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

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ABSTRACT

Changes in protein stability are commonly reported as changes in the melting temperature, ΔT_M, or as changes in unfolding free energy at a particular temperature, $\Delta\Delta G°$. Using data for 866 mutants from 16 proteins we examine the relationship between $\Delta\Delta G°$ and ΔT_M. A linear relationship is observed for each protein. The slopes of the plots of ΔT_M *vs* $\Delta\Delta G°$ for different proteins scale as N^{-1} where N is the number of residues in the protein. Thus, a given change in $\Delta G°$ causes a much larger change in T_M for a small protein relative to the effect observed for a large protein. The analysis suggests that reasonable estimates of $\Delta\Delta G°$ for a mutant can be obtained by interpolating measured values of T_M. The relationship between $\Delta\Delta G°$ and ΔT_M has implications for the design and interpretation of high-throughput assays of protein-ligand binding. So-called thermal shift assays rely upon the increase in stability which results from ligand binding to the folded state. Quantitative relationships are derived which show that the observed thermal shift, ΔT_M scales as N^{-1}. Hence thermal shift assays are considerably less sensitive for ligand binding to larger proteins.

INTRODUCTION

Protein stability can be defined in a variety of ways: through the unfolding free energy at a given temperature, $\Delta G°$, *via* the transition midpoint for thermal unfolding, T_M, by the resistance to proteolysis, or by *in vivo* stability. The most rigorous definition, from a thermodynamic perspective is to define $\Delta G°$ as a function of temperature, and if needed pressure. The Gibbs-Helmholtz equation is commonly used to define thermodynamic stability, but requires determination of T_M, ΔH_M, the enthalpy of unfolding at T_M, and $\Delta C_p°$, the change in heat capacity between the unfolded and folded states. In its simplest form $\Delta C_p°$ is assumed to be independent of temperature. These parameters are often not reported for proteins and instead values of T_M and $\Delta G°$ at one temperature are given. In some cases only T_M values are reported for mutants and it would be useful to be able to estimate changes in $\Delta G°$ from changes in T_M with high reliability.

Prior analysis has suggested an interesting relationship between changes in stability, $\Delta\Delta G°$ and changes in T_M, ΔT_M. Rees and Robertson showed that these quantities are related to each other, with the effect varying inversely with size of the protein.[1] Thus a small protein is predicted to exhibit a larger change in $\Delta G°$ for a given change in T_M and vice versa. Rees and Robertson examined mutational data for three proteins of different sizes to verify this relationship. Since their initial work considerably more data has been generated on the effects of mutations on both T_M and $\Delta G°$. In addition, thermal shift assays have become a popular tool for high-throughput screening of protein ligand interactions, and the scaling of $\Delta\Delta G°$ and ΔT_M have important implications for their interpretation and design. Briefly, thermal shift assays monitor an increase in T_M for a protein in the presence of a ligand which binds preferentially to the folded state; the increase in T_M results from the increase in $\Delta G°$ caused by ligand binding.[2-4] The method is attractive for its broad applicability and amenability to high-throughput screening, especially fluorescence-based thermal

shift assays, which use nonspecific, extrinsic dyes to detect protein unfolding. Recently, methods for cellular thermal shift assays have been developed, offering the promise of high-throughput *in vivo* drug screening.[5-8] The largest thermal shifts are known to be observed at significant molar excess of ligand. It is well understood how ligand binding affects $\Delta G°$, however the quantitative relationship between changes in T_M and ligand affinity is not well understood. In addition, sensitivity is also expected to depend on protein size, although this relationship has not been examined.

The prediction that changes in T_M are related to changes in ΔG in a way which scales as N^{-1}, where N is the number of residues has interesting implications for these types of experiments, since it will impact the sensitivity of the response to ligand binding. Here we analyze stability data, $\Delta G°$ and T_M for 866 mutants of 16 proteins and demonstrate a striking correlation between $\Delta\Delta G°$ and T_M. Plotting the experimental data reveals that for each protein there is a linear relationship between $\Delta\Delta G°$ and ΔT_M with the slope varying as N^{-1} where N is the number of residues in the protein. We examine the implications for thermal shift assays of ligand binding and show that small proteins are expected to have significantly larger shifts in T_M upon ligand binding than larger proteins. The analysis also illustrates how changes in T_M can be used to estimate changes in $\Delta G°$ with reasonable accuracy.

MATERIALS AND METHODS

Data was gathered from the ProTherm database as well as literature sources. For this analysis, selection criteria required that each entry have data for T_M, and $\Delta G°$ or sufficient data to calculate these values. All proteins in the dataset exhibit thermodynamically two-state and reversible unfolding. Only stability data at a single pH was included for each set of mutants.

RESULTS AND DISCUSSION

The temperature-dependent protein stability curve is described by the modified Gibbs-Helmholtz equation with the assumption that ΔC_p° is independent of T (1)

$$\Delta G(T) = \Delta H_M \left(1 - \frac{T}{T_M}\right) + \Delta C_p^\circ \left(T - T_M - T \ln\left(\frac{T}{T_M}\right)\right) \quad (1)$$

where T_M is the midpoint of the thermal unfolding transition, ΔH_M is the change in enthalpy upon unfolding at T_M and ΔC_p° is the difference in constant pressure heat capacity between the unfolded and folded states. Near the stability range of proteins, equation (1) is well approximated by a quadratic equation.[9-10] From this, a relationship can be derived to determine the maximum stability for a protein

$$\Delta G_{max} \cong \left(\frac{{\Delta H_M}^2}{2T_M \Delta {C_p}^\circ}\right) \quad (2)$$

where ΔG_{max} is the stability of a protein at the temperature of maximum stability (T_{max}), given by equation (3)

$$T_{max} \cong T_M - \left(\frac{\Delta H_M}{\Delta {C_p}^\circ}\right) \quad (3)$$

Rees and Robertson derived the following relationship between N, T_M and ΔG_{max} for any protein by substituting parameters for the chain-length dependent ΔH and ΔC_p° values of proteins determined by Robertson and Murphy into equation (2).[10-11] ΔH and ΔC_p° are approximately linear functions of N.

