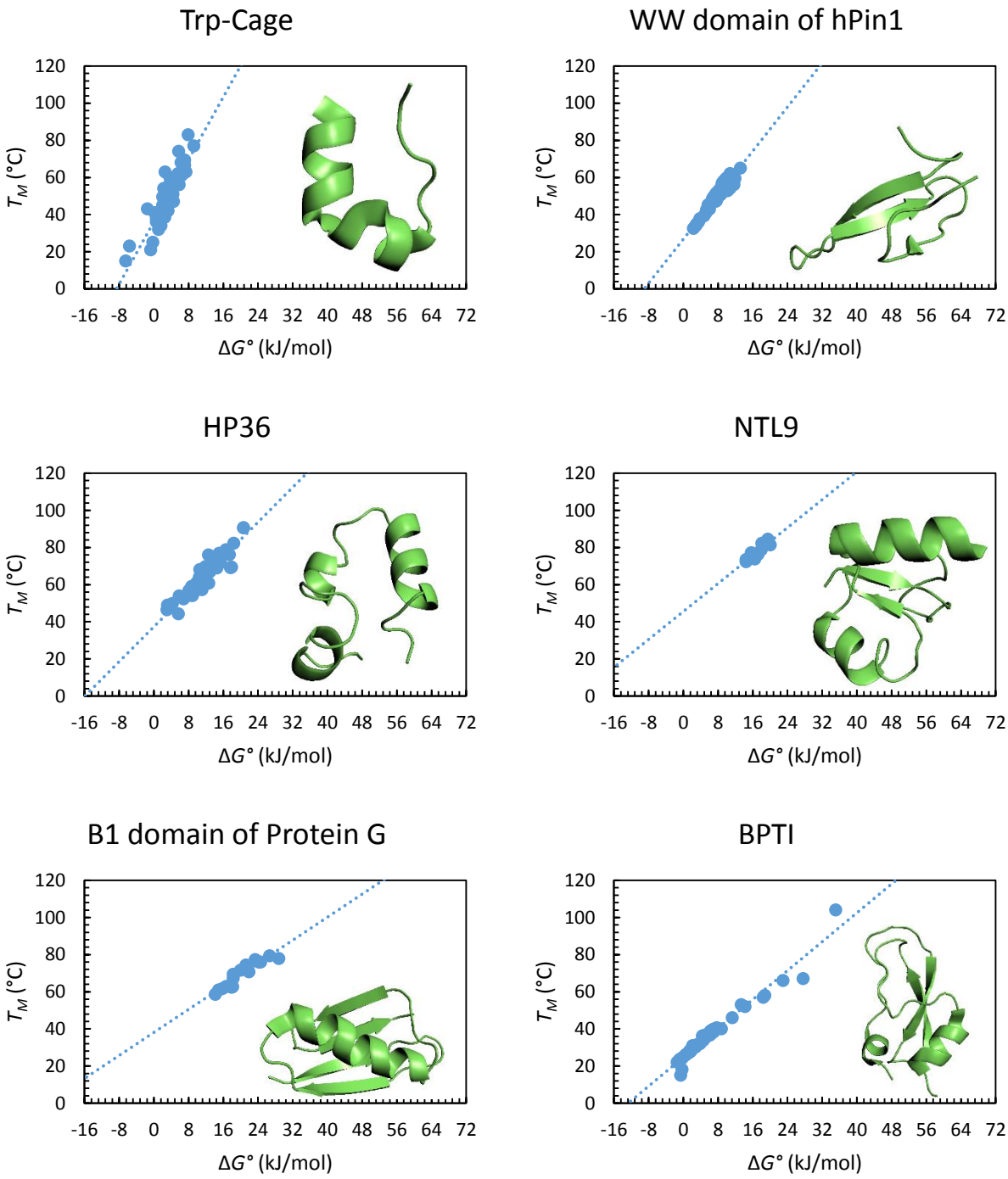
Supporting Information for

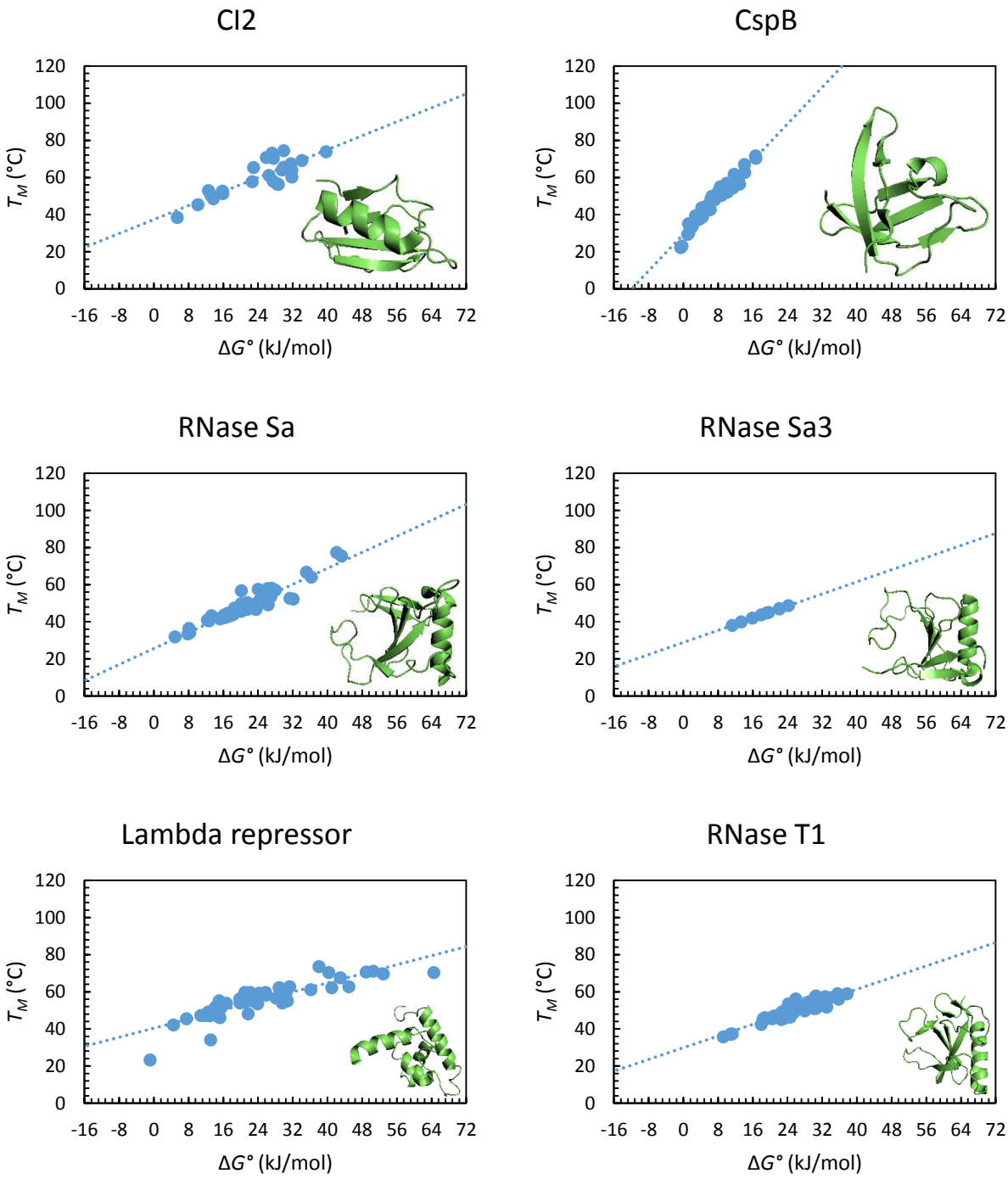
Size-Dependent Relationships Between Protein Stability and Thermal Unfolding Temperature Have Important Implications for Analysis of Protein Energetics and High-Throughput Assays of Protein-Ligand Interactions

Matthew D. Watson[1], Jeremy Monroe[1] and Daniel P. Raleigh[1,2,3]*

[1]Department of Chemistry and [2]Graduate Program in Biochemistry & Structural Biology, Stony Brook University, Stony Brook, New York 11794-3400, United States

[3]Research Department of Structural and Molecular Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom


Trp-Cage
WW domain of hPin1
HP36
NTL9
B1 domain of Protein G
BPTI
T_M (°C)
ΔG° (kJ/mol)
0
20
40
60
80
100
120
-16
-8
0
8
16
24
32
40
48
56
64
72


CI2
CspB
RNase Sa
RNase Sa3
Lambda repressor
RNase T1
T_M (°C)
ΔG° (kJ/mol)
0
20
40
60
80
100
120
-16
-8
0
8
16
24
32
40
48
56
64
72

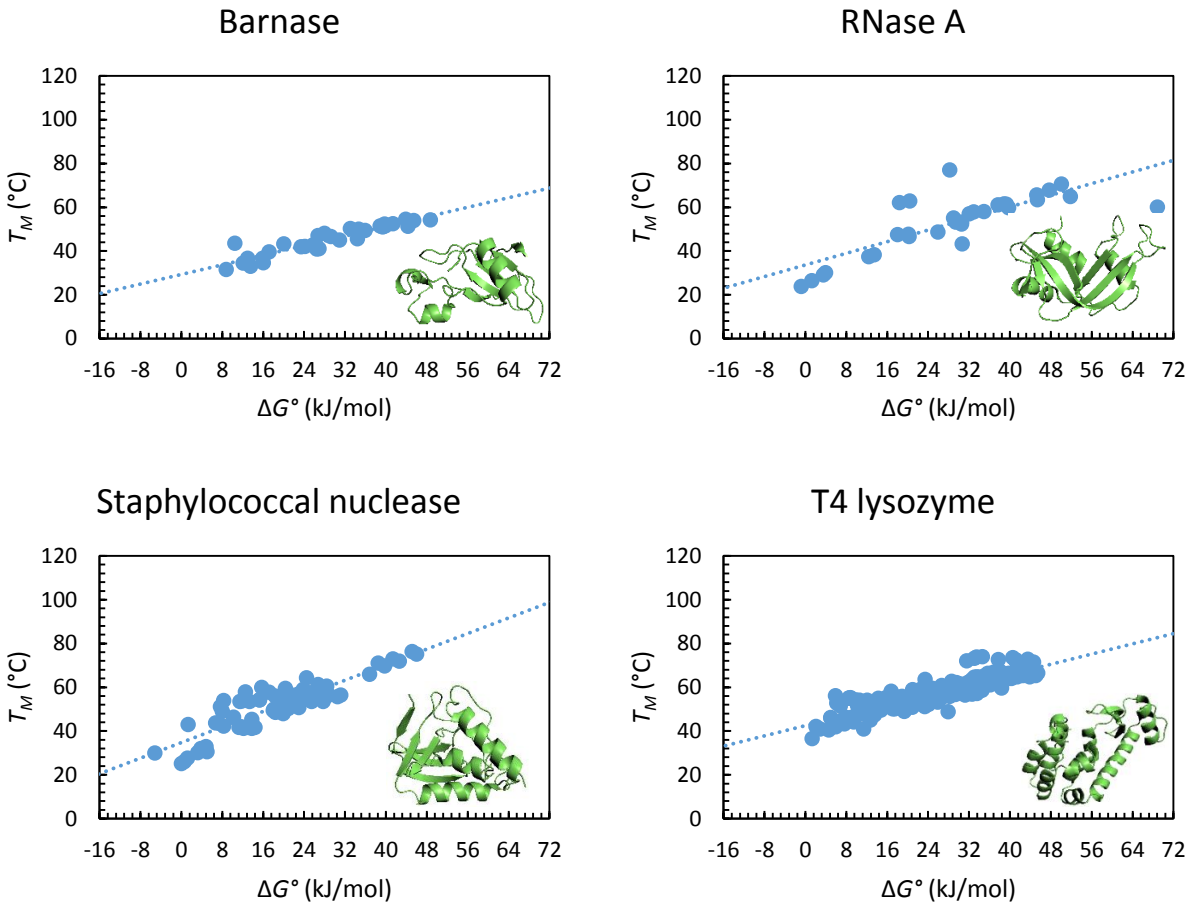


Figure S1. Plots of T_M *vs* $\Delta G°$ for mutants of all proteins included in the analysis

