Topologies of a Substrate Protein Bound to the Chaperonin GroEL

*The following commentary was written by Dr Clare Sansom and Professor Helen Saibil. The original article was published in the May 11 2007 issue of Molecular Cell [1]: Elad N, Farr GW, Clare DK, Orlova EV, Horwich AL & Saibil HR. Molecular Cell, **26, 415-426***

Molecular chaperones are proteins that assist other proteins to fold into their native, functional shapes, and prevent protein aggregation [2]. There are several families of chaperones, with different structures and different mechanisms. The best understood family, known as chaperonins, help fold newly synthesised proteins after they leave the ribosome, and also repair proteins that are damaged by misfolding. Helen Saibil FRS from Birkbeck College and her group have spent many productive years studying the structure of a ring-shaped bacterial chaperonin, GroEL with cryo-electron microscopy. Now, Saibil, with her Birkbeck colleague Elena Orlova and collaborators at Yale University and the Scripps Institute in the US, has shown how an unfolded form of one GroEL substrate, malate dehydrogenase (MDH), can bind to the inside surface of the chaperonin complex in several different conformations [1]. The researchers also observed that substrate binding caused changes in the conformation of the GroEL molecule itself.

The GroEL molecule consists of fourteen identical subunits, arranged symmetrically in two seven-membered rings. Each subunit has three domains; an equatorial domain closest to the interface between the rings, an intermediate domain, and an apical domain (Figure 1).

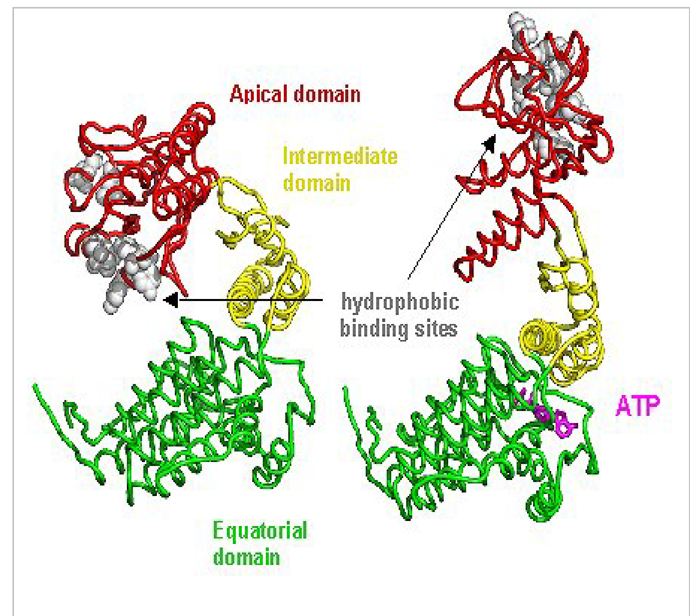

Figure 1: Picture of a GroEL subunit and its three domains. On the left: a GroEL subunit in the unliganded state (apo GroEL). On the right: a GroEL subunit in the conformation that binds ATP and GroES

The bound, unfolded substrate protein can only fold once the structure has been "capped" by the smaller GroES molecule, and ATP bound (Figure 2). GroEL has a hydrophobic surface that binds hydrophobic residues exposed on the exterior of the unbound substrate: this surface, in GroEL, is known to involve two helices on the inner surface of the apical domain, labelled H and I [3].