$$\Delta G_{max} \cong \left(\frac{0.0290N}{T_M}\right)(T_M - 282.6)^2 \text{ kJ/mol} \quad (4)$$

Rees and Robertson further demonstrate that equation (4) can be differentiated to determine the chain-length dependent relationship between ΔG_{max} and T_M, equation (5)

$$\frac{1}{N}\frac{d\Delta G_{max}}{dT_M} = 0.029 - \frac{2316}{{T_M}^2} \quad (5)$$

They examined three proteins in detail and demonstrated a linear relationship between $\Delta\Delta G_{max}$ and ΔT_M that scales as N^{-1} and predicted a value of 0.009 kJ mol^{-1} K^{-1} res^{-1} for the ratio of $\Delta\Delta G_{max}$ and ΔT_M for a protein with a T_M of 340 K. We collected thermodynamic data from the literature and a new dataset of 866 mutants of 16 proteins was compiled for which T_M and $\Delta G°$ data was available. Most of the available mutational stability data was reported at 298 K rather than at T_{max}, so $\Delta G°$ was plotted instead of ΔG_{max}. This is a reasonable substitution, since $T_{max} \approx 283$ K, independent of T_M, as noted by Rees and Robertson and the slope of the $\Delta G°$ *vs* T plot is small near T_{max} since the slope is 0 at T_{max}. In either case, the model assumes that changes in T_M are the result of changes in ΔG rather than changes in T_{max} or $\Delta C_p°$. This assumption is justified by Rees and Robertson, who observe that mutations tend to "pull up" or "push down" the protein stability curve, rather than shifting or broadening it, as well as by extensive work showing that $\Delta C_p°$ is strongly correlated to chain length.[11-13] Assuming that the change in stability results from a vertical shift of the Gibbs-Helmholtz plot, $\Delta G°$ at any temperature can be used since $\Delta\Delta G$ will be independent of the choice of T. In any case, any error due to the choice of 298 K as the reference temperature is expected to be small.

The proteins studied range in size from Trp cage at 20 residues to T4 lysozyme with 164 resides and include all-α, all-β and mixed α-β folds. Several of the domains contain disulfides while others do not (Table 1). Data for at least 9 mutants are available for each protein. The mutations include truncation of sidechains, the introduction or removal of charged residues and mutants which create cavities in the interior of proteins. These data were fitted to a linear equation to extract the value of the slope of a plot ΔT_M *vs* $\Delta\Delta G°$ for each protein. Representative plots for 8 proteins are shown in Figure 1 and the remainder are shown in supporting information (Figure S1). A strong linear correlation is observed for each protein, R^2 is greater than 0.85 for 10 of the 16 proteins and above

0.72 for five other proteins. The R^2 value for RNase A is the lowest at 0.6389. The p values for all but one protein are less than 7×10^{-8} and the other still has a p value less than 2×10^{-3}. Values of the slopes of ΔT_M *vs* $\Delta\Delta G$ for the different proteins scale as N^{-1}. Values of $\Delta T_M/\Delta\Delta G°$ were plotted against N^{-1} and fitted to a linear equation with a fixed intercept at the origin (Figure 2). The fit has R^2 and p values of 0.8726 and 2.34×10^{-11}, respectively. The relationship derived from the experimental data between chain length the change in T_M for a given stability change at 298 K is described by equation (6)

$$\frac{\Delta T_M}{\Delta\Delta G°} = \frac{90.72}{N} \text{ mol K kJ}^{-1}$$

$$\frac{1}{N}\frac{\Delta\Delta G°}{\Delta T_M} = 0.011 \text{ kJ mol}^{-1}\text{K}^{-1}\text{res}^{-1} \quad (6)$$

The value of 0.011 kJ mol^{-1} K^{-1} res^{-1} is in reasonable agreement with the value of 0.009 kJ mol^{-1} K^{-1} res^{-1} originally estimated by Rees and Robertson. The inverse correlation between chain length and the effect of stabilization at 298 K on T_M is apparent, demonstrating that the thermal stability of small proteins is more sensitive to changes in stability than in larger proteins. This is a consequence of the broad stability curves of small proteins resulting from the small $\Delta C_p°$ values of these systems. It should be noted that equation (6) is derived from direct fitting of the experimental data, and therefore is not dependent on the relationship between N, ΔG_{max}, ΔH_M and $\Delta C_p°$.

Equation (6) does more than predict ΔT_M for mutant proteins, the equation is an empirical relationship between stability and T_M. Thus, it can also be employed to predict changes in T_M observed upon the binding of a ligand to the folded or unfolded state of a protein of a given length, provided that the effects of ligand binding on stability mimic the thermodynamic effects of mutation. This is most likely the case for ligands that do not require a significant protein

conformational change to bind or lead to a large change in the heat capacity of the system. Many druglike ligands are small molecules which bind to sites which are accessible to solvent and equation (6) is likely to apply to this important class. The $\Delta G°$ of folding in the absence of ligand is given by equation (7)

$$\Delta G° = -RT \ln \frac{[F]}{[U]} \tag{7}$$

where R is the gas constant, $[F]$ is the equilibrium concentration of folded protein and $[U]$ is the equilibrium concentration of unfolded protein. In the presence of a single ligand that binds exclusively to the folded state, equation (7) becomes equation (8)[14-15]

$$\Delta G_L° = \Delta G° - RT \ln \left(1 + \frac{[L]}{K_D}\right) \tag{8}$$

where $\Delta G_L°$ is the Gibbs free energy of folding at 298 K in the presence of ligand, $[L]$ is the concentration of free ligand and K_D is the dissociation constant for ligand binding to the folded state. The change in free energy, $\Delta\Delta G°$ upon ligand binding is:

