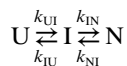


Supporting Information

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SI Text

The Φ -values were measured for the scheme



by

$$\Phi_{TS1} = \frac{\Delta\Delta G_{U-TS1}}{\Delta\Delta G_{U-N}} = \ln(k'_{UI}/k_{UI}) / \ln\left(\frac{(k'_{UI}/k'_{IU})(k'_{IN}/k'_{NI})}{(k_{UI}/k_{IU})(k_{IN}/k_{NI})}\right),$$

$$\Phi_{TS2} = \frac{\Delta\Delta G_{U-TS2}}{\Delta\Delta G_{U-N}} = \ln(k'_{UI}k'_{IU}k'_{IN}/k_{UI}k_{IU}k_{IN}) / \ln\left(\frac{(k'_{UI}/k'_{IU})(k'_{IN}/k'_{NI})}{(k_{UI}/k_{IU})(k_{IN}/k_{NI})}\right),$$

$$\Phi_I = \frac{\Delta\Delta G_{U-I}}{\Delta\Delta G_{U-N}} = \ln(k'_{UI}k_{IU}/k_{UI}k'_{IU}) / \ln\left(\frac{(k'_{UI}/k'_{IU})(k'_{IN}/k'_{NI})}{(k_{UI}/k_{IU})(k_{IN}/k_{NI})}\right),$$

and

where the prime denotes values for mutant protein.

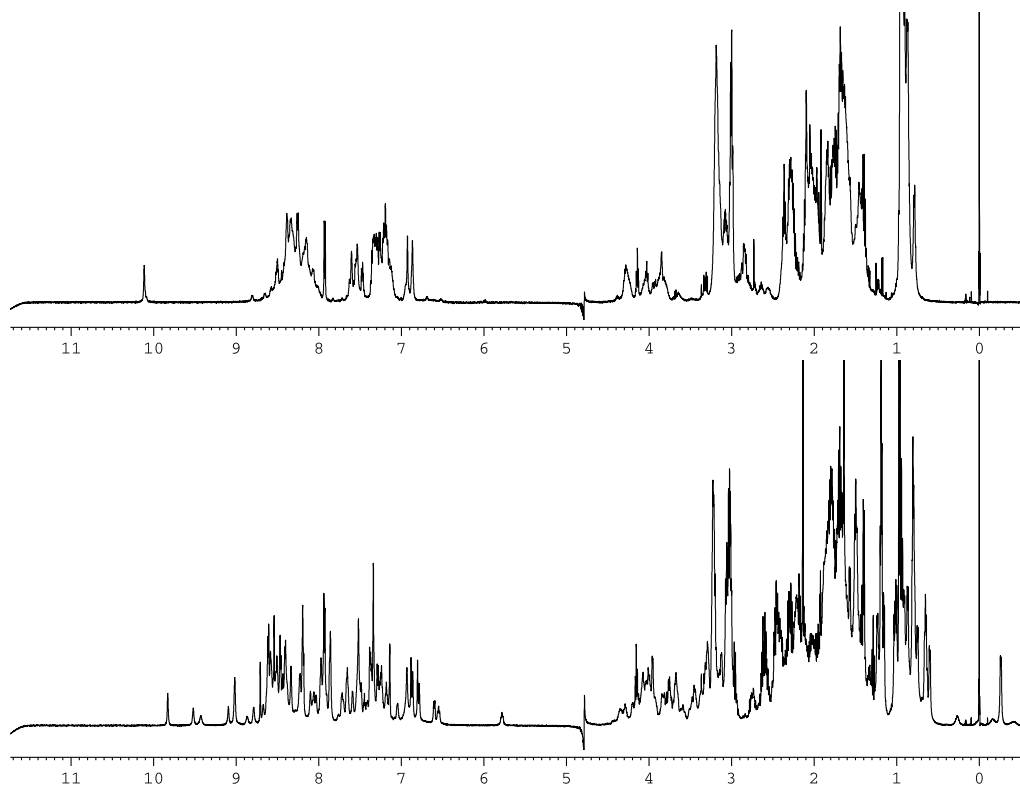


Fig. S1. One-dimensional NMR spectra of wild-type Pit1 (Lower) and peptide 16–60 (Upper, LERHFGHEHSKPSSQEIMRMAEELNLEKEVVRVWFNSNRRQREKRVK). All spectra were recorded in 50 mM acetate, pH 5.5, and 100 mM NaCl at 25 °C. Similar spectra were recorded for 16–60 at 500 mM acetate and pH 3 and pH 7 at both ionic strengths.

