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# The chaperonin folding machine

**Helen R. Saibil and Neil A. Ranson**

**Chaperonins are versatile molecular machines that assist the folding of a wide range of substrate proteins. They harness an ATPase cycle to control access of non-native proteins to hydrophobic binding sites. ATP binding promotes large conformational changes that partially bury the hydrophobic sites and initiate the binding of a co-chaperonin, creating closed and open cavities. Non-native proteins progress towards the native fold during their confinement in these cavities, and are then released by the allosteric action of ATP.**

The term 'molecular chaperones' covers a broad range of protein families whose common property is that they recognize non-native proteins and prevent their aggregation. Several chaperone families also play an active role in folding or unfolding using ATPase cycles to drive cycles of non-native protein binding and release by a range of mechanisms [1,2]. The best understood of these are the GroE chaperonins, an abundant subset of the molecular chaperones, which have a unique fold distinct from other ring-shaped ATPases [3].

Chaperonins are molecular machines that assist the folding of protein subunits. Chaperonin complexes provide folding chambers formed of flexible subunits that first bind and then twist, expand and engulf non-native proteins. Concerted domain rotations in each subunit are driven by an ATPase cycle and controlled by an intricate set of allosteric interactions that propagate through the entire molecular machine, making the bacterial chaperonins a model for complex allosteric systems [4,5]. How does the chaperonin accomplish all these steps, and how do these movements and the encapsulation they bring about help proteins to fold? We now have partial answers to

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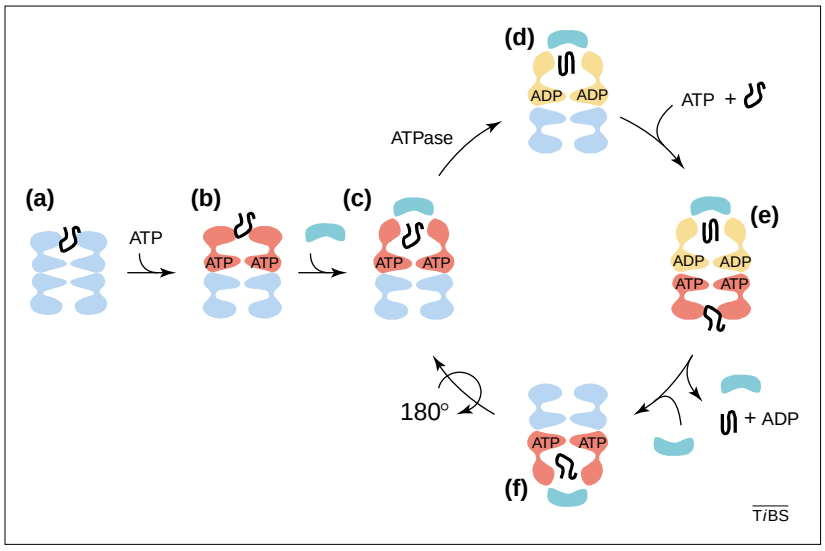


Fig. 1. GroEL ATPase and the folding cycle. The GroE complexes are shown as sections through the stacked-ring complexes. (a) GroEL has high affinity for non-native polypeptide substrate (black curved line). (b) ATP binds with positive co-operativity to one ring but negative co-operativity between rings, producing an altered conformation with reduced substrate affinity. (c) The ATP-bound ring rapidly binds GroES, simultaneously sequestering the hydrophobic binding sites and encapsulating the substrate in the folding chamber. There is a massive conformational change in the GroES-bound ring. (d) The substrate folds inside the chamber and ATP is hydrolysed. (e) ATP binding to the opposite ring primes the release of GroES and the trapped substrate. (f) A new substrate gets encapsulated. Adapted, with permission, from [14].

the first question and some interesting ideas about the second. In this article, we summarize the current state of understanding of chaperonin action, based on the well-characterized GroE system from *Escherichia coli*.

