Crystal structure of a symmetric football-shaped GroEL:GroES₂ complex determined at 3.8 Å reveals rearrangement between two GroEL rings

Ayumi Koike-Takeshita^{1,†}, Takatoshi Arakawa^{2,†}, Hideki Taguchi³ and Tatsuro Shimamura^{2,4}

¹ Department of Applied Bioscience, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi, Kanagawa 243-0292, Japan.

² Department of Cell Biology, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-Ku, Kyoto 606-8501, Japan.

³ Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, B-56, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan.

⁴ JST, Research Acceleration Program, Membrane Protein Crystallography Project, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.

[†] A. K.-T. and T. A. contributed equally to this work.

Correspondence to Hideki Taguchi: taguchi@bio.titech.ac.jp and Tatsuro Shimamura: t.shimamura@mfour.med.kyoto-u.ac.jp

Data deposition: The coordinates and structure factors will be deposited in the Protein Data Bank and released on publication.

Abstract

The chaperonin GroEL is an essential chaperone that assists in protein folding with the aid of GroES and ATP. GroEL forms a double ring structure, and both rings can bind GroES in the presence of ATP. Recent progress on the GroEL mechanism has revealed the importance of a symmetric 1:2 GroEL:GroES₂ complex (the “football”-shaped complex) as a critical intermediate during the functional GroEL cycle. We determined the crystal structure of the “football” GroEL:GroES₂ complex from *Escherichia coli* at 3.8 Å, using a GroEL mutant that is extremely defective in ATP hydrolysis. The overall structure of the “football” complex resembled the GroES-bound GroEL-ring of the asymmetric 1:1 GroEL:GroES complex (the “bullet” complex). However, the two GroES-bound GroEL-rings form a modified interface by a ~7 ° rotation about the 7-fold axis. As a result, the inter-ring contacts between the two GroEL rings in the football complex differed from those in the bullet complex. The differences provide a structural basis for the apparently impaired inter-ring negative cooperativity observed in several biochemical analyses.

Highlights:

- GroEL and GroES forms a symmetrical 1:2 GroEL:GroES₂ “football” complex.
- Crystal structure of the symmetrical “ football ” complex from E. coli.
- Unique inter-ring contacts between the two GroEL rings in the football complex.

Keywords: folding; chaperone; chaperonin; nanomachine; inter-ring contacts

Introduction

Chaperonins are a subclass of molecular chaperones capable of assisting protein folding in an ATP-dependent manner [1-3]. They have been divided into two distinct classes: group I chaperonins, such as bacterial GroEL, and group II chaperonins, such as archaeal thermosome [3]. Both chaperonin groups share a similar basic architecture that comprises two cylindrical rings, in which the subunits have three domains: an equatorial ATP-binding domain, an apical domain involved in substrate binding, and an intermediate domain with a hinge region connecting the equatorial and the apical domains [4, 5].

The best characterized chaperonin is *Escherichia coli* GroEL and its partner GroES. GroEL and GroES are the only indispensable chaperones for the viability of *E. coli*, and assist a wide spectrum of substrates in the cell [6, 7]. GroEL consists of two heptameric rings of identical 57-kDa subunits. GroES is a dome-shaped single heptameric ring of identical 10-kDa subunits [4, 8, 9]. ATP binding to the GroEL rings induces the positive cooperative upward movement of the intermediate and apical domains, leading to the formation of a GroEL ring that binds GroES (the *cis*-ring), which has a cavity for the encapsulation of the substrate protein [4]. Since GroEL is a double ring structure stacked back to back, two types of GroEL:GroES complexes are possible: asymmetric 1:1 and symmetric 1:2 GroEL:GroES complexes [10-14]. These are referred to as the “bullet” and “football” for the asymmetric and symmetric forms, respectively, based on their appearances in electron microscopic images [10].

Extensive efforts have been dedicated toward clarifying the roles of the asymmetric and symmetric complexes in the productive GroEL cycle. For years it has been widely accepted that the asymmetric complex is the functional intermediate during the productive GroEL cycle [15, 16]. GroEL alternates the folding-active rings, in which one GroES ring engages one GroEL ring at a time, resulting in the accumulation of the asymmetric “bullet” complex as the predominant species [16]. The origin for the asymmetric complex-based cycling has been explained by the nested allosteric behavior of GroEL: intra-ring positive cooperativity and inter-ring negative cooperativity [17].

