# Supporting Information for

X-ray Crystallographic Structure of a Giant Double-Walled Peptide Nanotube Formed by a Macrocyclic β-Sheet Containing $A\beta_{16-22}$

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**Supporting Figure and Table**

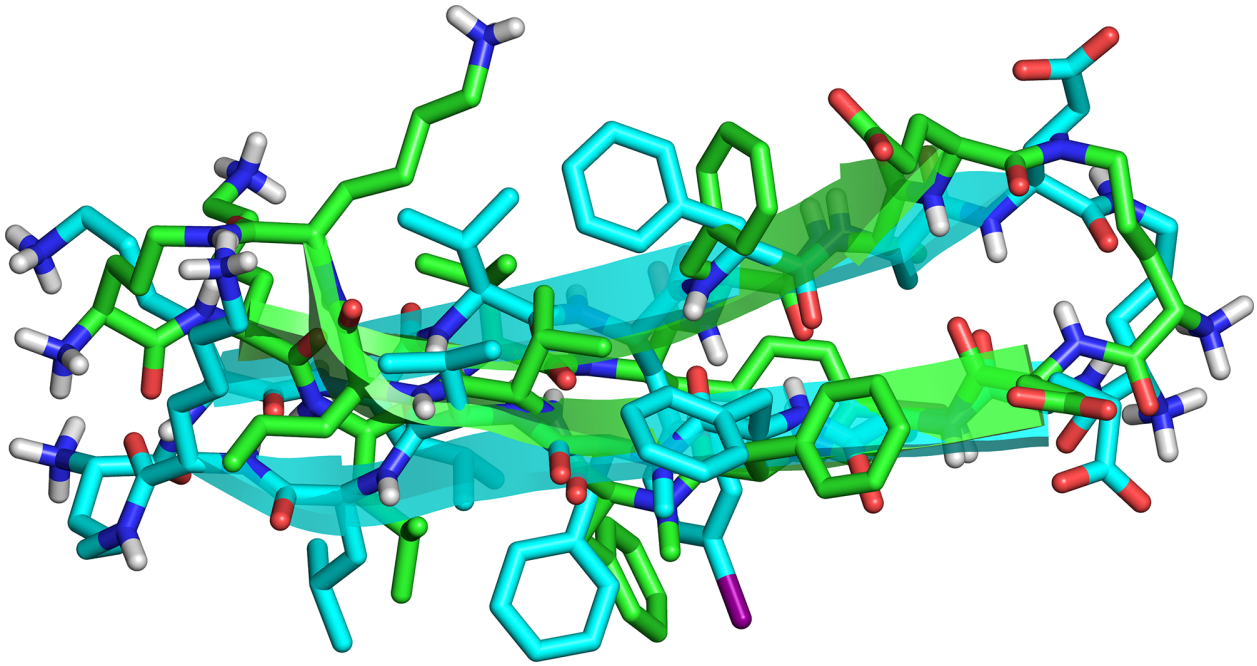


