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-Supplementary Information-

Effects of Incorporation of Azido Moieties into Hydrophobic Core of Coiled Coil Peptides

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General Procedure for Solid Phase Peptide Synthesis: Synthesis of the peptide library was performed by using an automatic synthesizer Titan 357 (AAPPTEC). 50 mg of ChemMatrix® resins (0.48 mmol/g) were swelled in NMP (1 ml) for 5 min in a Reaction Vessel (RV). With the liquid drained, 20% piperidine in NMP (1 ml, v/v) was added and the RV was vortexed for 3 min. The liquid was drained and a fresh solution of 20% piperidine in NMP (1 ml, v/v) was added and the RV was vortexed for another 12 min. The resulting beads were thoroughly washed by NMP (1 ml x 2), methanol (1 ml x 2) and DCM (1 ml × 2). With the resulting resins swelled with NMP (1 ml) for 15 min, Fmoc-protected amino acid (2.5 equiv, 0.2 M solution in NMP) was added to the RV, as well as TBTU (2.5 equiv, 0.2 M solution in NMP) and DIEA (5.0 equiv, 0.5 M in NMP). The resulting mixture was vortexed for 45 min. With the liquid drained, the resulting beads were thoroughly washed by NMP (1 ml × 3). The coupling step was repeated until the desired sequence of peptide attained. N-terminus was modified with acetyl group by

treatment of acetic anhydride (10 equiv, 0.5 M solution) and DIEA (20 equiv, 0.5 M in NMP) for 10 min. The resins were washed by NMP (1 ml × 3) and transferred in a 4 ml reactor equipped with a filter, using DCM (2 ml × 3). After the resins were dried under reduced pressure for 2 h, the peptide was cleaved in a cleavage cocktail of TFA-water-TIS (1.5 ml, 94/3/3, v/v) for 2 h on a 180-degree shaker, while all the acid-labile protective groups in the residues were also detached. The solution was collected and concentrated in a continuous flow of nitrogen and the crude peptides were precipitated in diethylether. The resulting white solid was then purified to >98% in purity by a preparative HPLC (Gilson) on a C_{18} reversed phase preparative column (Kromasil®, 21.2 mm x 250 mm) using water and acetonitrile with 0.1% trifluoroacetic acid as the mobile phase.

Table S1. Analytical HPLC conditions for peptides.

Peptide	HPLC condition *	Retention time
L8	5 – 60% A over 8 min	4.57 min
L6Z2	5 – 60% A over 8 min	3.83 min
L4Z4	5 – 60% A over 8 min	3.53 min
L2Z6	5 – 60% A over 8 min	3.78 min
Z8	5 – 60% A over 8 min	3.98 min

* A: CH_3CN/0.1%TFA, B: H_2O/0.1%TFA, %A + %B = 100%.

Table S2. ESI-MS Data of peptides.

Peptide	MS, calculated	ESI-MS, observed
L8	3560.24	1780.81 ($[M+2H]^{2+}$), 1187.96 ($[M+3H]^{3+}$), 890.96 ($[M+4H]^{4+}$), 712.93 ($[M+5H]^{5+}$)
L6Z2	3614.21	1807.31 ($[M+2H]^{2+}$), 1205.49 ($[M+3H]^{3+}$), 904.52 ($[M+4H]^{4+}$), 723.99 ($[M+5H]^{5+}$)
L4Z4	3668.18	1834.97 ($[M+2H]^{2+}$), 1223.48 ($[M+3H]^{3+}$), 918.11 ($[M+4H]^{4+}$), 734.58 ($[M+5H]^{5+}$)
L2Z6	3722.15	1861.8 ($[M+2H]^{2+}$), 1241.7 ($[M+3H]^{3+}$), 931.3 ($[M+4H]^{4+}$), 745.6 ($[M+5H]^{5+}$)
Z8	3776.12	1889.54 ($[M+2H]^{2+}$), 1259.56 ($[M+3H]^{3+}$), 944.99 ($[M+4H]^{4+}$), 756.36 ($[M+5H]^{5+}$)

