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Allosteric signalling of ATP hydrolysis in GroEL-GroES complexes

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Summary

The double ring chaperonin GroEL and its lid-like co-chaperonin GroES form asymmetric complexes that in the ATP-bound state mediate productive folding in a hydrophilic, GroES-encapsulated chamber, the so-called *cis* cavity. Upon ATP hydrolysis within the *cis* ring, the asymmetric complex becomes able to accept non-native polypeptide and ATP in the open, *trans* ring. Here we have examined the structural basis for this allosteric switch in activity by electron cryo-microscopy and single particle image processing. ATP hydrolysis does not change the conformation of the *cis* ring, but its effects are transmitted through an inter-ring contact and cause domain rotations in the mobile *trans* ring. These rigid-body movements in the *trans* ring lead to disruption of its *intra-ring* contacts, expansion of the entire ring and opening of both the nucleotide pocket and of the substrate-binding domains, admitting ATP and new substrate protein.

Introduction

Chaperonins are ubiquitous double-ring molecular machines that provide essential kinetic assistance to the folding of many proteins¹⁻⁴. Such assistance requires ATP, the binding of which is cooperative within a ring but negatively cooperative between rings^{5,6}. For the *E. coli* chaperonin GroEL, ATP binding to the seven equatorial domains at the base of a ring mediates a rigid-body elevation of ~10° and an anticlockwise twist of ~25° of its apical domains, that enables binding of the lid-like co-chaperonin GroES⁷. Such association induces a further 50° elevation and ~115° clockwise twisting of the apical domains, removing a hydrophobic, peptide-binding surface from the central cavity^{8,9}. This rearrangement alters the central cavity from predominantly hydrophobic to hydrophilic, effectively ejecting polypeptide into a GroES-encapsulated chamber where folding commences¹⁰. The ATP/GroES-bound, folding-active complex is the longest-lived state in the reaction cycle, with a lifetime dictated by the rate of ATP hydrolysis, which advances the assembly to a state which, while still folding-active, is weakened in affinity for GroES and thus prepared to discharge its ligands¹¹. Ligand release is allosterically triggered by ATP binding in the opposite ring. ATP thus directs cycles of polypeptide folding and release from each ring alternately¹², which have apparently evolved to strike a compromise

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between being long enough to allow substrate proteins a trial at encapsulated folding, but short enough to allow timely release of folded substrate proteins into the cell to accomplish work.

The fundamental conformational changes upon ATP hydrolysis that advance the GroEL-GroES machine through its cycle are poorly understood. The X-ray structure of GroEL-(ADP-AIF₃)₇-GroES, a folding-active state functionally emulating the ATP state, appears identical to that of GroEL-ADP₇-GroES₁₃. The identical appearance of the GroES-bound (*cis*) ring is understandable given that productive folding continues during the brief lifetime of the *cis*-ADP complex (before its ligands are discharged by ATP binding in *trans*). However, the lack of structural difference outside the nucleotide pocket cannot explain the conversion of the *trans* ring from a state with low affinity for substrate polypeptide (while ATP is present in the *cis* ring) to one that is the normal acceptor state for polypeptide (with ADP in the *cis* ring). There is also no accounting for why the *trans* ring of a complex with ATP in *cis* has low affinity for nucleotide, while the *trans* ring of a complex with ADP in *cis* binds ATP. An earlier cryo-EM study revealed changes at the interface between the two rings upon ATP binding to GroEL, suggesting that the crystal contacts were inhibiting structural changes that normally attend nucleotide binding and hydrolysis⁷. In order to examine the structural effects of ATP hydrolysis in the full GroEL-GroES system, we utilised single-particle cryo-EM and image processing to resolve the ATP and ADP states of the complex at intermediate resolution. We observe domain rotations in the *trans* ring upon *cis* ATP hydrolysis. These rotations alter the inter-ring contacts connected to the nucleotide pockets of both rings, and suggest a mechanistic explanation for the functional actions of *cis* ATP hydrolysis.