## Chaperonin functional cycle

The GroE proteins, both large (GroEL) and small (GroES), were found to be essential for growth of bacteriophage λ in *E. coli* [6]. A coherent picture began to form with two crucial discoveries: that the GroE heat-shock proteins were involved in protein assembly; and that they were homologous to Rubisco-binding protein in chloroplasts [7]. It was this link to protein assembly that launched the molecular-chaperone field.

Chaperonins are present in nearly all organisms and in all cases tested are essential for viability, presumably because they are required for the folding of some essential cellular proteins. They are grouped into two classes. Group I contains the eubacterial chaperonins (exemplified by *E. coli* GroEL and its lid-forming co-protein GroES) and homologous proteins from mitochondria and chloroplasts. They are promiscuous, binding most non-native proteins *in vitro*, and fold a significant subset of *E. coli* proteins *in vivo* [8]. Group II contains the eukaryotic chaperonin CCT and chaperonins from archaea, and its members operate without a GroES homologue. At least in the eukaryotic cytosol, group-II chaperonins are more selective in their clientele. They are clearly involved in the folding of the cytoskeletal proteins actin and tubulin [9], but there is also evidence that they interact functionally with several other proteins [10].

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Group-I chaperonins possess hydrophobic binding sites that line a cavity at either end of a barrel-shaped complex. This 'sticky' lining traps protein subunits or domains that are unfolded, partially folded or misfolded, and so have exposed hydrophobic surfaces (Fig. 1a). The binding of ATP to the chaperonin subunits initiates large conformational changes that both twist the binding sites away from the non-native protein (Fig. 1b) and simultaneously trigger the rapid binding of a co-chaperonin (Fig. 1c). Binding of the small co-chaperonin ring initiates a further large conformational change and caps a now-expanded folding chamber with a hydrophilic rather than a hydrophobic lining. Somehow, during these steps of displacement from the binding sites and encapsulation, the non-native protein is helped along its folding pathway (Fig. 1d) and, when the folding chamber is subsequently opened by the binding of ATP in the opposite ring, a folded protein subunit is released (Fig. 1e).

## Chaperonin structure

The 'gymnastic' ability of GroEL to contort itself into a wide range of different conformations is based on its structure [11,12] (Fig. 2). GroEL is a 14-mer of identical 58 kDa subunits arranged as two stacked rings. The whole assembly is held together by the back-to-back rings of equatorial domains that form most of the intra-ring and all of the inter-ring contacts. The equatorial domains also contain the nucleotide-binding site (Fig. 2a), near the hinge to the small, innocuous-looking intermediate domain. Although it lacks any binding sites, the intermediate domain turns out to be an important controlling element in the allosteric mechanism [12–14], and mutations in this domain have global effects on GroE function [15]. At the distal surface of each ring is the mobile apical domain, which contains the binding sites for substrate proteins and GroES. The apical domains form the lining of the cavity and are largely separated from the rest of the structure by gaps in the walls of the cylinder, although they are tethered to the intermediate domains of the neighbouring subunits by salt bridges. The presence of two hinge points in each subunit, the open ends of the complex, and windows in the walls of the complex allows scope for enormous rearrangements.

For group II chaperonins, the composition is more complex. Archaeal chaperonins consist of one, two or three different subunits and have eight- or ninefold symmetry. In eukaryotes, the cytosolic chaperonin CCT consists of two eight-membered rings, in which each subunit around the ring is a distinct gene product [9]. Chaperonins from group II have no co-protein, and the role of GroES appears to be fulfilled by a helical extension to the apical domain that closes the central cavity in certain nucleotide states.