**Figure S1.** Overlay of representative β-hairpin monomers that comprise the dimer and tetramer.

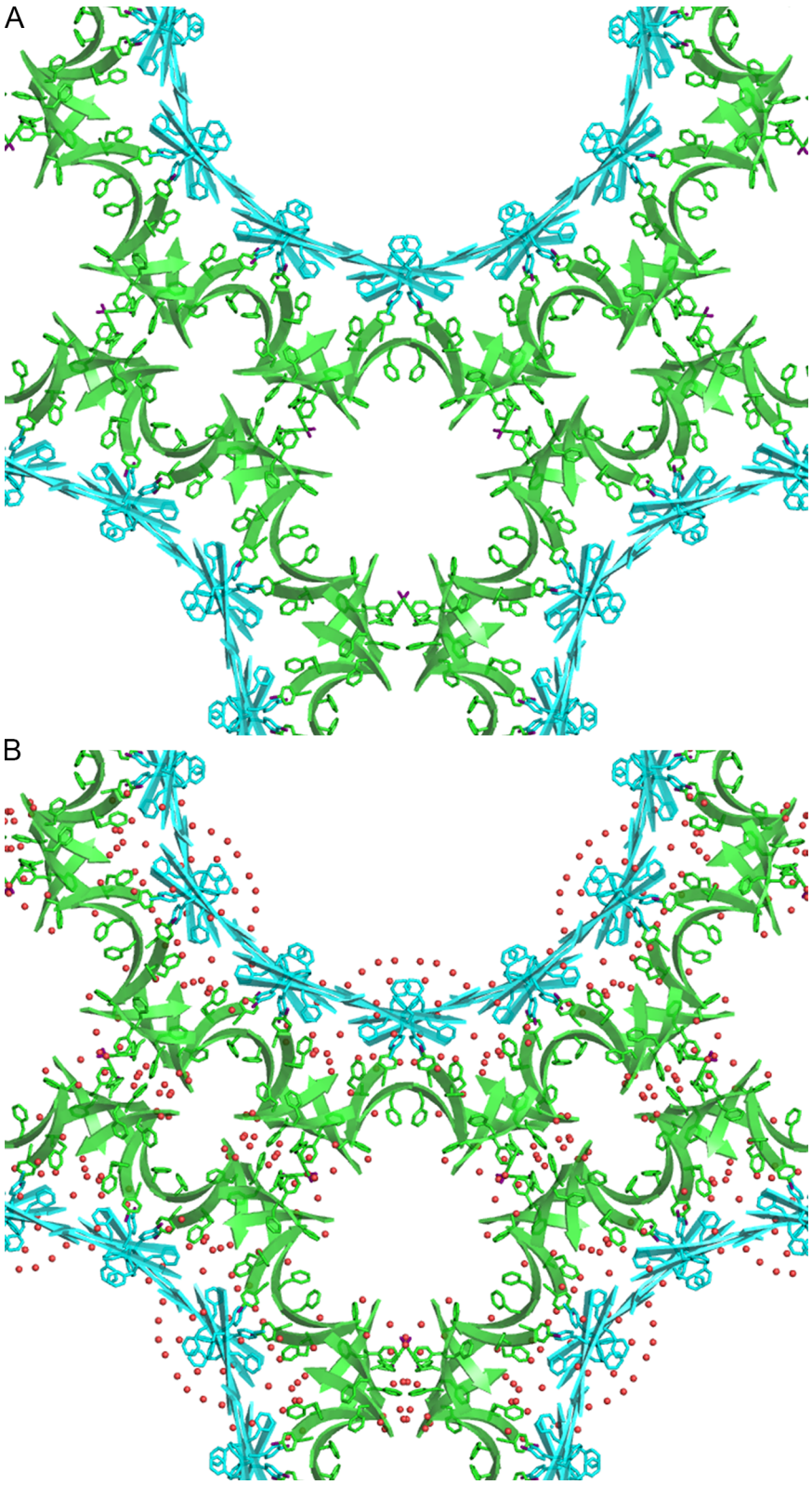



**Figure S2.** Junction between three nanotubes (A) without crystallographic water molecules and (B) with crystallographic water molecules.

