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Supporting Information

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Supporting Information

Stabilization of α-Helix by β-Sheet-mediated Self-Assembly of Macrocyclic Peptide

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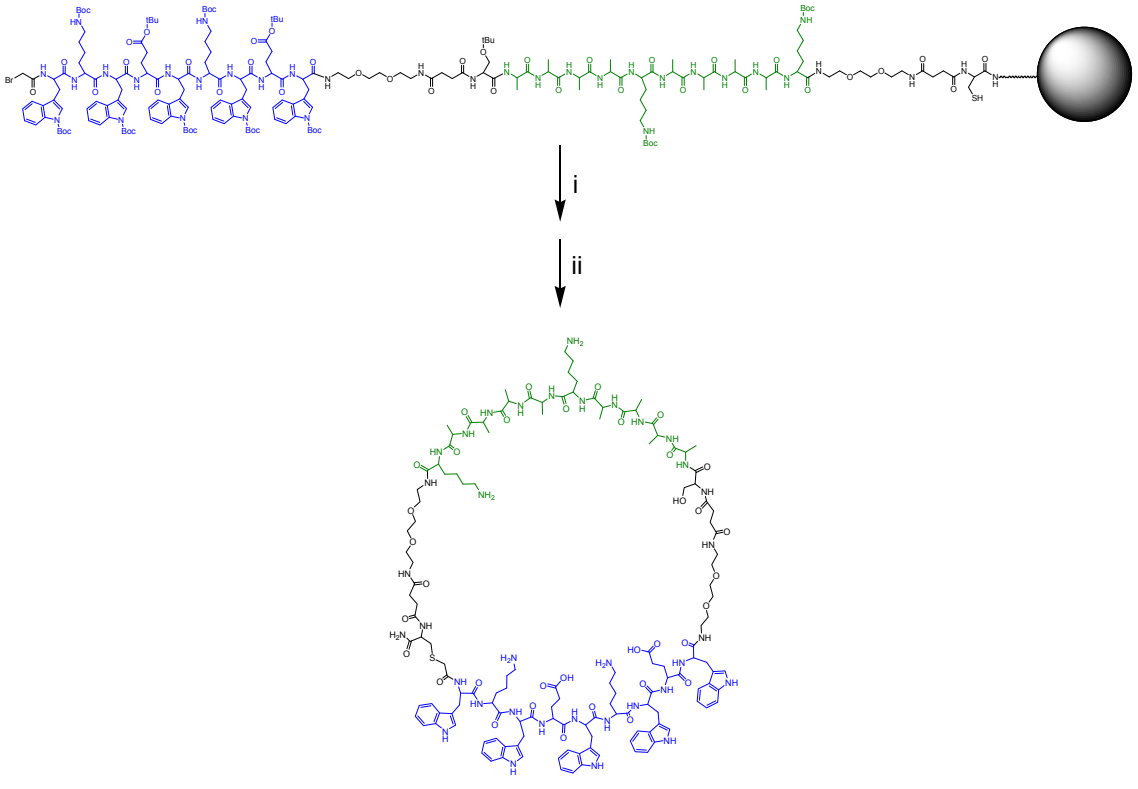
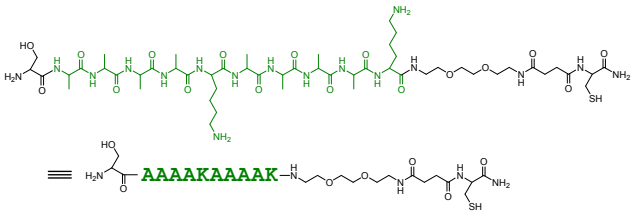
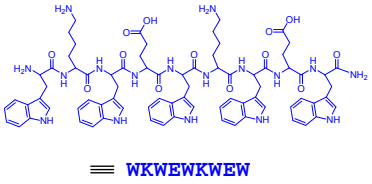


Peptide **4**

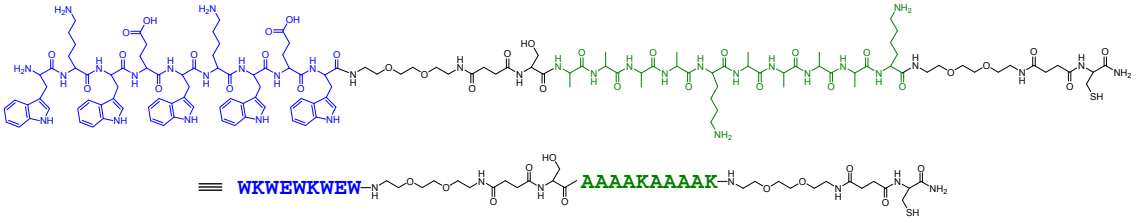
Figure S1. Synthesis of macrocyclic peptide. (i) 1% DIPEA in NMP; (ii) 95% TFA, 2.5% 1,2-ethanedithiol, 2.5% thioanisole.