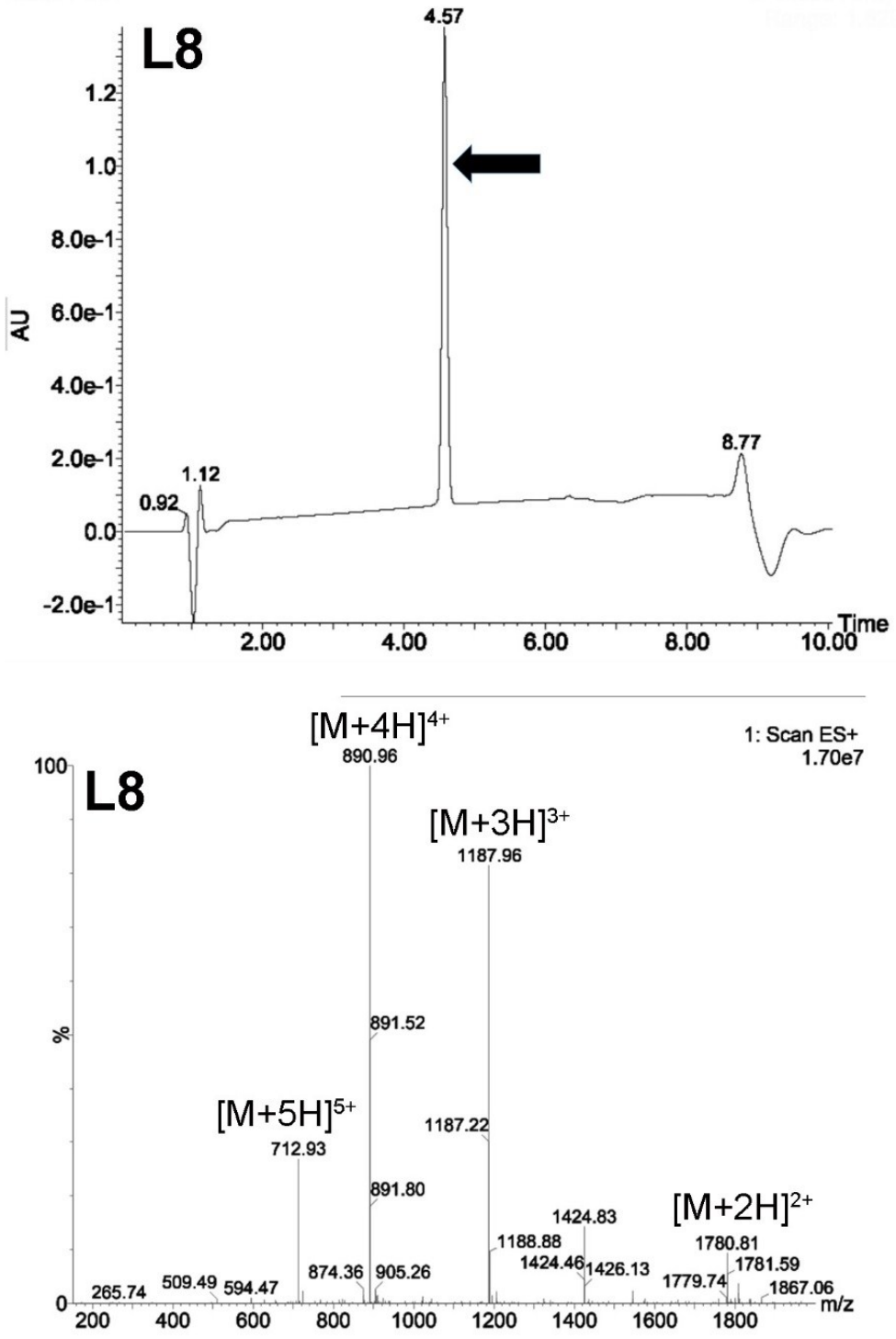

Fig. S1 Analytical HPLC traces (top) and ESI mass data (bottom) of **L8**.

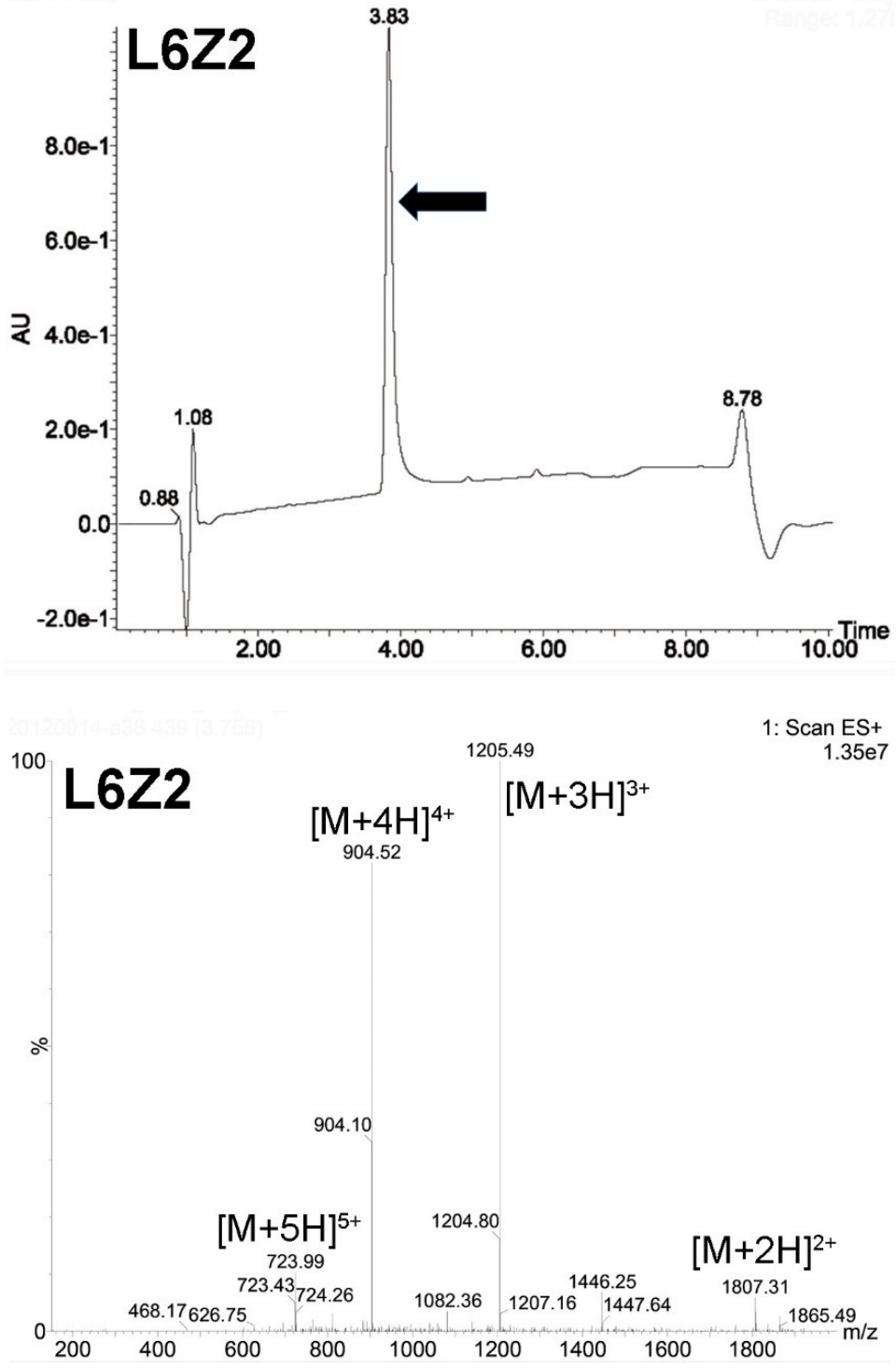

Fig. S2 Analytical HPLC traces (top) and ESI mass data (bottom) of **L6Z2**.