$$\Delta\Delta G_L° = -RT \ln \left(1 + \frac{[L]}{K_D}\right) \tag{9}$$

where $\Delta\Delta G_L°$ is the change in the Gibbs free energy of folding at 298 K upon the binding of ligand, which is related to ΔT_M by the linear relationship determined from the mutational dataset analysis (equation 6). A somewhat different equation applies when a ligand binds to both the folded and unfolded state, but the scaling of $\Delta\Delta G°$ and ΔT_M have the same implications and here we focus on the case where ligand binds only to the folded state. It is common in ligand binding assays to use a large molar excess of ligand such that the total ligand concentration $[L]_T$ is much greater than the total protein concentration $[P]_T$. In this limiting case $[L] \approx [L]_T$ and the curve can be approximated by a linear equation. However, it is straightforward to derive the quadratic solution for $[L]$ which is valid at all $[L]_T$ and $[P]_T$ (10)

$$[L] = \left(\frac{-([P]_T-[L]_T+K_D+K_DK_U)+\sqrt{([P]_T-[L]_T+K_D+K_DK_U)^2-4(-K_D-K_DK_U)}}{2}\right) \quad (10)$$

where K_U is the protein unfolding equilibrium constant in the absence of ligand. The value of K_U can be determined from the chain-length dependent stability equations parameterized by Robertson and Murphy or Sawle and Ghosh.[11, 16] However, this term is usually small compared to the other terms and can generally be excluded except in cases for proteins that are unstable in the absence of ligand (large K_U) or very weakly binding ligands (large K_D).

Equations (6), (9) and (10) can be combined by replacing $[L]$ in equation (9) with the expression in equation (10) and using equation (6) to express $\Delta\Delta G°$ to calculate the expected thermal shift for any N, $[P]_T$, $[L]_T$ and K_D. Representative plots are shown in Figure 3. Plotting $[L]_T/K_D$ normalizes $[L]_T$ relative to K_D. Several important considerations for thermal shift assays can be inferred from these plots. Firstly, thermal shifts which are much lower than the maximum achievable value are predicted when $[L]_T < [P]_T$, therefore it is preferable for the ligand to be in molar excess for the thermal shift to be most clearly observed; of course, significant thermal shifts can be observed for strongly binding ligands even at lower total ligand concentrations. When $[L]_T$ is normalized by K_D, it is apparent that the magnitude of the thermal shift is determined by the ratio of $[L]_T/K_D$. For example, when $[L]_T/K_D = 100$, $\Delta T_M = 1$ °C for a 100-residue protein for any K_D, provided that $[L]_T$ is at least tenfold higher than $[P]_T$. Consequently, thermal shift assays are not particularly sensitive to μM or weaker binding without employing high ligand concentrations.

Conversely, it is apparent from the sharp increase in the thermal shift where $[L]_T = [P]_T$ (indicated by red arrows in Figure 3) that ΔT_M is highly sensitive to the relative concentrations of protein and ligand when $[L]_T$ and $[P]_T$ are similar and larger than K_D (see for example Figure 3C, 3D). Consequently, significant loss of precision is expected to arise due to any error in the concentrations of protein and ligand under these conditions. This loss of precision is more likely

to be a problem in strongly binding systems because detection limits will necessitate protein concentrations much higher than K_D.

Most importantly, the analysis presented here predicts an inverse correlation between protein size and the magnitude of the expected thermal shift. In general, the analysis predicts that a protein will exhibit half the thermal shift experienced by a protein that is half as large. The model is validated by thermal shift assays from the literature, which report similar thermal shifts to those predicted.[17-18] Plots of thermal shift *versus* chain length at varying values of $[L]_T$ and $[P]_T$ also illustrate these predictions (Figure 4). Comparing plots of equal $[P]_T/[L]_T$, it is interesting to note that increasing $[P]_T$ and $[L]_T$ in tandem results in higher predicted thermal shifts due to the shift in the binding equilibrium, although this effect is much weaker than the effects of N, $[L]_T$ and K_D. It is important to note that the thermal shift model is based upon the chain-length dependence of stability and the relationship between ΔT_M and $\Delta\Delta G°$ for single domain proteins. The constant in equation (6) was derived considering the data sets available to us, as more data becomes available the constant may be revised, but the general conclusions are robust.

CONCLUSIONS

A dataset of mutational stability data for 866 mutants of 16 proteins was analyzed to derive a relationship between changes in $\Delta G°$ and T_M as a function of chain length. The strong linear correlation for each protein and the excellent correlation found between the slopes of the individual plots and N^{-1} indicates that changes in Gibbs free energy can be predicted from ΔT_M for a given protein provided that the parameters in equation (6) are known. From a practical perspective, $\Delta G°$ and T_M could be obtained for a subset of mutants and used to confirm linearity of the $\Delta G°$ *vs* T_M plot, allowing $\Delta G°$ to be estimated for other mutants. The data analyzed here for a range of

structurally distinct proteins provides confidence in the use of linear interpolation to estimate $\Delta\Delta G°$ values from changes in T_M.

The relationship between ΔT_M and $\Delta\Delta G°$ was used to model thermal stability changes upon ligand binding. The model predicts several important considerations for the design of thermal shift assays. Firstly, that thermal shift assays are less sensitive to μM or weaker binding affinities. Secondly, the full magnitude of the thermal shift is not observed unless the ligand is in molar excess; of course, in cases of strong binding affinities significant shifts can be observed even when the thermal shift is attenuated by the excess protein. In addition, thermal shift assays in more strongly binding systems are expected to be more prone to low precision when the concentrations of protein and ligand are very similar and larger than K_D due to the sensitivity of ΔT_M to the protein-to-ligand concentration ratio when the ratio is close to 1. A key observation is that there is a strong inverse correlation between chain length and the magnitude of the thermal shift, with the thermal shift being halved for every doubling of the protein length. Thus, thermal shift assays will be less sensitive for large proteins. There are some caveats to these conclusions; large, multidomain proteins may exhibit much larger thermal shifts than predicted if the assay monitors only the unfolding of a ligand binding domain. In addition, experimental thermal shift assays may actually be monitoring the conversion of monomeric folded proteins into an aggregated oligomeric unfolded form. In this case the equilibrium is more complicated. In some cases, thermal shift assays might be following the transition from the native state to a molten globule intermediate. The same general conclusions are expected if the thermodynamics of the native to molten globule transition scale with N in the same manner as the native to unfolded transition does since the predicted dependence of the thermal shift upon the size of a protein is based upon the relationship between the thermodynamics of protein unfolding and size.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Thermodynamic stability data for mutants included in the analysis (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ABBREVIATIONS

