Supplementary Information for

Enthalpically driven peptide stabilization by protective osmolytes

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Experimental procedures

Peptide of sequence AcKKYTVSINGKKITVSI at 98% purity was purchased from CA Peptide Research Inc. Stock solutions of peptides were prepared as ca. 1mg in 1mL of solution, and their concentrations were monitored by tryrosin absorbance. All osmolytes, except glycerol (that was from Frutarom, Israel), were purchased from Fluka, Germany at a purity of ≥ 99%. Osmolalities of osmolyte solutions were measured on a Wescor 5520 vapor pressure osmometer.

Circular dichroism experiments

The experimental procedure to determining the folding free energy of the model peptide by Circular dichroism (CD) follows closely the procedure first presented, and further validated by comparison to NMR-based methods, by Searle and coworkers[1, 2]. We briefly describe the methodology, and then show how we have been able to validate and extend its use to peptide solutions that also contain added sugar and polyols acting as osmolytes.

CD spectra were collected on a Jasco J-810 spectrometer. Peptide concentrations in all CD analysis were diluted from stock solution to 100μM and buffered to pH 7 by 10mM phosphate buffer. Samples containing peptide were placed in 1 or 2mm path length quartz cells (Starna, CA) and titrated using osmolytes stock solutions of 1-3m concentration. Typically, ten scans were acquired over the 200-260 nm wavelength range at 298K. The resulting ellipticity spectra were averaged and baseline-corrected by background subtraction. The isodichroic point at 209nm allows to treat the folding as a two state process. The limiting value for the fully folded (*N*) state elipticity at 215 nm was determined as $\theta = -8.53\times10^{-3}$ deg cm^2 dmol^{-1} from the peptide θ value in 50% methanol at T=273K (Fig. S1b). Based on Searle et al[2] and our own data, θ reaches its limiting value under these conditions. Based on NMR measurements as well as the CD

signal for a random sequence of the same amino acids that did not show evidence for folding[2], it was also shown that the limiting ellipticity value for the unfolded (*D*) state is 0 deg cm^2 dmol^{-1}. We then used solution ellipticities at 215nm to determine the free energy of folding $\Delta G^0_{D\rightarrow N} = -RT\ln([N]/[D])$ under various conditions, where [*N*] and [*D*] represent the concentrations of native and non-native structures, *T* is absolute temperature, and R the ideal gas constant. This method was previously used to not only find the folding free energy, but also shown to be valid for assessing changes of the free energy with temperature, thereby allowing a van't Hoff analysis to derive ΔH and ΔS for folding from these CD spectral data.

Determining the effect of osmolytes on baseline signals

To extend the use of the CD based determination of the peptide folding free energy, we first verified that osmolytes do not alter the isodchroic point. Indeed, the same isodichroic point at 209nm is observed for all osmolytes used here, as demonstrated for trehalose in Fig S1. Moreover, we find that chemically different osmolytes at different concentrations, which are found by this method to exert the same effect on folding $\Delta\Delta G$, show CD spectra that completely overlap, as seen in Fig S2. This further supports the assumption that the CD signal assigned to the two peptide states, *D* and *N*, are not altered in the presence of this wide concentration range of different osmolytes, and that these osmolytes only change the relative populations of each state.

To further support the use of the previously determined base spectra of the *D* and *N* states for use in the presence of osmolytes, we examined the effect of trehalose on $\Delta\Delta G$ in the presence and absence of 1M GuHCl at *T*=278. Known to be a denaturant, GuHCl significantly reduces the folded population of the peptide from 50.3% in aqueous solution (pH 5.5 and *T*=278) to 39.2% in the presence of 1M GuHCl (pH 5.5 and *T*=278). Perhaps because of their weak per-mole action, it has been documented in numerous cases that the effect of protecting osmolytes (such as trehalose) and denaturants (such as GuHCl) on protein folding are additive [3]. Indeed, Fig S3 clearly shows that the effect of trehalose (seen as the slope of the two curves) is identical within experimental error in the presence and absence of GuHCl, -1540(±118) and 1486(±154) J/mol, respectively. Note

