Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

## Unique behavior of α-helix in bending deformation

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Table of Contents

## Materials and Methods

***Materials.*** gBlock gene fragments for the cloning of target genes were obtained from Integrated DNA Technologies (USA). The pTWIN2 vector was purchased from New England Biolabs (USA). *E. coli* strains for cloning and protein expression were obtained from New England Biolabs (USA) and Novagen (USA). 2-Cholorotrityl chloride resin and Fmoc-amino acids were purchased from Novabiochem (Germany) and AAPPTec (USA), respectively. All other general chemicals for solid-phase peptide synthesis (SPPS) were obtained from Thermo Fisher Scientific (USA) and Samchun (Korea).

***Intein-mediated cyclization of bacterially expressed peptides.*** Target genes for **C64** and **C118** were cloned into the Sap I sites of the pTWIN2 vector for intein-mediated cyclization. The precursors of **C64** and **C118** were expressed in *E. coli* strains lysY and Rosetta(DE3), respectively. For **C64**, transformed lysY cells were grown in Luria-Bertani (LB) medium containing ampicillin (100 μg/mL) at 37 °C. When the $OD_{600}$ reached 0.5, 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the cells to induce the protein expression for 8 h at 25 °C. For **C118**, transformed Rosetta(DE3) cells were grown in LB medium containing ampicillin (100 μg/mL) at 37 °C. When the $OD_{600}$ reached 0.4, 0.5 mM IPTG was added to the cells for 5 h at 37 °C. After harvesting, the cells were resuspended in lysis buffer (20 mM Na-HEPES, pH 8.5, 500 mM NaCl, 1 mM EDTA), and the lysate was centrifuged at 10,000 ×g for 1 h. Then, the clarified cell extract in supernatant was applied to a chitin column at a flow rate of 1 mL/min. Unbound proteins were washed off from the column using 100 mL of lysis buffer. On-column cleavage of intein1 was induced by equilibrating the chitin beads in a buffer containing 20 mM Na-HEPES, pH 6.5, 500 mM NaCl, and 1 mM EDTA. The column was incubated overnight at room

temperature to allow the cleavage reaction to proceed. After washing the column to remove impurities, both on-column cleavage of intein2 and the peptide cyclization were induced by equilibrating the chitin beads in a buffer containing 20 mM HEPES, pH 8.5, 500 mM NaCl, 50 mM 2-mercaptoethanesulfonic acid, and 1 mM EDTA. The column was incubated overnight at 4 °C. Then, the cyclic and linear peptides were eluted from the chitin column. The peptides were purified by reverse-phase high-performance liquid chromatography (RP-HPLC) using a water/acetonitrile gradient containing 0.1% trifluoroacetic acid (TFA). The molecular weight was characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; LRF20, Bruker Daltonics, Germany).

***SPPS and cyclization.*** Peptides were elongated on 2-chlorotrityl chloride (CTC) resin by following a standard Fmoc SPPS protocol. To attach the first amino acid to the CTC resin, the resin was treated with 5 equiv. of the first amino acid and 10 equiv. of diisopropylethylamine (DIPEA) dissolved in 2 mL of methylene chloride (MC) in a glass vial, and the mixture was shaken for 3 h. Further elongation was performed in a solid-phase extraction tube using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxide hexafluorophosphate (HATU) as a coupling reagent. Cyclic peptides were synthesized by the head-to-tail cyclization of protected linear peptide fragments. One syringe was filled with the protected linear peptide fragment (20 μmol, 1 equiv.) and DIPEA (16 equiv.) in 20 mL of dimethylformamide (DMF), while the other syringe was filled with HATU (20 μmol, 1 equiv.) in 20 mL of DMF. The two solutions were simultaneously added at a rate of 0.05 mL/min to a stirred solution of 20 mL of DMF in a round-bottom flask using a syringe pump. Final deprotection was performed in a cleavage cocktail (TFA: triisopropylsilane: water = 95: 2.5: 2.5), and the peptide was triturated with *tert*-

butyl methyl ether (3×). The peptides were purified by RP-HPLC using a water/acetonitrile gradient containing 0.1% TFA. The molecular weight was determined by MALDI-TOF MS.

***Circular dichroism (CD) spectroscopy.*** CD spectra were recorded using a Chirascan CD spectrometer equipped with a Peltier temperature controller (Applied Photophysics, UK). The spectra were obtained from 190 nm to 260 nm using a 2-mm pathlength cuvette. In the temperature ramping experiment, the temperature was varied from 10 ℃ to 80 ℃, and the measurements were repeated more than twice. The peptide concentration was typically 30 μM. Most CD studies were performed in distilled water.

***Atomic force microscopy (AFM).*** Two microlitres of the sample at a concentration of 0.5 μM was loaded on a disc-shaped mica surface. Immediately after loading, the mica was mounted on a spin coater (ACE-200, Dong-Ah Trade Corp, Korea). The spin speed was ramped to 3000 rpm over 5 s, and the speed was maintained for 30 s. The mica was dried at room temperature for approximately 10 min. AFM scans were conducted in tapping mode with a Park NX10 instrument (Park Systems, Korea). The scanning was performed at a scan rate of 0.3 Hz and a Z-gain of 1. The images were processed using XEN software.

