Supporting information for:

Square channels formed by a peptide derived from transthyretin

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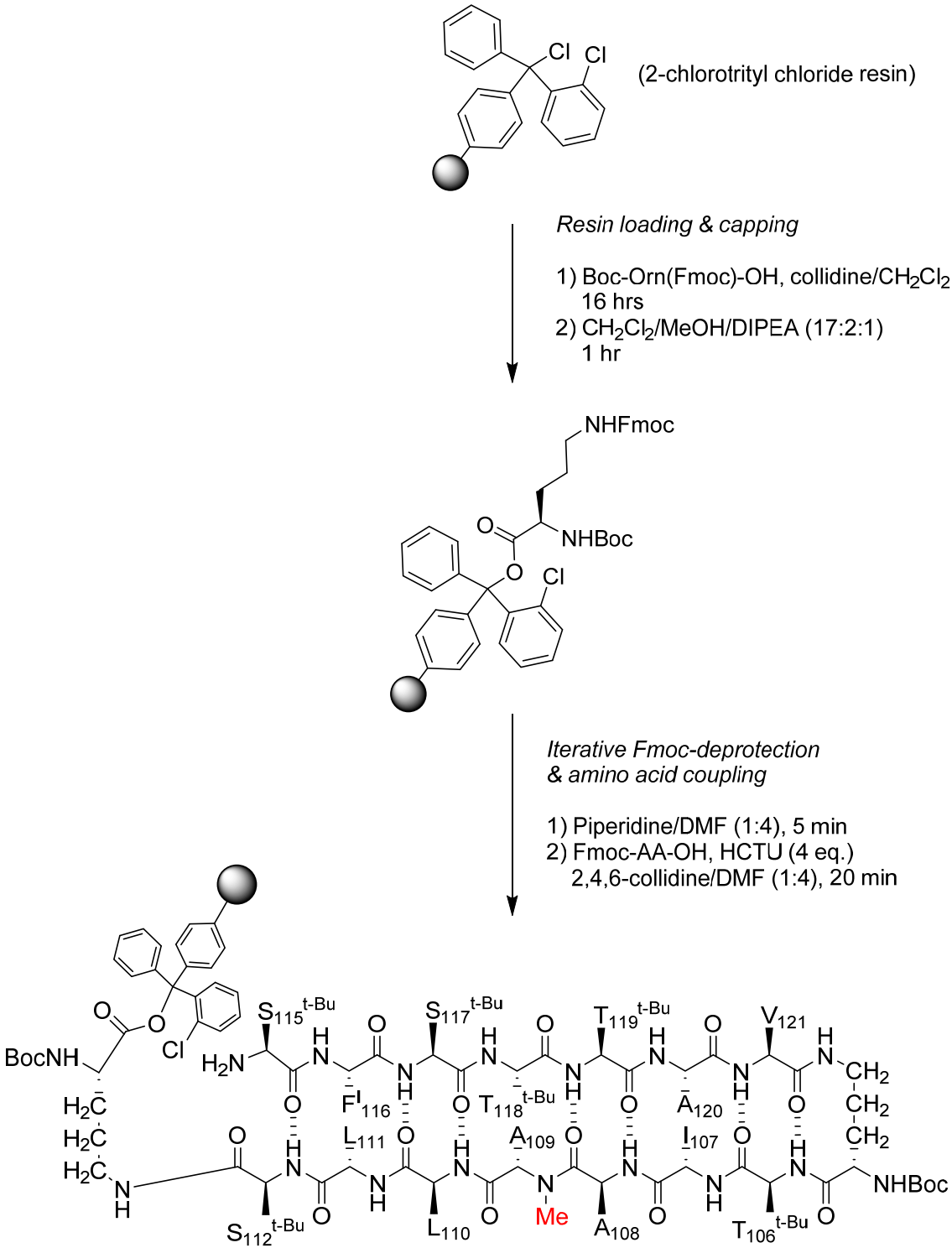
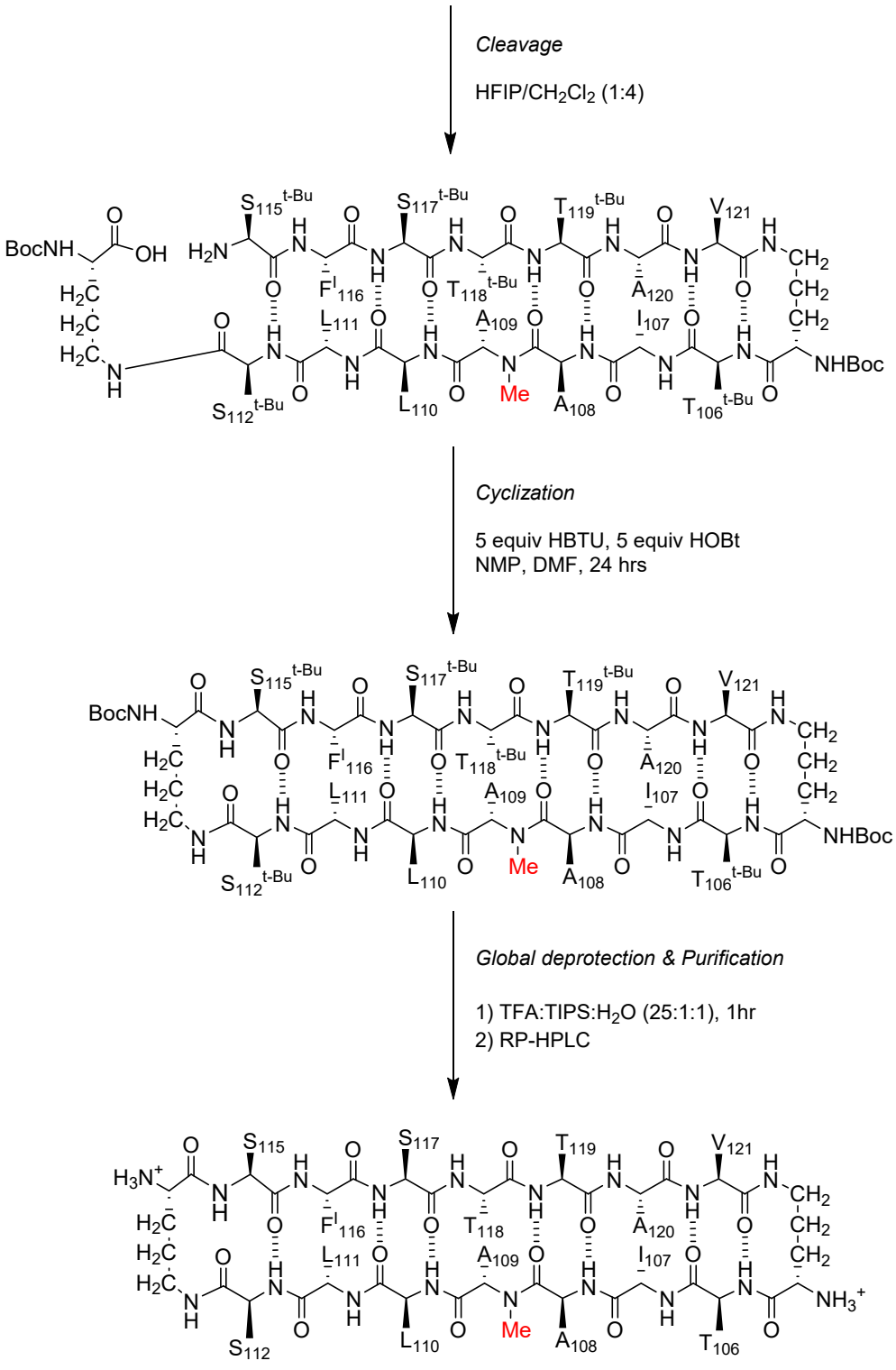
Scheme S1. Synthesis of peptides **1**





