Supporting Information

for

Exploring the temperature dependence of β-hairpin peptide self-assembly

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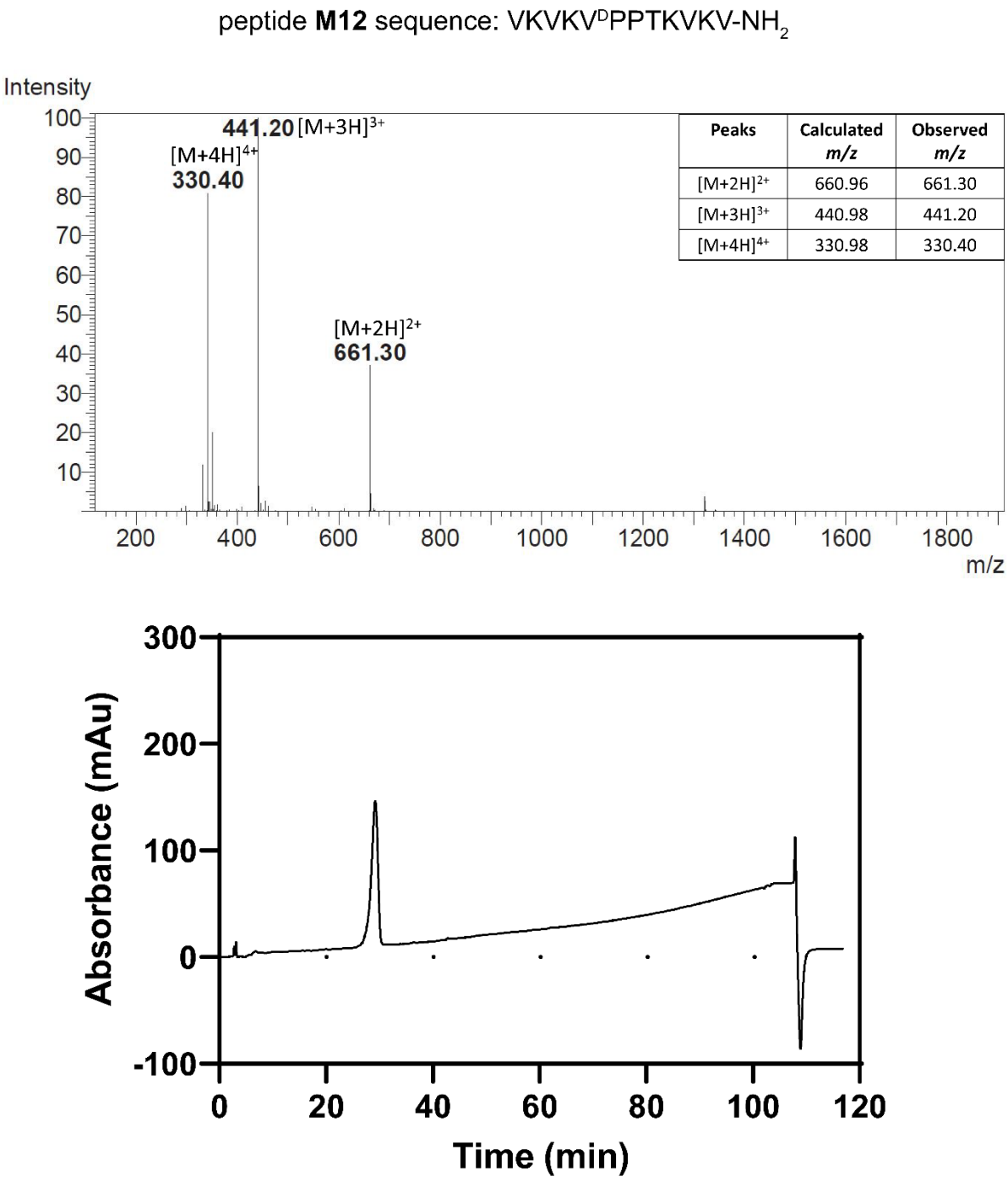
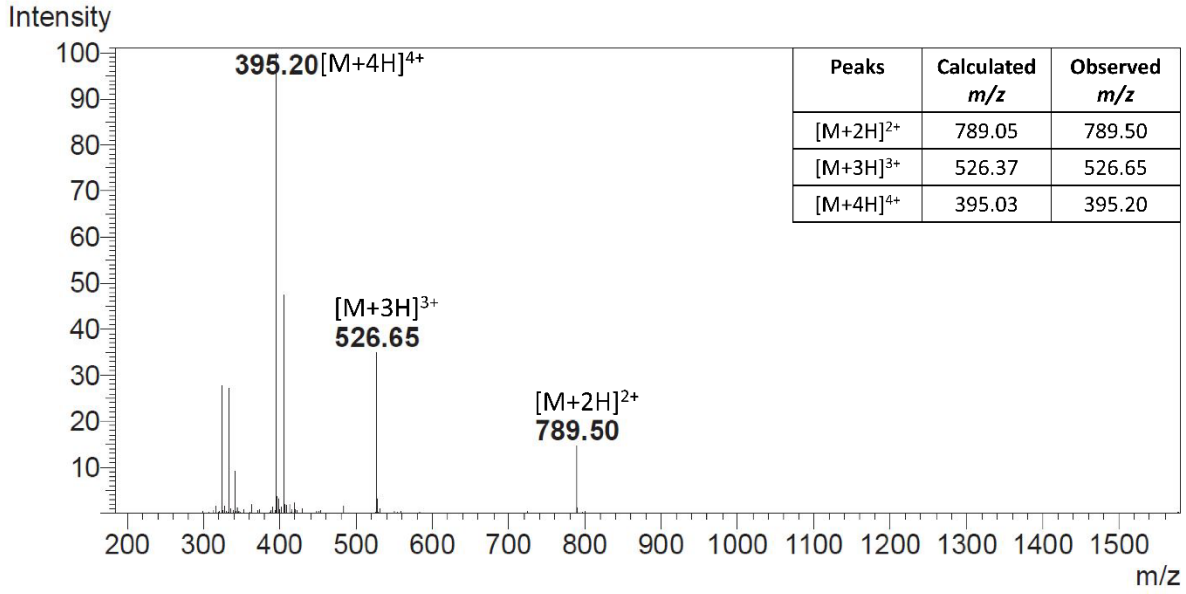


Figure S1. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M12**.

peptide **M14** sequence: KVKVKV^{D}PPTKVKVK-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	789.05	789.50
$[M+3H]^{3+}$	526.37	526.65
$[M+4H]^{4+}$	395.03	395.20

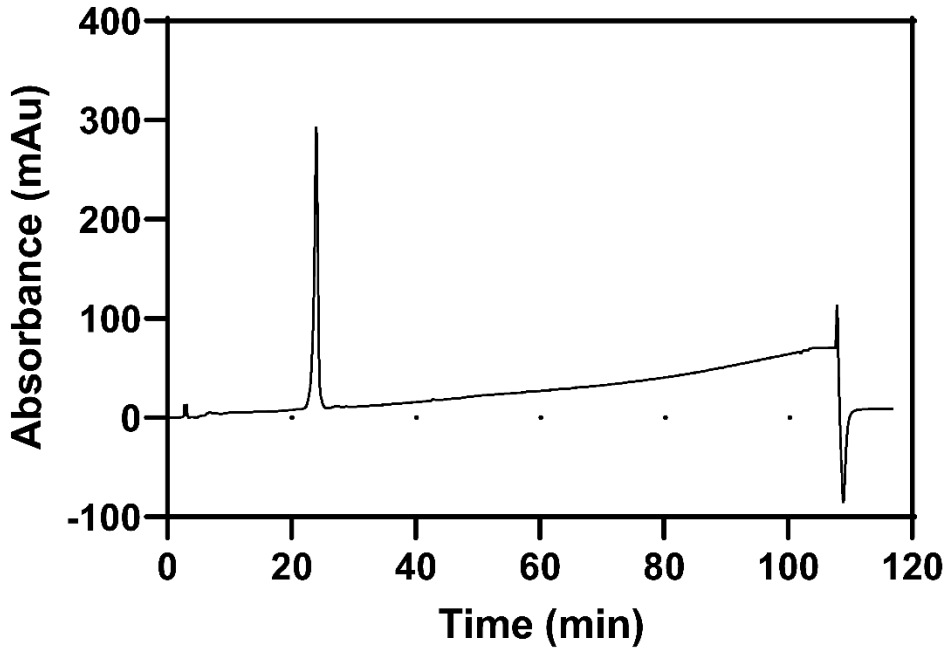
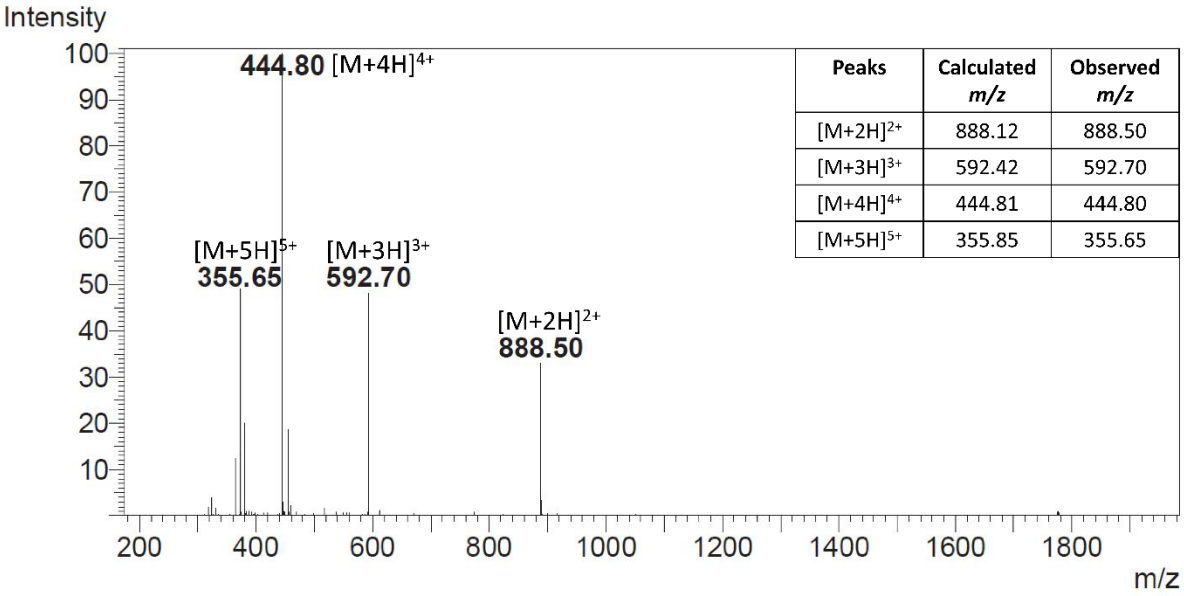


