Electronic Supplementary Information (ESI) for

Chaperonin GroEL hydrolyses ortho-nitrophenyl *β*-galactoside

P. K. Hashim,[a,b]* Hisham M. Dokainish,[c] and Nobuyuki Tamaoki[a,b]

a. *Research Institute for Electronic Science, Hokkaido University, Kita 20, Nishi 10, Kita-Ku, Sapporo, Hokkaido, 001-0020, Japan*

b. *Graduate School of Life Science, Hokkaido University, Kita 10, Nishi 8, Kita-ku, Sapporo, Hokkaido, Japan, 060-0810*

c. *Center of Research and Education on drug discovery, Faculty of pharmaceutical sciences, Hokkaido University, Kita 12, Nishi 6, Kita-ku, Sapporo, Hokkaido 060-0812, Japan*

Correspondence should be addressed to P. K. H. (E-mail: hashim@es.hokudai.ac.jp)

Table of contents

1. Chemicals and instrumentation
2. Protein expression and isolation
3. Experimental procedures
4. SDS-PAGE analysis of GroEL
5. The rate of ONPG hydrolysis at different concentrations
6. ONPG hydrolysis by $GroEL_{WT}$ or β-gal
7. ONPG hydrolysis using a new batch of $GroEL_{WT}$
8. ONPG spontaneous hydrolysis without $GroEL_{WT}$
9. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of ATP
10. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of lactose
11. Molecular docking
12. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of GroEL mutant

1. Chemicals and instrumentation

All chemicals and biochemical reagents were purchased from commercial sources (Merck; Wako Pure Chemical industries; Tokyo Chemical industries; Kanto Chemicals, Thermo Fisher Scientific) and used without further purification. Absorption spectra were recorded by Shimadzu UV-1800 or JASCO spectrophotometer.

2. Protein expression and isolation

We prepared $GroEL_{WT}$ and $GroEL_{52//398}$ according to the method analogous to previous reports [S1, S2]. *E. coli* GroEL mutants were generated using QuikChange site-directed mutagenesis (Stratagene). $GroEL_{52//398}$ was additionally mutated with cysteines at its apical domain (All C to A, K311C, L314C). Briefly, bacterial cells bearing the plasmid were collected and sonicated in Tris-HCl buffer A (25 mM, 1 mM EDTA, 0.5 mM DTT, pH 8.0) and subjected to ultracentrifugation (40,000 g, 45 min, 4 °C). Then, the supernatant was mixed with 10% saturated ammonium sulfate and loaded on a hydrophobic column (Butyl-Toyopearls). Elution was performed with Tris-HCl buffer A containing 10% saturated ammonium sulfate until no protein was detected (Bradford assay). Then, the elution buffer was gradually changed from a mixture of buffer A with 20% saturated ammonium sulfate and 10% (v/v) MeOH to a mixture of Tris buffer A with 10–0% saturated ammonium sulfate. GroEL started to elute out when the buffer was changed to buffer A with 5% saturated ammonium sulfate. All fractions were collected and subjected to SDS-PAGE to identify those containing GroEL. The selected fractions were precipitated by 65% saturated ammonium sulfate. The precipitate was collected by centrifugation, redissolved into buffer B (20 mM Tris-HCl, pH 7.5, 100 mM Na_2SO_4, 20% MeOH), and subjected to size exclusion chromatography (Sepharose CL-4B) using buffer B as an eluent. The fractions containing GroEL were identified by SDS-PAGE. GroEL solutions were combined, concentrated via ultracentrifugation with an Amicon® Ultra-15 mL (100K), and dialyzed overnight (dialysis buffer: 20 mM Tris-HCl, pH 7.5, 100 mM Na_2SO_4) 3 times. Glycerol (10%, v/v) and DTT (0.5 mM) were added to the final dialysis solution. The resulting protein solution was distributed to aliquots (~100 μL), flash-frozen by liquid N_2, and stored at –20 °C.