Table S1. Kinetic constants from free fit of chevron plots to three-state equations

	$k_{UI} \times 10^{-5}, s^{-1}$	$-m_{UI},$ kcal mol ⁻¹ M ⁻¹	$k_{IU} \times 10^{-4}, s^{-1}$	$m_{IU},$ kcal mol ⁻¹ M ⁻¹	$k_{IN} \times 10^{-4}, s^{-1}$	$-m_{IN},$ kcal mol ⁻¹ M ⁻¹	k_{NI}, s^{-1}	$m_{NI},$ kcal mol ⁻¹ M ⁻¹
WT	2.96 ± 0.14	0.19 ± 0.07	2.22 ± 1.50	0.58 ± 0.13	1.61 ± 0.09	0.10 ± 0.07	255 ± 46	0.30 ± 0.02
I8V	1.80 ± 0.15	0.28 ± 0.02	5.99 ± 0.85	0.52 ± 0.04	1.92 ± 0.16	0.00 ± 0.01	1019 ± 124	0.23 ± 0.01
S9A	2.42 ± 0.06	0.10 ± 0.06	0.41 ± 0.45	1.34 ± 0.34	1.41 ± 0.05	0.40 ± 0.07	2260 ± 219	0.19 ± 0.02
I10A	3.50 ± 0.15	0.16 ± 0.04	0.31 ± 0.35	0.79 ± 0.20	1.44 ± 0.06	0.22 ± 0.04	420 ± 115	0.17 ± 0.03
I10G	3.56 ± 0.13	0.11 ± 0.03	0.03 ± 0.04	1.40 ± 0.29	1.49 ± 0.06	0.32 ± 0.03	968 ± 168	0.14 ± 0.02
A11G	2.67 ± 0.19	0.30 ± 0.12	1.24 ± 0.97	0.87 ± 0.18	1.30 ± 0.09	0.08 ± 0.13	1,170 ± 286	0.18 ± 0.04
A12G	1.66 ± 0.56	0.38 ± 0.30	7.05 ± 5.09	0.40 ± 0.19	2.04 ± 0.68	0.02 ± 0.31	938 ± 169	0.30 ± 0.03
K13A	3.08 ± 0.05	0.10 ± 0.01	0.15 ± 0.11	0.71 ± 0.11	1.69 ± 0.03	0.28 ± 0.01	171 ± 34	0.23 ± 0.02
D14A	3.42 ± 0.16	0.14 ± 0.05	0.58 ± 0.80	0.57–0.20	1.49 ± 0.06	0.23 ± 0.05	77 ± 47	0.37 ± 0.06
D14G	2.32 ± 0.09	0.07 ± 0.06	1.13 ± 0.80	0.87 ± 0.15	1.18 ± 0.04	0.15 ± 0.06	604 ± 78	0.26 ± 0.02
A15G	2.06 ± 1.21	0.16 ± 0.41	2.59 ± 11.57	0.68 ± 1.23	1.30 ± 0.82	0.38 ± 0.49	1,642 ± 685	0.24 ± 0.07
E17A	2.47 ± 0.14	0.00 ± 0.03	0.58 ± 0.95	0.60 ± 0.31	1.67 ± 0.09	0.45 ± 0.07	3,611 ± 1,228	0.20 ± 0.05
R18A	3.18 ± 0.08	0.28 ± 0.04	1.67 ± 0.74	0.66 ± 0.09	1.78 ± 0.05	0.18 ± 0.05	1,250 ± 147	0.18 ± 0.02
R18G	1.88 ± 0.25	0.34 ± 0.08	4.27 ± 1.46	0.61 ± 0.12	1.83 ± 0.27	0.00 ± 0.03	1,943 ± 655	0.23 ± 0.05
F20A	1.68 ± 0.55	0.28 ± 0.36	6.19 ± 5.46	0.53 ± 0.20	1.33 ± 0.39	0.00 ± 0.33	3,592 ± 1,807	0.25 ± 0.10
G21A	2.73 ± 0.98	0.25 ± 0.20	10.97 ± 9.49	0.23 ± 0.13	2.32 ± 0.85	0.12 ± 0.20	75 ± 35	0.27 ± 0.04
E22A	3.58 ± 0.10	0.18 ± 0.04	0.40 ± 0.36	0.97 ± 0.20	1.71 ± 0.05	0.24 ± 0.04	791 ± 125	0.23 ± 0.02
E22G	3.65 ± 0.28	0.36 ± 0.12	3.42 ± 2.86	0.75 ± 0.26	1.82 ± 0.18	0.10 ± 0.13	2,170 ± 407	0.19 ± 0.03
S24A	2.33 ± 0.14	0.16 ± 0.03	0.23 ± 0.11	0.96 ± 0.10	0.73 ± 0.03	0.00 ± 0.02	403 ± 99	0.26 ± 0.03
S24G	4.12 ± 0.11	0.09 ± 0.04	0.35 ± 0.52	1.00 ± 0.33	1.46 ± 0.05	0.28 ± 0.05	1,141 ± 335	0.25 ± 0.04
P26A	0.39 ± 1.06	0.00 ± 0.00	15.18 ± 14.91	0.20 ± 0.23	9.15 ± 25.11	0.20 ± 1.16	615 ± 858	0.39 ± 0.16