Figure S2. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M14**.

peptide **M16** sequence: VKVKVKV^{D}PPTKVKVKV-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	888.12	888.50
$[M+3H]^{3+}$	592.42	592.70
$[M+4H]^{4+}$	444.81	444.80
$[M+5H]^{5+}$	355.85	355.65

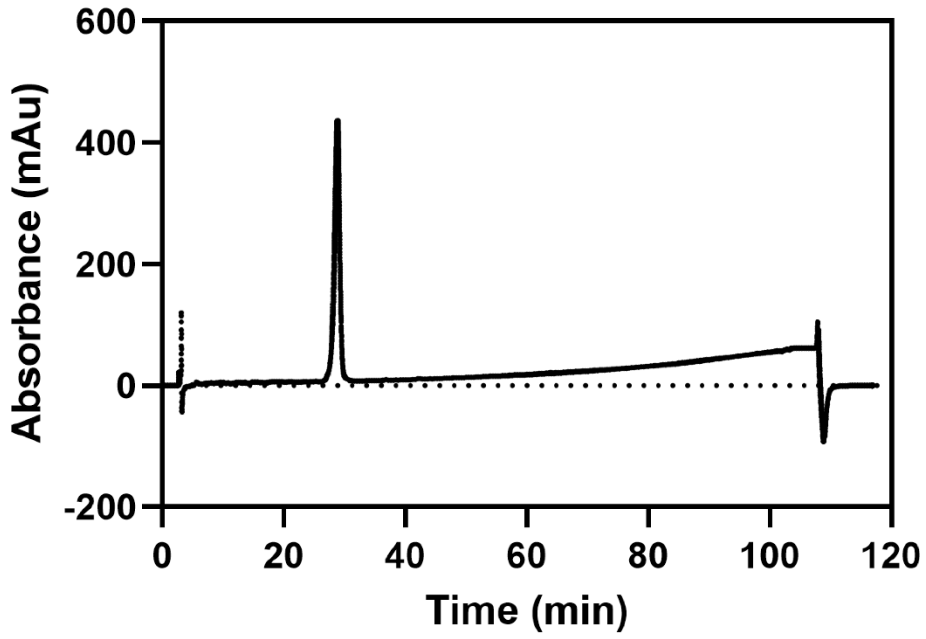
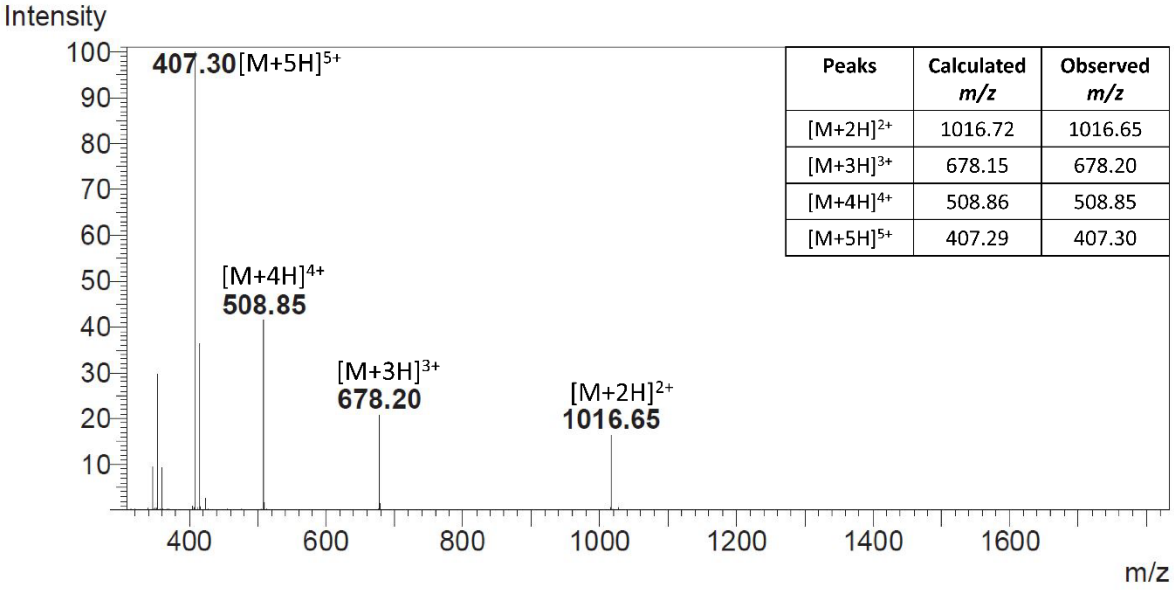


Figure S3. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M16**.

peptide **M18** sequence: KVKVKVKV^{D}PPTKVKVKVK-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	1016.72	1016.65
$[M+3H]^{3+}$	678.15	678.20
$[M+4H]^{4+}$	508.86	508.85
$[M+5H]^{5+}$	407.29	407.30

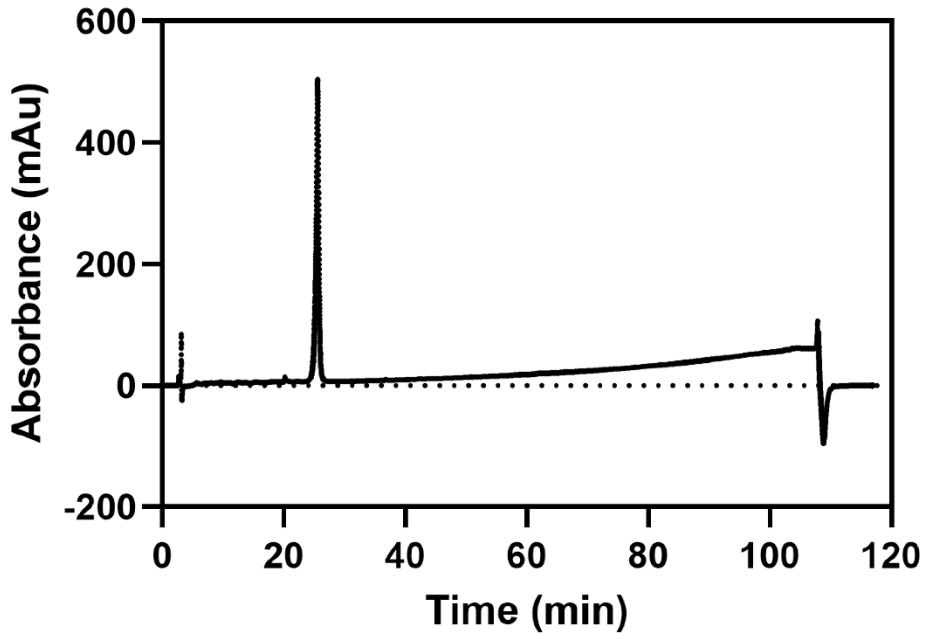
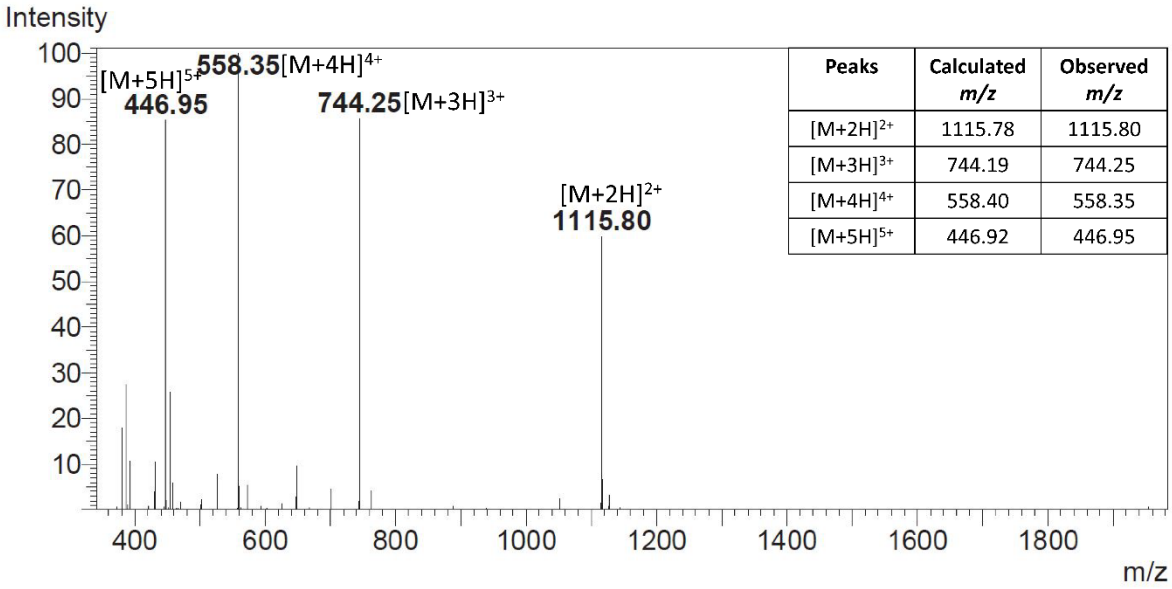