Table S1. Mutants of Trp-cage.[1-5]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
TC12b	36	1.3	Biochemistry (2014) 53, 6011-6021
TC11b1	40	2.4	Biochemistry (2014) 53, 6011-6021
TC9b	51	4.4	Biochemistry (2014) 53, 6011-6021
R16Norvaline (nva)	36	0.6	Biochemistry (2014) 53, 6011-6021
control (NAUYUQWLKDGGPSSGRAA)	23	-5.6	Biochemistry (2014) 53, 6011-6021
TC10b	41	1.6	Biochemistry (2014) 53, 6011-6021
TC10b P19A	15	-6.5	Biochemistry (2014) 53, 6011-6021
TC10b S14A	21	-0.7	Biochemistry (2014) 53, 6011-6021
TC10b P17A	46	2.6	Biochemistry (2014) 53, 6011-6021
TC10b P18A	47	4.3	Biochemistry (2014) 53, 6011-6021
TC10b P12W/P18A	40	1.9	Biochemistry (2014) 53, 6011-6021
TC13b	68	6.3	Biochemistry (2014) 53, 6011-6021
TC13b P12W	77	9.2	Biochemistry (2014) 53, 6011-6021
TC13b P12W/P18A	50	2.1	Biochemistry (2014) 53, 6011-6021
tr-TC16b	60	5.4	Biochemistry (2014) 53, 6011-6021
tr-TC16b R16nva	50	2.1	Biochemistry (2014) 53, 6011-6021
TC16b	74	5.7	Biochemistry (2014) 53, 6011-6021
TC16b R16nva	63	2.6	Biochemistry (2014) 53, 6011-6021
TC16b P19A	43	-1.5	Biochemistry (2014) 53, 6011-6021
TC10b	56	5.3	Biochemistry (2011) 50, 1143-1152
TC10b	40	1.9	Biochemistry (2011) 50, 1143-1152
D9E	57	4.6	Biochemistry (2011) 50, 1143-1152

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
D9E	41	0.2	Biochemistry (2011) 50, 1143-1152
D9E/R16E	56	3.6	Biochemistry (2011) 50, 1143-1152
D9E/R16E	25	-0.2	Biochemistry (2011) 50, 1143-1152
D9E/R16K	60	3.7	Biochemistry (2011) 50, 1143-1152
R16K	54	2.3	Biochemistry (2011) 50, 1143-1152
D9E/R16O	52	2.9	Biochemistry (2011) 50, 1143-1152
R16O	45	2.0	Biochemistry (2011) 50, 1143-1152
R16O	38	0.6	Biochemistry (2011) 50, 1143-1152
TC10b P17A	42	3.3	Protein Eng. Des. Sel. (2008) 21, 171-185
TC10b P17G	32	1.1	Protein Eng. Des. Sel. (2008) 21, 171-185
TC10b P18L	34	1.7	Protein Eng. Des. Sel. (2008) 21, 171-185
TC10b P18A	47	4.6	Protein Eng. Des. Sel. (2008) 21, 171-185
TC10b P12A	43	3.1	Protein Eng. Des. Sel. (2008) 21, 171-185
TC10b P12A/P18A	39	2.6	Protein Eng. Des. Sel. (2008) 21, 171-185
TC5b WT	46	3.3	J. Am. Chem. Soc. (2011) 133, 18750-18759
TC10b G10 (D)A	67	7.1	J. Am. Chem. Soc. (2011) 133, 18750-18759
TC10b (D)N	56	5.9	J. Am. Chem. Soc. (2011) 133, 18750-18759
TC10b (D)Q	69	7.1	J. Am. Chem. Soc. (2011) 133, 18750-18759
TC10b K8A	61	6.4	Org. Biomol. Chem. (2008) 6, 4287-4289
TC10b S13A	63	7.4	Org. Biomol. Chem. (2008) 6, 4287-4289
TC10b G15(D)A	62	5.6	Org. Biomol. Chem. (2008) 6, 4287-4289
TC16b	83	7.9	Org. Biomol. Chem. (2008) 6, 4287-4289

Table S2. Mutants of hPin1 WW domain.[6]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
F25A	32.5	2.35	Protein Sci. (2009) 18, 1806-1813
Y23A	33.9	2.85	Protein Sci. (2009) 18, 1806-1813
T29G	34.4	2.98	Protein Sci. (2009) 18, 1806-1813
W11F	35.0	3.14	Protein Sci. (2009) 18, 1806-1813
N26D	36.0	3.55	Protein Sci. (2009) 18, 1806-1813
L7A	37.8	3.82	Protein Sci. (2009) 18, 1806-1813
R14A	39.2	4.78	Protein Sci. (2009) 18, 1806-1813
A31G	40.9	5.21	Protein Sci. (2009) 18, 1806-1813
F25L	42.5	5.32	Protein Sci. (2009) 18, 1806-1813
T29D	42.9	6.01	Protein Sci. (2009) 18, 1806-1813
L7V	44.0	5.94	Protein Sci. (2009) 18, 1806-1813
T29A	44.3	5.74	Protein Sci. (2009) 18, 1806-1813
Y23L	45.3	5.72	Protein Sci. (2009) 18, 1806-1813
I28G	47.2	7.53	Protein Sci. (2009) 18, 1806-1813
P8A	47.4	6.62	Protein Sci. (2009) 18, 1806-1813
S16G	47.6	7.68	Protein Sci. (2009) 18, 1806-1813
P8G	47.7	7.19	Protein Sci. (2009) 18, 1806-1813
L7NVa	48.8	6.80	Protein Sci. (2009) 18, 1806-1813
G20A	48.9	6.94	Protein Sci. (2009) 18, 1806-1813
G10A	49.0	7.47	Protein Sci. (2009) 18, 1806-1813
L7I	49.3	7.99	Protein Sci. (2009) 18, 1806-1813
S32G	50.1	7.15	Protein Sci. (2009) 18, 1806-1813

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
E35A	50.3	7.52	Protein Sci. (2009) 18, 1806-1813
H27G	50.5	8.51	Protein Sci. (2009) 18, 1806-1813
T29S	50.8	8.57	Protein Sci. (2009) 18, 1806-1813
R21A	50.9	8.59	Protein Sci. (2009) 18, 1806-1813
Y24F	51.4	7.63	Protein Sci. (2009) 18, 1806-1813
R21G	51.5	7.58	Protein Sci. (2009) 18, 1806-1813
M15A	51.8	8.11	Protein Sci. (2009) 18, 1806-1813
E12A	52.6	9.50	Protein Sci. (2009) 18, 1806-1813
Y24W	52.9	8.17	Protein Sci. (2009) 18, 1806-1813
W34A	52.9	10.24	Protein Sci. (2009) 18, 1806-1813
P9G	53.1	8.42	Protein Sci. (2009) 18, 1806-1813
Q33A	53.1	8.51	Protein Sci. (2009) 18, 1806-1813
N30A	53.3	8.85	Protein Sci. (2009) 18, 1806-1813
S16A	54.0	8.65	Protein Sci. (2009) 18, 1806-1813
V22A	54.2	10.77	Protein Sci. (2009) 18, 1806-1813
I28A	54.2	9.66	Protein Sci. (2009) 18, 1806-1813
Δ3,4P37	55.1	8.77	Protein Sci. (2009) 18, 1806-1813
P9A	56.0	10.10	Protein Sci. (2009) 18, 1806-1813
S19G	56.0	11.67	Protein Sci. (2009) 18, 1806-1813
S18G	56.5	11.61	Protein Sci. (2009) 18, 1806-1813
R36A	56.7	9.05	Protein Sci. (2009) 18, 1806-1813
S32A	56.9	8.99	Protein Sci. (2009) 18, 1806-1813
S19A	57.1	9.85	Protein Sci. (2009) 18, 1806-1813

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
R17G	57.3	9.19	Protein Sci. (2009) 18, 1806-1813
H27A	57.7	9.88	Protein Sci. (2009) 18, 1806-1813
W34F	58.0	9.61	Protein Sci. (2009) 18, 1806-1813
S38G	58.2	9.43	Protein Sci. (2009) 18, 1806-1813
S18A	58.4	11.22	Protein Sci. (2009) 18, 1806-1813
wt	58.6	10.47	Protein Sci. (2009) 18, 1806-1813
R17A	58.8	10.56	Protein Sci. (2009) 18, 1806-1813
S38A	58.8	9.79	Protein Sci. (2009) 18, 1806-1813
K6A	59.4	11.95	Protein Sci. (2009) 18, 1806-1813
K13A	59.6	9.91	Protein Sci. (2009) 18, 1806-1813
F25Y	62.0	10.81	Protein Sci. (2009) 18, 1806-1813

Table S3. Mutants of HP36.[7-16]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-HP36	73.0	13.47	Xiao, S. thesis
N68A/K70M-HP36	90.6	20.67	Xiao, S. thesis
N68A/K70M/P62A-HP36	71.8	15.36	Xiao, S. thesis
N68A/K70M/W64A-HP36	78.7	16.61	Xiao, S. thesis
N68A/K70M/W64L-HP36	74.4	14.98	Xiao, S. thesis
N68A/K70M/W64K-HP36	74.9	15.02	Xiao, S. thesis
N68A/K70M/P62A/W64L-HP36	67.9	11.46	Xiao, S. thesis
WT-HP35	70.0	13.81	J. Mol. Biol. (1996) 260, 126–134
N27H-HP36	68.9	14.53	J. Mol. Biol. (2003) 329, 625–630
N27H/F35A-HP36	66.9	12.92	J. Mol. Biol. (2003) 329, 625–630
WT-HP35	69.1	17.70	J. Am. Chem. Soc. (2016) 138, 6498-6505
N19β3N-HP35	44.2	5.65	J. Am. Chem. Soc. (2016) 138, 6498-6505
W23β3W-HP35	59.4	11.21	J. Am. Chem. Soc. (2016) 138, 6498-6505
Q26β3Q-HP35	57.4	10.17	J. Am. Chem. Soc. (2016) 138, 6498-6505
K30β3K-HP35	57.2	11.09	J. Am. Chem. Soc. (2016) 138, 6498-6505
N19ACPC-HP35 (t-(1S,2S)-2-aminocyclopentyl-1-carboxylic acid)	54.0	8.91	J. Am. Chem. Soc. (2016) 138, 6498-6505
W23ACPC-HP35 (t-(1S,2S)-2-aminocyclopentyl-1-carboxylic	56.9	8.91	J. Am. Chem. Soc. (2016) 138, 6498-6505

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
acid)			
Q26ACPC-HP35 (t-(1S,2S)-2-aminocyclopentyl-1-carboxylic acid)	57.1	9.00	J. Am. Chem. Soc. (2016) 138, 6498-6505
K30APC (t-(3R,4S)-4-aminopyrrolidine-3-carboxylic acid)	69.4	17.91	J. Am. Chem. Soc. (2016) 138, 6498-6505
WT-HP36	61.1	11.13	Biopolymers (2015) 103, 627-637
F51Z-HP36 (4-fluoroPhe)	66.0	11.51	Biopolymers (2015) 103, 627-637
F51X-HP36 (4-methylPhe)	60.8	10.29	Biopolymers (2015) 103, 627-637
F58Z-HP36 (4-fluoroPhe)	60.2	10.00	Biopolymers (2015) 103, 627-637
F58X-HP36 (4-methylPhe)	57.7	8.79	Biopolymers (2015) 103, 627-637
F51Z/F58Z-HP36 (4-fluoroPhe, 4-methylPhe)	63.2	10.67	Biopolymers (2015) 103, 627-637
F51Z/F58X-HP36 (4-flouroPhe, 4-methylPhe)	64.3	11.67	Biopolymers (2015) 103, 627-637
F51X/F58Z-HP36 (4-methylPhe, 4-fluoroPhe)	62.7	11.25	Biopolymers (2015) 103, 627-637
K24Nle-HP36	76.9	15.06	Proc. Natl. Acad. Sci. USA (2005) 102, 7517–7522
A18V-HP35	61.9	11.23	J. Mol. Biol. (2006) 359, 546–553
A18S-HP35	63.9	10.46	J. Mol. Biol. (2006) 359, 546–553
N68A/K70M/F47L-HP36	69.6	11.92	Biochemistry (2009) 48, 4607-4616
N68A/K70M/F51L-HP36	76.0	12.59	Biochemistry (2009) 48, 4607-4616
N68A/K70M/F58L-HP36	60.9	10.96	Biochemistry (2009) 48, 4607-4616
N68A/K70M/F47L/F51L-HP36	68.1	10.63	Biochemistry (2009) 48, 4607-4616
N68A/K70M/F47L/F58L-HP36	48.9	3.14	Biochemistry (2009) 48, 4607-4616
N68A/K70M/F51L/F58L-HP36	46.4	3.01	Biochemistry (2009) 48, 4607-4616