However, recent detailed mechanistic analyses have changed the situation

and prompted reconsideration of the GroEL cycle, including the symmetric complex [18-24]. Although both the asymmetric and symmetric cycles are possible, the presence of substrate proteins facilitates the formation of the symmetric football complex [20, 23], suggesting the critical role of the symmetric cycle in the productive protein-folding cycle of GroEL. It would be of interest to know that the inter-ring negative cooperativity is compromised in the symmetric complex. Therefore, a structural characterization of the symmetric complex is necessary to understand the GroEL mechanism.

We now report the crystal structure of the “football” GroEL:GroES₂ complex using a new GroEL mutant that is deficient in ATP hydrolysis. The “football” structure revealed unique features of the inter-ring contacts of GroEL, and thus provides insights into the differences between the symmetric and asymmetric GroEL cycles.

Results

Crystallization using a long-lived symmetric GroEL:GroES₂ complex.

The symmetric GroEL:GroES₂ complex transiently exists in the GroEL ATPase cycle, and decays after ATP hydrolysis [18, 19, 23, 24]. For the crystallization, it was necessary to prepare a long-lived symmetric GroEL:GroES₂ complex that maintains the ATP-bound state for at least more than a few days. We previously demonstrated that an ATPase-defective GroEL mutant in which Asp398 was replaced with Ala (GroEL³⁹⁸) [15], forms the symmetric complex in the presence of ATP and GroES [18], but the half-life of the symmetric complex is around 30 minutes [15, 16, 18], which is not sufficient for crystallization.

We found that Asp52, which is strictly conserved in chaperonin family including group II chaperonins, of the equatorial domain was also critical for the ATP hydrolysis of GroEL¹. Briefly, although ATPase activity of single GroEL mutant, GroEL⁵², is ~20% of wild type GroEL, that of a double mutant, in which both Asp52 and Asp398 are replaced with Ala (GroEL^{52/398}), is lower than that of GroEL³⁹⁸; less than 0.1% of that of wild-type GroEL. As expected, in the presence of GroES and

¹ Manuscript on the biochemical characterization of GroEL⁵² and GroEL^{52/398} has been submitted and is under revision.

ATP, GroEL^{52/398} forms a very stable symmetric GroEL:GroES₂ complex, in which denatured rhodanese can fold to the native state as wild-type GroEL or GroEL³⁹⁸, indicating that the function of GroEL^{52/398} to encapsulate the substrate proteins is normal. Importantly, GroEL^{52/398} retains the football structure with a half-life of ~150 h (~6 days). The long-lived symmetric complex allowed us to crystallize the symmetric football complex.

Overall structure.

The overall structure of the symmetric GroEL^{52/398}:GroES₂ complex is presented in **Fig. 1**. The complex shows the “football”-like shape as observed in the EM images (*e.g.* [25]). The football structure is formed by the back-to-back arrangement of two GroES-bound *cis*-rings, with the longest dimensions of 240 Å (along the 7-fold axis) by 140 Å (normal to the 7-fold axis). The football structure binds 14 ATP molecules at the nucleotide binding sites, where the γ -phosphate binds at the N-terminal end of helix D (residues 89-109) (**Supplementary Fig. 1**) (*see Methods*). The conformations of the two GroES-bound *cis*-rings are similar with root-mean-square deviations (rmsd) of the Ca atoms of 0.6 Å (**Supplementary Fig. 2**). The structures of the GroES subunit and the three domains in the GroEL subunit are similar, with small rmsd values of 0.2-0.8 Å (**Supplementary Fig. 3a,b**). The rmsd value for the GroEL subunit is slightly larger (rmsd 2.0 Å), because there are several variations in the twist angles between the apical domain and the intermediate domain in the GroEL subunit (**Supplementary Fig. 3c**). As compared with the GroES-bound *cis*-ring in the bullet complex structure (PDB code:1PCQ, 1PF9, 1AON) [4, 9], no significant structural differences were observed in the GroEL and GroES subunits (**Supplementary Fig. 4**). It should be noted the equatorial regions of the *cis*-rings in both the football and bullet structures also are similar with an rmsd of 1.1 Å.

