Supporting information for:

Interpenetrating Cubes in the X-Ray Crystallographic Structure of a Peptide Derived from Medin$_{19–36}$

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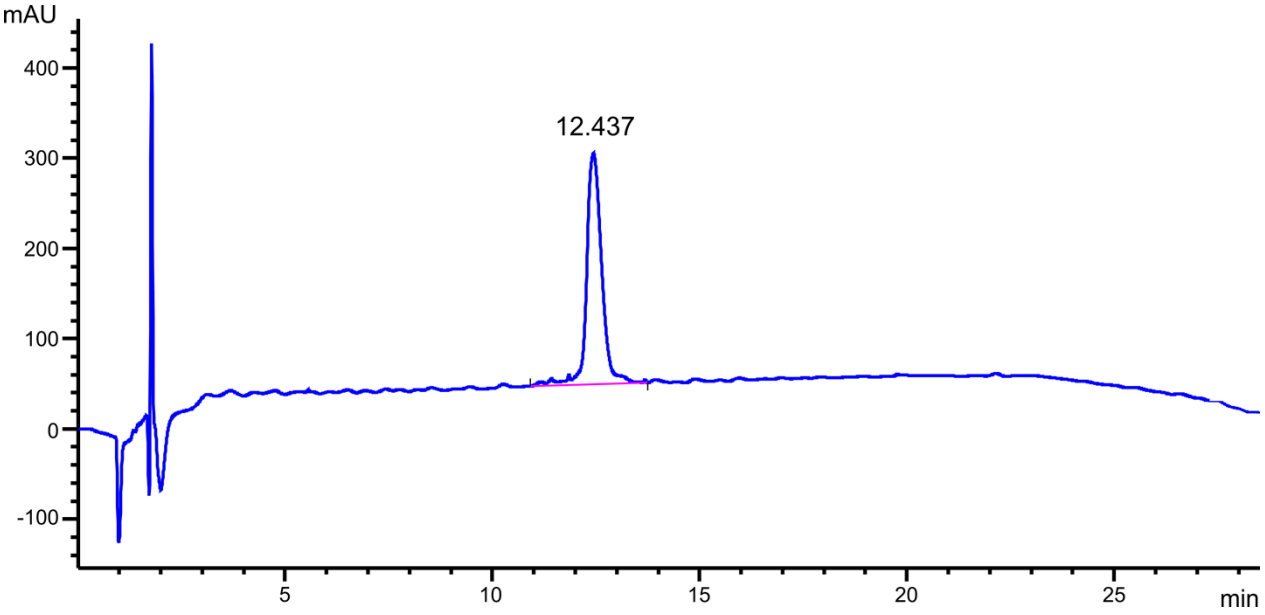