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
N68A/K70M/F47L/F51L/F58L-HP36	49.8	4.27	Biochemistry (2009) 48, 4607-4616
N68A/K70M/W64L-HP36	74.4	14.98	Biochemistry (2009) 48, 4607-4616
WT-HP36	61.1	9.92	Biochemistry (2010) 49, 4255–4263
P62Hyp-HP36 (4R,2S)-4-hydroxyproline	59.0	8.70	Biochemistry (2010) 49, 4255–4263
P62Flp-HP36 (4R,2S)-4-fluoroproline	53.8	5.86	Biochemistry (2010) 49, 4255–4263
P62Mop-HP36 (4R,2S)-4-methoxyproline	56.8	8.08	Biochemistry (2010) 49, 4255–4263
P62hyp-HP36 (4S,2S)-4-hydroxyproline	54.1	8.08	Biochemistry (2010) 49, 4255–4263
P62flp-HP36 (4S,2S)-4-fluoroproline	60.8	12.68	Biochemistry (2010) 49, 4255–4263
P62mop-HP36 (4S,2S)-4-methoxyproline	52.2	6.86	Biochemistry (2010) 49, 4255–4263
WT-HP36	73.0	13.47	Biochemistry (2007) 46, 7497-7505
N68A-HP36	76.1	17.41	Biochemistry (2007) 46, 7497-7505
K70M-HP36	82.2	18.45	Biochemistry (2007) 46, 7497-7505
N68A/K70M-HP36	90.6	20.67	Biochemistry (2007) 46, 7497-7505

Table S4. Mutants of NTL9.[17]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	References
N42FCN/Y25F-NTL9	73.43	14.5	Peran, I. thesis
N42FCN/Y25W-NTL9	72.28	14.5	Peran, I. thesis
K2FCN/Y25F-NTL9	76.20	17.2	Peran, I. thesis
K2FCN/Y25W-NTL9	73.71	16.5	Peran, I. thesis
K2FCN/Y25F/Q33F-NTL9	81.40	20.0	Peran, I. thesis
K2FCN/Y25F/Q33W-NTL9	84.47	19.5	Peran, I. thesis
K2FCN/Y25F/K51F-NTL9	82.22	18.1	Peran, I. thesis
Y25W-NTL9	77.08	15.6	Peran, I. thesis
WT-NTL9	78.70	18.0	Peran, I. thesis

Table S5. Mutants of the B1 domain of Protein G.[18]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
B1 A6A53	79.3	26.69	Structure (1999) 7, 1333-1343
B1 AE53	77.8	28.83	Structure (1999) 7, 1333-1343
B1 A6R	77.2	23.35	Structure (1999) 7, 1333-1343
B1 A6E	76.0	24.18	Structure (1999) 7, 1333-1343
B1 A6E/A53E	75.9	24.60	Structure (1999) 7, 1333-1343
B1 A6E/A53R	74.3	21.25	Structure (1999) 7, 1333-1343
B1 A6K	74.0	21.25	Structure (1999) 7, 1333-1343
B1 A6K/A53E	73.8	21.71	Structure (1999) 7, 1333-1343
B1 A6K/A53R	72.2	21.25	Structure (1999) 7, 1333-1343

Table S6. Mutants of BPTI.[19-20]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
C14A/C30A/C38A/C51A-BPTI	39.2	7.00	J. Mol. Biol. (1995) 249, 388-397
R1A/C14A/C30A/C38A/C51A-BPTI	35.3	5.14	J. Mol. Biol. (1995) 249, 388-397
P2A/C14A/C30A/C38A/C51A-BPTI	29.4	2.20	J. Mol. Biol. (1995) 249, 388-397
D3A/C14A/C30A/C38A/C51A-BPTI	40.6	7.73	J. Mol. Biol. (1995) 249, 388-397
F4A/C14A/C30A/C38A/C51A/M52L-BPTI	18.0	-0.30	J. Mol. Biol. (1995) 249, 388-397
L6A/C14A/C30A/C38A/C51A-BPTI	34.6	4.71	J. Mol. Biol. (1995) 249, 388-397
E7A/C14A/C30A/C38A/C51A-BPTI	28.0	1.78	J. Mol. Biol. (1995) 249, 388-397
P8A/C14A/C30A/C38A/C51A/M52L-BPTI	36.7	5.54	J. Mol. Biol. (1995) 249, 388-397
P9A/C14A/C30A/C38A/C51A-BPTI	33.7	4.58	J. Mol. Biol. (1995) 249, 388-397
Y10A/C14A/C30A/C38A/C51A-BPTI	30.2	2.18	J. Mol. Biol. (1995) 249, 388-397
T11A/C14A/C30A/C38A/C51A/M52L-BPTI	39.0	6.74	J. Mol. Biol. (1995) 249, 388-397
G12A/C14A/C30A/C38A/C51A-BPTI	26.1	0.58	J. Mol. Biol. (1995) 249, 388-397
P13A/C14A/C30A/C38A/C51A-BPTI	30.2	2.48	J. Mol. Biol. (1995) 249, 388-397
K15A/C14A/C30A/C38A/C51A-BPTI	36.6	5.02	J. Mol. Biol. (1995) 249, 388-397
R17A/C14A/C30A/C38A/C51A-BPTI	37.3	5.93	J. Mol. Biol. (1995) 249, 388-397
I18A/C14A/C30A/C38A/C51A-BPTI	28.3	1.51	J. Mol. Biol. (1995) 249, 388-397
I19A/C14A/C30A/C38A/C51A-BPTI	24.1	-0.37	J. Mol. Biol. (1995) 249, 388-397
R20A/C14A/C30A/C38A/C51A-BPTI	26.0	0.60	J. Mol. Biol. (1995) 249, 388-397
F22A/C14A/C30A/C38A/C51A-BPTI	24.5	-0.18	J. Mol. Biol. (1995) 249, 388-397
N24A/C14A/C30A/C38A/C51A-BPTI	23.4	-0.83	J. Mol. Biol. (1995) 249, 388-397

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
K26A/C14A/C30A/C38A/C51A-BPTI	39.1	7.16	J. Mol. Biol. (1995) 249, 388-397
G28A/C14A/C30A/C38A/C51A-BPTI	31.9	3.85	J. Mol. Biol. (1995) 249, 388-397
L29A/C14A/C30A/C38A/C51A-BPTI	39.5	6.92	J. Mol. Biol. (1995) 249, 388-397
Q31A/C14A/C30A/C38A/C51A-BPTI	31.7	3.37	J. Mol. Biol. (1995) 249, 388-397
T32A/C14A/C30A/C38A/C51A-BPTI	38.8	6.29	J. Mol. Biol. (1995) 249, 388-397
V34A/C14A/C30A/C38A/C51A-BPTI	30.2	2.85	J. Mol. Biol. (1995) 249, 388-397
Y35A/C14A/C30A/C38A/C51A/M52L-BPTI	30.9	2.19	J. Mol. Biol. (1995) 249, 388-397
G36A/C14A/C30A/C38A/C51A-BPTI	23.8	-0.47	J. Mol. Biol. (1995) 249, 388-397
G37A/C14A/C30A/C38A/C51A-BPTI	22.0	-1.43	J. Mol. Biol. (1995) 249, 388-397
R39A/C14A/C30A/C38A/C51A-BPTI	39.2	6.62	J. Mol. Biol. (1995) 249, 388-397
K41A/C14A/C30A/C38A/C51A-BPTI	36.2	4.43	J. Mol. Biol. (1995) 249, 388-397
R42A/C14A/C30A/C38A/C51A-BPTI	35.6	5.11	J. Mol. Biol. (1995) 249, 388-397
N44A/C14A/C30A/C38A/C51A/M52L-BPTI	15.0	-0.63	J. Mol. Biol. (1995) 249, 388-397
K46A/C14A/C30A/C38A/C51A-BPTI	39.8	7.03	J. Mol. Biol. (1995) 249, 388-397
S47A/C14A/C30A/C38A/C51A-BPTI	27.3	1.40	J. Mol. Biol. (1995) 249, 388-397
E49A/C14A/C30A/C38A/C51A-BPTI	37.9	6.50	J. Mol. Biol. (1995) 249, 388-397
D50A/C14A/C30A/C38A/C51A-BPTI	36.6	6.13	J. Mol. Biol. (1995) 249, 388-397
M52A/C14A/C30A/C38A/C51A-BPTI	27.1	1.16	J. Mol. Biol. (1995) 249, 388-397
R53A/C14A/C30A/C38A/C51A-BPTI	38.3	7.02	J. Mol. Biol. (1995) 249, 388-397
T54A/C14A/C30A/C38A/C51A-BPTI	38.5	6.74	J. Mol. Biol. (1995) 249, 388-397
G56A/C14A/C30A/C38A/C51A-BPTI	37.8	6.46	J. Mol. Biol. (1995) 249, 388-397

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
G57A/C14A/C30A/C38A/C51A-BPTI	37.7	6.60	J. Mol. Biol. (1995) 249, 388-397
WT-BPTI	104.0	35.15	Biochemistry (1997) 36, 5323-5335
C30A/C51A-BPTI	66.0	23.01	Biochemistry (1997) 36, 5323-5335
C30V/C51A-BPTI	67.0	27.61	Biochemistry (1997) 36, 5323-5335
C30G/C51A-BPTI	53.0	13.39	Biochemistry (1997) 36, 5323-5335
C30T/C51A-BPTI	58.0	18.83	Biochemistry (1997) 36, 5323-5335
C30S/C51A-BPTI	57.0	18.41	Biochemistry (1997) 36, 5323-5335
C30A/C51S-BPTI	52.0	14.23	Biochemistry (1997) 36, 5323-5335
C30S/C51S-BPTI	46.0	11.30	Biochemistry (1997) 36, 5323-5335
C30G/C51M-BPTI	40.0	8.79	Biochemistry (1997) 36, 5323-5335

Table S7. Mutants of Chymotrypsin Inhibitor 2.[21-23]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-CI2*	64.0	29.55	Biochemistry (1993) 32, 11259-11269
WT-CI2*	73.8	39.69	Biochemistry (1993) 32, 11259-11269
L27A-CI2*	48.5	13.72	Biochemistry (1993) 32, 11259-11269
V38A-CI2*	61.1	26.57	Biochemistry (1993) 32, 11259-11269
I39V-CI2*	57.7	22.69	Biochemistry (1993) 32, 11259-11269
I39V-CI2*	67.3	31.64	Biochemistry (1993) 32, 11259-11269
I48V-CI2*	69.1	34.16	Biochemistry (1993) 32, 11259-11269
I48A-CI2*	52.5	15.80	Biochemistry (1993) 32, 11259-11269
V66A-CI2*	45.3	10.18	Biochemistry (1993) 32, 11259-11269
L68A-CI2*	52.3	15.85	Biochemistry (1993) 32, 11259-11269
V70A-CI2*	51.3	15.90	Biochemistry (1993) 32, 11259-11269
I76V-CI2*	65.4	29.95	Biochemistry (1993) 32, 11259-11269
I76A-CI2*	38.3	5.41	Biochemistry (1993) 32, 11259-11269
I76A-CI2*	50.3	13.20	Biochemistry (1993) 32, 11259-11269
I48A/I76V-CI2*	52.8	12.62	Biochemistry (1993) 32, 11259-11269
WT-CI2	74.3	29.96	Protein Eng. (1994) 7, 103-108
A43E-CI2	73.0	27.28	Protein Eng. (1994) 7, 103-108
A45E-CI2	70.2	27.57	Protein Eng. (1994) 7, 103-108
A43K/A45E-CI2	70.7	25.94	Protein Eng. (1994) 7, 103-108
A44P-CI2	65.2	23.01	Protein Eng. (1994) 7, 103-108
WT-CI2	63.8	31.84	Protein Eng. (1994) 7, 777-782
SA31-CI2	58.0	27.53	Protein Eng. (1994) 7, 777-782

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
EA33EA34-CI2	60.2	31.84	Protein Eng. (1994) 7, 777-782
SG31EA33EA34-CI2	56.6	28.41	Protein Eng. (1994) 7, 777-782
SA31EA33EA34-CI2	56.3	28.70	Protein Eng. (1994) 7, 777-782

