Supporting Information

Wensley et al. 10.1073/pnas.1201793109

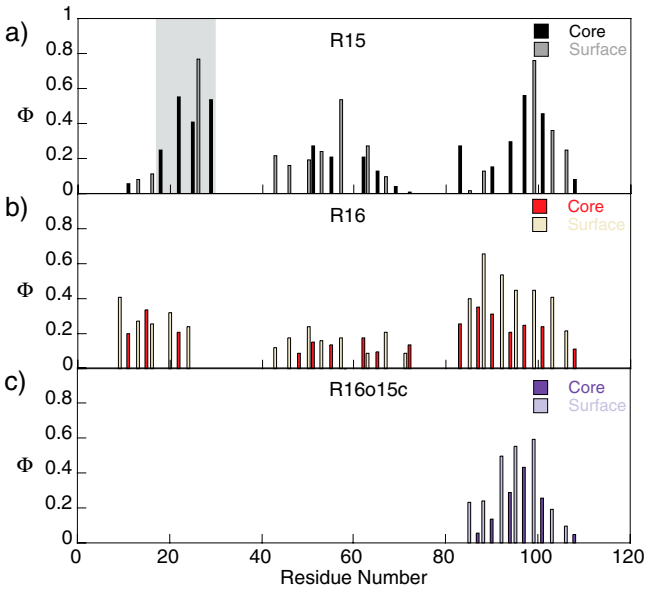

Fig. S1. Core swapped versions of R16 fold by a mechanism more like R15. (Data taken from refs. 1–3).

1. Scott KA, Randles LG, Clarke J (2004) The folding of spectrin domains II: phi-value analysis of R16. *J Mol Biol* 344:207–221.
2. Wensley BG, Gärtner M, Choo W, Batey S, Clarke J (2009) Different members of a simple three-helix bundle protein family have very different folding rate constants and fold by different mechanisms. *J Mol Biol* 390:1074–1085.
3. Wensley BG, et al. (2010) Experimental evidence for a frustrated energy landscape in a three-helix-bundle protein family. *Nature* 463:685–688.