At the heart of the chaperonin mechanism are the hydrophobic binding sites facing into the central cavity of the complex. Twists of the GroEL apical

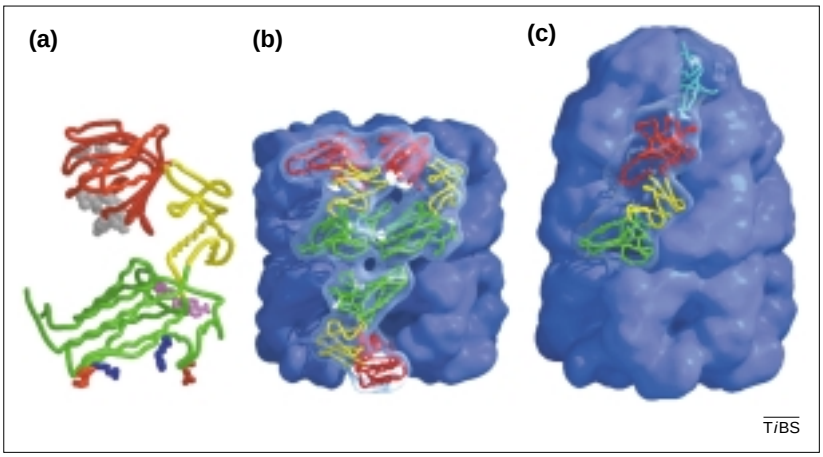


Fig. 2. Crystal structures of *Escherichia coli* GroEL (Hsp60) and its complex with ADP and GroES (Hsp10). (a) The three domains of the GroEL subunit are colour coded – green for the equatorial domain, yellow for the intermediate domain and red for the apical domain. The equatorial domain contains the nucleotide-binding site, shown occupied, as well as the two inter-ring contacts at the base of the molecule (negatively charged residues in red, positive in blue). The apical domain contains the hydrophobic binding sites (grey, space-filling representation) for non-native polypeptides. ATP is shown in pink. (b) The structure of the unbound GroEL 14-mer, with three subunits shown at the front [11]. The hydrophobic binding sites face into the end cavities. (c) The GroEL–ADP–GroES complex [12], with one GroEL subunit and one GroES subunit (cyan).

domains observed in the nucleotide-bound states move the binding sites away from the cavity lining and thus reduce their accessibility to substrate proteins. The first detailed structural information about how peptides bind came from the serendipitous binding of an N-terminal tag to the binding site of the adjacent molecule in a crystal of GroEL apical domains [16] (Fig. 3). Subsequent crystal structures of other peptides and also of the GroES mobile loop