S27A	2.11 ± 0.30	0.00 ± 0.03	3.57 ± 2.13	0.83 ± 0.20	1.07 ± 0.15	0.42 ± 0.07	991 ± 185	0.23 ± 0.03
S28A	3.18 ± 0.13	0.17 ± 0.05	0.51 ± 0.57	0.68 ± 0.18	1.85 ± 0.08	0.18 ± 0.05	380 ± 118	0.24 ± 0.03
S28G	2.62 ± 0.14	0.15 ± 0.09	1.14 ± 1.24	0.85 ± 0.25	0.99 ± 0.05	0.12 ± 0.10	337 ± 81	0.29 ± 0.03
I31V	2.31 ± 0.30	0.31 ± 0.05	5.33 ± 1.59	0.53 ± 0.08	1.32 ± 0.17	0.00 ± 0.01	309 ± 114	0.40 ± 0.05
M32A	3.02 ± 0.07	0.15 ± 0.02	0.21 ± 0.09	0.83 ± 0.07	1.52 ± 0.03	0.27 ± 0.01	228 ± 57	0.24 ± 0.02
M32G	2.43 ± 0.14	0.24 ± 0.09	1.83 ± 1.32	0.71 ± 0.16	0.62 ± 0.04	0.02 ± 0.10	336 ± 64	0.20 ± 0.02
M34A	1.27 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	3.09 ± 4.53	0.79 ± 0.05	0.39 ± 0.10	295 ± 542	0.53 ± 0.40
A35G	2.02 ± 0.36	0.00 ± 0.01	6.45 ± 2.81	1.05 ± 0.25	0.57 ± 0.11	0.18 ± 0.09	491 ± 120	0.25 ± 0.04
E37A	2.18 ± 0.05	0.19 ± 0.05	0.73 ± 0.37	1.09 ± 0.14	1.27 ± 0.03	0.13 ± 0.05	1,320 ± 87	0.21 ± 0.01
E37G	1.97 ± 0.61	0.00 ± 0.08	18.62 ± 4.72	0.82 ± 0.10	1.81 ± 0.56	0.01 ± 0.08	393 ± 285	0.54 ± 0.16
N39A	2.44 ± 0.21	0.23 ± 0.06	3.20 ± 1.17	0.76 ± 0.11	0.96 ± 0.10	0.00 ± 0.03	635 ± 218	0.23 ± 0.05
L40V	2.67 ± 0.07	0.20 ± 0.04	0.21 ± 0.23	1.14 ± 0.29	1.17 ± 0.03	0.25 ± 0.04	657 ± 109	0.33 ± 0.02
E41A	2.80 ± 0.16	0.23 ± 0.10	1.59 ± 1.54	0.74 ± 0.22	2.09 ± 0.13	0.15 ± 0.10	1,234 ± 249	0.20 ± 0.02
E41G	1.67 ± 1.25	0.06 ± 0.56	6.44 ± 10.65	0.72 ± 0.41	2.77 ± 2.11	0.00 ± 0.60	867 ± 650	0.32 ± 0.11
K42A	2.57 ± 1.39	0.22 ± 0.14	7.05 ± 12.60	0.05 ± 0.16	3.3 ± 1.78	0.15 ± 0.15	11 ± 12	0.40 ± 0.08
E43A	2.37 ± 0.13	0.31 ± 0.03	7.21 ± 0.81	0.60 ± 0.04	1.17 ± 0.07	0.00 ± 0.02	829 ± 88	0.25 ± 0.02
E43G	2.32 ± 0.18	0.32 ± 0.07	4.70 ± 1.54	0.79 ± 0.16	0.90 ± 0.09	0.00 ± 0.04	782 ± 88	0.26 ± 0.02
V44A	2.92 ± 0.13	0.27 ± 0.03	1.13 ± 0.30	1.01 ± 0.08	1.67 ± 0.08	0.00 ± 0.01	1,887 ± 186	0.15 ± 0.01
R46A	1.81 ± 0.05	0.12 ± 0.03	0.35 ± 0.22	0.68 ± 0.11	0.94 ± 0.03	0.02 ± 0.03	385 ± 100	0.23 ± 0.02
V47A	2.66 ± 0.07	0.14 ± 0.01	0.01 ± 0.01	1.23 ± 0.22	2.42 ± 0.06	0.27 ± 0.01	892 ± 141	0.19 ± 0.02
V47G	1.97 ± 0.05	0.19 ± 0.04	0.58 ± 0.45	0.65 ± 0.15	1.71 ± 0.04	0.23 ± 0.04	663 ± 180	0.31 ± 0.04
F49A	11.93 ± 3.73	0.25 ± 0.46	50.33 ± 38.67	0.88 ± 0.22	1.09 ± 0.33	0.00 ± 0.10	4,108 ± 1,000	0.21 ± 0.06
S50A	3.72 ± 0.12	0.16 ± 0.03	0.19 ± 0.17	0.84 ± 0.15	1.65 ± 0.06	0.22 ± 0.03	450 ± 92	0.18 ± 0.02
S50G	2.44 ± 0.22	0.37 ± 0.04	6.36 ± 1.24	0.58 ± 0.07	1.61 ± 0.15	0.00 ± 0.00	862 ± 162	0.24 ± 0.03