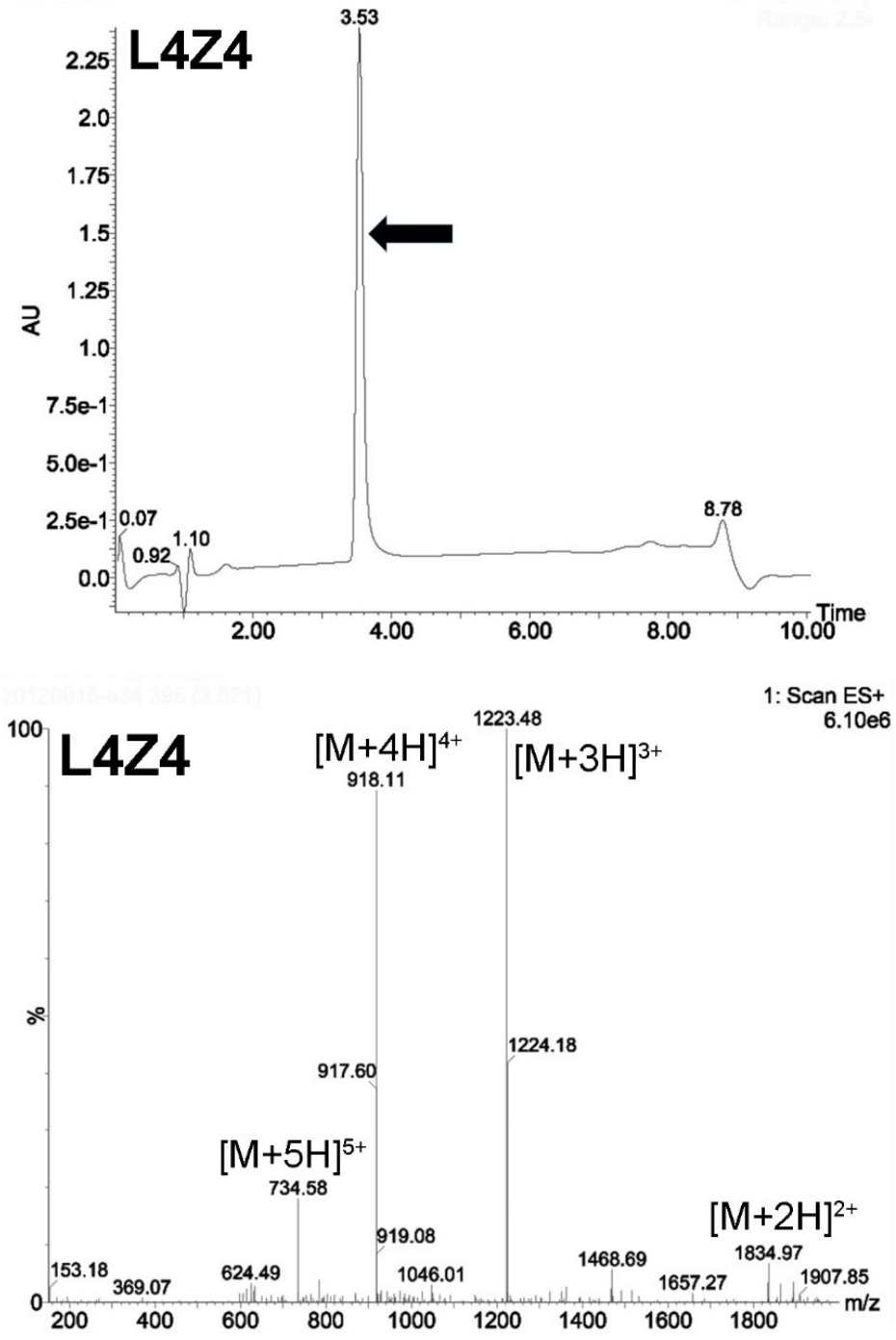

Fig. S3 Analytical HPLC traces (top) and ESI mass data (bottom) of **L4Z4**.