T, temperature; T_M, midpoint of protein thermal unfolding transition; $\Delta G°$, Gibbs free energy change upon unfolding under standard conditions; N, number of residues; ΔH_M, enthalpy change upon unfolding at T_M; $\Delta C_p°$, constant pressure heat capacity change upon unfolding; $\Delta G(T)$, temperature-dependent Gibbs free energy change upon unfolding; T_{max}, temperature of maximum stability; ΔG_{max}, Gibbs free energy change at T_{max}; R, gas constant; [F], equilibrium concentration of folded protein; [U], equilibrium concentration of unfolded protein; $\Delta G_L°$, Gibbs free energy change upon folding in the presence of ligand at 298 K; K_D, dissociation constant; [L], equilibrium concentration of free ligand; $\Delta\Delta G_L°$, change in the Gibbs free energy change upon folding upon the binding of ligand at 298 K; $[L]_T$, total equilibrium concentration of ligand; $[P]_T$, total equilibrium concentration of protein; K_U, equilibrium constant of protein unfolding.

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FIGURES

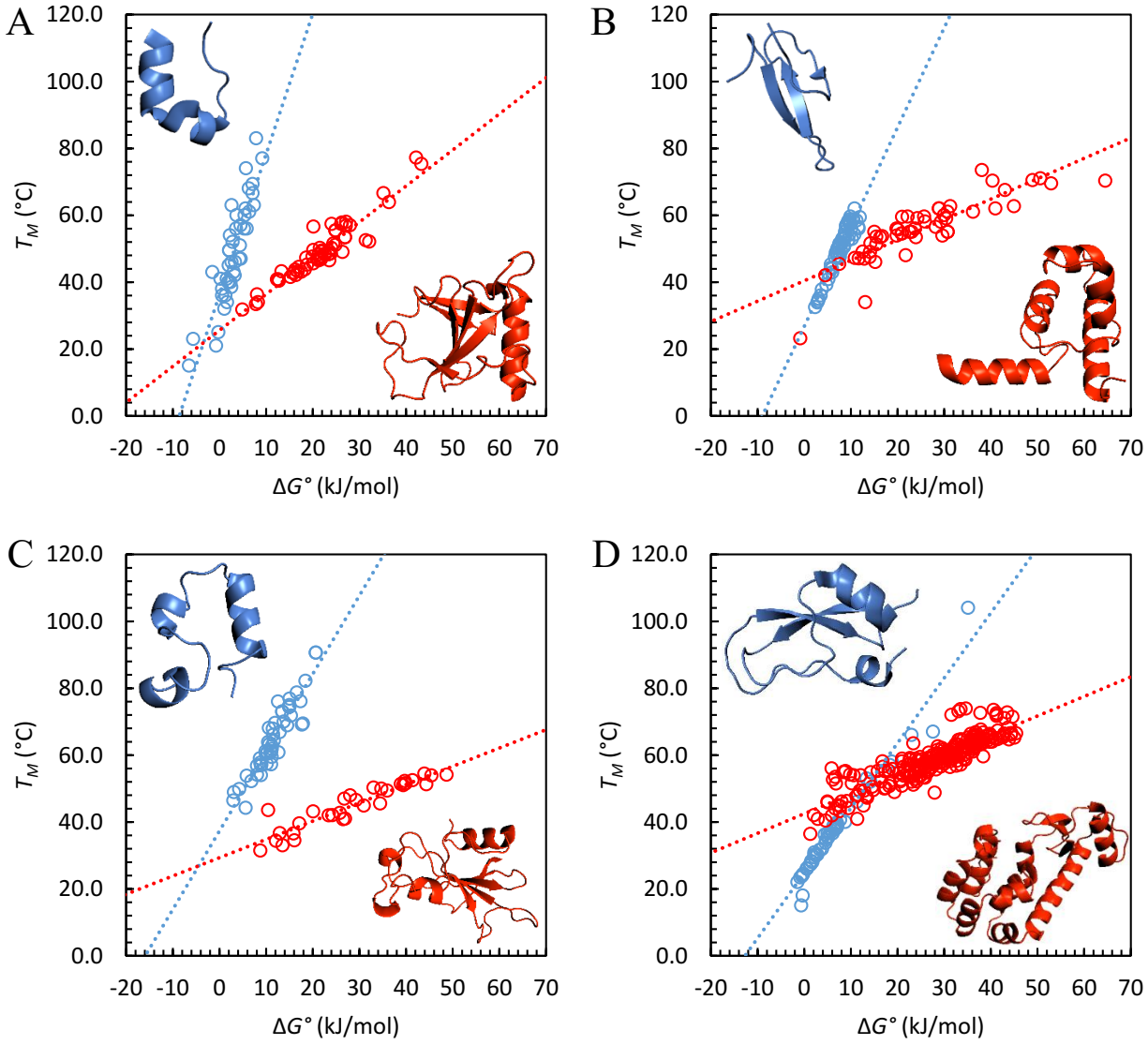


Figure 1. There is a strong linear correlation between $\Delta G°$ and T_M for mutants of single domain proteins. **(A)** Trp-cage (blue, 20 residues) and RNase Sa (red, 96 residues). **(B)** hPin1 WW domain (blue, 34 residues) and lambda repressor (red, 102 residues). **(C)** HP36 (blue, 36 residues) and barnase (red, 110 residues). **(D)** BPTI (blue, 58 residues) and T4 lysozyme (red, 164 residues). Plots for an additional 8 proteins are given in the supporting information (Figure S1).