Table S8. Mutants of Cold shock protein B.[24]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT*	55.3	9.71	J. Mol. Biol. (2007) 366, 842–856
6H-WT*	49.7	7.70	J. Mol. Biol. (2007) 366, 842–856
WT	52.3	9.63	J. Mol. Biol. (2007) 366, 842–856
E3R	70.2	16.74	J. Mol. Biol. (2007) 366, 842–856
E3R*	71.6	16.69	J. Mol. Biol. (2007) 366, 842–856
6H-E3R*	66.9	14.10	J. Mol. Biol. (2007) 366, 842–856
E3Q	62.6	14.18	J. Mol. Biol. (2007) 366, 842–856
K5E*	22.2	-0.62	J. Mol. Biol. (2007) 366, 842–856
K5Q*	38.6	4.40	J. Mol. Biol. (2007) 366, 842–856
6H-K5Q*	37.6	3.80	J. Mol. Biol. (2007) 366, 842–856
N10D	57.7	12.04	J. Mol. Biol. (2007) 366, 842–856
N10K*	41.1	4.42	J. Mol. Biol. (2007) 366, 842–856
E12K*	50.2	8.78	J. Mol. Biol. (2007) 366, 842–856
K13E*	52.7	8.76	J. Mol. Biol. (2007) 366, 842–856
K13Q*	54.0	10.70	J. Mol. Biol. (2007) 366, 842–856
E19K*	50.5	8.68	J. Mol. Biol. (2007) 366, 842–856
E19Q*	53.7	10.12	J. Mol. Biol. (2007) 366, 842–856
V20Q*	39.5	4.56	J. Mol. Biol. (2007) 366, 842–856
6H-V20Q*	30.1	1.26	J. Mol. Biol. (2007) 366, 842–856
V20Q/E3R*	58.2	12.71	J. Mol. Biol. (2007) 366, 842–856
6H-V20Q/E3R*	51.6	8.94	J. Mol. Biol. (2007) 366, 842–856
V20E/E3R*	43.0	5.23	J. Mol. Biol. (2007) 366, 842–856

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
6H-V20E/E3R*	34.7	2.20	J. Mol. Biol. (2007) 366, 842–856
V20K*	37.2	3.40	J. Mol. Biol. (2007) 366, 842–856
6H-V20K*	29.3	1.00	J. Mol. Biol. (2007) 366, 842–856
V20K/E3R*	54.6	9.99	J. Mol. Biol. (2007) 366, 842–856
6H-V20K/E3R*	47.9	7.20	J. Mol. Biol. (2007) 366, 842–856
E21K*	54.3	11.46	J. Mol. Biol. (2007) 366, 842–856
E21Q*	52.5	8.15	J. Mol. Biol. (2007) 366, 842–856
E21Q/E19Q*	52.0	10.16	J. Mol. Biol. (2007) 366, 842–856
D24K*	50.9	7.71	J. Mol. Biol. (2007) 366, 842–856
D24N*	48.4	6.41	J. Mol. Biol. (2007) 366, 842–856
D25K*	35.0	1.24	J. Mol. Biol. (2007) 366, 842–856
D25K/E19Q*	39.4	2.83	J. Mol. Biol. (2007) 366, 842–856
D25Q	44.2	5.31	J. Mol. Biol. (2007) 366, 842–856
D25Q/E19Q*	43.6	4.27	J. Mol. Biol. (2007) 366, 842–856
K39E*	52.8	8.52	J. Mol. Biol. (2007) 366, 842–856
K39Q*	54.9	8.57	J. Mol. Biol. (2007) 366, 842–856
E24K*	55.3	11.46	J. Mol. Biol. (2007) 366, 842–856
E42Q*	54.1	8.58	J. Mol. Biol. (2007) 366, 842–856
E43K*	56.3	12.96	J. Mol. Biol. (2007) 366, 842–856
E43Q*	55.5	11.68	J. Mol. Biol. (2007) 366, 842–856
S48K*	61.6	11.75	J. Mol. Biol. (2007) 366, 842–856
6H-S48K*	56.8	9.97	J. Mol. Biol. (2007) 366, 842–856
S48E	53.0	9.32	J. Mol. Biol. (2007) 366, 842–856

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
E50K*	49.7	6.70	J. Mol. Biol. (2007) 366, 842–856
6H-E50K*	49.7	6.63	J. Mol. Biol. (2007) 366, 842–856
E50Q	42.6	6.22	J. Mol. Biol. (2007) 366, 842–856
E53K*	53.7	10.21	J. Mol. Biol. (2007) 366, 842–856
E53Q*	56.6	12.06	J. Mol. Biol. (2007) 366, 842–856
N55K*	54.3	9.41	J. Mol. Biol. (2007) 366, 842–856
N55D*	59.2	12.66	J. Mol. Biol. (2007) 366, 842–856
R56Q	55.3	11.37	J. Mol. Biol. (2007) 366, 842–856
K65E*	38.2	3.29	J. Mol. Biol. (2007) 366, 842–856
6H−K65E*	33.4	2.00	J. Mol. Biol. (2007) 366, 842–856
K65Q*	48.0	6.43	J. Mol. Biol. (2007) 366, 842–856
6H-K65Q*	44.4	5.27	J. Mol. Biol. (2007) 366, 842–856
E3R/F15A/ D25K/F27A*	44.0	6.05	J. Mol. Biol. (2007) 366, 842–856
E3R/F15A/E19K/F27A*	39.0	4.09	J. Mol. Biol. (2007) 366, 842–856
E3R/F15A/ D25K/F27A*	23.0	-0.31	J. Mol. Biol. (2007) 366, 842–856

Table S9. Mutants of RNase Sa.[25-31]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-RNase Sa	48.4	22.68	Biochemistry (1998) 37, 16192-16200
N39D-RNase Sa	43.2	16.82	Biochemistry (1998) 37, 16192-16200
N39S-RNase Sa	40.4	12.76	Biochemistry (1998) 37, 16192-16200
N39A-RNase Sa	40.8	12.34	Biochemistry (1998) 37, 16192-16200
WT-RNase Sa	47.2	21.51	Protein Sci. (1999) 8, 1843-1849
D25K-RNase Sa	50.2	24.02	Protein Sci. (1999) 8, 1843-1849
E74K-RNase Sa	51.1	24.89	Protein Sci. (1999) 8, 1843-1849
WT-RNase Sa	48.4	23.47	J. Mol. Biol. (2001) 312, 393-404
Y30F-RNase Sa	49.6	20.13	J. Mol. Biol. (2001) 312, 393-404
Y49F-RNase Sa	47.6	19.96	J. Mol. Biol. (2001) 312, 393-404
Y51F-RNase Sa	40.8	12.68	J. Mol. Biol. (2001) 312, 393-404
Y52F-RNase Sa	36.3	8.08	J. Mol. Biol. (2001) 312, 393-404
Y55F-RNase Sa	46.5	21.30	J. Mol. Biol. (2001) 312, 393-404
Y80F-RNase Sa	43.2	13.31	J. Mol. Biol. (2001) 312, 393-404
Y81F-RNase Sa	44.5	17.03	J. Mol. Biol. (2001) 312, 393-404
Y86F-RNase Sa	47.3	18.62	J. Mol. Biol. (2001) 312, 393-404
WT-RNase Sa	48.4	23.47	J. Biol. Chem. (2003) 278, 31790-31795
T18V-RNase Sa	43.7	16.28	J. Biol. Chem. (2003) 278, 31790-31795
T56V-RNase Sa	42.1	16.40	J. Biol. Chem. (2003) 278, 31790-31795
T67V-RNase Sa	48.3	21.30	J. Biol. Chem. (2003) 278, 31790-31795

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
T82V-RNase Sa	42.7	15.90	J. Biol. Chem. (2003) 278, 31790-31795
T5V-RNase Sa	48.4	22.55	J. Biol. Chem. (2003) 278, 31790-31795
T16V-RNase Sa	49.4	21.92	J. Biol. Chem. (2003) 278, 31790-31795
T59V-RNase Sa	42.8	15.94	J. Biol. Chem. (2003) 278, 31790-31795
T72V-RNase Sa	47.6	21.76	J. Biol. Chem. (2003) 278, 31790-31795
V2T-RNase Sa	45.2	18.79	J. Biol. Chem. (2003) 278, 31790-31795
V36T-RNase Sa	43.8	18.33	J. Biol. Chem. (2003) 278, 31790-31795
V43T-RNase Sa	46.8	21.80	J. Biol. Chem. (2003) 278, 31790-31795
V57T-RNase Sa	33.4	7.87	J. Biol. Chem. (2003) 278, 31790-31795
WT-RNase Sa	49.0	26.40	Protein Sci. (2003) 12, 2367-2373
Q38A-RNase Sa	52.5	31.38	Protein Sci. (2003) 12, 2367-2373
E41K-RNase Sa	46.5	23.56	Protein Sci. (2003) 12, 2367-2373
E54Q-RNase Sa	43.1	17.57	Protein Sci. (2003) 12, 2367-2373
R65A-RNase Sa	45.6	20.13	Protein Sci. (2003) 12, 2367-2373
E74K-RNase Sa	52.1	32.13	Protein Sci. (2003) 12, 2367-2373
H85Q-RNase Sa	49.1	22.05	Protein Sci. (2003) 12, 2367-2373
WT-RNase Sa	47.8	21.88	J. Mol. Biol. (2005) 354, 967-978
D79F-RNase Sa	57.8	26.28	J. Mol. Biol. (2005) 354, 967-978
D79Y-RNase Sa	57.4	24.06	J. Mol. Biol. (2005) 354, 967-978
D79A-RNase Sa	57.0	27.99	J. Mol. Biol. (2005) 354, 967-978
D79I-RNase Sa	57.4	26.11	J. Mol. Biol. (2005) 354, 967-978

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
D79R-RNase Sa	56.8	27.11	J. Mol. Biol. (2005) 354, 967-978
D79L-RNase Sa	56.6	20.17	J. Mol. Biol. (2005) 354, 967-978
D79K-RNase Sa	55.4	24.89	J. Mol. Biol. (2005) 354, 967-978
D79W-RNase Sa	55.4	24.89	J. Mol. Biol. (2005) 354, 967-978
D79H-RNase Sa	53.4	26.94	J. Mol. Biol. (2005) 354, 967-978
D79N-RNase Sa	53.4	26.94	J. Mol. Biol. (2005) 354, 967-978
D79E-RNase Sa	47.0	21.05	J. Mol. Biol. (2005) 354, 967-978
Q94K-RNase Sa	48.6	23.93	J. Mol. Biol. (2005) 354, 967-978
D33A-RNase Sa	31.8	4.94	J. Mol. Biol. (2005) 354, 967-978
WT-RNase Sa	49.3	23.47	Protein Sci. (2010) 19, 1044-1052
D25K/E74K-RNase Sa	51.5	24.43	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G-RNase Sa	66.6	35.19	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/D79F-RNase Sa	77.2	42.17	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/I92D-RNase Sa	34.0	8.28	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/D79A-RNase Sa	75.3	43.30	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/D79F/I92D-RNase Sa	50.2	21.55	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/I70D/E74K/T76P/Q77G/D79F-RNase Sa	41.5	15.23	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/D79F/I92A-RNase Sa	63.9	36.36	Protein Sci. (2010) 19, 1044-1052
D25K/S31P/S42G/S48P/E74K/T76P/Q77G/D79F/Y80A-RNase Sa	58.0	27.15	Protein Sci. (2010) 19, 1044-1052

Table S10. Mutants of RNase Sa3.[27]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-RNase Sa3	46.9	22.18	J. Mol. Biol. (2001) 312, 393-404
Y11F-RNase Sa3	45.0	19.66	J. Mol. Biol. (2001) 312, 393-404
Y33F-RNase Sa3	48.6	24.27	J. Mol. Biol. (2001) 312, 393-404
Y54F-RNase Sa3	38.0	11.30	J. Mol. Biol. (2001) 312, 393-404
Y55F-RNase Sa3	39.8	13.39	J. Mol. Biol. (2001) 312, 393-404
Y58F-RNase Sa3	44.6	19.25	J. Mol. Biol. (2001) 312, 393-404
Y83F-RNase Sa3	41.9	15.90	J. Mol. Biol. (2001) 312, 393-404
Y84F-RNase Sa3	43.6	17.99	J. Mol. Biol. (2001) 312, 393-404
Y89F-RNase Sa3	47.0	22.18	J. Mol. Biol. (2001) 312, 393-404