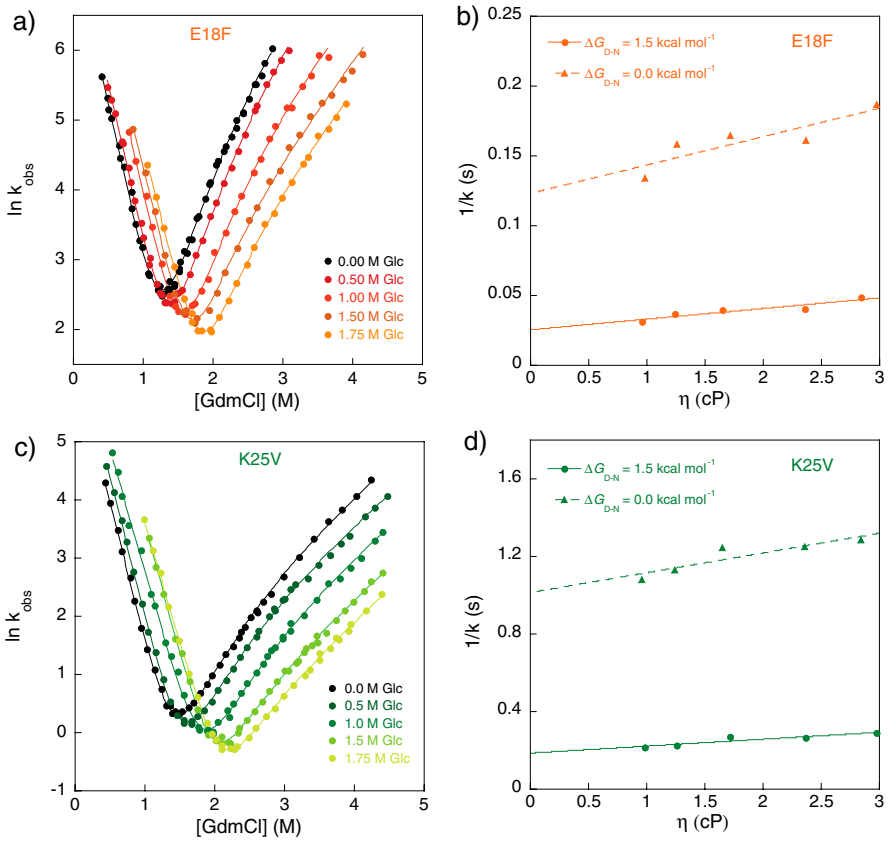

Fig. S2. Kinetics determined in increasing concentrations of glucose. (*A* and *C*) Chevron plots of E18F and K25V at increasing concentrations of glucose. (*B* and *D*) Plots of solvent viscosity vs. $1/k$ for R16 E18F and K25V at $\Delta G_{D\text{-}N} = 1.5$ and 0.0 kcal mol^{-1}, used to determine σ.

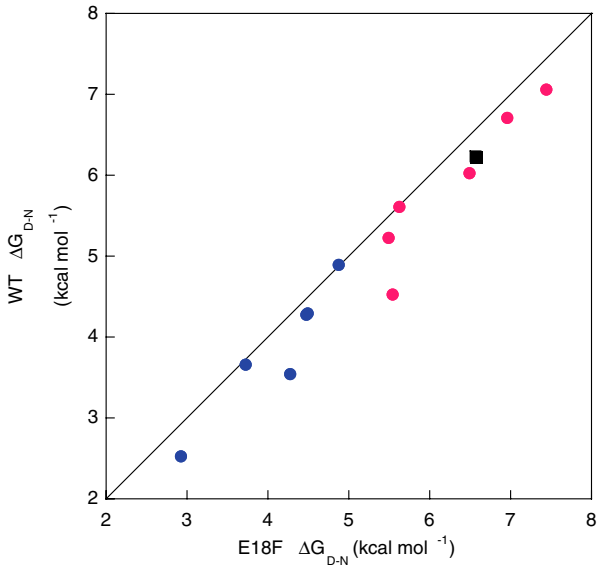

Fig. S3. The mutation E18F does not affect $\Delta G^{H_2O}_{D\text{-}N}$ for the R16 Φ-value mutations Core mutations are shown as blue circles and surface mutations as pink circles. Wild-type (i.e., wild-type R16 and the pseudo-wild-type E18F) data shown as a black square. Only positions where the same mutation was made in both wild-type R16 and E18F are shown. WT data are taken from ref. 1.

1. Scott KA, Randles LG, Clarke J (2004) The folding of spectrin domains II: phi-value analysis of R16. *J Mol Biol* 344:207–221.