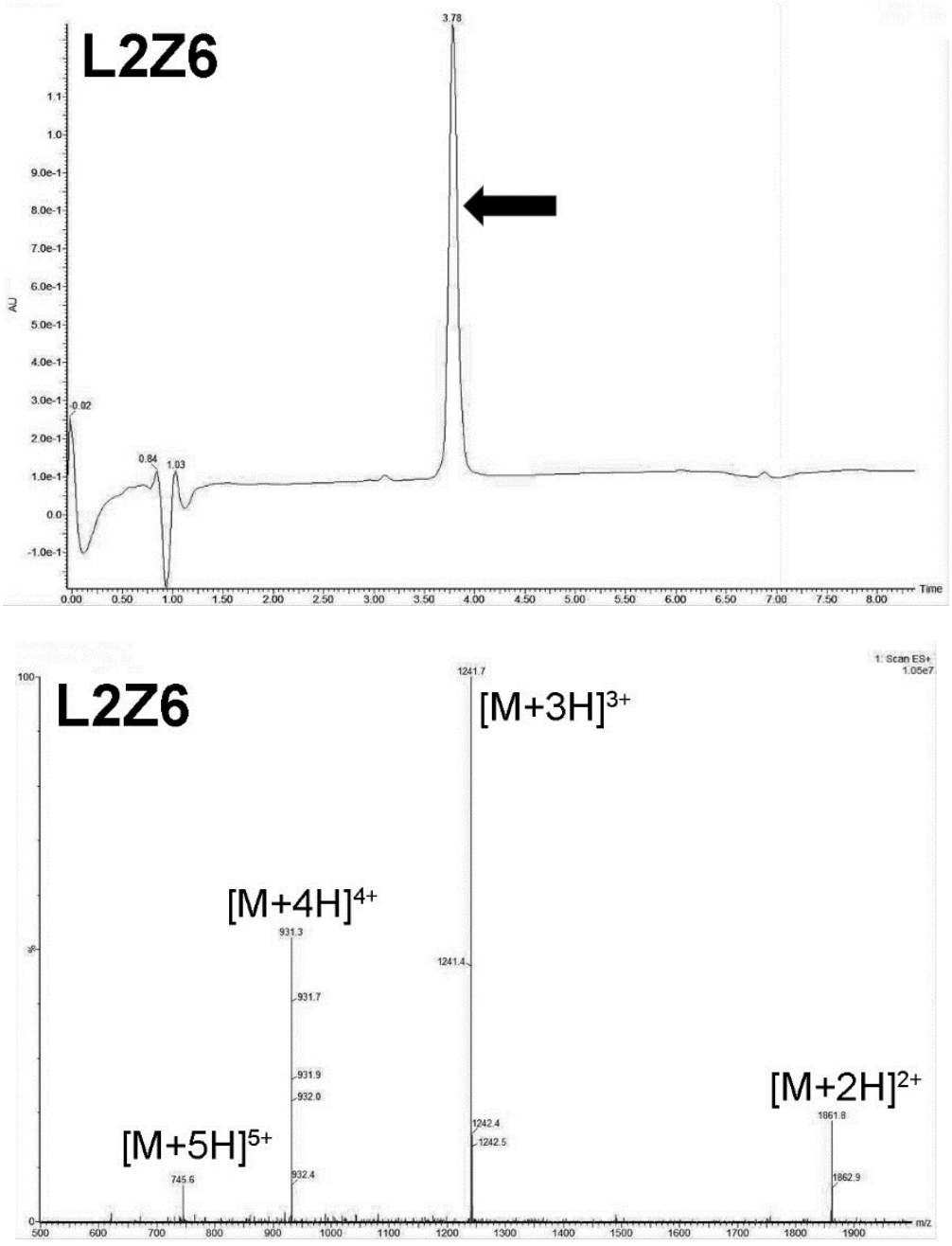

Fig. S4 Analytical HPLC traces (top) and ESI mass data (bottom) of **L2Z6.**

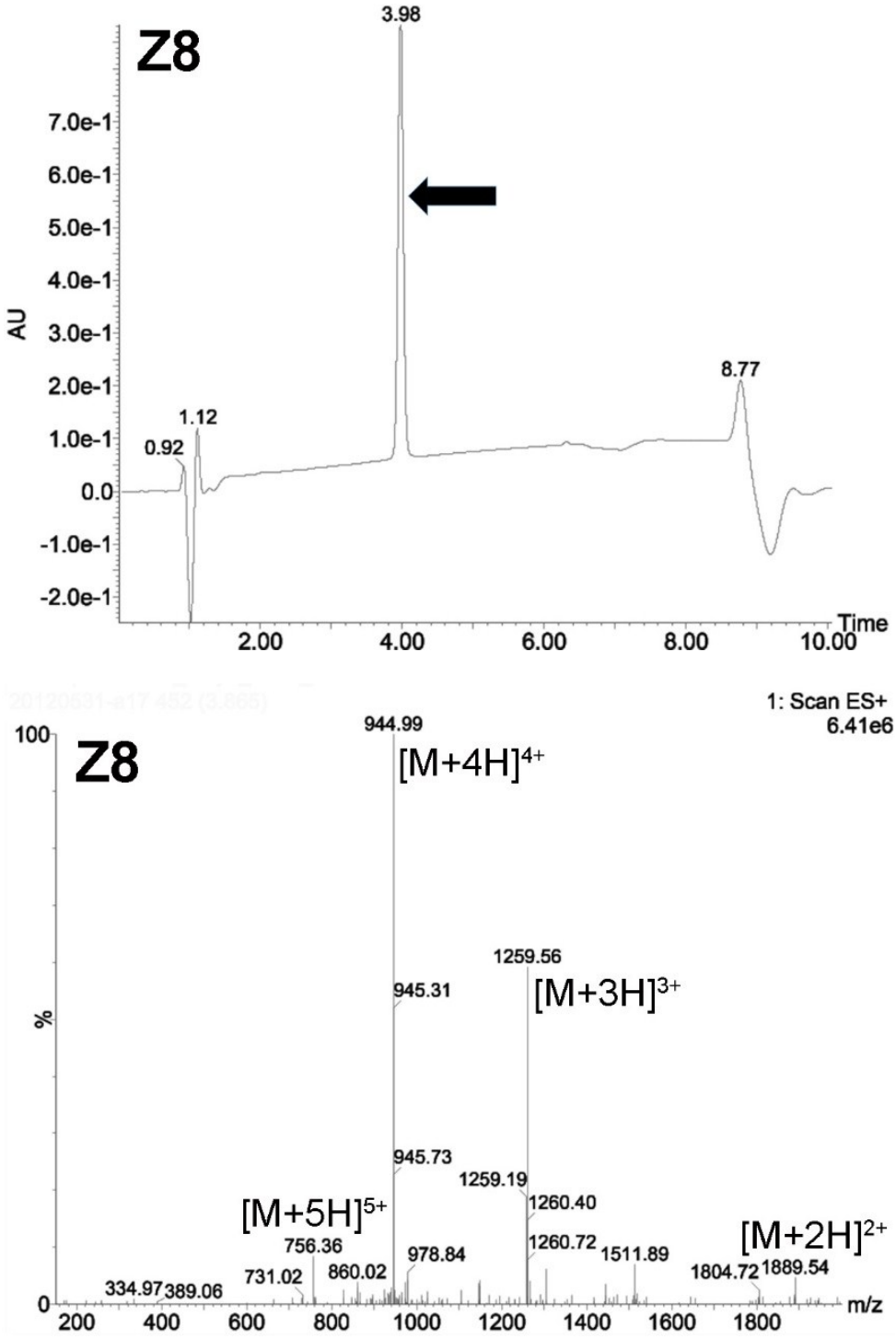

Fig. S5 Analytical HPLC traces (top) and ESI mass data (bottom) of **L8**.

Circular dichroism analysis. CD spectra were recorded on Jasco-815 circular dichroism spectrometer equipped with temperature controller. Concentrations of peptide solutions were determined by measuring tryptophan absorbance in 6 M Gdn·HCl (extinction coefficient 5690 $M^{-1} \cdot cm^{-1}$ at 280 nm).[S1] All sample solutions made in 10 mM phosphate (pH 7.40, 137 mM NaCl, 2.7 M KCl) were equilibrated for 24 h at 3 ºC before the measurements. A Quartz Cell of 1 mm path length was used. For wavelength scan, temperature was increased to the desired points at a rate of 1 °C/min and the sample solutions were equilibrated for 10 min before the measurements. Spectra were recorded from 260 to 190 mm. Spectra were baseline corrected before converting to mean residue ellipticities (MREs, [θ]) and MRE was calculated as follows;

$$[\theta] = \theta/(10 \cdot N \cdot c \cdot l)$$

θ represents the ellipticity in millidegrees, N the number of amino acid residues, c the molar concentration in $mol \cdot L^{-1}$, and l the cell path length in cm.[S2]

The α-helix was calculated based on an ellipticity value of 38,250 in MRE for a 100% helical 30-residue peptide derived from the equation 42,500·(1 3/(# residues)).[S3] Thermal denaturation curves were obtained at peptide concentrations of 25 (or 265) μM in 10 mM phosphate at pH 7.4 with 137 mM NaCl and 2.7 mM KCl. Molar ellipticity at 222 nm was measured as a function of temperature in steps of 0.5 °C in a range of 1 to 95 ºC at a heating rate of 30 °C/h. Data were collected over 8 s per point. The T_m's were determined from the points, at which 50% helicity are lost. To obtain fully soluble fractions from peptide emulsions, peptide solutions containing aggregates were centrifuged at 12,000 x g for 10 min separating it into clear supernatants and white pellets.