***Fourier transform infrared (FTIR).*** Ten microlitres of the peptide sample (10 μM) was cast from the solution onto a ZnSe window and dried at room temperature. The solution casting processes were repeated five times. The FT-IR spectra were recorded using a Vertex 70 spectrometer (Bruker, Germany). The typical scanning range was 1800 $cm^{-1}$ to 1500 $cm^{-1}$. The spectra were deconvoluted using Origin software.

# A

| Name | Sequence |
|---|---|
| **L20** | EAAAKEAAARETFSDLWKLL |
| **L25** | EAAAREAAAKEAAARETFSDLWKLL |
| **L26** | KEAAAREAAAKEAAARETFSDLWKLL |
| **L27** | AKEAAAREAAAKEAAARETFSDLWKLL |
| **L28** | AAKEAAAREAAAKEAAARETFSDLWKLL |
| **L29** | AAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L30** | EAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L31** | REAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L32** | AREAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L33** | AAREAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L34** | AAAREAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L35** | EAAAREAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L40** | EAAAKEAAAREAAAKEAAAREAAAKEAAARETFSDLWKLL |
| **L64** | CRAAEAAAKEAAAREAAAKEAAARETFSDLWKLLEAAAKEAAAREAAAKEAAAREAAAKAAMRM |
| **L118** | CRAAEAAAKEAAAREAAAKEAAARETFSDLWKLLEAAAKEAAAREATAKEAAAREAAAKDARAKEAAA REAAAKEAAAREAAAKARADEASAKEAAAREAAAKEAAAREAAAKAAMRM |