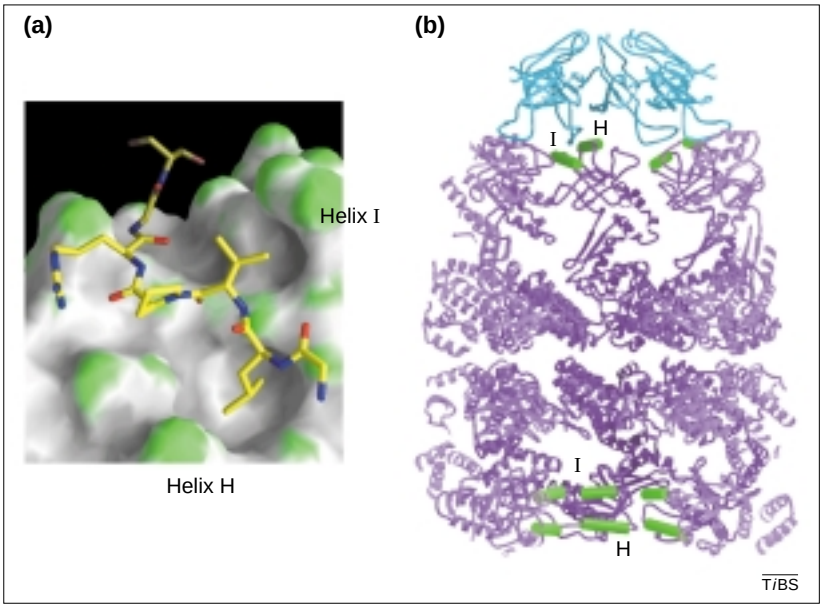


Fig. 3. The structure of an extended polypeptide binding to the hydrophobic patch on the apical domain of GroEL. (a) The molecular surface of the GroEL apical domain, showing hydrophobic binding sites on helices H and I, is coloured according to surface curvature. Convex, concave and flat regions are shown in green, grey and white, respectively. Bulky side chains on the bound peptide insert into hydrophobic cavities on the GroEL apical domain. Reproduced, with permission, from [16]. (b) The location of helices H and I on a ribbon diagram of the rear three subunits of the GroEL–GroES–ADP crystal structure [12]. The view is from inside the complex. GroEL is shown in purple, with helices H and I shown as green cylinders. GroES is shown in cyan.

show very similar binding [17]. The bound peptide is extended and there are pockets for binding hydrophobic side chains as well as some polar interactions, consistent with nonspecific binding to two of the three regions originally mapped out by mutagenesis [15,18].

### Role of ATP

What happens when ATP binds? Biochemically, the effects are dramatic. The binding of non-native substrates is weakened enough for some proteins to be released and folded simply by ATP-driven cycles of binding and release. Kinetic measurements reveal the complexity of the system. ATP binding is positively co-operative within the seven subunits of one ring (Fig. 1b). Although the oligomer is made of two chemically identical rings, there is negative co-operativity between them, inhibiting ATP binding in the second ring [19–21]. This makes the two rings asymmetric and causes them to act sequentially in the ATPase cycle [22,23]. In the crystal structure of GroEL with an ATP analogue bound [24], this asymmetry and the large conformational changes expected from low-resolution electron-microscopy maps [25] were not observed. The only explanation consistent with all the observations is that conditions in the crystal lattice prevent the conformational changes seen in solution. Such a situation is surprising but not unprecedented. Indeed, the archaeal thermosome (a group-II chaperonin) shows a similar effect, in which the crystal structures of different nucleotide-bound states have very similar conformations [26].

The biggest event in the chaperonin cycle, rapidly triggered by ATP binding, is the formation of an enclosed cavity upon GroES binding (Fig. 2c). The GroEL apical domains swing 60° upwards and twist through 90° to contact loops dangling down from the GroES lid [12,25,27]. The open hydrophobic cavity is converted to an expanded but enclosed chamber lined with hydrophilic residues. The radical change in surface property comes about because of the 90° twist, which buries the hydrophobic patches in the walls of the chamber. Somehow, the displacement of the substrate from its binding sites, combined with encapsulation in a GroEL–ATP–GroES cavity, provides the assistance needed for folding. This key interaction between the chaperonin and its most reluctant substrates, such as Rubisco and mitochondrial malate dehydrogenase (MDH), is still not understood. Encapsulating these proteins in the GroEL–ADP–GroES complex (Fig. 1d) does not suffice to fold them [22]. The signal to release the GroES and thus the contents of the chamber is ATP binding to the opposite ring (Fig. 1e). This long-range allosteric interaction might involve a strained conformation of the open GroEL ring [14].

The crystal structure of the GroEL–ADP–GroES complex revealed the hinge rotations that form part of the GroEL functional cycle, and described the