3. Experimental procedures

We performed ONPG assay by following a typical procedure for the enzyme β-gal. Briefly, $GroEL_{WT}$ (5.2 μL, 3.6 μM stock solution) was added into a freshly prepared buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, pH 6.8) containing ONPG (13.3 mM, 400 μL) and incubated for 1–4 h at 25 °C before taking absorbance spectra. To measure the initial rate of hydrolysis, absorbance changes at 420 nm was monitored immediately after mixing $GroEL_{WT}$ and ONPG. For the calculation of Michaelis–Menten constant, the initial rate of hydrolysis was performed using a diluted ONPG solution (0.4–13.3 mM, 400 μL).

4. SDS-PAGE analysis of GroELs

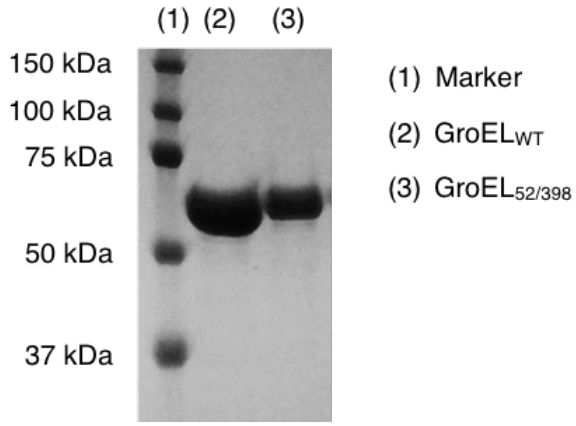


Figure S1: SDS-PAGE (12%) profiles (stained with CBB R-250 followed by silver stain plus) of GroELs ran in tris buffer (25 mM tris base, 192 mM glycine, 0.1% SDS buffer). Loading amount; 36 ng/well (GroEL$_{WT}$) and 28 ng/well (GroEL$_{52/398}$). A band (~60 kDa) corresponding to a GroEL subunit was observed for both GroEL$_{WT}$ and GroEL$_{52/398}$.