Figure S4. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M18**.

peptide **M20** (MAX1) sequence: VKVKVKVKV^{D}PPTKVKVKVKV-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	1115.78	1115.80
$[M+3H]^{3+}$	744.19	744.25
$[M+4H]^{4+}$	558.40	558.35
$[M+5H]^{5+}$	446.92	446.95

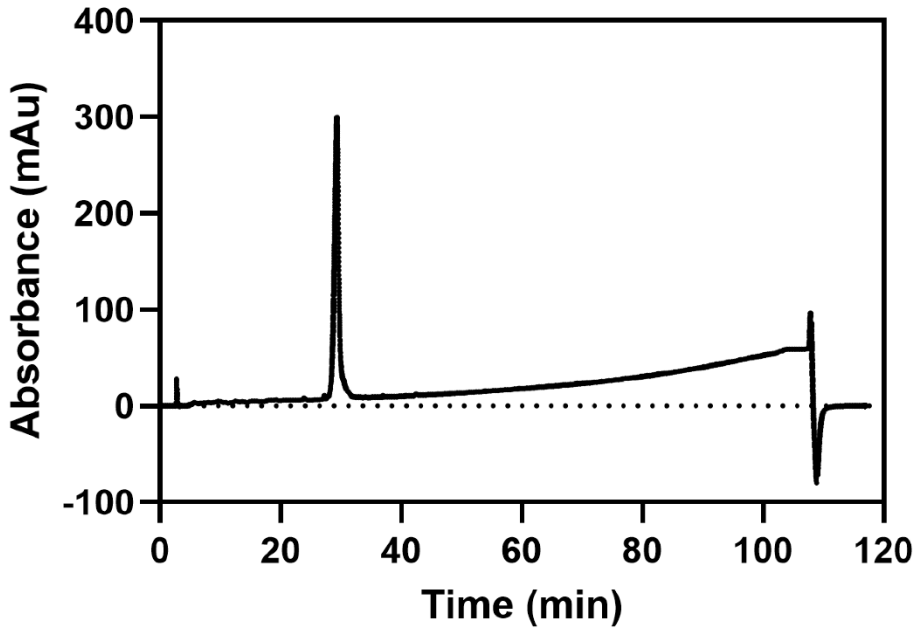
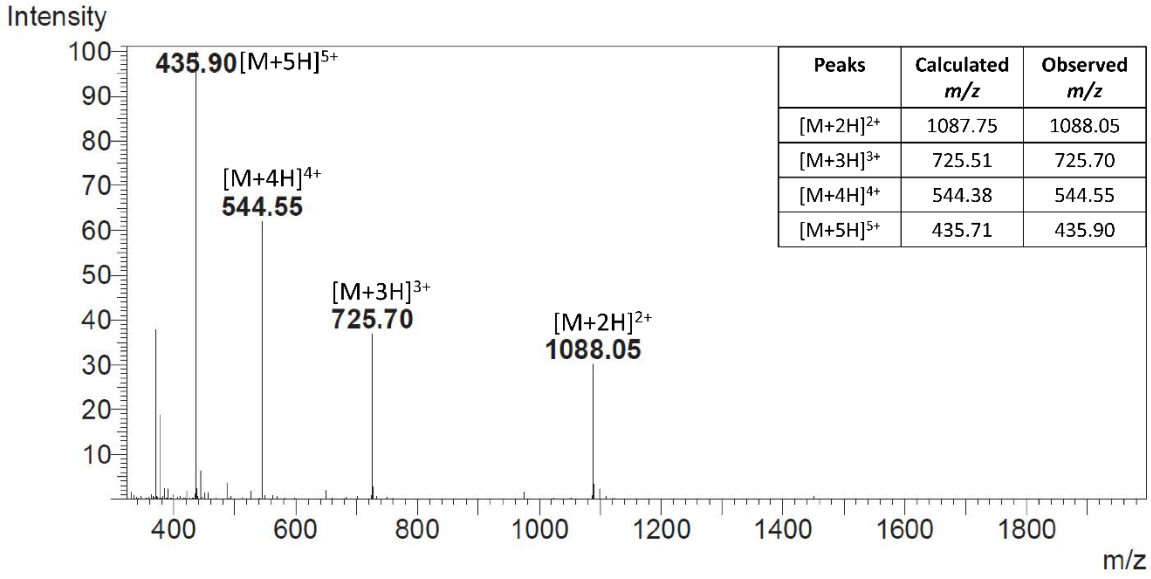


Figure S5. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M20** (MAX1).

peptide **M20A** sequence: AKVKVKVKV^{D}PPTKVKVKVKA-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	1087.75	1088.05
$[M+3H]^{3+}$	725.51	725.70
$[M+4H]^{4+}$	544.38	544.55
$[M+5H]^{5+}$	435.71	435.90

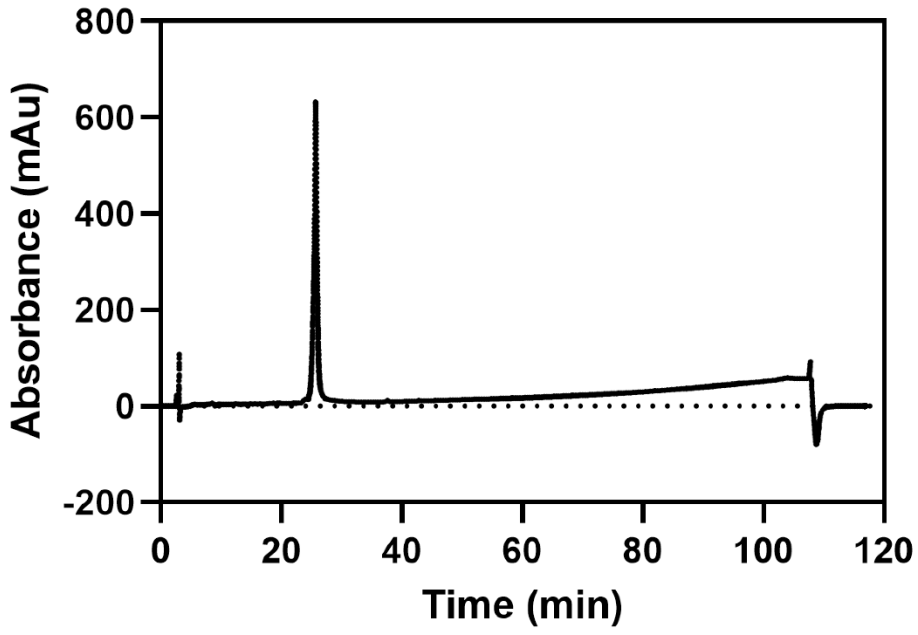
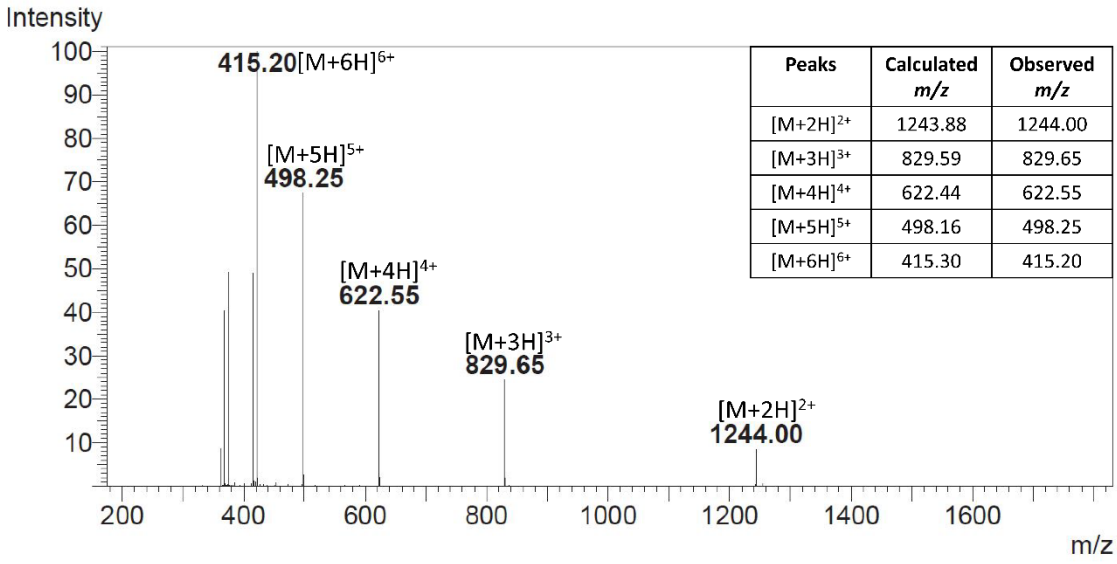


Figure S6. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M20A**.

