SUPPORTING INFORMATION

MALDI-TOF

Table S.I. MALDI-TOF data for **17-H-6** showing the expected and measured mass of the polypeptide.

Sequence	Expected Mass	Measured Mass
$[AAAQEAAAAQAAAQAEAAQAAQ]_6$	14770 Da	14659 Da

HPLC

Reverse-phase HPLC was run using a Delta600 HPLC (Waters, Milford, MA) equipped with a Symmetry300 C4 analytical column. The elution buffer was a gradient starting at 100% 0.1% trifluoroacetic acid in water and ending with 70% 0.1% trifluoroacetic acid in acetonitrile.

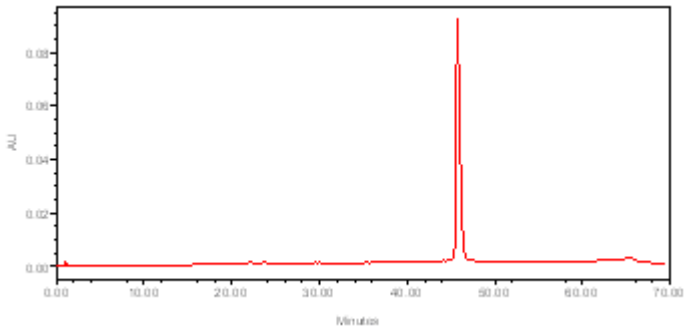

Figure S.1. HPLC of purified 17-H-6.

Amino Acid Analysis

Table S.II. Amino acid analysis of **17-H-6** shows the observed composition of the polypeptide is within 10% error of the expected composition.

	Expected mol%	Observed mol%
Asx	0.00	0.12
Ser	1.26	1.16
Glx	26.42	28.38
Gly	4.40	4.72
Ala	57.23	57.54
Met	1.89	0.99
Ile	0.63	0.64
Tyr	0.63	0.64
His	7.55	7.71

Helical Wheel Plots

Helical wheel plots of the sequences 17-H-1 and 35-H-1 are shown in Figure S.2. The glutamic acid residues are on the same side of the helix, as shown for Figure S.2.a. The glutamine residues are positioned around the backbone to reduce patches of high hydrophobicity.

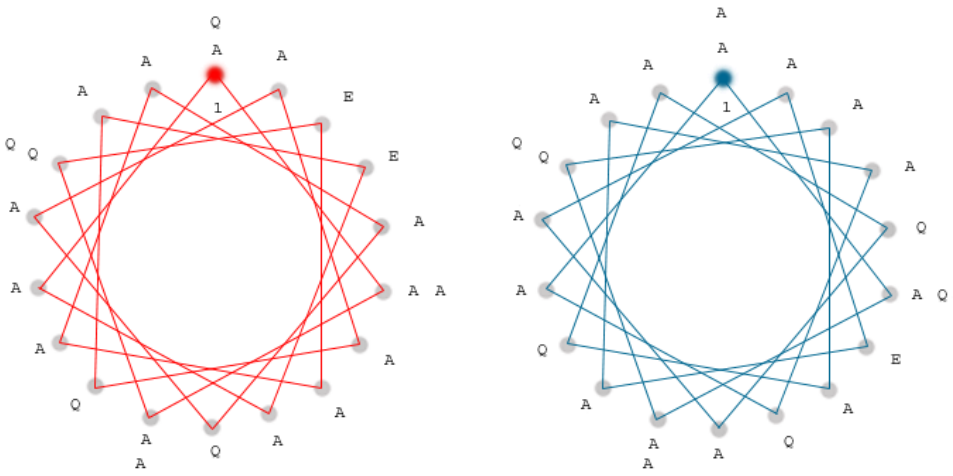

Figure S.2. Helical wheel plots for a) 17-H-1 and b) 35-H-1.

Fractional Helicity Calculation

The fractional helicity is calculated using the following equation, where $[\theta_n]_{222}$ represents the MRE of an idealized 100% helical peptide of length n, $[\theta_\infty]_{222}$ is the limit of $[\theta_n]_{222}$ at very large n, and x is a length correction value that is set to 2.5. The value given to $[\theta_\infty]_{222}$ was -61,000 deg cm^2 dmol^{-1} (Miller, *J. Am. Chem. Soc.*, 2002).

$$[\theta_n]_{222} = [\theta_\infty]_{222}\left(1 - \frac{x}{n}\right)$$

Computational prediction of helical content

The prediction algorithm, Agadir, can be used to predict the helical content of amino acid sequences (Lacroix, *J. Mol. Biol.*, 1998; Munoz, *Biopolymer*, 1997; Munoz, *J. Mol. Biol.*, 1995). Specific conditions such as pH, ionic strength and temperature are incorporated into the calculation. The standard deviation associated with the calculation is reported as 6%. For the

calculations involving **17-H-3**, **17-H-6** and **35-H-6**, a pH of 7.4 and the ionic strength of 0.150 M were set as the solution conditions. The predictions for the polypeptides at 5 °C and 25 °C are displayed in Table S.III.

