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Supporting Information for

A diminished hydrophobic effect inside the GroEL/ES cavity contributes to protein substrate destabilization

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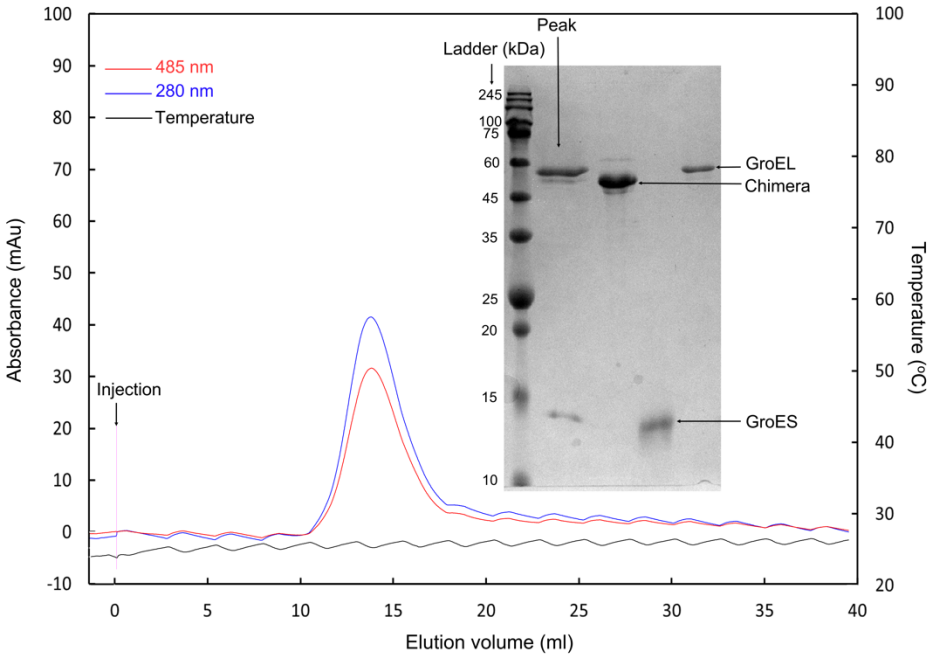

Fig. S1. Stability of GroEL-GroES$_2$ football-shaped complexes containing the chimera of the I61V mutant of DHFR$_{Mp}$ fused to eGFP. A sample of BeF$_x$-stabilized 'football' complexes with the encapsulated chimera was kept at room temperature overnight and then applied to a Sepharose 6 10/300 gel-filtration column as described (1). Elution was monitored by measuring protein and eGFP absorbances at 280 (blue) and 485 (red) nm, respectively. Only one protein-containing fraction was observed and no free chimera could be detected. The baseline fluctuations are due to temperature fluctuation (black trace). SDS-PAGE analysis of this fraction (inset) showed that it contains GroEL, GroES and the chimera. Taken together, these results show that the BeF$_x$-stabilized GroEL-GroES$_2$ football-shaped complexes remained intact and that the chimera did not escape outside during the overnight incubation. These experiments were repeated four times.

SI Reference

1. I. Korobko, H. Mazal, G. Haran, A. Horovitz, Measuring protein stability in the GroEL chaperonin cage reveals massive destabilization. eLife 9, e56511 (2020).