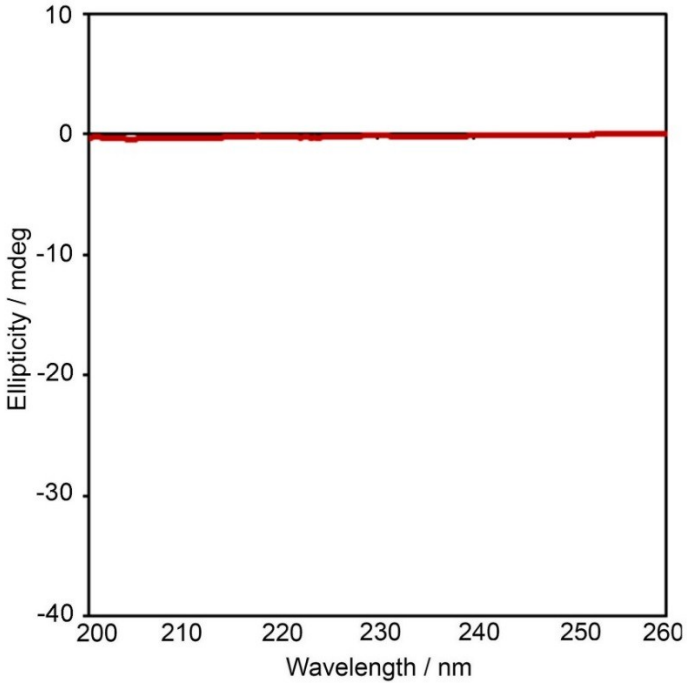

Fig. S6 CD spectra of azidobutyric acid at 25 °C (0.4 mM in PBS).

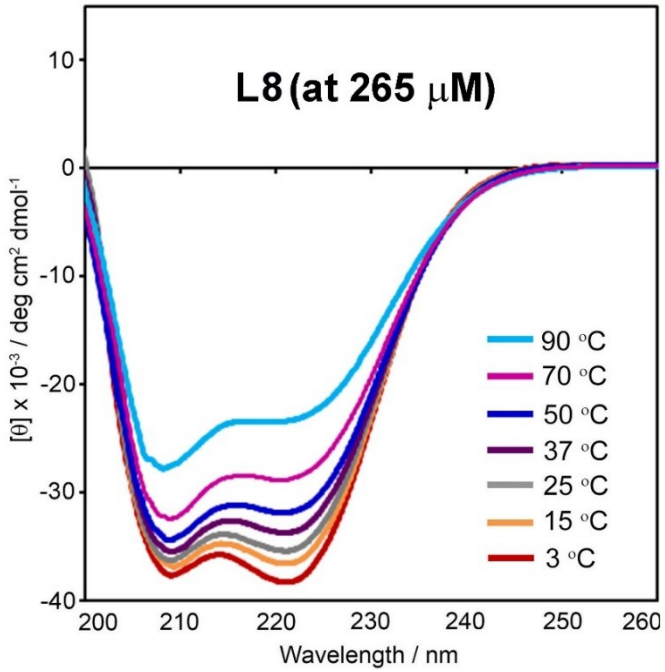

Fig. S7 CD spectra of **L8** at 3 °C (265 μM in PBS).

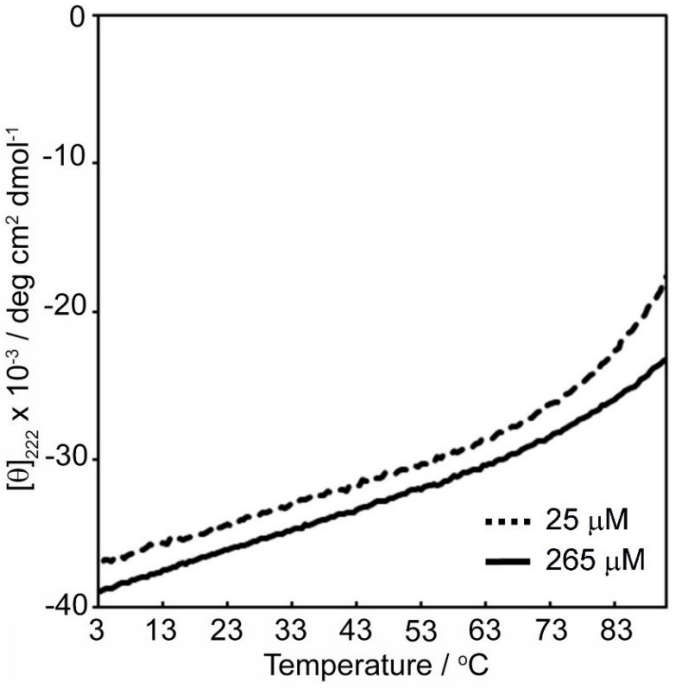

Fig. S8 Thermal denaturation profiles of **L8**.

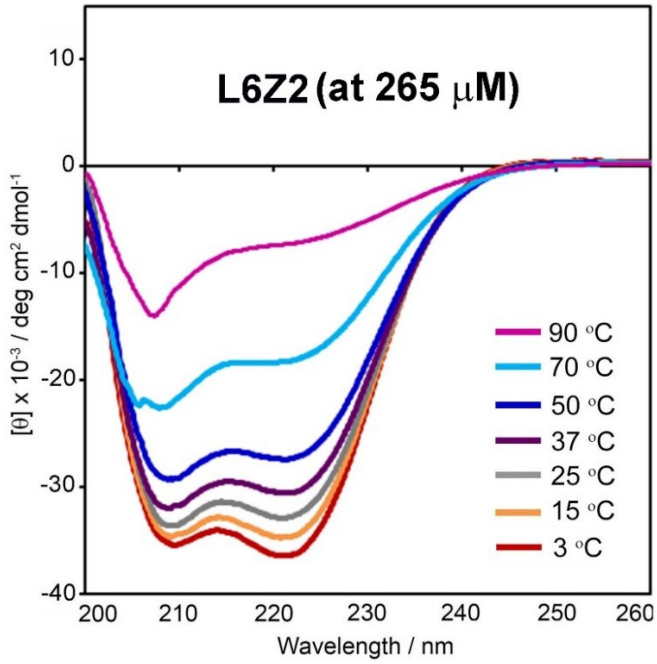

Fig. S9 CD spectra of **L6Z2** at 3 °C (265 μM in PBS).