Peptide **1** (TFA salt)

Procedure for the synthesis of peptide 1

Loading resin: 2-Chlorotrityl chloride resin (300 mg, 1.2 g/mol) was added to a Bio-Rad Poly-Prep column. The resin was swollen for 15 minutes in CH_2Cl_2, and the solvent was drained. A 8 mL solution of Boc-Orn(Fmoc)-OH (0.6 equiv, 80 mg, 0.22 mmol) in 4% 2,4,6-collidine in CH_2Cl_2 was added to the resin. The resin was left on a rocker overnight (~16 hours). The solution was drained and a solution of CH_2Cl_2/MeOH/DIPEA (17:2:1) was added to the resin to cap unreacted sites. The suspension was rocked for one hour. The solution was drained and transferred into a reaction vessel for synthesizer or hand-coupling.

Peptide synthesis: The iterative procedure of Fmoc-deprotection and amino acid coupling was done on the PS3 synthesizer or manually as follows: (1) Fmoc-deprotection with 5 mL of 20% piperidine in DMF (5 minutes); (2) washing the resin 3 times with 5 mL DMF; (3) amino acid coupling with 4 equiv of appropriate amino acid (Fmoc-AA-OH) and 4 equiv of HCTU, in 20% 2,4,6-collidine in DMF for 20 minutes. Alanine 108, which follows *N*-methylalanine 109, was coupled twice with 4 equiv of HATU and 4 equiv of HOAt (for one hour per coupling). (4) washing the resin 3 times with 5 mL DMF; (5) final deprotection of Fmoc from the last amino acid coupled; (6) washing 3 times with 5 mL DMF.

Cleavage of the peptide from resin: Resin was transferred to a Bio-Rad Poly-Prep column, washed with 10 mL of CH_2Cl_2, and dried under N_2. A solution of 8 mL of 20% hexafluoroisopropanol (HFIP) in CH_2Cl_2 was added to the resin and agitated for 40 minutes. The filtrate was drained into a 250 mL round bottom flask and the resin was washed with 5 mL CH_2Cl_2. [The resin turns red upon an addition of HFIP solution and turns light yellow/green upon washing with CH_2Cl_2.] An additional 8 mL of 20% HFIP in CH_2Cl_2 was added and followed by the same procedure. The combined solution was concentrated *in vacuo*, yielding a yellow thin film around the flask.

Cyclization of the peptide: The linear peptide, HOBt (4 equiv), and HBTU (4 equiv) was dissolved in 125 mL DMF and stirred. After 10 minutes of stirring, 0.3 mL of *N*-methylmorpholine was added to the solution and stirred overnight under N_2. The solution was concentrated *in vacuo* to affords the crude cyclic peptide as a yellow oil.

Global deprotection and isolation: The crude cyclized peptide was dissolved in a 20 mL solution of TFA/TIPS/H_2O (25:1:1) and stirred for 1 hour. The solution was concentrated *in vacuo*. The crude peptide was dissolved in 20% ACN in water and filtered through a 0.2 μm syringe filter. The peptide was purified with a C18 column coupled to Beckman preparative HPLC. The fractions were collected over a gradient of 20-60% ACN in water each containing 0.1% TFA. The pure fractions that contained the desired peptide were combined, concentrated *in vacuo*, frozen, and lyophilized to afford the peptide as a white fluffy solid.

Crystallization procedure for peptide 1

Initial screening: Peptide **1** was screened in 864 crystallization conditions using Hampton Research crystallization kits (Crystal Screen, Index, and PEG/ION). This screen was performed using the hanging drop vapor diffusion method in 96-well plates with three 150-nL drops per well. In each hanging drop, a 10 mg/mL solution of peptide **1** in filtered deionized water (18 MΩ) was combined with the crystallization buffer in 1:1, 1:2, and 2:1 ratio. The 96-well plates were set up with a TTP Labtech Mosquito pipetting robot. The 96-well plates were examined daily under a microscope for next seven days to check for crystal growths.

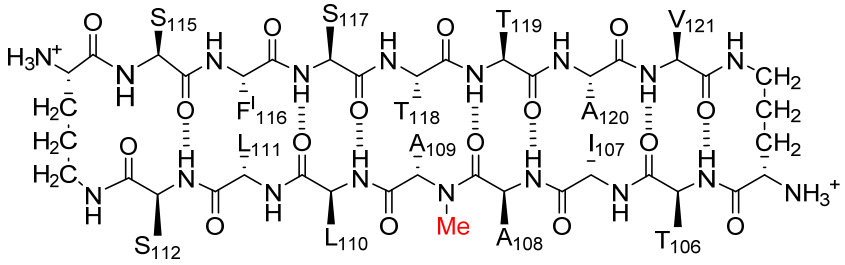
Optimization: Crystallization conditions that grew crystals in the initial screening were optimized further in 4x6 Hampton VDX 24-well plates. The hanging drop vapor diffusion method was used. Each well in optimization plates was set up to contain 1 mL of crystallization buffer based on conditions from the initial screening by varying pH and percent co-solvents. Each hanging drop on siliconized glass cover slides from Hampton Research contained of 10 mg/mL solution of peptide **1** in water and crystallization buffer in 1:1 ratio (2 μL drop), 1:2 ratio (3 μL drop), and 2:1 ratio (3 μL drop). The cover slides were inverted and pressed down onto the 24-well plates with silicon grease to provide a sealed environment in each well.