Results

Solution structures of GroEL-GroES complexes

To generate an ATP-bound complex that is stable on the time scale of a cryo-EM experiment, we used the D398A substitution in GroEL. GroEL(D398A) has normal affinity for ATP but ~2% of the wild type ATPase activity^{9,11}. Since ATP and GroES binding support productive folding, it retains the ability to refold stringent protein substrates¹². GroEL(D398A) was previously used to characterize the GroEL-ATP₇ state⁷. Here, the sample of GroEL(D398A), GroES and ATP was markedly more heterogeneous than the one in ADP, with several clearly distinct types of GroE complex shown in Figure 1. While the largest structural class corresponds to the asymmetric GroEL-GroES complexes reconstructed in this study (~70%), ATP binding weakens the GroEL ring-ring interface, and promotes formation of single ring GroEL (8%) or single ring-GroES complexes (3.5%) that occur extremely rarely in ADP (<1% total). In addition, symmetric GroEL-GroES₂ complexes (16%) are only found in the presence of ATP. Finally, ~5% of GroEL particles are observed without bound GroES under both conditions.

Shown in Figure 2a is the cryo-EM structure of GroEL(D398A)-ATP₇-GroES, at 7.7 Å resolution (0.5 Fourier shell correlation) from a final dataset of ~16,300 images. The cryo-EM map of GroEL-ADP₇-GroES at 8.7 Å resolution (from ~7,600 images) is shown in Figure 2b. A plot of the resolution curves for both maps is shown in Supplementary figure 1.

Atomic structure fitting reveals rigid body movements

Initial docking of either GroEL-(ADP·AIF₃)₇-GroES or GroEL-ADP₇-GroES crystal structures as a single rigid body into our maps showed good fits for the *cis* (GroES-bound) ring, but poor fitting of the *trans* (open) ring of either EM structure. We attributed these differences between the cryo-EM and X-ray structures to crystal lattice constraints in the X-

ray-determined GroEL-GroES complexes, which pack in a head-to-tail arrangement with the GroES “head” abutting into the open, *trans* ring of the adjacent complex in the crystal lattice (Supplementary figure 2). The cryo-EM structures were therefore analyzed by separating the subunits into their constituent domains and fitting as seven rigid bodies (a GroES subunit and the GroEL apical, intermediate and equatorial domains for both *cis* and *trans* rings), using the program URO14. Sections through the cryo-EM maps with the fitted atomic models are shown in Figure 2c,d. α -helices are resolved as tubes and β -sheets as layers of density, although at this resolution, the individual β -strands are not resolved. The quality of the cryo-EM maps and accuracy of fitting is demonstrated by the hole in the 10 kDa β -barrel of the GroES subunit (Figure 3a), identifiable helices, sheets and loops of the GroEL subunit (Figure 3b) and by the α -helices in the equatorial domain (Figure 3c). Some disordered regions are present in the map of the ATP-bound state, especially the GroES mobile loops that bind to GroEL, the *trans*-intermediate domains, and the *trans*-apical helical hairpin (helices K & L). With the use of rigid-body fitting into the intermediate resolution maps reported here, we are not modelling any local deformations. However, the excellent correspondence between the EM density and the domain atomic structures clearly support previous observations that conformational changes in GroEL are achieved predominantly through rigid-body movements about hinge regions between the apical-intermediate and intermediate-equatorial domains of GroEL.

Structural changes after ATP hydrolysis

Comparison of the fitted coordinates for the ATP and ADP-bound GroEL-GroES complexes shows that the *cis* rings are almost identical, but that differences exist in all three domains of the *trans* rings. The most striking observation is found within the *trans* ring, where the main intra-ring contact between adjacent equatorial domains, an inter-subunit β -sheet interaction (residues 4-25, 37-51 and 519-523), is disrupted in the ADP-bound complex (Figure 4a,b). A sideways tilt of the *trans* equatorial domains (in the view of Figure 4c,d, looking at the inside surfaces of the complexes) shears the interface between them pulling apart the β -sheet contacts. Remarkably, the 5 Å inter-strand distance in the ATP complex widens to 9-10 Å in the ADP state (Figure 4e).

This disruption of this inter-subunit β -sheet interaction within the *trans* ring is also observed in a complex of GroEL with ADP and the GroES homologue gp31 (DKC, HRS, P Bakkes, H van Heerikhuizen, S van der Vies, in preparation). These changes explain earlier biochemical results. For example, the observation that GroEL(C519S) partially dissociates into monomers in 5 mM ADP is likely to be related to our observed effect in the ADP complex¹⁵.