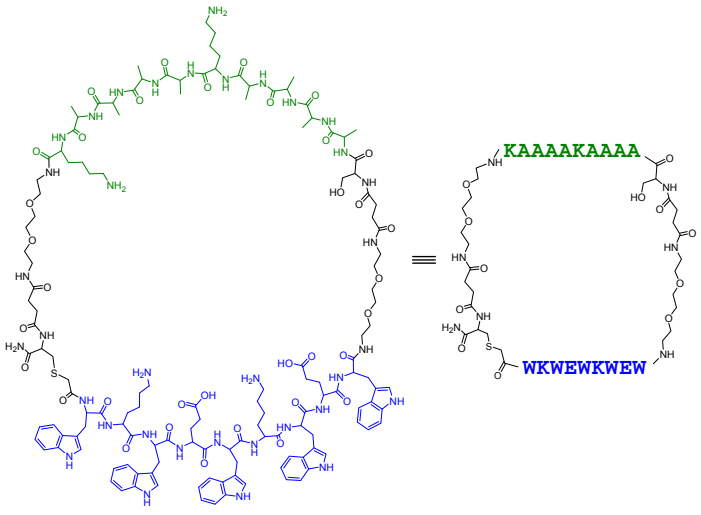
Peptide **1**



Peptide **2**



Peptide **3**



Peptide **4**

Figure S2. Structures of peptide **1**, **2**, **3**, and **4**.

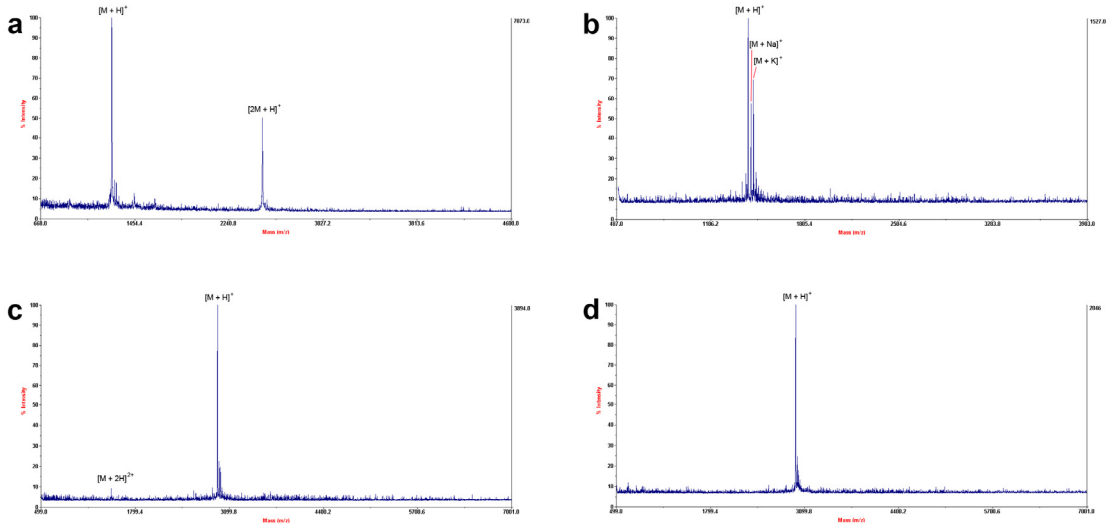


Figure S3. MALDI-TOF MS spectra of a) peptide **1**, b) peptide **2**, c) peptide **3**, and d) peptide **4**. Matrix: α-cyano-4-hydroxycinnamic acid.