The fast and slow phases for each mutant were fitted simultaneously to the standard equations for three-state chevron plots. Experiments were performed in 50 mM acetate, 100 mM NaCl (pH 5.5) at 25.0 °C; k_{UI} is the rate constant for U → I, etc.

Table S2. Kinetic parameters from global fit of chevron plots to three-state equations

	$k_{UI} \times 10^{-5}, s^{-1}$	$k_{IU} \times 10^{-4}, s^{-1}$	$k_{IN} \times 10^{-4}, s^{-1}$	k_{NI}, s^{-1}	$\Delta G_{(U-N)kin}, kcal/mol$
WT	3.19 ± 0.09	0.90 ± 0.18	1.69 ± 0.05	480 ± 28	4.22 ± 0.12
I8V	2.43 ± 0.11	2.10 ± 0.33	1.66 ± 0.07	1017 ± 50	3.10 ± 0.10
S9A	1.98 ± 0.09	2.78 ± 0.37	1.58 ± 0.07	1679 ± 69	2.49 ± 0.09
I10A	3.45 ± 0.11	0.35 ± 0.09	1.50 ± 0.05	225 ± 15	5.20 ± 0.15
I10G	3.43 ± 0.08	0.69 ± 0.04	1.51 ± 0.04	428 ± 10	4.43 ± 0.04
A11G	2.28 ± 0.09	1.65 ± 0.27	1.61 ± 0.07	781 ± 39	3.35 ± 0.11
A12G	2.09 ± 0.07	2.02 ± 0.29	1.42 ± 0.05	1317 ± 60	2.79 ± 0.10
K13A	3.27 ± 0.09	0.19 ± 0.05	1.56 ± 0.04	163 ± 11	5.76 ± 0.17
D14A	3.46 ± 0.09	0.27 ± 0.07	1.38 ± 0.04	254 ± 17	5.24 ± 0.16
D14G	2.48 ± 0.10	1.92 ± 0.31	1.40 ± 0.06	730 ± 36	3.26 ± 0.10
A15G	1.86 ± 0.09	3.03 ± 0.43	1.23 ± 0.07	1,580 ± 65	2.29 ± 0.10
E17A	2.19 ± 0.10	2.91 ± 0.38	1.65 ± 0.08	4,266 ± 553	2.00 ± 0.12
R18A	2.88 ± 0.09	0.94 ± 0.18	1.77 ± 0.06	782 ± 46	3.87 ± 0.12
R18G	2.03 ± 0.10	2.23 ± 0.34	1.76 ± 0.09	1,846 ± 93	2.64 ± 0.10
F20A	2.07 ± 0.09	2.78 ± 0.40	1.11 ± 0.06	3,838 ± 161	1.81 ± 0.10
G21A	4.33 ± 0.13	0.54 ± 0.14	1.47 ± 0.05	123 ± 8	5.42 ± 0.16
E22A	3.20 ± 0.10	1.00 ± 0.19	1.76 ± 0.06	693 ± 41	3.97 ± 0.12
E22G	3.29 ± 0.14	2.40 ± 0.45	1.91 ± 0.09	1482 ± 83	3.06 ± 0.12
S24A	2.51 ± 0.18	0.43 ± 0.07	1.08 ± 0.06	491 ± 24	4.24 ± 0.11
S24G	4.09 ± 0.08	1.30 ± 0.08	1.47 ± 0.04	1,223 ± 25	3.51 ± 0.04
P26A	2.94 ± 0.12	1.51 ± 0.14	1.18 ± 0.06	1,810 ± 80	2.87 ± 0.07
S27A	1.84 ± 0.15	6.04 ± 0.74	1.18 ± 0.10	962 ± 42	2.14 ± 0.10