Figure S1. Analytical HPLC trace for peptide **1.**

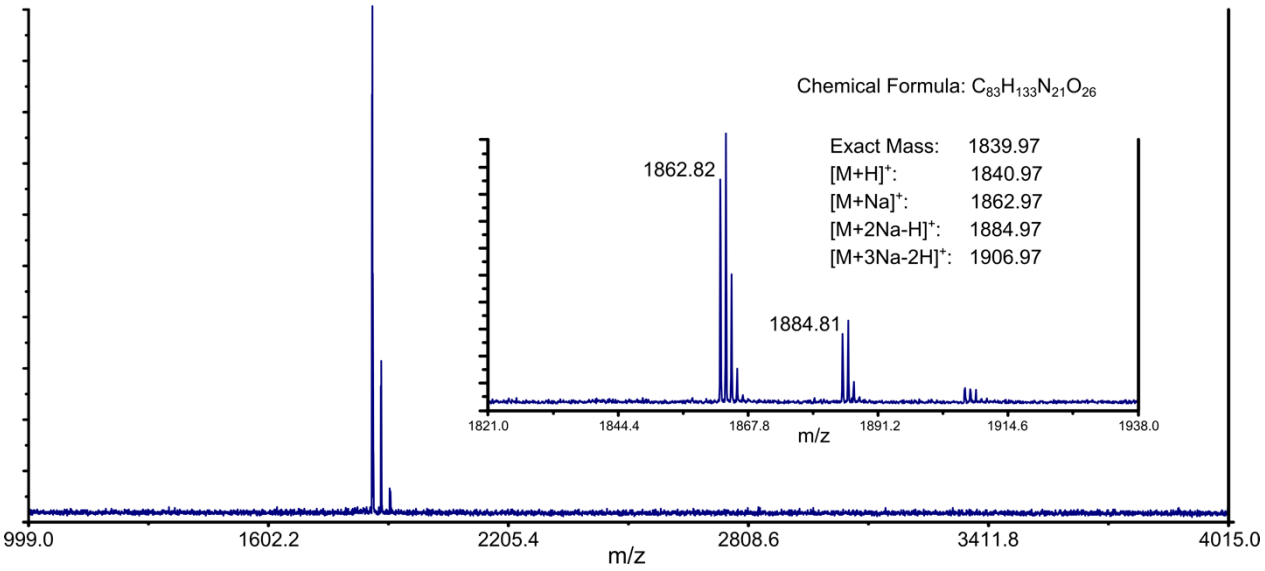

Figure S2. MALDI-TOF mass spectrum of peptide **1.**

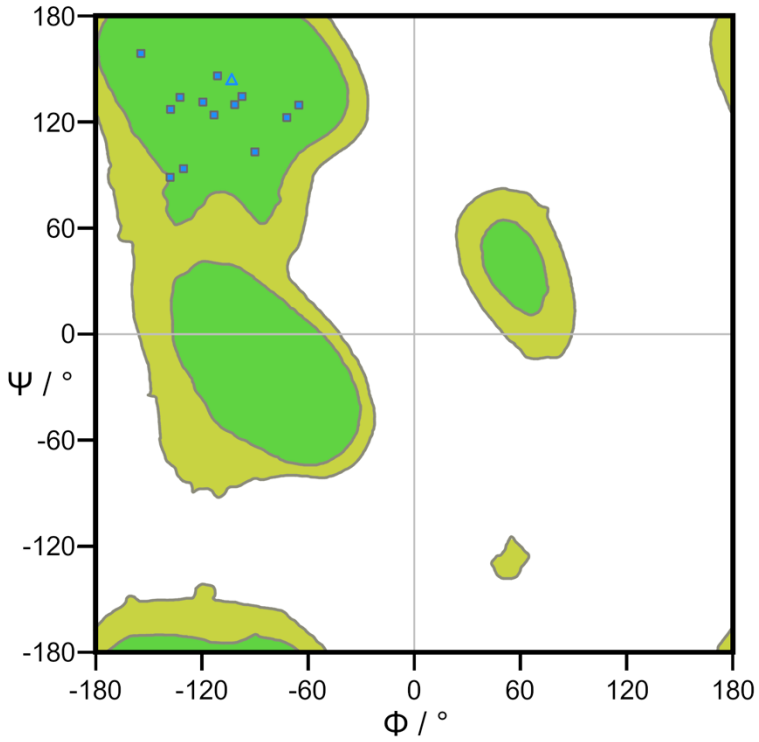

Figure S3. Ramachandran plot illustrating the φ and ϕ angles of residues D_{19}–D_{25} and E_{31}–T_{37} of peptide **1**. Preferred areas (0.2% probability) are shown in green and acceptable areas (2% probability) are shown in yellow-green. The glycine residue is shown as a triangle.