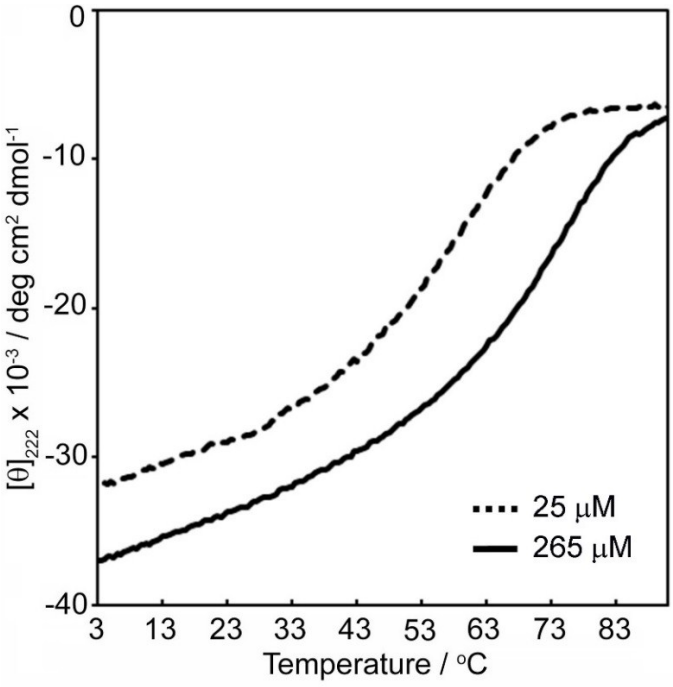

Fig. S10 Thermal denaturation profiles of **L6Z2**.

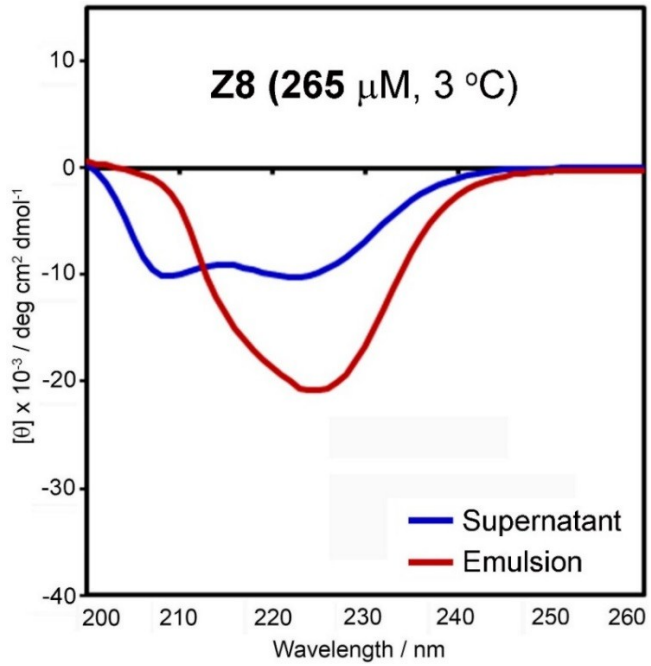

Fig. S11 CD spectra of **Z8** at 3 °C (265 μM in PBS).

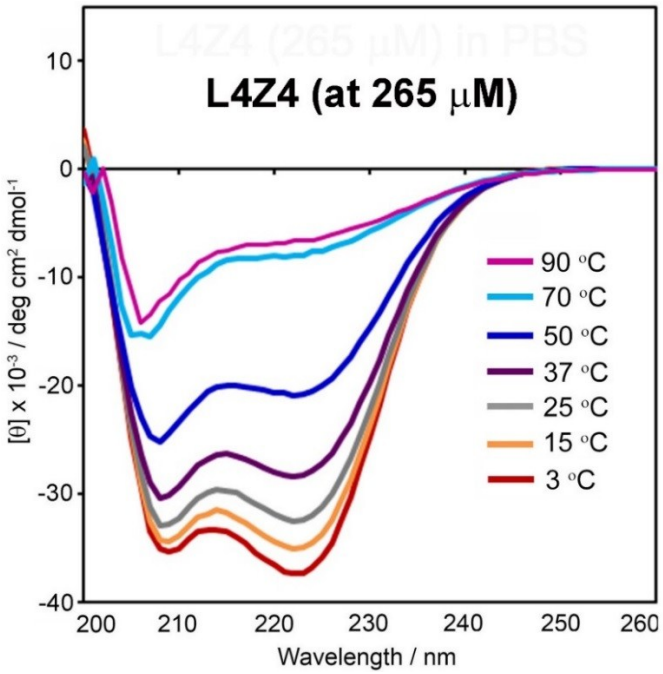

Fig. S12 CD spectra of **L4Z4** (265 μM in PBS).

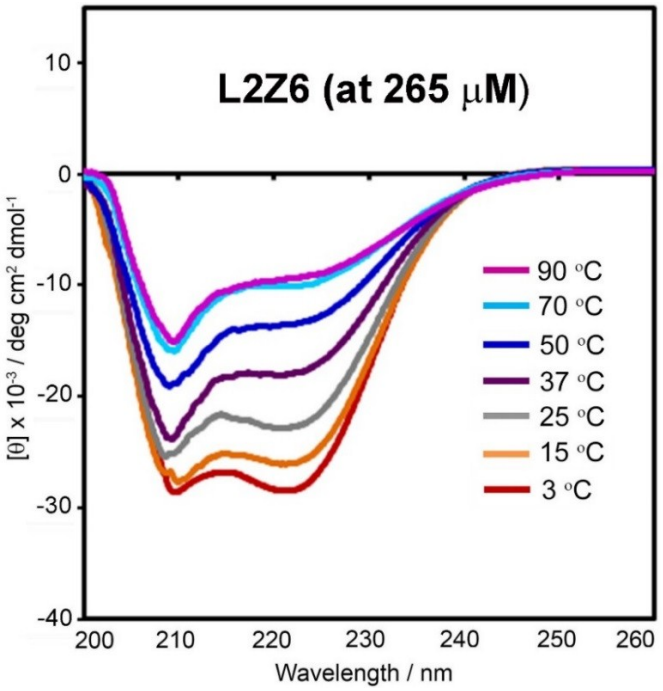

Fig. S13 CD spectra of **L2Z6** (265 μM in PBS).