Table S.III. Computational Prediction of Helicity by Agadir.

With fusion sequences	**5 °C**	**25 °C**
17-H-3	65 %	47 %
17-H-6	80 %	68 %
35-H-6	84 %	76 %
No fusion sequences	**5 °C**	**25 °C**
17-H-3	82 %	56 %
17-H-6	91 %	75 %
35-H-6	92 %	80 %

Table S.IV. Fusion sequences for the polypeptide families **17-H-X** and **35-H-X**.

	N-terminal	**C-terminal**
17-H-X	MGHHHHHHHHHHSSGHIHM	AGGYGGMG
35-H-X	MGHHHHHHHHHHSSGHINM	A

Fitting of $[\theta]_{222}$ vs. temperature

The following equation defines the native fraction of the protein as a function of temperature,

$$f(T) = \frac{[f_N + m_N T] + \left[f_D + m_D T \left\{ \exp\left[\frac{\Delta H_{VH}}{R} \left(\frac{1}{T_m} - \frac{1}{T} \right) \right] \right\} \right]}{1 + \exp\left[\frac{\Delta H_{VH}}{R} \left(\frac{1}{T_m} - \frac{1}{T} \right) \right]}$$

where f_N and f_D are the fraction of native protein before and after the transition, respectively, m_N and m_D are the slopes of the pre-transition and post-transition curves (Kedracka-Krok, *Eur. J. Biochem.*, 2003). This equation was used to perform a nonlinear curve fitting of the data (using Sigma Plot 9.0 Software), and the results of the fit give the van't Hoff enthalpy (ΔH_{vH}) and transition temperature (T_m) values. Normalized ellipticity was determined by dividing each ellipticity value to the maximum absolute ellipticity value. An example of the fit calculated for

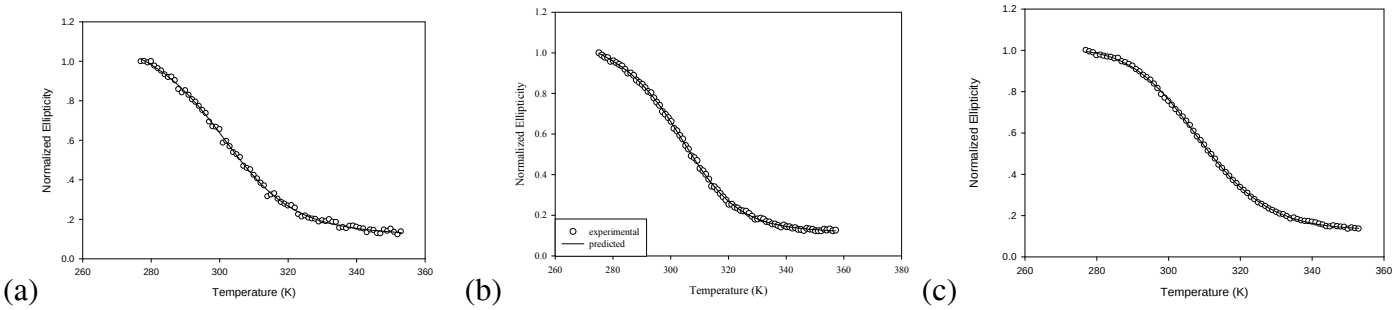

Figure S.3. Two-state model fits for determining the T_m and ΔH_{vH} for the polypeptides. a) 7 μM **17-H-3**, b) 10 μM **17-H-6**, c) 11 μM **35-H-6**.

the thermal denaturation of **17-H-6** (10 μM) in pH 7.4 PBS is shown in Figure S.3. The fit to this particular sample has an R^2 value of 0.9997, indicating a good fit to the data. All samples showed similarly good fits, and the ΔH_{vH} and T_m values reported are the averages of the data for at least duplicate measurements of a given polypeptide. Statistical comparisons were made of these averages via ANOVA analysis; the ΔH_{vH} and T_m values were found to be statistically the same via this analysis ($p > 0.05$).

