SUPPORTING INFORMATION

Disassembly/Reassembly Strategy for the Production of Highly Pure GroEL, a Tetradecameric Supramolecular Machine, Suitable for Quantitative NMR, EPR and Mutational Studies.

Marielle A. Wälti[1] and G. Marius Clore[1,*]

[1]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520

6 Supplementary Figures

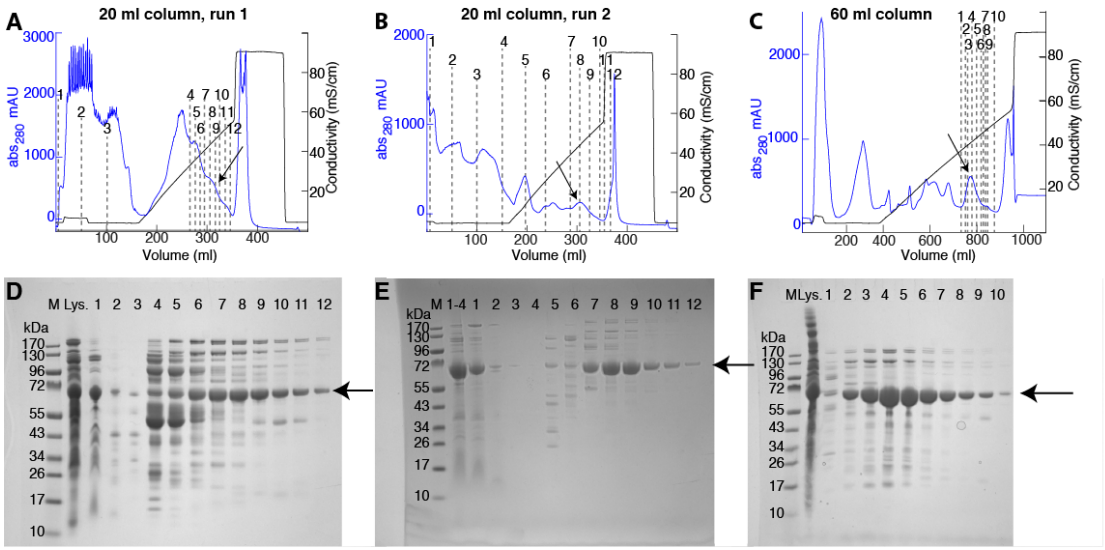

Fig. S1. Analysis of fractions obtained from ion exchange chromatography during the course of the standard GroEL purification. Elution profiles from the first (A) and second (B) runs through a standard 20 ml ion exchange column compared to the elution profile from the self-packed 60 ml ion exchange column (C). The arrows indicate the fractions containing GroEL. The protein was loaded and washed in 50 mM Tris pH 8, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM DTT, and eluted with a gradient from 0 to 0.56 M NaCl (black line). The elution profile shows many overlapping peaks in (A) and (B), but much better separation in (C). (D), (E) and (F), respective coomassie-stained SDS-PAGE (4-12% w/v) gels. (D) Lane 1: sample after cell lysis (Lys.); lanes 2-13: fractions (indicated with corresponding numbers) from the elution profile shown in (A). (E) Lane 1: flow-through from run 1, which was reloaded for a second run (corresponding to fractions 1-3 from run 1); lane 2-13: fractions from the elution profile shown in (B). (F) Lane 1: sample after cell lysis (Lys.); lane 2-11: fractions from the elution profile shown in (C). The band at around 60 kDa is attributed to GroEL (subunit theoretical MW = 57.1 kDa, indicated by arrows). M is the molecular weight standard with masses indicated in kDa.

