The Helical Alanine Controversy: An $(Ala)_6$ Insertion Increases Helicity Dramatically

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SUPPORTING INFORMATION

For our Trp-cage thermodynamic analyses, the melting temperature (T_m) is defined as the temperature at which the peptide is 50% folded. Oftentimes, the more stable Trp-cage analogs had T_m's higher than 325 K, the highest temperature at which we normally run NMR experiments. To determine the T_m thus requires an extrapolation from the two highest temperature points in order to determine the 50% folded temperature. This process is less than ideal, especially in those cases where the extrapolation is relatively large. Alternatively, we calculated the temperature at which the peptide is 70% folded ($T_{70\%}$). This point lies within the relatively linear region of the melting profile. Also, all of the peptides are 30% unfolded at temperatures less than 325 K. Therefore, $T_{70\%}$ was always an interpolation within the linear region of the melting curves.

In our previous NMR melting studies of Trp-cage species, the slopes of the f_F *versus* T plots for diverse species with T_m's spanning from 20° to 65°C are parallel in the $f_F = 0.70 – 0.20$ range. This implies that there should be a near constant value for ($T_m – T_{70\%}$) and a nearly linear relationship between melting points and ΔG_U. Figures S1 – S2 show the correlations between the two melting measures and ΔG_U. It would appear that a general rule of 0.34 kJ/mol fold stability change for a 1 °C change in melting temperature is applicable for these systems. From the correlations shown in Figure S1, the ΔG_U of TC11b1, which could not be determined using H/D exchange experiments, is calculated from the T_m.

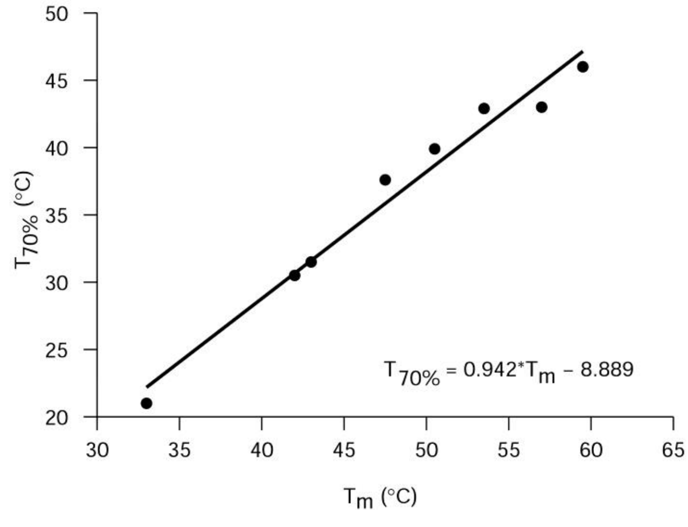

Figure S1. Correlation between T_m and $T_{70\%}$. A slope of ~1 demonstrates a constant difference between T_m and $T_{70\%}$.

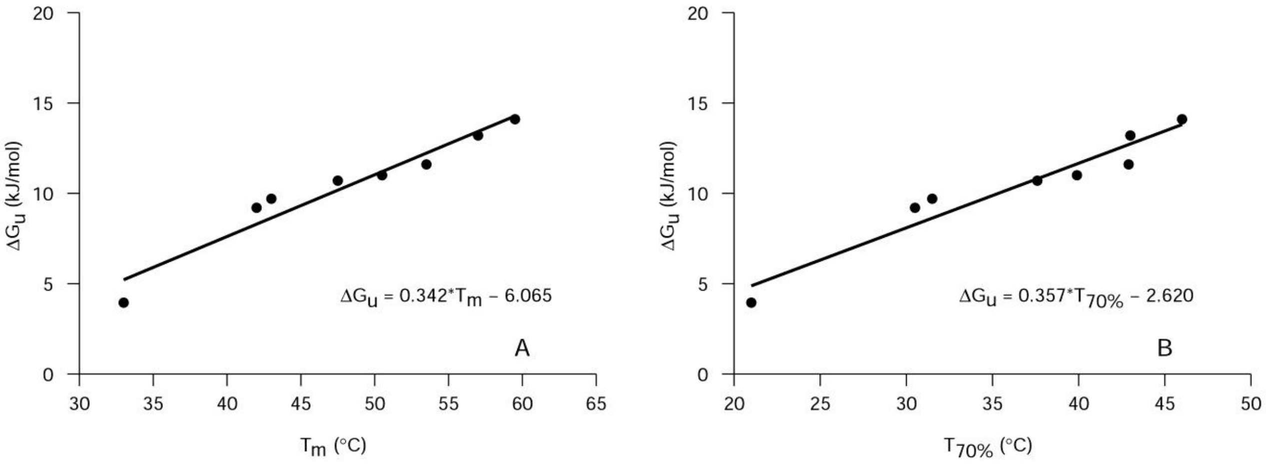

Figure S2. Correlation between ΔG_U and T_m (A) or $T_{70\%}$ (B).

Almost all of the ΔG_U data in Figure S2 was computed using protection factors. Only one point, TC12b (ΔG_U = 4.0 kJ/mol), was calculated from CSD data. Ignoring the TC12b point, predicts a 0.26 kJ/mol stability change per 1 °C change in melting temperature: $\Delta G_U = 0.26\bullet(Tm - 1.75)$. Only one point in Figure 5 relies on this correlation, excluding that point, or revising it based on $\Delta G_U = 0.26\bullet(Tm - 1.75)$, lowers the alanine propagation value estimate to 1.59 (or 1.51), still well above the Scheraga and Kemp values of 1.03 – 1.15.

We can also derive an alanine propagation value directly from a plot of $T_{70\%}$ versus x for the TC11bx series of peptides (Figure S3). The slope for this correlation is 3.18°C/Ala-insertion. Based on the calibration line in panel B of Figure S2 this yields $w_{\text{Ala}} = 1.62$, exactly the same value as obtained by the correlation in Figure 5.

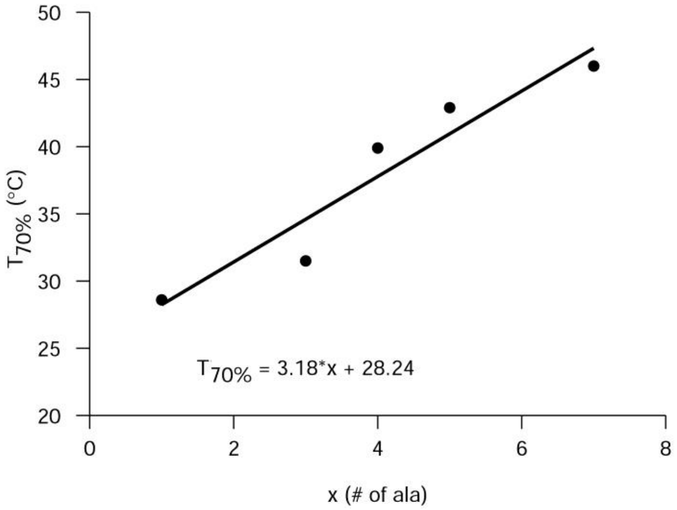

Figure S3. Increase of $T_{70\%}$ upon addition of alanine residues. The slope represents the $\Delta T_{70\%}$ per alanine and can be used to calculate the $\Delta\Delta G_U$ per alanine.