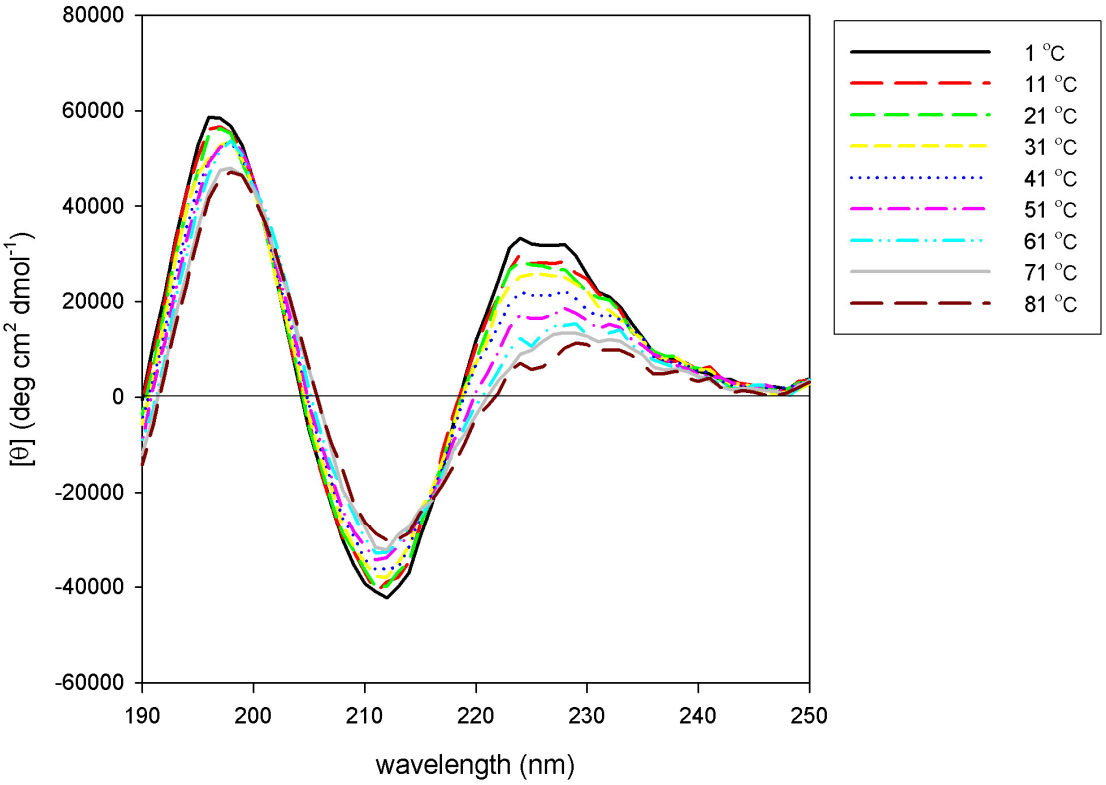
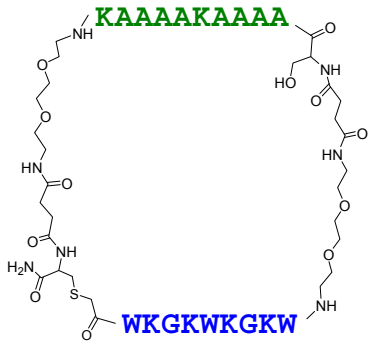
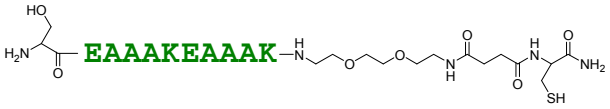


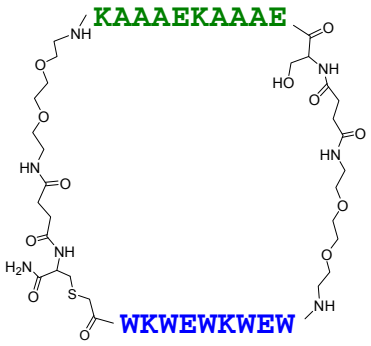
Figure S4. CD spectra of peptide **2** at various temperatures.



Peptide **5**



Peptide **6**



Peptide **7**

Figure S5. Structures of peptide **5**, **6**, and **7**.