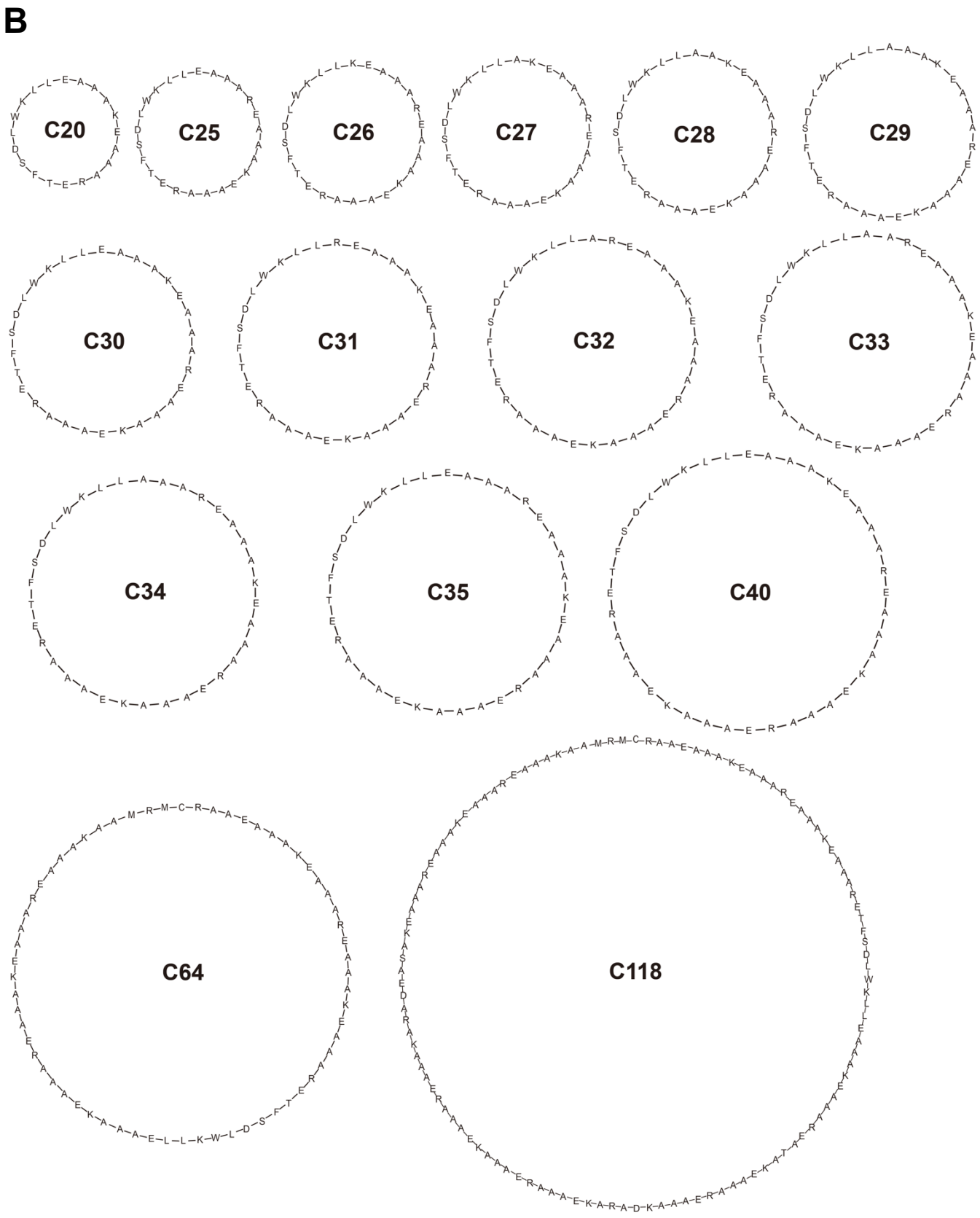
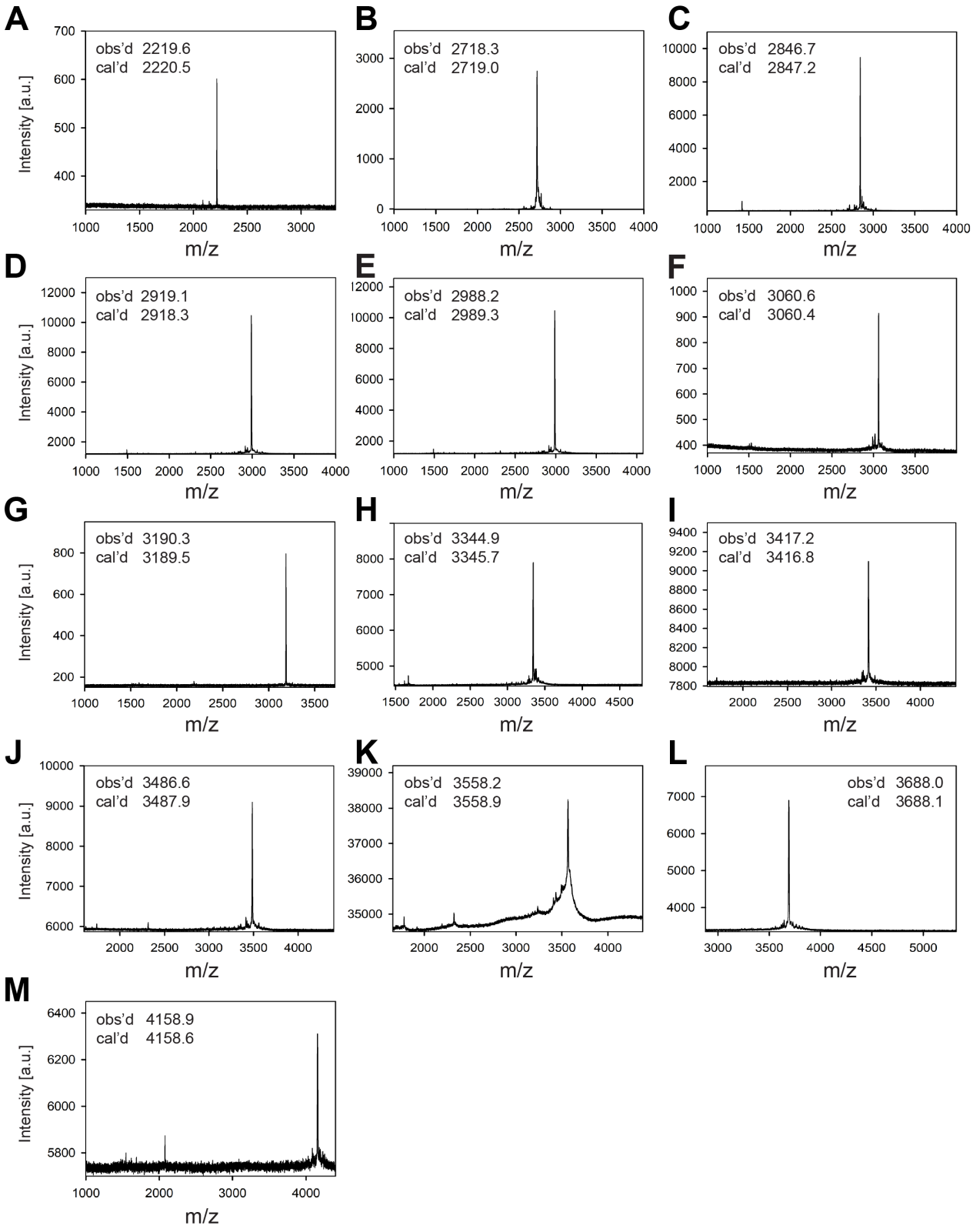



**Figure S1.** Sequences (A) linear peptides. (B) cyclic peptides.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cal'd 2220.5
B
obs'd 2718.3
cal'd 2719.0
C
obs'd 2846.7
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D
obs'd 2919.1
cal'd 2918.3
E
obs'd 2988.2
cal'd 2989.3
F
obs'd 3060.6
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G
obs'd 3190.3
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H
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I
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J
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K
obs'd 3558.2
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L
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M
obs'd 4158.9
cal'd 4158.6
Intensity [a.u.]
m/z