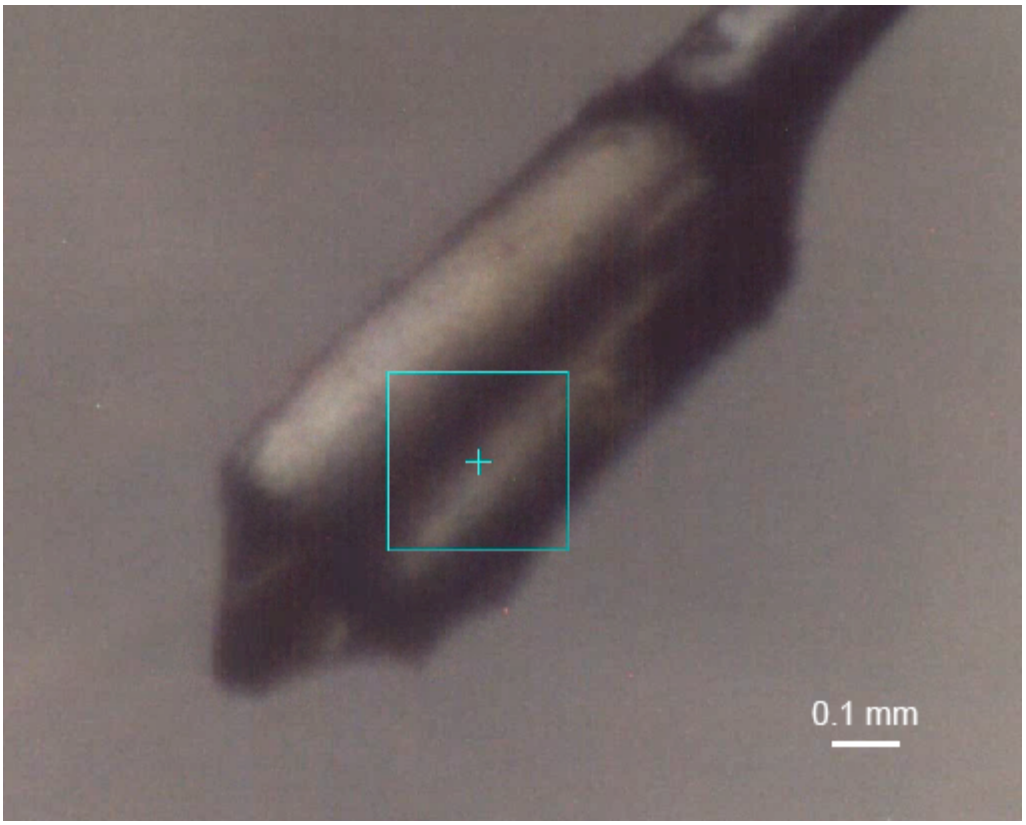



**Figure S3.** A hexagonal pointed prism crystal formed by macrocyclic β-sheet **1**.

**Table S1.** Crystallographic properties, crystallization conditions, and data collection and model refinement statistics for macrocyclic β-sheet **1**.

| Macrocyclic β-sheet **1** | |
|---|---|
| PDB ID | 5VF1 |
| Space group | $P6_122$ |
| *a*, *b*, *c* (Å) | 121.22 121.22 29.96 |
| α, β, λ (˚) | 90 90 120 |
| Peptides per asymmetric unit | 6 |
| Crystallization conditions | 0.1 M bis-Tris at pH 5.5, 0.2 M ammonium acetate, and 45% v/v 2-methyl-2,4-pentanediol |
| Wavelength (Å) | 1.54 |
| Resolution (Å) | 19.74–2.1 (2.175–2.1) |
| Total reflections | 15963 (1511) |
| Unique reflections | 7990 (776) |
| Multiplicity | 2.0 (2.0) |
| Completeness (%) | 99.73 (100.00) |
| Mean I/σ | 36.78 (6.54) |
| Wilson B factor | 30.01 |
| $R_{merge}$ | 0.05179 (0.1482) |
| $R_{measure}$ | 0.07324 (0.2096) |
| $CC_{1/2}$ | 0.994 (0.917) |
| $CC^*$ | 0.999 (0.978) |
| $R_{work}$ | 0.2240 (0.2782) |
| $R_{free}$ | 0.2812 (0.3000) |
| Number of non-hydrogen atoms | 866 |
| $RMS_{bonds}$ | 0.009 |
| $RMS_{angles}$ | 1.45 |
| Ramachandran favored (%) | 100 |
| Ramachandran outliers (%) | 0 |
| Clashscore | 8.27 |
| Average B-factor | 49.41 |
| Number of TLS groups | 6 |

Statistics for the highest-resolution shell are shown in parentheses.

**Materials and Methods**

*General information*: All Fmoc-protected amino acids including unnatural amino acids, Fmoc-*N*-methyl phenylalanine-OH, Fmoc-*p*-iodo-phenylalanine-OH, and Boc-ornithine(Fmoc)-OH, were purchased from LC Sciences, Chem-Impex, and GL Biochem. 2-Chlorotrityl chloride resin was purchased from Chem-Impex. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (ACN) were purchased from Fischer Scientific. Water was purified with Barnstead NANOpure Diamond lab water purification system. All other solvents and chemicals were purchased from Alfa Aesar and Sigma Aldrich. All amino acids, resins, solvents, and chemicals were used as received, with the exception that dichloromethane (DCM) and *N*,*N*-dimethylformamide (DMF) was dried by passage through dry alumina under argon.[1] Abbreviations: ACN, acetonitrile; TFA, trifluoroacetic acid; DCM, dichloromethane; MeOH, methanol; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; HCTU, 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HATU, (1-bis(dimethylamino)methylene)-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate); HOBt, hydroxybenzotriazole; HBTU, *N*,*N*,*N'*,*N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uranium hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TIPS, triisopropylsilane.

