Supplementary Table 1: Parameters for the various models employed derived from the fitting to experimental heat capacity profile of PDD.

Mean-Field Model $\Delta S_{res}^{conf} = 0.0165$ kJ/(mol.K) $k_{\Delta Cp} = 4.3$	Index	$\Delta C_{p,res}$ J/(mol.K)	$k_{\Delta H}$	ΔH_{res}^{385} kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	1	0	1.89	6.12	6.87	0.0304	0.977	1 (327.6)	0.1224	0.3772
	2	10	2.01	6.13	7.10	0.0247	0.794	1.86 (326.8)	0.1491	0.0777
	3	20	2.15	6.13	7.30	0.0194	0.624	3.3 (326.2)	0.2695	2.062e-4
	4	30	2.34	6.14	7.45	0.0147	0.473	5.8 (326.1)	0.4837	9.130e-9
Muñoz Eaton Model - Exact Solution PDB id: 2PDD NN = 1 → includes nearest neighbors NN = 0 → excludes nearest neighbors	Index	**Cut-off(Å)/NN**	ΔS_{res}^{conf} kJ/(mol.K)	**ΔH_{cont}** kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	5	4.0/1	-0.0205	-0.3292	7.11	0.0167	0.537	0.88 (323)	0.6684	1.77e-11
	6	4.5/1	-0.0176	-0.1761	6.67	0.0168	0.594	2.37 (326)	0.3392	1.043e-5
	7	5.0/1	-0.0147	-0.1018	7.40	0.0236	0.759	3.2 (327)	0.2696	6.210e-4
	8	6.0/1	-0.0124	-0.0483	7.48	0.0264	0.849	5.25 (327)	0.3501	4.528e-5
	9	4.0/0	-0.0152	-0.6694	7.45	0.0229	0.736	5.3 (327)	0.3532	2.157e-5
	Index	**Baseline Used**	**f**	**Σα**	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)**	**SLS**	**P$_h$**

				kJ/mol				kJ/mol		
Variable Barrier Model FB → Freire Baseline	10	FB - σ	0.84	120.5	5.89	0.0251	0.81	-0.11 (321.4)	0.8349	3.19e-13
	11	FB	1.0	102.7	6.14	0.0311	1.00	0.13 (322.9)	0.2900	6.142e-4
	12	FB + σ	1.0	138.1	6.39	0.0371	1.183	1.10 (322.0)	0.7934	1.63e-12
	13	Floating Baseline	1.0	139.2	6.65	0.0307	0.987	1.13 (322.7)	0.1238	0.3592
	14	Baseline 2	0.72	143.1	7.10	0.0247	0.794	1.79 (323.6)	0.1900	0.0162
	15	Baseline 1	1.0	142.5	6.87	0.0304	0.978	1.96 (322.6)	0.1524	0.1195
	16	Baseline 3	0.43	148.2	7.30	0.0194	0.824	2.16 (325.2)	0.2531	1.701e-3
	17	Baseline 8	0.48	144.5	7.45	0.0229	0.736	4.32 (327.0)	0.2888	2.628e-4
	18	Baseline 4	0.1 (fixed)	167.1	7.45	0.0147	0.473	4.47 (331.1)	0.3748	5.946e-7
Muñoz Eaton Model - Single Sequence	Index	$\mathbf{a_{ASA}}$ J/(mol.K.Å^2)	ΔS_{res}^{385} kJ/(mol.K)	$\Delta H_{res}^{T_{ref}}$ kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	19	0	-0.0186	-6.16 (385 K)	6.64	0.0297	0.955	0	0.6244	1.612e-9
	20	0.05	-0.0181	-5.69 (322 K)	7.03	0.0273	0.878	0.35 (322.2)	0.2693	1.100e-3

Approximation PDD ID: 2PDD	21	0.11	-0.0177	-5.18 (322 K)	7.05	0.0238	0.765	1.70 (320.2)	0.1592	0.0445
	22	0.19	-0.0177	-4.72 (322 K)	8.02	0.0198	0.637	3.32 (318.2)	0.2277	1.200e-3
	23	0.29	-0.0182	-4.28 (322 K)	8.60	0.0146	0.473	6.00 (316.2)	0.4006	2.211e-7

* The gray shaded boxes are the free parameters for the corresponding model.

* The yellow shaded boxes are the best fit parameters from each model.

