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# Structural and functional analysis of allostery in chaperonins

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Protein folding in the cell is assisted by a family of proteins named 'molecular chaperones'. Our research focuses on a subfamily of molecular chaperones named chaperonins that are divided into type I found in eubacteria, mitochondria and chloroplasts and type II found in archaea and the eukaryotic cytosol. Type I chaperonins, such as GroEL from *E. coli*, consist of 14 identical subunits that form two heptameric rings. They have helper-proteins such as GroES from *E. coli*. Type 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 type I and type II chaperonins assist protein folding in an ATP-regulated manner. The ATPase activity of chaperonins involves complex allosteric regulation. The focus of our research is to understand the molecular basis of allosteric transitions in chaperonins and how they relate to their function. Kinetic analysis led us to propose a nested allosteric model for GroEL. According to this model, intra-ring positive cooperativity in ATP binding is due to a concerted inter-conversion of each ring of GroEL 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). A second level of allostery is reflected in inter-ring negative cooperativity. Recently, we showed that the allosteric transition of the D155A GroEL mutant (Fig. 1) is converted from concerted to sequential. In other words, ATP binding induces a break in symmetry in this mutant. Kinetic data for CCT shows that it also undergoes two ATP-induced allosteric transitions that may be sequential and not concerted. Specific questions we are now addressing are:

(i) What is the relationship between allostery in the GroE system and its folding function? Several years ago we reported the first data on the relationship between GroEL-assisted protein folding rates and allostery in GroEL. A linear relationship was found between the folding rate of mouse dihydrofolate reductase (mDHFR) and the rate of the T to R transition. We also found linear relationships between the folding rate of mDHFR and the extent of inter-ring negative cooperativity. These experiments demonstrated that protein folding by GroEL is coupled to cooperative ATP binding. We are now examining whether converting the allosteric transition of GroEL from concerted to sequential affects the rate of assisted protein folding.

(ii) What is the pathway(s) of ATP-induced allosteric transitions of GroEL? The atomic-resolution structures of the relatively stable end states of many allosteric proteins are known but the pathways by which they inter-convert are generally not known. We are addressing this issue using GroEL as a model system. One approach of ours is to employ linear free energy relationships of physical organic chemistry such as the Brönsted plot. Our data so far suggests that in the transition-state of the T to R reaction of GroEL, the inter-subunit R197-E386 salt-link is broken, thus enabling rotation of subunits in the plane of the ring, but that the upward shift of the apical domains has not yet taken place. Our data have also shown that there are at least two pathways for the transition between the T and R states. We are also interested in developing bioinformatic methods (see: http://bioportal.weizmann.ac.il/cmutatd/) that may shed light on pathways of information transfer in allosteric proteins.

(iii) What is the mechanism and function of allostery in CCT? We have shown that CCT displays positive intra-ring cooperativity and negative inter-ring cooperativity with respect to ATP.

Virtually nothing is known about the mechanism of allosteric transitions in CCT and the role of allostery in its function. One attractive hypothesis is that the subunit heterogeneity of CCT facilitates sequential progression of conformational changes around the ring that, in turn, facilitates sequential folding of protein domains. We are carrying out electron microscopy (in collaboration with Dr. S. Wolf from the WIS) and kinetic experiments on ATP binding to CCT to examine such a model. We are also trying to purify CCT from yeast so that structure-function studies of this molecule can be initiated. This work is in collaboration with Prof. K. Willison from Chester Beatty Laboratories, London.

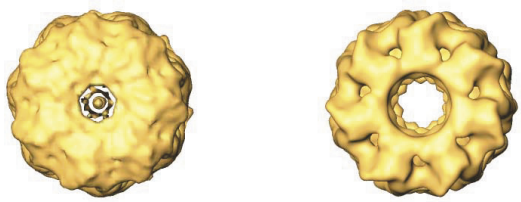

**Fig. 1** *cryo-EM structures of wild-type (right) and the D155A GroEL mutant (left) determined by single-particle reconstruction at 14Å resolution*

### *Selected Publications*


Yifrach, O. and Horovitz, A. (1995) Nested co-operativity in the ATPase activity of the oligomeric chaperonin GroEL. Biochemistry, 34, 5303-5308.

Yifrach, O. and Horovitz, A. (2000) Coupling between protein folding and allostery in the GroE chaperonin system. Proc. Natl. Acad. Sci. U. S. A., 97, 1521-1524.

Fridmann, Y., Ulitzur, S., and Horovitz, A. (2000) In vivo and in vitro function of GroEL mutants with impaired allosteric properties. J. Biol. Chem., 275, 37951-37956.

Kafri, G., Willison, K. R., and Horovitz, A. (2001) Nested allosteric interactions in the cytoplasmic chaperonin containing TCP-1. Protein Sci., 10, 445-449.

Horovitz, A., Fridmann, Y., Kafri, G., and Yifrach, O. (2001) Review: Allostery in chaperonins. J. Struct. Biol., 135, 104-114.

Fridmann, Y., Kafri, G., Danziger, O., and Horovitz, A. (2002) Dissociation of the GroEL-GroES asymmetric complex is accelerated by increased cooperativity in ATP binding to the GroEL ring distal to GroES. Biochemistry, 41, 5938-5944.

Kass, I. and Horovitz, A. (2002) Mapping pathways of allosteric communication in GroEL by analysis of correlated mutations. Proteins: Struct. Funct. & Genet., 48, 611-617.

Horovitz, A., Amir, A., Danziger, O., and Kafri, G. (2002) Phi-value analysis of heterogeneity in pathways of allosteric transitions: evidence for parallel pathways of ATP-induced conformational changes in a GroEL ring. Proc. Natl. Acad. Sci. U. S. A., 99, 14095-14097.

Kafri, G. and Horovitz, A. (2003) Transient kinetic analysis of ATP-induced allosteric transitions in the eukaryotic chaperonin containing TCP-1. J. Mol. Biol., 326, 981-987.

Danziger, O., Rivenzon-Segal, D., Wolf, S. G., and Horovitz, A. (2003) Conversion of the allosteric transition of GroEL from concerted to sequential by the single mutation Asp-155→Ala. Proc. Natl. Acad. Sci. U. S. A., 100, 13797-13802.

Amir, A. and Horovitz, A. (2004) Kinetic analysis of ATP-dependent inter-ring communication in GroEL. J. Mol. Biol., 338, 979-988.



***Acknowledgement:***

Our work is supported by the Israel Science Foundation. Amnon Horovitz is incumbent of the Carl and Dorothy Bennett Professorial Chair in Biochemistry

Our work was supported by a research grant from the Lord Sieff of Brompton Memorial Fund