peptide **M22** sequence: KVKVKVKVKV^{D}PPTKVKVKVKVK-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	1243.88	1244.00
$[M+3H]^{3+}$	829.59	829.65
$[M+4H]^{4+}$	622.44	622.55
$[M+5H]^{5+}$	498.16	498.25
$[M+6H]^{6+}$	415.30	415.20

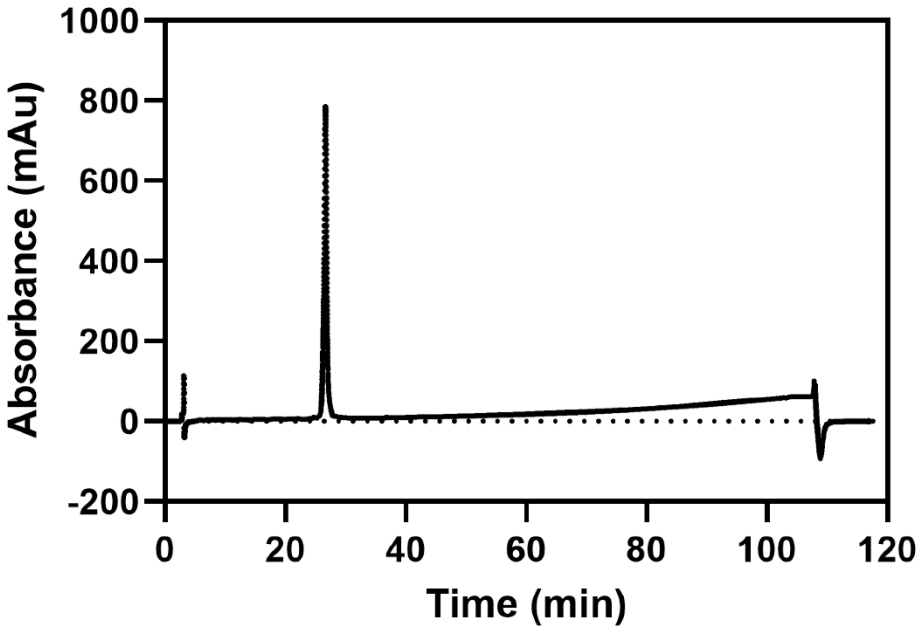
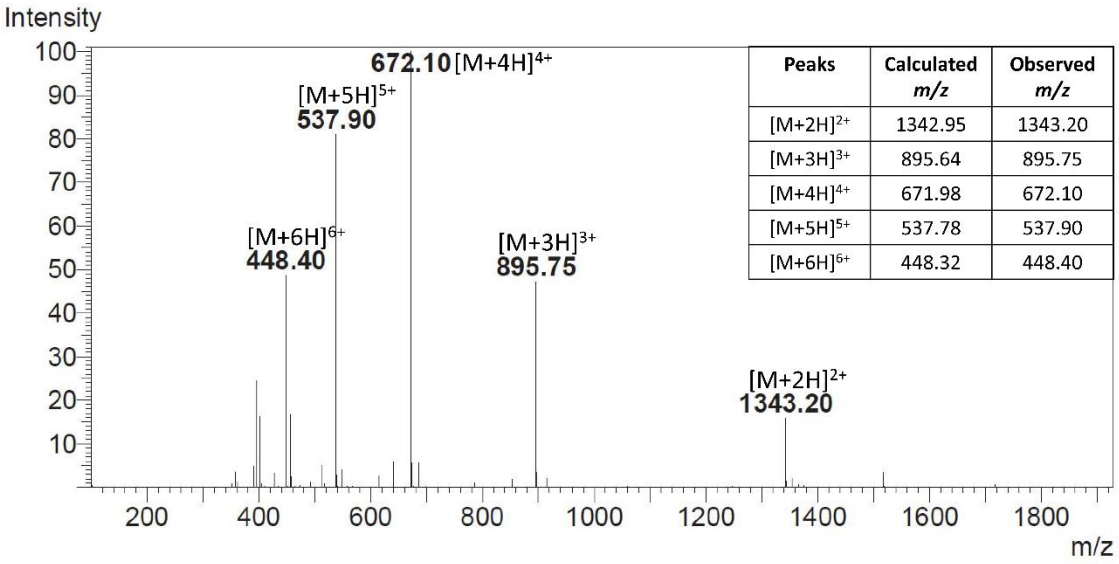


Figure S7. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M22**.

peptide **M24** sequence: VKVKVKVKVK^{D}PPTKVKVKVKVKV-NH_2



Peaks	Calculated *m/z*	Observed *m/z*
$[M+2H]^{2+}$	1342.95	1343.20
$[M+3H]^{3+}$	895.64	895.75
$[M+4H]^{4+}$	671.98	672.10
$[M+5H]^{5+}$	537.78	537.90
$[M+6H]^{6+}$	448.32	448.40

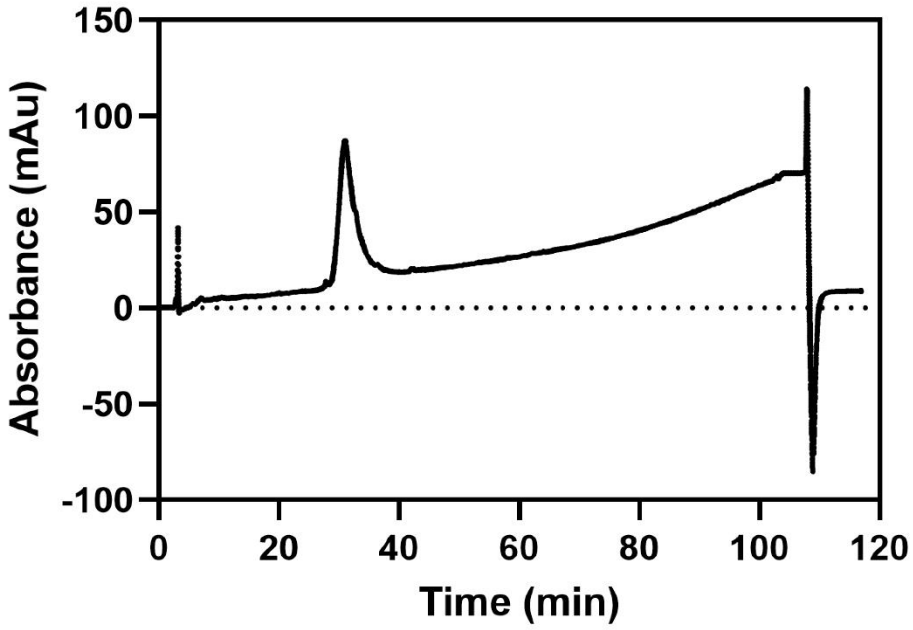


Figure S8. Sequence, RP-HPLC trace, and ESI (+) mass spectrum of purified peptide **M24**.

Supplementary Table 1. OPLS parameters for partial charges of residue atomic groups used in SAC and SAH calculations The original table of OPLS values for all residue atomic groups may be found in: Tirado-Rives, J.; Jorgensen, W. L. The OPLS Potential Functions for Proteins. Energy Minimizations for Crystals of Cyclic Peptides and Crambin. *Journal of the American Chemical Society* **1988**, *110* (6), 1657-1666.

residue	atomic group	*q*	residue	atomic group	*q*
main chains					
Ala	N	-0.570	Pro	N	-0.570
	H(N)	0.370		CH^{α}	0.285
	CH^{α}	0.200		C	0.500
	C	0.500		O	-0.500
	O	-0.500			
side chains					
Ala	CH_3^{β}	0.0	Thr	CH^{β}	0.265
				O^{γ}	-0.700
Val	CH^{β}	0.0		$H^{\gamma}(O)$	0.435
	CH_3^{γ}	0.0		CH_3^{γ}	0.0
Pro	CH_2^{β}	0.0	Lys	CH_2^{β}	0.0
	CH_2^{γ}	0.0		CH_2^{γ}	0.0
	CH_2^{δ}	0.285		CH_2^{δ}	0.0
				CH_2^{ε}	0.310
				N^{ζ}	-0.300
				$H^{\zeta}(N)$	0.330
Termini					
N-terminal amine	N	-0.300	C-terminal amide	C	0.500
	H(N)	0.330		O	-0.500
				N	-0.850
				H(N)	0.425

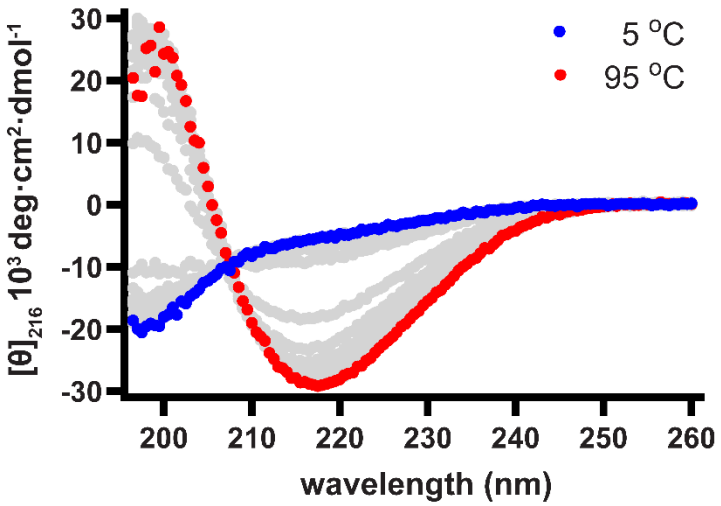


Figure S9. Temperature dependent CD spectrum of peptide **M16**; 150 μM peptide, 125 mM Boric acid, 10 mM NaCl, pH 9.0, 30-minute equilibration time at each temperature step. For clarity, only values recorded at 5°C (blue) and 95°C (red) are highlighted.

