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Supplemental Information

Contact Order Is a Determinant for the Dependence of GFP Folding on the Chaperonin GroEL

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SI Materials and Methods

Construction of circular permutants. The N- and C-termini of the wild-type eGFP gene were first fused by constructing a 5′-phosphorylated PCR fragment using the forward and reverse primers 5'-GGTACCGGTGTTAGCAAGGGCGAGGAGCTG-3' and 5'-GCCGGAACCCTTATACAGCTCGTCCATGCC-3' (5'-phosphorylated), and then self-ligation. The circular gene was then used as a template for the construction of circular permuted variant using the Restriction Free (RF) cloning method. The primers used for PCR amplification and introducing new termini are given in Table S1. The resulting PCR product was a linear DNA fragment containing the permuted eGFP gene flanked by vector specific regions at both termini. This PCR product was used as megaprimer for integration into the destination vector pET28a.

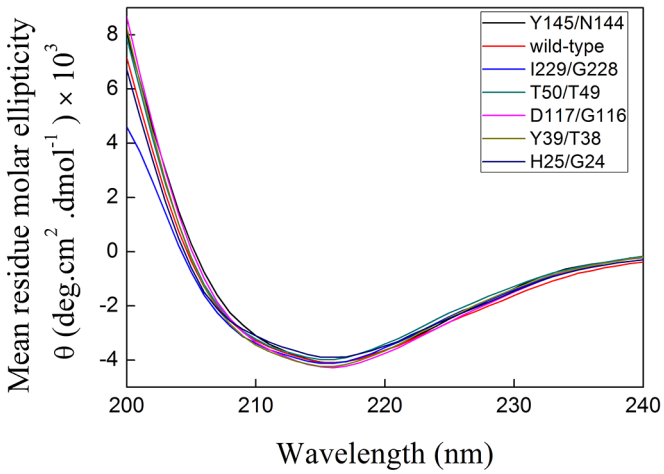

Figure S1. Far-UV CD spectra of the different eGFP permutants. The far-UV CD spectra from 200 to 240 nm of wild-type eGFP and the permutants were recorded at 25 °C. Three consecutive scans were averaged and then corrected by subtracting the spectra of buffer alone. See Materials and Methods for further details.

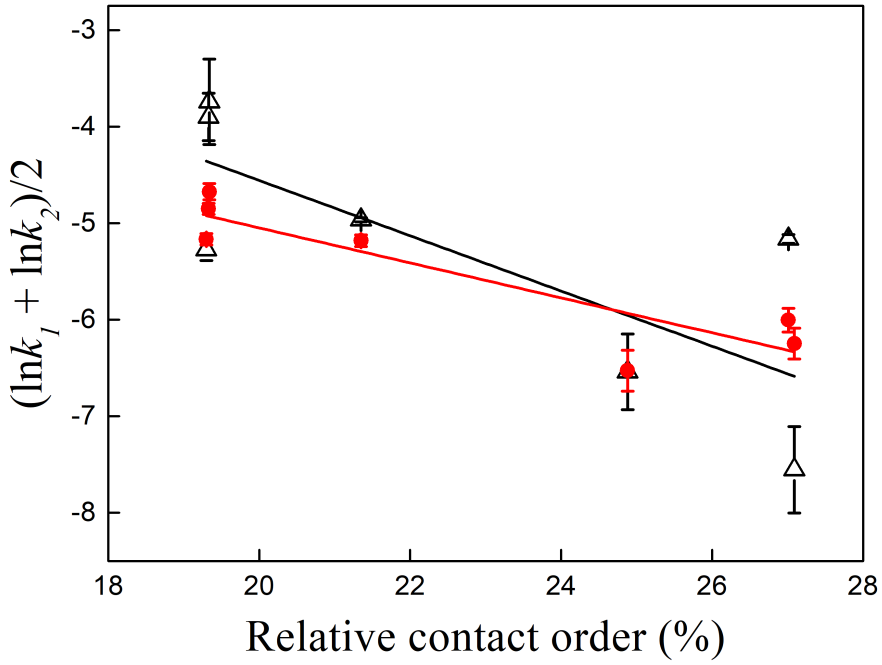

Figure S2. Plots of the average of the logarithms of the folding rate constants of eGFP variants as a function of their respective contact orders. The experiments were carried out in the presence of 125 (black) or 42.5 nM (red) eGFP concentrations. The rate constants k_1 and k_2 were determined from fits of the spontaneous refolding progress curves to a double-exponential equation. Error bars show the standard deviations of three independent refolding experiments.