Table S11. Mutants of lambda repressor.[32-37]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-λRep	55.7	20.08	Biochemistry (1992) 31, 4324-4333
M40V/V47L-λRep	51.3	15.48	Biochemistry (1992) 31, 4324-4333
V36L/M40V/V47I-λRep	53.6	15.48	Biochemistry (1992) 31, 4324-4333
V36I/M40V/V47L-λRep	53.4	16.32	Biochemistry (1992) 31, 4324-4333
V36I/M40V/V47I-λRep	53.7	16.74	Biochemistry (1992) 31, 4324-4333
V36I-λRep	59.1	24.27	Biochemistry (1992) 31, 4324-4333
V36L/M40L/V47I-λRep	59.6	22.18	Biochemistry (1992) 31, 4324-4333
V36F/M40L-λRep	51.6	14.23	Biochemistry (1992) 31, 4324-4333
M40A-λRep	47.1	11.72	Biochemistry (1992) 31, 4324-4333
V36F/M40F/V47I-λRep	47.2	10.88	Biochemistry (1992) 31, 4324-4333
V36F/M40F/V47F-λRep	45.4	7.53	Biochemistry (1992) 31, 4324-4333
L18A/M40A-λRep	23.2	-0.84	Biochemistry (1992) 31, 4324-4333
V36F/M40F/V47I/L65F-λRep	49.1	12.55	Biochemistry (1992) 31, 4324-4333
WT-λRep	53.4	23.96	Proteins (1986) 1, 43-46
G46A-λRep	56.5	28.20	Proteins (1986) 1, 43-46
G48A-λRep	58.1	25.87	Proteins (1986) 1, 43-46
G46A/G48A-λRep	59.6	25.74	Proteins (1986) 1, 43-46
WT-λRep	55	30.75	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
K4Q-λRep	56	19.80	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
Q33S-λRep	55	30.75	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
Q33Y-λRep	61	36.21	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
Q44L-λRep	58	29.32	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
Q44Y-λRep	56	23.76	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
S45L-λRep	55	23.07	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
A49V-λRep	49	14.11	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
Y22H-λRep	34	13.09	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
A66T-λRep	42	4.55	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
I84S-λRep	46	15.25	Proc. Natl. Acad. Sci. USA (1984) 81, 5685-5689
WT-λRep	55.0	15.06	Biochemistry (1990) 29, 7563-7571
P78A-λRep	48.0	21.76	Biochemistry (1990) 29, 7563-7571
G46A/G48A/P78A-λRep	55.0	22.59	Biochemistry (1990) 29, 7563-7571
G46A/G48A-λRep	62.0	28.87	Biochemistry (1990) 29, 7563-7571
WT-λRep	53.9	19.85	Biochemistry (1988) 27, 7571-7574
Y88C-λRep	62.7	31.31	Biochemistry (1988) 27, 7571-7574
G46A/G48A-λRep	62.0	41.00	Biochemistry (1988) 27, 7571-7574
G46A/G48A/Y88C-λRep	70.3	40.36	Biochemistry (1988) 27, 7571-7574
WT-λRep	53.9	29.66	Biochemistry (1988) 27, 7571-7574
Y88C-λRep	62.7	44.98	Biochemistry (1988) 27, 7571-7574
G46A/G48A-λRep	62.0	28.94	Biochemistry (1988) 27, 7571-7574
G46A/G48A/Y88C-λRep	70.3	64.56	Biochemistry (1988) 27, 7571-7574
D14A/Y22W/Q33Y/G46A/G48A-λ(6-85)	73.5	38.11	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/G46A/G48A-λ(6-85)	71.0	50.62	Biochemistry (2004) 43, 13018-13025

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
Y22W/Q33Y/A37G-λ(6-85)	59.5	21.01	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/G46A/S45A/G48A-λ(6-85)	69.5	52.94	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/G46A/S45A/G48A/S79A-λ(6-85)	70.5	48.95	Biochemistry (2004) 43, 13018-13025
Y22W-λ(6-85)	61.0	30.73	Biochemistry (2004) 43, 13018-13025
Y22W/G46A/G48A-λ(6-85)	67.5	43.04	Biochemistry (2004) 43, 13018-13025
Y22W/A37G-λ(6-85)	54.5	21.01	Biochemistry (2004) 43, 13018-13025
Y22W/A63V-λ(6-85)	60.0	29.96	Biochemistry (2004) 43, 13018-13025
Y22W/A37G/A49G-λ(6-85)	47.0	12.89	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/A37G/A49G/A81G-λ(6-85)	47.5	14.06	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/A37G/A49G-λ(6-85)	54.5	20.88	Biochemistry (2004) 43, 13018-13025
Y22W/Q33Y/M42G/S45A/G46A/G48A/S79A-λ(6-85)	59.0	30.44	Biochemistry (2004) 43, 13018-13025

Table S12. Mutants of RNase T1.[25-26, 38-43]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-RNase T1	49.3	23.01	J. Biol. Chem. (1989) 264, 11621-11625
Q25K-RNase T1	51.7	28.87	J. Biol. Chem. (1989) 264, 11621-11625
E58A-RNase T1	46.0	18.83	J. Biol. Chem. (1989) 264, 11621-11625
Q25K/E58A-RNase T1	48.8	22.59	J. Biol. Chem. (1989) 264, 11621-11625
WT-RNase T1	50.9	30.12	Biochemistry (1992) 31, 725-732
Y11F-RNase T1	44.9	22.59	Biochemistry (1992) 31, 725-732
Y42F-RNase T1	54.3	31.38	Biochemistry (1992) 31, 725-732
Y56F-RNase T1	48.8	25.10	Biochemistry (1992) 31, 725-732
Y57F-RNase T1	49.6	28.03	Biochemistry (1992) 31, 725-732
Y68F-RNase T1	46.9	20.92	Biochemistry (1992) 31, 725-732
S12A-RNase T1	47.7	24.27	Biochemistry (1992) 31, 725-732
S17A-RNase T1	52.6	31.38	Biochemistry (1992) 31, 725-732
S64A-RNase T1	46.3	24.69	Biochemistry (1992) 31, 725-732
N9A-RNase T1	48.8	25.52	Biochemistry (1992) 31, 725-732
N36A-RNase T1	50.9	30.54	Biochemistry (1992) 31, 725-732
N44A-RNase T1	45.4	20.50	Biochemistry (1992) 31, 725-732
N81A-RNase T1	42.3	17.99	Biochemistry (1992) 31, 725-732
WT-RNase T1	57.6	30.54	Biochemistry (1994) 33, 10725-10730
Y45W/W59Y-RNase T1	53.1	24.69	Biochemistry (1994) 33, 10725-10730
W59Y-RNase T1	53.4	24.27	Biochemistry (1994) 33, 10725-10730
Y45W-RNase T1	56.0	25.94	Biochemistry (1994) 33, 10725-10730
WT-RNase T1	50.8	25.52	Biochemistry (1998) 37, 16192-16200

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
N44D-RNase T1	45.3	18.41	Biochemistry (1998) 37, 16192-16200
N44S-RNase T1	45.8	20.08	Biochemistry (1998) 37, 16192-16200
N44A-Rnase T1	45.6	19.25	Biochemistry (1998) 37, 16192-16200
WT-RNase T1	50.8	25.52	Biochemistry (1999) 38, 13379-13384
D76N-RNase T1	37.0	10.88	Biochemistry (1999) 38, 13379-13384
D76S-RNase T1	37.2	11.30	Biochemistry (1999) 38, 13379-13384
D76A-RNase T1	35.6	9.20	Biochemistry (1999) 38, 13379-13384
WT-RNase T1	52.3	29.29	Protein Sci. (1999) 8, 1843-1849
D49A-RNase T1	54.0	32.22	Protein Sci. (1999) 8, 1843-1849
D49Y-RNase T1	52.4	30.54	Protein Sci. (1999) 8, 1843-1849
D49F-RNase T1	52.7	31.80	Protein Sci. (1999) 8, 1843-1849
D49W-RNase T1	51.2	28.45	Protein Sci. (1999) 8, 1843-1849
WT-RNase T1	55.5	33.05	Protein Sci. (1999) 8, 1843-1849
D49H-RNase T1	58.9	35.56	Protein Sci. (1999) 8, 1843-1849
WT-RNase T1	51.6	33.05	Eur. J. Biochem. (1996) 241, 516-524
P73V-RNase T1	45.8	23.43	Eur. J. Biochem. (1996) 241, 516-524
WT-RNase T1	57.2	32.70	Eur. J. Biochem. (1994) 220, 527-534
W59Y-RNase T1	54.3	28.80	Eur. J. Biochem. (1994) 220, 527-534
Y24W-RNase T1	58.8	37.80	Eur. J. Biochem. (1994) 220, 527-534
Y24W/W59Y-RNase T1	56.6	33.90	Eur. J. Biochem. (1994) 220, 527-534
Y42W-RNase T1	56.6	32.10	Eur. J. Biochem. (1994) 220, 527-534
Y42W/W59Y-RNase T1	53.7	28.30	Eur. J. Biochem. (1994) 220, 527-534
Y45W-RNase T1	55.9	35.80	Eur. J. Biochem. (1994) 220, 527-534

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
Y45W/W59Y-RNase T1	52.8	28.90	Eur. J. Biochem. (1994) 220, 527-534
H40T-RNase T1	56.7	33.80	Eur. J. Biochem. (1994) 220, 527-534
H40T/W59Y-RNase T1	53.9	28.80	Eur. J. Biochem. (1994) 220, 527-534
H92A-RNase T1	55.9	30.10	Eur. J. Biochem. (1994) 220, 527-534
W59Y/H92A-RNase T1	53.2	26.20	Eur. J. Biochem. (1994) 220, 527-534

Table S13. Mutants of barnase.[44-46]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-brn	53.9	45.46	Biochemistry (1989) 28, 4914-4922
I96V-brn	51.5	39.95	Biochemistry (1989) 28, 4914-4922
I88V-brn	51.0	39.42	Biochemistry (1989) 28, 4914-4922
I96A-brn	44.9	30.97	Biochemistry (1989) 28, 4914-4922
I88A-brn	42.7	25.82	Biochemistry (1989) 28, 4914-4922
L14A-brn	42.0	24.21	Biochemistry (1989) 28, 4914-4922
WT-brn	54.1	48.70	J. Mol. Biol. (1999) 286, 1471-1485
G53A-brn	45.5	34.48	J. Mol. Biol. (1999) 286, 1471-1485
G52A-brn	40.8	26.53	J. Mol. Biol. (1999) 286, 1471-1485
G53V-brn	34.5	16.07	J. Mol. Biol. (1999) 286, 1471-1485
G52V-brn	33.0	13.56	J. Mol. Biol. (1999) 286, 1471-1485
G53Δ-brn	41.0	26.94	J. Mol. Biol. (1999) 286, 1471-1485
WT-brn	54.5	43.93	Biochemistry (2004) 43, 3346-3356
H102A-brn	52.3	39.75	Biochemistry (2004) 43, 3346-3356
D8G-brn	51.5	39.75	Biochemistry (2004) 43, 3346-3356
D8A-brn	52.5	41.42	Biochemistry (2004) 43, 3346-3356
D12G/H102A-brn	50.0	34.73	Biochemistry (2004) 43, 3346-3356
D12A/H102A-brn	51.3	44.35	Biochemistry (2004) 43, 3346-3356
Y13G/H102A-brn	36.7	12.97	Biochemistry (2004) 43, 3346-3356
Q15G/H102A-brn	50.3	33.05	Biochemistry (2004) 43, 3346-3356
Q15A/H102A-brn	51.4	38.91	Biochemistry (2004) 43, 3346-3356
Y17G/H102A-brn	42.0	23.43	Biochemistry (2004) 43, 3346-3356

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
Y17A-brn	49.4	35.98	Biochemistry (2004) 43, 3346-3356
I55G/H102A-brn	47.0	26.78	Biochemistry (2004) 43, 3346-3356
R72G/H102A-brn	46.6	29.29	Biochemistry (2004) 43, 3346-3356
E73G/H102A-brn	39.6	17.15	Biochemistry (2004) 43, 3346-3356
I88G/H102A-brn	31.5	8.79	Biochemistry (2004) 43, 3346-3356
L89G/H102A-brn	43.6	10.46	Biochemistry (2004) 43, 3346-3356
L95G/H102A-brn	43.2	20.08	Biochemistry (2004) 43, 3346-3356
I96G/H102A-brn	36.6	15.90	Biochemistry (2004) 43, 3346-3356
Y97G/H102A-brn	34.4	12.13	Biochemistry (2004) 43, 3346-3356
T100G/H102A-brn	48.0	28.03	Biochemistry (2004) 43, 3346-3356