that the presence of 1M GuHCl shifts the pH to 5.5, so that in experiments in the absence of GuHCl, shown in Fig S3, solutions were buffered to pH 5.5 by 10mM sodium acetate buffer. Assuming that also for this peptide the effect of GuHCl and trehalose are additive, our findings serve as additional important support of the CD based method for evaluating $\Delta\Delta G$. In particular, shifting the initial equilibrium state of the peptide and finding the same $\Delta\Delta G$ due to osmolyte (trehalose) could only be achieved if the baseline values of the CD signal were correctly resolved. Had they not been properly resolved, a shift would appear in the derived values of $\Delta\Delta G$ in the presence *versus* the absence of GuHCl, even if in reality the effects of trehalose and GuHCl were completely additive. From this analysis, we can estimate our errors in determined $\Delta\Delta G$ as 10% at most from their actual value. Note, however, that even larger errors in $\Delta\Delta G$ would not change our qualitative conclusions presented in this communication.

Temperature dependence of peptide stability

A Jasco J023A Peltier device was used to control the temperature of sample cells. 600 µL Samples containing 100 µM peptide and 10 mM phosphate buffer, together with various concentrations of different osmolytes (glycerol, trehalose, and sorbitol) in the 0-1.5 Osm range, were placed in 2mm quartz cells (Starna, CA). Ellipticities of these peptide solution were scanned at 215nm from 278 to 333K in 5K increments. Full scans over the 200-260nm range were performed at 298K after full scans at 278 and 333K, to ensure that folding is completely reversible over the temperature range studied. Figure 1c and S4 show the variation of folding free energy ΔG with temperature, in the presence of different osmolyte concentrations.

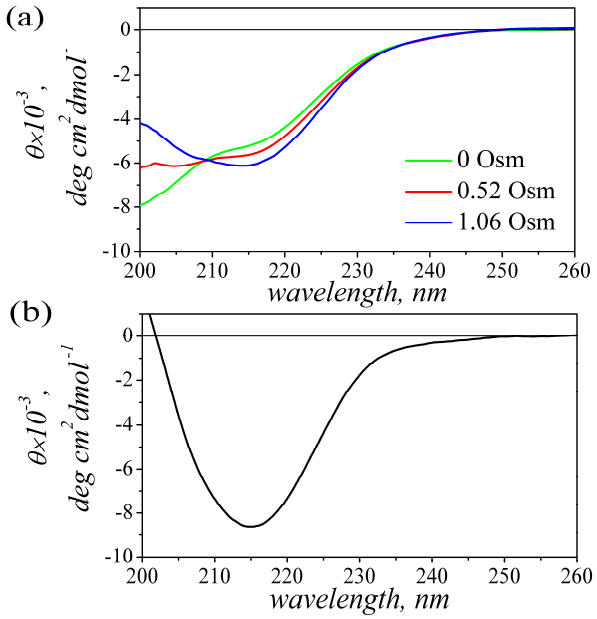

Figure S1. Circular dichroism spectra of the model peptide a) measured in the presence of 0, 0.52 and 1.06 Osm trehalose at pH 7, *T*=298K and [peptide] = 100µM. b) measured at 50% v/v methanol at pH 7, *T*=273K and [peptide] = 100µM.

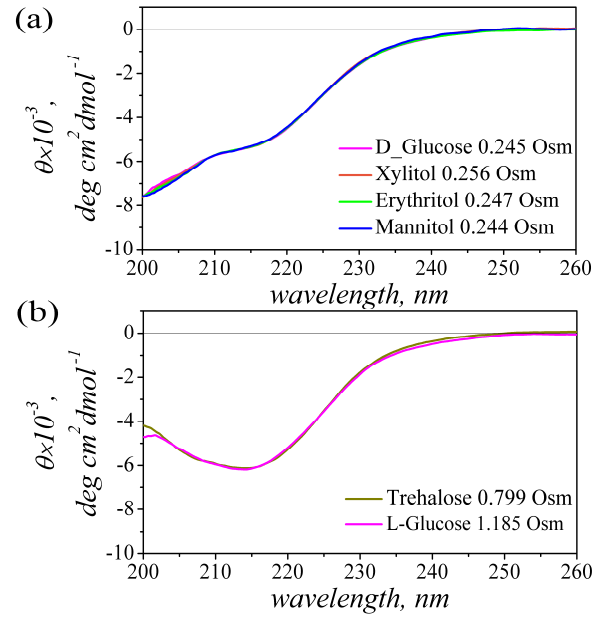

Figure S2. Circular dichroism spectra of the model peptide under different conditions but same $\Delta\Delta G$ are identical a) CD ellipticity measured in the presence of different osmolytes at similar concentrations at pH 7, *T*=298K and [peptide] = 100µM. b) Ellipticity measured in the presence of different osmolytes at different concentrations at pH 7, *T*=298K and [peptide] = 100µM. In each case the osmolytes that are found to exert the same effect on folding, $\Delta\Delta G$, also show identical spectra.