The origin and consequences of this distortion of the *trans* ring become apparent when the inter-ring interface and *trans* ring are examined as a unit. In Figure 5, a central slice through this region is shown with some key elements highlighted. In the ATP state (Figure 5a), the D helices (magenta) of the two rings are offset, seen by the separation of opposing Ala109 residues (magenta spheres). In the ADP state (Figure 5b), the *trans* equatorial domains pivot about the fixed Glu461 contact (gold spheres) so that the D helices (cyan) come more into alignment, seen by the closer approach of Ala109 residues. This rotation pulls apart the β -strand contact (green). The two complexes are overlaid in Figure 5c, and the interface, contact and substrate binding sites are enlarged in separate panels. The *trans* apical cavity opens in a jaw-like movement after ATP hydrolysis. The movements are most clearly illustrated by side and end-view movies of the two states in alternation (Supplementary videos 1 and 2 respectively).

Discussion

Routes of allosteric transmission

These observations permit a more complete description of the GroEL allosteric cycle. Figure 6 summarizes the movements of key structural elements in allosteric transmission through the GroEL ATPase cycle. In unliganded GroEL (figure 6a)¹⁶, the D helices (gold) are in close contact at the inter-ring interface and the substrate binding sites (gray surfaces) line the central cavity. The intra-ring contacts are formed by the β -sheet interaction between adjacent equatorial domains, shown in the *trans* ring (magenta/blue strands). After ATP binding⁷ (Figure 6b), the inter-ring interface is weakened. The substrate binding sites are partly removed from the cavity by an anti-clockwise twist of the apical domains. Upon GroES binding (Figure 6c), a tilt of the *cis* equatorial domains weakens the inter-ring contact at Ala109 (see also Supplementary Figure 3). The weakened interface is consistent with the presence of single rings in the GroES/ATP preparation (Figure 1). Furthermore, the *trans* ring becomes more closed (bold curved arrows). ATP hydrolysis and formation of the ADP/GroES-bound state (Figure 6d) cause a rotation of the *trans* equatorial domain, bringing helix D in *trans* more into alignment with its counterpart in *cis* and thus re-tightening the Ala109 inter-ring contact. An additional consequence of this domain movement is the disruption of the intra-ring β -sheet contact (magenta/blue) between adjacent *trans* equatorial domains, leading to an expansion of the whole *trans* ring. The expanded and slightly less twisted apical domains (bold curved arrows) then provide the new acceptor state for polypeptide substrate in *trans*¹².

Finally, ATP binding in *trans* to GroEL-ADP₇-GroES forms the transient GroES-ADP₇-GroEL-ATP₇ complex, from which the *cis* ligands are discharged¹². We previously examined this transient complex by rapid mixing of GroEL-ADP₇-GroES complexes with ATP in the presence of single ring GroEL to trap any released GroES, preventing such molecules from rebinding to the ATP-bound *trans* ring. That work yielded a low resolution map⁷. Re-examination of atomic structure fitting to this complex allows us to conclude that it is very similar to the GroEL-ATP₇-GroES complex. In particular, the expansion and strand separation of the *trans* ring seen in the GroEL-ADP₇-GroES precursor are reversed upon ATP binding in *trans* (Figure 6e). This ATP-driven switching of the *trans* ring back to the “closed” state must be the trigger that ejects the ligands from the *cis* ring. The nature of the *cis* ring movements involved in this ejection step remain to be observed.

With respect to substrate binding in the open *trans* ring, movements of the *trans* apical domains are critical to determining when the *trans* ring becomes an acceptor for new substrate¹². In the GroEL-ATP₇-GroES complex, the *trans* apical ring is twisted and closed, so that the hydrophobic binding sites for non-native proteins are poorly accessible. In the ADP-bound structure, the *trans* apical domains are shifted radially outward and are 5° less twisted, resulting in a central binding cavity that is ~15 Å larger in diameter (end view movie, Supplementary Figure 5), consistent with the formation of the substrate acceptor state.

Proposed relay helix mechanism

Helix D (residues 89-109) runs from the ATP binding site at the top of the rigid equatorial domain, through the body of the domain to the inter-ring interface, where Ala109, Lys105 and Glu434 form a contact with the equivalent residues in a subunit from the equatorial domain of the opposite ring (Supplementary Figure 3). This provides a mechanism for direct communication from each of the *cis* nucleotide-binding pockets, through the ring interface, to the *trans* nucleotide-binding sites^{7,8,17}. We therefore term it the relay helix, in analogy to the relay helix in myosin and kinesin motor proteins, although there is no structural

homology aside from the presence of a nucleotide binding site P-loop at the N-terminus of the relay helix 18. In these motor proteins, the relay helix is proposed to exert mechanical force to produce further conformational changes.

