Supporting Information:

Temperature Regulates Stability, Ligand Binding (Mg^{2+} and ATP) and Stoichiometry of GroEL/GroES Complexes

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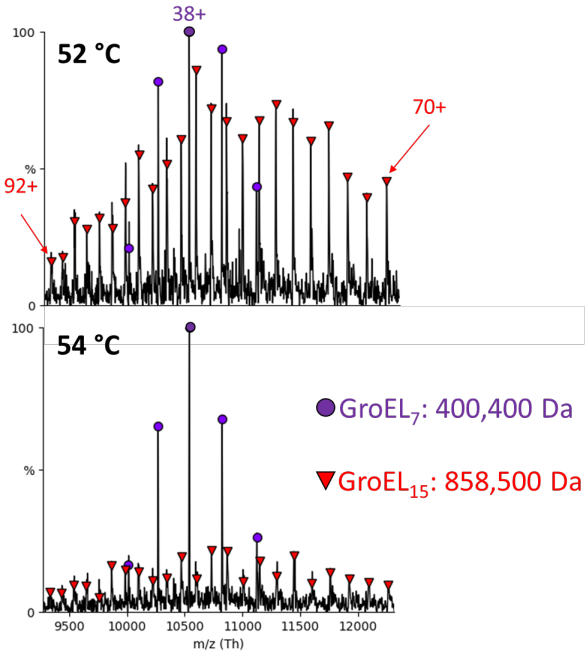

***Figure S1*. Temperature stability of GroEL$_{14}$.** Mass spectra (**Figure 2**) of GroEL acquired at 52 and 54 °C provide evidence for thermal decomposition of GroEL$_{14}$. The most abundant signals in the m/z range from ~9,000 to 12,500 are assigned to GroEL$_7$ (36^+ to 40^+) and a series GroEL$_{15}$ ions (charge states 70^+ to 92^+); mass and charge assignments were initially determined using ProteinMetric Intact Mass™ program. Solution conditions used to acquire the mass spectra differ only in the temperature of the ESI solution. The most abundant ions detected at 52 °C correspond to GroEL$_7$ (blue circles) and GroEL$_{15}$ (red triangles), and at 54 °C the signals for GroEL$_7$ dominate the spectrum. The GroEL$_7$ charge state distribution (CSD) is consistent with a native-like heptamer, whereas the CSD for GroEL$_{15}$ is broad and consistent with non-native oligomers. The increased relative abundances for GroEL$_7$ are attribute to a reduction in the abundances GroEL$_{15}$ signals at 54° C spectrum vis-à-vis an increased abundance of GroEL$_7$. This assignment is based on comparisons of signal-to-noise ratios of the two spectra. At higher temperatures the signals for GroEL$_{15}$ are lower, possibly indicating that higher temperatures promote higher order oligomers that precipitate from solution.

The mass assignments for GroEL$_7$ and GroEL$_{14}$-GroEL$_1$ ions is supported by excellent agreement between calculated isotopically averaged mass and experimentally measured mass of 400.47 kDa vs. 400.40 kDa, and 858.5 ± 1 kDa vs. 858.0 kDa, respectively.

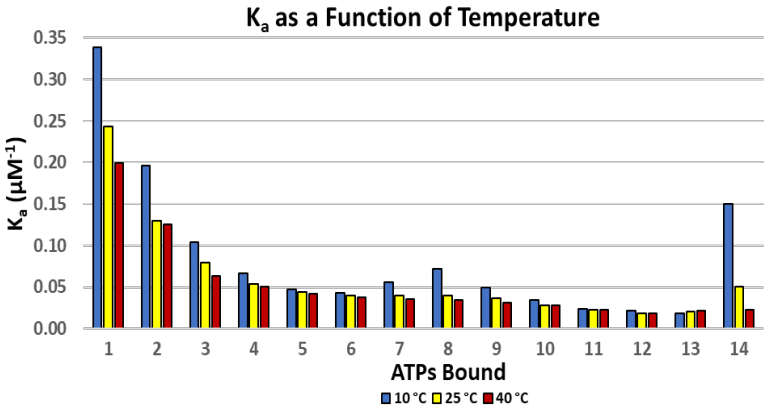

***Figure S2*. ATP binding is favored at low temperatures.** Calculated macroscopic association constants (K_a) for binding of ATP to GroEL 10 °C (blue), 25 °C (yellow), and 40 °C (red). These data show that ATP is more tightly bound at cold solution temperatures and more loosely bound, comparatively, at higher solution temperatures. Note that the 14th ATP binds with higher affinity, at cold temperatures, than many of the other preceding binding events.

Calculation of Association Constants (K_a)

The association constants were calculated using the equation 1 and then were statistically corrected using equation 2.[1, 2] The statistical correction accounts for the number of sites available for a ligand (ATP in this case) to bind or dissociate. Sharon *et al.* discuss these equations in much more detail, especially in the supplemental information.[3]

$$K_i^{app} = \frac{I_i}{I_{i-1}[S]_{free}} \quad (1)$$

$$K_i^{app} = \frac{N-i+1}{i} K_i^{int} \quad (2)$$

Where K_i^{app} is the apparent binding constant, I_i is the intensity of the ith bound ATP, I_{i-1} is the intensity of the preceding binding, and $[S]_{free}$ is the free ligand concentration. Where N is the number of total binding sites, i is the number of bound ATPs, and K_i^{int} is the intrinsic binding constant.

References:

1. Garces, J. L.; Rey-Castro, C.; David, C.; Madurga, S.; Mas, F.; Pastor, I.; Puy, J., Model-Independent Link between the Macroscopic and Microscopic Descriptions of Multidentate Macromolecular Binding: Relationship between Stepwise, Intrinsic, and Microscopic Equilibrium Constants. *Journal of Physical Chemistry B* **2009,** *113* (46), 15145-15155.
2. Garces, J. L.; Acerenza, L.; Mizraji, E.; Mas, F., A hierarchical approach to cooperativity in macromolecular and self-assembling binding systems. *J Biol Phys* **2008,** *34* (1-2), 213-35.
3. Dyachenko, A.; Gruber, R.; Shimon, L.; Horovitz, A.; Sharon, M., Allosteric mechanisms can be distinguished using structural mass spectrometry. *Proc Natl Acad Sci U S A* **2013,** *110* (18), 7235-9.