Inter-ring contacts.

In the bullet structure, the *en bloc* inward tilt of the *cis*-ring and the complementary outward tilt of the *trans*-ring were observed, as compared with unliganded GroEL [4]. This type of complementary tilt cannot be attained at the

interface of the two symmetric GroES-bound *cis*-rings in the football structure. Instead, the two *cis*-rings form a modified interface by a $\sim 7^\circ$ rotation about the 7-fold axis (**Fig. 2a**). As a consequence, the inter-ring contact surface area ($2208 \text{ \AA}^2$) became smaller than that of the bullet structure ($2856 \text{ \AA}^2$) (**Supplementary Table 1**). In the football structure, one GroEL subunit in one ring interacts with two neighboring GroEL subunits in the opposite ring at “*Left*” and “*Right*” sites (**Fig. 2b**), as also seen in the GroEL structures [4, 9]. In the bullet structure (1PCQ) [9], the “*Right*” site contains two Arg452-Glu461 salt bridges and van der Waals contacts between Ser463, Val464 and Asn467 on the two adjacent Q helices (residues 462-471). In the football structure, the Arg452, Glu461 Ser463, Val464 and Asn467 residues are suitably located to form symmetric contacts similar to those observed in the bullet structure (**Fig. 2c and Supplementary Fig. 5**). Indeed, the contact surface area for “*Right*” site ($195 \text{ \AA}^2$) is similar to that of the bullet structure ($198 \text{ \AA}^2$) (**Supplementary Table 1**). The “*Left*” site in the football structure was more affected by the $\sim 7^\circ$ rotation, as compared with that in the bullet structure, with the reduction of the contact surface area from $210 \text{ \AA}^2$ to $121 \text{ \AA}^2$ (**Supplementary Table 1**). The bullet structure forms a van der Waals contacts between two D helices. Lys105, Ala108 and Ala109 in the *cis*-ring, and Ala109, Gly110 and Met111 in the *trans*-ring mainly contribute to the contacts. Notably, helix D forms the ATP binding site at its N-terminus and is supposed to be essential for the negative cooperativity between the two GroEL rings [26]. Additionally, the bullet structure contains a salt bridge between Arg445 in the *cis*-ring and Glu434 in the *trans*-ring. In the football structure, the contact pattern between the two D helices was modified by the $\sim 7^\circ$ rotation and only Ala108 and Ala109 are involved in the contacts (**Fig. 2c and Supplementary Fig. 6**). As a result, the contact surface area around the C terminus of helix D decreased to $\sim 60\%$ in the football structure ($73 \text{ \AA}^2$ and $122 \text{ \AA}^2$ for football and bullet structures, respectively) (**Supplementary Table 1**). In addition, the two A helices (residues 10-28) form symmetric contacts at Asn10 and Val14. The football structure lacks the salt bridge between Arg445 and Glu434 observed in the bullet structure (**Fig. 2d**), since the distance between these residues is too far for salt bridge formation.

Equatorial split of the symmetric complex in native gel electrophoresis.

The altered inter-GroEL ring contacts in the football structure reminded us of a preliminary biochemical observation of the quaternary structure change of the GroEL:GroES complex in native gel electrophoresis. As shown in **Fig. 3**, the GroEL^{52/398} tetradecamer dissociated in the presence of ATP and GroES in a native gel. The dissociated GroEL accumulated at a position around a single-ring GroEL variant, suggesting that the dissociation can be interpreted as a split at the plane between two GroEL rings, which was previously demonstrated for the GroEL from *Thermus thermophilus* [27]. A similar, albeit inefficient, dissociation reaction was also observed with GroEL³⁹⁸ and GroEL⁵² (**Fig. 3**). Strikingly, GroEL^{WT} also dissociated in the presence of GroES, ATP and fluoroberyllate (BeFx) in the native electrophoresis (**Supplementary Fig. 7**), indicating that the dissociation was not attributed to a special feature of GroEL^{52/398}. Rather, all of the conditions where we observed the dissociation of GroEL rings were common to the formation of the symmetric complex. We note that we did not observe the split *E. coli* GroEL ring in electron microscopy or gel filtration analysis under the same conditions, suggesting that the split phenomenon was caused by the electrophoretic conditions.