S28A	3.15 ± 0.08	0.34 ± 0.08	1.96 ± 0.05	382 ± 25	5.01 ± 0.15
S28G	2.60 ± 0.10	1.50 ± 0.13	1.11 ± 0.04	486 ± 14	3.54 ± 0.06
I31V	2.47 ± 0.10	2.19 ± 0.35	1.13 ± 0.05	890 ± 42	2.94 ± 0.10
M32A	3.06 ± 0.08	0.30 ± 0.07	1.52 ± 0.04	247 ± 15	5.18 ± 0.15
M32G	2.60 ± 0.09	0.98 ± 0.09	0.73 ± 0.03	261 ± 7	3.91 ± 0.07
M34A	1.23 ± 0.04	0.68 ± 0.12	0.64 ± 0.03	1,137 ± 65	2.73 ± 0.12
A35G	1.69 ± 0.19	10.82 ± 1.29	0.76 ± 0.09	518 ± 21	1.85 ± 0.12
E37A	1.91 ± 0.06	1.92 ± 0.13	1.46 ± 0.04	1,051 ± 17	2.92 ± 0.05
E37G	1.62 ± 0.27	23.23 ± 1.07	2.30 ± 0.36	1,500 ± 44	1.40 ± 0.14
N39A	2.61 ± 0.11	2.59 ± 0.39	1.11 ± 0.05	643 ± 32	3.06 ± 0.10
L40V	2.20 ± 0.08	1.02 ± 0.19	1.12 ± 0.04	1,119 ± 55	3.18 ± 0.12
E41A	2.72 ± 0.10	1.22 ± 0.22	2.22 ± 0.08	883 ± 51	3.75 ± 0.12
E41G	2.21 ± 0.39	5.13 ± 2.35	2.77 ± 0.50	1,632 ± 78	2.54 ± 0.31
K42A	3.38 ± 0.06	0.02 ± 0.00	2.43 ± 0.04	125 ± 4	7.58 ± 0.06
E43A	2.72 ± 0.13	3.78 ± 0.54	1.08 ± 0.05	896 ± 40	2.64 ± 0.10
E43G	2.33 ± 0.20	3.65 ± 0.98	0.93 ± 0.05	907 ± 16	2.48 ± 0.17
V44A	2.77 ± 0.16	1.90 ± 0.18	2.41 ± 0.14	970 ± 36	3.49 ± 0.08
R46A	1.89 ± 0.04	0.28 ± 0.02	0.91 ± 0.02	362 ± 5	4.40 ± 0.04
V47A	2.55 ± 0.05	0.14 ± 0.01	2.39 ± 0.04	555 ± 14	5.29 ± 0.04
V47G	1.84 ± 0.04	0.40 ± 0.08	1.56 ± 0.04	971 ± 56	3.92 ± 0.13
F49A	9.77 ± 0.84	67.25 ± 3.45	1.49 ± 0.12	3,881 ± 112	1.02 ± 0.08
S50A	3.55 ± 0.09	0.29 ± 0.07	1.73 ± 0.05	237 ± 16	5.38 ± 0.16
S50G	2.64 ± 0.10	3.02 ± 0.19	1.47 ± 0.05	900 ± 23	2.94 ± 0.05

The fast and slow phases for each mutant were fitted simultaneously to the standard equations for three-state chevron plots, and the results for all mutants were simultaneously fitted assuming that each rate constant had a common value throughout the series. Values reported at 0 M urea. The global m values ($kcal\ mol^{-1}\ M^{-1}$) obtained from the fit were m_{UI} , -0.14; m_{IU} , 0.73; m_{IN} , -0.23; and m_{NI} , 0.24.