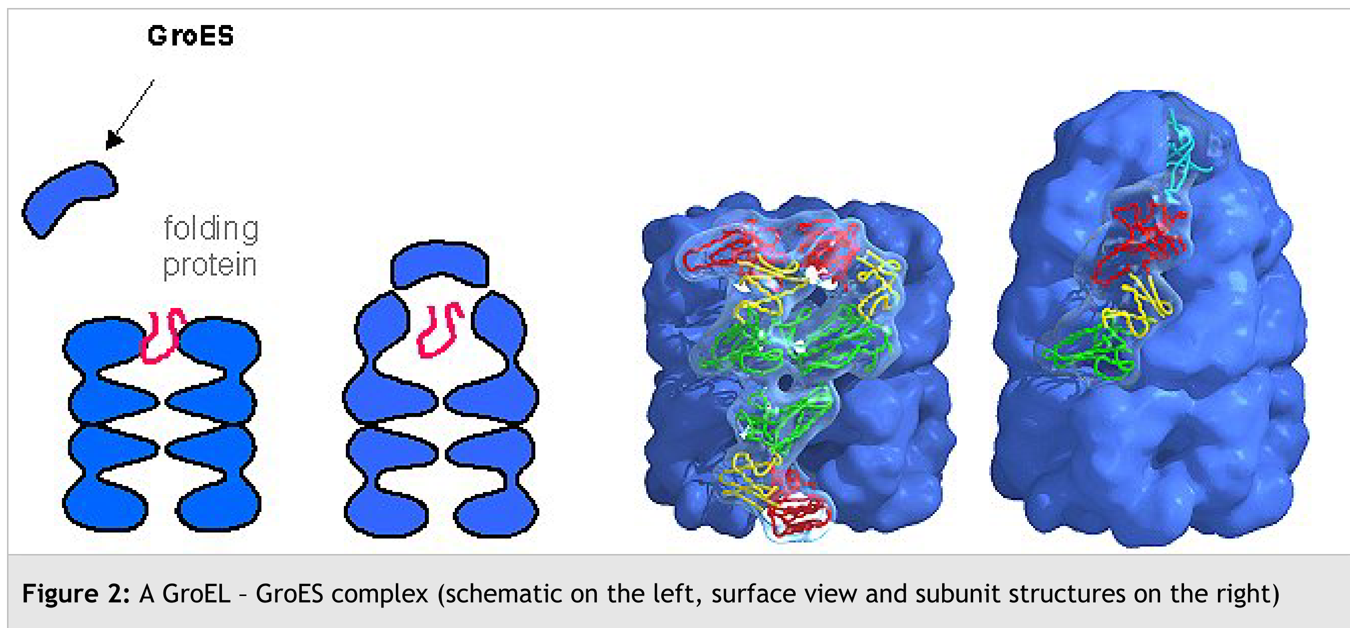

Figure 2: A GroEL – GroES complex (schematic on the left, surface view and subunit structures on the right)

Saibil and her colleagues first reconstructed the three-dimensional structure of the GroEL-MDH complexes from cryo-electron micrographs of a set of 8000 such particles, immobilised on a thin carbon film. Atomic coordinates of a single GroEL monomer [4] were "docked" into the resulting electron density. Extra electron density, which could not be attributed to any GroEL monomer, was assumed to come from the substrate, MDH. This was observed inside the cavity, adjoining the apical domain, and close to helix I. However, this extra density was far less than would be expected for a protein of the mass of MDH, indicating that the substrate density was disordered and had been "smeared out" by the application of symmetry.

To confirm this, the images were classified into subsets using a technique called multivariate statistical analysis (MSA; [5]). Ultimately, a dataset of 40,000 images could be separated into five major classes, each corresponding to a different characteristic conformation of the GroEL-MDH complex. Three contained extra density that could be confidently assigned to substrate, bound at different positions to the apical domain of GroEL (Figure 3). In two of these, substrate density was deep inside the chaperonin cavity, in contact with helix I in one case and helices H and I in the other. The third showed a larger volume of substrate density at the inlet to the central cavity (Figure 3), in a shape reminiscent of the native MDH monomer, which might indicate a partially folded substrate conformation. No substrate density was visible in the other two classes of structure. However, in all five classes the ring structure had become asymmetric, indicating that substrate had bound to each, causing conformational change. The three or four consecutive subunits that bind GroEL are seen to cluster together, leaving gaps in other parts of the ring of apical domains.

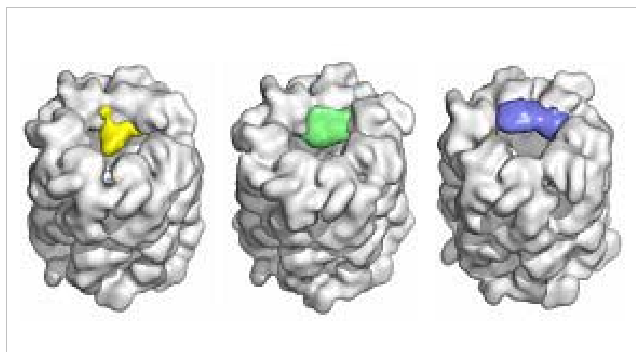

Figure 3: Three different observed conformational classes of the MDH-GroEL complex

Further experiments indicated that unfolded substrate molecules may make contact with any surface within the GroEL central cavity. Another GroEL substrate, dihydrofolate reductase (DHFR), containing a single cysteine residue, was bound to a number of functional mutant GroEL complexes, in each of which a different residue had also been mutated to cysteine. Disulphide crosslinks were formed in all the cases where the mutated GroEL residue was located on the interior surface of the cavity or around its inlet. Therefore, although helices H and I of the apical domains are important surfaces for substrate binding, unfolded protein may be found in the whole of the cavity and even some of the upper surface of the apical domain.

This important study, therefore, gives us the first complete three-dimensional image of the first step of GroEL-assisted protein folding: the binding of the unfolded protein into the chaperonin complex. It reveals a preference for binding deep inside the cavity, compatible with the subsequent displacement of substrate protein by the GroES co-chaperonin, and gives a clear picture of the disruption of the symmetrical GroEL complex on substrate binding.

References

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