* b_0 = 0.0311 kJ/ (mol K^2) is the Freire Baseline (FB) slope for PDD

Supplementary Table 2: Low-temperature heat capacity baseline slopes*

Index	Protein	dC_p/dT kJ/(K^2 g)	MW	Source	Index	Protein	dC_p/dT kJ/(K^2 g)	MW	Source
1	BPTI	5.65E-03	6525	S1	17	RNase H (T. ter)	8.67E-03	18621	S4
2	Barnase	6.09E-03	12328	S1	18	RNase H (E. coli)	10.84E-03	17552	S4
3	Myoglobin	6.96E-03	17801	S1	19	Thioredoxin (E. coli)	8.08E-03	11760	S5
4	Lysozyme	7.13E-05	14307	S1	20	Thioredoxin h	7.95E-03	12600	S6
5	Cytochrome C	7.83E-03	12600	S1	21	α-lactalbumin	7.74E-03	14200	S7
6	Ubiquitin	7.83E-03	8539	S1	22	Engrailed	11.30E-03	7583	S1
7	T_4 lysozyme	7.83E-03	18860	S1	23	Mat α2	12.44E-03	9755	S1
8	RNase T1	8.00E-03	11068	S1	24	Antennapedia	12.87E-03	8595	S1
9	RNase A	9.57E-03	13725	S1	25	LZ-GCN4	15.22E-03	8070	S1
10	Staph. Nuclease	6.82E-03	16000	S2	26	HMGD-74	13.39E-03	8354	S1
11	Interleukin 1β	4.75E-03	17391	S2	27	HMG SOX5	12.53E-03	9085	S1
12	Ovalbumin	7.74E-03	44000	S2	28	NHP6A	18.00E-03	10708	S1
13	Chymiotrypsinogen	7.32E-03	8539	S2	29	SRY	23.04E-03	10234	S1
14	ROP	7.78E-03	6510	S3	30	Lef-79	23.74E-03	9310	S1

15	Tendamistat	4.48E-03	7960	S3	31	Zn-finger TFIIIA	15.04E-03	12040	S1
16	SH_3 domain	6.57E-03	6700	S3					

* The proteins in red are DNA binding domains

Supplementary References

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S5) Georgescu RE, Garcia-Mira MM, Tasayco ML, Sanchez-Ruiz JM (2001), European Journal of Biochemistry 268:1477-1485.

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S7) Hendrix T, Griko YV, Privalov PL (2000), Biophysical Chemistry 84:27-34.

Supplementary Table 3: Parameters for the various models employed derived from the fitting to experimental heat capacity profile of gpW.

Mean-Field Model $\Delta S_{res}^{conf} = 0.0165$ kJ/(mol.K) $k_{\Delta Cp} = 4.3$	Index	$\Delta C_{p,res}$ J/(mol.K)	$k_{\Delta H}$	ΔH_{res}^{385} kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	1	0	1.94	6.45	8.62	0.0692	1.512	2.0 (345)	2.5905	3.795e-4
	2	10	2.01	6.44	9.04	0.0613	1.337	2.8 (345)	2.9859	1.400e-3
	3	20	2.08	6.44	9.42	0.0536	1.171	3.9 (345)	3.8298	1.100e-3
	4	30	2.17	6.44	9.75	0.0465	1.016	5.0 (345)	5.1483	1.965e-4
	5	40	2.28	6.43	10.0	0.0399	0.871	7.0 (343)	7.0043	7.944e-6
Muñoz Eaton Model - Exact Solution PDB id: 1HYW NN = 1 → includes nearest neighbors NN = 0 → excludes nearest neighbors	Index	**Cut-off(Å)/NN**	ΔS_{res}^{conf} kJ/(mol.K)	**ΔH_{cont}** kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	6	3.6/1	-0.0216	-0.4512	10.4	0.0295	0.644	7.0 (344)	5.0113	4.571e-5
	7	4.0/1	-0.0167	-0.2358	9.85	0.0505	1.103	6.1 (344)	3.6603	1.900e-3
	8	4.5/1	-0.0152	-0.1354	9.82	0.0542	1.183	6.6 (344)	4.2762	5.140e-4
	9	5.0/1	-0.0129	-0.0799	9.67	0.0611	1.334	7.0 (344)	5.8664	1.408e-5
		Baseline	**f**	**Σα**	**a**	**b**	**b/b$_0$**	**β (T$_0$)**	**SLS**	**P$_h$**