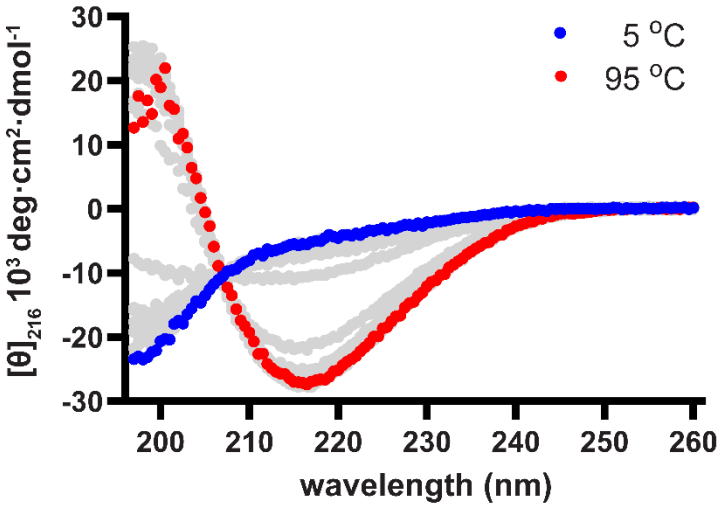


Figure S10. Temperature dependent CD spectrum of peptide **M18**; 150 μM peptide, 125 mM Boric acid, 10 mM NaCl, pH 9.0, 30-minute equilibration time at each temperature step. For clarity, only values recorded at 5°C (blue) and 95°C (red) are highlighted.

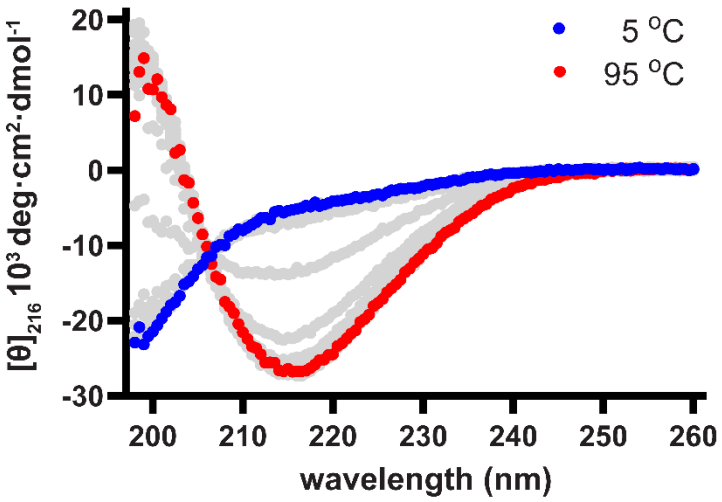


Figure S11. Temperature dependent CD spectrum of peptide **M20** (MAX1); 150 μM peptide, 125 mM Boric acid, 10 mM NaCl, pH 9.0, 30-minute equilibration time at each temperature step. For clarity, only values recorded at 5°C (blue) and 95°C (red) are highlighted.

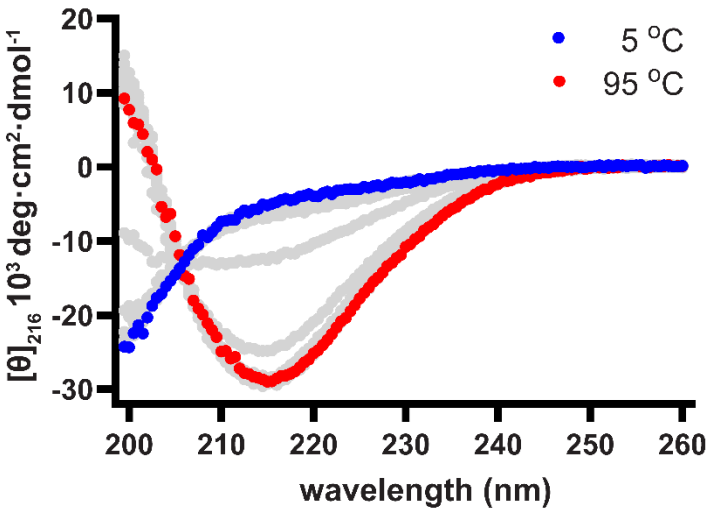


Figure S12. Temperature dependent CD spectrum of peptide **M22**; 150 μM peptide, 125 mM Boric acid, 10 mM NaCl, pH 9.0, 30-minute equilibration time at each temperature step. For clarity, only values recorded at 5°C (blue) and 95°C (red) are highlighted.

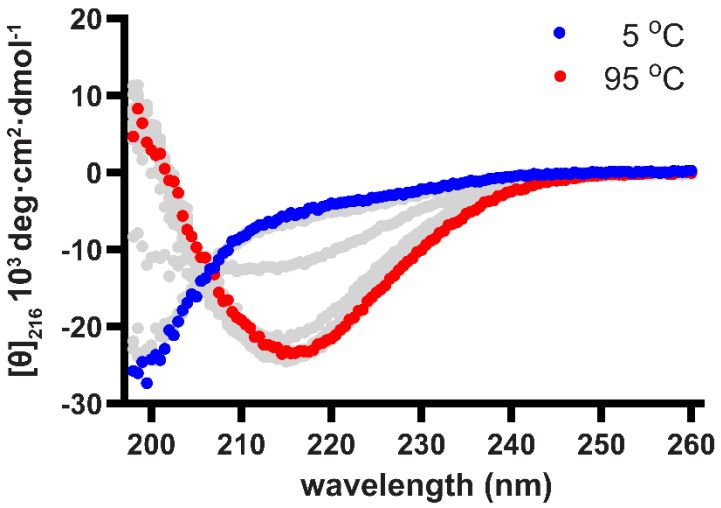


Figure S13. Temperature dependent CD spectrum of peptide **M24**; 150 μM peptide, 125 mM Boric acid, 10 mM NaCl, pH 9.0, 30-minute equilibration time at each temperature step. For clarity, only values recorded at 5°C (blue) and 95°C (red) are highlighted.

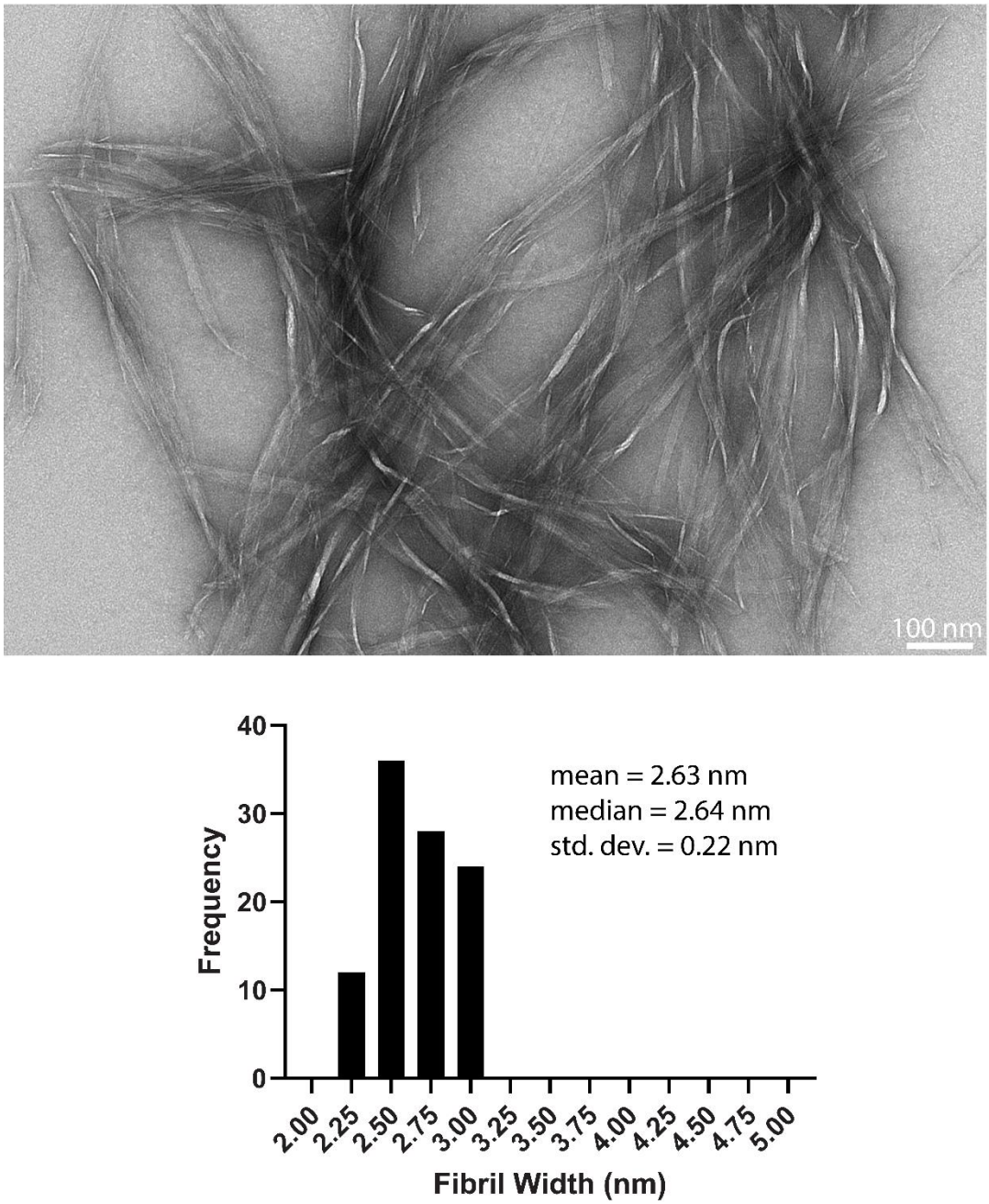


Figure S14. Population frequency distribution for observed fibril diameters analyzed by TEM (13 bins, 0.25 nm each) for Peptide **M16** (n = 50 analyzed fibrils). Prepared from 1 wt.% gels formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0.