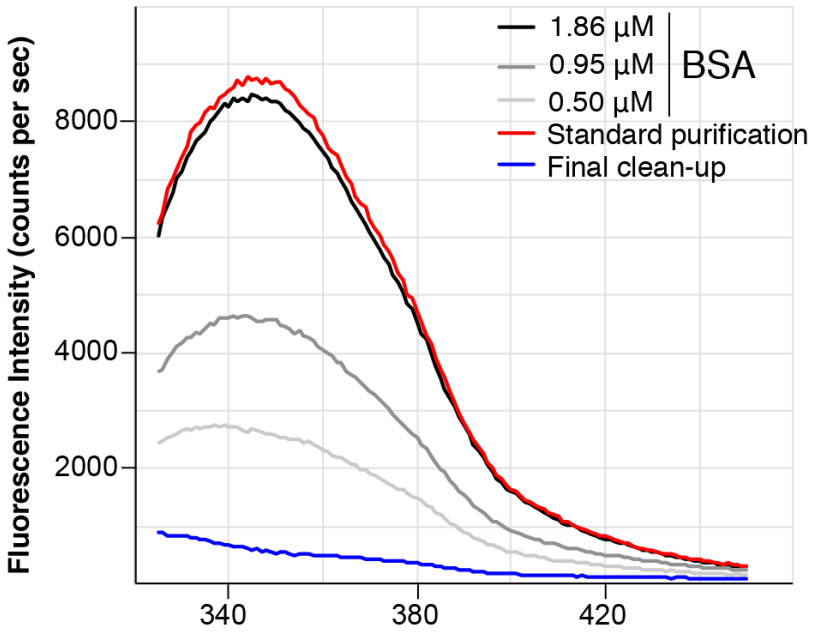

Fig. S2. Tryptophan fluorescence to determine GroEL purity. A standard calibration curve was performed with a dilution series of BSA (6 points between 0 and 2 µM), illustrated in the figure by three concentrations (0.5, 0.95 and 1.86 µM). The amount of protein impurities per GroEL tetradecamer ($GroEL_{14}$) was estimated as 1.5-3 mol impurities per mol $GroEL_{14}$ with large sample to sample variability before the final clean-up (red curve) and 0.04-0.06 mol impurities per mol $GroEL_{14}$ after the final clean-up (blue curve).

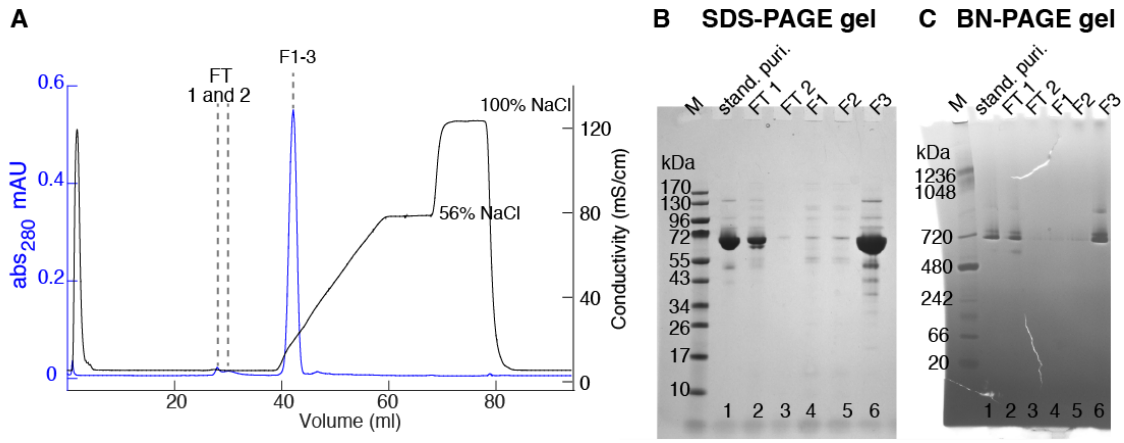

Fig. S3. Disassembly and reassembly of GroEL without chaotropic agents using an ion exchange column and MgADP. The elution profile is shown in (A) and the corresponding SDS PAGE and BN-PAGE analyses are shown in (B) and (C), respectively. 5 mM Mg-ATP was added to GroEL^{E315C} after the standard purification. The sample was injected onto a 5 ml ion exchange column (HiTrap^{TM} QFF) in 10 mM Tris, pH 7.4, 10 mM $MgCl_2$, 2 mM DTT, 1 mM TCEP and 1 mM ADP at a flow rate of 2 ml/min. GroEL^{E315C} was eluted with a gradient from 0-0.56 M NaCl within 4 column volumes. The column was washed for 2 column volumes at 0.56 M NaCl, followed by another 2 column volumes at 1 M NaCl. (B) and (C) Lane1: GroEL^{E315C} after the standard purification (stand. puri.) with 5 mM Mg-ADP; lanes 2 and 3: flow-through; lanes 4-6: GroEL^{E315C} containing fractions. M is the molecular weight standard with masses indicated in kDa.

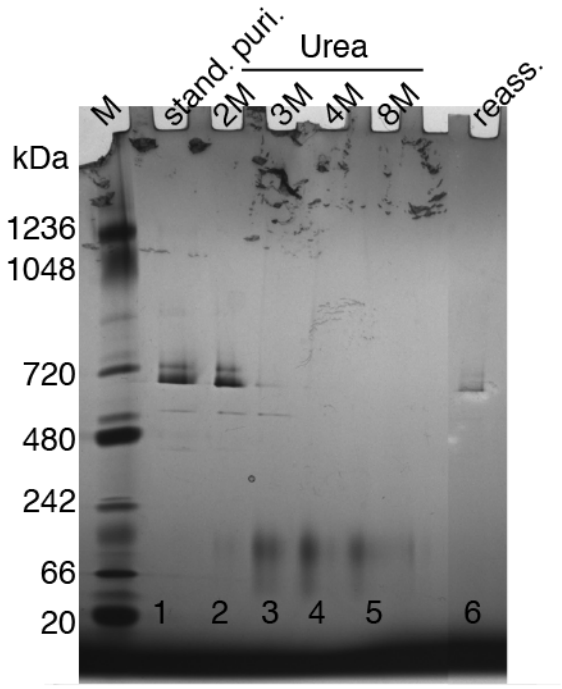

Fig. S4. BN-PAGE analysis of the disassembly/reassembly reaction. Lane 1: $GroEL_{14}$ (illustrated by the mutant $GroEL^{E315C}$) after the standard purification (stand. puri.) with a band at 720 kDa. Lanes 2-5: different amounts of urea used to disassemble GroEL into monomers. Lane 6: the sample in 3 M urea was reassembled back into $GroEL_{14}$ by the addition of $(NH_4)_2SO_4$ and ATP (see Results section of main paper for details). M is the molecular weight standard with the masses indicated in kDa.