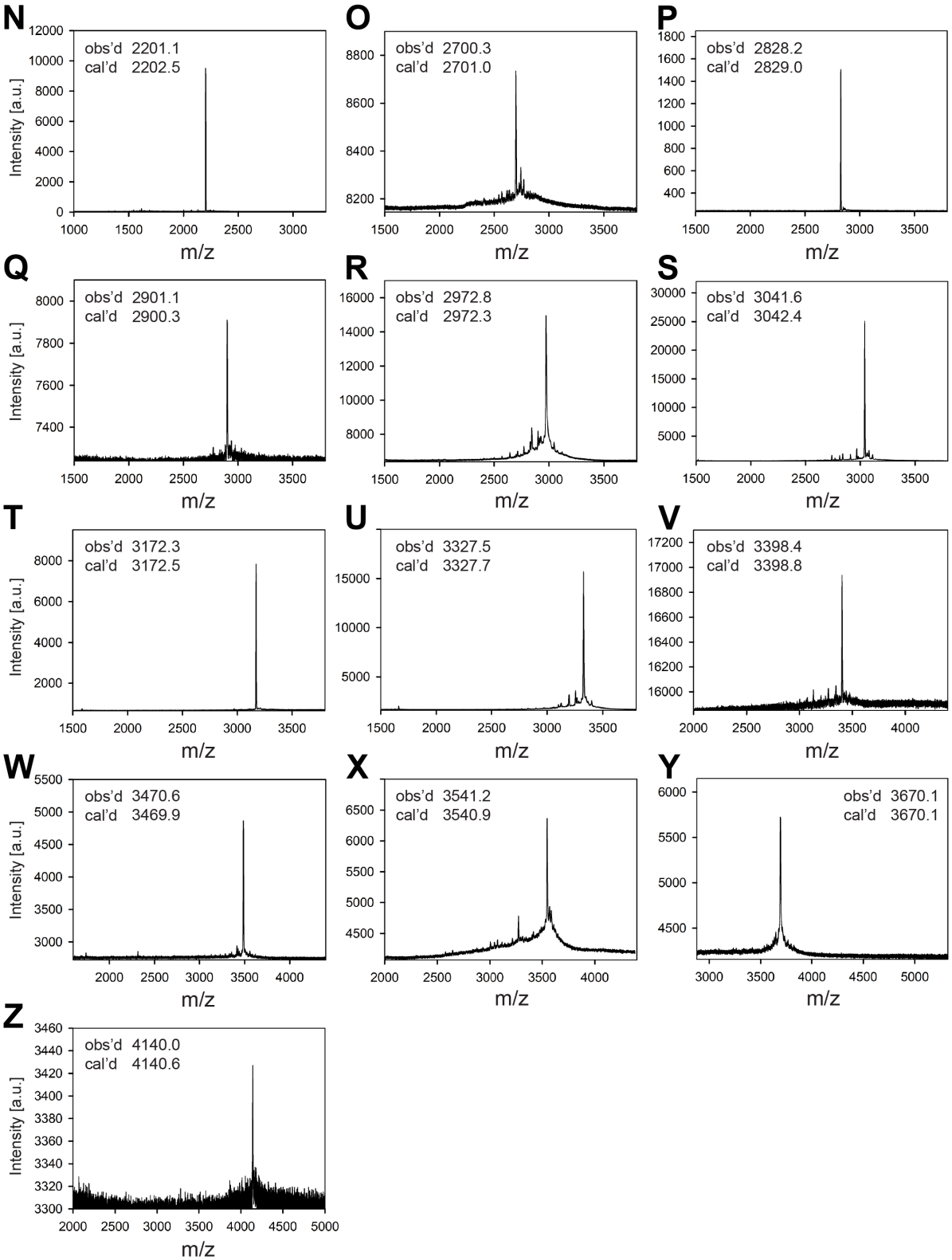


**Figure S2**. MALDI-TOF MS spectra. (A) **L20**. (B) **L25**. (C) **L26**. (D) **L27**. (E) **L28**. (F) **L29**. (G) **L30**. (H) **L31**. (I) **L32**. (J) **L33**. (K) **L34**. (L) **L35**. (M) **L40**. (N) **C20**. (O) **C25**. (P) **C26**. (Q) **C27**. (R) **C28**. (S) **C29**. (T) **C30**. (U) **C31**. (V) **C32**. (W) **C33**. (X) **C34**. (Y) **C35**. (Z) **C40**.