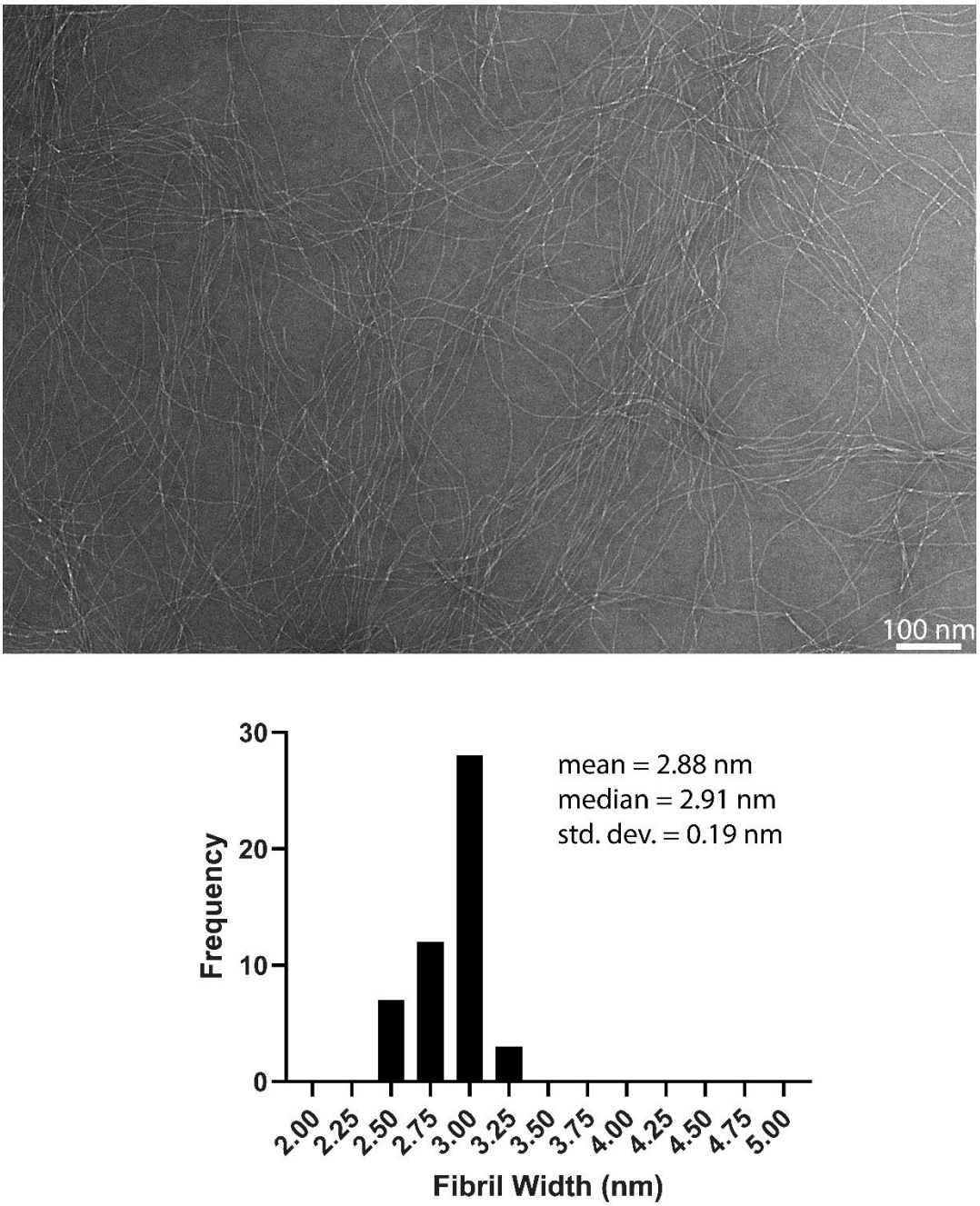


Figure S15. Population frequency distribution for observed fibril diameters analyzed by TEM (13 bins, 0.25 nm each) for Peptide **M18** (n = 50 analyzed fibrils). Prepared from 1 wt.% gels formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0.

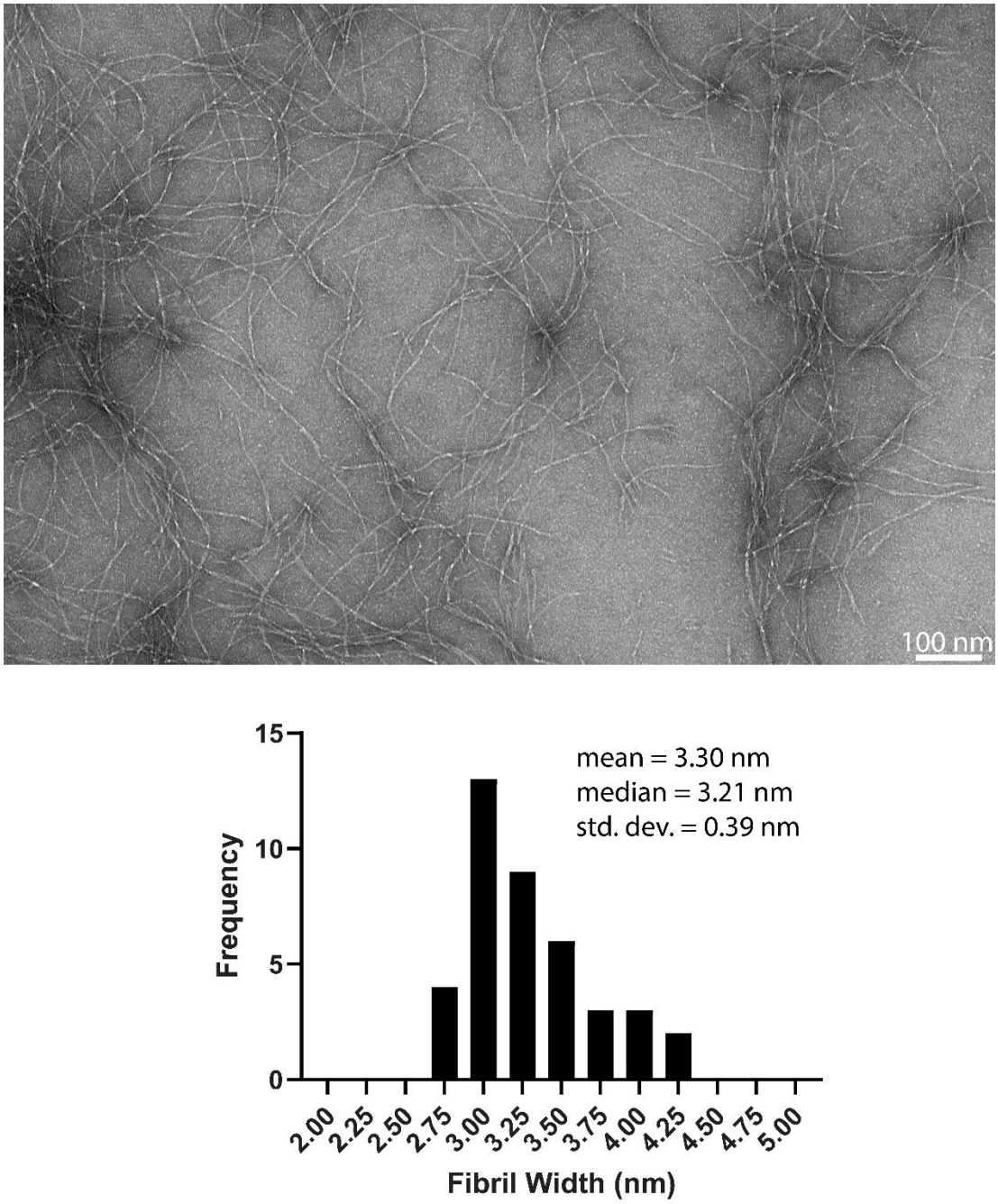


Figure S16. Population frequency distribution for observed fibril diameters analyzed by TEM (13 bins, 0.25 nm each) for Peptide **M20** (MAX1) (n = 40 analyzed fibrils). Prepared from 1 wt.% gels formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0.