Variable Barrier Model FB → Freire Baseline	Index	**Used**		kJ/mol	kJ/(mol.K)	kJ/(mol.K^2)		kJ/mol		
	10	FB - σ	0.54	129.5	8.67	0.0369	0.806	-0.04 (338.8)	2.5058	8.400e-3
	11	FB	0.56	192.1	9.04	0.0458	1.000	0.50 (341.3)	0.4271	0.3969
	12	FB + σ	0.56	221.7	9.41	0.0547	1.194	2.73 (343.4)	2.2314	0.0131
	13	Floating Baseline	0.62	194.1	8.89	0.0490	1.068	0.54 (341.4)	0.3954	0.3931
	14	Baseline 1	0.96	216.6	8.62	0.0692	1.511	2.92 (341.9)	3.8473	5.007e-5
	15	Baseline 2	0.74	218.3	9.04	0.0613	1.338	2.60 (342.7)	2.8388	1.800e-3
	16	Baseline 3	0.54	222.0	9.42	0.0536	1.170	2.53 (343.3)	1.9321	0.0239
	17	Baseline 4	0.33	230.3	9.75	0.0465	1.015	2.90 (344.4)	1.2663	0.1023
Muñoz Eaton Model - Single Sequence Approximation	Index	**a_{ASA}** J/(mol.K.Å^2)	ΔS_{res}^{385} kJ/(mol.K)	$\Delta H_{res}^{T_{ref}}$ kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b_0**	**β (T_0)** kJ/mol	**SLS**	**P_h**
	18	0	-0.0193	-6.72	8.29	0.0654	1.428	0.00	3.069	4.936e-4
	19	0.105 (floating)	-0.0180	-5.81 (340 K)	9.40	0.0600	1.310	1.92 (339)	1.1972	0.0320
	20	0.2	-0.0173	-5.18 (340 K)	10.4	0.0542	1.183	3.87 (337)	2.001	0.0201

PDD ID: 1HYW	21	0.3	-0.0170	-4.68 (340 K)	11.3	0.0476	1.039	6.09 (335)	3.640	2.200e-3

* b_0 = 0.0458 kJ/ (mol K^2) is the FB slope for gpW

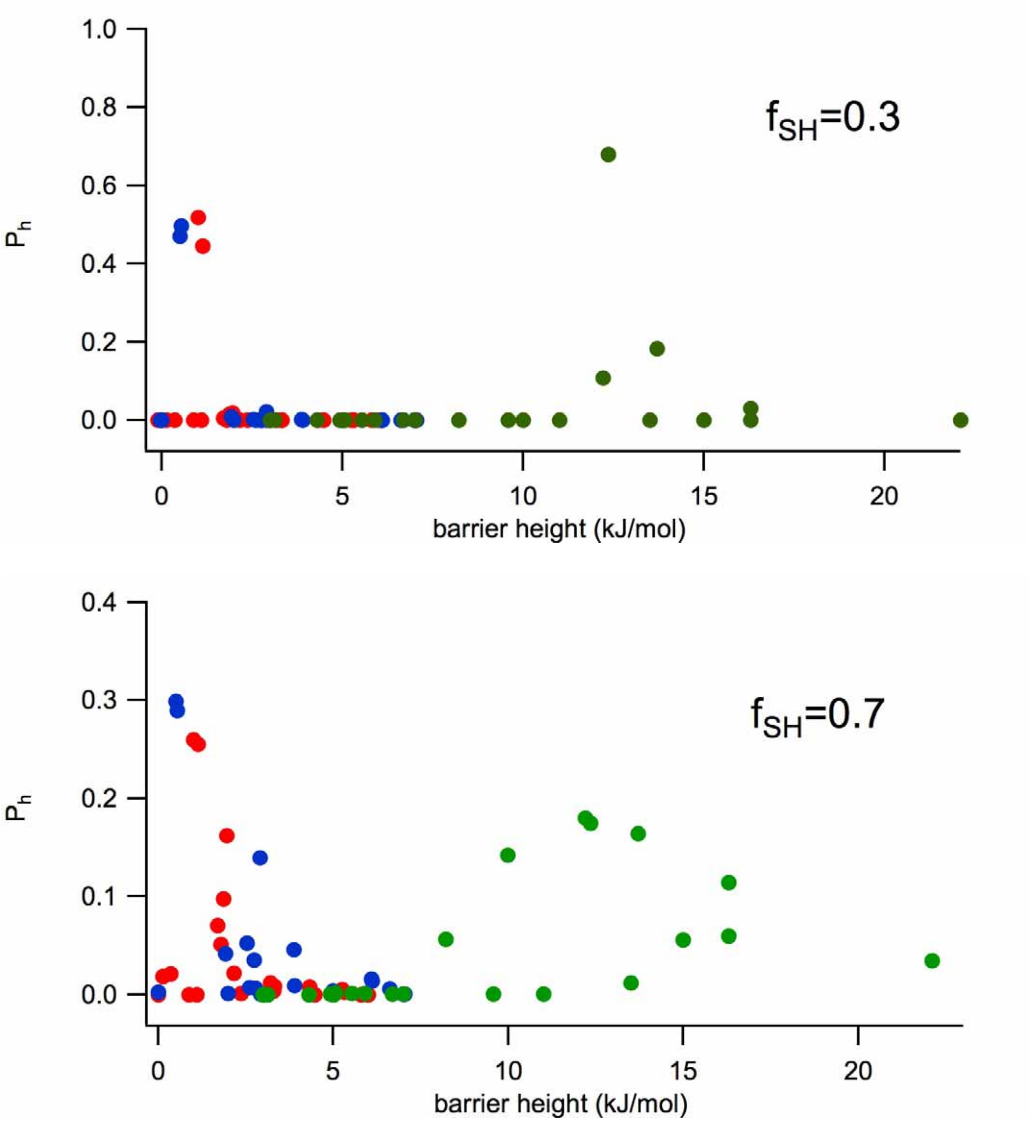
Supplementary Table 4: Parameters for the various models employed derived from the fitting to experimental heat capacity profile of SH3.