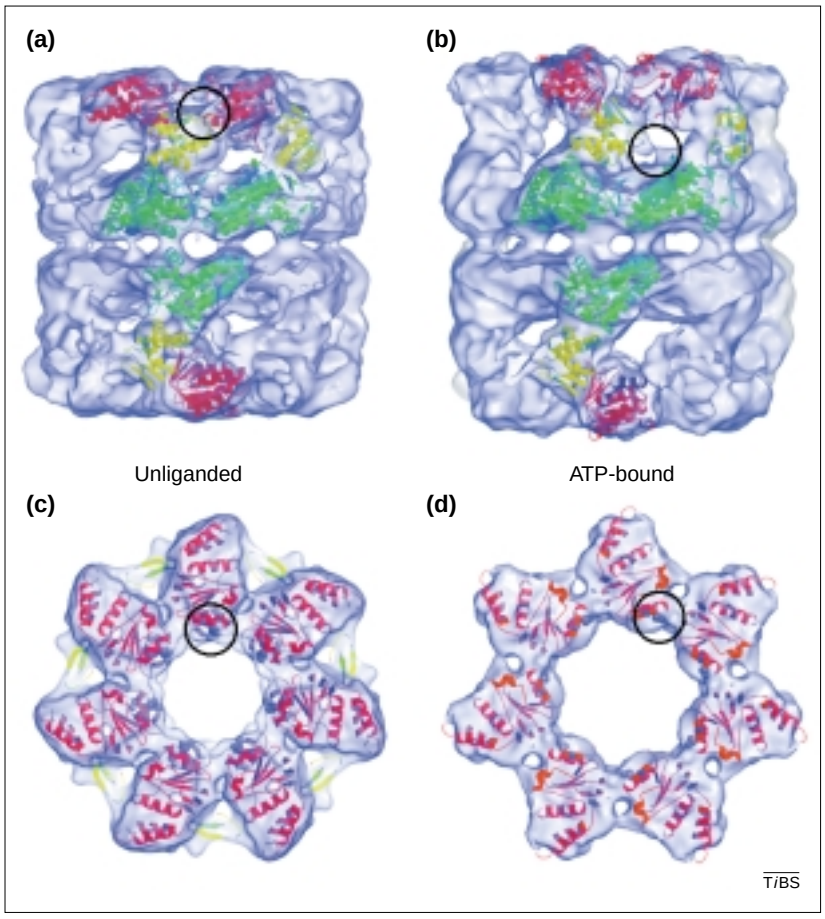


Fig. 4. Allosteric structural changes caused by ATP binding to GroEL. (a,b) Unbound GroEL and GroEL (D398A)–ATP. Cryo-electron-microscopy density maps are shown as transparent blue surfaces and the domains of three subunits, docked into the maps as rigid bodies, are colour coded – green for the equatorial domain, yellow for the intermediate domain and red for the apical domain. Notice the contact between adjacent intermediate and apical domains in the top ring of (a), and the switch to a contact with the adjacent equatorial domain in (b) (black circles). The ATP-bound complex is asymmetric and extended vertically. The inter-ring interface is also distorted. (c,d) Top views of the same structures. ATP binding causes a large anticlockwise twist of the apical domains. The hydrophobic residues (circled) become less accessible in the ATP-bound state (d). Adapted, with permission, from [14].

enormous rearrangement of the apical domains upon GroES binding [12]. The GroEL–ADP–GroES structure also showed how the intermediate domain closes down over the nucleotide-binding pocket, allowing a crucial interaction between Asp398 from the intermediate domain and the $Mg^{2+}$ coordinating the ADP. This observation led to the design of the GroEL mutant Asp398Ala, which is still able to function *in vitro* but has a severely reduced ATPase rate [22]. The Asp398→Ala mutation has turned out to be an extremely useful tool that gives a relatively long-lived ATP-bound state for both structural and functional studies. To understand the role of ATP in triggering the machine, it is necessary to examine the structural consequences of ATP binding, before GroES is allowed to bind.

An intermediate-resolution cryo-electron-microscopy structure revealed a few unexpected twists and turns on the pathway to the GroES-bound state [14] (Fig. 4). First, the intermediate domain rotates downwards, similar to the prediction of molecular dynamics simulation [13], forcing the loss of the original salt bridge to the neighbouring apical domain but forming a new contact to the neighbouring equatorial domain (Fig. 4b). The freed apical domain twists 25° but in the 'wrong' direction (i.e. opposite to its twist in the GroES-bound complex) (Figs 4d,5b,c). The significance of this extra travel of the binding sites in the full trajectory is unknown, but it shows that the movements are even larger than previously thought. The twist is large enough partially to bury the hydrophobic sites (Fig. 4d). In addition, opposing movements between the two back-to-back rings of equatorial domains distort the ring–ring interface (Fig. 4a,b). This provides a possible mechanism for propagating changes in the ATP-binding site to the opposite ring. The enormous conformational rearrangement undergone by a GroEL subunit upon binding of nucleotide and then co-protein is shown in Fig. 5; the conformational changes are easier to follow in a movie (http://archive.bmn.com/supp/tibs/saibil.html).