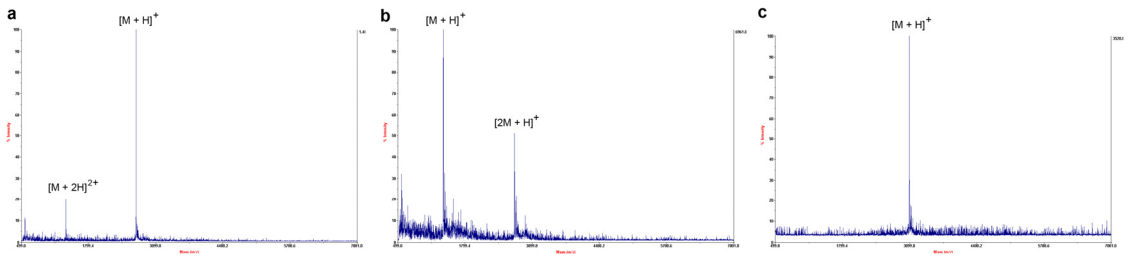


Figure S6. MALDI-TOF MS spectra of a) peptide **5**, b) peptide **6**, and c) peptide **7**. Matrix: α-cyano-4-hydroxycinnamic acid.

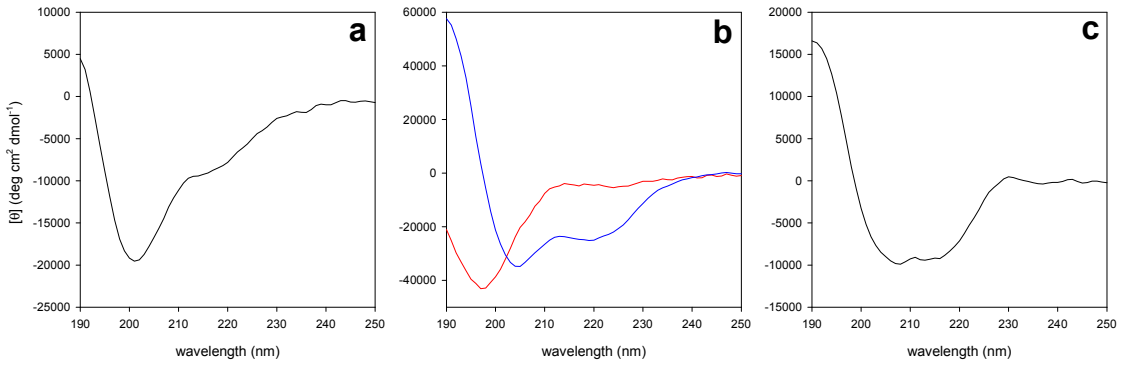


Figure S7. CD analyses of secondary structures. a) Peptide **5** in 75 mM KF at 1 °C. b) Peptide **6** in 75 mM KF at 1 °C (red). Peptide **6** in 75 mM KF/30% TFE at 1 °C (blue). c) Peptide **7** in 75 mM KF at 1 °C.

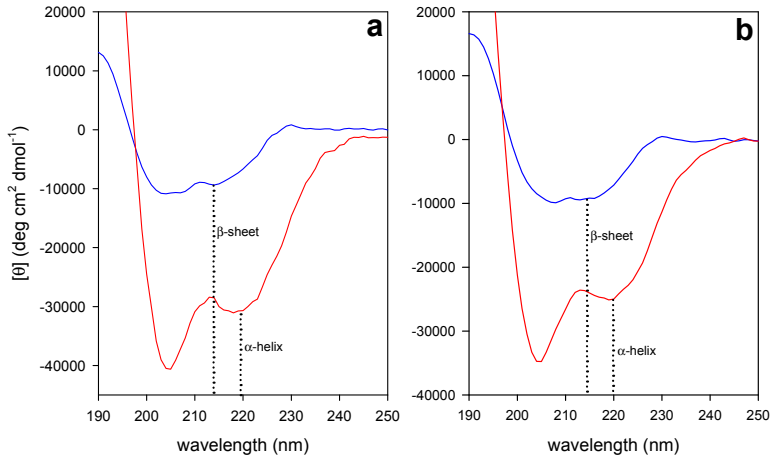


Figure S8. Superposition of CD spectra. a) Peptide **1** in 75 mM KF/30% TFE at 1 °C (red). Peptide **4** in 75 mM KF at 1 °C (blue). b) Peptide **6** in 75 mM KF/30% TFE at 1 °C (red). Peptide **7** in 75 mM KF at 1 °C (blue).