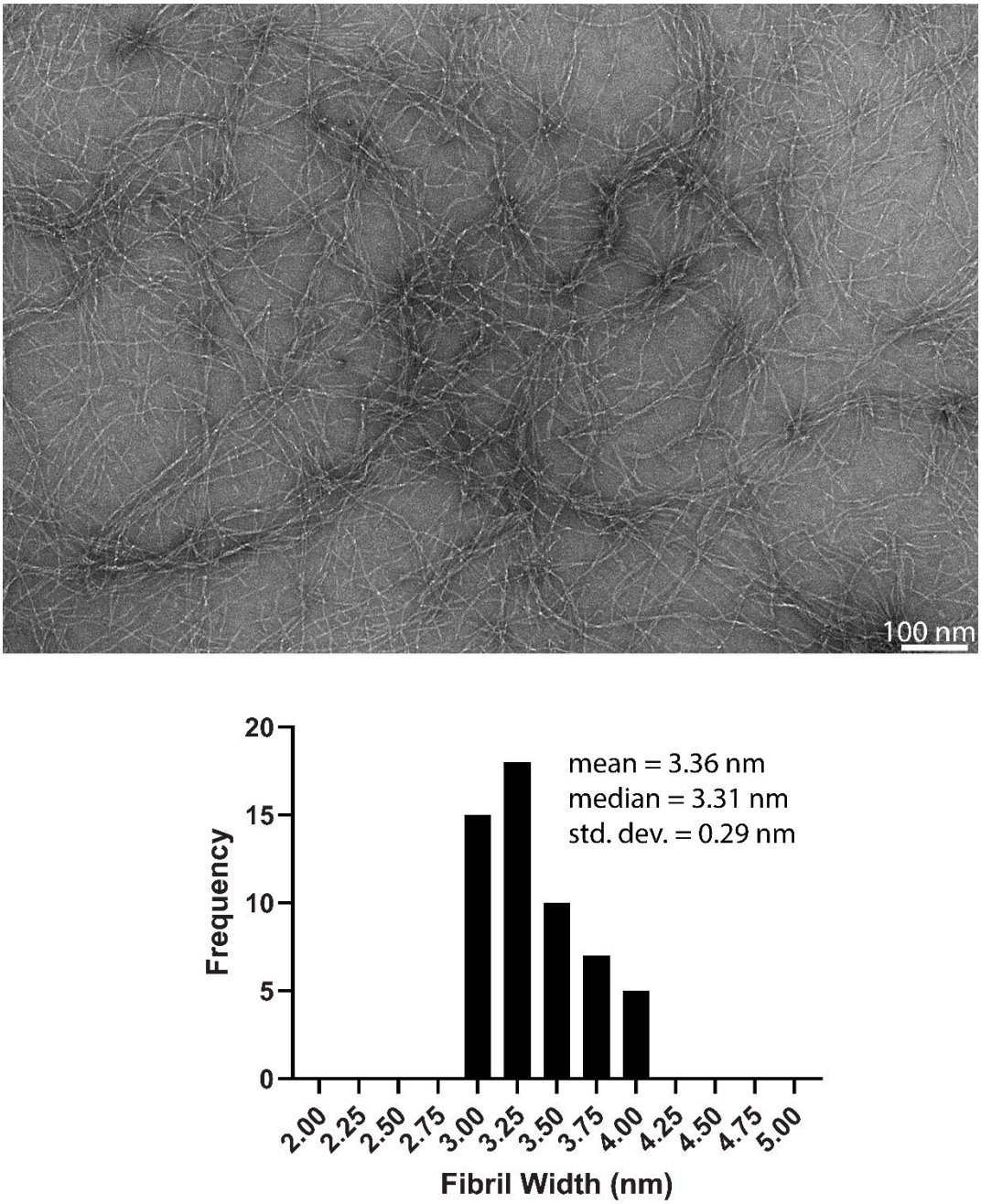


Figure S17. Population frequency distribution for observed fibril diameters analyzed by TEM (13 bins, 0.25 nm each) for Peptide **M22** (n = 55 analyzed fibrils). Prepared from 1 wt.% gels formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0.

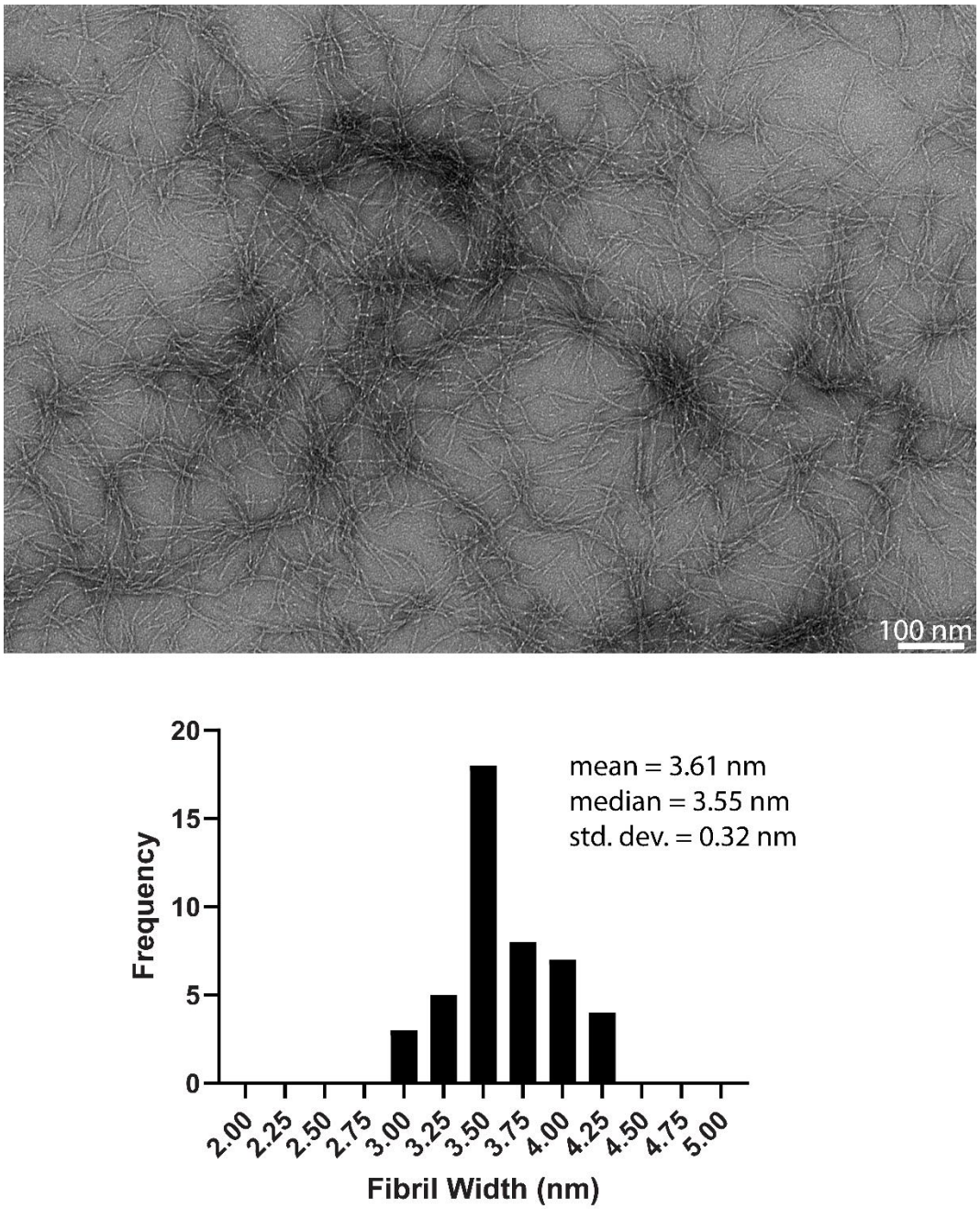


Figure S18. Population frequency distribution for observed fibril diameters analyzed by TEM (13 bins, 0.25 nm each) for Peptide **M24** (n = 45 analyzed fibrils). Prepared from 1 wt.% gels formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0.

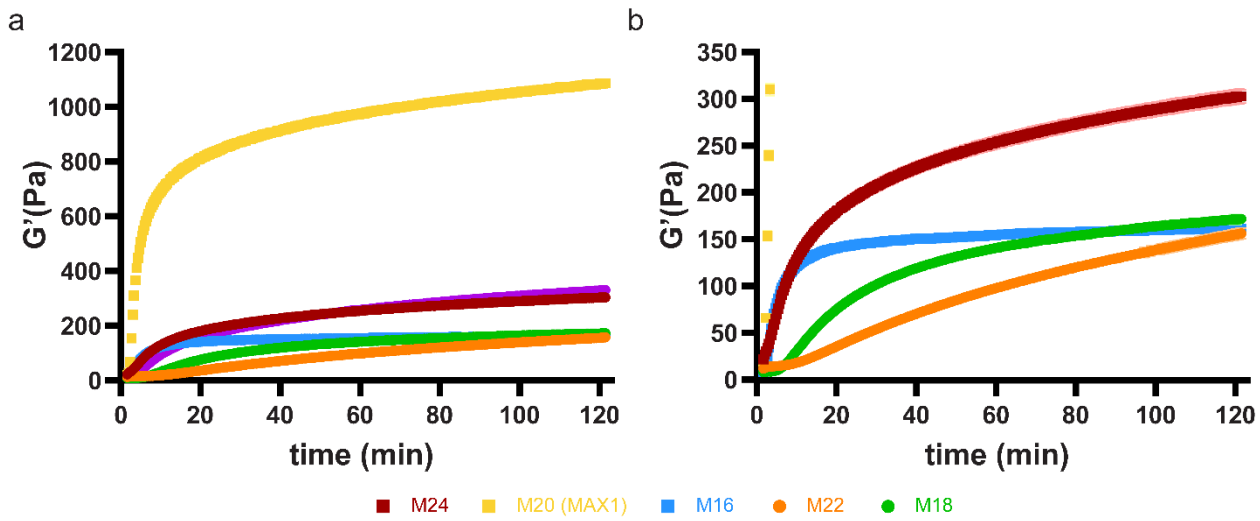


Figure S19. (a) Dynamic time sweep oscillatory shear rheology data measuring the storage moduli, G' (Pa), of gels formed by peptides **M16**, **M18**, **M20 (MAX1)**, **M22**, and **M24**. 3.1 mM hydrogels, 37°C, 125 mM Boric acid, 10 mM NaCl, pH 9.0, a constant strain of 0.2%, and frequency of (6 rad/s). Data are presented as mean ± SD (n = 3). (b) A focused view of data presented in panel (a).

