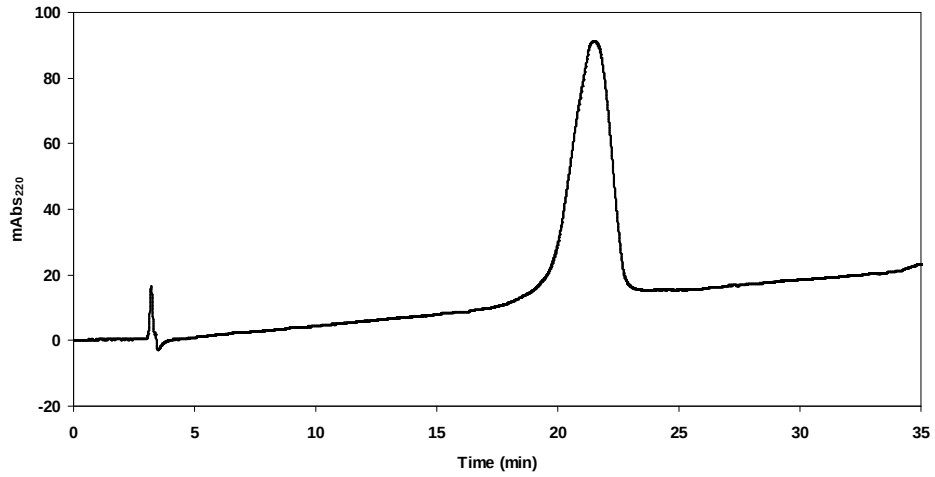
Analytical HPLC (Vydac C18) of MAX1

5% to 100% B over 95 minutes

(Solvent A is 0.1% TFA and

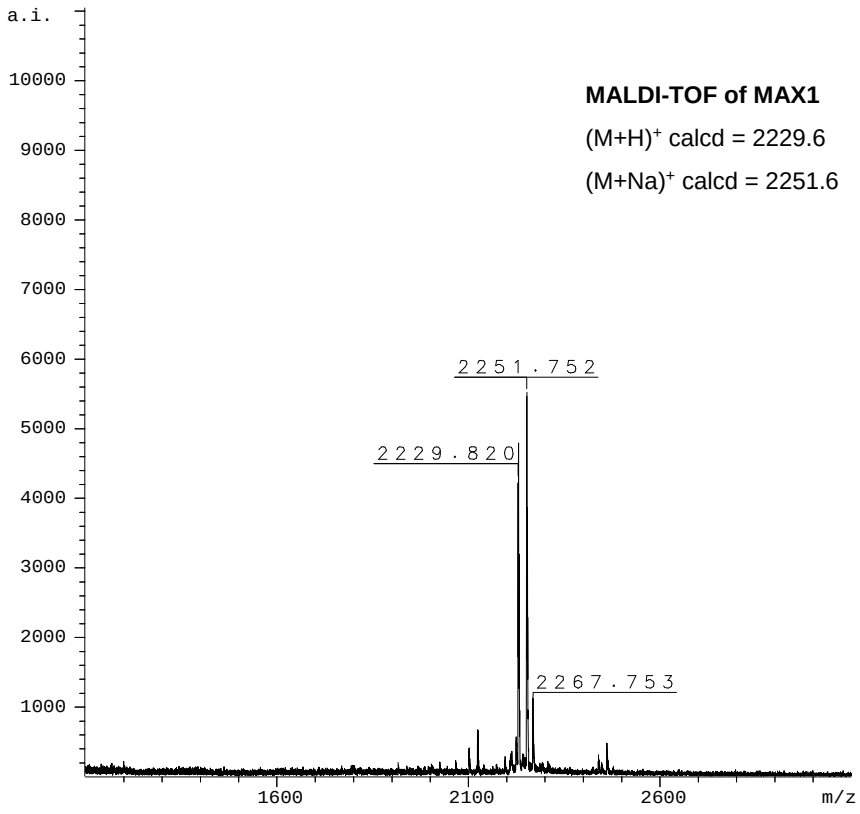
Solvent B is 90% Acetonitrile, 10% Water, 0.1%TFA)