Discussion

The existence of the symmetric “football” GroEL:GroES₂ complex was well recognized, even in the early stages of GroEL studies (*e.g.* [10-12]). However, its transient nature as an intermediate during the ATPase cycle of GroEL has made it difficult not only to characterize the importance of the complex in the GroEL function, but also to prepare the homogeneous population for detailed structural analyses. In this report, we solved the crystal structure of the symmetric GroEL:GroES₂ complex using an extremely ATPase defective GroEL mutant, GroEL^{52/398}.

In the GroEL cycle, the negative cooperativity is proposed to be transmitted through the contacts between two D helices [26]. For the formation of the 14 ATP-bound football complex, the negative cooperativity should be reduced. The present crystal structure of the symmetric complex provides a structural basis for the impaired inter-ring negative cooperativity. As a result of the ~7 ° rotation about the 7-fold axis, the two helices D in the football structure are skewed, whereas the two

helices are arranged nearly antiparallel (**Supplementary Fig. 6**). By the skew, the contacts of two helices D are formed only at the C terminus of the helix in the football structure and the contact surface area are decreased to ~60% compared with the bullet structure. The decreased contact surface area would be too small for the efficient transmission of the inter-ring negative cooperativity, allowing the formation of the football complex. In addition, the lack of the Arg445-Glu434 salt bridge in the football structure would also contribute to the disturbance of the negative cooperativity.

Although the symmetric structure shown here was solved with an ATPase-defective GroEL mutant, GroEL^{52/398}, to prepare the intermediate species during the ATPase cycle, the structure provides insights into the productive wild-type GroEL cycling [19, 21, 22]. A single-molecule study on the decay process of the football complex to the bullet complex, during GroEL^{WT} cycling in the presence of ATP, revealed that the initially bound GroES does not always dissociate from the football complex prior to the dissociation of the second GroES, *i.e.* the dissociation of GroES from the football can occur in a random order [21]. The random order of GroES dissociation cannot be explained by the prevailing GroEL cycling model, in which GroEL alternates the folding-active rings, suggesting an impaired inter-ring negative cooperativity during the GroEL cycle including the football complex. Therefore, the football structure shown here is consistent with the random order cycling revealed by the single-molecule study.

We showed an equatorial split of the GroEL rings under the conditions where GroEL forms the symmetric complex (**Fig. 3** and **Supplementary Fig. 7**). This split would be attributed to weakened ring-ring interactions in the football complex that has smaller contact surface area and lacks a salt bridge at the inter-ring interface compared with the bullet structure.. Since the split was not observed in our electron microscopic or gel filtration analyses, the split might be exaggerated under electrophoretic conditions. However, the split phenomenon would be related, at least in part, to several previous observations on the quaternary structure changes of group I chaperonins including *E. coli* GroEL. First, we previously showed that the incubation of *E. coli* (*Ec*) GroEL and *T. thermophilus* (*Tth*) GroEL in the presence of ATP forms a hybrid between *Ec* GroEL₇:*Tth* GroEL₇ [28]. A hybrid between *Ec*

GroEL at 42 °C was also reported [29]. These observations suggested that *Ec* GroEL should split during the ATPase cycle. Second, Llorca *et al.* reported that ~10 % of the GroEL under heat shock conditions exists in the form of single rings, as observed by electron microscopy [30]. Importantly, they also showed that the dissociation of GroES was inhibited at high temperatures, suggesting that the negative cooperativity between the two GroEL rings gradually diminishes as the temperature increases. Finally, some group I chaperonins, such as mitochondrial Hsp60 and *Thermoanaerobacter brockii* (*Tbr*) GroEL, were purified as a single ring forms [31, 32]. Importantly, *Tbr* GroEL₇ dimerizes to the double ring form, *Tbr* GroEL₁₄, in the presence of ATP and the cochaperonin *Tbr* GroES [32]. Taken together, the next challenge to elucidate in the GroEL cycling is the incorporation of single-ring GroEL, after the split from the football complex, which was previously suggested by Todd *et al.* [32, 33].

