SI Appendix

Experimental Data on the Rop-Dimer

Mutant	Activity *in*		T_m/	ΔG/	C_m/	m/	Relative		Structure
	vitro	*vivo*	(°C)	($\frac{kcal}{mol}$)	M	($\frac{kcal}{mol}$ M)	k_F	k_U	
WT	Y	Y	64	-7.7	3.3	2.4	1	1	AP (X-Ray, NMR)
$Ala_2Leu_2 - 2$	Y	Y	72	-7.7	2.5	3.2	3.2	18	AP (*in vitro* activity)
$Ala_2Leu_2 - (3+6)$	Y	-	72	-8.4	2.7	3.2	7.5	8.3	AP (*in vitro* activity)
$Ala_2Leu_2 - (2+7)$	Y	-	85	-8.7	2.7	3.4	120	7100	AP (*in vitro* activity)
$Ala_2Leu_2 - (1+8)$	Y	-	54	-6.3	2.3	2.9	160	110	AP (*in vitro* activity)
$Ala_2Leu_2 - 4$	Y	P	68	-5.8	2.5	2.3	1.5	28	AP (*in vitro* activity)
$Ala_2Leu_2 - 6$	Y	N	82	-8.1	2.7	2.7	310	31000	AP (*in vitro* activity)
$Ala_2Leu_2 - 8$	Y	N	91	-7.5	3.0	2.8	610	50000	AP (*in vitro* activity)
$Ala_2Leu_2 - 6$-rev	Y	-	85	-10.3	3.4	3.1	85	670	AP (*in vitro* activity)
$Ala_2Leu_2 - 8$-rev	Y	N	91	-9.9	3.4	3.1	92	2700	AP (*in vitro* activity)
$Leu_2Ala_2 - (2+7)$	Y	-	85	-12.8	3.6	3.7	10	18	AP (*in vitro* activity)
$Ala_2Ile_2 - 6$	N	N	83	-5.1	-	-	-	-	P
$Ala_{31}Pro$	P	-	-	-	-	-	-	-	bisecting U (X-Ray)
$Leu_2Ala_2 - 8$	N	-	*	-12.8	4.1	3.2	-	-	unknown
$Ala_2Met_2 - 8$	N	-	48	-3.1	0.73	4.9	-	-	unknown
$Leu_4 - 8$	N	-	*	>30	6.7	4.6	-	-	unknown
$Ala_4 - 8$	-	-	<2	-	-	-	-	-	unknown
$Ala_2Val_2 - 8$	-	-	-	-	-	-	-	-	unknown

Synopsis of the experimental data on the Rop-dimer and its mutants. T_F is the folding temperature at which the protein is equally often in the folded and unfolded states. The free-energy difference ΔG has been calculated from chemical unfolding experiments by denaturants as have been the folding and unfolding rates k_F and k_U. The mutants are named according to the number and location of reconstructed hydrophobic core amino acid pairs as illustrated in the main part. $Ala_2Leu_2 - 2$ means that only the innermost two layers, 4 and 5, have been rebuild with Ala in the (a) and Leu at the (d) position, $Ala_2Leu_2 - (3+6)$ means that the pair of the layers 3 and 6 has been rebuild. $Ala_2Leu_2 - 4$ combines these two rebuilds. In general these mutations increase thermodynamic stability. The chemical stability does not behave the same way and tends to be lower. Different topologies have been determined by experiments, but for most mutants the actual structure is concluded from its activity and helical content. (* no transition observable within the temperature range accessible).

Mathematical Description

A mathematical description for our model is

$$V(\vec{X}) = \sum_{\text{Atoms}} K_m(x - \bar{x})^2 + \sum_{\text{Bonds}} K_r(r - r_0)^2 + \qquad (1)$$

$$\sum_{\text{Angles}} K_\theta(\theta-\theta_0)^2 + \sum_{\text{Dihedrals}} K_\phi^{(n)}\left[1-\cos\left(n(\phi-\phi_0)\right)\right] +$$
$$\sum_{i<j-3}\left(\epsilon_{ij}\left[5\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-6\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{10}\right]+\bar{\epsilon}_{ij}\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}\right)$$

$\vec{X}$ describes a conformation of the proteins C_α atoms with the bond lengths of neighboring atoms r, the angles θ, the dihedral angles ϕ and the distance r_{ij} between any two atoms. The used constants are $K_\mathrm{r} = 100\,k_\mathrm{B}T/\text{Å}^2$, $K_\theta = 20\,k_\mathrm{B}T$, $K_\phi^1 = 1\,k_\mathrm{B}T$ and $K_\phi^3 = 0.5\,k_\mathrm{B}T$. An attractive interaction $\epsilon_{ij} = 1k_\mathrm{B}T$, $\bar{\epsilon}_{ij} = 0$ is assigned to natively interacting residues, while $\epsilon_{ij} = 0$, $\bar{\epsilon}_{ij} = 1\,k_\mathrm{B}T$ enforces a hard-sphere potential for non-natively interacting residues. σ_{ij} gives the native distances between the interacting residues in case of native interaction or is set to 4 Å for noninteracting residues. To allow the monomers disassociation but preventing them from moving too far apart, a weak center of mass force was additionally introduced with the force constant $K_\mathrm{m} = 25 \cdot 10^{-6}\,k_\mathrm{B}T/\text{Å}^2$ and $\bar{x} = \sum_{\text{Atoms}} x/N$ (the x's give the positions of the atoms).