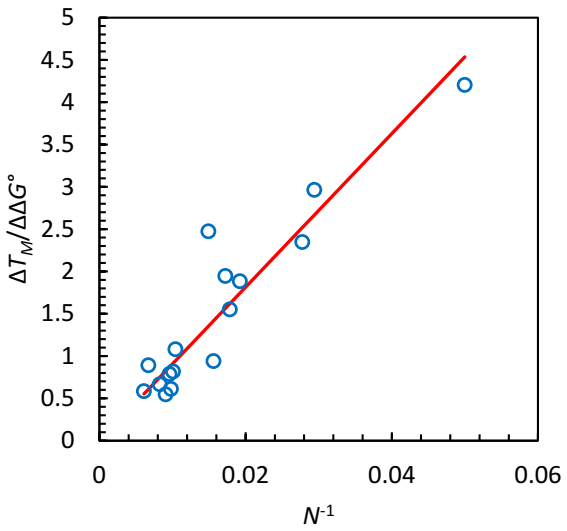


Figure 2. The slope of T_M *vs.* $\Delta G°$ plots correlates linearly with N^{-1} ($R^2 = 0.8726$, $p = 2.34\times10^{-11}$).

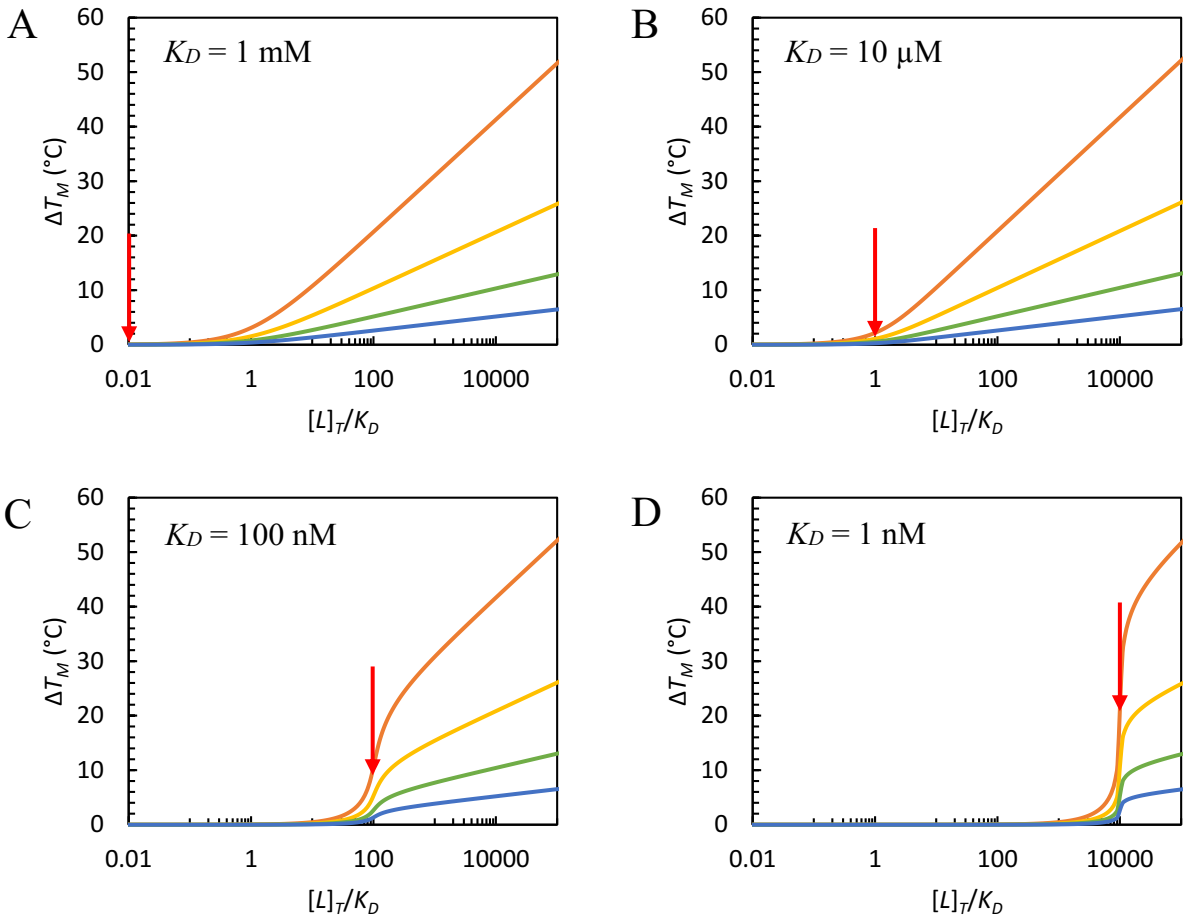


Figure 3. The thermal shift resulting from binding of a ligand with a K_D of **(A)** 1 mM **(B)** 10 µM **(C)** 100 nM and **(D)** 1 nM. Individual curves represent proteins of length 50 (orange), 100 (yellow), 200 (green) and 400 (blue). Red arrows indicate $[L]_T$ = 10 µM, which also corresponds to the protein concentration used to generate the plots. This concentration was chosen to agree with typical experimental methods from the literature.[17-18]