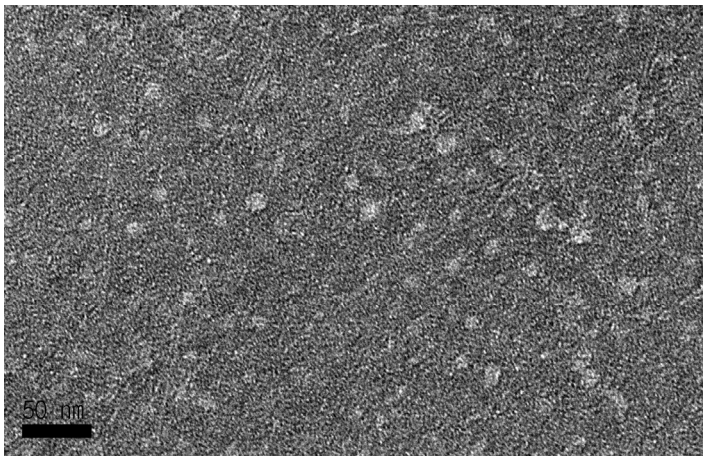
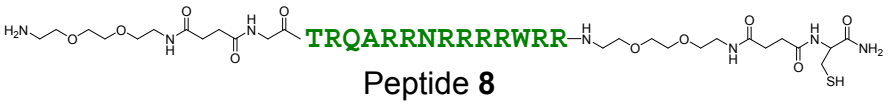


Figure S9. TEM image of peptide **7** nano-aggregates. Similarly to peptide **4**, peptide **7** forms spherical objects of about 10 nm in diameter as a major population.

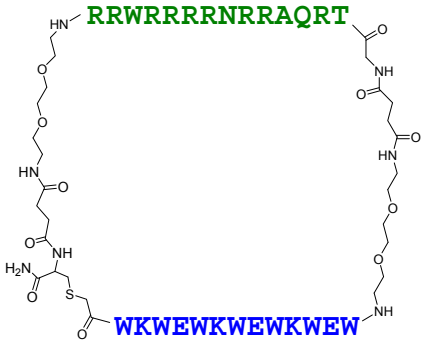


Peptide **8**

WKWEWKWEWKWEG

Peptide **9**

Peptide **10**



Peptide **11**

Figure S10. Stabilization of bioactive Rev peptide by the macrocyclization approach. Structures of peptide **8**, **9**, **10**, and **11**.

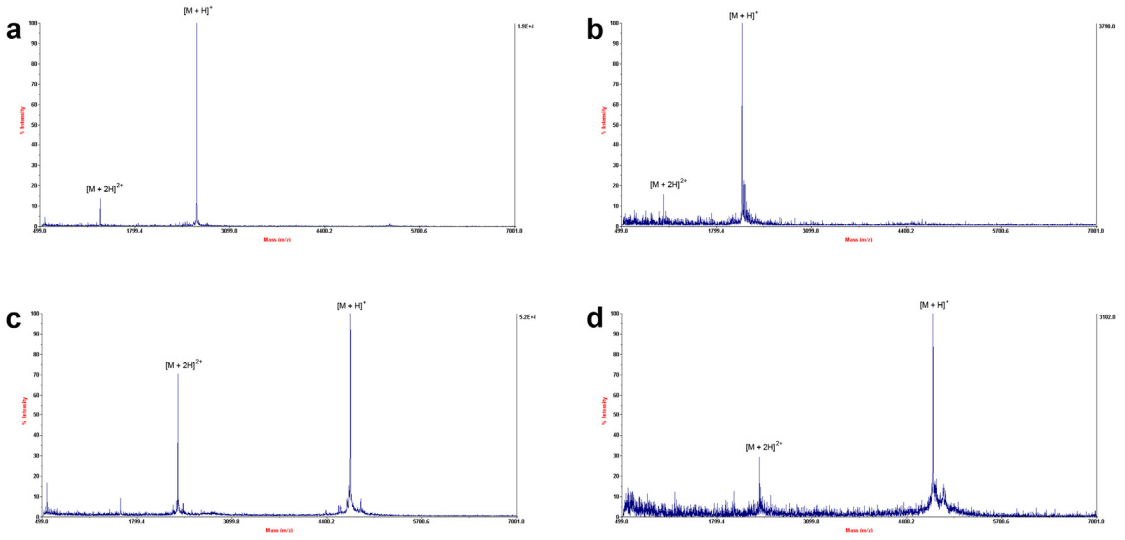


Figure S11. MALDI-TOF MS spectra of a) peptide **8**, b) peptide **9**, c) peptide **10**, and d) peptide **11**. Matrix: α-cyano-4-hydroxycinnamic acid.