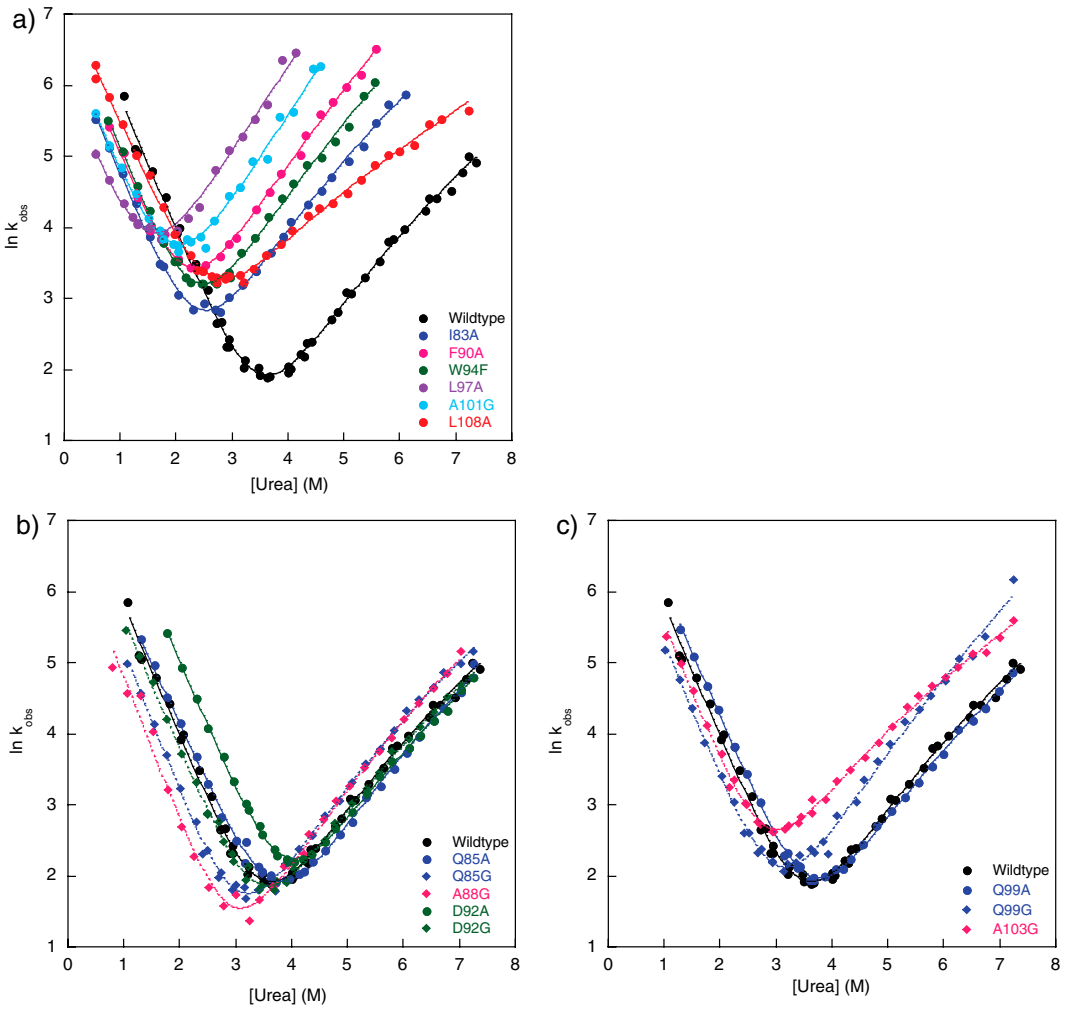

Fig. S4. Chevron plots and fits for the R16 E18F Φ-value mutants. (*A*) Core mutants. (*B* and *C*) Surface mutations where each residue was mutated to Ala and Gly and the two compared. No data were collected when $k > 600\ s^{-1}$.

