Figure S1  Prevention of rhodanese aggregation by SR-EL charge mutants.

Prevention of rhodanese (Rho) aggregation *in vitro* was measured at a 4-fold molar excess of SR-EL or SR-EL charge variants (see Materials and methods). Aggregation after 10 min of rhodanese dilution from denaturant in the absence of chaperonin was set to 1.
Figure S2  Inhibition of spontaneous refolding of WT-MBP.

Inhibition of spontaneous refolding of WT-MBP (0.25 μM) \textit{in vitro} was measured at an equimolar ratio of GroEL (or size variants) to WT-MBP (A) or at a 2-fold molar excess of GroEL or the size variants (B) or at a 4-fold molar excess of SR-EL or SR-EL size variants (C) in the absence of ATP. Refolding of GuHCl-denatured WT-MBP at 25°C upon 100-fold dilution into buffer A was set to 1.
Inhibition of spontaneous refolding of DM-MBP in vitro was measured as in Figure S2 at a 2-fold molar excess of GroEL (or size variants) to DM-MBP (A) or at a 4-fold molar excess of SR-EL or SR-EL size variants (B). Refolding of GuHCl-denatured DM-MBP at 25°C upon 100-fold dilution into buffer A was set to 1.
Figure S4  ATPase activities of GroEL size-mutants measured at different ATP concentrations.

The oligomer concentration of GroEL or GroEL size variants was ~0.5 μM. The ATPase activity was measured at 25°C using a coupled enzymatic assay essentially as described by Poso, D. et al. (2004, J. Mol. Biol., 338, 969-977) and ATP concentrations between 0 and 1000 μM. Buffer conditions in the assay were 50 mM BisTris pH 7.5, 100 mM KCl, 10 mM MgCl₂. Note that under these assay conditions the ATPase activity of the GroEL tail extension-mutants was found to be ~3-fold stimulated relative to WT-GroEL (see Figure 7A for comparison).
Figure S5 Kinetic model for the simulation of rhodanese folding rate dependent on GroEL ATPase activity (see Figure 7E).

1. The binding of unfolded rhodanese to GroEL occurs with a rate constant of 2x10^7 M^{-1} s^{-1}.

2. The step of encapsulation by GroES is dependent on the rate of ATP and GroES binding to GroEL. Since the rate of ATP binding is ~50 fold faster than GroES binding under these conditions, the rate of formation of the encapsulated complex is limited by the rate of GroES association, i.e 1x10^6 M^{-1} s^{-1}.

3. The fraction of molecules that undergo productive folding in the residence time in the GroEL cavity is determined by the rate of ATP hydrolysis (0.07 s^{-1}), and by the rate constant of rhodanese folding of 2.5x10^{-3} s^{-1}. The rate of ATP hydrolysis controls the dissociation of GroES and thus the residence time of substrate inside the GroEL/GroES cage. The rate of ATP hydrolysis is assumed to be independent of the conformation of the protein inside the GroEL cavity. Note that unfolding of rhodanese upon GroEL-binding or upon GroES binding to the GroEL-rhodanese complex does not result in a partitioning between rapid and slow folding pathways upon subsequent displacement of substrate into the GroEL-GroES cage, based on the absence of a rapid folding phase upon single-round encapsulation (Figure 6C and Brinker et al., 2001).

4. Protein that has not reached native state within this residence time is transiently released and rapidly recaptured for another round of encapsulation and folding.

5. Native folded molecules are also released from GroEL with a rate determined by the rate of ATP hydrolysis.