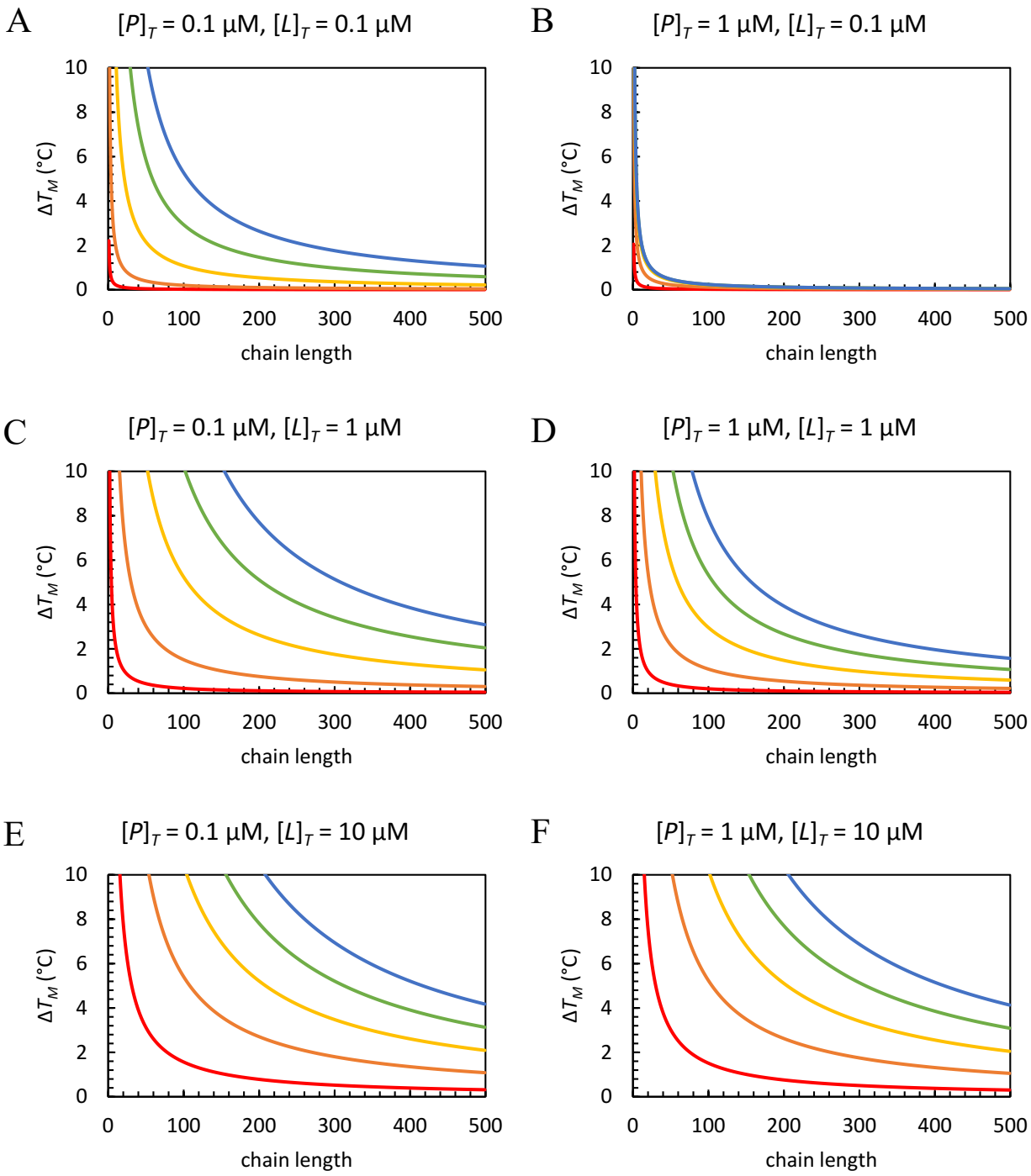


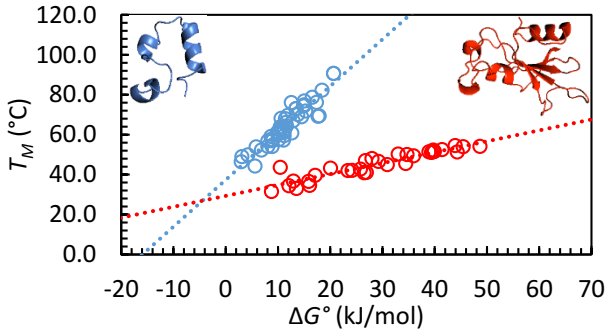
Figure 4. Chain length dependence of the thermal shift for a ligand with a K_D of 10 µM (red), 1 µM (orange), 100 nM (yellow), 10 nM (green) and 1 nM (blue). Curves were calculated at total protein concentrations of 0.1 and 1 µM and total ligand concentrations of 0.1, 1 and 10 µM.

TABLES

Table 1. Summary of proteins in the mutational dataset.

Protein	N	$\Delta T_M/\Delta\Delta G°$ (K mol/kJ)	Number of Data Points	R^2	p value	PDB
Trp-Cage[19-23]	20	4.2023	44	0.7782	2.53×10^{-15}	2JOF
hPin1 WW[24]	34	2.9628	56	0.9187	1.18×10^{-31}	2NC3
HP36[25-34]	36	2.3459	49	0.8604	1.01×10^{-21}	1VII
NTL9[35]	52	1.8823	9	0.7847	1.53×10^{-3}	2HBB
B1 domain of Protein G[36]	56	1.5507	19	0.8652	8.13×10^{-9}	2GB1
BPTI[37-38]	58	1.9451	51	0.9573	3.28×10^{-35}	5PTI
CI2[39-41]	64	0.9397	25	0.7275	6.12×10^{-8}	1COA
CspB[42]	67	2.4721	60	0.9445	4.04×10^{-38}	1CSP
RNase Sa[43-49]	96	1.0783	60	0.8826	1.17×10^{-28}	1RGG
RNase Sa3[43]	99	0.8162	9	0.9997	1.73×10^{-13}	1MGR
λ Repressor[50-55]	102	0.6100	53	0.7420	1.27×10^{-16}	1LMB
RNase T1[44-45, 56-61]	104	0.7865	50	0.8545	1.14×10^{-21}	9RNT
Barnase[62-64]	110	0.5466	32	0.8646	1.45×10^{-14}	1BNI
RNase A[65-71]	121	0.6645	31	0.6389	6.97×10^{-8}	2E3W
Staph. Nuclease[72-81]	149	0.8905	76	0.7727	1.64×10^{-25}	1STN
T4 Lysozyme[82]	164	0.5843	242	0.7752	9.69×10^{-80}	2LZM

TOC GRAPHIC


120.0
100.0
80.0
60.0
40.0
20.0
0.0
T_M (°C)
-20 -10 0 10 20 30 40 50 60 70
$\Delta G°$ (kJ/mol)