5. The rate of ONPG hydrolysis at different concentrations

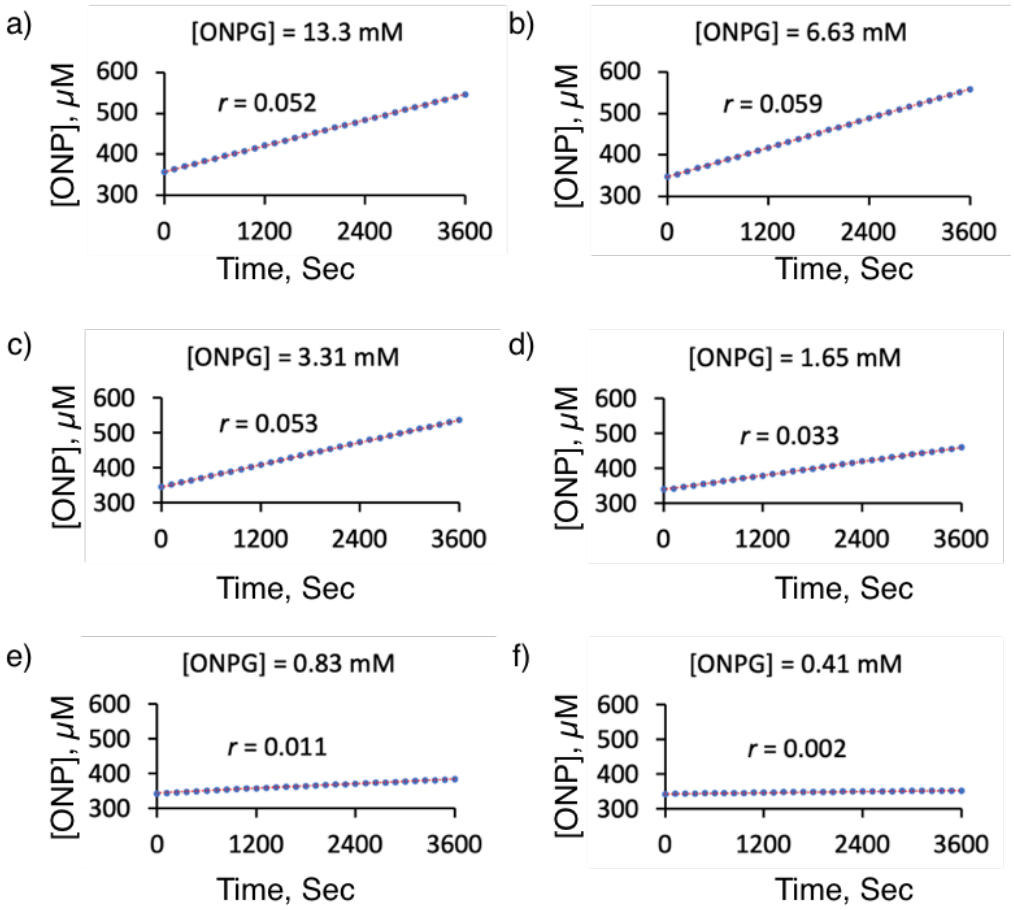


Figure S2: Graph showing the formation of ONP over time (blue dot, red line = fit) due to the hydrolysis of ONPG by $GroEL_{WT}$ (0.5 μM). The absorbance at 420 nm was converted to the amount of ONP using a standard curve. The unit of initial rate (r) is μM/Sec.

6. ONPG hydrolysis by GroEL$_{WT}$ or β-gal

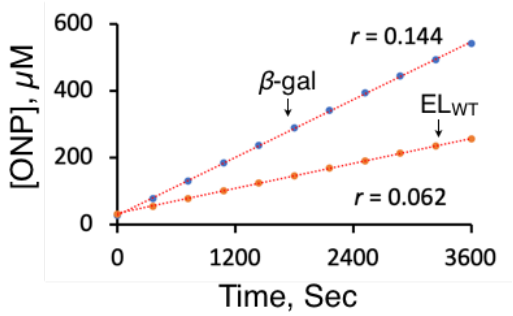


Figure S3: Graph showing the formation of ONP over time (blue dot, red line = fit) due to the hydrolysis of ONPG (1.3 mM) by GroEL$_{WT}$ (0.5 μM) or β-gal (0.5 μM). The absorbance at 420 nm was converted to the amount of ONP using a standard curve. The unit of initial rate (r) is μM/Sec.

7. ONPG hydrolysis using a new batch of GroEL$_{WT}$

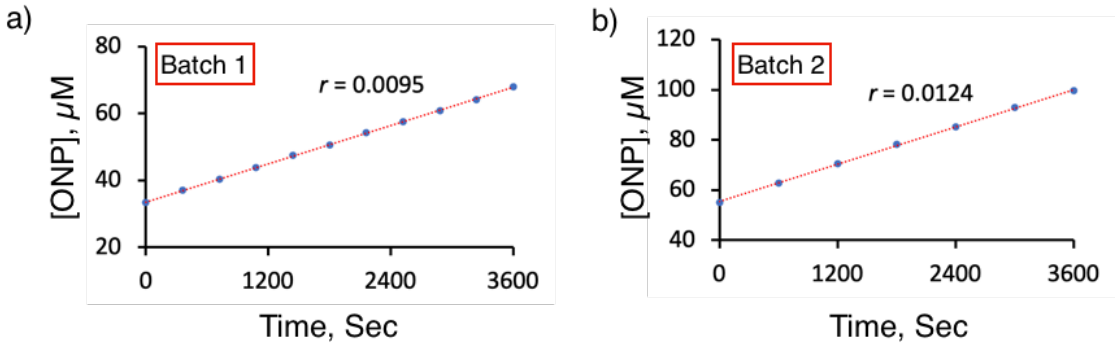


Figure S4: Graph showing the formation of ONP over time (blue dot, red line = fit) due to the hydrolysis of ONPG by GroEL$_{WT}$ (0.1 μM) isolated from bacterial expression in different batch. ONPG (6.63 mM), EL$_{WT}$ (100 nM) (a) and ONPG (13.4 mM), EL$_{WT}$ (100 nM) (b). The unit of initial rate (r) is μM/Sec.