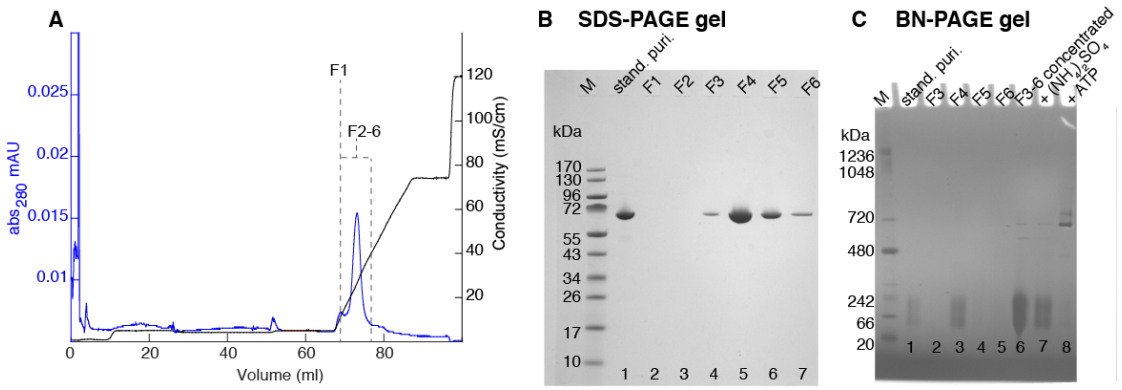

Fig. S5. Analysis of the final clean-up using ion exchange chromatography in urea. Elution profile (A) and corresponding SDS-PAGE (B), and BN-PAGE (C) analysis. GroEL, after the standard purification in 3 M urea, was loaded onto a 5 ml ion exchange column (HiTrap^{TM} QFF) and washed in 10 mM Tris, pH 7.4, 10 mM $MgCl_2$, 2 mM DTT, 1 mM TCEP, and 3 M urea at a flow rate of 2.5 ml/min. GroEL^{E315C} was eluted with a gradient from 0 to 0.56 M NaCl within 4 column volumes. The column was washed for 2 column volumes at 0.56 M NaCl followed by another 2 column volumes at 1 M NaCl. (B) Lane1: GroEL^{E315C} after the standard purification (stand. puri.) incubated overnight at 4 °C in 3 M urea; lanes 2-7: the collected fractions as shown in (A). (C) Lane1: GroEL^{E315C} after the standard purification (stand. puri.) incubated overnight at 4 °C in 3 M urea; lanes 2-4: the GroEL containing fractions as shown in (A); lane 5: the pooled and concentrated GroEL sample; lanes 7-8: GroEL reassembly by the addition of $(NH_4)_2SO_4$ (lane 7) and ATP (lane 8) (for details see Results Section in main text). M is the molecular weight standard with the masses indicated in kDa.

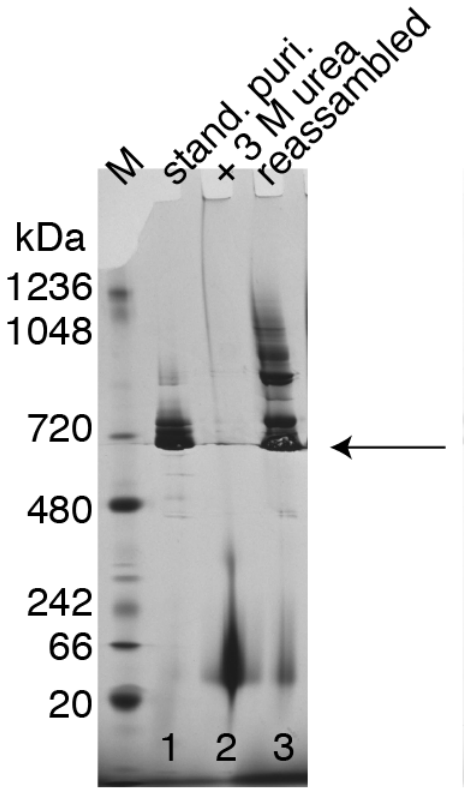

Fig. S6. BN-PAGE analysis of reassembled GroEL without site-specific spin labeling. Lane 1: $GroEL^{E315C}$ 14mer after the standard purification; lane 2: GroEL monomer after the addition of 3 M urea; lane 3: reassembly of GroEL before site specific spin-labeling. Reassembly was achieved by the addition of $(NH_4)_2SO_4$ and ATP and results in higher mass species, probably due to cross-linking of different GroEL molecules by the free cysteines. M is the molecular weight standard with masses indicated in kDa. The band at 720 kDa depicts the $GroEL_{14}$ (indicated by the arrow) and the band at around 50 kDa is the monomer.