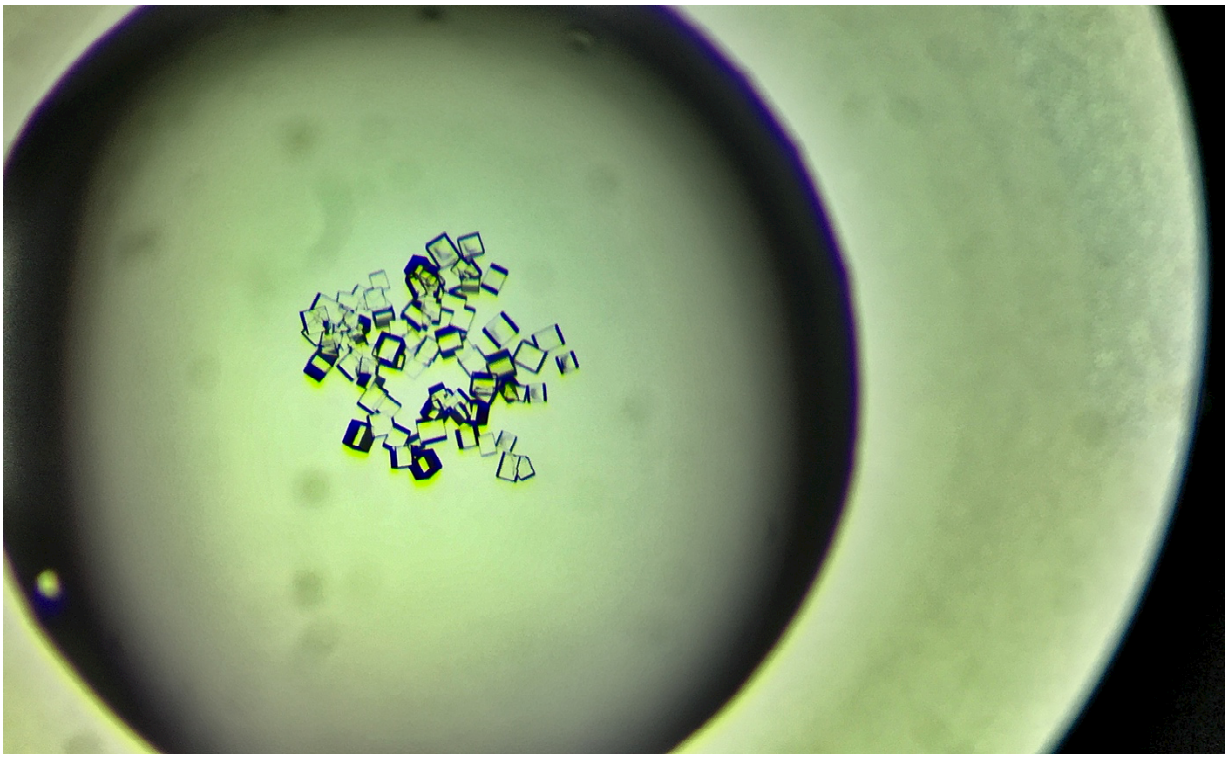

Figure S4. Crystals of peptide **1** within a crystallization drop.

General Information[1]

All Fmoc-protected amino acids including the unnatural amino acid, Boc-ornithine(Fmoc)-OH were purchased from Chem-Impex or Anaspec. 2-Chlorotrityl chloride resin was purchased from Chem-Impex. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (MeCN) were purchased from Fischer Scientific. Water was purified to 18 MΩ with a ThermoFischer GenPure Pro 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) were dried by passage through dry alumina under argon. Analytical HPLC chromatograms were obtained using an Agilent 1260 Infinity II HPLC equipped with Phenomenex bioZen C18 column (150 mm × 4.6 mm, 2.6 μm particle size). A 1 mL/min flow rate was used with peak detection at 214 nm, all monitored using the provided HPLC OpenLAB software. Preparative-scale purification of peptides was done using an Agilent Zorbax SB-C18 PrepHT column (21.2 mm x 250 mm, 7 μm particle size) on a Rainin Dynamax HPLC with a flow rate of 7.0 mL/min, monitored at 214 nm with the accompanying DA Rainin HPLC software. MALDI mass spectrometry was performed using an Applied Biosystems SCIEX TOF/TOF under reflector positive ion mode using 2,5-dihydroxybenzoic acid matrix. Spectra were analyzed using the accompanying TOF/TOF Series Explorer software.

Abbreviations:

DCM dichloromethane
DIPEA diisopropylethylamine
DMF *N*,*N*-dimethylformamide
HATU *N*,*N*,*N*′,*N*′-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate
HBTU *N*,*N*,*N*′,*N*′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
HCTU *N*,*N*,*N*′,*N*′-tetramethyl-O-(6-chlorobenzotriazol-1-yl)uronium hexafluorophosphate
HFIP 1,1,1,3,3,3-hexafluoro-2-propanol
HPLC high-performance liquid chromatography
HOBt hydroxybenzotriazole
MALDI matrix-assisted laser desorption ionization
MeOH methanol
MeCN acetonitrile

TFA trifluoroacetic acid

TIPS triisopropylsilane

Synthesis of Peptide 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 by analytical HPLC and MALDI mass spectrometry.