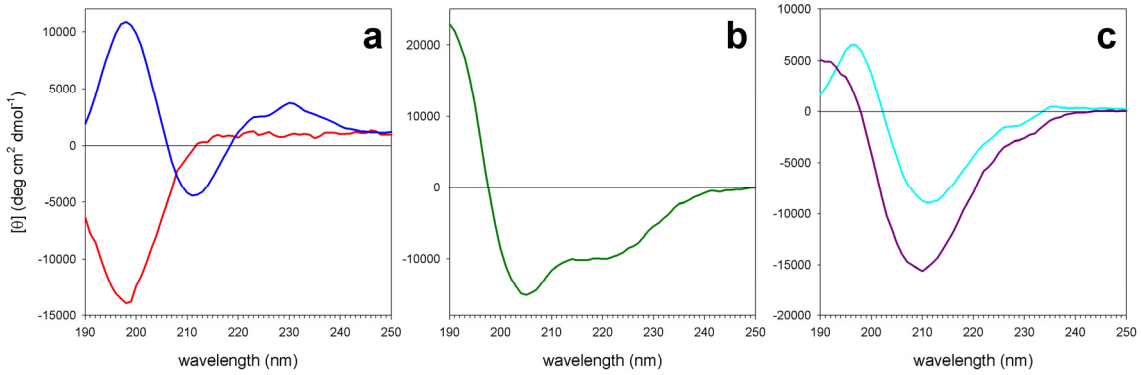


Figure S12. CD analyses of secondary structures. a) Peptide **8** (red) and peptide **9** (blue) in 75 mM KF at 1 °C. b) Peptide **8** in 75 mM KF, 60% TFE at 1 °C. c) Peptide **10** (light blue) and peptide **11** (purple) in 75 mM KF at 25 °C.

CD spectrum of peptide **8** (see Figure S10 for structure), a Rev peptide-based molecule, reveals that it is random coil in solution even at low temperature (Figure S12a). Similarly to peptide **1**, peptide **8** formed partially stabilized α-helical structure only when TFE was supplemented as a cosolvent and at low temperature (1 °C), with double minimum at 205 nm and 221 nm and a strong positive band around 190 nm (Figure S12b). Peptide **9**, a β-sheet forming peptide, was shown to consist predominantly of β-sheet structure as peptide **2** (Figure S12a). Macrocyclic peptide **11**, which contains minimal Rev peptide sequence (14 amino acids), was designed to have a longer β-sheet forming segment than peptide **4** to compensate for the increase in the α-helical segment length. Compared to acyclic homologue **10**, which is mostly in β-sheet conformation with a negative band at 212 nm, a positive band at 197 nm, and a crossover point at 202 nm, the blueshifted crossover point (~ 4 nm) and a positive maximum around 190 nm indicate the stabilization of α-helix[17] in the macrocyclic peptide **11** (Figure S12c).

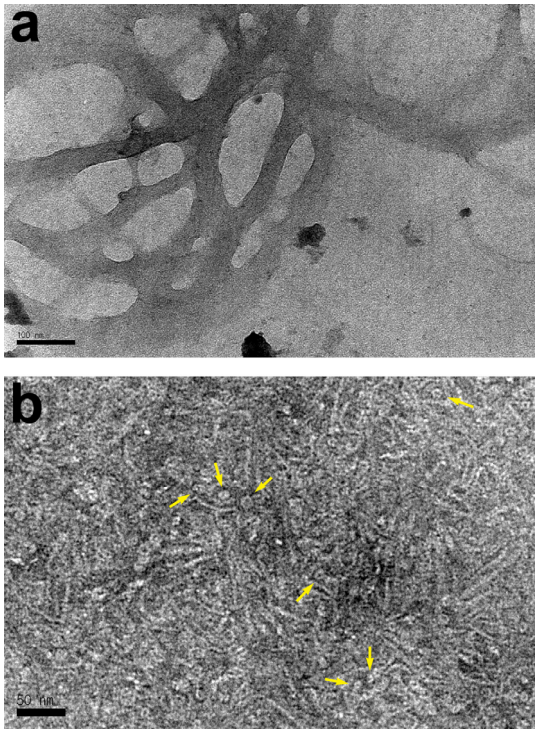


Figure S13. TEM image of a) peptide **9** and b) peptide **11**. Some of the spherical objects are marked with arrows.

