

A chaperonin complex with a newly folded protein encapsulated in the folding chamber

The following commentary was written by Dr Clare Sansom, Prof Helen Saibil and Dr Daniel Clare. The original article was published in the 1 January 2009 issue of *Nature* [1]: Clare DK, Bakkes P, van Heerikhuizen H, van der Vies SM and Saibil HR. *Nature*, 2009, 457, 107-111.

Molecular chaperones are proteins that assist other proteins to fold into their native, functional, shapes, and prevent protein aggregation [2]. There are many types of chaperone: one chaperone family, known as the chaperonins, are barrel-shaped proteins that enclose newly formed proteins as they are released from the ribosome to fold into their native conformations, or refold proteins denatured by heat or other stresses. Electron microscopist Helen Saibil FRS from Birkbeck College, a core member of the Institute of Structural and Molecular Biology, and her group have made some of the most important structural studies of chaperonins, focusing particularly on one system, GroEL/GroES, which is found in eubacteria, mitochondria and chloroplasts. Fourteen GroEL subunits assemble into two seven-membered rings stacked back-to-back. Each ring encloses a protein folding cavity lined by hydrophobic amino acids. Once a protein substrate has been bound in this cavity, the chaperonin ring binds ATP and becomes capped by a co-chaperonin, GroES, in a dramatic series of conformational changes that encapsulate the substrate inside a hydrophilic folding chamber. Now, working with collaborators at Free University Medical Center in Amsterdam, the Netherlands, Saibil and Dan Clare, a postdoctoral researcher in her group, have visualised the structure of a newly folded viral protein about to be released from its chaperonin cavity.

The bacteriophage T4 is a virus that infects bacteria such as *E. coli*. Its genome encodes a gene for a co-chaperonin, homologous to GroES, called gp31. The cavity formed by GroEL and gp31 is similar to but taller than the one formed by GroEL and GroES [3]. The major protein component of the T4 head, gp23, will fold into its native conformation only when it is enclosed in a folding chamber formed from GroEL and gp31; it cannot fold in a GroEL-GroES cavity.

Saibil and her colleagues constructed ternary complexes of the substrate gp23 with gp31 and GroEL by adding gp31 and the non-hydrolysable ATP analogue ADP-AIF₃ to a binary complex of GroEL and denatured (unstructured) gp23. They then reconstructed the structures of both the binary and ternary complexes from datasets of 30-35,000 particles of each preparation. Expecting that, as with the previous studies of malate dehydrogenase [4], the gp31 substrate would form a heterogeneous set of complexes with the chaperonins, the group used a technique called multivariate statistical analysis to sort the images of binary and ternary complexes into classes and determine a separate structure of each class, without imposing any symmetry [5].

The structures of the binary gp23-GroEL complexes revealed classes with substrate bound to one or both of the GroEL rings. The ternary structures showed substrate bound to the open GroEL ring (the *trans* ring) only, or

bound to both the open and the gp31-capped (*cis*) rings [Figure 1]. Interestingly, the empty folding chamber of the ternary complex with substrate bound in the open (*trans*) ring only was compressed, whereas the folding chamber occupied by substrate was stretched and distorted. Remarkably, the major domain of the bound substrate protein was represented by particularly well-defined density, showing that upon folding it became trapped in the chamber in a specific orientation.

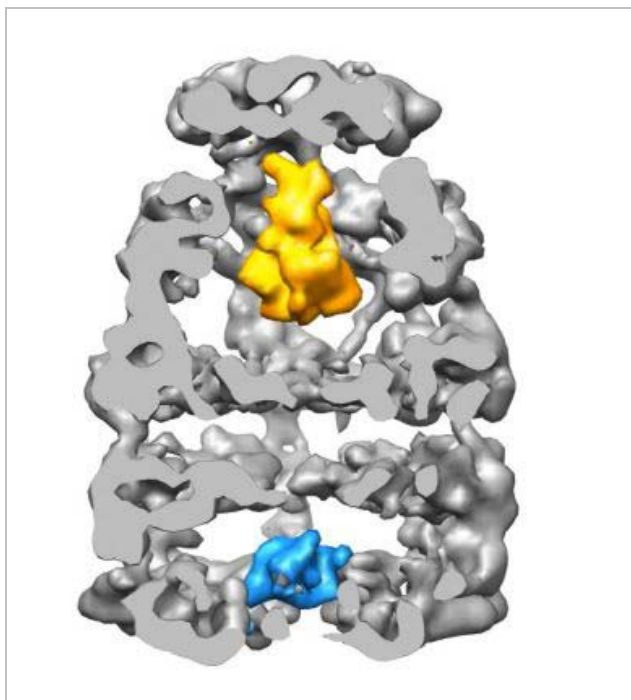


Figure 1: Cut-away view of the GroEL-Gp31-Gp23 complex, showing a subunit of Gp23 with its major domain folded inside the chaperonin chamber (orange), and partial density of a non-native Gp23 subunit bound in the open chaperonin cavity (cyan).

In all the structures of binary and ternary complexes with substrate bound, the electron density associated with the substrate was found deep in the cavity and in contact with at least five of the seven GroEL subunits; the GroEL subunits retained a symmetrical structure similar to that of the empty complex. This is in contrast with the group's earlier findings, in which the smaller GroEL substrate malate dehydrogenase was seen to bind to one side of the cavity with the GroEL

subunits bunching close to substrate and so losing their seven-fold symmetry [4].

It is hard to trap a definitive structure for malate dehydrogenase, or, indeed, for most substrates, in the open GroEL cavity because the non-native protein is very disordered and the non-bound regions are free to move. However, the enclosed cavity - even the GroEL-gp31 cavity, which is larger than that of native GroEL-GroES - can only just accommodate the elongated gp23 molecule in its newly-folded conformation, and this puts physical constraints on the substrate's movement which made its structure easier to determine. The stretching and distorting of the chaperonin cavity with gp23 bound is also reminiscent of the way a growing baby stretches the womb. This structure of a newly folded protein almost literally about to be born will make an important contribution to our understanding of chaperoned protein folding.

References

- [1] Clare DK, Bakkes P, van Heerikhuizen H, van der Vies SM & Saibil HR. Chaperonin complex with a newly folded protein encapsulated in the folding chamber. *Nature*, 2009, **457**, 107-111.
- [2] Saibil HR. Molecular chaperones: containers and surfaces for folding, stabilising or unfolding proteins. *Current Opinion in Structural Biology*, 2000, **10**, 251-258; Saibil HR. Chaperone machines in action. *Current Opinion in Structural Biology*, 2008, **18**, 35-42.
- [3] van der Vies SM, Gatenby AA & Georgopoulos C. Bacteriophage T4 encodes a co-chaperonin that can substitute for E coli GroES in protein folding. *Nature*, 1994, **368**, 6544-656; Clare D, Bakkes PJ, van Heerikhuizen H, van der Vies S & Saibil HR An expanded protein folding cage in the GroEL-gp31 complex. *J Mol Biol*, 2006, **358**, 905-911.
- [4] Elad N, Farr GW, Clare DK, Orlova EV, Horwich AL & Saibil HR. Topologies of a substrate protein bound to the chaperonin GroEL. *Molecular Cell*, 2007, **26**(3), 415-26.
- [5] Elad N, Clare DK, Saibil HR & Orlova EV. Detection and separation of heterogeneity in molecular complexes by statistical analysis of their two-dimensional projections. *J. Struct. Biol.*, 2008, **162**, 108-120.