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

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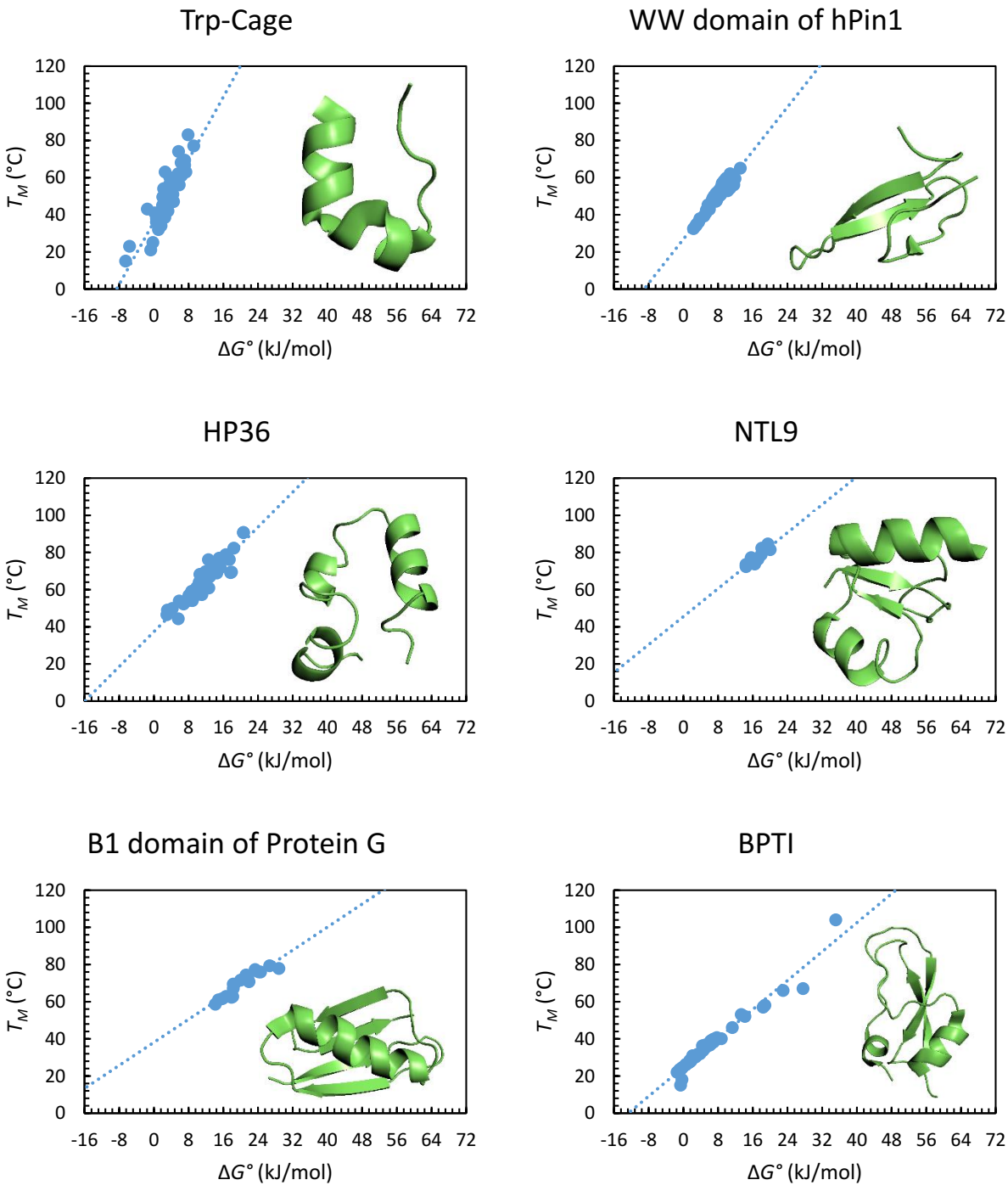
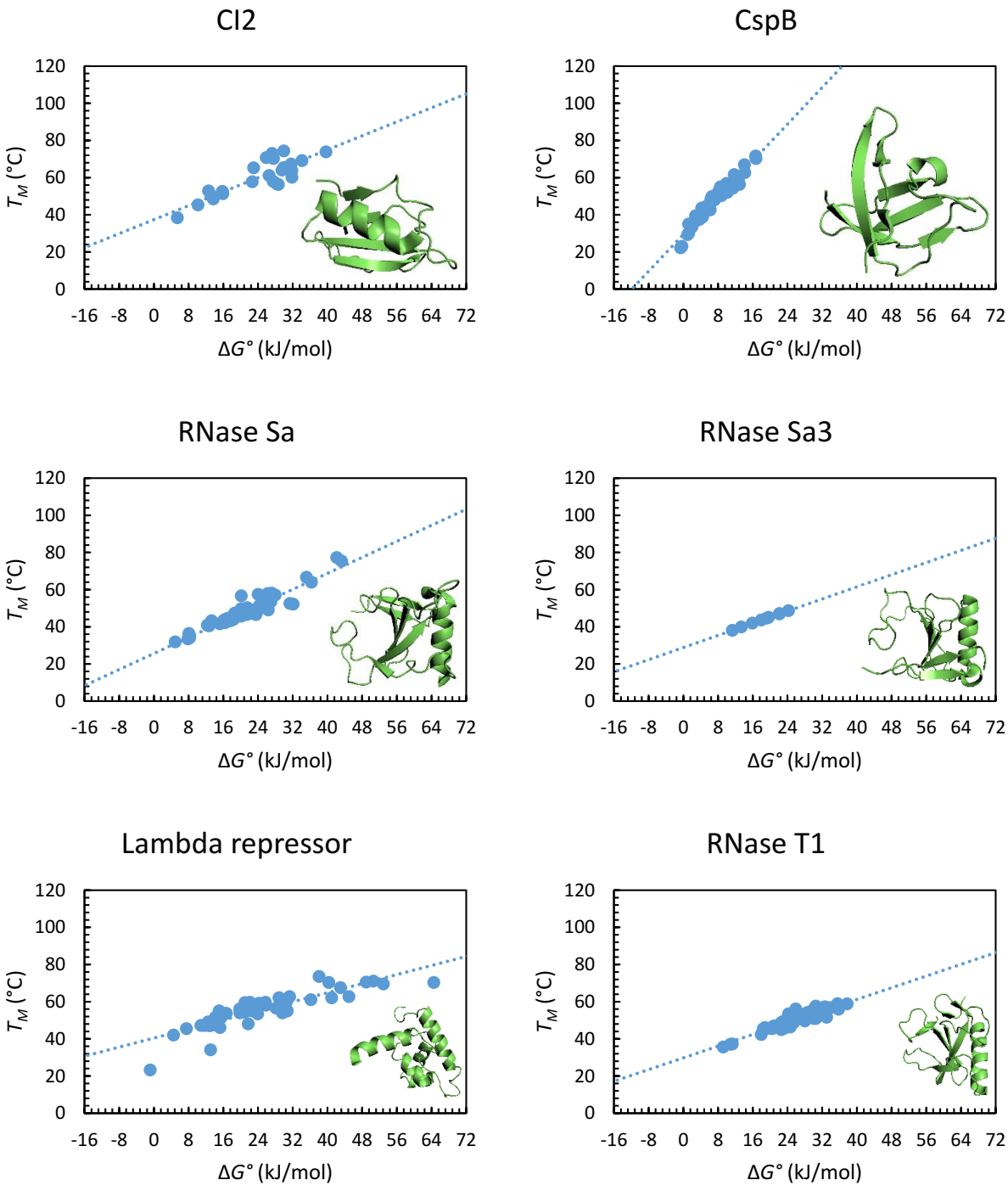