8. ONPG spontaneous hydrolysis without $GroEL_{WT}$

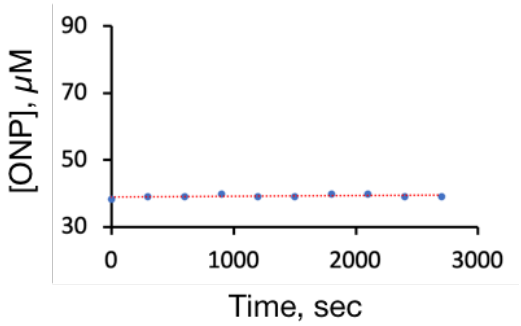


Figure S5: Graph showing no spontaneous hydrolysis of ONPG over time (blue dot, red line = fit) in absence of $GroEL_{WT}$.

9. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of ATP

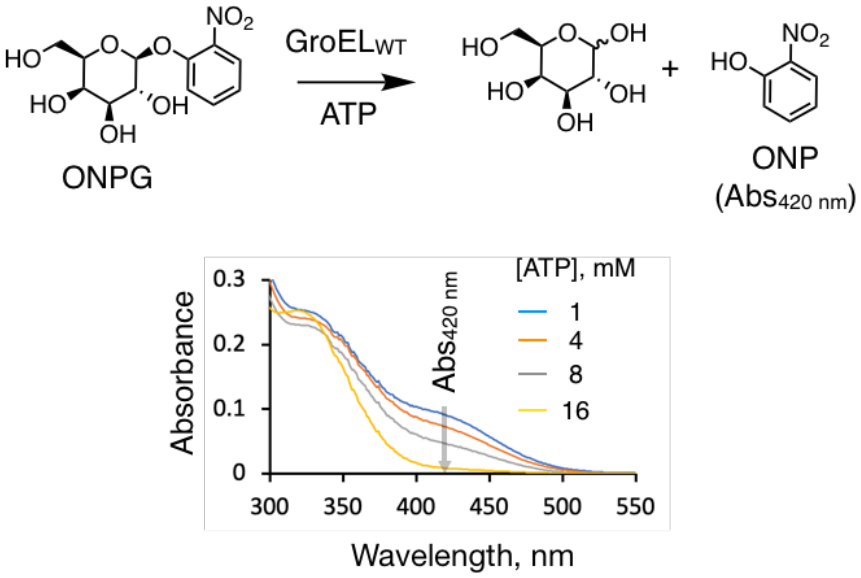


Figure S6: Absorption spectra showing the hydrolysis of ONPG by $GroEL_{WT}$ (0.05 μM) in the presence of ATP. Upon increasing the concentration of ATP (1–16 mM), a decrease in absorbance at 420 nm was observed.

10. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of lactose

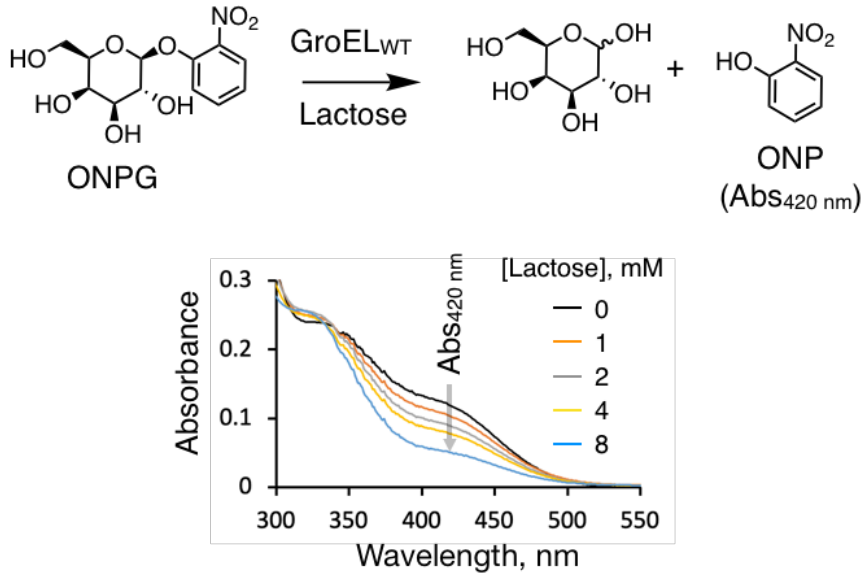
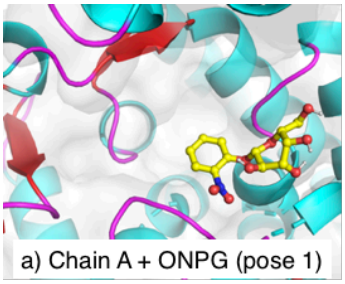
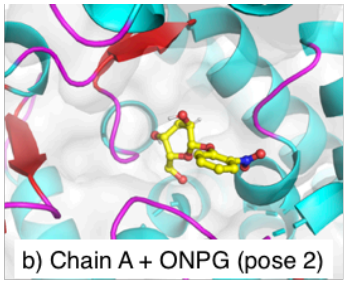