	Index	$\Delta C_{p,res}$ J/(mol.K)	$k_{\Delta H}$	ΔH_{res}^{385} kJ/mol	a kJ/(mol.K)	b kJ/(mol.K^2)	b/b$_0$	β (T$_0$) kJ/mol	SLS	P$_h$
Mean-Field Model $\Delta S_{res}^{conf} = 0.0165$ kJ/(mol.K) $k_{\Delta Cp} = 4.3$	1	0	2.03	6.41	8.51	0.0607	1.2541	3.0 (344.1)	12.324	5.84e-34
	2	10	2.10	6.41	8.82	0.0545	1.1260	4.3 (344.1)	7.2408	1.17e-19
	3	20	2.19	6.41	9.10	0.0486	1.0041	5.9 (344)	3.6700	1.018e-9
	4	30	2.31	6.40	9.34	0.0430	0.8884	8.2 (345)	1.4466	1.200e-3
	5	35	2.38	6.40	9.44	0.0404	0.8347	9.9 (345)	0.8245	0.0499
	6	40	2.49	6.40	9.55	0.0377	0.7789	12.2 (344)	0.5328	0.2390
	7	41.9 (floating)	2.54	6.39	9.59	0.0366	0.7562	13.7 (344)	0.5080	0.2431
	8	45	2.63	6.39	9.67	0.0348	0.7190	16.3 (344)	0.5646	0.1310
	9	50	2.88	6.37	9.83	0.0312	0.6446	22.1 (344)	0.8755	9.700e-3
Muñoz Eaton Model - Exact Solution	Index	**Cut-off(Å)/NN**	ΔS_{res}^{conf} kJ/(mol.K)	ΔH_{cont} kJ/mol	a kJ/(mol.K)	b kJ/(mol.K^2)	b/b$_0$	β (T$_0$) kJ/mol	SLS	P$_h$

