Allostery in molecular machines: the chaperonins GroEL and CCT

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'Molecular chaperones' assist protein folding *in vivo* and *in vitro*. Our research focuses on a subfamily of molecular chaperones named chaperonins that are divided into two groups: group I found in eubacteria, mitochondria and chloroplasts and group II found in archaea and the eukaryotic cytosol. Group I chaperonins, such as GroEL from *E. coli*, consist of 14 identical subunits that form two heptameric rings. They function in conjunction with helper-proteins such as GroES from *E. coli*. Group II chaperonins consist of two eight- or nine-membered rings that are made up of two types of subunits in the case of the archaeal thermosome or eight different subunits in the case of the cytoplasmic eukaryotic chaperonin containing TCP-1 (CCT). Both groups of chaperonins assist protein folding in an ATP-regulated manner that involves complex allosteric regulation. The focus of our research is to understand the molecular basis of allosteric transitions, in general, and those in chaperonins in particular. Specific questions we are currently addressing are:

(i) What is the relationship between allostery in chaperonins and their folding function? Our previous work showed that each chaperonin ring is in equilibrium between a T state (with low affinity for ATP and high affinity for protein substrates) and an R state (with high affinity for ATP and low affinity for protein substrates) that is reflected in cooperative ATP binding. In addition, there is a second level of allostery between rings that is reflected in inter-ring negative cooperativity in ATP binding. We have shown that the intra-ring allosteric transitions of GroEL are concerted whereas those of CCT are sequential. Given that the efficiency of machines is path-dependent, we have been interested in investigating the impact of the mechanism of allosteric switching (concerted vs. sequential) on the folding function of chaperonins. Our working hypothesis (which is supported by lattice model simulations that were carried out in collaboration with Prof. R. Unger, Bar-Ilan University) has been that sequential conformational changes facilitate domain-by-domain folding and, thus, enable CCT to better assist folding of multi-domain proteins that are more common in eukaryotes. By contrast, the concerted allosteric transitions of GroEL facilitate protein substrate release in an all-or-none manner folding, thereby enhancing the folding efficiency of single-domain proteins that are more common in prokaryotes. In the case of GroEL, this hypothesis has been tested experimentally and confirmed (in collaboration with Prof. G. Haran, WIS) by monitoring the folding of designed two-domain proteins, in the presence of wild-type GroEL and a mutant we discovered that undergoes sequential conformational changes. Work in collaboration with Prof. K. Willison from Chester Beatty Laboratories (London) is underway to test this hypothesis for CCT and to address other open questions about this chaperonin that remains poorly understood.

(ii) What are the pathway(s) of ligand-induced allosteric transitions of proteins? The atomic-resolution structures of the relatively stable end states of different allosteric proteins are known but the pathways by which they inter-convert are generally not. We are addressing this issue using both computational and experimental approaches. One computational approach is based on analysis of correlated mutations in protein families that, in principle, can reveal such pathways provided that mutations that reflect energetic connectivity can be distinguished from those due to common ancestry. We have recently developed a method that distinguishes between these two types of correlated mutations. This method is now being tested on real and lattice model proteins (in collaboration with Prof. R. Unger, Bar-Ilan University) to determine whether it can provide insights into mechanisms of allosteric communication. Our experimental approach is based on linear free energy relationships of physical organic chemistry such as the Brönsted plot. Our data so far suggests that GroEL switches between the T and R states via at least two parallel pathways and that, in the transition states of the T→R reaction, the inter-subunit R197-E386 salt-link is broken thus enabling rotation of subunits in the plane of the ring, but the upward shift of the apical domains has not yet taken place.

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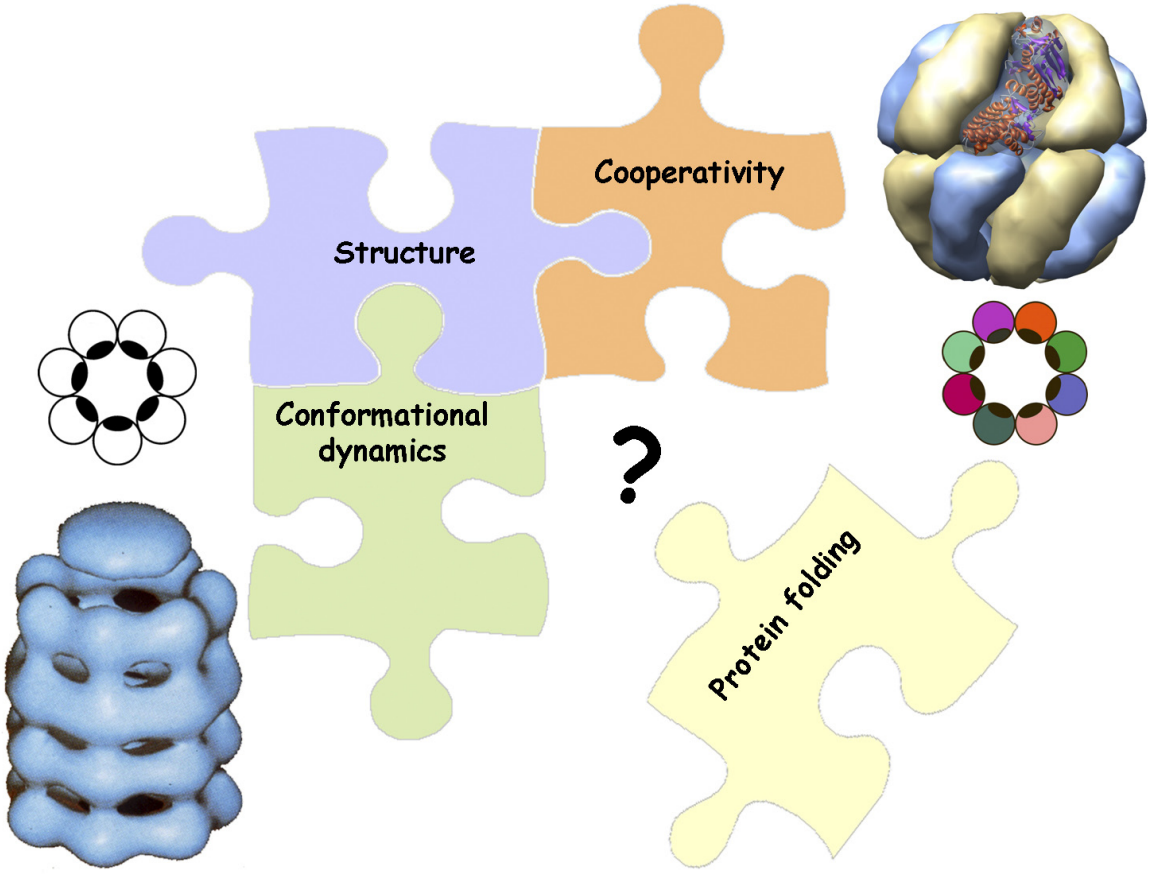

Fig. 1: *Our group is interested in the interplay between chaperonin-mediated protein folding and the conformational dynamics of chaperonins that are under allosteric control. The cryo-EM structures of the type I E. coli chaperonin GroEL in complex with GroES and the type II chaperonin from archaea named thermosome are shown at the bottom left and top right, respectively. Type I and II chaperonins are comprised of homo- (left) and hetero-oligomeric (right) rings, respectively, with a cavity where protein folding takes place.*

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