsZ-interacting proteins. A second region is located ~30 residues proximal to the C-terminus, in a linker region connecting the polymerization domain to the C-terminal conserved core region. We determined that amino acid residues from both regions are important for FtsZ degradation in the absence and presence of GTP, the condition that promotes polymerization. We also showed that the amount of FtsZ degraded by ClpXP in vitro is reduced in the presence of excess MinC, an inhibitor of FtsZ polymerization. Our results suggest that MinC competes with ClpXP for the FtsZ C-terminus and prevents unfolding and degradation by ClpXP. These studies indicate that two negative regulators of FtsZ assembly, ClpXP and MinC, both utilize the FtsZ C-terminal region for destabilization and/or degradation of FtsZ polymers.

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Molecular Mechanism of Allosteric Communication in Hsp70 Revealed by Molecular Dynamics Simulations

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Investigating ligand-regulated allosteric coupling between protein domains is fundamental to understand cell-life regulation. The Hsp70 family of chaperones represents an example of proteins in which ATP binding and hydrolysis at the Nucleotide Binding Domain (NBD) modulate substrate recognition at the Substrate Binding Domain (SBD).
Herein, a comparative analysis of an allosteric (Hsp70-DnaK) and a non-allosteric structural homolog (Hsp110-Sse1) of the Hsp70 family is carried out through molecular dynamics simulations, starting from different conformations and ligand-states. Analysis of ligand-dependent modulation of internal fluctuations and local deformation patterns highlights the structural and dynamical changes occurring at residue level upon ATP-ADP exchange, which are connected to the conformational transition between closed and open structures. By identifying the dynamically responsive protein regions and specific cross-domain hydrogen-bonding patterns that differentiate Hsp70 from Hsp110 as a function of the nucleotide, we propose a molecular mechanism for the allosteric signal propagation of the ATP-encoded conformational signal. This study opens up the way to introducing rational mutations able to control the chaperone functional dynamics. Moreover, the residue based insight into the intra molecular communication mechanism can be exploited for designing new allosteric drugs.

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A Molecular Dynamics Study of the Flexibility and Protein-Binding of the Trigger Factor Chaperone in Solution

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Trigger factor (TF) is a chaperone, found in bacterial cells (and chloroplasts), that interacts with nascent polypeptide chains to guide protein folding, and prevent spontaneous misfolding and aggregation. While its crystal structure has been resolved, the solution structure and dynamics is largely unknown. using all-atom and coarse-grained molecular dynamics (MD) simulations in combination with Markov State Modeling (MSM) on the extended crystal structure of TF in solution, we show that the collective motions of its tertiary domains, hinged about inter-domain linkers with minimal or no loss in secondary structure, leads to a substantially different conformation in solution, with a relatively stable compact collapsed state and several intermediate metastable states. These states are the results of domain-pair formations that are triggered by burial of hydrophobic residues and stabilized by hydrophilic contacts. A hydrophobic probes method, which uses methane-like Lennard-Jones particles to identify hydrophobic patches, is employed to characterize these contacts as well as the surface hydrophobicity of TF. The flexibility and promiscuity of TF facilitate its chaperone action on a variety of substrate proteins; however, its direct influence, or that of chaperones in general, on protein-folding pathways remains poorly understood. We employ steered MD to complement AA-MD simulations to understand the relatively unknown binding mechanism of maltose binding protein (MBP) to TF. We show that, in solution, TF, by conserving secondary structural elements in the folding intermediates of MBP, stabilizes native contacts in MBP and prevents the protein aggregation. Contrary to the current viewpoint, our results also illustrate a crucial role for N-terminal of TF in its binding with protein substrates through hydrophilic interactions.

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Inter-Subunit Coordination in Hsp104, a Protein Disaggregase

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Hsp104, a hexameric AAA+ ATPase from yeast, disaggregates diverse structures including stress-induced amorphous aggregates, yeast prions, and disease associated amyloid conformers. However, the mechanism by which these substrates are remodeled is unknown. We have shown that Hsp104 hexamers adapt different mechanisms of intersubunit collaboration to disaggregate amorphous aggregates versus amyloid. To resolve amorphous aggregates, Hsp104 subunits collaborate non-co-operatively via probabilistic substrate binding and ATP hydrolysis. To disaggregate amyloid, several subunits co-operatively engage substrate and hydrolyze ATP. We also found that inter-subunit co-operativity is dictated by the stability of the substrate: Hsp104 hexamers deploy more subunits to disaggregate Sup35 prion strains with more stable 'cross-β' cores. Surprisingly, the mechanism of inter-subunit co-operativity is not conserved, as the prokaryotic homologue of Hsp104, ClpB, collaborates differently than Hsp104 and couples probabilistic substrate binding to co-operative ATP hydrolysis.