## Folding mechanisms

Clever experiments have been designed to test the requirements for concerted domain movements and also for multiple interactions of a substrate with the apical domains. Constructs that lock the apical domain down onto the equatorial domain within a subunit prevent the movements required for ATP hydrolysis, substrate release and negative co-operativity [5,28,29]. It seems likely that apical domain twisting is needed for the ATP-induced release of bound substrate. ATP hydrolysis is catalysed by the downward rotation of the intermediate domain, inserting Asp398 into the ATP-binding site. It is possible that the cross-linked state prevents this movement. Even a single cross-linked subunit in a ring disrupts negative co-operativity between rings and the ability of the ring to release bound substrate [29]. In a remarkable feat of genetic engineering, Horwich and colleagues constructed a complete ring of GroEL with all subunits as a continuous chain, making use of the proximity of C and N termini in a disordered region inside the GroEL cylinder [30]. Variants of this construct were created with selected apical domains rendered dysfunctional for protein binding by known point mutations. By screening the activity of a panel of such constructs, they found that at least three adjacent, functional apical domains are necessary for productive folding of substrates such as Rubisco and MDH. This requirement strongly suggests that these substrates are bound to multiple hydrophobic sites in the cavity.

The mechanism by which chaperonins aid the protein-folding reaction has been the subject of heated debate. Could the chaperonin cage simply act as a passive isolation chamber ('Anfinsen cage'), improving the efficiency of the folding process by preventing aggregation of non-native proteins?