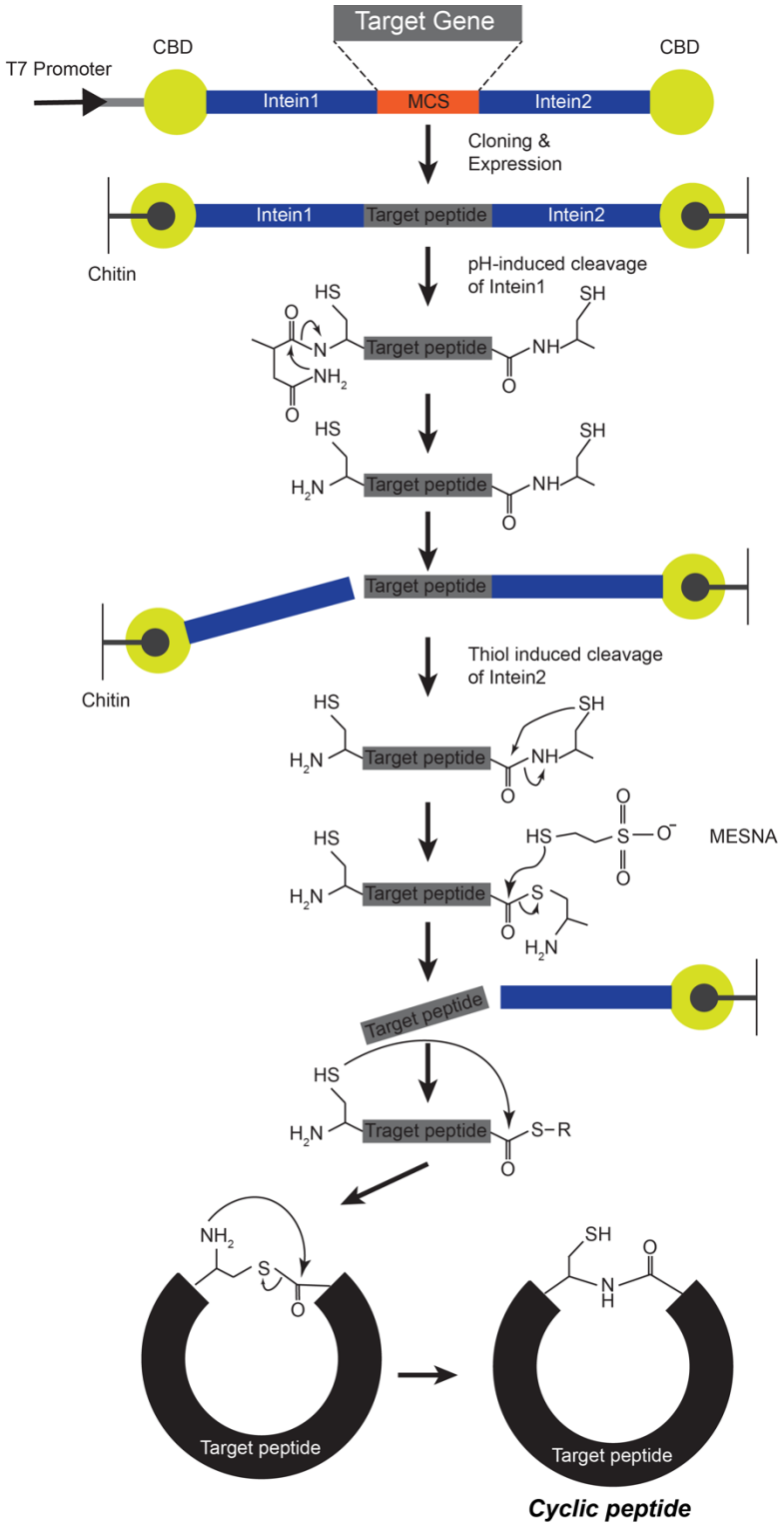


**Figure S3**. Mechanism of intein-mediated peptide cyclization. Extremely large cyclic peptides (**C64** and **C118**) were prepared using this method.