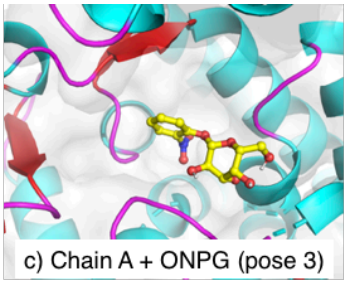
Figure S7: Absorption spectra showing the hydrolysis of ONPG by $GroEL_{WT}$ (0.05 μM) in the presence of lactose. Upon increasing the concentration of lactose (1–8 mM), a decrease in absorbance at 420 nm was observed.

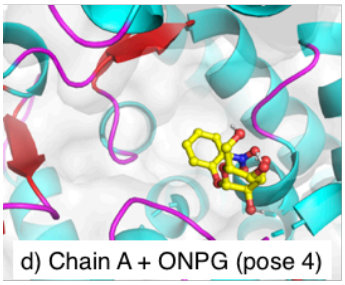
11. Molecular docking

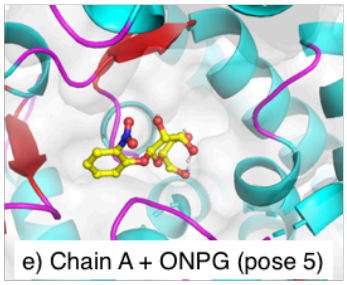
Docking studies were performed using Auto Dock Vina. Chain A from PDB (1KP8) was used as receptor site. We employed flexible docking approach where the active site residues were flexible in simulation. This includes T89, T90, T91, T30, L31, V499, D87, D495, I493, N479, I454, T500, L419 and K51.

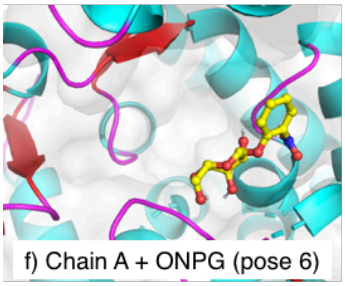


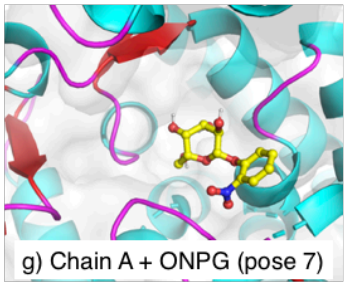












Pose	Affinity (kcal/mol)
1	-6.608
2	-6.474
3	-6.452
4	-6.445
5	-6.342
6	-6.307
7	-6.104
8	-6.089

Figure S8: Scheme showing the docked ONPG ligands in the ATP binding site of GroEL (chain A). The affinity value corresponding to poses 1–7 is shown in the table.