For GroEL, we propose that the allosteric signals are transmitted electrostatically by the relay helix dipoles. After ATP and GroES binding, the *cis* ring is held in a more restricted conformation, but the *trans* ring is more mobile. It seems likely that the reduction of negative charge in the *cis* ring binding sites upon hydrolysis of ATP to ADP changes the balance of electrostatic forces at the ring-ring interface, where the opposing relay helix dipoles meet. Since the equatorial domains in the *trans* ring are less constrained, changes in the electrostatic forces that alter the alignment between the opposing helices would cause the *trans* equatorial domains to tilt. This change in tilt is propagated through the *trans* ring.

Subsequent ATP binding in *trans* reverses the conformational changes in the *trans* ring, tending to reverse the change in the inter-ring contacts. In this transient ADP/ATP occupied state, the *trans* ring must exert a force on the *cis* ring to revert it to the unliganded state and eject GroES and ligands from the folding chamber. Therefore the distorted conformation of the *trans* ring of the ADP complex is poised to accept new substrate and ATP and to take over as the allosteric regulator of the *cis* ring. Such a mechanism ensures that GroES binding is not reversed during the folding step – it always binds strongly and its release must be triggered through electrostatic communication by ATP binding in *trans*.

Methods

Proteins and reagents

GroEL(D398A), wild-type GroEL and GroES were prepared as described previously^{10,11}. All EM experiments were carried out in a standard buffer containing 12.5 mM HEPES (pH 7.5), 5 mM KCl and 5 mM MgCl₂. All buffers and reagents were from Sigma (Poole, Dorset, UK.).

Specimen preparation and EM data collection

Grids with holey carbon films were prepared and vitrified by standard methods¹⁹. For GroEL-ATP₇-GroES, a solution containing 1 μM GroEL(D398A), 0.9 μM GroES and 100 μM ATP was frozen within ~10s of ATP addition. All images were collected using an FEI-TF20 microscope and Gatan-626 cryo-transfer holder. Micrographs were recorded on Kodak SO-163 film at 50,000x magnification, with 0.1 s pre-exposure, 0.2 s pause, and 1 s exposure. The total accumulated dose was ~15 e⁻ Å⁻².

Image processing

Images were digitised using a Z/I-Imaging Photoscan-TD microdensitometer (7 μm step, 1.4 Å pixel⁻¹). Defocus and astigmatism were determined using CTFFIND3 (ref 20). Micrographs with noticeable drift or astigmatism were discarded. Particles were interactively selected using Ximdisp21, and excised into 512×512 pixel boxes. Images were then phase corrected for the effects of the contrast transfer function (CTF), cropped to 320×320 boxes, band-pass filtered (285-4.2 Å) and normalized.

Unless otherwise stated, all processing steps were performed in spider-v9.03 (ref 22) with FFTW libraries²³. GroEL-GroES complexes were extracted from both ATP and ADP datasets using a simple sorting protocol in which a single side projection was generated from the crystal structure of the ADP-bound state⁹ (1aon). All images were then iteratively aligned to a Fourier filtered (midpoint 35 Å) version of this side view.

A preliminary classification using multivariate statistical analysis (MSA) in IMAGIC24 confirmed the expected heterogeneity in the samples. To partition the images into their respective structural classes, further alignment was undertaken using six references (corresponding to a GroE end view, GroEL-GroES₂, GroEL-GroES, GroEL, single ring-GroEL & single ring-GroEL-GroES), derived from the classification of the entire dataset. Reclassification revealed no obviously mis-aligned or out of register images.

Images corresponding to the GroEL-ATP₇-GroES and GroEL-ADP₇-GroES complexes were refined by a projection matching protocol derived from that previously described^{8,7,25}. 155 reference views were generated, corresponding to sets of azimuthal rotations around the 7-fold axis, with the 7-fold axis tipped progressively out of the image plane (maximum tip 12.86°). The crystal structure of GroEL-ADP₇-GroES, filtered to 35 Å, was used as a starting model for both datasets. (The same results were obtained with a starting model derived from the image data by angular reconstitution). After multi-reference alignment (MRA), the resulting aligned image dataset was tightly masked and classified by MSA, creating a large number of small classes (with ~3 or 4 images per class). With sufficient data, such classes contain images corresponding to the same molecular view, and the averaging provides improved signal-to-noise. The corresponding classes were created in SPIDER and aligned to the original set of reference views. The final orientation (for each refinement round) of each class was determined by a local angular search ($\pm \sim 1.2^\circ$). Finally, images belonging to each unique orientation were combined into a single average for calculation of the reconstruction. Refinement parameters for both datasets are shown in Table 1.