*Synthesis of macrocyclic β-sheet **1***:[2] The synthesis of macrocyclic β-sheet **1** involved the following sequence of operations: (1) resin loading, (2) solid-phase amino acid couplings, (3) cleavage of the linear peptide from the resin, (4) solution-phase cyclization of the linear peptide, (5) global deprotection of acid-labile protecting groups, and (6) purification with preparative reverse-phase HPLC. The purified peptide was characterized with analytical HPLC and ESI-MS.

(1) *Resin loading*: 2-Chlorotrityl chloride resin (0.300 g, 1.12 mmol/g) was added to a 10-mL Bio-Rad Poly-Prep chromatography column (8 mm x 40 mm). The resin was suspended in dry DCM (10 mL) and allowed to swell undisturbed for 30 min. The solution was drained from the resin using nitrogen and a solution of Boc-ornithine(Fmoc)-OH (70.0 mg, 0.17 mmol) in 20% (v/v) 2,4,6-collidine in dry DCM (5 mL) was added immediately. The suspension was gently agitated for 12 h. The solution was then drained using nitrogen and washed with dry DCM (2x). After washing, a mixture of DCM/MeOH/DIPEA (8.5:1:0.5, 10 mL) was added immediately. The suspension was gently agitated for 1 h to cap any unreacted sites on the resin. The resin was washed with DMF (2x) and dried by passing nitrogen through the chromatography column. The resin loading was determined to be 0.11 mmol (0.33 mmol/g, 65%) based on UV analysis (290 nm) of the Fmoc cleavage product.

(2) *Solid-phase amino acid couplings*: The Boc-ornithine(Fmoc) loaded resin was transferred to a solid-phase peptide synthesizer reaction vessel designed for an automated peptide synthesizer (Protein Technologies). The resin was subjected to cycles of automated amino acid couplings using Fmoc-protected amino acid building blocks. The linear peptide was synthesized from the *C*-terminus to the *N*-terminus. Each coupling consisted of: (1) Fmoc deprotection with 20% (v/v) piperidine in DMF for 5 min; (2) resin washing with DMF (3x); (3) activation of the Fmoc-protected amino acid (0.44 mmol, 4 equiv) with 20% (v/v) 2,4,6-collidine in DMF (5 mL) in the presence of HCTU (0.44 mmol, 4 equiv); (4) coupling of the activated Fmoc-protected amino acid; (5) resin washing with DMF (3x). All amino acid couplings took 20 min except for phenylalanine that followed the *N*-methyl-phenylalanine. The phenylalanine that followed the *N*-methyl-phenylalanine (0.44 mmol, 4 equiv) was coupled twice for 1 h each with HATU (0.44 mmol, 4 equiv) and HOAt (0.44 mmol, 4 equiv) in 20% (v/v) 2,4,6-collidine in DMF (5 mL),

with no Fmoc deprotection in between the two coupling reactions. [This modification ensured complete amino acid coupling onto a more sterically hindered, secondary amine.] After the last amino acid was coupled, and its Fmoc protecting group deprotected, the resin was transferred from the peptide synthesizer reaction vessel to a new Bio-Rad Poly-Prep chromatography column. The resin was washed with DCM (3x), and dried by passing nitrogen through the chromatography column.

(3) *Cleavage of the linear peptide derivative of macrocyclic β-sheet **1***: The protected linear peptide derivative of macrocyclic β-sheet **1** was cleaved from the resin by subjecting the resin to a cleavage solution of 20% (v/v) HFIP in DCM (5 mL). The resin and the suspension immediately turned red. The suspension was gently agitated for 1 h. The suspension was filtered, and the filtrate was collected in a 250-mL round-bottom flask. The resin was washed with additional cleavage solution (5 mL) and then with DCM (2x 5 mL) until the resin was no longer red. The combined filtrates were concentrated under reduced pressure to give a colorless oil. The crude product was dried using a vacuum pump to afford the protected linear peptide derivative of macrocyclic β-sheet **1**, which was cyclized without purification.

(4) *Solution-phase cyclization of the linear peptide derivative of macrocyclic β-sheet **1***: The protected linear peptide derivative of macrocyclic β-sheet **1** was dissolved in dry DMF (125 mL) in the same 250-mL round-bottom flask as the previous step. HOBt (0.074 g, 0.55 mmol, 5 equiv) and HBTU (0.208 g, 0.55 mmol, 5 equiv) were added to the solution. The reaction mixture was then stirred under nitrogen at room temperature for 10 min. DIPEA (0.3 mL, 1.5 mmol, 14 equiv) was added to the solution and the mixture was stirred under nitrogen at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure to give a

brown oil. The crude product was dried using a vacuum pump to afford the protected cyclic peptide derivative of macrocyclic β-sheet **1**.