Resin Loading

2-Chlorotrityl chloride resin (0.300 g, 1.6 mmol/g, 100-200 mesh) was added to a 10-mL Bio-Rad Poly-Prep chromatography column (8 mm x 40 mm). The resin was suspended in dry DCM (8 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 (150.0 mg, 0.33 mmol) in 20% (v/v) 2,4,6-collidine in dry DCM (8 mL) was added immediately. The suspension was gently agitated for 24 h. The solution was then drained using nitrogen and washed with dry DCM (3x). After washing, a mixture of DCM/MeOH/DIPEA (8.5:1:0.5, 8 mL) was added immediately. The suspension was gently agitated for 30 min to cap any unreacted sites on the resin. The resin was washed with DMF (3x) and dried by passing nitrogen through the chromatography column. The resin loading was determined to be 0.61 mmol/g based on UV analysis (290 nm) of the Fmoc cleavage product.

Solid-Phase Peptide Synthesis

The Boc-ornithine(Fmoc) loaded resin was transferred to a solid-phase peptide synthesizer reaction vessel designed for the Protein Technologies PS3 automated peptide synthesizer. 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 (2x); (2) resin washing with DMF (3x); (3) activation of the Fmoc protected amino acid (4 equiv) with 20% (v/v) 2,4,6-collidine in DMF (8 mL) in the presence of HCTU (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 the tryptophan that followed the *N*-methyl-leucine. The tryptophan that followed the *N*-methyl-leucine (4 equiv) was coupled twice for 1 h each with HATU (4 equiv) in 20% (v/v) 2,4,6-collidine in DMF (8 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 column.

Protected Cleavage of the Linear Peptide

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 (8 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 (8 mL) and agitated for another 30 min. The suspension was filtered and the filtrate collected into the same round-bottom flask as the previous filtrate. The suspension was then washed with DCM (3 x 2 mL) until the resin was no longer red with all washes collected into the round-bottom flask. The combined filtrates were concentrated under reduced pressure to give a clear film. The protected linear peptide derivative of macrocyclic β-sheet **1** was then cyclized without prior purification.

Cyclization

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 (0.150 g, 1.11 mmol, 6.2 equiv) and HBTU (0.300 g, 0.79 mmol, 4.4 equiv) were dissolved in 8 mL of dry DMF in a test tube to which 300 μL of 2,4,6-collidine was added and the solution mixed until homogenous. The solution was then added to the flask and the mixture was stirred under nitrogen at room temperature for 96 h. The reaction mixture was concentrated under reduced pressure to give a white solid. The crude product was immediately subjected to global deprotection.

Global Deprotection of Acid-Labile Protecting Groups and Ether Precipitation

The protected cyclic peptide derivative of macrocyclic β-sheet **1** was dissolved in a mixture of TFA/TIPS/H_2O (9.0:0.5:0.5, 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. During the 1 h deprotection, two 50-mL conical tubes containing 40 mL of dry ether each were chilled on dry ice. Following the 1 h time, the peptide solution was split between the two conical tubes of ether. The tubes were then centrifuged at 500 *x g* for 15 min. The ether supernatant was poured off and the pelleted peptides dried under nitrogen for 15-20 min.

Purification of Peptide 1

The crude peptide pellets were dissolved in 15% MeCN/H_2O (4 mL), and the solution was centrifuged at 500 *x g* for 5 min to pellet any insoluble material. The supernatant was then filtered through a 0.2 μm nylon syringe filter. The crude peptide was purified by reverse-phase HPLC following a gradient whereby the percentage of MeCN in the mobile phase was increased at a rate of 0.2%/min. Elution occurred at ca. 28% MeCN/H_2O. The pure fractions were lyophilized to afford 52.6 mg (13.9 % based on resin loading) of peptide **1** as the trifluoroacetate salt as a white powder.