Table S3. Mutants of Pit1 chosen for Φ -analysis, their interactions and accessible solvent exposed area (ASA)

Mutant	Location	% ASA	Residue (no. of contacts removed)	Overall interaction probed
I8V	N term	18.3	Thr6(4), Thr7(3), Leu16(1),Leu40(3), Val44(5), Trp48(7)	packing of the hydrophobic core
S9A	N cap	71.3	Ile8(4), Ile10(5), Ala11(5), Ala12(4), Lys13(1)	degree of formation of H1
I10A	helix 1	100	Ile8(1), Ser9(7), Ala11(6), Asp14(4)	local interactions within H1
I10G	helix 1	100	Ile8(2), Ser9(12), Ala11(10),Ala12(1), Lys13(1), Asp14(7)	degree of formation of H1 -Ala → Gly scan
A11G	helix 1	100	Ser9(5), Ile10(6), Ala12(4), Lys13(1), Asp14(4)	degree of formation of H1 -Ala → Gly scan
A12G	helix 1	9.2	Ile8(6), Ser9(6), Ile10(4), Ala11(5), Lys13(4), Asp14(1),Leu16(2), Leu38(5), Leu40(3)	packing of the hydrophobic core
K13A	helix 1	71.2	Ile8(3), Ser9(4), Ile10(7), Ala12(2), Asp14(11), Leu16(1), Glu17(2), Trp48(7)	local interactions within H1 and between H1 and H3
D14A	helix 1	65.6	Ile10(15), Ala11(14), Ala12(3), Lys13(15), Ala15(4)	local interactions within H1
D14G	helix 1	65.6	Ile10(19), Ala11(19), Ala12(7),Lys13(21), Ala15(8), Leu16(1)	degree of formation of H1 -Ala → Gly scan
A15G	helix 1	29.1	Ala11(2), Ala12(4), Lys13(3), Asp14(5), Leu16(6), Glu17(1), Arg18(3), His19(4), Met34(1), Glu37(3), Leu38(3)	packing of the hydrophobic core
E17A	helix 1	47.6	Lys13(1), Leu16(6), Arg18(8), Phe20(26), Gly21(5), Trp48(4), Arg52(22)	local interactions within H1 and between H1 and H3
R18A	helix 1	69.1	Asp14(5), Ala15(11), Glu17(2), His19(20), Glu22(14)	local interactions within H1
R18G	helix 1	69.1	Asp14(7), Ala15(16), Leu16(4),Glu17(7), His19(27), Glu22(18)	degree of formation of H1 -Ala → Gly scan
F20A	helix 1	8.1	Leu16(3), Glu17(20), His19(6), Gly21(9), His23(5), Ser24(24), Lys25(2), Trp48(10), Phe49(24),Arg52(35), Arg53(11), Glu56(8)	packing of the hydrophobic core
G21A	helix 1	74.1	N/A	local interactions within H1
E22A	helix 1	61.4	Arg18(29), His19(29), Phe20(4),Gly21(5), His23(4)	local interactions within H1
E22G	helix 1	61.4	Arg18(32), His19(34), Phe20(8),Gly21(9), His23(9)	degree of formation of H1 -Ala → Gly scan
S24A	loop	56	Phe20(10), His23(2), Lys25(3),Phe49(2), Arg53(8), Glu56(3)	local interactions within the loop
S24G	loop	56	Phe20(10), His23(2), Lys25(3),Phe49(2), Arg53(8), Glu56(3)	degree of the loop between H1 and H2 formation
P26A	loop	0.3	His19(1), His23(18), Ser24(9), Lys25(10), Ser27(6), Glu30(8), Ile31(7), Met34(2), Phe49(14), Arg53(4)	packing of the hydrophobic core
S27A	N cap	60	Pro26(4), Ser28(5), Gln29(9), Glu30(7), Ile31(1)	degree of formation of H2
S28A	helix 2	91.3	Ser27(2), Gln29(2), Ile31(1)	local interactions within H2
S28G	helix 2	91.3	Ser27(2), Gln29(2), Ile31(1)	degree of formation of H2 -Ala → Gly scan