Peptide **9** was found to form even thicker and more extended fibers/ribbons than peptide **2** (Figure S13a). This higher degree of lateral association is likely due to the increased β-sheet chain length. In contrast, peptide **11** forms discrete 1D fibers about 50 nm in length along with 2D spherical objects as a minor population (Figure S13b). The less efficient formation of spherical objects by peptide **11** compared to the efficiency of spherical objects formation by peptide **4** and peptide **7** might be related to the variation in the macrocyclic structure such as the increase of the β-sheet chain length.

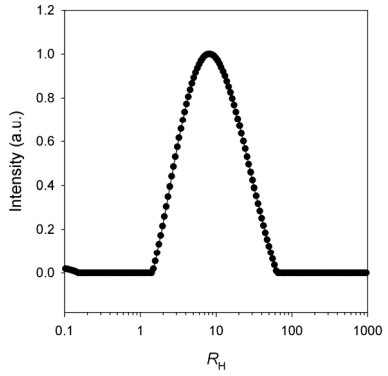


Figure S14. R_H distribution of peptide **11** by DLS.

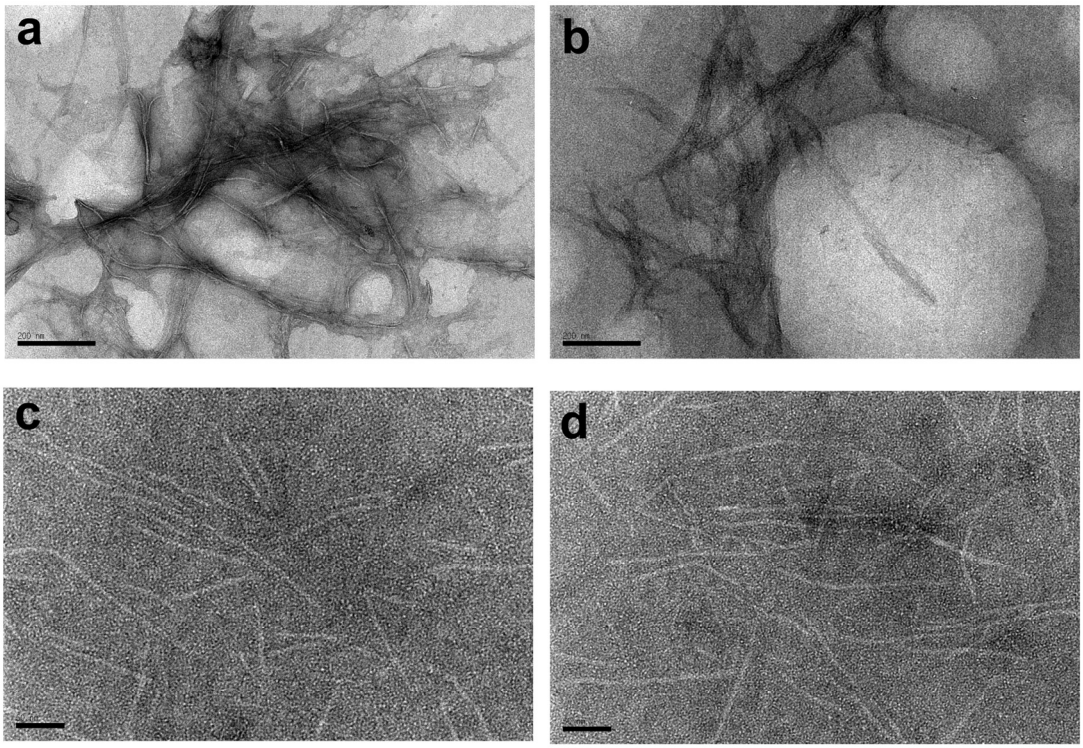


Figure S15. Negative stain TEM images. a) and b) peptide **2**. c) and d) peptide **3**.

