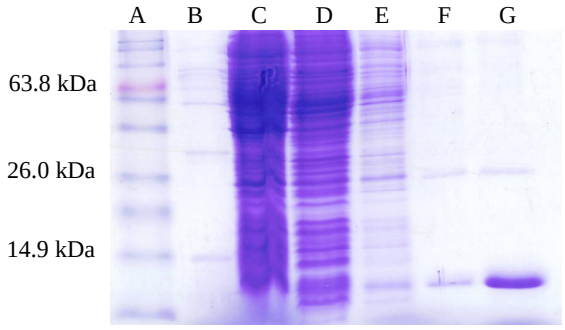
Energy Minimization Calculations. Energy minimization calculations were conducted with the BioSym Insight II molecular modeling program from Accelrys (San Diego, CA) to determine appropriate amino acid sequences for protein design. Minimizations were performed on various alanine-rich sequences in an explicit water solvent. The molecule was minimized by both steepest descent and conjugate gradient minimization processes. The energy minimization calculations were terminated when the total energy of the molecule varied by less than 0.10kJ/mol between iterations, a criterion set in the program to indicate the convergence of the calculations to a minimum energy.

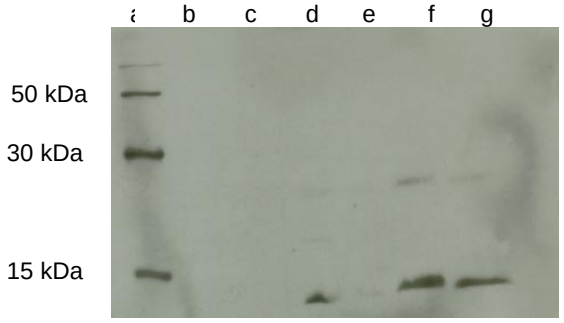
SDS-PAGE analysis of the immobilized metal chelate chromatography purification of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$. (A) Molecular weight markers; corresponding molecular weights are shown to the left of the figure. (B-E) Flow through and wash fractions obtained during purification. (F-G) Elution fractions of pH 5.9 and pH 4.5 8M urea buffer containing purified protein. The presence of low intensity bands at higher molecular weights in lanes F and G in the figure below were observed only in the purification of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$



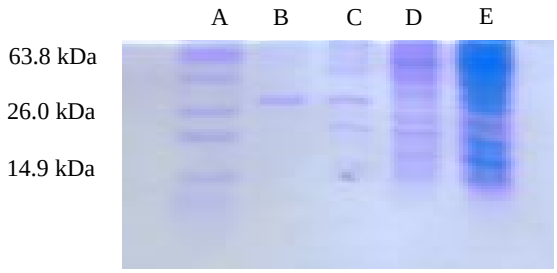
and not in the purification of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$. The presence of the higher molecular weight bands suggests the formation of well-defined aggregates under the purification

conditions and does not indicate contamination, which was confirmed via Western blot analysis of the SDS-PAGE gel and amino acid analysis (see below).

Western blot analysis of the immobilized metal chelate affinity chromatography purification of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$. Western blot analysis of the SDS-Page purification gel of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$ was performed using a Penta-His HRP conjugate for chemiluminescence. The lanes assignments are (a) molecular weight markers with corresponding molecular weights shown to the left of the figure. (b-c) Flow through and wash fractions obtained during purification. (d-e) Elution fractions of pH 5.9 and (f-g) pH 4.5 8M urea buffer containing purified protein. The presence of the lower intensity, high molecular weight band in the elution buffers indicate an aggregated species of the protein.



SDS-PAGE analysis of the immobilized metal chelate chromatography purification of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$. (A) Molecular weight markers; corresponding molecular weights are shown to the left of the figure. (B-C) Elution fractions of pH 4.5 and pH 5.9 8M urea buffer containing purified protein. (D-E) Wash and flow through fractions obtained during purification.