Table S1. Equilibrium and kinetic parameters for R16 and R17 mutants

Domain	$[Den]_{50\%}$ (M)	m^{eqb}_{D-N} (kcal mol^{-1}M^{-1})	$\Delta G^{H_2O}_{D-N}$ (kcal mol^{-1})	$k_f^{H_2O}$ (s^{-1})	m_{k_f} (M^{-1})	$k_u^{H_2O}$ (s^{-1})	m_{k_u} (M^{-1})	$\Delta G^{H_2O}_{kin}$ (kcal mol^{-1})
R16								
R16 WT*	3.3 (±0.1)	1.9 (±0.1)	6.4 (±0.2)	126 (±2)	2.0 (±0.1)	3.2×10^{-3} ($\pm 1 \times 10^{-4}$)	1.2†	6.3 (±0.3)
Individual substitutions in A helix of R16 to residue found in R15								
R16 E18F	3.4 (±0.1)	1.9 (±0.1)	6.5 (±0.1)	2,200 (±200)	1.8 (±0.1)	6.4×10^{-2} ($\pm 9 \times 10^{-3}$)	1.2†	6.2 (±0.1)
R16 E19D	2.5 (±0.1)	2.0 (±0.1)	4.9 (±0.1)	81 (±4)	2.0 (±0.1)	1.10×10^{-2} ($\pm 8 \times 10^{-4}$)	1.2†	5.3 (±0.1)
R16 I22L	2.5 (±0.1)	2.0 (±0.1)	4.9 (±0.1)	139 (±5)	2.0 (±0.1)	3.9×10^{-2} ($\pm 2 \times 10^{-3}$)	1.2†	4.8 (±0.1)
R16 K25V	3.3 (±0.1)	1.9 (±0.1)	6.4 (±0.1)	630 (±50)	1.8 (±0.1)	1.7×10^{-2} ($\pm 3 \times 10^{-3}$)	1.2†	6.2 (±0.1)
R16 V29L	3.6 (±0.1)	1.9 (±0.1)	7.0 (±0.1)	313 (±9)	1.8 (±0.1)	3.0×10^{-3} ($\pm 3 \times 10^{-4}$)	1.2†	6.9 (±0.1)
Other substitutions of E18								
R16 E18A	3.1 (±0.1)	2.2 (±0.1)	6.0 (±0.1)	900 (±60)	2.0 (±0.1)	0.05 (±0.01)	1.2†	5.8 (±0.1)
R16 E18L	4.1 (±0.1)	1.8 (±0.1)	7.9 (±0.1)	3,800 (±200)	1.8 (±0.1)	1.4×10^{-2} ($\pm 1 \times 10^{-3}$)	1.2†	7.4 (±0.1)
R16 E18Q	3.3 (±0.1)	2.1 (±0.1)	6.2 (±0.1)	280 (±10)	2.0 (±0.1)	9.2×10^{-3} ($\pm 9 \times 10^{-4}$)	1.2†	6.1 (±0.1)
Other substitutions of K25								
R16 K25M	3.9 (±0.1)	2.2 (±0.1)	7.4 (±0.1)	1,490 (±50)	1.7 (±0.1)	7.1×10^{-3} ($\pm 1 \times 10^{-3}$)	1.2†	7.3 (±0.1)
R16 K25A‡	3.2 (±0.1)	1.8 (±0.1)	6.2 (±0.1)	400 (±10)	1.9 (±0.1)	1.2×10^{-2} ($\pm 9 \times 10^{-4}$)	1.2†	6.2 (±0.1)
R16 E18A	3.1 (±0.1)	2.2 (±0.1)	6.0 (±0.1)	900 (±60)	2.0 (±0.1)	0.05 (±0.01)	1.2†	5.8 (±0.1)
R17								
R17 WT*	3.1 (±0.1)	2.0 (±0.1)	6.0 (±0.2)	30 (±2)	2.3 (±0.1)	4.0×10^{-4} ($\pm 3 \times 10^{-5}$)	1.5 (±0.1)	6.6 (±0.3)
Substitutions at positions E18 and K25 in A helix of R17 to residue found in R15								
R17 E18F	2.2 (±0.1)	1.9 (±0.1)	4.2 (±0.1)	170 (±20)	1.8 (±0.1)	0.37 (±0.04)	1.2 (±0.1)	3.6 (±0.1)
R17 K25V	3.4 (±0.1)	1.9 (±0.1)	6.3 (±0.2)	84 (±3	2.2 (±0.1)	1.2×10^{-3} ($\pm 1 \times 10^{-4}$)	1.2 (±0.1)	6.6 (±0.1)
R17 E18F/K25V	2.0 (±0.1)	1.5 (±0.1)	3.1 (±0.1)	210 (±60)	1.5 (±0.2)	3.7 (±0.5)	0.9 (±0.1)	2.4 (±0.2)
R16 K25M	3.9 (±0.1)	2.2 (±0.1)	7.4 (±0.1)	1,490 (±50)	1.7 (±0.1)	7.1×10^{-3} ($\pm 1 \times 10^{-3}$)	1.2†	7.3 (±0.1)
R16 K25A‡	3.2 (±0.1)	1.8 (±0.1)	6.2 (±0.1)	400 (±10)	1.9 (±0.1)	1.2×10^{-2} ($\pm 9 \times 10^{-4}$)	1.2†	6.2 (±0.1)

*R15, R16, and R17 data are taken from Scott et al. (1). These data are included for comparison.
†This is the globally fitted R16 unfolding *m* value determined from the R16 Φ-value data set (2).
‡R16 K25A data taken from in Scott et al. (2).