METHODS

Synthesis. Peptide was synthesized on Rink amide MBHA resin using standard Fmoc protocols on an Applied Biosystems model 433A peptide synthesizer. Standard amino acid protecting groups were employed except cysteine, in which an acid-labile methoxytrityl (Mmt) group was used. The oligoethylene glycol-based linker, *N*-(Fmoc-8-amino-3,6-dioxaoctyl)succinamic acid (Fmoc-PEG_2-Suc-OH), was purchased from Anaspec. The peptide-attached resin (20 μmol of N-terminal amine groups) was swollen in *N*-methyl-2-pyrrolidone (NMP) for 30 min. For cyclization, bromoacetic acid was first coupled to the N-terminal part of the resin-bound peptide. Before addition to the resin, a mixture of bromoacetic acid (28 mg, 200 μmol) and *N*,*N'*-diisopropylcarbodiimide (15.5 μL, 100 μmol) in NMP was incubated for 10 min for carboxyl activation. The reaction was continued for 1 h with shaking at room temperature, in a 6 mL polypropylene tube with a frit (Restek). The resin was then washed successively with NMP and dichloromethane (DCM). For orthogonal deprotection of the Mmt group from the cysteine, the resin was treated with 1% trifluoroacetic acid (TFA) in DCM several times (1 min × ~5). Intramolecular cyclization reaction was performed in 3 mL of 1% diisopropylethylamine (DIPEA) in NMP overnight with shaking at room temperature. The resin was then successively washed with NMP and acetonitrile, and dried *in vacuo*. The dried resin was treated with cleavage cocktail (TFA：1,2-ethanedithiol：thioanisole; 95 : 2.5 : 2.5) for 3 h, and was triturated with *tert*-butyl methyl ether. The peptides were purified by reverse-phase HPLC (water–acetonitrile with 0.1% TFA). The molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the peptides was >95% as determined by analytical HPLC. Concentration was determined spectrophotometrically in 8 M urea using a molar extinction coefficient of tryptophan (5,500 $M^{-1}cm^{-1}$) at 280 nm.

Circular dichroism. CD spectra were measured using a JASCO model J-810 spectropolarimeter equipped with temperature controller. Spectra were recorded from 250 nm to 190 nm using a 0.1 cm path-length cuvette. Scans were repeated seven times and averaged. Molar ellipticity was calculated per amino acid residue. Peptide concentration was 20 μM. Peptides were typically prepared in 75 mM KF solution unless described otherwise. Sample solutions were incubated at least for 2 days before measurement, and essentially the same CD spectra were obtained after prolonged incubation, indicating thermodynamic equilibrium states. Essentially the same results were obtained when the peptides were prepared in phosphate-

buffered saline (PBS), indicating that the data obtained can be extrapolated to physiological conditions.

Transmission electron microscopy. Two μL of sample was placed onto a carbon-coated copper grid and dried completely. Then 2 μL of 2 % (w/v) uranyl acetate solution was added for 1 min and excess solution was wicked off by filter paper. Sample concentrations were typically 5 - 20 μM in 75 mM KF. The specimen was observed with a JEOL-JEM 2010 instrument operating at 120 kV and the image was recorded with a SC 1000 CCD camera (Gatan). The data were analyzed with DigitalMicrograph software.

Dynamic light scattering (DLS). DLS experiment was performed at room temperature with LV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. Before measurement, the sample was centrifuged at 16,110 × g for 20 min to sediment any dust particles. Sample concentrations were typically 5 - 20 μM in 75 mM KF. The size distribution was determined by using a constrained regularization method.

Infrared spectroscopy. For FT-IR measurement, 100 μL of the sample (300 μM in 75 mM KF) was cast from the solution onto ZnSe window. Twenty thousand scans were acquired on a Bruker Equinox 55 FT-IR spectrometer.

Reference

[17] B. Czarnik-Matusewicz, S. Pilorz, *J. Mol. Struct*. **2006**, *799*, 211-220.

The complete reference from the main paper

[3a] H. Zhang, Q. Zhao, S. Bhattacharya, A. A. Waheed, X. Tong, A. Hong, S. Heck, F. Curreli, M. Goger, D. Cowburn, E. O. Freed, A. K. Debnath, *J. Mol. Biol*. **2008**, *378*, 565-580.