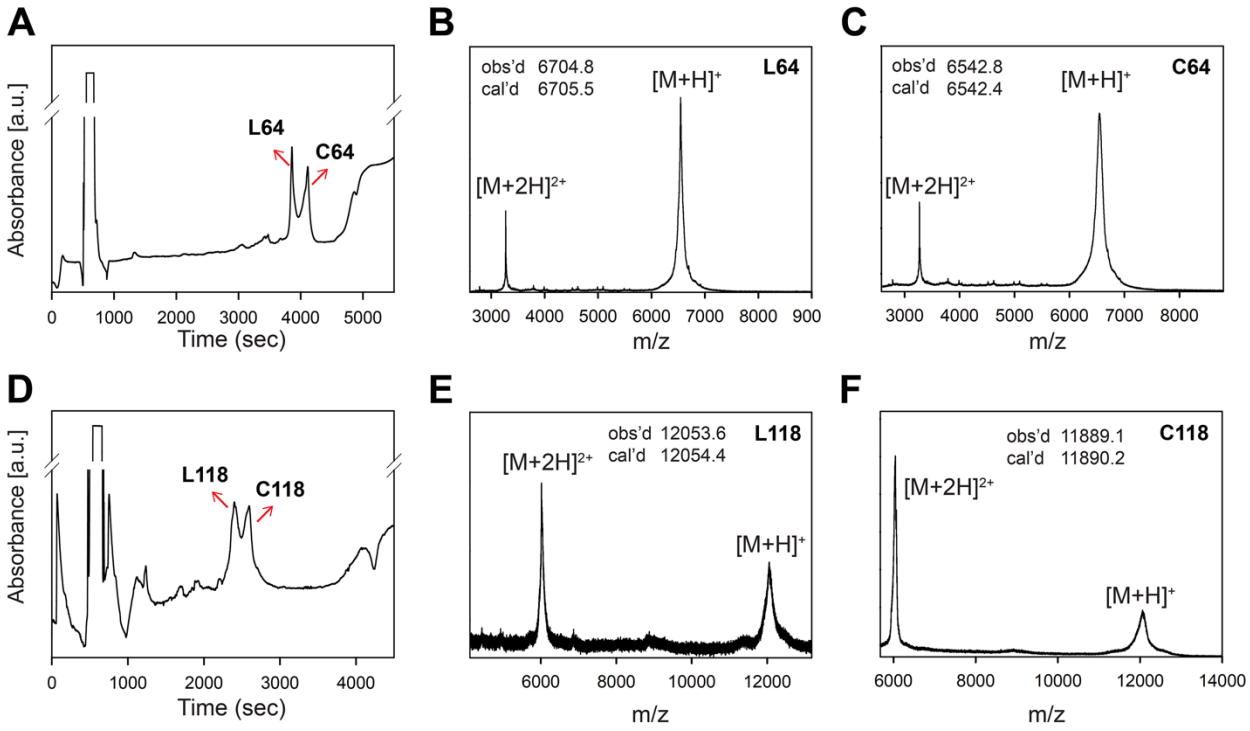


**Figure S4**. Purification and characterization of linear and cyclic peptides by intein-mediated ligation of bacterially expressed peptides. (A) HPLC chromatogram. MALDI-TOF MS spectra of (B) **L64** and (C) **C64**. (D) HPLC chromatogram. MALDI-TOF MS spectra of (E) **L118** and (F) **C118**.

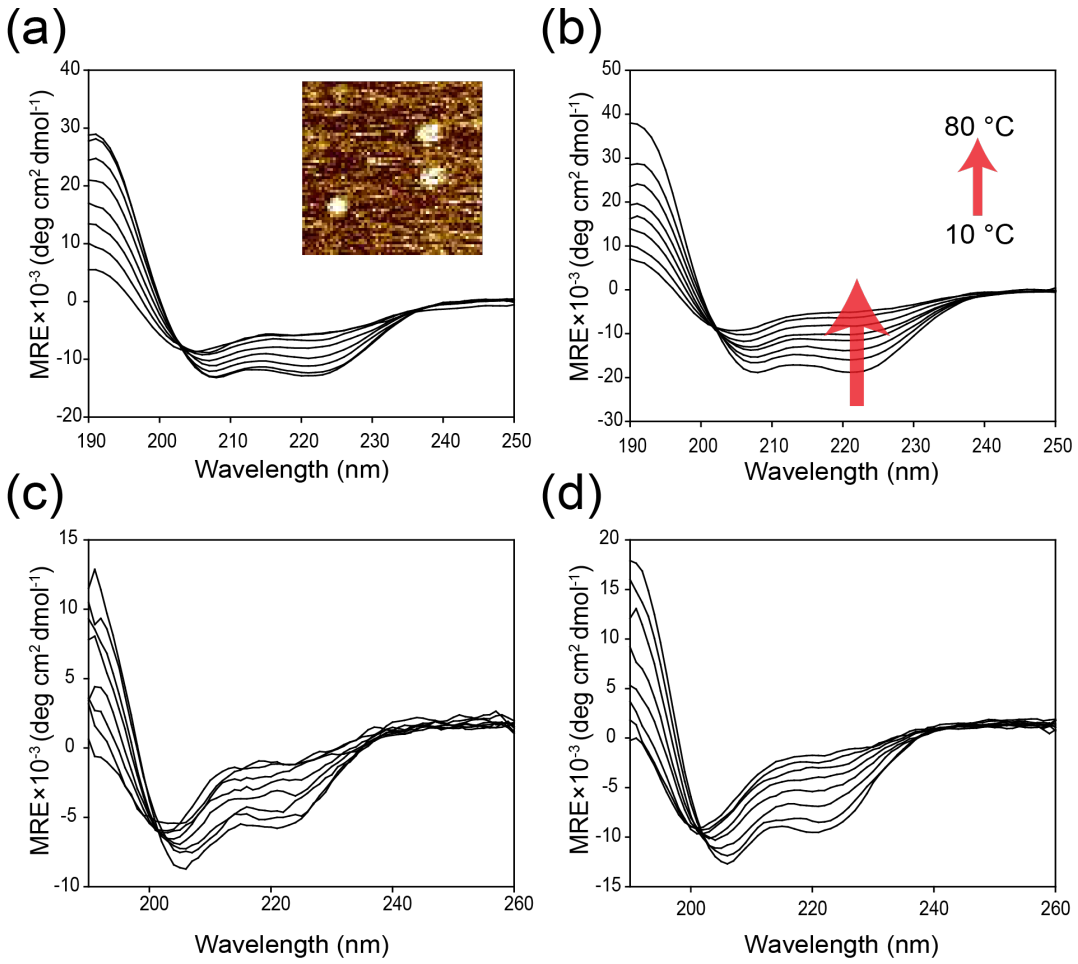


**Figure S5**. CD spectra of (A) **C64**, (b) **L64**, (c) **C40**, and (d) **L40**. An inset in (A): An AFM image of **C64**.