Although the recent re-emergence of the football complex has focused attention on the critical role of the symmetric complex during the GroEL cycle, the bullet and football models are not mutually exclusive. Rather, GroEL would switch the cycles according to demand, as shown by previous studies in which the substrate proteins facilitated the formation of the football complex [20, 23].

Materials and Methods

Reagents and proteins.

GroEL^{WT}, GroEL mutants, and GroES were purified as previously described [34].

Crystallization.

The GroEL:(GroES)₂-ATP₁₄ complex was formed in HKM buffer (20 mM HEPES-KOH, (pH 7.4), 100 mM KCl, and 5 mM MgCl₂) with 1 mM ATP by mixing separately purified GroEL^{52/398} and GroES, at concentrations of 22.5 mg/ml (28 μM) and 5.1 mg/ml (70 μM), respectively. Protein concentrations were determined by the bicinchoninic acid assay (Pierce). Prior to crystallization, the unbound GroES was removed by washing repeatedly with the same buffer, using a ultrafiltration device (Amicon Ultra MWCO 100kDa; Millipore). The complex was concentrated to 20 mg/ml and subjected to crystallization. Crystals were grown by the hanging drop vapor diffusion method, by mixing 1.5 μl of protein sample with 1.5 μl of reservoir solution and equilibrating the mixture at 293 K. Hexahedral-shaped crystals appeared within 2 days, in the reservoir solution composed of 0.1 M HEPES-NaCl (pH 7.5), 35% PEG 550 MME, and 1 mM ATP. Crystals with typical dimensions of 0.15 x 0.15 x 0.4 mm were harvested within 6 days and immediately frozen in liquid N₂.

Data collection and refinement.

Diffraction data were collected at 100 K on beamline BL32XU at SPring-8. The crystal belonged to the space group *P*2₁ with one football-shaped molecule in the asymmetric unit. The data were integrated and scaled to 3.774 Å resolution with the HKL2000 program suite [35]. The structure was determined by molecular replacement with the program PHASER [36], using the GroES-bound *cis*-ring of the bullet-shaped GroEL-GroES-ADP/AlF₃ structure (PDB code:1PCQ) [9] as a search model. Two repetitive searches resulted in covering the asymmetric unit with the models with two *cis*-rings placed in back-to-back locations (**Supplementary Fig. 8**). The refinement processes were mostly performed by the program phenix.refine in the PHENIX software suite [37]. Considering that there are two- and seven-fold non-crystallographic symmetries (NCS) according to an estimation of the self-rotation function calculated by the program MOLREP [38], NCS positional restraints for the

GroEL and GroES subunits were applied in the initial rounds, so that the 7-fold symmetry was maintained. In the latter rounds of refinement, the positional restraints were released and the torsion NCS was applied. The three (apical, intermediate, equatorial) domains of the GroEL subunit and the GroES subunit were independently defined as a TLS group during the whole refinement process. The refinement was concluded when R_{free} was minimized without the divergence of the R_{work} value. Strong electron density (5 sigma) was observed in the nucleotide-binding pocket of each GroEL subunit in the *Fo-Fc* omit map (**Supplementary Fig. 1**). Since strongly positive residual *Fo-Fc* density appeared when we modeled an ADP in the pocket, we modeled an ATP in the density. In addition, two strong and spherical electron densities were detected near the ATP molecule in the *Fo-Fc* omit map (**Supplementary Fig. 1**). We modeled them as K^+ and Mg^{2+} ions because the positions of the densities are similar to the positions of these ions at the nucleotide binding site in the bullet structure [4]. The N-terminal first residue, the C-terminal 21 residues of GroEL, and the C-terminal 36 residues of GroES were not included in the model, as they lacked interpretable densities. The final model contains 2 GroES molecules (14 subunits), 2 GroEL molecules (14 subunits), 14 ATP molecules, 14 Mg^{2+} ions and 14 K^+ ions per chaperonin molecule. Statistics for data collection and refinement are provided in **Table 1**. Coordination of the structure was verified using the programs Molprobity [39] and Polygon. Molecular graphic images were prepared using the program PyMOL (version 0.99rc6, Schrödinger, LLC). Superposition of molecules and rmsd calculations were performed with the CEAlign plugin command of PyMOL.