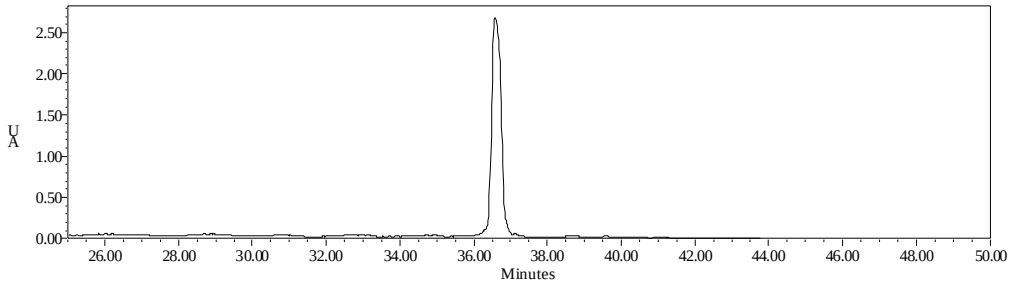


Analysis of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$.

Amino Acid Analysis. Amino acid analysis was performed at the University of Iowa by Brian Morrison. Average results from the amino acid analysis of nine samples are tabulated below; the standard deviation in the values for the primary amino acids is $\leq$ 10%. The determined mole percentages for the primary amino acid residues are within 10% of the expected mole percentages, confirming the composition of the protein.

Expected Residues	Expected mol %	Observed mol %
Asx	0.93	1.86
Ser	1.85	2.51
Glx	20.37	21.09
Gly	1.85	1.71
Ala	62.04	59.78
Met	1.85	0.00
Ile	0.92	0.54
His	10.19	9.72
Others	0	2.80

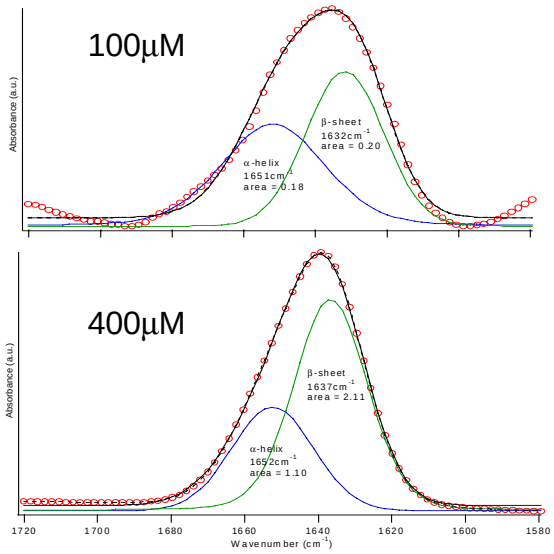
HPLC.



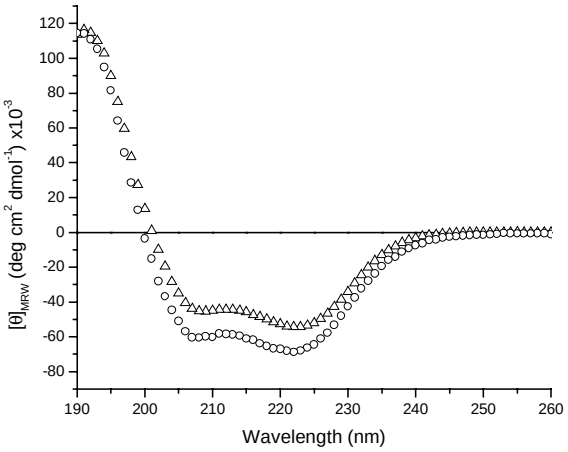
MALDI-TOF.

Protein	Expected Mass	Measured Mass
$[(AAAQ)_5(AAAE)(AAAQ)_5]_2$	9889 Da	9759 Da

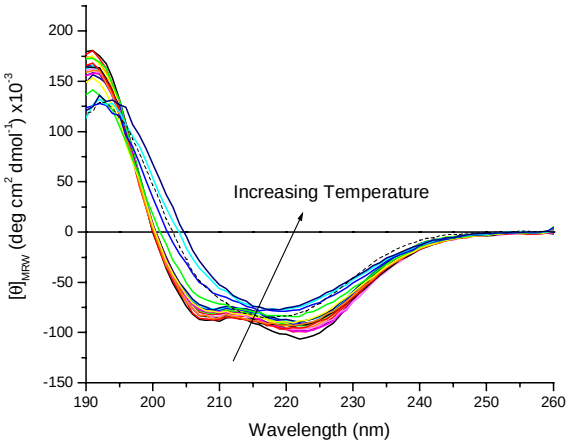
Infrared spectra of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$. Because the tendency for β-sheet formation is also concentration-dependent, characterization of the protein via infrared spectroscopy at higher concentrations (100μM and 400μM) in pH 2.3, 10mM phosphate D_20 buffer was conducted. Results yield spectra with a prominent Amide I vibration which can be deconvoluted into two peaks at approximately 1651cm^{-1} and 1633cm^{-1}. The positions of these two peaks suggest a combination of α-helical and β-sheet conformations at higher concentrations. The relative areas of the two peaks (1651cm^{-1}:1633cm^{-1}) are approximately 3:1 at 100μM and 0.75:1 at 400μM, indicating an increase in β-sheet character in these proteins at higher concentrations. The concentration dependence of β-sheet formation in these proteins is also consistent with the known behavior of β-sheet forming proteins such as silk.