(5) G*lobal deprotection of acid-labile protecting groups of macrocyclic β-sheet **1**:* The protected cyclic peptide derivative of macrocyclic β-sheet **1** was dissolved in a mixture of TFA/TIPS/$H_2O$ (9.5:0.5:0.5, 10 mL) in the same 250-mL round-bottom flask as the previous step. The reaction mixture was then stirred under nitrogen at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure to give a brown oil. The crude product was dried using a vacuum pump to afford the crude macrocyclic β-sheet **1**.

(6) *Purification of macrocyclic β-sheet **1** with preparative reverse-phase HPLC*: The crude macrocyclic β-sheet **1** was dissolved in 20% ACN/$H_2O$ (10 mL), and the solution was centrifuged at 3,000 rpm for 5 min to pellet any insoluble material. [The presence of a small amount of insoluble pellet is normal.] The supernatant was then filtered through a 0.2 μm syringe filter. The crude macrocyclic β-sheet **1** was purified by reverse-phase HPLC. The purification was performed on a Agilent Zorbax SB-C18 PrepHT column (21.2 mm x 250 mm, 7-μm particle size) on a Beckman HPLC with a flow of 15.0 mL/min. The UV detector was set to 214 nm. The gradient started with 20% to 30% ACN/$H_2O$ for 10 min, followed by 30% to 40% ACN/$H_2O$ for 20 min, and end with 40% to 50% ACN/$H_2O$ for 30 min. The peptide eluted around 42% ACN/$H_2O$. The pure fractions were lyophilized to afford macrocyclic β-sheet **1** as a white powder.

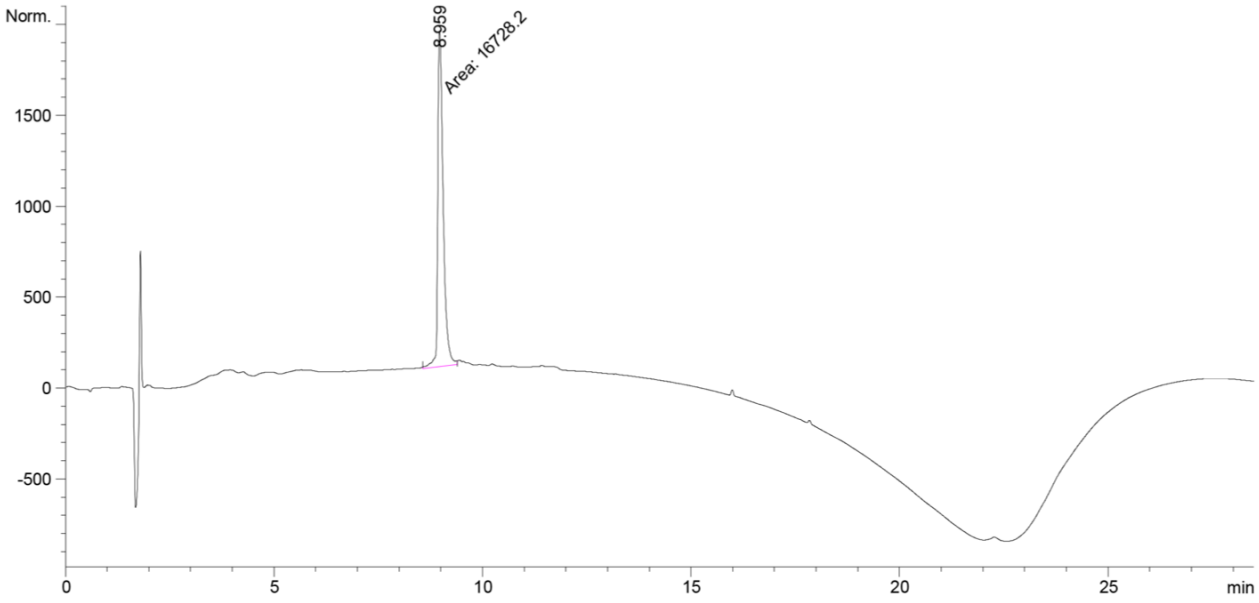
*Crystallization of macrocyclic β-sheet **1**:*[3] A 10 mg/mL stock solution of macrocyclic β-sheet **1** was prepared using purified water that was filtered through a 0.2 μm syringe filter. Three 96-