Crystal Screening[3]

A 10 mg/mL stock solution of peptide was prepared using 18 MΩ water. Two 96-well screening kits (Index and Crystal) from Hampton Research were used to screen crystallization conditions. The peptide was screened against 576 crystal growing conditions with a nanoliter liquid handling instrument (TTP Labtech Mosquito) using the 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 0.02 M calcium chloride dihydrate, 0.1 M sodium acetate trihydrate pH 4.6, and 30% v/v (+/-)-2-methyl-2,4-pentanediol were identified (Crystal, A1). Optimization of the crystal growing conditions using Hampton VDX 24-well plates and varying the pH of the sodium acetate and the concentration of the (+/-)-2-methyl-2,4-pentanediol resulted in larger crystal growth in 0.02 M calcium chloride dihydrate, 0.1 M sodium acetate trihydrate pH 6.5, and 30% v/v (+/-)-2-methyl-2,4-pentanediol. The best X-ray diffracting crystals grew as cubes. To facilitate crystallographic phasing, crystals were also grown in 0.02 M barium chloride, 0.1 M

sodium acetate trihydrate pH 6.0, and 30% v/v (+/-)-2-methyl-2,4-pentanediol to afford the same cubic crystals.

X-Ray Crystallography

Diffraction data for the barium salt of peptide **1** were collected at the Advanced Light Source at Lawrence Berkeley National Laboratory beamline 8.2.2. The dataset was indexed and integrated with XDS,[4] scaled and merged with pointless and aimless in CCP4.[5] The crystallographic phase determination was done with Autosol.[6] The structure was refined using phenix.refine,[7] with manipulation of the model performed using Coot.[8]

Diffraction data for the calcium salt of peptide **1** were collected using a Bruker Microstar APEX II CCD diffractometer equipped with Cu-K_α source (1.54178 Å) up to a resolution of 1.32 Å. The dataset was processed with SAINT (Bruker), and scaled using SADABS. The structure was solved through isomorphous replacement using the previously refined structure of the barium salt, and refined with phenix.refine. Data collection and refinement statistics are shown below in Supplementary Table 1.

Supplementary Table 1. Crystallographic properties, crystallization conditions, data collection, and model refinement statistics for the calcium salt of peptide **1**.

peptide	**1**
PDB ID	7JRH
Wavelength / Å	1.54178
Resolution range / Å	21.24 - 1.321 (1.368 - 1.321)
Space group	I23
Unit cell parameters	42.486 42.486 42.486 90 90 90
Total reflections	51865 (2021)
Unique reflections	3117 (315)
Multiplicity	16.6 (6.4)
Completeness / %	99.74 (99.83)
Mean I/σ(I)	30.81 (3.32)
Wilson B-factor	15.04
R_{merge}	0.04568 (0.3153)
R_{meas}	0.04648 (0.3423)
R_{pim}	0.008085 (0.1275)
$CC_{1/2}$	1.000 (0.925)
CC*	1.000 (0.980)
Reflections used in refinement	3117 (315)
Reflections used for R_{free}	311 (35)
R_{work}	0.1779 (0.3195)
R_{free}	0.2106 (0.2877)
$CC_{(work)}$	0.987 (0.659)
$CC_{(free)}$	0.937 (0.695)
Number of non-hydrogen atoms	143
macromolecules (peptide)	130
ligands (Ca^{2+})	2
solvent (H_2O)	11
Protein residues	16
RMS(bonds)	0.011
RMS(angles)	1.62
Ramachandran favored (%)	100.00
Ramachandran allowed (%)	0.00
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	7.97
Average B-factor	18.59
macromolecules	18.04
ligands	22.62
solvent	24.31

References:

1. The chemicals and instruments required for the synthesis of macrocyclic β-sheet **1** are similar to those used in our laboratory's previous publications. This information was either adapted from or taken verbatim from Chen, K. H.; Corro, K. A.; Le, S. P.; Nowick, J. S. X-ray Crystallographic Structure of a Giant Double-Walled Peptide Nanotube Formed by a Macrocyclic β-Sheet Containing $A\beta_{16-22}$. *J. Am. Chem. Soc.* **2017**, *139*, 8102-8105.

2. Macrocyclic β-sheet **1** was synthesized following a protocol similar to those published previously by our laboratory. The procedures were either adapted from or taken verbatim from: Spencer, R.; Chen, K. H.; Manuel, G.; Nowick, J. S. Recipe for β-Sheets: Foldamers Containing Amyloidogenic Peptide Sequences. *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. Stabilization, Assembly, and Toxicity of Trimers Derived from Aβ. *J. Am. Chem. Soc.* **2017**, *139*, 966–975.

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