Accession numbers

The atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession number 3WVL.

Acknowledgements

We thank S. Iwata for supporting the project. We thank the beamline staffs of BL32XU at SPring-8 for the X-ray data collection. The experiments were performed with the approval numbers of SPring-8 (2011B1136, 2012A1472, 2013A1379, 2013B1184). This work was supported by Grants-in-Aid for Scientific Research (20870035 to A. K-T., 24370044 and 26102725 to T.S., and 19058002 and 17049009 to H.T.) from JSPS and MEXT, Japan. This work was also supported by a grant of Takeda Science Foundation (to H.T.), Uehara Memorial Foundation (to H.T.), National Project on Protein Structural and Functional Analyses from MEXT (to T.S.) and Research Acceleration Program of JST (to T.S.).

References

- [1] Thirumalai D, Lorimer GH. Chaperonin-mediated protein folding. *Annu Rev Biophys Biomol Struct.* 2001;30:245-269.
- [2] Taguchi H. Chaperonin GroEL meets the substrate protein as a "load" of the rings. *J Biochem (Tokyo).* 2005;137:543-9.
- [3] Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU. Molecular chaperone functions in protein folding and proteostasis. *Annu Rev Biochem.* 2013;82:323-55.
- [4] Xu Z, Horwich AL, Sigler PB. The crystal structure of the asymmetric GroEL-GroES-(ADP)₇ chaperonin complex. *Nature.* 1997;388:741-750.
- [5] Ditzel L, Lowe J, Stock D, Stetter KO, Huber H, Huber R, et al. Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. *Cell.* 1998;93:125-38.
- [6] Kerner MJ, Naylor DJ, Ishihama Y, Maier T, Chang HC, Stines AP, et al. Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. *Cell.* 2005;122:209-20.
- [7] Fujiwara K, Ishihama Y, Nakahigashi K, Soga T, Taguchi H. A systematic survey of in vivo obligate chaperonin-dependent substrates. *EMBO J.* 2010;29:1552-64.
- [8] Hunt JF, Weaver AJ, Landry SJ, Gierasch L, Deisenhofer J. The crystal structure of the GroES co-chaperonin at 2.8Å resolution. *Nature.* 1996;379:37-45.
- [9] Chaudhry C, Farr GW, Todd MJ, Rye HS, Brunger AT, Adams PD, et al. Role of the gamma-phosphate of ATP in triggering protein folding by GroEL-GroES: function, structure and energetics. *EMBO J.* 2003;22:4877-87.
- [10] Taguchi H, Konishi J, Ishii N, Yoshida M. A chaperonin from a thermophilic bacterium, *Thermus thermophilus*, that controls refolding of several thermophilic enzymes. *J Biol Chem.* 1991;266:22411-22418.
- [11] Azem A, Kessel M, Goloubinoff P. Characterization of a functional GroEL14(GroES7)₂ chaperonin hetero-oligomer. *Science.* 1994;265:653-6.
- [12] Schmidt M, Rutkat K, Rachel R, Pfeifer G, Jaenicke R, Viitanen P, et al. Symmetric Complexes of GroE Chaperonins as Part of the Functional Cycle. *Science.* 1994;265:656-659.

