Supporting Information

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SI Materials and Methods

Construction of GroEL$^{D83A/R197A}$. The double mutant D83A/R197A was constructed on the plasmid pGEL1 (gift from E. Eisenstein, Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Shady Grove, MD). Mutagenesis was done with the Stratagene QuikChange Kit according to the supplied protocol using the following PAGE-purified primers from Qiagen: D83A (GCCTCTAAAGCAAACGCAGCTGCAGGCGACGGTACC), R197A (GGTATGCAGTTCGACGCTGGCTACCTGTCTCCTTAC), and their reverse compliment (Integrated DNA Technology). For FRET analysis, a third mutation E315C was inserted into the plasmid coding both WT GroEL and GroEL$^{D83A/R197A}$ using the primer (GCTGGAAAAAGCAACCCTGTGCGACCTAGGTCAGGCTAAACG). All mutagenesis was confirmed by sequencing.

Purification of GroEL^{WT}, GroEL^{E315C}, GroEL$^{D83A/R197A}$, GroEL$^{D83A/R197A/E315C}$, GroES^{WT}, and GroES^{98C}. These proteins were prepared, purified, and labeled as previously described (1). Typically, GroEL preparations contained <0.2 mol contaminating substrate protein per $GroEL_{14}$ (i.e., <10% of the rings may be contaminated with an ensemble of substrate proteins). The concentration of purified GroEL was measured at 280 nm using the extinction coefficient of 9,600 $cm^{-1}M^{-1}$.

Purification and Labeling of Phosphate Binding Protein A197C. Phosphate binding protein was purified and labeled with N-[2-(1-maleimidyl)ethyl]-7-(diethylamino)coumarin-3-carboxamide as previously described (2).

Steady State and Pre-Steady State Measurements of ATP Hydrolysis. The steady state hydrolysis of ATP was measured at 37 °C as previously described (3). The presteady state measurements were carried out on an Applied Photophysics SX18MV-R stopped flow apparatus at 37 °C as previously described (3).

Labeling GroEL^{E315C} and GroES^{98C}. A slight modification of the method described by Rye et al. (4) was used to label GroEL^{E315C} with IAEDANS (5-((((2-iodoacetyl)amino)ethyl)amino) naphthalene-1-sulfonic acid) and GroES^{98C} with fluorescein-5-maleimide. GroEL^{E315C} labeled to varying extents (40–90%) with IAEDANS was used.

Stopped Flow Measurements of FRET. All FRET measurements using GroEL^{E315C}, GroEL$^{D83A/R197A/E315C}$, and GroES^{98C} were conducted at 37 °C as previously described (1).

Preparation of Unfolded α-Lactalbumin. Unfolded α-lactalbumin was prepared as described (5).

Structure Analysis. ***Crystallization.*** The relaxed **R-ADP** state GroEL was made by mixing 190 μM GroEL$^{D83A/R197A}$ and 5 mM ATP in 50:20:200 buffer (50 mM Tris·acetate, pH 7.5, 20 mM $MgCl_2$, 200 mM KCl). GroEL and ATP mixture were incubated at room temperature until ATP was all converted to ADP. The **R-ADP** samples were screened for crystallization condition by using the sitting drop vapor diffusion method (drop: 0.5 μL; well solution: 100 μL). Diffraction quality crystals were grown at the screen condition with well solution that consists of 34% MPD (2-methyl 2,4-pentanediol) (vol/vol), 0.1 M acetic acid (pH 5.5) and 20 mM $CaCl_2$. Crystals were optimized by the hanging drop vapor diffusion method (drop: 6 μL; well solution: 1 mL). Cube-shaped crystals began to show up after 3–4 d in drops containing a 2:1 mixture of protein sample and well solution; 1 wk after the crystallization trays were set up, single crystals were removed from mother liquor and soaked in well solution for 5 s before being mounted and flash-frozen by liquid nitrogen.

Data collection and structural determination. Diffraction data were collected at the beamline 22-ID located at the Advanced Photon Source, Argonne National Laboratory; 300 frames with 0.5° oscillation were collected at 100 K. The data were indexed and integrated using HKL2000 (6). The structure of the **R-ADP** was solved by segmented molecular replacement using apo GroEL [Protein Data Bank (PDB) ID code 1XCK] as the search model. Three domains in the apo GroEL were dissected and fitted sequentially. The first search ensemble consisted of seven equatorial domains and seven intermediate domains. Then, search ensembles 2–8 were added one at a time, and each ensemble contained one apical domain. In structure refinement, noncrystallographic symmetry was turned off, and each subunit was refined independently. Molecular replacement and structure refinement were carried out using AutoMR, Refine, and Coot in Phenix suites (7). The coordinates after refinement were deposited in the PDB as ID code 4KI8.

