Materials and Methods

Materials

DH10B cells *(endA1 recA1 galE15 galK16 nupG rpsL* Δ*lacX74 Φ80lacZ* Δ*M15 araD139* Δ*(ara,leu)7697 mcrA* Δ(*mrr-hsdRMS-mcrBC*) λ^-) and MC1000 cells (F^- *araD139* Δ(*araA-leu)7679* (*codB-lac)X74 galE15 galK16 rpsL150 relA1 thi*) were used for protein over-expression and visualization, respectively. The evolved *M. jannaschi* tRNA and synthetase constructs have been reported previously.[1] One of the plasmids (pBKcouRS) contains a constitutively expressed copy of the synthetase while the other (pBADJYgroEL129TAG) constitutively expresses the tRNA as well as the *groE* operon with *groES* and the mutated *groEL* gene both under the *araBAD* promoter. The *groEL* coding sequence was cloned into pBADJY using standard molecular biology techniques. The *amber* mutation was introduced using a previously reported modified quick-change protocol.[2]

CouAA incorporation and protein purification

DH10B cells transformed with pBKCouRS and pBADJYgroEL129TAG were grown to an OD_{600} 1.0 in terrific broth (TB) at 37°C, induced with 0.2% arabinose, coumarin amino acid added to 1 mM, and grown at 37°C for five hours in the dark. Large scale $GroEL_{129CouAA}$ expression was carried out in DH10B cells on a 200 mL scale and protein synthesis was induced with 0.2 % arabinose in the presence of 1 mM coumarin amino acid for eight hours in the dark. $GroEL_{129CouAA}$ was purified as previously reported for wild type GroEL except that light was excluded throughout the purification procedure.[3]

ATPase assay

ATPase activity was measured using a standard malachite green based assay.[4] For both wtGroEL and $GroEL_{129Cou}$, assays were conducted with 1 μM chaperonin tetradecamer and 1 mM ATP for 10 minutes.

MDH refolding assay

MDH refolding was measured by forming a binary complex between 1.1 μM GroEL and 1 μM MDH denatured with 6M guanidine•HCl followed by addition of 1.5 μM GroES and 2 mM ATP to initiate the reaction. Refolding extent was then calculated by quenching the reaction at desired times with EDTA and measuring MDH activity by loss of absorbance at 340 nm due to NADH consumption. The details of this procedure have been reported previously.[5]

In vivo visualization and FRAP

MC1000 cells transformed with the pBKCouRS and pBADJYgroEL129TAG plasmids were grown in M9 minimal medium to an OD_{600} 0.4-0.6 and induced with 0.02% - 0.2% L-arabinose in the presence of 1 mM coumarin amino acid. Importantly, the amount of arabinose used did not lead to differences in $GroEL_{129CoAA}$ localization. Immediately prior to visualization, cells were washed once with M9 to remove unincorporated coumarin amino acid and placed on an agarose pad. Studies demonstrated nearly complete removal of coumarin fluorescence when 200 μL of cell culture was washed with the same volume of coumarin amino acid free minimal media (data not shown). In the present studies, cells were washed twice with coumarin amino acid free minimal medium equal to the cell culture volume (200 μL) to ensure complete removal of unincorporated fluorophore. In vivo fluorescence was visualized using a DAPI filter set (excitation 345 nm and emission 458 nm) in a Nikon E1000 microscope with a DIC

100X objective and a Hamamatsu Orca-ER LCD camera, or using a Nikon E80i microscope with a DIC 100X objective and an Andor iXon+ camera.

Photobleaching experiments were performed using a Photonic Instruments Micropoint Laser system equipped with a 364 nm laser dye set at 10 to 15 pulses with maximum laser intensity. Images were taken and processed with Metamorph and ImageJ softwares.

References

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Supplemental Figure 1. GroEL-YFP over-expression induces chromosome condensation. A *trc* promoter driven GroEL-YFP fusion was induced in MC1000 cells grown in minimal media for two hours in the presence of 100 μM IPTG and placed on an agarose pad for visualization. *In vivo* fluorescence was visualized using a Nikon E1000 microscope with a DIC 100X objective and a Hamamatsu Orca-ER LCD camera. A) DIC

image of cells. B) Overlay of fluorescence images false colored blue (DAPI) and red (YFP).

Supplemental movie 1. Heat shock of cells expressing $GroEL_{129CouAA}$. Fluorescence images of cells growing on an M9 agarose pad at 45°C.