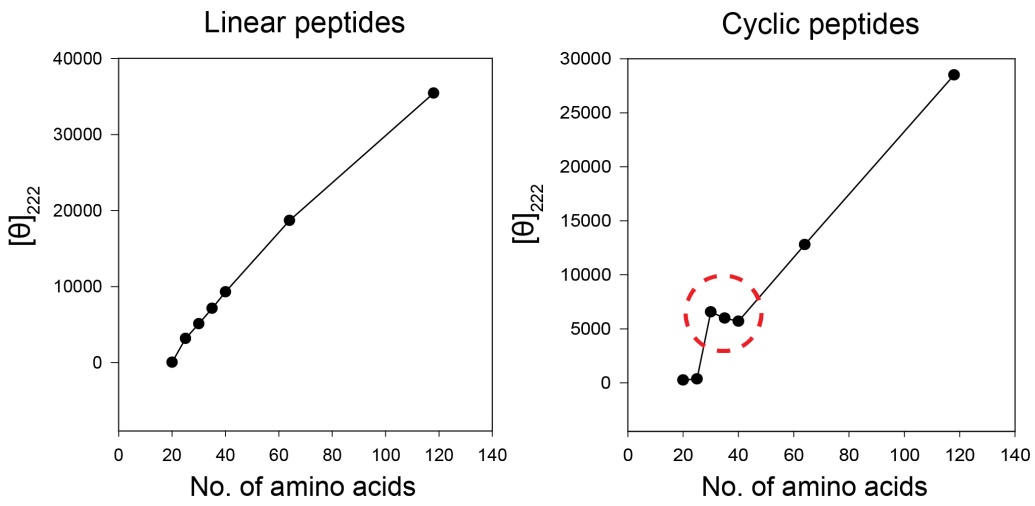


**Figure S6**. A break in continuity for cyclic peptides. A plot of $[\theta]_{222}$ as a function of peptide size.

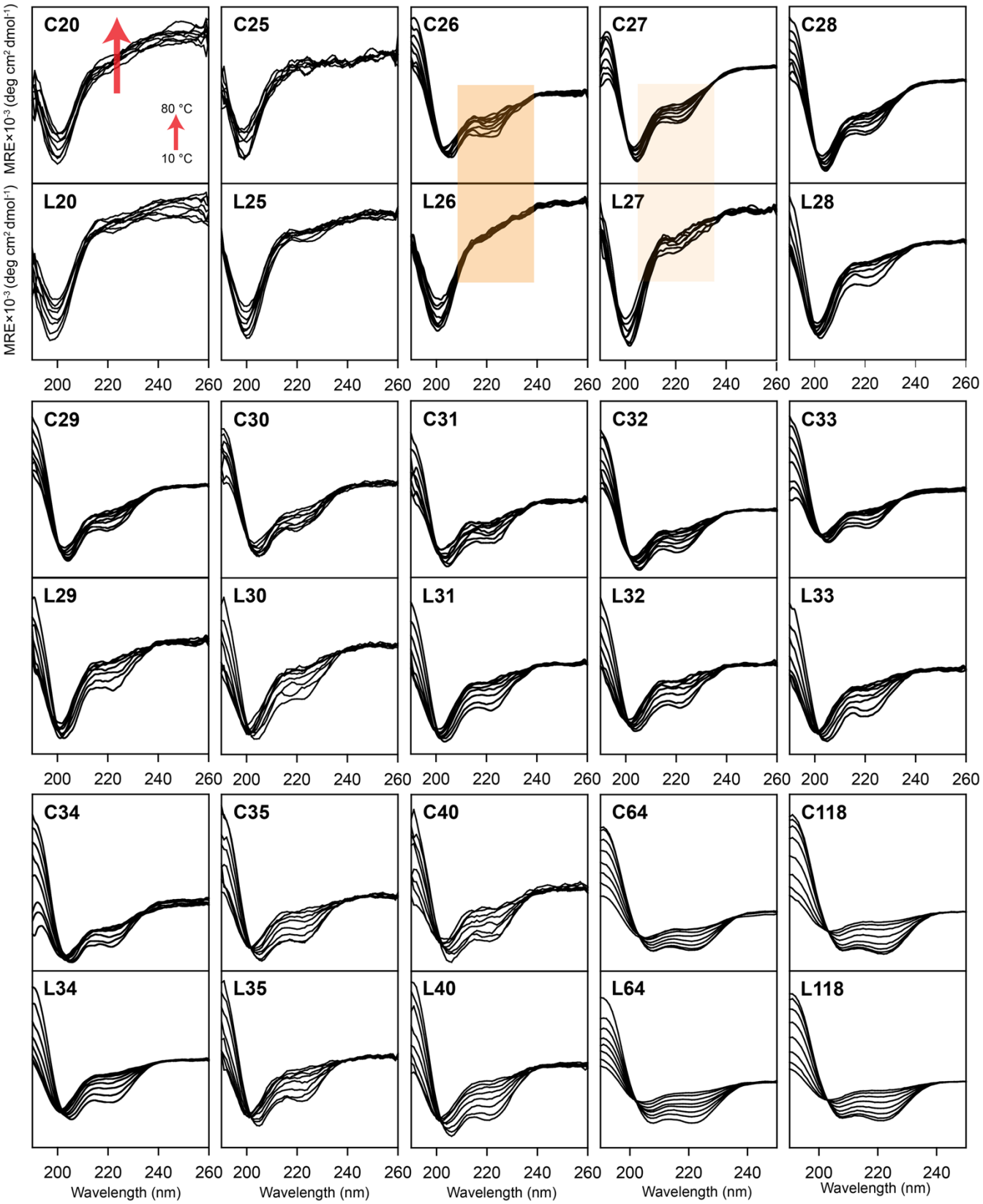


**Figure S7.** CD spectra of cyclic and linear peptides.

**Table S1.** Quantitative description of thermal behaviours as determined from the CD data

| $[\theta]_{222/208}$ | Cyclic | | | Linear | | |
|---|---|---|---|---|---|---|
| **Length** | **10 °C** | **80 °C** | **$\Delta[\theta]_{222/208}$[a]** | **10 °C** | **80 °C** | **$\Delta[\theta]_{222/208}$** |
| 25-mer | *n.d.*[b] | *n.d.* | *n.d.* | *n.d.* | *n.d.* | *n.d.* |
| 26-mer | 0.7366 | 0.4663 | 0.2703 | *n.d.* | *n.d.* | *n.d.* |
| 27-mer | 0.6796 | 0.5989 | 0.0807 | 0.4323 | 0.3258 | 0.1065 |
| 28-mer | 0.6888 | 0.5836 | 0.1052 | 0.6146 | 0.4471 | 0.1675 |
| 29-mer | 0.7084 | 0.551 | 0.1574 | 0.6439 | 0.3593 | 0.2846 |