Three-dimensional reconstruction

In later rounds of refinement, tight masking of each input view was used before calculation of the 3D reconstruction (using the SPIDER command BP RP). To achieve this, a 3D mask was derived from the 3D model calculated in the previous round of refinement, and projected at the orientation of each input view to provide a set of specific 2D masks. Full amplitude and phase CTF correction was incorporated into the final maps⁷, which were also sharpened by suppression of low frequency components. A smooth band-pass filter was applied to the maps to give 90% reduction of amplitudes poorer than 14 Å and zeroing of amplitudes exceeding 6 Å. Refinement statistics for both structure determinations are given in Table 1.

Atomic structure fitting

Atomic coordinates for GroEL-(ADP.AIF₃)₇-GroES (1pcq) or GroEL-ADP₇-GroES (1aon) were fitted into the ATP and ADP-bound cryo-EM maps respectively, as seven independent rigid-body domains using the program URO (see legend to figure 2)¹⁴. The fitted coordinates, with hinges rejoined and after energy minimization in AMBER26, for both GroEL-ATP₇-GroES and GroEL-ADP₇-GroES have been deposited in the PDB (2C7C and 2C7D respectively). The EM maps for both complexes have been deposited in the EMDB (1180 and 1181 respectively). For this study, we have refined the fitting of GroEL(D398A)-ATP (ref 7) (EMD1047) on the basis of comparisons with the new structures determined here. A 4% reduction in map scale and improved atomic structure fitting with URO14 shows domain movements that are qualitatively the same as originally reported. Refitted coordinates corresponding to GroEL-ATP₇ (1GR6) are deposited as PDB 2C7E.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Complex	ATP data (%)	ADP data (%)
GroEL-GroES	67.8%	93.5%
GroEL-(GroES) ₂	15.8%	0.4%
GroEL	5.0%	5.0%
SR	7.8%	} ~1.0%
SR-GroES	3.5%	

Figure 1.
Distribution of protein complexes found in the ATP and ADP samples. Figures given are as a percentage of all side views imaged.

Figure 2. Solution structures of GroEL-ATP₇-GroES and GroEL-ADP₇-GroES

(a) Surface representation of the side view of the GroEL-ATP₇-GroES complex. (b) surface representation of the side view of the GroEL-ADP₇-GroES complex. (c,d) Central sections through the cryo-EM maps are shown as a semi-transparent surface in either gold (c, ATP) or blue (d, ADP), with the atomic coordinates for the GroEL equatorial (green: residues 3-136 & 410-524), intermediate (yellow: residues 137-191 & 374-409), apical (red: residues 192-373, except for 353-361 at the tip of the mobile helical hairpin in the *trans* ring), and GroES (magenta) fitted in. The 7-fold axis of the GroEL-GroES oligomer is vertical and in the image plane for all figures. The resolution of the ATP and ADP-bound maps are 7.7 Å and 8.7 Å respectively, determined by Fourier shell correlation (FSC) at a cut-off of 0.5. FSC curves for both reconstructions are shown in supplementary figure 1. Figures 2-6 (and supplementary figures 2-5) were created with Pymol (www.pymol.org).

Figure 3. Quality of EM maps and atomic structure fitting

(a) A section through the atomic coordinates for GroES fitted into the EM-derived electron density of GroEL-ATP₇-GroES. The hole in the β -barrel that forms the body of the 10 kDa. subunit is clearly resolved. (b) A section through a single GroEL subunit in the *cis* (GroES-bound) ring with ADP·AlF₃ in the nucleotide binding site shown in CPK-color space-filling mode. Although small regions corresponding to loops are not well resolved, the length and orientation of all secondary structure elements fit the EM density extremely well, regardless of their radius from the 7-fold axis (which is vertical and in the plane of the figure). (c) A section through the equatorial domains of the *cis* ring, perpendicular to the 7-fold axis. The helical segments of the equatorial domain are clearly resolved. In all panels, the EM-derived electron density for the GroEL-ATP₇-GroES complex is contoured as a semi-transparent gold surface.