I31V	helix 2	7.7	Pro26(7), Ser27(4), Ser28(3), Glu30(1), Met32(1), Lys42(2), Val45(5), Arg46(7), Phe49(4), Arg53(1)	packing of the hydrophobic core
M32A	helix 2	58.8	Ser28(3), Gln29(5), Ile31(3), Arg33(5), Ala35(2), Glu36(12), Lys42(12)	local interactions within H2 and between H2 and H3
M32G	helix 2	58.8	Ser28(3), Gln29(5), Ile31(3), Arg33(5), Ala35(2), Glu36(12), Lys42(12)	degree of formation of H2 -Ala → Gly scan
M34A	helix 2	3.1	Ala15(3), Leu16(12), His19(23),Phe20(1), Pro26(3), Glu30(9), Ile31(10), Arg33(5), Ala35(2), Glu37(4), Leu38(3), Val45(4), Phe49(10)	packing of the hydrophobic core
A35G	helix 2	5.5	Ile31(4), Met32(7), Arg33(4), Met34(5), Glu36(5), Glu37(1), Asn39(1), Leu40(5), Glu41(4), Lys42(6), Val45(4)	packing of the hydrophobic core
E37A	helix 2	49.9	Ala15(4), His19(9), Arg33(6),Met34(22), Glu36(2), Leu38(22)	local interactions within H2 and between H1 and H2
E37G	helix 2	49.9	Arg33(9), Met34(28), Ala35(4),Glu36(7), Leu38(29), Asn39(1)	degree of formation of H2 -Ala → Gly scan
N39A	turn/C cap	100	Ala35(2), Glu36(4), Leu38(7), Leu40(6)	degree of formation of H2
L40V	turn	12.8	Thr6(1), Thr7(1), Ile8(3), Ala12(1), Leu38(3), Asn39(2),Glu41(1), Val44(3), Val45(1)	packing of the hydrophobic core
E41A	turn/N cap	89.5	Leu40(11), Lys42(3), Glu43(1), Val44(4)	degree of formation of H3
E41G	turn	89.5	Leu40(16), Lys42(8), Glu43(6), Val44(8)	degree of formation of the turn
K42A	helix 3	44.1	Ser27(2), Ser28(15), Gln29(1), Ile31(20), Met32(24), Ala35(1), Glu41(2), Glu43(6), Val45(2), Arg46(6)	local interactions within H3 and between H2 and H3
E43A	helix 3	66	Glu41(1), Lys42(6), Val44(7), Arg46(29), Val47(11)	local interactions within H3
E43G	helix 3	66	Glu41(5), Lys42(12), Val44(12),Val45(1), Arg46(33), Val47(12)	degree of formation of H3 -Ala → Gly scan
V44A	helix 3	19.9	Thr6(9), Ile8(3), Leu40(12), Glu41(10), Lys42(3), Glu43(8), Val45(6), Trp48(1)	packing of the hydrophobic core
R46A	helix 3	50.9	Ile31(6), Lys42(8), Val45(2),Glu43(36), Val47(23), Ser50(8)	local interactions within H3 and between H2 and H3
V47A	helix 3	53.8	Glu43(9), Val44(5), Val45(1), Arg46(14), Trp48(6), Ser50(4), Asn51(7)	local interactions within H3
V47G	helix 3	53.8	Glu43(15), Val44(9), Val45(4),Arg46(23), Trp48(10), Phe49(1),Ser50(7), Asn51(10)	degree of formation of H3 -Ala → Gly scan
F49A	helix 3	0.1	Leu16(15), His19(11), Phe20(33),His23(7), Ser24(16), Lys25(8),Pro26(28), Ile31(10), Met34(11),Val45(16), Arg46(8), Trp48(32),Ser50(4), Arg52(3), Arg53(15)	packing of the hydrophobic core
S50A	helix 3	43	Arg46(10), Val47(6), Trp48(2),Phe49(5), Asn51(1), Arg53(4)	local interactions within H3
S50G	helix 3	43	Arg46(14), Val47(12), Trp48(5),Phe49(10), Asn51(7), Arg52(1),Arg53(10)	local interactions within H3