Table S14. Mutants of RNase A.[47-53]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT-RNase A	63.3	45.44	Protein Sci. (1996) 5, 1697-1703
Y97F-RNase A	53.2	29.46	Protein Sci. (1996) 5, 1697-1703
Y97A-RNase A	29.0	3.51	Protein Sci. (1996) 5, 1697-1703
Y97G-RNase A	30.0	3.97	Protein Sci. (1996) 5, 1697-1703
WT-RNase A	61.3	39.33	Protein Sci. (1997) 6, 1682-1693
N67D-RNase A	61.5	38.91	Protein Sci. (1997) 6, 1682-1693
N67isoD-RNase A	55.0	29.04	Protein Sci. (1997) 6, 1682-1693
WT-RNase A	58.0	34.94	J. Biol. Chem. (2002) 277, 17538-17543
F46V-RNase A	46.5	20.29	J. Biol. Chem. (2002) 277, 17538-17543
F46E-RNase A	26.4	1.30	J. Biol. Chem. (2002) 277, 17538-17543
F46K-RNase A	23.7	-0.79	J. Biol. Chem. (2002) 277, 17538-17543
WT-RNase A	59.7	39.79	Biochemistry (2003) 42, 10651-10658
F46L-RNase A	48.6	25.94	Biochemistry (2003) 42, 10651-10658
F46V-RNase A	47.6	20.21	Biochemistry (2003) 42, 10651-10658
F46A-RNase A	37.3	12.43	Biochemistry (2003) 42, 10651-10658
WT-RNase A	61.1	37.74	Protein Sci. (2007) 16, 1609-1616
S75A-RNase A	53.2	30.59	Protein Sci. (2007) 16, 1609-1616
S75T-RNase A	52.2	30.59	Protein Sci. (2007) 16, 1609-1616
S75C-RNase A	43.2	30.67	Protein Sci. (2007) 16, 1609-1616
S75R-RNase A	38.2	13.47	Protein Sci. (2007) 16, 1609-1616
WT-RNase A	65.6	45.31	Biophys. Chem. (2009) 141, 21-28
A4C/V118C-RNase A	70.5	50.08	Biophys. Chem. (2009) 141, 21-28
R10C/R33C-RNase A	56.9	32.09	Biophys. Chem. (2009) 141, 21-28

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
M30C/N44C-RNase A	47.4	18.03	Biophys. Chem. (2009) 141, 21-28
V43C/R85C-RNase A	67.8	47.74	Biophys. Chem. (2009) 141, 21-28
H105C/V124C-RNase A	64.9	51.84	Biophys. Chem. (2009) 141, 21-28
I107C/A122C-RNase A	57.8	32.97	Biophys. Chem. (2009) 141, 21-28
WT-RNase A	62.8	20.46	Biochemistry (2006) 45, 10795-10806
A4S	77.0	28.28	Biochemistry (2006) 45, 10795-10806
A5S	62.0	18.45	Biochemistry (2006) 45, 10795-10806
S123A	60.0	68.87	Biochemistry (2006) 45, 10795-10806

Table S15. Mutants of staphylococcal nuclease.[54-63]

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
wild type (w/o phosphate buffer)	50.6	22.98	Biochemistry (1991) 30, 1193-1199
wild type (w phosphate buffer)	52.1	22.99	Biochemistry (1991) 30, 1193-1199
P I17G	55.6	30.56	Biochemistry (1991) 30, 1193-1199
P I17T	51.2	20.36	Biochemistry (1991) 30, 1193-1199
H124L	56.4	31.18	Biochemistry (1991) 30, 1193-1199
K116G	54.4	27.80	Biochemistry (1991) 30, 1193-1199
nuclease-conA	32.8	4.84	Biochemistry (1991) 30, 1193-1199
nuclease-con A-S28G	30.5	5.02	Biochemistry (1991) 30, 1193-1199
nuclease-conA-S28G	26.0	0.60	Biochemistry (1991) 30, 1193-1199
wild type (w Pi).	53.4	27.79	Biochemistry (1991) 30, 1193-1199
V66L	55.7	22.98	Biochemistry (1991) 30, 1193-1199
V66L + G88V	57.6	17.18	Biochemistry (1991) 30, 1193-1199
G88V	55.9	21.74	Biochemistry (1991) 30, 1193-1199
V66L + G79S + G88V	53.4	11.49	Biochemistry (1991) 30, 1193-1199
A69T	41.2	12.16	Biochemistry (1991) 30, 1193-1199
118M + A90S	41.6	14.41	Biochemistry (1991) 30, 1193-1199
WT	54.1	15.33	Protein Sci. (1994) 3, 952-959
V66L	59.8	15.74	Protein Sci. (1994) 3, 952-959
V66A	42.2	8.31	Protein Sci. (1994) 3, 952-959
V66W	46.3	10.25	Protein Sci. (1994) 3, 952-959
WT	54.1	15.33	Protein Sci. (1994) 3, 952-959
WT	53.4	25.52	Biochemistry (1988) 27, 4761-4768

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
V66L	55.7	23.43	Biochemistry (1988) 27, 4761-4768
G88V	55.9	22.18	Biochemistry (1988) 27, 4761-4768
V66L/G88V	57.6	16.32	Biochemistry (1988) 27, 4761-4768
V66L/G79S/G88V	53.4	13.39	Biochemistry (1988) 27, 4761-4768
A69T	41.2	13.81	Biochemistry (1988) 27, 4761-4768
I18M/A90S	41.6	11.30	Biochemistry (1988) 27, 4761-4768
L7A	50.9	20.99	Biochemistry (1995) 34, 2034-2041
V23A	41.6	12.97	Biochemistry (1995) 34, 2034-2041
K24G	47.9	19.92	Biochemistry (1995) 34, 2034-2041
L137A	45.3	13.78	Biochemistry (1995) 34, 2034-2041
K70W	59.4	20.38	J. Mol. Biol. (1993) 232, 718-724
G88W	49.9	18.12	J. Mol. Biol. (1993) 232, 718-724
H124L	55.9	18.37	Biochemistry (1996) 35, 10328-10338
H124L/Q80C/K116C (oxidized)	57.9	12.55	Biochemistry (1996) 35, 10328-10338
H124L/Q80C/K116C (reduced)	51.1	7.66	Biochemistry (1996) 35, 10328-10338
G79S/H124L/Q80C/K116C (oxidized)	53.8	11.92	Biochemistry (1996) 35, 10328-10338
G79S/H124L/Q80C/K116C (reduced)	43.0	1.34	Biochemistry (1996) 35, 10328-10338
H124L/G79C/N118C (oxidized)	64.3	24.48	Biochemistry (1996) 35, 10328-10338
H124L/G79C/N118C (reduced)	48.9	8.03	Biochemistry (1996) 35, 10328-10338
H124L/D77C/N118C (oxidized)	53.9	8.28	Biochemistry (1996) 35, 10328-10338
H124L/D77C/N118C (reduced)	29.9	-5.19	Biochemistry (1996) 35, 10328-10338
G29C	48.4	18.41	Protein Sci. (1995) 4, 2545-2558

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
G50C	50.4	19.66	Protein Sci. (1995) 4, 2545-2558
E57C	52.1	20.08	Protein Sci. (1995) 4, 2545-2558
A60C	49.4	17.99	Protein Sci. (1995) 4, 2545-2558
K70C	50.2	20.92	Protein Sci. (1995) 4, 2545-2558
K78C	53.4	21.34	Protein Sci. (1995) 4, 2545-2558
R105C	41.9	11.72	Protein Sci. (1995) 4, 2545-2558
A112C	50.2	19.66	Protein Sci. (1995) 4, 2545-2558
K134C	50.1	20.08	Protein Sci. (1995) 4, 2545-2558
D21K	61.2	26.78	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A	65.9	36.82	J. Mol. Biol. (2000) 303, 125-130
D21N	60.5	28.45	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I	69.7	39.75	J. Mol. Biol. (2000) 303, 125-130
T33V	55.3	24.27	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I/T33V	70.9	38.49	J. Mol. Biol. (2000) 303, 125-130
T41I	57.2	25.52	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I/S59A	71.8	42.68	J. Mol. Biol. (2000) 303, 125-130
S59A	56.2	24.69	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I/T33V	72.9	41.42	J. Mol. Biol. (2000) 303, 125-130
P117G	55.6	26.78	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I/D21K	76.2	45.19	J. Mol. Biol. (2000) 303, 125-130
H124L	56.4	28.87	J. Mol. Biol. (2000) 303, 125-130
P117G/H124L/S128A/T41I/D21N	75.0	46.02	J. Mol. Biol. (2000) 303, 125-130

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
S128A	57.3	25.52	J. Mol. Biol. (2000) 303, 125-130
E73G/D77G	30.0	3.23	J. Biol. Chem. (2001) 276, 46039-46045
E73G/E75G	27.5	1.23	J. Biol. Chem. (2001) 276, 46039-46045
E75G/D77G	32.0	3.82	J. Biol. Chem. (2001) 276, 46039-46045
E73G/E75G/D77G	25.1	0.04	J. Biol. Chem. (2001) 276, 46039-46045
W140H	59.0	23.85	J. Mol. Biol. (2004) 338, 383-400
W140F	56.9	23.01	J. Mol. Biol. (2004) 338, 383-400
W140Y	56.4	20.92	J. Mol. Biol. (2004) 338, 383-400
W140L	43.6	6.69	J. Mol. Biol. (2004) 338, 383-400
F76W/W140H	56.6	21.34	J. Mol. Biol. (2004) 338, 383-400

Table S16. Mutants of T4 lysozyme.[64]

Mutation	T_M (°C)	ΔG° (kJ/mol)
T21C/S38D/L99A/M102E/E108V/S117V/T142C/N114D (“L99A/M102E/St oxidized”)	58.1	16.80
M102K/WT*	45.0	7.23
V87I/I100V/M102L/V103I/M106I/V111A/M120Y/L133F/V149I/T152V/WT* ("Core 10")	59.1	22.70
V87I/I100V/V103I/M106I/V111A/M120Y/L133F/V149I/T152V/WT* ("L102M/Core 10")	58.3	24.35
I78V/V87M/L118I/M120Y/L133F/V149I/T152V/WT* ("Core 7")	55.7	24.76
I78V/V87M/M120Y/L133F/V149I/T152V/WT* ("I118L/Core 7")	56.0	24.83
V87I/I100V/M102L/V103I/M106I/M120Y/L133F/V149I/T152V/WT* ("A111V/Core 10")	60.7	26.72
V87I/I100V/M102L/M106I/V111A/M120Y/L133F/V149I/T152V/WT* ("I103V/Core 10")	61.5	28.59
I78V/L118I/M120Y/L133F/V149I/T152V/WT* ("M87V/Core 7")	60.5	31.60
L118I/WT*	62.4	34.72
M120Y/WT*	65.4	36.64
I78V/WT*	63.4	36.80
V87I/WT*	64.7	37.04
I100V/WT*	64.4	37.97
V103I/WT*	64.0	38.37
L133F/WT*	64.8	38.54
M106I/WT*	66.1	39.76
R96W	53.8	21.35
R96Y	53.4	22.73

Mutation	T_M (°C)	ΔG° (kJ/mol)
R96C	58.9	23.98
R96P	51.1	23.40
R96F	55.1	26.55
R96V	60.2	29.77
R96L	58.0	29.74
R96M	59.5	30.09
R96A	61.5	31.78
R96I	58.7	31.19
R96S	59.6	31.85
D89A/R96H	56.4	31.28
R96G	59.5	32.69
R96H	58.3	32.76
K85A/R96H	56.8	32.64
R96D	57.1	33.57
R96T	59.0	34.27
R96N	58.6	34.55
K85A	65.6	36.67
D89A	65.3	40.12
R96E	59.6	38.38
R96K	66.4	42.87
R96Q	65.2	45.07
M102L/WT*	63.2	32.48

Mutation	T_M (°C)	ΔG° (kJ/mol)
V111A/WT*	48.8	27.97
V149I/WT*	65.2	37.61
G113E/WT*	65.9	36.71
N40D/WT*	66.3	39.80
R119H/WT*	64.4	39.44
R80K/R119H/WT*	64.0	39.81
R14K/WT*	65.1	41.07
R80K/WT*	64.7	41.47
A41D/WT*	65.9	42.75
E22K/WT*	66.5	44.44
N163D/WT*	64.7	43.91
A129M/F153A/WT*	52.6	20.74
L121A/A129L/WT*	62.5	32.40
L121A/A129M/WT*	62.6	33.82
R96-[A]/WT*	36.4	1.32
R148-[AAAA]/WT*	42.0	2.11
I150-[A]/WT*	40.9	2.92
R8Δ/WT*	45.8	5.03
R148-[TT]/WT*	44.7	7.74
R148-[VP]/WT*	43.2	7.66
R8-[A]/WT*	46.1	8.03
R148-[DS]/WT*	46.2	9.14

Mutation	T_M (°C)	ΔG° (kJ/mol)
R148-[AA]/WT*	47.7	11.23
A73-[AAA]/WT*	49.3	11.93
R148-[AAA]/WT*	49.3	12.88
A73-[AA]/WT*	47.2	13.52
R148-[D]/WT*	50.5	14.60
R148-[S]/WT*	49.5	14.80
N68-[A]/WT*	52.1	16.44
R148-[A]/WT*	51.5	18.43
E64-[A]/WT*	54.7	20.36
S44Δ/WT*	58.1	20.99
R119Δ/WT*	55.1	21.56
R119-[A]/WT*	58.6	22.68
D127-[A]/WT*	55.1	22.33
A73Δ/WT*	53.8	22.46
D127Δ/WT*	56.8	23.76
F4-[A]/WT*	56.8	24.57
A73-[A]/WT*	52.8	24.00
V131-[A]/WT*	57.3	25.47
Y88-[A]/WT*	53.9	25.06
E108-[A]/WT*	58.1	26.01
A73-[L]/WT*	56.2	27.25
V75-[A]/WT*	57.4	30.00