MALDI-TOF of MAX1

$(M+H)^+$ calcd = 2229.6

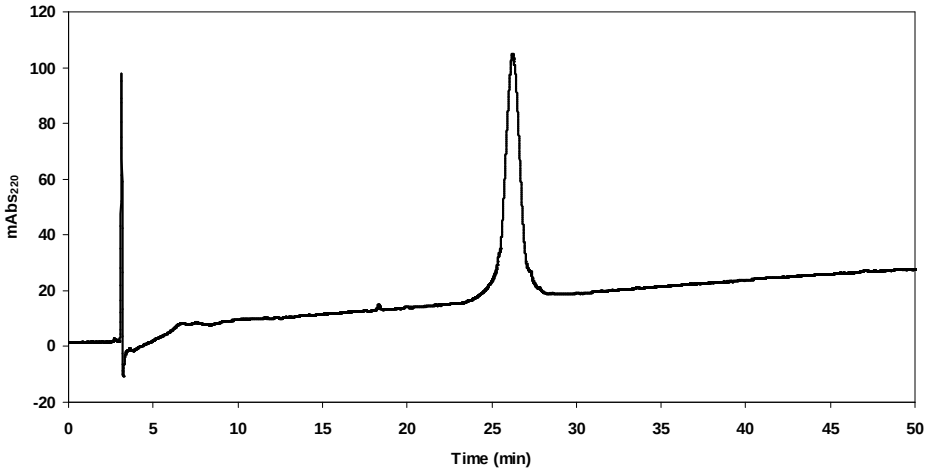
$(M+Na)^+$ calcd = 2251.6

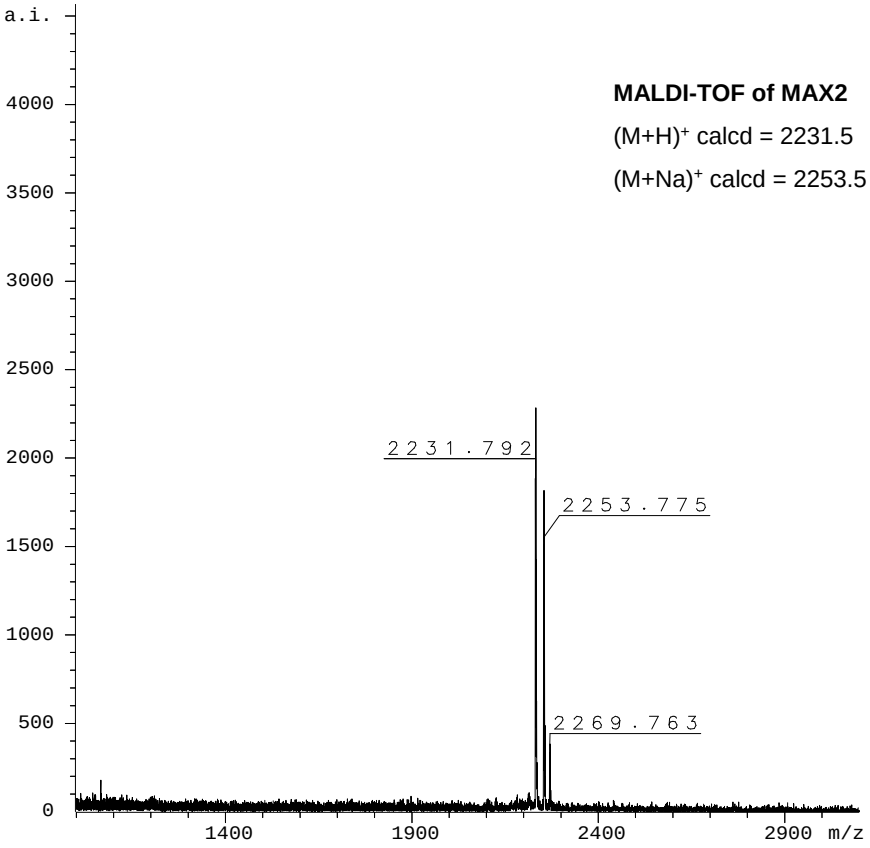


Analytical HPLC (Vydac C18) of MAX2

0% to 100% B over 100 minutes

(Solvent A and B defined in above)