Table S4. Φ -values at individual positions in Pit1

	Position	Interaction, s	$\Delta\Delta G_{(U-D)}$, kcal/mol	Φ_{TS1}	Φ_I	Φ_{TS2}
I8V	N terminus	H1/core	-1.12	0.14 ± 0.03	0.59 ± 0.09	0.60 ± 0.09
S9A	N cap H1	H1	-1.73	0.16 ± 0.02	0.55 ± 0.05	0.57 ± 0.05
I10A	H1	H1	0.98	0.05 ± 0.03	0.61 ± 0.13	0.54 ± 0.11
K13A	H1	H1	1.54	0.01 ± 0.01	0.62 ± 0.09	0.59 ± 0.08
D14A	H1	H1	1.02	0.05 ± 0.02	0.75 ± 0.15	0.63 ± 0.13
E17A	H1	H1/H2	-2.22	0.1 ± 0.02	0.41 ± 0.03	0.42 ± 0.03
R18A	H1	H1	-0.35	ND	ND	ND
F20A	H1	core	-2.41	0.11 ± 0.01	0.38 ± 0.03	0.49 ± 0.03
G21A	H1	H1	1.2	0.15 ± 0.03	0.4 ± 0.07	0.33 ± 0.06
E22A	H1	H1	-0.25	ND	ND	ND
S24A	loop	H1/H3	0.02	ND	ND	ND
S24G	loop	H1/H3	-0.71	-0.21 ± 0.05	0.1 ± 0.03	0.22 ± 0.05
P26A	loop	core	-1.35	0.04 ± 0.02	0.26 ± 0.04	0.42 ± 0.05
S27A	N cap H2	H2	-2.08	0.16 ± 0.03	0.7 ± 0.06	0.8 ± 0.07
S28A	H2	H2	0.79	-0.01 ± 0.03	0.72 ± 0.18	0.83 ± 0.21
I31V	H2	core	-1.28	0.12 ± 0.03	0.53 ± 0.07	0.71 ± 0.09
M32A	H2	H2/H3	0.96	-0.03 ± 0.02	0.66 ± 0.14	0.59 ± 0.12
M34A	H2	core	-1.49	0.38 ± 0.05	0.27 ± 0.04	0.66 ± 0.08
E37A	H2	H1/H2	-1.3	0.23 ± 0.03	0.58 ± 0.06	0.64 ± 0.07
N39A	C cap H2/turn	H2	-1.16	0.10 ± 0.03	0.64 ± 0.09	0.85 ± 0.12
L40V	turn	core	-1.04	0.21 ± 0.04	0.28 ± 0.05	0.52 ± 0.09
E41A	N cap H1/turn	H3	-0.47	0.20 ± 0.09	0.58 ± 0.22	0.24 ± 0.10
E41G	N cap H1/turn	H3	-1.68	0.13 ± 0.07	0.74 ± 0.16	0.57 ± 0.13
K42A	H3	H2/H3	3.36	0.01 ± 0.01	0.70 ± 0.03	0.76 ± 0.03
E43A	H3	H3	-1.58	0.06 ± 0.02	0.60 ± 0.06	0.77 ± 0.08
V44A	H3	core	-0.73	0.11 ± 0.06	0.72 ± 0.15	0.43 ± 0.10
R46A	H3	H2/H3	0.18	ND	ND	ND
V47A	H3	H3	1.07	-0.12 ± 0.02	0.89 ± 0.11	1.08 ± 0.13
F49A	H3	core	-3.2	-0.21 ± 0.02	0.59 ± 0.03	0.61 ± 0.03
S50A	H3	H3	1.16	0.06 ± 0.02	0.63 ± 0.11	0.64 ± 0.11
S50G	H3	H3	-1.28	0.09 ± 0.02	0.65 ± 0.07	0.71 ± 0.08

Calculated from data from global analysis at 0 M denaturant (Table S2). Errors are the standard errors calculated from those propagated mathematically from the errors determined for each rate constant. ND, not determined

Table S5. Φ -values for Ala → Gly scanning of helices

	Position	Interaction, s	$\Delta\Delta G_{(U-N)}$, kcal/mol	Φ_{TS1}	Φ_I	Φ_{TS2}
A10G*	H1	H1	-0.77	0.00 ± 0.03	0.51 ± 0.11	0.51 ± 0.11
A11G [†]	H1	H1	-0.87	0.23 ± 0.05	0.64 ± 0.12	0.67 ± 0.13
A12G [†]	H1	H1/core	-1.43	0.18 ± 0.03	0.51 ± 0.06	0.58 ± 0.07
A14G*	H1	H1	-1.98	0.10 ± 0.02	0.69 ± 0.07	0.68 ± 0.07
A15G [†]	H1	H1/core	-1.93	0.17 ± 0.02	0.54 ± 0.05	0.63 ± 0.05
A18G*	H1	H1	-1.23	0.17 ± 0.04	0.58 ± 0.08	0.58 ± 0.08
A22G*	H1	H1	-0.91	-0.02 ± 0.03	0.56 ± 0.11	0.50 ± 0.10
A28G*	H2	H2	-1.47	0.08 ± 0.02	0.68 ± 0.08	0.9 ± 0.1
A32G*	H2	H2/H3	-1.27	0.08 ± 0.02	0.63 ± 0.08	0.97 ± 0.13
A35G [†]	H2	core	-2.37	0.16 ± 0.03	0.78 ± 0.07	0.98 ± 0.08
A37G*	H2	H1/H2	-1.52	0.07 ± 0.07	1.04 ± 0.12	0.86 ± 0.11
A47G*	H3	H3	-1.37	0.14 ± 0.02	0.57 ± 0.06	0.76 ± 0.07

*Calculated from the data for Table S2 using the values of wild type → Ala and wild type → Gly.

[†]Wild-type protein has Ala at these positions.