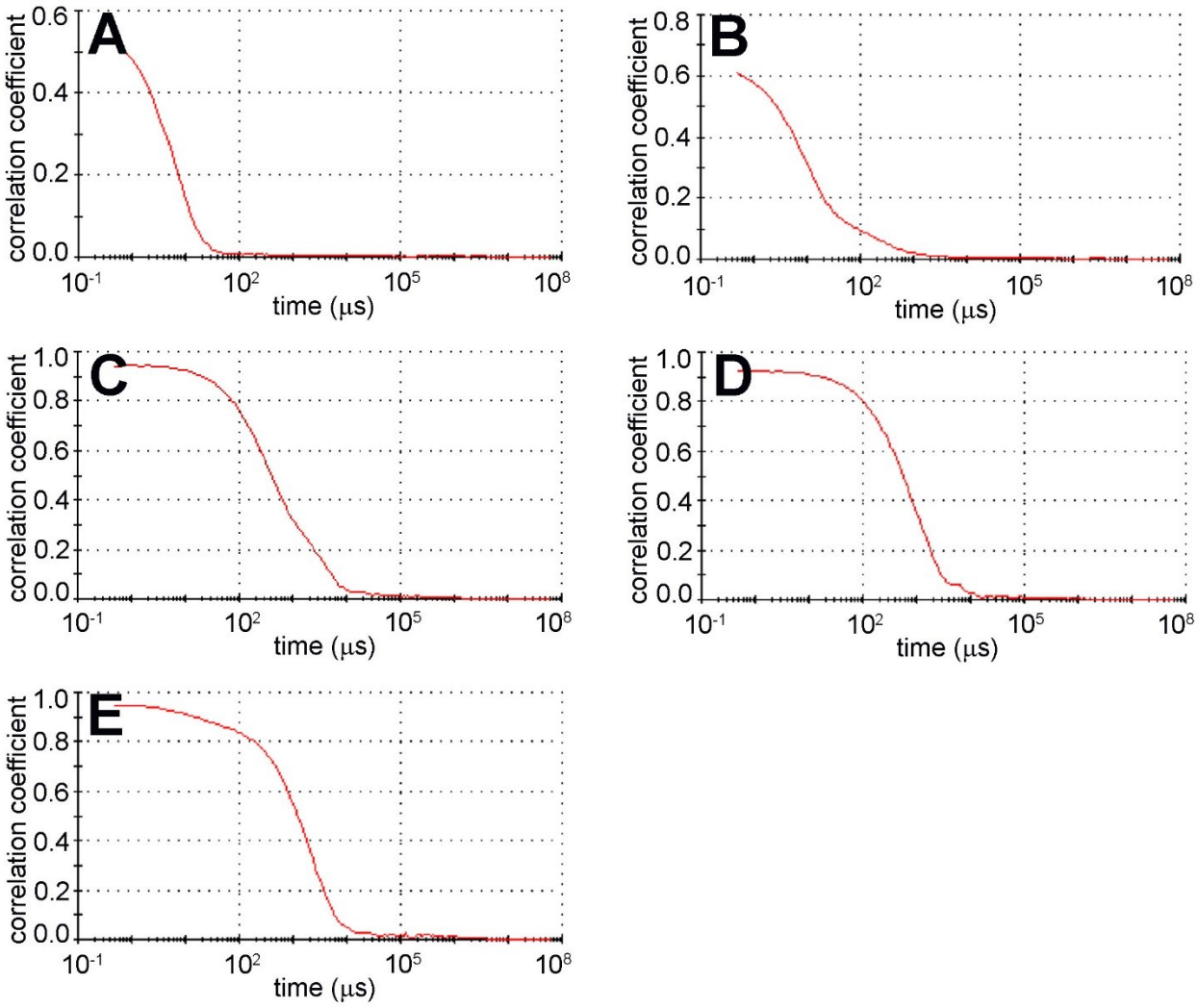

Fig. S14 DLS correlograms of (A) **L8** (3 °C, 7 days), (B) **L6Z2** (3 °C, 7 days), (C) **L4Z4** (3 °C, 7 days), (D) **L4Z4** (3 °C, 14 days), (E) **L4Z4** (3 °C, 30 days).

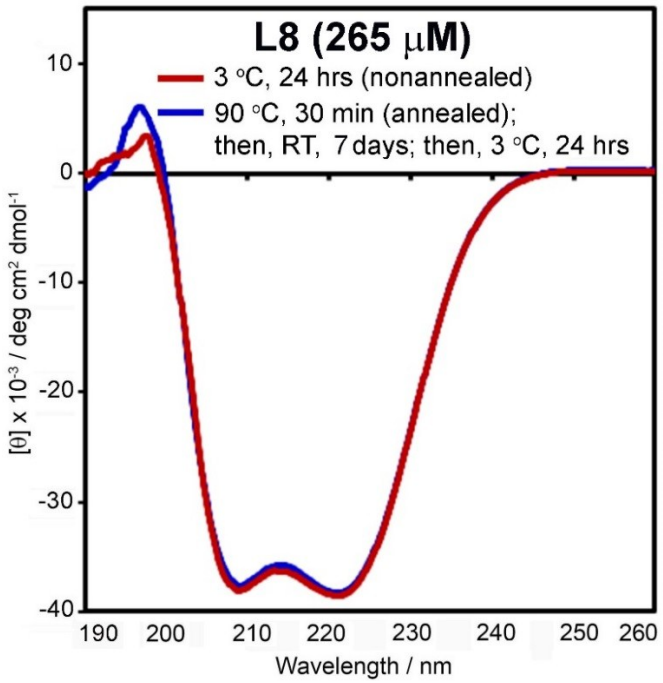

Fig. S15 CD spectra of **L8** before and after annealing at 3 °C (265 μM in PBS).

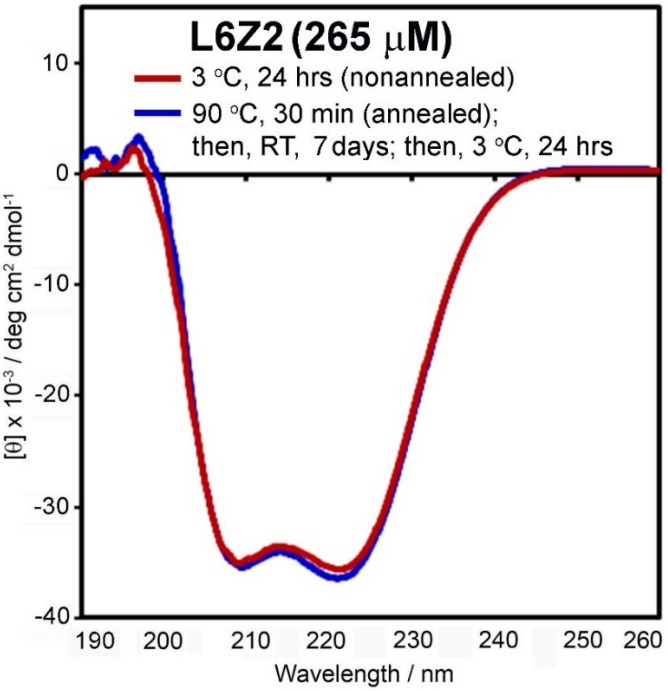

Fig. S16 CD spectra of **L6Z2** before and after annealing at 3 °C (265 μM in PBS).