Structure details. Two heptametrical rings in the **R-ADP** are related by crystallographic symmetry, and the asymmetrical unit contains one of two rings. Electron density is weak from residue 221 to 226 in chain C and chain G because of high flexibility in those regions. Data collection and refinement statistics are show in Table S1. The figures that depict structures of the **R-ADP** were prepared using Pymol. The signature plots of R, H, and θ were prepared using Prism.

Conformational Analysis of GroEL-ATPγS$_{14}$. We quantified the difference between the **R-ADP** and GroEL-ATPγS_{14} (7), which was considered to be in the R state, using ΔR and ΔH as described in the text. Although the contacts between nucleotides and nucleotide binding pocket are almost identical in these two structures (Fig. S1), the intermediate and apical domains of the two have distinctive conformations (Fig. S2, dashed pink and cyan lines). The difference between the **R-ADP** and GroEL-ATPγS_{14} may arise from differences in crystallization methods. Although we cocrystallize ADP and GroEL, ATPγS was soaked after the crystal had formed, and lattice forces restrained any nucleotide-induced large conformational rearrangements. Therefore, GroEL-ATPγS_{14} is almost identical to the nucleotide-free taut (**T**) state, with a small difference in the height of apical domain (~2 Å) (Fig. S2, solid red and blue lines).

1. Grason JP, Gresham JS, Widjaja L, Wehri SC, Lorimer GH (2008) Setting the chaperonin timer: The effects of K^+ and substrate protein on ATP hydrolysis. *Proc Natl Acad Sci USA* 105(45): 17334–17338.
2. Brune M, et al. (1998) Mechanism of inorganic phosphate interaction with phosphate binding protein from Escherichia coli. *Biochemistry* 37(29):10370–10380.
3. Grason JP, Gresham JS, Lorimer GH (2008) Setting the chaperonin timer: A two-stroke, two-speed, protein machine. *Proc Natl Acad Sci USA* 105(45):17339–17344.
4. Rye HS, et al. (1999) GroEL-GroES cycling: ATP and nonnative polypeptide direct alternation of folding-active rings. *Cell* 97(3):325–338.
5. Yifrach O, Horovitz A (1996) Allosteric control by ATP of non-folded protein binding to GroEL. *J Mol Biol* 255(3):356–361.
6. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
7. Adams PD, et al. (2010) PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66(Pt 2):213–221.

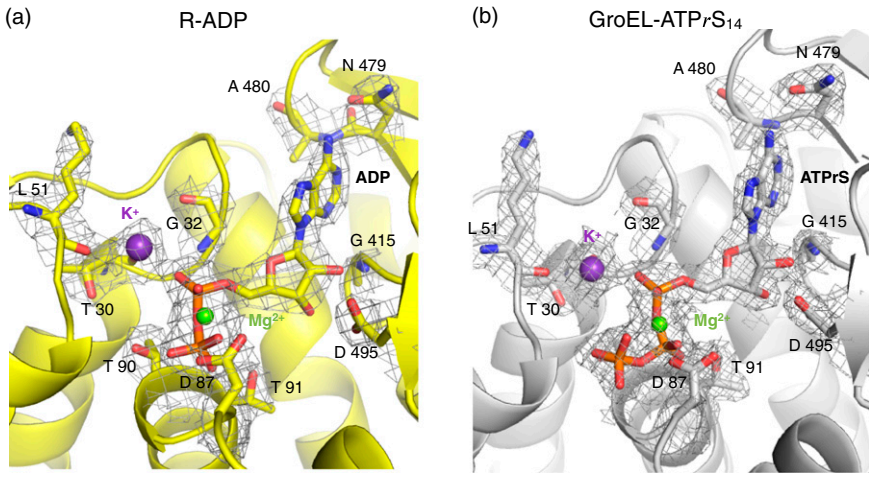

Fig. S1. Contact between nucleotides and the nucleotide binding residues in (*A*) **R-ADP** (yellow) and (*B*) GroEL-ATPγS_{14} (gray; PDB ID code 1KP8). Despite the difference in the nucleotide (KMgADP or KMgATPγS), the nucleotide binding residues (shown as sticks) in two structures adopt very similar conformations. The electron density maps (2Fo-Fc) are contoured at 1.5 σ.