- [13] Sparrer H, Rutkat K, Buchner J. Catalysis of protein folding by symmetric chaperone complexes. *Proc Natl Acad Sci U S A*. 1997;94:1096-100.
- [14] Taguchi H, Tsukuda K, Motojima F, Koike-Takeshita A, Yoshida M. BeF(x) stops the chaperonin cycle of GroEL-GroES and generates a complex with double folding chambers. *J Biol Chem*. 2004;279:45737-43.
- [15] Rye HS, Burston SG, Fenton WA, Beechem JM, Xu Z, Sigler PB, et al. Distinct actions of *cis* and *trans* ATP within the double ring of the chaperonin GroEL. *Nature*. 1997;388:792-798.
- [16] Rye HS, Roseman AM, Chen S, Furtak K, Fenton WA, Saibil HR, et al. GroEL-GroES cycling: ATP and nonnative polypeptide direct alternation of folding-active rings. *Cell*. 1999;97:325-338.
- [17] Horovitz A, Willison KR. Allosteric regulation of chaperonins. *Curr Opin Struct Biol*. 2005;15:646-51.
- [18] Koike-Takeshita A, Yoshida M, Taguchi H. Revisiting the GroEL-GroES reaction cycle via the symmetric intermediate implied by novel aspects of the GroEL(D398A) mutant. *J Biol Chem*. 2008;283:23774-81.
- [19] Sameshima T, Ueno T, Iizuka R, Ishii N, Terada N, Okabe K, et al. Football- and bullet-shaped GroEL-GroES complexes coexist during the reaction cycle. *J Biol Chem*. 2008;283:23765-73.
- [20] Sameshima T, Iizuka R, Ueno T, Funatsu T. Denatured proteins facilitate the formation of the football-shaped GroEL-(GroES)₂ complex. *Biochem J*. 2010;427:247-54.
- [21] Sameshima T, Iizuka R, Ueno T, Wada J, Aoki M, Shimamoto N, et al. Single-molecule study on the decay process of the football-shaped GroEL-GroES complex using zero-mode waveguides. *J Biol Chem*. 2010;285:23159-64.
- [22] Takei Y, Iizuka R, Ueno T, Funatsu T. Single-molecule observation of protein folding in symmetric GroEL-(GroES)₂ complexes. *J Biol Chem*. 2012;287:41118-25.
- [23] Ye X, Lorimer GH. Substrate protein switches GroE chaperonins from asymmetric to symmetric cycling by catalyzing nucleotide exchange. *Proc Natl Acad Sci U S A*. 2013;110:E4289-97.
- [24] Yang D, Ye X, Lorimer GH. Symmetric GroEL:GroES₂ complexes are the protein-folding functional form of the chaperonin nanomachine. *Proc Natl Acad Sci U S A*. 2013;110:E4298-305.
- [25] Grallert H, Buchner J. Review: a structural view of the GroE chaperone cycle. *J Struct Biol*. 2001;135:95-103.
- [26] Roseman AM, Chen S, White H, Braig K, Saibil HR. The chaperonin ATPase cycle: mechanism of allosteric switching and movements of substrate-binding domains in GroEL. *Cell*. 1996;87:241-251.
- [27] Ishii N, Taguchi H, Sasabe H, Yoshida M. Equatorial split of holo-chaperonin from *Thermus thermophilus* by ATP and K⁺. *FEBS Lett*. 1995;362:121-125.
- [28] Taguchi H, Amada K, Murai N, Yamakoshi M, Yoshida M. ATP-, K⁺-dependent heptamer exchange reaction produces hybrids between GroEL and chaperonin from *Thermus thermophilus*. *J Biol Chem*. 1997;272:18155-60.
- [29] Burston SG, Weissman JS, Farr GW, Fenton WA, Horwich AL. Release of both native and non-native proteins from a *cis*-only GroEL ternary complex. *Nature*. 1996;383:96-99.

- [30] Llorca O, Galan A, Carrascosa JL, Muga A, Valpuesta JM. GroEL under heat-shock. Switching from a folding to a storing function. *J Biol Chem.* 1998;273:32587-94.
- [31] Viitanen PV, Lorimer GH, Seetharam R, Gupta RS, Oppenheim J, Thomas JO, et al. Mammalian mitochondrial chaperonin 60 functions as a single toroidal ring. *J Biol Chem.* 1992;267:695-698.
- [32] Todd MJ, Walke S, Lorimer G, Truscott K, Scopes RK. The single-ring *Thermoanaerobacter brockii* chaperonin 60 (Tbr-EL7) dimerizes to Tbr-EL14.Tbr-ES7 under protein folding conditions. *Biochemistry.* 1995;34:14932-14941.
- [33] Ishii N, Sato T. Anisotropic intersubunit and inter-ring interactions revealed in the native bullet-shaped chaperonin complex from *Thermus thermophilus*. *Biochim Biophys Acta.* 2013;1830:2907-16.
- [34] Motojima F, Makio T, Aoki K, Makino Y, Kuwajima K, Yoshida M. Hydrophilic residues at the apical domain of GroEL contribute to GroES binding but attenuate polypeptide binding. *BiochemBiophysResCommun.* 2000;267:842-849.
- [35] Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods in Enzymology.* 1997;276:307-326.
- [36] McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. Phaser crystallographic software. *J Appl Crystallogr.* 2007;40:658-674.
- [37] Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr.* 2010;66:213-21.
- [38] Vagin A, Teplyakov A. An approach to multi-copy search in molecular replacement. *Acta Crystallogr D Biol Crystallogr.* 2000;56:1622-4.
- [39] Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr.* 2010;66:12-21.