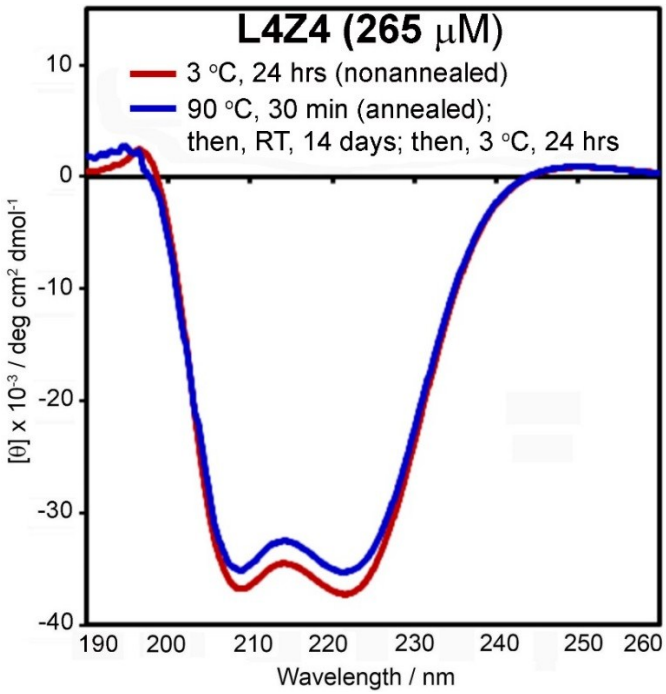

Fig. S17 CD spectra of **L4Z4** before and after annealing at 3 °C (265 μM in PBS).

Dynamic light scattering (DLS). All DLS measurements of particle size were performed at room temperature with a Malvern Instrument Zetasizer Nano ZS (Malvern Instruments, Westborough, MA, USA) equipped with a He-Ne laser (λ = 633 nm, max 4 mW) and operated at a scattering angle of 173°. 1 mL of peptide suspension was placed in a 12 mm polystyrene cuvette for all measurements. An automatic algorithm was employed to determine the number of scans required.

Labeling L4Z4 aggregates with fluorescein isothiocyanate (FITC). 265 μM of **L4Z4** solution (1 mL) was prepared in PBS by filtering 200 nm membrane and was kept at 3 °C for two weeks. To this solution containing L4Z4 aggregates, 10 μL FITC (1mg/ml) was added and the resulting mixture was gently shaken for 2 hours. Amicon® Ultra 10K device was used to remove

unconjugated dye and centrifuged the device 12,000 × g for approximately 5 minutes to remove unbounded dye (repeated five times). To recover the final/concentrated solute, the Amicon® Ultra filter device was placed upside down in a clean microcentrifuge tube and spun for 2 minutes at 1,000 × g to transfer the sample from the device to the tube.

Confocal microscopy. The samples were examined using a Zeiss LSM DUO laser scanning microscope (Carl Zeiss, Oberkochen, Germany). The digital photographs were processed with LSM Image Brower software. The Argon laser line with the wavelength of 488 nm was used as the excitation light source for green fluorescence signals. Signal pass through the filter of BP505/550 were collected to generate green fluorescence images. Some green fluorescence images were acquired by wide field fluorescence imaging. Signals produced through the Zeiss filter Set 09 (Ex: 450-490, Em: LP 515; Carl Zeiss, Oberkochen, Germany) were captured by Zeiss AxioCam HRm high resolution digital camera (Carl Zeiss, Oberkochen, Germany).

Transmission electron Microscopy (TEM). TEM experiments were performed on a FEI Tecnai G^2 F20 electron microscope operated at 200 kV. All TEM sampling was carried out at room temperature except for the sample in Figure 3C (For the sample in Figure 3C, sample solutions were cooled to 4 °C and TEM sampling was carried out in a cold room (4 °C)). TEM sample was prepared by first dispensing a drop of peptide solution onto a 400 Mesh Cu grid pre-coated with holey thin carbon film procured from Pacific Grid-Tech. 1 min later, solution was wick off using a filter paper. Next, a drop of distilled water was dispensed onto the grid, wick off immediately. This step was repeated one more time. Finally, a drop of 2 wt% phosphotungstenic acid was

dispensed onto the grid and allowed to stand for one minute before wicking the solution off using filter paper.

Fourier transform infrared (FT-IR) spectroscopy. 0.5 mL of Peptide solution containing aggregates (total peptide concentration: 265 μM) was centrifuged at 12,000 x g for 10 min separating it into a clear supernatant and white pellet. Clear supernatant was frozen at -78 °C and lyophilized. The white pellet was further rinsed with 50 μl of deionized (DI) water, dispersed, and centrifuged at 12,000 x g for 1 min (repeated three times). The pellet was then redispersed in 50 μl of DI water, frozen at -78 °C, and lyophilized. FT-IR measurements of peptide samples were carried out using a Perkin Elmer FT-IR Spectrum 100 attached with a PIKE Miracle Single Reflection ATR accessory between 2000 and 1000 cm^{-1} at a spectral resolution of 4 cm^{-1}, and the number of scans was 4. Samples were placed on a germanium stage and pressed before every measurement.

Scanning Electron Microscopy (SEM). Lyophilized peptide pellets described in the FT-IR section were placed on a carbon tape which is attached to a sample mount. Samples were then sputter-coated with platinum using the JFC-1600 High resolution sputter coater. The SEM images were obtained on JEOL JSM-7400F at an accelerating voltage of 5 kV and an emission current of 10 uA.

References

S1. Gill, S. C.; Von Hippel, P. H. *Anal. Biochem.* **1989**, *182*, 319-326.

S2. Srinivasan, M.; Lu, D.; R. Eri, Brand, D. D.; Haque, A.; Blum, J. S., *J. Biol. Chem.* **2005,** *280*, 10149-10155.

S3. Zimenkov, K.Y.; Dublin, S. N.; Ni, R.; Tu, R. S.; Breedveld, V.; Apkarian, R. P.; Conticello, V. P., *J. Am. Chem. Soc.* **2006,** *18*, 6770-6771