Analytical ultracentrifugation of polypeptides

The concentration studies of **17-H-3** and **35-H-6** display a linear relationship between ln c_r (expressed as natural log of absorbance units at 230 nm) and $r^2/2$.

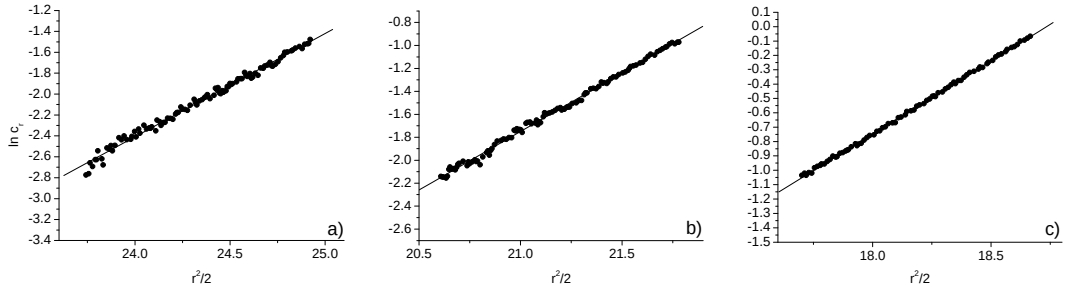

Figure S.4. AUC of **17-H-3** at polypeptide concentrations of a) 3.8 μM, b) 7.6 μM, and c) 19 μM. Samples were spun at 30000 rpm for 22 hours prior to absorbance measurements being recorded.

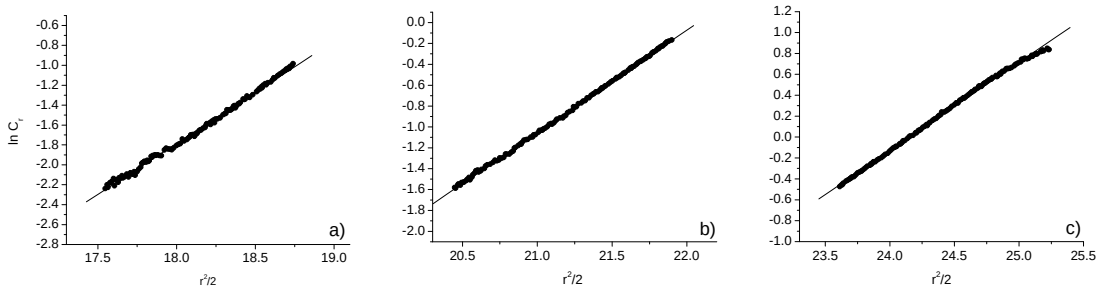

Figure S.5. AUC of **36-H-6** at polypeptide concentrations of a) 7 μM, b) 18 μM, and c) 36 μM. Samples were spun at 25000 rpm for 22 hours prior to absorbance measurements being recorded.

Non-denaturing PAGE of polypeptides

Samples of each polypeptide were run on a non-denaturing polyacrylamide gel at the same concentration used in AUC experiments for confirmation of the existence of a single species at the low concentrations. Results of the non-denaturing PAGE are shown in Figure S.6. Each of the three protein polymers runs as a single band, indicating a single species in the sample. Although the molecular weight of the molecules cannot be directly determined using the non-native PAGE results, the relationship of the band positions to one another can provide information as to the relative size of the molecules. The **17-H-3** band has migrated furthest down the gel, indicating a lower molecular weight. Although **17-H-6** and **35-H-6** have similar molecular weights as determined by mass spectroscopy, the two bands migrate differently, which may be a result of differences in helicity. The **35-H-6** polypeptide has a higher helicity than **17-H-6**, which may reduce its ability to migrate into the gel matrix.

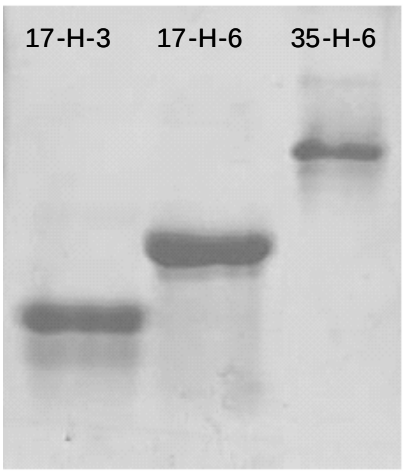

Figure S.6. Non-denaturing polyacrylamide gel electrophoresis of alanine-rich polypeptides at concentrations of 19 μM, 17 μM, and 18 μM for 17-H-3, 17-H-6 and 35-H-6, respectively.