Figure Legends

Figure 1. Overall structure of the symmetric *E. coli* [GroEL^{52/398}:GroES₂] complex. (a) Representations of Ca traces are colored separately by chain. The $2F_o - F_c$ electron density map (1 σ contour) is depicted by a *blue* mesh. (b) Central side view slice through GroEL:GroES:ATP₁₄. The atomic coordinates of the subunits that form the walls of the double chamber are overlaid on the molecular surface section (*cyan*). Ribbon representations are colored according to the subdomain architecture (GroEL equatorial, intermediate and apical domains are colored *blue*, *green* and *pink*, and GroES is in *yellow*). Signature helices of GroEL (H: residues 234-241, I: 256-268 and M: 387-409) are highlighted in *red*, *orange* and *cyan*, respectively.

Figure 2. Inter-ring contacts. (a) Clockwise rotation of the GroEL ring. Top view of the equatorial domains in the opposite ring, viewed from the superposed *cis*-rings. The panel displays cylinder models of the football structure (*magenta*) overlaid on the ADP-AlF₃-bound bullet structure (1PCQ [9]; *cyan*). The ring of the football structure is rotated clockwise around the 7-fold symmetry axis of the molecule, as indicated. (b) Molecular surface of the football structure highlighting one subunit (*green*) interacting with two adjacent subunits in the opposite ring (*magenta* and *orange*) at the “Left” and “Right” sites. (c) A close-up view of the “Left” and “Right” sites. The view is from the outer periphery of the rings. The residues located within 4 Å from the opposite ring are depicted as sticks. (d) A schematic representation of the three equatorial domains (*blue* rectangles) involved in the inter-ring contacts viewed from the outside of the rings. Arginines, Glutamates and residues in van der Waals contacts are shown as *blue*, *red* and *grey* boxes, respectively.

Figure 3. Equatorial split of GroEL mutants in native gel electrophoresis. Blue-native PAGE analysis of GroEL^{WT} and GroEL mutants (GroEL^{52/398}, GroEL³⁹⁸ and GroEL⁵²)-GroES complexes formed in the absence or the presence of ATP. GroEL and GroES were mixed at 1:4 ratio in HKM buffer containing 1 mM dithiothreitol and, where indicated, 1 mM ATP. After 5 minutes, the mixtures were loaded on a 3-12% Bis-Tris Protein Gels (Invitrogen). For comparison, single-ring GroEL (SR, *lane 1*)

was also analyzed.

Table 1. Data collection and refinement statistics

GroEL^{52/398}-GroES (PDB ID: 3WVL)	
Data collection	
Space group	$P 2_1$
Cell dimensions	
a, b, c (Å)	160.28, 217.12, 198.48
β (°)	98.02
Resolution (Å)	49.6 - 3.8 (3.9 - 3.8) *
R_{merge}	0.126(0.503)
$I / \sigma I$	7.7 (2.0)
Completeness (%)	95.7 (93.4)
Redundancy	2.6 (2.3)
Refinement	
Resolution (Å)	49.6 - 3.8 (3.9 - 3.8)
No. reflections	122258
$R_{\text{work}} / R_{\text{free}}$	0.220 (0.289) / 0.247 (0.305)
No. atoms	
Protein	64078
Ligand/ion	462
B -factors	
Protein	180.5
Ligand/ion	118.7
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.76
Ramachandran plot	
Favored (%)	94.6
Outliers (%)	0.91

*Values in parentheses are for highest-resolution shell.

a

b

Fig. 1

Fig. 2

Fig. 3

Graphical Abstract

a

b