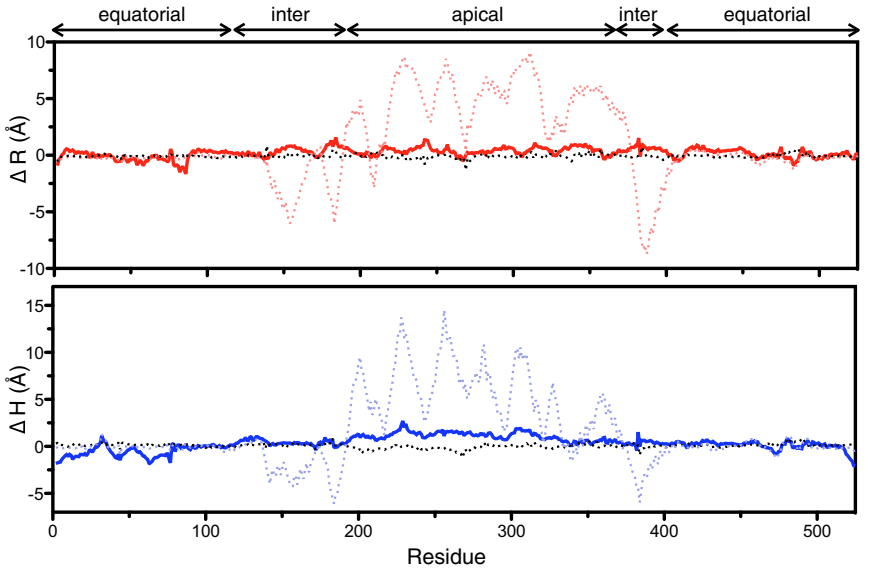

Fig. S2. Conformation differences between GroEL-ATPγS_{14} and the **T** or **R-ADP** state are analyzed in similar ways as describe in the text. The conformational difference in each residue is quantified by (*Upper*) ΔR and (*Lower*) ΔH. $\Delta R = \langle R \rangle - \langle R' \rangle$, where R is the distance to the sevenfold axle in GroEL-ATPγS_{14}, R' is the corresponding distance in the **T** (red solid trace; PDB ID code 1XCK) or **R-ADP** (pink dashed trace) state, and <> denotes average over seven subunits. Similarly, $\Delta H = \langle H \rangle - \langle H' \rangle$, where H is the height in GroEL-ATPγS_{14} and H' is the corresponding height in a known state (**T** in blue solid trace and **R-ADP** in cyan dashed trace). ΔR and ΔH between two **T** state structures (black dashed lines; PDB ID codes 1XCK and 2NWC) serve as the negative controls, showing that two crystal structures of the same conformational state of GroEL have only minor differences.

Table S1. Data collection and refinement statistics

Space group	C121
Unit cell *a*/*b*/*c* (Å)	235.222/141.655/156.693
Resolution range (Å)	46.17–2.722 (2.819–2.722)
Rmerge (%)	5.2 (41.1)
Total number of reflections	120,262 (10,710)
Redundancy	3.1 (3.1)
Completeness (%)	95.62 (85.28)
I/r (I)	13.98 (3.13)
No. of residues/asymmetry unit	3,668
No. of protein atoms	27,012
No. of ligand atoms	368
No. of water atoms	597
R (%)	0.1663 (0.2310)
Rfree (%)	0.2030 (0.2834)
Test set size (%), selection	5, radom
rmsd	
Bond lengths (Å)	0.005
Bond angles (°)	0.95
Ramachandran plot	
Most favored (%)	97.1
Allowed (%)	2.71
Outlier (%)	0.11
Average B factors (Å^2)	
Protein	88.2
Ligand	81.8
Water	63.2

Table S2. Domain averaged B factors for the T (PDB ID code 1XCK), R-ADP, and R″ (PDB ID code 1AON) states

	R-ADP	T	R″
Equatorial	41.4	29.2	22.5
Intermediate	85.0	42.7	55.9
Apical	131.2	60.5	98.9

Table S3. Possible intersubunit salt bridges in the R-ADP and T (PDB ID code 1XCK) states

	Each interface in R-ADP								
	AB	BC	CD	DE	EF	FG	GA	R-ADP average	T
Arg197-Cβ Glu386-Cδ	16.6	17.3	20.4	16.8	24.2	20.4	17.3	19.0	4.6*
Lys80-Nζ Glu386-Cδ	12.2	13.6	14.8	16.9	19.7	13.2	13.7	14.9	17.8
Glu255-Cδ Lys207-Nζ	17.3	17.2	28.8	17.7	28.6	22.9	12.8	20.8	4.0*
Glu255-Cδ Lys245-Nζ	4.2*	7.7	16.7	10.8	22.9	6.3†	12.1	11.5	16.4
Glu257-Cδ Lys245-Nζ	14.7	9.8	12.6	9.1	25.1	14.6	20.8	15.2	17.5

Residues were too far to interact, except where noted.
*Possible strong salt bridge.
†Possible weak salt bridge.