Trp-Cage
WW domain of hPin1
HP36
NTL9
B1 domain of Protein G
BPTI
T_M (°C)
ΔG° (kJ/mol)
0
20
40
60
80
100
120
-16
-8
0
8
16
24
32
40
48
56
64
72


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

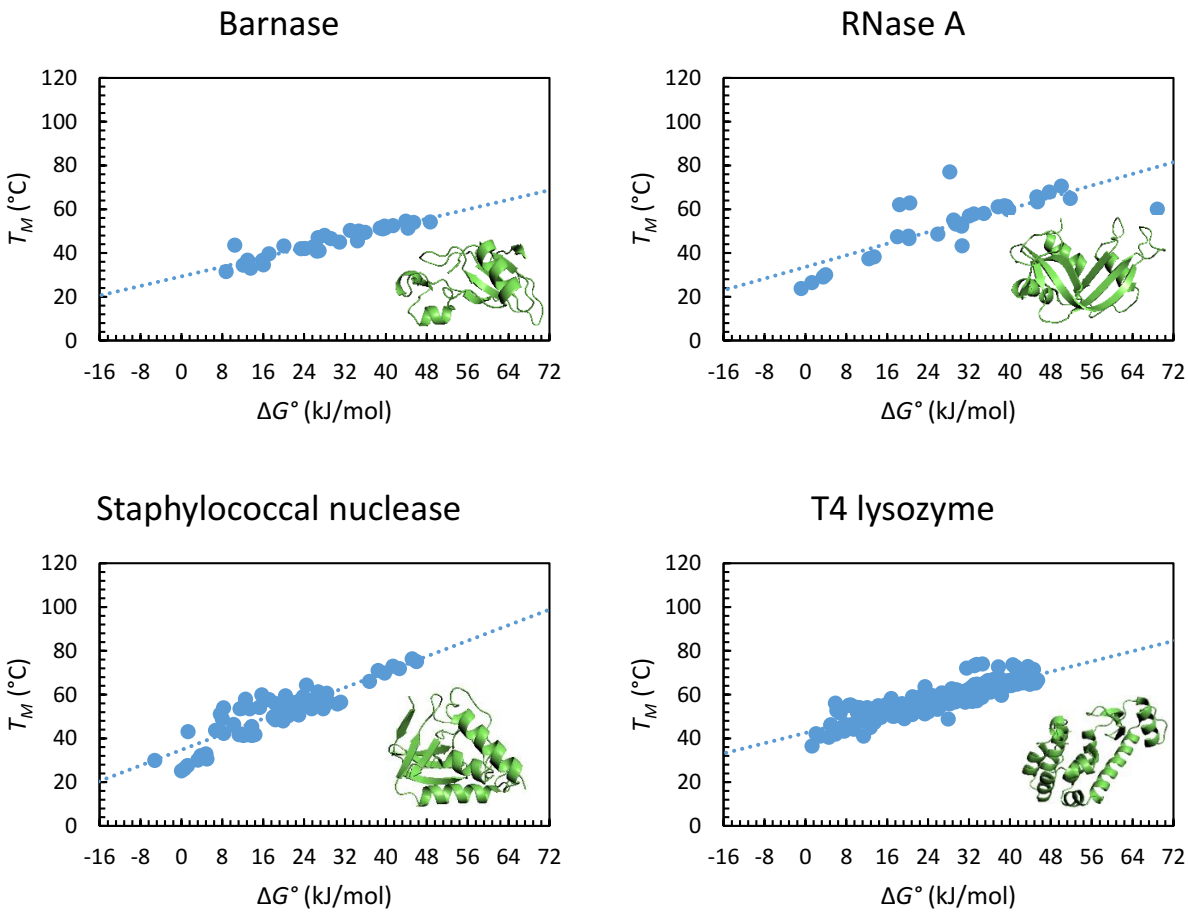


Figure S1. Plots of T_M *vs* $\Delta G°$ for mutants of all proteins included in the analysis

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)-	56.9	8.91	J. Am. Chem. Soc. (2016) 138, 6498-6505

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
2-aminocyclopentyl-1-carboxylic 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/M52 L-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

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
WT	53.4	25.52	Biochemistry (1988) 27, 4761-4768
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

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
H124L/D77C/N118C (reduced)	29.9	-5.19	Biochemistry (1996) 35, 10328-10338
G29C	48.4	18.41	Protein Sci. (1995) 4, 2545-2558
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

Mutation	T_M (°C)	$\Delta G°$ (kJ/mol)	Reference
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
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

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