MALDI-TOF of MAX2

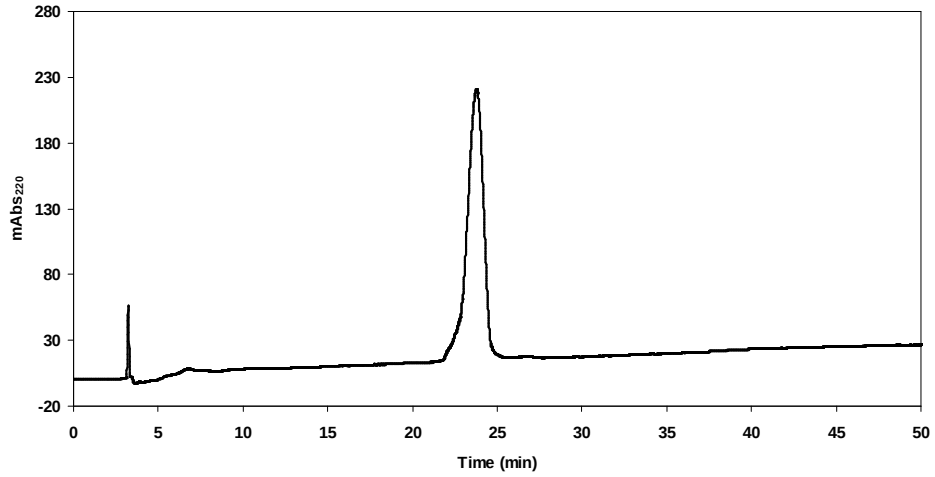
$(M+H)^+$ calcd = 2231.5

$(M+Na)^+$ calcd = 2253.5

Analytical HPLC (Vydac C18) of MAX3

0% to 100% B over 100 minutes

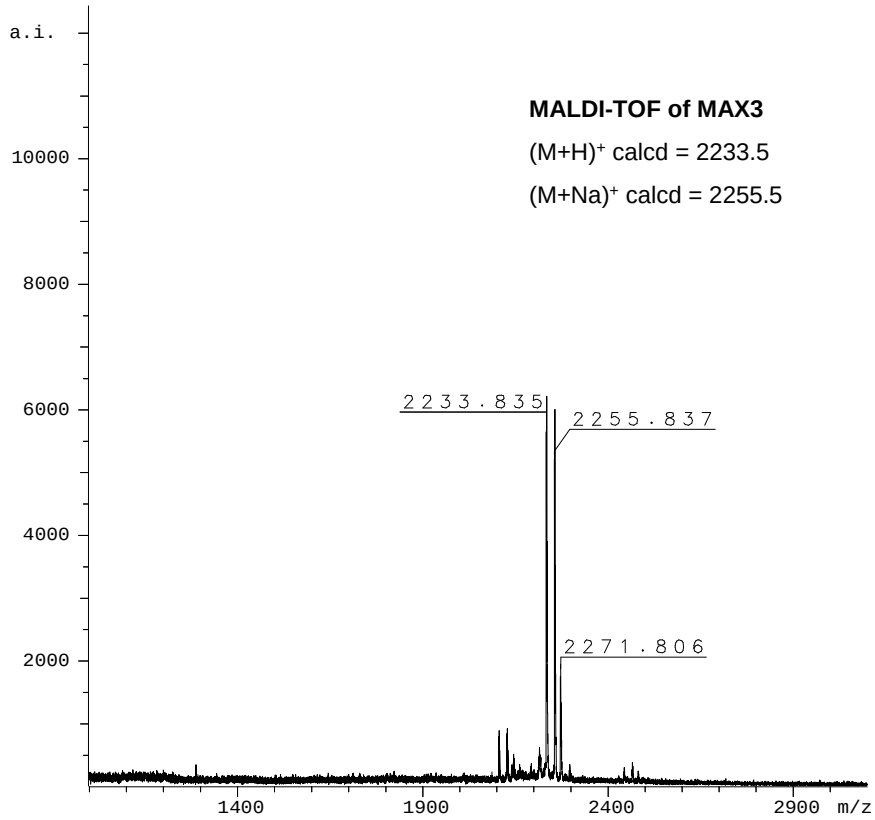
(Solvent A and B defined in above)

