

Table S2. Comparison of Folding Rate Constants (k_F), Activation Energy of Folding (ΔG_F^{Ea}), and the Entropy Barrier ($T\Delta S_F$) for Spontaneous and GroEL/ES-assisted Refolding of DapA and Spontaneous Refolding of MsNanA, Related to Figure S3E

	$k_F * 10^{-3}$ (s ⁻¹)*	ΔG_F^{Ea} (kJ mol ⁻¹)	ΔH_F (kJ mol ⁻¹)	$T\Delta S_F$ (kJ mol ⁻¹)
DapA spontaneous 25°C	3.0 ± 0.2	87.2	0	-87.2
DapA spontaneous 10°C	1.5 ± 0.3	84.5	96.8	12.3
DapA GroEL/ES-assisted 25°C	106 ± 10	78.5	66.6	-11.9
DapA GroEL/ES-assisted 10°C	25 ± 3	77.8	66.6	-11.2
MsNanA spontaneous 25°C	23 ± 2	82.3	67.8	-14.5
MsNanA spontaneous 10°C	4.9 ± 0.2	81.7	67.8	-13.9

* The apparent refolding rate in buffer B (for DapA) or buffer D (for MsNanA) (3 independent experiments).

According to transition state theory, the rate constant of folding, k_F , of a two-state reaction is defined by the Eyring (or Arrhenius) equation (2):

$$k_F = \frac{k_B T}{h} e^{-\frac{\Delta G_F^{Ea}}{RT}}, \quad (2)$$

where ΔG_F^{Ea} is the activation energy of folding, k_B is Boltzmann's constant (1.381×10^{-23} JK⁻¹), and h is Planck's constant (6.626×10^{-34} Js), R is the gas constant (8.31 JK⁻¹mol⁻¹) and T is temperature in K.

Using the definition of the free energy:

$$\Delta G_F^{Ea} = \Delta H_F - T\Delta S_F, \quad (3)$$

the Eyring equation can be rewritten as

$$\ln(k_F) = \left(\ln\left(\frac{k_B T}{h}\right) + \frac{\Delta S_F}{R} \right) - \frac{\Delta H_F}{R} \left(\frac{1}{T}\right). \quad (4)$$

As $\ln(T)$ depends weakly on $1/T$ in the range measured, equation (4) is approximately linear with the slope equal to $-\frac{\Delta H_F}{R}$ and an intercept that depends on ΔS_F . Hence, the enthalpy and entropy contribution to the barrier between the intermediate state (I) and the transition state (TS) can be extracted from the graph. The results are given in Table S2.