| 30-mer | 0.6307 | 0.4364 | 0.1943 | 0.6049 | 0.2404 | 0.3645 |
| 31-mer | 0.7342 | 0.5285 | 0.2057 | 0.7003 | 0.4137 | 0.2866 |
| 32-mer | 0.7642 | 0.4877 | 0.2765 | 0.7291 | 0.4451 | 0.284 |
| 33-mer | 0.7888 | 0.5386 | 0.2502 | 0.7198 | 0.4665 | 0.2533 |
| 34-mer | 0.7969 | 0.5782 | 0.2187 | 0.7789 | 0.4517 | 0.3272 |
| 35-mer | 0.761 | 0.3439 | 0.4171 | 0.7487 | 0.3402 | 0.4085 |
| 40-mer | 0.7187 | 0.3231 | 0.3956 | 0.7919 | 0.3144 | 0.4775 |
| 64-mer | 0.9749 | 0.7058 | 0.2691 | 0.9945 | 0.599 | 0.3955 |
| 118-mer | 1.091 | 0.6035 | 0.4875 | 1.0706 | 0.5885 | 0.4821 |

[a] $\Delta[\theta]_{222/208} = [\theta]_{222/208}$ at 10 °C – $[\theta]_{222/208}$ at 80 °C
[b] *n.d.* = not determined (because the helicity of the peptides was too small to give meaningful interpretation)

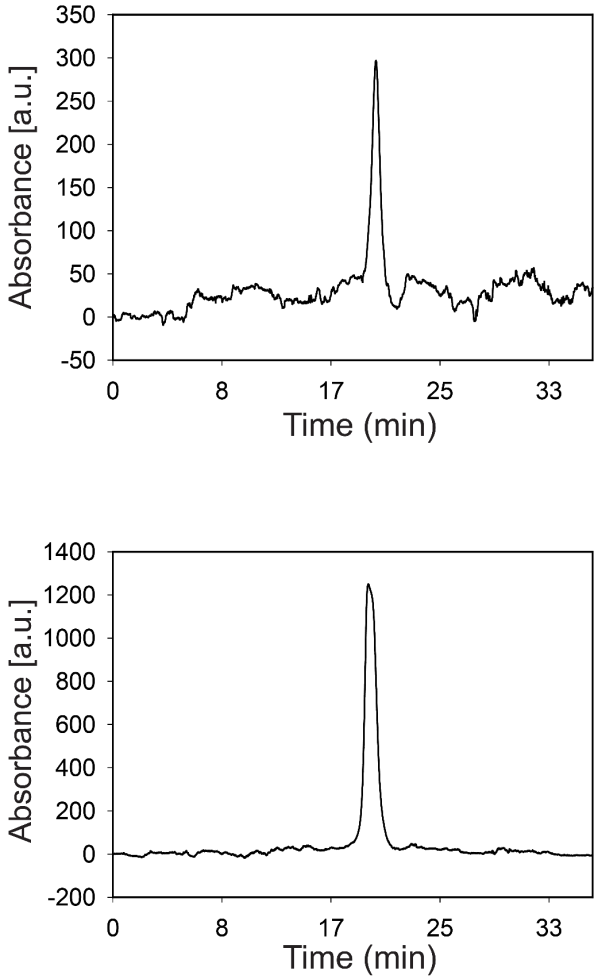


**Figure S8.** Size exclusion chromatography data for **C26** (top) and **L26** (bottom).