Figure 4. Disruption of intra-ring contacts between the equatorial domains of the *trans* ring
 (a) The interface between neighbouring equatorial domains in the *trans* ring of the ATP-bound complex. The EM-derived electron density is shown as a gold mesh, with the adjacent equatorial domains in blue and magenta. In the ATP complex (and all crystal structures of GroEL complexes) two β -strands from each subunit form a 4-stranded β -sheet that is a major contact holding the ring of equatorial domains together (highlighted by a black rectangle). (b). The corresponding view of the ADP-bound complex (with EM density shown as a blue mesh and equatorial domains in green and orange) shows that a small ($\sim 3^\circ$) rotation of the equatorial domain results in the two strands from the orange subunit moving upward (in this view) and the two strands from the green subunit moving downward, pulling apart the β -sheet contact. (c & d) Structure of the rear half of the ring of equatorial domains from the ATP(c) and ADP-bound (d) complexes. The 7-fold axis is vertical and in the image plane for c & d, and these views are reached by tipping a & b forward by $\sim 30^\circ$. (e) A comparison of the intra-strand (main chain) distances in the ATP (blue/magenta) and ADP (green/gold) complexes showing that the strand separation increases by 4-5 Å upon ATP hydrolysis.

Figure 5. ATP hydrolysis in *cis* causes radial expansion of the *trans* ring

(a & b) The inter-ring interface and *trans* ring of the ATP and ADP bound complexes. The *cis* equatorial (Eq) domain, together with the *trans* equatorial, intermediate and apical (Ap) domains are shown as a gray cartoon for both structures. The structural elements involved in communicating nucleotide binding are colored as follows: relay helix D (running from the nucleotide binding site to the Ala109 inter-ring contact) and substrate binding residues are magenta for ATP and blue for ADP. Glu461 at the second inter-ring contact is gold for ATP and orange for ADP. Finally, the β -strand contact in the *trans* equatorial ring is purple for ATP and green for ADP. (c) An overlay of ATP and ADP bound structures. ATP binding causes an offset between the relay helices across the ring interface. After ATP hydrolysis, the relay helices become realigned. The *trans* equatorial domains pivot about the Glu461 inter-ring contact, disrupting the β -strand intra-ring contact. The radial expansion is propagated throughout the *trans* ring, resulting in an opening of the substrate binding cavity.

Figure 6. Movements of key structural elements in the GroEL ATPase cycle

(a) The structures important in the allosteric mechanism are shown superimposed on the shape of an axial section through unliganded GroEL. The hydrophobic substrate-binding sites (gray space filling) line the end cavities in the high affinity state for non-native substrates, and apposed helix D's (gold) form a close contact via Ala109 between the rings. The intra-ring β -strand contacts are shown (red/blue) in the lower ring. (b) GroEL-ATP₇ (EMD1047; Ref. 7). ATP binding to the upper ring causes the whole complex to elongate, with twisting of the apical domains so that the substrate binding sites are partly rotated out of the end cavity. The inter-ring interface is much weaker. (c) GroEL-ATP₇-GroES. Tilt of the equatorial domains in *cis* causes an offset of helix D, thus moving apart the Ala109 residues across the inter-ring contact. The large rotation of the *cis* apical domains moves the substrate binding sites away from the cavity lining. The *trans* ring becomes more closed. (d) GroEL-ADP₇-GroES. Rotation of the *trans* equatorial domains brings the helix D contact back into register but disrupts the intra-ring β -strand contact. The whole *trans* ring expands radially, creating the new substrate acceptor state. (e) ATP₇-GroEL-ADP₇-GroES. At the lower resolution to which this state was captured (EMD1046; Ref. 7), the conformation appears the same as the GroEL-ATP₇-GroES state. The *trans* ring contracts and the intra-ring contact is re-established.

Table 1

Image processing statistics

	GroEL-ADP ₇ -GroES	GroEL-ATP ₇ -GroES
Number of micrographs	66	189
Defocus range used (μm)	1.4 – 3.3	1.1-3.2
Total number of particles in initial selection	10,273	20,666
Number of unique orientations	388	459
Total number of particles in final map	7,591	16,281
Resolution (FSC=0.5)	8.7 Å	7.7 Å
Resolution (3σ)	6.3 Å	4.7 Å