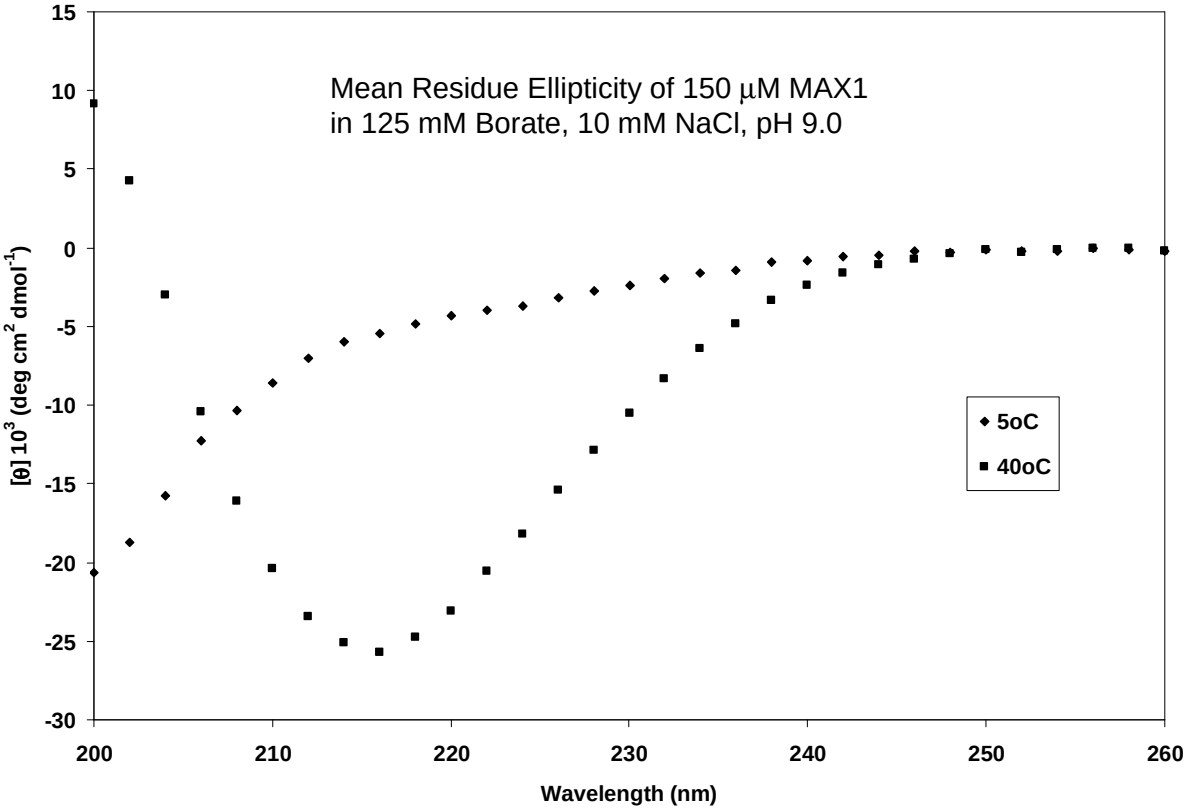


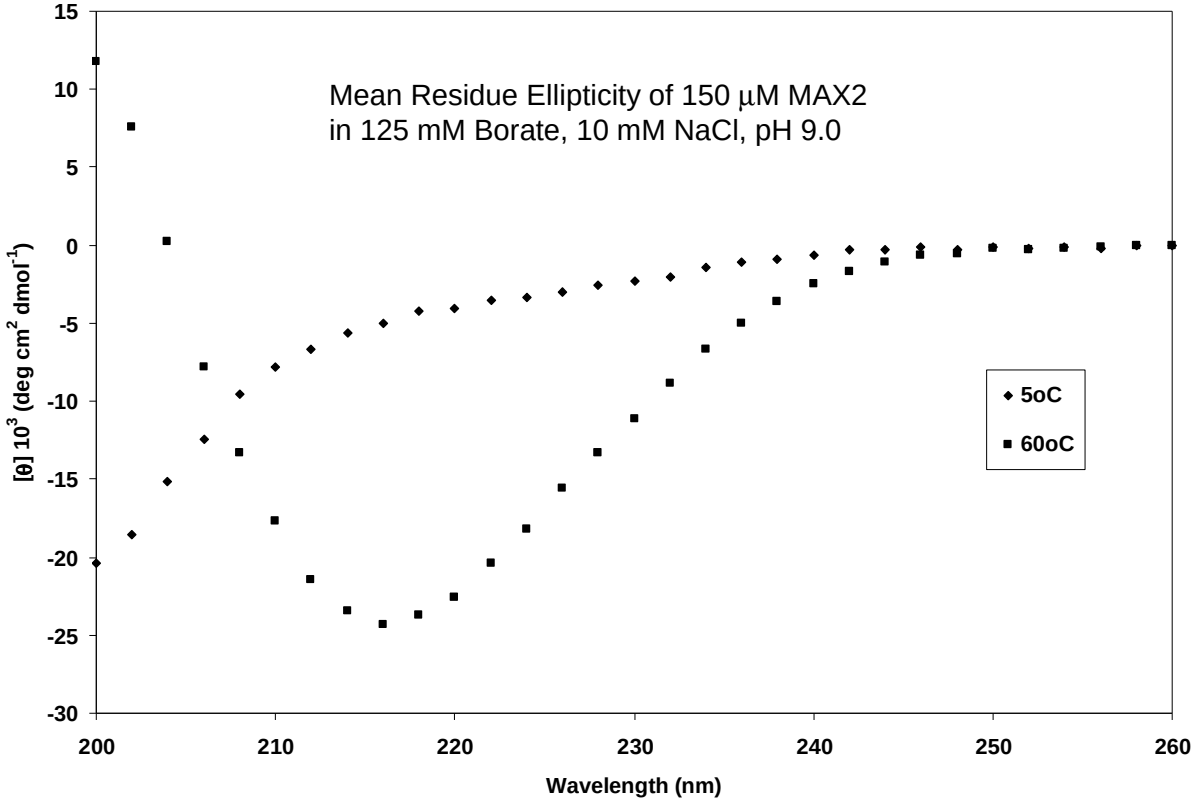
MALDI-TOF of MAX3

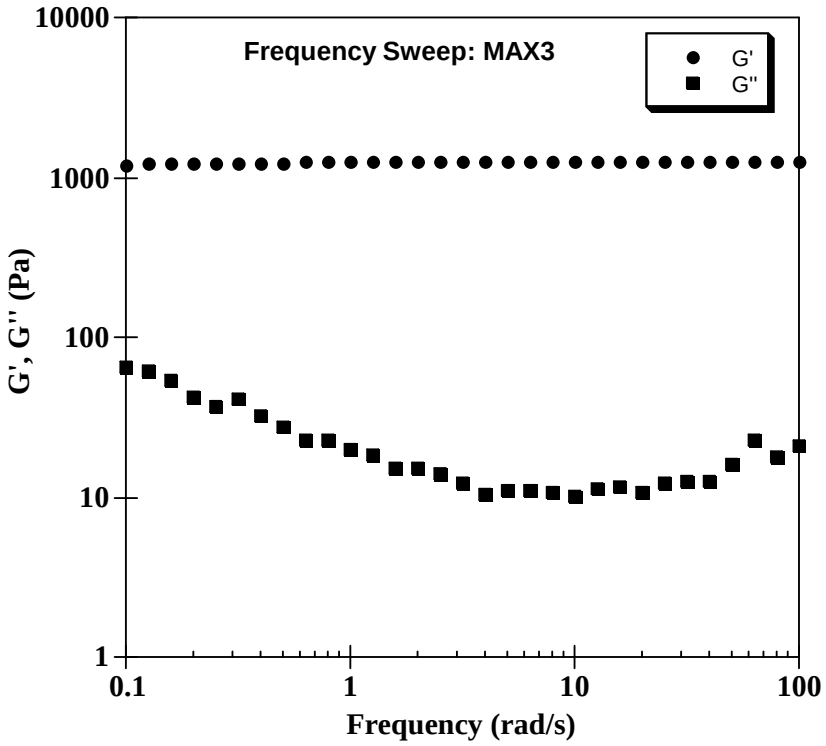
$(M+H)^+$ calcd = 2233.5

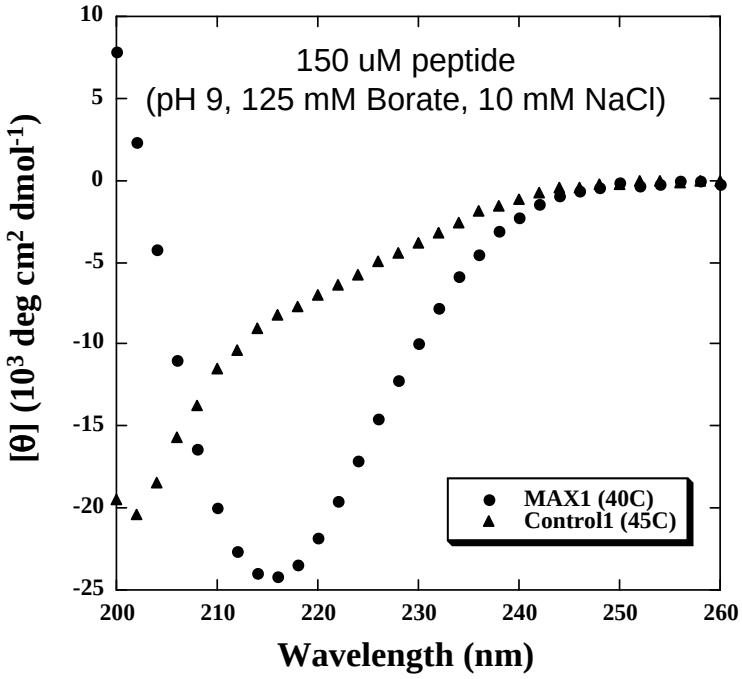
$(M+Na)^+$ calcd = 2255.5




Mean Residue Ellipticity of 150 μM MAX1
in 125 mM Borate, 10 mM NaCl, pH 9.0
[θ] 10^3 (deg cm^2 dmol^-1)
Wavelength (nm)
5oC
40oC
15
10
5
0
-5
-10
-15
-20
-25
-30
200
210
220
230
240
250
260


Mean Residue Ellipticity of 150 μM MAX2
in 125 mM Borate, 10 mM NaCl, pH 9.0
[θ] 10^3 (deg cm^2 dmol^-1)
Wavelength (nm)
5oC
60oC
15
10
5
0
-5
-10
-15
-20
-25
-30
200
210
220
230
240
250
260





MAX1: VKVKVKVKV^{D}PPTKVKVKVKV-CONH$_2$

Control1: VKVKVKVKV^{L}PPTKVKVKVKV-CONH$_2$

Experimental Section

General Methods and Material. Trifluoroacetic acid (TFA), piperidine, thioanisole, ethanedithiol, boric acid and anisole were purchased from Acros. Appropriately side-chain protected Fmoc-amino acids, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-Hydroxybenzotriazole (HOBT) were purchased from Novabiochem.