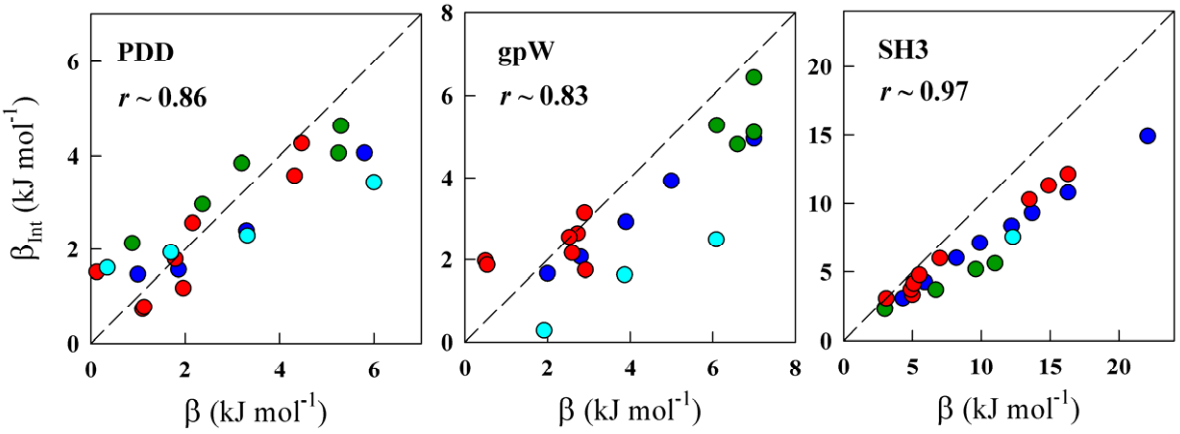
PDB id: 1SHG NN = 1 → includes nearest neighbors NN = 0 → excludes nearest neighbors	10	3.5/1	-0.0202	-0.6412	9.30	0.0405	0.8368	3.0 (342)	6.1550	1.0e-16
	11	4.0/1	-0.0144	-0.2348	9.15	0.0559	1.1550	6.7 (342)	3.8905	1.82e-10
	12	4.5/1	-0.0123	-0.1238	9.14	0.0607	1.2541	9.6 (342)	3.4645	1.592e-9
	13	5.0/1	-0.0115	-0.0721	9.14	0.0621	1.2831	11.0 (342)	3.5359	8.23e-10
Variable Barrier Model FB → Freire Baseline	Index	**Baseline Used**	**f**	**Σα** kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b$_0$**	**β (T$_0$)** kJ/mol	**SLS**	**P$_h$**
	14	FB - σ	0.44	250.5	9.16	0.0390	0.8058	3.1 (343.3)	6.4882	1.04e-17
	15	FB	0.06	244.5	9.55	0.0484	1.0000	14.9 (354.8)	1.5435	7.459e-4
	16	Baseline 1	0.80	235.1	8.51	0.0607	1.2541	5.0 (342.1)	5.1584	3.39e-14
	17	Baseline 2	0.64	238.3	8.82	0.0545	1.1260	4.9 (343.1)	4.0387	7.93e-11
	18	Baseline 3	0.48	242.7	9.10	0.0486	1.0041	5.1 (344.1)	3.4962	3.069e-9
	19	Baseline 4	0.33	249.3	9.34	0.0430	0.8884	5.5 (345.5)	3.5036	2.482e-9
	20	Baseline 6 (large errors)	0.16	260.9	9.55	0.0377	0.7789	7.0 (348.1)	3.9374	9.73e-11
	21	Grid analysis on β fixing FB slope	0.06	239.6	9.69	0.0484	1.0000	16.3 (355.3)	1.5082	9.334e-4

	22	Two-state Fit Baseline (large errors)	0.06	250.7	9.80	0.0427	0.8822	13.5 (354.4)	2.2966	5.195e-6
Muñoz Eaton Model - Single Sequence Approximation	Index	**a_{ASA}** J/(mol.K.Å^2)	ΔS_{res}^{385} kJ/(mol.K)	$\Delta H_{res}^{T_{ref}}$ kJ/mol	**a** kJ/(mol.K)	**b** kJ/(mol.K^2)	**b/b_0**	**β (T_0)** kJ/mol	**SLS**	**P_h**
PDD ID: 1SHG	23	0.39	-0.0172	-4.23 (340 K)	12.0	0.0364	0.7521	12.3 (335.6)	0.4583	0.3245

* b_0 = 0.0484 kJ/ (mol K^2) is the FB slope for SH3



Supplementary Figure 1. Bayesian determination of the thermodynamic folding barrier for PDD using multiple models. The plots shown are analogous to panel (d) in figures 4 and 5 of the main text, except that calculations have been carried out with relative Shannon entropy values equal to 0.3 and 0.7. Color refers to the protein: PDD (red), gpW (blue), SH3 (green).



Supplementary Figure 2. Agreement between absolute estimate of barriers (β) and that calculated considering the differences in curvature from the integration of probability density (β_{int}) (MF: blue, ME-ES: green, VB: red, ME-SSA: cyan). The dashed line corresponds to the 1:1 correlation line and *r* is the linear correlation coefficient.

To account for the differences in the curvature of the folded, unfolded and barrier top extrema, the corresponding reaction coordinate values (F_{min}, U_{min} and B_{max}, respectively) were first identified from the one-dimensional free-energy profiles. The upper (*u*) and lower (*l*) limits for the transition-state ensemble along the reaction co-ordinate *x* were calculated from:

$$x_u = B_{\max} + \left((F_{\min} - B_{\max}) / z \right)$$

$$x_l = B_{\max} - \left((B_{\max} - U_{\min}) / z \right)$$

where *z* is a parameter that determines the resultant width on either side of the barrier. The area within the limits is integrated in the probability density plots and referenced to that corresponding to the folded and unfolded states to calculate β_{int}. The value of *z* was set to 2 for the MF, ME-ES and VB models that display similar overall features. However, *z* was defined as 3.5 for the ME-SSA calculation as the unfolded state is not captured well in the one-dimensional representation.