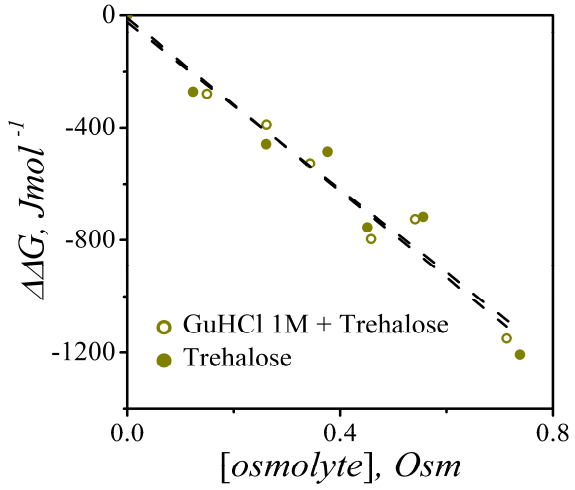

Figure S3. Effect of trehalose concentration on β-hairpin stability in the presence and absence of denaturant. Folding free energy, $\Delta\Delta G_{D\to N}$, is shown versus osmolyte concentration in the presence and absence of GuHCl 1M at T=278K and pH 5.5.

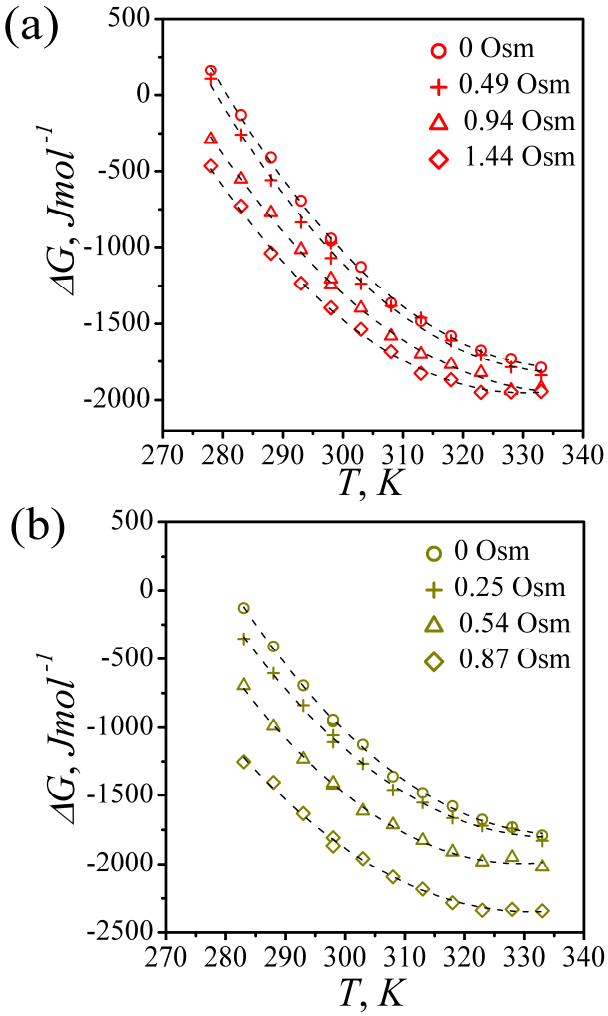

Figure S4. Effect of osmolyte concentration and temperature on β-hairpin stability. Folding free energy, ΔG, is shown versus temperature, T, at different (a) glycerol and (b) trehalose concentrations. Errors are expected to be on the order of 10% of the measured value.

References

1. A. J. Maynard, G. J. Sharman and M. S. Searle, J. Am. Chem. Soc., 1998, 120, 1996-2007.
2. B. Ciani, M. Jourdan and M. S. Searle, J. Am. Chem. Soc., 2003, 125, 9038-9047.
3. D. Harries and J. Rosgen, in Biophysical Tools for Biologists: Vol 1 in Vitro Techniques, 2008, vol. 84, pp. 679.