Peptide Synthesis. Peptides were prepared on PAL amide resin via automated Fmoc peptide synthesis employing an ABI 433A peptide synthesizer and HBTU/HOBT activation. The resulting dry resin-bound peptides were cleaved and side-chain deprotected using TFA:thioanisole:ethanedithiol: anisole (90:5:3:2) cocktail. Crude peptide was purified by RP-HPLC (preparative Vydac C18 peptide/protein column) employing a linear gradient from 12% to 100% B over 176 min where solvent A is 0.1% TFA in water and solvent B is 90% acetonitrile, 10% water and 0.1% TFA. MAX1, MS (MALDI-TOF):2229.8 [$(M+H)^+$, calcd 2229.6]; MAX2, MS (MALDI-TOF):2231.8 [$(M+H)^+$, calcd 2231.5]; MAX3, MS (MALDI-TOF):2233.8 [$(M+H)^+$, calcd 2233.5].

Circular Dichroism Studies. CD spectra were collected on an AVIV model 215 spectropolarimeter. Wavelength scans were recorded using a 2 nm step size and a 2 sec averaging time. Thermal denaturation experiments were monitored at 218 nm from 5 to 95 ºC in 5 degree steps with a 10 min equilibration time and data averaging for 60 seconds at each temperature. All peptide solutions were prepared at pH 9.0 (125 mM Borate, 10 mM NaCl) and spectra were collected in 1 mm quartz cells. Peptide samples were prepared by dilution of millimolar aqueous stock solutions with water to half the final volume and chilling in an ice bath, followed by an equal volume addition of cold stock buffer solution (250 mM Borate, 20 mM NaCl, pH 9.0). Samples were then transferred from the ice bath directly to 1 mm quartz cells previously equilibrated to 5ºC in the spectropolarimeter. Concentrations of peptide solutions were determined by absorbance at 220 nm (e = 15750 cm-1 M-1). $\in_{220}$ was determined by amino acid analysis. Mean residue ellipticity [θ] was calculated using the equation [θ] = (θobs/10*lc*)/*r*, where θ obs is the measured ellipticity in millidegrees, *l* is the length of the cell (cm), *c* is the concentration (M) and *r* is the number of residues. Solutions of control peptides for CD were prepared according to the same procedure at room temperature and spectra were collected in 0.1 mm pathlength

Procedure for Hydrogel Preparation. Generally, alkaline solutions of MAX1 will undergo gelation at room temperature and concentrations of 1 wt% or greater. Extremely uniform gels can be prepared according to the example procedure given below for the preparation of 300 mL of a 1 wt% hydrogel: To a solution of 3.0 mg of MAX1 in 150 mL of water was added 150 mL of a buffer stock solution (250 mM Borate, 20 mM NaCl, pH 9.0) resulting in a 1 wt% solution of peptide (pH 9.0, 125 mM Borate, 10 mM NaCl). After minimal pipette mixing, this solution is allowed to stand for a few minutes until gelation is complete. Basic solutions of MAX2 and MAX3 do not gel until they are heated beyond their respective T_{Gels}.

Rheology. Dynamic time and frequency sweep rheology experiments were performed on a Rheometrics Ares rheometer with a 25 mm diameter parallel plate geometry. Self-assembly and consequent gel network formation via temperature step changes of a 2 wt % solution (mixture of equal volume of an aqueous stock solution of peptide and equal volume of stock buffer solution) was monitored by dynamic time sweep experiments. These time sweep experiments were done at 6 rad/sec frequency and 5 % strain (previously determined to be in the linear regime). Temperature step changes between 75 and 5 °C were controlled by a peltier temperature controller. To minimize evaporation loses the parallel plate is covered with mineral oil. When the temperature increased from 5 to 75 °C, approximately 1 hour was allowed in order for the gel to equilibrate before the measurements were taken. In the cooling cycles (75 to 5 °C) a longer time was allowed (~2 hours) due to hysteresis in the disassembly of the gels. Dynamic frequency sweep measurements were done at 75 °C to monitor G' (storage) and G" (loss) moduli at 5 % strain and between frequencies 100 and 0.1 rad/sec in order to quantify equilibrium behavior.