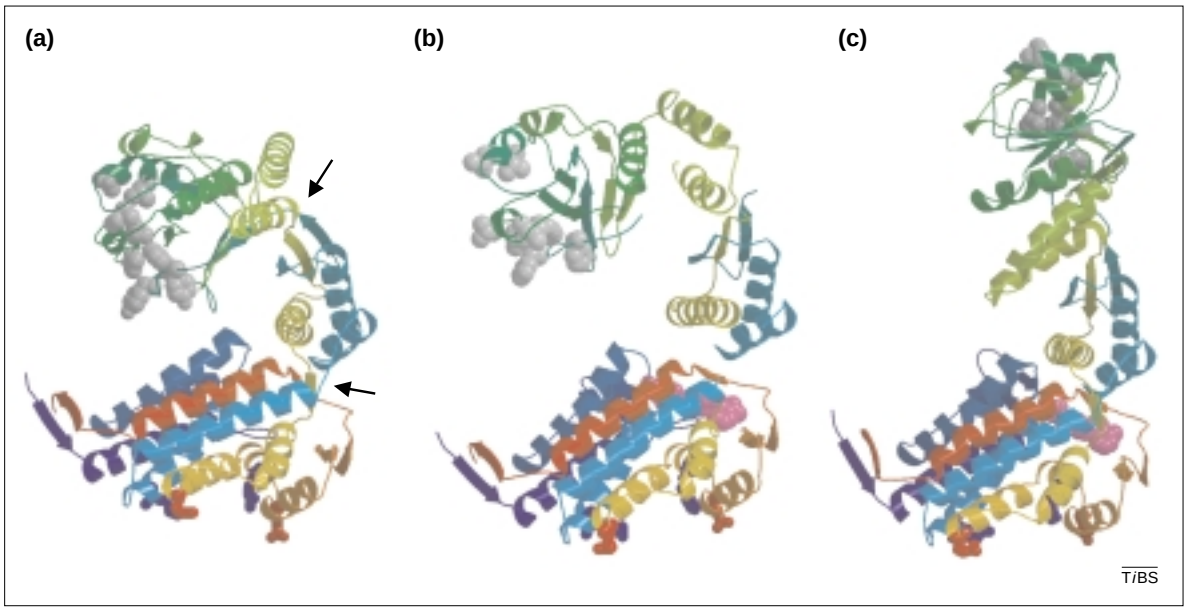


Fig. 5. Range of conformational changes in the GroEL subunit, showing the unbound GroEL (a) [11], GroEL–ATP (b) [14] and GroEL–ADP–GroES (c) [12] forms. The chain is colour coded from blue to red from N- to C-terminus. The domains move as rigid bodies about the two hinge points marked with arrows in (a). Hydrophobic substrate-binding residues are shown in grey and nucleotides in pink. In the ATP-bound state (b), the binding sites twists towards the viewer but, in the GroES-bound state (c), the apical domain is twisted in the opposite direction so that the binding sites face away from the direction of view. In (b), the hinge residues are omitted because the local conformation is unknown. Transitions between these states are available as a movie (http://archive.bmn.com/supp/tibs/saibil.html).

Or could a more complex process occur, with GroEL going through cycles of protein binding and release that rip apart misfolded proteins and allow them to refold in free solution (iterative annealing)? Is there an active mechanism assisting folding inside the cage?

In another elegant study, a version of GroEL was designed that was specifically labelled with biotin on the apical-domain protein-binding surfaces [31]. If streptavidin is added to this system, it binds to the biotinylated apical domains and blocks access to the central cavity, sterically preventing the binding of non-native proteins. This allowed an ingenious experiment to be devised in which, after the normal ejection of a substrate protein from the GroEL cavity, re-entry is prevented. Addition of streptavidin immediately halted further folding of Rubisco (50 kDa), showing that normal folding occurs while Rubisco is encapsulated in the GroEL–GroES cavity. Interestingly, for conditions under which spontaneous refolding of Rubisco is permissible, encapsulation in the GroEL–GroES cavity increased the rate of renaturation. This acceleration in folding suggests that the chaperonin cage can block off-pathway misfolding steps and favour productive folding.

Do the complex twisting and capping motions through which the chaperonins are driven do something beyond confining folding proteins? Requirements for the folding of different GroEL substrates are diverse and reflect a range of mechanisms, depending on the size and physicochemical properties of the substrate. Even proteins with the same native fold but different sequences can have quite different folding requirements. The proposal of forced unfolding or iterative annealing suggests that multivalently bound, misfolded Rubisco gets pulled apart as the apical domains go through their motions, to be released into the cavity and to have a chance to collapse into a productive folding conformation [32]. However, hydrogen-exchange experiments have not identified such effects on the substrate MDH [33].

Furthermore, a protein is now known that is too large to fit into the enclosed cavity but nevertheless requires the full GroEL, GroES and ATP system for efficient refolding [34]. The mitochondrial enzyme aconitase requires the full chaperonin system for its folding but it is not encapsulated. Rather, it appears to interact with chaperonins in a way distinct from those discussed above. Apo-aconitase undergoes multiple rounds of binding and release in the open cavity, with GroEL acting as a 'holding tank' for the apoenzyme until its metal cofactor binds and the holoenzyme is formed. Release of the protein is triggered allosterically by GroES binding to the opposite ring. This release signal is transmitted ~150 Å from the site of GroES binding to the apical domains of the aconitase-bound ring.

## Conclusion

Owing to their conformational flexibility, chaperonins can help the folding of a wide range of substrate proteins from either open or closed rings. They exemplify a new kind of molecular machine, in which domains twist through large angles and containers alternately get created and discharged, coupled with alternation of hydrophobic/hydrophilic

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surface character inside the binding cavity. By contrast, another molecular machine, ATP synthase, is a rotary motor with sequential rather than concerted binding of ATP. In chaperonins, ATP binding is used to discharge bound ligands and to create the folding chamber. The network of long-range allosteric effects is being mapped but we have not yet reached an understanding of what really happens to folding proteins inside either the closed or the open cavities.

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