Mutation	T_M (°C)	ΔG° (kJ/mol)
N140-[A]/WT*	59.6	30.54
K147-[A]/WT*	59.3	30.48
T115-[A]/WT*	60.5	31.60
A73-[V]/WT*	60.0	34.36
N144-[A]/WT*	63.2	36.76
L164-[AAAA]/WT*	65.8	39.21
R95A/WT*	50.7	18.27
L99G/WT*	44.9	12.73
L99G/E108V/WT*	48.9	19.37
L99A/WT*	53.7	23.53
L99A/E108V/WT*	56.6	29.35
E108V/WT*	67.6	37.88
I27M/L33M/WT*	55.0	8.53
L33A/WT*	56.5	16.92
L66A/WT*	55.2	17.34
I17A/WT*	58.9	20.98
L84A/WT*	54.8	23.80
M102A/M106A/WT*	54.5	23.73
I17M/WT*	59.4	24.87
L99A/WT*	53.6	24.61
V149A/WT*	56.3	26.08
L33M/WT*	60.0	27.54

Mutation	T_M (°C)	ΔG° (kJ/mol)
F153A/WT*	55.6	26.69
L121A/WT*	59.1	27.42
M102A/WT*	57.1	27.06
F104A/WT*	57.8	27.62
L118A/WT*	56.3	27.27
L7A/WT*	59	29.12
L91A/WT*	57.9	29.30
M106A/WT*	60.1	30.20
I50A/WT*	61.1	31.26
I100A/WT*	58.2	31.47
M6A/WT*	60.8	33.46
V103A/WT*	60.9	33.93
I78A/WT*	61.8	36.43
V111A/WT*	62.4	36.58
V111M/WT*	63.3	36.78
V87A/WT*	61	36.21
F153M/WT*	63.7	37.35
L99M/WT*	64.0	40.31
I58T/WT*	55.2	17.34
V149S/WT*	52.1	20.63
Q105M/WT*	62.6	28.66
N101A/WT*	61.6	29.97

Mutation	T_M (°C)	ΔG° (kJ/mol)
V149C/WT*	59.8	29.71
T152A/WT*	61.3	30.84
T152S/WT*	59.8	31.46
T152I/WT*	64.3	36.00
T152C/WT*	63.9	36.90
A93T/WT*	65.3	42.61
T151S/WT*	66.1	44.32
T26S/WT*	66.5	45.45
A129M/WT*	60.1	28.44
A129L/WT*	62.1	29.56
L84M/WT*	60.4	28.47
I78M/WT*	61.6	31.80
V103M/WT*	62.2	31.89
L91M/WT*	63.3	35.83
I100M/WT*	60.8	35.25
L121M/WT*	63.2	37.71
L118M/WT*	63.5	38.26
I27M/WT*	55.2	8.87
V149M/WT*	57.5	28.38
V87M/WT*	59.0	29.55
I50M/WT*	64.7	33.60
F104M/WT*	64.5	34.07

Mutation	T_M (°C)	ΔG° (kJ/mol)
L66M/WT*	62.6	34.29
V149I/WT*	65.3	35.13
V149G/WT*	50.0	17.13
T152V/WT*	66.1	37.23
G30F	63.5	23.42
S117I/N132I	72.0	31.57
S117A/N132M	73.1	33.07
S117A/N132I/WT*	73.7	33.53
S117I/N132M/WT*	73.9	34.67
S117I	72.6	37.78
S117V	73.5	40.60
E11M	72.5	41.24
N132F	71.7	41.26
N132M	72.0	41.26
E11A	71.0	42.94
E11F	72.7	43.54
N132I	71.4	44.65
I78M/L84M/L91M/L99M/I100M/V103M/L118M/L121M/L133M/WT* ("9b-M")	40.4	4.61
L84M/V87M/L91M/L99M/I100M/V103M/G110R/V111M/L118M/L121M/L133M/WT* ("10a-M")	41.9	5.95
I17M/I27M/L33M/WT*	56.0	5.90
I27M/L33M/WT*	54.9	8.56

Mutation	T_M (°C)	ΔG° (kJ/mol)
L84M/L91M/L99M/I100M/V103M/L118M/L121M/L133M/WT* ("8b-M")	44.1	9.48
L84M/V87M/L91M/L99M/I100M/V103M/G110R/V111M/L118M/L121M/L133M/WT*, SeMet ("10a-sM")	48.1	11.56
I78M/L84M/L91M/L99M/L118M/L121M/L133M/WT* ("7c-M")	47.7	13.31
L84M/L91M/L99M/L118M/L121M/L133M/V149M/I150M/F153M/WT* ("9a-M")	40.9	11.40
L84M/V87M/L91M/L99M/L111M/L118M/L121M/L133M/WT* ("8a-M")	50.1	17.48
L84M/L91M/L99M/L118M/L121M/F153M/WT* ("6a-M")	52.0	23.04
L84M/L91M/L99M/L118M/L121M/L133M/WT* ("6b-M")	52.1	24.12
L84M/L91M/L99M/F153M/WT* ("4a-M")	54.8	26.46
L84M/L91M/L99M/L133M/WT* ("4b-M")	55.7	28.28
L118M/L121M/WT* ("2-M")	60.0	33.26
T157I/W158L/WT*	55.0	22.69
W158L/WT*	60.7	30.75
T157I/WT*	59.6	31.85
D20A/WT*	64.3	36.98
E11N/WT*	64.5	33.09
E11H/WT*	65.2	36.12
D20N/WT*	68.2	43.74
D20S/WT*	66.7	38.32
D20T/WT*	67.3	41.50
L133M/WT*	64.1	37.43

Mutation	T_M (°C)	ΔG° (kJ/mol)
L84M/L91M/L99M/L118M/L121M/L133M/F153M/WT* ("7a-M")	50.8	20.96
I78M/L84M/L91M/L99M/I100M/V103M/L118M/L121M/L133M/WT*, SeMet ("9b-sM")	46.1	4.99
L84M/L91M/L99M/L118M/L121M/L133M/F153M/WT*, SeMet ("7a-sM")	56.5	21.32
L84M/L91M/L99M/L118M/L121M/WT*, SeMet ("5-sM")	56.8	24.17
L84M/L91M/L99M/L118M/L121M/WT* ("5-M")	53.2	25.93
G110R/V111M/WT*	63.3	36.78
L84M/V87M/L91M/L99M/G110R/V111M/L118M/L121M/L133M/F153M/WT* ("9a-M")	47.6	13.29
L84M/V87M/L91M/L99M/G110R/V111M/L118M/L121M/L133M/F153M/WT*, SeMet ("9a-sM")	55.0	24.61
L84M/L91M/L99M/WT*, SeMet ("3-sM")	58.7	25.24
L84M/L91M/L99M/WT* ("3-M")	56.5	26.12
L84M/L91M/L99M/V111M/L118M/L121M/L133M/WT* ("7b-M")	53.4	25.64
L84M/L91M/L99M/V111M/L118M/L121M/L133M/WT*, SeMet ("7b-sM")	57.8	26.79
WT* (SeMet)	66.3	35.72
K48-[AAAA]/WT*	52.7	6.19
A42K/WT*	54.2	9.85
S44-[AAA]/L46A/WT*	54.0	12.11
N40-[AA]/K48-[LP]/WT*	55.4	18.11
N40-[SLD]/L46A/WT*	57.1	22.17
K48-[LP]/WT*	57.9	26.38

Mutation	T_M (°C)	ΔG° (kJ/mol)
S44-[AAAA]/WT*	57.9	26.80
N40-[AAAA]/WT*	58.8	28.65
N40L/K43A/S44-[A]/WT*	59.8	32.32
L39A/WT*	62.7	36.64
K48-[AAA]/WT*	51.5	6.82
S44-[A]/WT*	53.5	6.69
K48-[A]/WT*	54.0	10.63
K48-[AA]/WT*	54.9	14.29
S44-[AA]/WT*	55.3	15.80
N40-[A]/WT*	57.4	22.61
S44-[AAA]/WT*	59.2	26.57
K48-[HP]/WT*	58.2	27.27
S44A-[AA]/WT*	60.8	30.31
N40-[AAA]/WT*	60.5	31.15
N40L-[A]/WT*	61.4	34.03
N40-[AA]/WT*	62.3	34.69
N40-[ES]/WT*	63.6	36.36
N40-[SLD]/WT*	63.3	36.77
G77A	65.6	36.17
A82P	66.8	36.78
L133D/WT*	44.0	6.66
N40A/K43A/S44A/E45A/L46A/D47A/K48A/WT*	51.5	10.23

Mutation	T_M (°C)	ΔG° (kJ/mol)
L46A/WT*	55.8	18.91
K43A/WT*	59.1	27.43
K48A/WT*	60.5	28.49
D47A/WT*	59.5	29.64
N40A/S44A/E45A/D47A/K48A/WT*	60.6	31.63
S44A/WT*	63.2	32.96
E45A/WT*	62.2	35.15
N40A/WT*	63.1	35.79
E45A/K48A/WT*	62.2	36.54

REFERENCES

(1) Byrne, A.; Williams, D. V.; Barua, B.; Hagen, S. J.; Kier, B. L.; Andersen, N. H., Folding dynamics and pathways of the trp-cage miniproteins. *Biochemistry* **2014,** *53* (38), 6011-6021.

(2) Williams, D. V.; Byrne, A.; Stewart, J.; Andersen, N. H., Optimal salt bridge for trp-cage stabilization. *Biochemistry* **2011,** *50* (7), 1143-1152.

(3) Barua, B.; Lin, J. C.; Williams, V. D.; Kummler, P.; Neidigh, J. W.; Andersen, N. H., The trp-cage: Optimizing the stability of a globular miniprotein. *Protein Eng Des Sel* **2008,** *21* (3), 171-185.

(4) Rodriguez-Granillo, A.; Annavarapu, S.; Zhang, L.; Koder, R. L.; Nanda, V., Computational design of thermostabilizing d-amino acid substitutions. *J Am Chem Soc* **2011,** *133* (46), 18750-18759.

(5) Williams, D. V.; Barua, B.; Andersen, N. H., Hyperstable miniproteins: Additive effects of d- and l-ala mutations. *Org Biomol Chem* **2008,** *6* (23), 4287-4289.

(6) Jager, M.; Dendle, M.; Kelly, J. W., Sequence determinants of thermodynamic stability in a ww domain--an all-beta-sheet protein. *Protein Sci* **2009,** *18* (8), 1806-1813.

(7) McKnight, C. J.; Doering, D. S.; Matsudaira, P. T.; Kim, P. S., A thermostable 35-residue subdomain within villin headpiece. *J Mol Biol* **1996,** *260* (2), 126-134.

(8) Kubelka, J.; Eaton, W. A.; Hofrichter, J., Experimental tests of villin subdomain folding simulations. *J Mol Biol* **2003,** *329* (4), 625-630.

(9) Kreitler, D. F.; Mortenson, D. E.; Forest, K. T.; Gellman, S. H., Effects of single alpha-to-beta residue replacements on structure and stability in a small protein: Insights from quasiracemic crystallization. *J Am Chem Soc* **2016,** *138* (20), 6498-6505.

(10) Hsu, W. L.; Shih, T. C.; Horng, J. C., Folding stability modulation of the villin headpiece helical subdomain by 4-fluorophenylalanine and 4-methylphenylalanine. *Biopolymers* **2015,** *103* (11), 627-637.

(11) Chiu, T. K.; Kubelka, J.; Herbst-Irmer, R.; Eaton, W. A.; Hofrichter, J.; Davies, D. R., High-resolution x-ray crystal structures of the villin headpiece subdomain, an ultrafast folding protein. *Proc Natl Acad Sci U S A* **2005,** *102* (21), 7517-7522.

(12) Kubelka, J.; Chiu, T. K.; Davies, D. R.; Eaton, W. A.; Hofrichter, J., Sub-microsecond protein folding. *J Mol Biol* **2006,** *359* (3), 546-553.

(13) Xiao, S.; Bi, Y.; Shan, B.; Raleigh, D. P., Analysis of core packing in a cooperatively folded miniature protein: The ultrafast folding villin headpiece helical subdomain. *Biochemistry* **2009,** *48* (21), 4607-4616.