Circular dichroism spectra, collected at 8°C, of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$ (ρ) and $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ (☑) in pH 2.3, 10mM phosphate buffer.



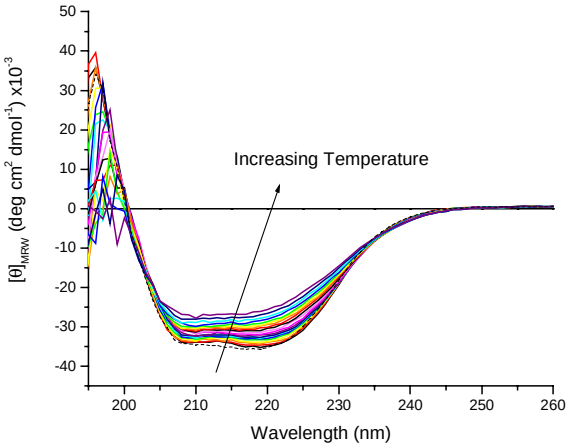
Circular dichroism spectra of the temperature dependence of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ conformation in 10mM phosphate buffer, pH 2.3.



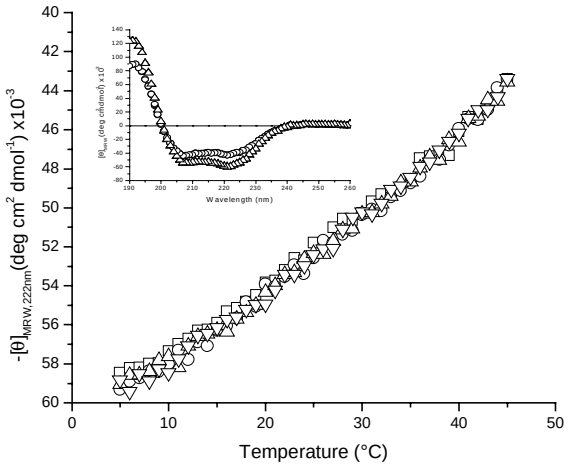
The temperature dependent CD spectra of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ show conformational changes with increasing temperature that are similar to those observed for $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$. The MRE values in these CD spectra were determined by using the mass of the protein dissolved in the buffer used for analysis. The conformational change to β-sheet is clearly defined and irreversible. Variations in the purification conditions of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ were shown to alter the temperature dependence of the conformational changes of the protein, and could be used to favor either helical or β-sheet conformations; the details of this behavior will be summarized in a separate report.

Circular dichroism spectra of the temperature dependence of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ conformation in 10mM phosphate, 150mM NaCl buffer, pH 2.3. CD spectra were recorded in increments of 4°C, from 4°C to 80°C. Differences in MRE values of this sample as compared to other samples of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ arise from differences in purification methods. Although the absolute MRE values are lower here than for previous samples, the increased

stability of the helical conformation relative to the initial conformation are clearly indicated by a smaller relative change in MRE values (and the lack of a blue shift in the data) relative to the $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$ samples.



Circular dichroism confirmation of reversibility of structural changes of $[(AAAQ)_5(AAAE)(AAAQ)_5]_2$ in 10mM phosphate, pH 2.3 buffer below 45°C. The overlapping $[\theta]_{222}$ values from four cycles over 7 hours are indicative of reversibility over this temperature range for extended periods of time. The wavelength spectra shown in the inset were taken at 5°C (▩) to 45°C (ρ) to demonstrate that α-helical structure is maintained during heating with complete reversibility upon cooling and cycling.



Circular dichroism characterization of $[(AAAQ)_5(AAAE)(AAAQ)_5]_6$ at 37°C in pH 2.3, 10mM phosphate, 150mM NaCl buffer. The longer protein also maintains helical structure for up to 14 hours with no apparent loss in helicity.