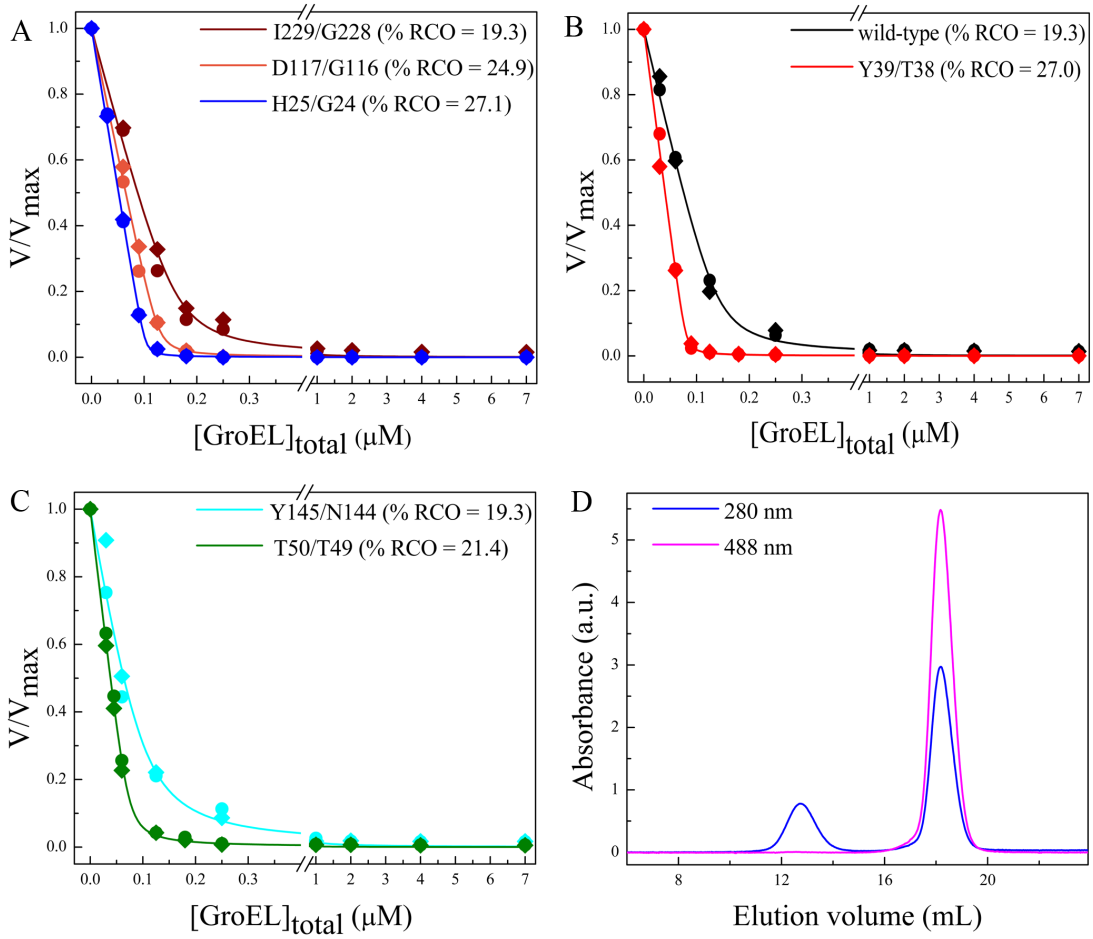

Figure S3. Kinetic partitioning of the eGFP variants between spontaneous folding and binding to GroEL. *(A-C)* The extent of retardation of folding of the different eGFP permutants was measured as a function of total GroEL concentration. The slopes of the initial linear changes in fluorescence as a function of time, V, upon refolding were divided by the slope in the absence of GroEL, V_{max}, and plotted as a function of the total oligomeric concentration of GroEL. The data points obtained from two independent experiments for each eGFP permutant are represented by open circles (○) and filled diamonds (♦), respectively, and shown using the color code in Fig. 1. The data were subjected to global fitting using Eq. 1 that yielded the estimates and standard errors of all the parameters. The N- and C- termini and % RCO of the respective permutants are indicated. (*D*) GroEL was mixed with equal amounts of all the eGFP variants in their native state in the MOPS refolding buffer without Tween. The mixture was then subjected to size-exclusion chromatography on a Superose 6 column. Elution was monitored by measuring the absorbance at 488 nm (where eGFP absorbs) and 280 nm. No co-elution of GroEL and eGFP variants was observed, thereby indicating that the native eGFP variants do not bind GroEL.

Table S1: List of primers used to introduce new termini. Gene specific parts are marked in bold and the vector-specific parts are underlinerd.

New termini	Primers	Primer sequence
H25/G24	Forward	ATGGGCAGCAGCCATCATCATCATCATCAC**CACAAGTTCAGCGTGTCCGG**
	Reverse	TCAGTGGTGGTGGTGGTGGTGCTCGAGTTTA**GCCGTTTACGTCGCCGTCC**
Y39/T38	Forward	ATGGGCAGCAGCCATCATCATCATCATCAC**TACGGCAAGCTGACCCTGAAG**
	Reverse	AGTGGTGGTGGTGGTGGTGCTCGAGTTTA**GGTGGCATCGCCCTCGCCCTC**
T50/T49	Forward	ATGGGCAGCAGCCATCATCATCATCATCAC**ACCGGCAAGCTGCCCGTGC**
	Reverse	TCTCAGTGGTGGTGGTGGTGGTGCTCGAGTTTA**GGTGCAGATGAACTTCAGGGTC**
D117/G116	Forward	TGGGCAGCAGCCATCATCATCATCATCAC**GACACCCTGGTGAACCGCATC**
	Reverse	CAGTGGTGGTGGTGGTGGTGCTCGAGTTTA**GCCCTCGAACTTCACCTCGG**
Y145/N144	Forward	GGCAGCAGCCATCATCATCATCATCAC**TACAACAGCCACAACGTCTATAT**
	Reverse	CAGTGGTGGTGGTGGTGGTGCTCGAGTTTA**GTTGTACTCCAGCTTGTGCC**
I229/G228	Forward	TGGGCAGCAGCCATCATCATCATCATCAC**ATCACTCTCGGCATGGACGAG**
	Reverse	CAGTGGTGGTGGTGGTGGTGCTCGAGTTTA**CCCGGCGGCGGTCACGAAC**