well screening kits were purchased from Hampton Research (Index, Crystal, and PEG/Ion). The peptide was screened against 864 crystal growing conditions using a nanoliter liquid handling instrument (TTP Labtech Mosquito) and its default "three drops" method to facilitate nanoliter-scaled crystallization (2:1, 1:1, and 1:2 peptide to well solution with a total volume of 150 nL). Crystal growing conditions of 0.1 M bis-Tris pH 5.5, 0.2 M ammonium acetate, and 45% v/v 2-methyl-2,4-pentanediol were identified (Index, E2). Attempts to further optimize crystal growing conditions using Hampton VDX 24-well plates and varying the pH of the bis-Tris buffer and the concentration of ammonium acetate and 2-methyl-2,4-pentanediol resulted in no further improvements. The best X-ray diffracting crystal grew as hexagonal pointed prism and is shown in Figure S1.

*X-ray crystallography of macrocyclic β-sheet **1***:[4] Crystal diffraction data were collected using a Rigaku MicroMax 007HF X-ray diffractometer with a copper source at 1.54Å wavelength at low temperature under a stream of liquid nitrogen vapor. The dataset was scaled with XDS. Coordinates of the anomalous scattering atom, iodine, were determined by Hyss within the PHENIX software suite. An initial electron density map was generated by Autosol followed by initial generation of the model with Autobuild. Subsequent repetitions of model building in PyMOL and refinement in Coot were conducted. The X-ray crystallographic assembly of macrocyclic β-sheet **1** was solved and refined in space group $P6_122$. The X-ray crystallographic model refinement statistics are summarized in Table S1.

## Characterization of macrocyclic β-sheet 1

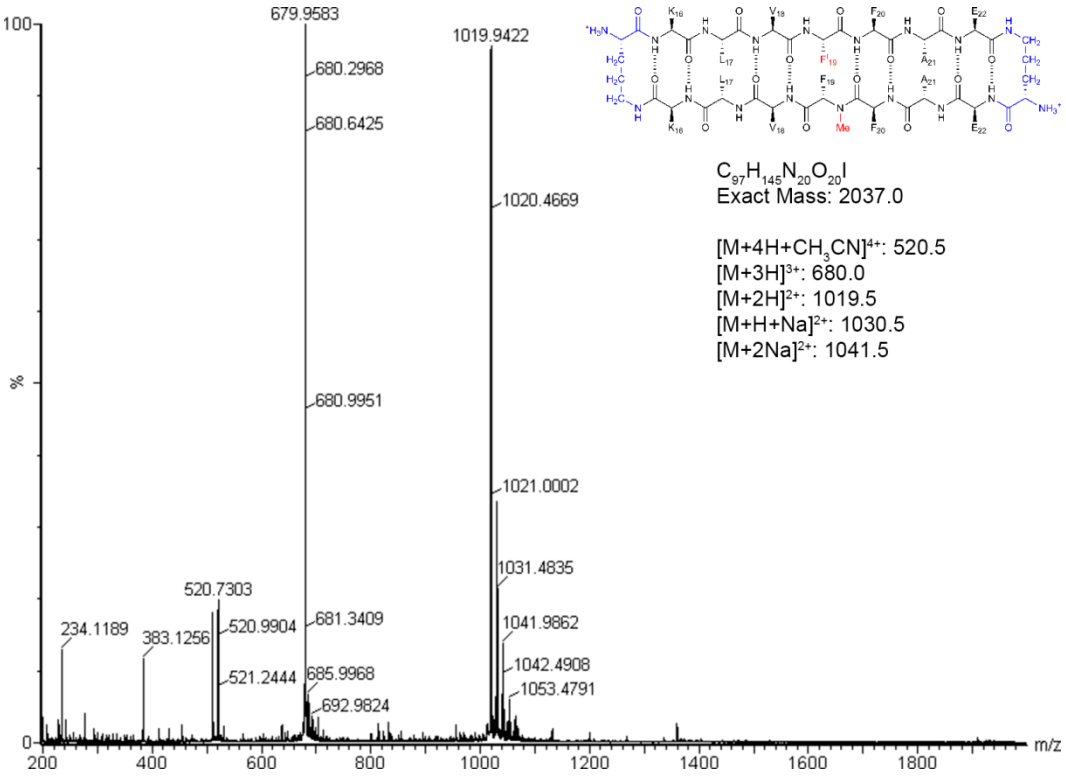
*Reverse-phase HPLC spectra of macrocyclic β-sheet **1***:

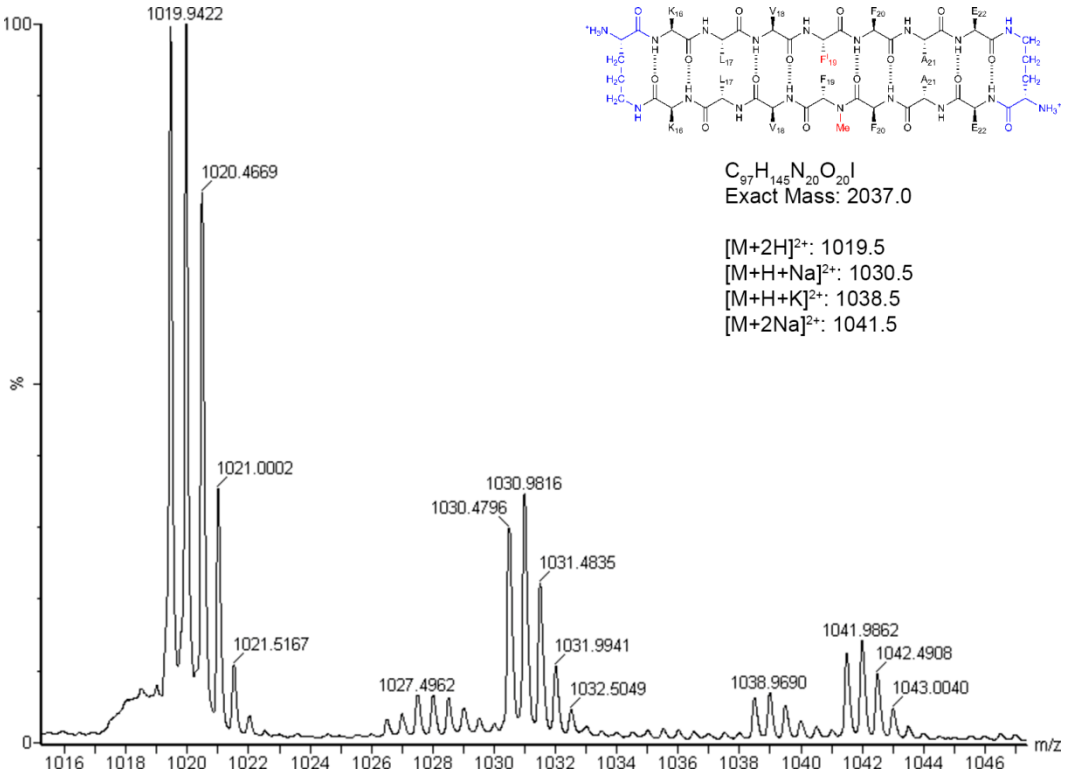



Signal 1: VWD1 A, Wavelength=214 nm

| Peak # | RetTime [min] | Type | Width [min] | Area mAU *s | Height [mAU ] | Area % |
|---|---|---|---|---|---|---|
| 1 | 8.959 | MM | 0.1508 | 1.67282e4 | 1849.00122 | 100.0000 |
| Totals : | | | | 1.67282e4 | 1849.00122 | |

*Electrospray-ionization mass spectra of macrocyclic β-sheet **1***:



*References*:

(1) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

(2) Macrocyclic β-sheet **1** was synthesized following a protocol similar to those published previously. The procedures were either adapted from or taken verbatim from Spencer, R.; Chen, K. H.; Manuel, G.; Nowick, J. S. *Eur. J. Org. Chem*. **2013**, *17*, 3523–3528.

(3) Macrocyclic β-sheet **1** was crystallized following a protocol similar to those published previously. The procedures were either adapted from or taken verbatim from Kreutzer, A. G.; Yoo, S.; Spencer, R. K.; Nowick, J. S. *J. Am. Chem. Soc*. **2017**, *139*, 966–975.

(4) The X-ray diffraction data of macrocyclic β-sheet **1** was processed following a protocol similar to those published previously. The procedures were either adapted from or taken verbatim from Kreutzer, A. G.; Yoo, S.; Spencer, R. K.; Nowick, J. S. *J. Am. Chem. Soc*. **2017**, *139*, 966–975.