1. Scott KA, Batey S, Hooton KA, Clarke J (2004) The folding of spectrin domains I: Wild-type domains have the same stability but very different kinetic properties. *J Mol Biol* 344:195–205.
2. Scott KA, Randles LG, Clarke J (2004) The folding of spectrin domains II: phi-value analysis of R16. *J Mol Biol* 344:207–221.

Table S2. Viscosity parameters

Domain	$\Delta G_{D\text{-}N}$ (kcal mol^{-1})	k	Slope	error	σ (cP)	error
R15*	1.5	k_f	**0.77**	0.05	**0.25**	0.21
R15*	1.5	k_u	**0.83**	0.04	**0.09**	0.14
R15*	0	$k_f = k_u$	**0.64**	0.03	**0.42**	0.13
R15*		Weighted average:	**0.72**	0.02	**0.26**	0.09
R16*	1.5	k_f	**0.17**	0.04	**3.43**	1.24
R16*	1.5	k_u	**0.28**	0.05	**3.58**	1.32
R16*	0	$k_f = k_u$	**0.16**	0.03	**6.26**	2.30
R16*		Weighted average:	**0.19**	0.02	**3.87**	0.84
R16 E18F	1.5	k_f & k_u	**0.27**	0.03	**3.34**	1.20
R16 E18F	0	$k_f = k_u$	**0.20**	0.03	**6.07**	2.59
R16 E18F		Weighted average:	**0.24**	0.02	**3.82**	1.09
R16 K25V	1.5	k_f & k_u	**0.19**	0.03	**5.23**	1.75
R16 K25V	0	$k_f = k_u$	**0.11**	0.01	**9.95**	3.18
R16 K25V		Weighted average:	**0.12**	0.01	**6.33**	1.53

*R15 and R16 data taken from Wensley et al. (1).

1. Wensley BG, et al. (2010) Experimental evidence for a frustrated energy landscape in a three-helix-bundle protein family. *Nature* 463:685–688.

Table S3. Kinetic and thermodynamic parameters for R16 E18F wild type (WT) and mutants in urea

Mutation	$\Delta G_{D\text{-}N}^{H_2O}$ (kcal mol^{-1})	$\Delta\Delta G_{D\text{-}N}^{H_2O}$ (kcal mol^{-1})*	$k_{f,TS1}^{2\ M}$ (s^{-1})	$\Phi_{f,TS1}^{2\ M}$ *†
WT	6.6 (±0.1)	-	55 (±6)	-
Core mutants				
I83A	4.5 (±0.1)	2.1 (±0.1)	19 (±2)	0.30
F90A	4.3 (±0.1)	2.3 (±0.1)	23 (±5)	0.23
W94F	4.5 (±0.1)	2.1 (±0.1)	25 (±4)	0.23
L97A	2.9 (±0.1)	3.6 (±0.1)	10 (±3)	0.28
A101G	3.7 (±0.1)	2.9 (±0.1)	19 (±4)	0.22
L108A	4.9 (±0.1)	1.7 (±0.1)	62 (±5)	-0.04
Surface mutants				
Q85A	6.8 (±0.1)	-	64 (±6)	-
Q85G	5.9 (±0.1)	0.9 (±0.1)	25 (±3)	0.62
A88G	5.5 (±0.1)	1.1 (±0.1)	16 (±4)	0.67
D92A	7.4 (±0.1)	-	160 (±10)	-
D92G	6.5 (±0.1)	1.0 (±0.1)	46 (±4)	0.76
Q99A	7.0 (±0.1)	-	73 (±5)	-
Q99G	5.6 (±0.1)	1.3 (±0.1)	30 (±5)	0.40
A103G	5.5 (±0.1)	1.0 (±0.1)	43 (±5)	0.14

*For Ala-Gly helix scanning positions values of $\Delta\Delta G_{D\text{-}N}^{H_2O}$, $\Phi_{f,TS1}^{2\ M}$, $\Phi_{f,TS2}^{2\ M}$ and are shown against the appropriate glycine mutant.

†Errors in the Φ-values were propagated form errors of the fits of the data and are <0.1.