(14) Zheng, T. Y.; Lin, Y. J.; Horng, J. C., Thermodynamic consequences of incorporating 4-substituted proline derivatives into a small helical protein. *Biochemistry* **2010,** *49* (19), 4255-4263.

(15) Bi, Y.; Cho, J. H.; Kim, E. Y.; Shan, B.; Schindelin, H.; Raleigh, D. P., Rational design, structural and thermodynamic characterization of a hyperstable variant of the villin headpiece helical subdomain. *Biochemistry* **2007,** *46* (25), 7497-7505.

(16) Xiao, S. Protein folding and stability: Distinguishing folded from unfolded state effects. Stony Brook University, Stony Brook, NY, 2012.

(17) Peran, I. Dynamics of protein unfolded states. Stony Brook University, Stony Brook, NY, 2017.

(18) Merkel, J. S.; Sturtevant, J. M.; Regan, L., Sidechain interactions in parallel beta sheets: The energetics of cross-strand pairings. *Structure* **1999,** *7* (11), 1333-1343.

(19) Yu, M. H.; Weissman, J. S.; Kim, P. S., Contribution of individual side-chains to the stability of bpti examined by alanine-scanning mutagenesis. *J Mol Biol* **1995,** *249* (2), 388-397.

(20) Liu, Y.; Breslauer, K.; Anderson, S., "Designing out" disulfide bonds: Thermodynamic properties of 30-51 cystine substitution mutants of bovine pancreatic trypsin inhibitor. *Biochemistry* **1997,** *36* (18), 5323-5335.

(21) Jackson, S. E.; Moracci, M.; elMasry, N.; Johnson, C. M.; Fersht, A. R., Effect of cavity-creating mutations in the hydrophobic core of chymotrypsin inhibitor 2. *Biochemistry* **1993,** *32* (42), 11259-11269.

(22) de Prat Gay, G.; Johnson, C. M.; Fersht, A. R., Contribution of a proline residue and a salt bridge to the stability of a type i reverse turn in chymotrypsin inhibitor-2. *Protein Eng* **1994,** *7* (1), 103-108.

(23) elMasry, N. F.; Fersht, A. R., Mutational analysis of the n-capping box of the alpha-helix of chymotrypsin inhibitor 2. *Protein Eng* **1994,** *7* (6), 777-782.

(24) Gribenko, A. V.; Makhatadze, G. I., Role of the charge-charge interactions in defining stability and halophilicity of the cspb proteins. *J Mol Biol* **2007,** *366* (3), 842-856.

(25) Hebert, E. J.; Giletto, A.; Sevcik, J.; Urbanikova, L.; Wilson, K. S.; Dauter, Z.; Pace, C. N., Contribution of a conserved asparagine to the conformational stability of ribonucleases sa, ba, and t1. *Biochemistry* **1998,** *37* (46), 16192-16200.

(26) Grimsley, G. R.; Shaw, K. L.; Fee, L. R.; Alston, R. W.; Huyghues-Despointes, B. M.; Thurlkill, R. L.; Scholtz, J. M.; Pace, C. N., Increasing protein stability by altering long-range coulombic interactions. *Protein Sci* **1999,** *8* (9), 1843-1849.

(27) Pace, C. N.; Horn, G.; Hebert, E. J.; Bechert, J.; Shaw, K.; Urbanikova, L.; Scholtz, J. M.; Sevcik, J., Tyrosine hydrogen bonds make a large contribution to protein stability. *J Mol Biol* **2001,** *312* (2), 393-404.

(28) Takano, K.; Scholtz, J. M.; Sacchettini, J. C.; Pace, C. N., The contribution of polar group burial to protein stability is strongly context-dependent. *J Biol Chem* **2003,** *278* (34), 31790-31795.

(29) Yakovlev, G. I.; Mitkevich, V. A.; Shaw, K. L.; Trevino, S.; Newsom, S.; Pace, C. N.; Makarov, A. A., Contribution of active site residues to the activity and thermal stability of ribonuclease sa. *Protein Sci* **2003,** *12* (10), 2367-2373.

(30) Trevino, S. R.; Gokulan, K.; Newsom, S.; Thurlkill, R. L.; Shaw, K. L.; Mitkevich, V. A.; Makarov, A. A.; Sacchettini, J. C.; Scholtz, J. M.; Pace, C. N., Asp79 makes a large, unfavorable contribution to the stability of rnase sa. *J Mol Biol* **2005,** *354* (4), 967-978.

(31) Fu, H.; Grimsley, G.; Scholtz, J. M.; Pace, C. N., Increasing protein stability: Importance of deltac(p) and the denatured state. *Protein Sci* **2010,** *19* (5), 1044-1052.

(32) Lim, W. A.; Farruggio, D. C.; Sauer, R. T., Structural and energetic consequences of disruptive mutations in a protein core. *Biochemistry* **1992,** *31* (17), 4324-4333.

(33) Hecht, M. H.; Sturtevant, J. M.; Sauer, R. T., Stabilization of lambda repressor against thermal denaturation by site-directed gly----ala changes in alpha-helix 3. *Proteins* **1986,** *1* (1), 43-46.

(34) Hecht, M. H.; Sturtevant, J. M.; Sauer, R. T., Effect of single amino acid replacements on the thermal stability of the nh2-terminal domain of phage lambda repressor. *Proc Natl Acad Sci U S A* **1984,** *81* (18), 5685-5689.

(35) Reidhaar-Olson, J. F.; Parsell, D. A.; Sauer, R. T., An essential proline in lambda repressor is required for resistance to intracellular proteolysis. *Biochemistry* **1990,** *29* (33), 7563-7571.

(36) Stearman, R. S.; Frankel, A. D.; Freire, E.; Liu, B. S.; Pabo, C. O., Combining thermostable mutations increases the stability of lambda repressor. *Biochemistry* **1988,** *27* (19), 7571-7574.

(37) Yang, W. Y.; Gruebele, M., Rate-temperature relationships in lambda-repressor fragment lambda 6-85 folding. *Biochemistry* **2004,** *43* (41), 13018-13025.

(38) Shirley, B. A.; Stanssens, P.; Steyaert, J.; Pace, C. N., Conformational stability and activity of ribonuclease t1 and mutants. Gln25----lys, glu58----ala, and the double mutant. *J Biol Chem* **1989,** *264* (20), 11621-11625.

(39) Shirley, B. A.; Stanssens, P.; Hahn, U.; Pace, C. N., Contribution of hydrogen bonding to the conformational stability of ribonuclease t1. *Biochemistry* **1992,** *31* (3), 725-732.

(40) Fabian, H.; Schultz, C.; Backmann, J.; Hahn, U.; Saenger, W.; Mantsch, H. H.; Naumann, D., Impact of point mutations on the structure and thermal stability of ribonuclease t1 in aqueous solution probed by fourier transform infrared spectroscopy. *Biochemistry* **1994,** *33* (35), 10725-10730.

(41) Giletto, A.; Pace, C. N., Buried, charged, non-ion-paired aspartic acid 76 contributes favorably to the conformational stability of ribonuclease t1. *Biochemistry* **1999,** *38* (40), 13379-13384.

(42) Schindler, T.; Mayr, L. M.; Landt, O.; Hahn, U.; Schmid, F. X., The role of a trans-proline in the folding mechanism of ribonuclease t1. *Eur J Biochem* **1996,** *241* (2), 516-524.

(43) Schubert, W. D.; Schluckebier, G.; Backmann, J.; Granzin, J.; Kisker, C.; Choe, H. W.; Hahn, U.; Pfeil, W.; Saenger, W., X-ray crystallographic and calorimetric studies of the effects of the mutation trp59-->tyr in ribonuclease t1. *Eur J Biochem* **1994,** *220* (2), 527-534.

(44) Kellis, J. T., Jr.; Nyberg, K.; Fersht, A. R., Energetics of complementary side-chain packing in a protein hydrophobic core. *Biochemistry* **1989,** *28* (11), 4914-4922.

(45) Axe, D. D.; Foster, N. W.; Fersht, A. R., An irregular beta-bulge common to a group of bacterial rnases is an important determinant of stability and function in barnase. *J Mol Biol* **1999,** *286* (5), 1471-1485.

(46) Vu, N. D.; Feng, H.; Bai, Y., The folding pathway of barnase: The rate-limiting transition state and a hidden intermediate under native conditions. *Biochemistry* **2004,** *43* (12), 3346-3356.

(47) Eberhardt, E. S.; Wittmayer, P. K.; Templer, B. M.; Raines, R. T., Contribution of a tyrosine side chain to ribonuclease a catalysis and stability. *Protein Sci* **1996,** *5* (8), 1697-1703.

(48) Catanzano, F.; Graziano, G.; Capasso, S.; Barone, G., Thermodynamic analysis of the effect of selective monodeamidation at asparagine 67 in ribonuclease a. *Protein Sci* **1997,** *6* (8), 1682-1693.

(49) Salamanca, S.; Villegas, V.; Vendrell, J.; Li, L.; Aviles, F. X.; Chang, J. Y., The unfolding pathway of leech carboxypeptidase inhibitor. *J Biol Chem* **2002,** *277* (20), 17538-17543.

(50) Kadonosono, T.; Chatani, E.; Hayashi, R.; Moriyama, H.; Ueki, T., Minimization of cavity size ensures protein stability and folding: Structures of phe46-replaced bovine pancreatic rnase a. *Biochemistry* **2003,** *42* (36), 10651-10658.

(51) Johnson, R. J.; Lin, S. R.; Raines, R. T., Genetic selection reveals the role of a buried, conserved polar residue. *Protein Sci* **2007,** *16* (8), 1609-1616.

(52) Pecher, P.; Arnold, U., The effect of additional disulfide bonds on the stability and folding of ribonuclease a. *Biophys Chem* **2009,** *141* (1), 21-28.

(53) Gotte, G.; Donadelli, M.; Laurents, D. V.; Vottariello, F.; Morbio, M.; Libonati, M., Increase of rnase a n-terminus polarity or c-terminus apolarity changes the two domains' propensity to swap and form the two dimeric conformers of the protein. *Biochemistry* **2006,** *45* (36), 10795-10806.

(54) Eftink, M. R.; Ghiron, C. A.; Kautz, R. A.; Fox, R. O., Fluorescence and conformational stability studies of staphylococcus nuclease and its mutants, including the less stable nuclease-concanavalin a hybrids. *Biochemistry* **1991,** *30* (5), 1193-1199.

(55) Carra, J. H.; Anderson, E. A.; Privalov, P. L., Thermodynamics of staphylococcal nuclease denaturation. Ii. The a-state. *Protein Sci* **1994,** *3* (6), 952-959.

(56) Shortle, D.; Meeker, A. K.; Freire, E., Stability mutants of staphylococcal nuclease: Large compensating enthalpy-entropy changes for the reversible denaturation reaction. *Biochemistry* **1988,** *27* (13), 4761-4768.

(57) Carra, J. H.; Privalov, P. L., Energetics of denaturation and m values of staphylococcal nuclease mutants. *Biochemistry* **1995,** *34* (6), 2034-2041.

(58) Gittis, A. G.; Stites, W. E.; Lattman, E. E., The phase transition between a compact denatured state and a random coil state in staphylococcal nuclease is first-order. *J Mol Biol* **1993,** *232* (3), 718-724.

(59) Hinck, A. P.; Truckses, D. M.; Markley, J. L., Engineered disulfide bonds in staphylococcal nuclease: Effects on the stability and conformation of the folded protein. *Biochemistry* **1996,** *35* (32), 10328-10338.

(60) Byrne, M. P.; Stites, W. E., Chemically crosslinked protein dimers: Stability and denaturation effects. *Protein Sci* **1995,** *4* (12), 2545-2558.

(61) Chen, J.; Lu, Z.; Sakon, J.; Stites, W. E., Increasing the thermostability of staphylococcal nuclease: Implications for the origin of protein thermostability. *J Mol Biol* **2000,** *303* (2), 125-130.

(62) Leung, K. W.; Liaw, Y. C.; Chan, S. C.; Lo, H. Y.; Musayev, F. N.; Chen, J. Z.; Fang, H. J.; Chen, H. M., Significance of local electrostatic interactions in staphylococcal nuclease studied by site-directed mutagenesis. *J Biol Chem* **2001,** *276* (49), 46039-46045.

(63) Maki, K.; Cheng, H.; Dolgikh, D. A.; Shastry, M. C.; Roder, H., Early events during folding of wild-type staphylococcal nuclease and a single-tryptophan variant studied by ultrarapid mixing. *J Mol Biol* **2004,** *338* (2), 383-400.

(64) Baase, W. A.; Liu, L. J.; Tronrud, D. E.; Matthews, B. W., Lessons from the lysozyme of phage t4. *Protein Science* **2010,** *19* (4), 631-641.