Materials and Methods

Methods and protocols for synthesis, purification, and study of peptides **M12**, **M14**, **M16**, **M18**, **M20**, **M20A**, **M22**, and **M24** were adapted from methods previously reported by our group.[1-3] All chemicals were used as received, unless otherwise noted, from commercial vendors. Deionized water (18.25 MΩ) was obtained from a Millipore Milli-Q water purification system. HPLC grade acetonitrile and deionized water were used to prepare analytical and preparative reverse-phase HPLC buffers: Std A (0.1% TFA in Milli-Q water) and Std B (0.1% TFA in 80:20 acetonitrile: Milli-Q water). Analytical reverse-phase HPLC was performed on an Agilent 1260 Infinity II instrument equipped with a Vydac 218TP 5μm C18 column, at a flow rate of 0.25 mL/min and linear gradient of 1.0% Std B per min from 0 to 100% Std B. Preparative reverse-phase HPLC was performed on a Waters 600 HPLC system equipped with a Vydac 218TP 10μm C18 column, at a flow rate of 8.0 mL/min. Analytical reverse-phase LC/MS was performed on a Shimadzu UFLC HPLC system equipped with a Luna 5μm C18 100Å column. LC/MS grade water and acetonitrile, each containing 0.1% formic acid, were used for analytical reverse-phase LC/MS. Columns were heated to 40°C in the analytical and preparative methods used to isolate or characterize each peptide. All peptides were prepared and used as the trifluoroacetate salts and were assumed to have one trifluoracetic acid molecule per amine group on each peptide. The PyMol Molecular Graphics System, Version 2.5.2 Schrödinger, LLC was used to generate molecular representations of peptides.[4] The FreeSASA Python Module 2.2.1 was used to calculate solvent accessible surface area.[5]

Peptide Synthesis and Purification

All peptides were synthesized using microwave assisted Fmoc-based solid phase peptide synthesis (SPPS), using a CEM Liberty Blue peptide synthesizer. Each synthesis was carried out at 0.1 mmol scale using: ProTide Rink Amide resin (CEM, 0.55 – 0.88 mmol/g, 100 – 200 mesh), standard Fmoc-protected amino acids (0.2 M in DMF, 5 eq), piperidine (20% v/v in DMF), OxymaPure (1.0 M in DMF, 5 eq), N,N'-Diisopropylcarbodiimide (DIC) (0.5 M in DMF, 5 eq), O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. (HCTU) (0.45 M in DMF, 5 eq), and N,N-Diisopropylethylamine (DIEA) (2.0 M in NMP, 10 eq). All Fmoc deprotection cycles were carried out at 90°C for 65 seconds and then washed four times with DMF. All couplings, unless specified, were carried out at 90°C for 4 minutes using OxymaPure and DIC. To ensure complete couplings and to limit the growth of deletion sequences during synthesis, the three residue -Val-Lys-Val- segment that follows ^{D}Pro was coupled using HCTU and DIEA at room temperature. The valine immediately following ^{D}Pro was triple-coupled, 30 minutes for each coupling. Then the two following residues, lysine and valine, were each double-coupled with 30 minutes for each coupling. Following completion of the synthesis, the resin was then rinsed four times with DCM and then allowed to dry for 1 hour under vacuum. Peptides were then cleaved from the resin with a cleavage cocktail comprising trifluoroacetic acid (TFA)/ triisopropylsilane (TIPS)/ water (9:0.5:0.5) for 3 hours. Following cleavage, the crude peptide TFA solution was precipitated with ice cold diethyl ether, dried under vacuum, dissolved in water, frozen and lyophilized prior to purification.

Semi-preparative reverse-phase high performance liquid chromatography (RP-HPLC) was used to purify all peptides, (instrument, column, and mobile-phases described in the materials section). Crude, lyophilized peptide was dissolved in Std A at ~4 mg/mL and purified in 5 mL

volumes per purification run. Pure fractions were collected, combined and lyophilized furnishing each peptide as a TFA salt. Peptide purity (LC and LC/MS) was then assessed (instrument, column, and mobile-phases described in the materials section).

Methodology to Calculate Solvent Accessible Charge (SAC) and Solvent Accessible Hydrophobicity (SAH)

Determination of both solvent accessible charge (SAC) and solvent accessible hydrophobicity (SAH) for models of the unfolded state of peptides **M12**, **M14**, **M16**, **M18**, **M20** (MAX1), **M20A**, **M22**, and **M24** required that the solvent accessible surface area (SASA) be calculated first. SASA was calculated using the FreeSASA Python Module 2.2.1.[5] using the Shrake and Rupley algorithm[6], with "n-slices" set to 200. The default ProtOr atomic group radii were used for all calculations using FreeSASA.[7] The output from the FreeSASA calculation was a (.pdb)file with SASA values printed in the ATOM records "temperature factor" column.

SAH and SAC were calculated using **equation (3)** and **(4)** respectively from the main text as follows:

$$SAH = \sum_{i}^{atoms} q_b \times A_i \qquad (3)$$

$$SAC = \sum_{i}^{atoms} q \times A_i \qquad (4)$$

With (A_i) as the SASA per atomic group as output by FreeSASA, (q_b) a binary 0 (polar groups) or 1 (nonpolar) value for charge, and (q) the charge per atomic group as referenced from the OPLS parameters.[8] Charge values for the atomic groups of residues found in peptides **M12**, **M14**, **M16**, **M18**, **M20** (MAX1), **M20A**, **M22**, and **M24** are found in **Supplementary Table 1**. Care was taken to ensure that charge values for atomic groups containing heteroatoms were used as the sum of the

heteroatom and the covalently attached protons, e.g an amine is represented as the sum of one nitrogen (-0.300) and three protons (0.330). For the molecular representation of solvent accessible hydrophobicity in the unfolded state of **M20** (MAX1), SAH values for each atomic group were printed in the ATOM records "temperature factor" column, with "spectrum b" used to generate the gradient in PyMol. Before values of SAC were printed to the "temperature factor" column, negative values were normalized to the most negative SAC atomic group and positive values normalized to the most positive SAC atomic group. This ensures that gradient representations of potential charge run from red (most negative) to white (no charge) to blue (most positive).

Circular Dichroism Spectroscopy

All circular dichroism (CD) spectroscopy experiments were carried out on a Jasco J-1500 Circular Dichroism Spectrophotometer with Spectra Manage software (v. 2.14.06). Each peptide was dissolved in ice-cold ultrapure water to make a 2x stock solution (300 μM for solution-phase experiments or 3.08 mM for gel-phase experiments). The 2x stock was then diluted 1:1 with ice-cold buffer (250 mM Boric acid, 20 mM NaCl pH 9.0 or 100 mM BTP 300 mM NaCl pH 7.4). The 1X solution was then transferred to high precision quartz cuvette with a light path of 1 mm for μM concentrations or 0.1 mm for higher (mM) concentrations. Wavelength scans were taken between 260 and 200 nm, at 1 nm intervals. For the CD spectra reported in **Figure 2a** temperatures (7°C, 22°C, 37°C, 62°C) were held constant over the length of each experiment, with 10-minute equilibration periods between each measurement. For all other CD spectra, the temperature was increased from 2°C to 92°C in increments of 5°C, with 30-minute equilibrations between each measurement. Mean residue ellipticity, $[\theta]$ (deg cm^2 dmol^{-1}) was calculated as follows:

$$[\theta] = \frac{\theta_{obs}}{10 \cdot l \cdot c \cdot r}$$

Where (θobs) is the measured ellipticity (mdeg), (l) is the path length of the cell (0.1 cm), (c) is the molar concentration of the peptide (M), and (r) is the number of amino acid residues in the peptide.

Rheological Characterization of Peptide Gelation and Viscoelastic Properties

Rheology of all gels was performed using a HR20 rheometer (TA Instruments) equipped with a 25 mm parallel plate geometry with a 500 μM gap. Ice-cold peptide in 125 mM Boric acid, 10 mM NaCl, pH 9.0 (300 μL, 10 mg/mL) were loaded onto the pre-cooled (5 °C) stage. The geometry was then lowered to the defined gap, and the temperature was then raised to 37 °C over 1 minute. The storage (G') and loss modulus (G'', not reported) were monitored for 2 hours at 0.2% strain and 6 rad/s. To prevent drying artifacts, mineral oil (ASTM oil standard) was applied to the geometry and stage prior to analysis.

Transmission Electron Microscopy

Carbon-coated copper grids (400-square mesh, CF400-CU; Electron Microscopy Science, Inc.) were treated with plasma air for 30 seconds using a PELCO easiGlow™ glow discharge cleaning system (Ted Pella, Inc.) to render the carbon film more hydrophilic. A 10 μL droplet of fibrils, prepared from a 1 wt.% gel formed in 125 mM Boric acid, 10 mM NaCl, pH 9.0. was then deposited on top of a plasma-treated grid and allowed to sit for 1 minute. Then, the excess solution was wicked away with filter paper, leaving a thin film of the sample atop the grid. A 7 μL droplet of uranyl acetate (2 wt% in MilliQ water) was then added on top of the grid for negative staining and blotted away after 30 seconds. Grids were allowed to dry for at least 3 hours before imaging on an FEI Tecnai 12 Twin transmission electron microscope operating at 80 kV acceleration voltage. All images were recorded using an AMT XR16 charge-coupled device (CCD) bottom-

mount camera (Advanced Microscopy Techniques, Corp.). Fibril diameters were measured using ImageJ software.

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