12. UV absorption spectra showing the decrease of absorbance at 420 nm in the presence of GroEL mutant

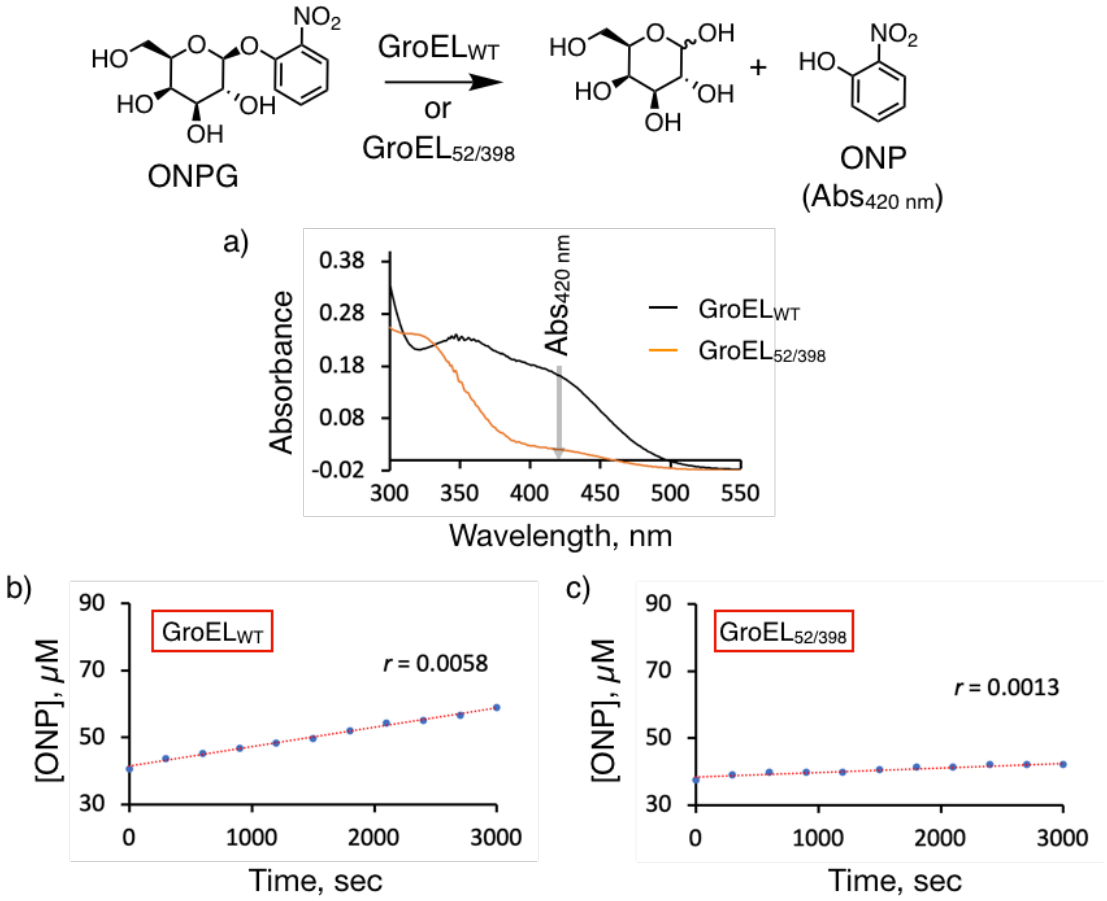


Figure S9: Absorption spectra showing the hydrolysis of ONPG by $GroEL_{WT}$ (0.05 μM) and $GroEL_{52/398}$ (0.05 μM) (a). The absorbance at 420 nm was significantly decreased for the $GroEL_{52/398}$ mutant. Graphs showing the formation of ONP over time (blue dot, red line = fit) due to the hydrolysis of ONPG by $GroEL_{WT}$ (47 nM) (b) and $GroEL_{52/398}$ (47 nM) (c). The unit of initial rate (r) is μM/Sec.

13. References

[S1] Sim, S.; Niwa, T.; Taguchi, H.; Aida, T. *J*. *Am*. *Chem*. *Soc*. **2016,** *138,* 11152

[S2] A. Koike-Takeshita, K. Mitsuoka, H. Taguchi. *J Biol Chem.* 2014, 289, 30005