Data collection and data processing: Data was collected on a Rigaku Micromax-007 HF diffractometer with a Cu rotating anode at 1.54 Å wavelength. The data were integrated, scaled, and merged using iMosflm. Hybrid structure search (HySS) in the Phenix software suite was used to determine the coordinates of the anomalous signal. The electron density maps were generated using the coordinates of the iodine anomalous signal as initial positions in Autosol. Manipulation of the model coordinates was done in Coot. Models were refined with phenix.refine. Table S1 shows the refinement statistics for peptide **1**.

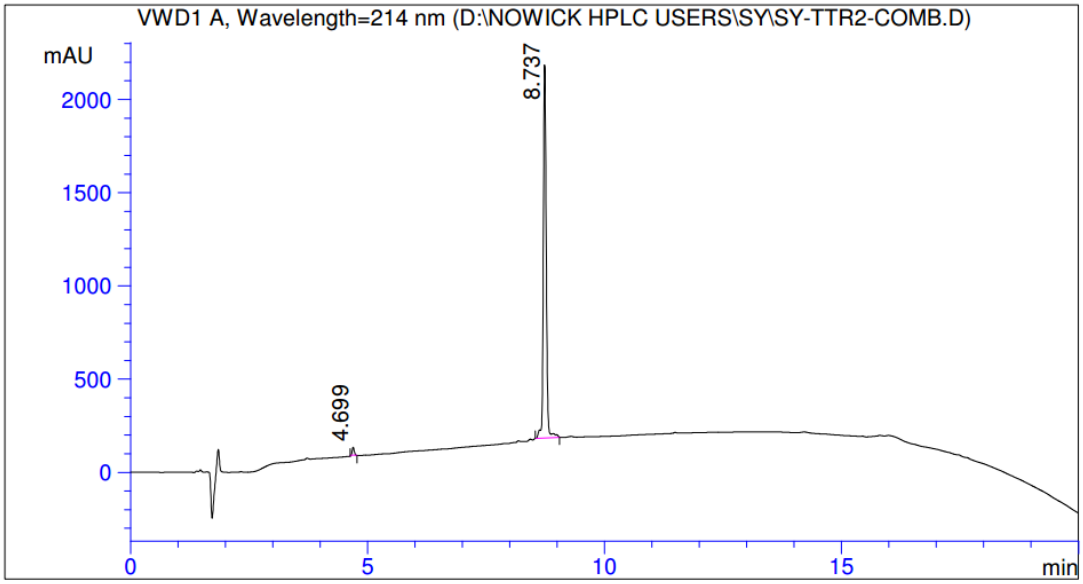
Table S1. **Table S1. Data collection and refinement statistics for peptide 1**

PDB ID	5HPP
space group	$P4_32_12$
a, *b*, *c* (Å)	42.3, 42.3, 16.3
α, β, λ (°)	90, 90, 90
peptide per asymmetric unit	1
crystallization conditions	0.1 M NaOAc buffer at pH 5.3, 0.2 M $CaCl_2$, and 31% isopropanol
wavelength (Å)	1.54
resolution (Å)	21.15–2.083 (2.157–2.083)
total reflections	2053 (200)
unique reflections	1027 (98)
multiplicity	2.0 (2.0)
completeness (%)	100 (100)
mean I/σ	13.92 (5.49)
Wilson B factor	14.51
R_{merge}	0.04945 (0.1101)
$R_{measure}$	0.06993 (0.1557)
$CC_{1/2}$	0.994 (0.956)
CC^*	0.999 (0.989)
R_{work}	0.1661 (0.1637)
R_{free}	0.1843 (0.2251)
number of non-hydrogen atoms	129
RMS_{bonds}	0.018
RMS_{angles}	1.30
average B-factor	19.33
Number of TLS groups	1

HPLC and ESI-MS of peptide 1

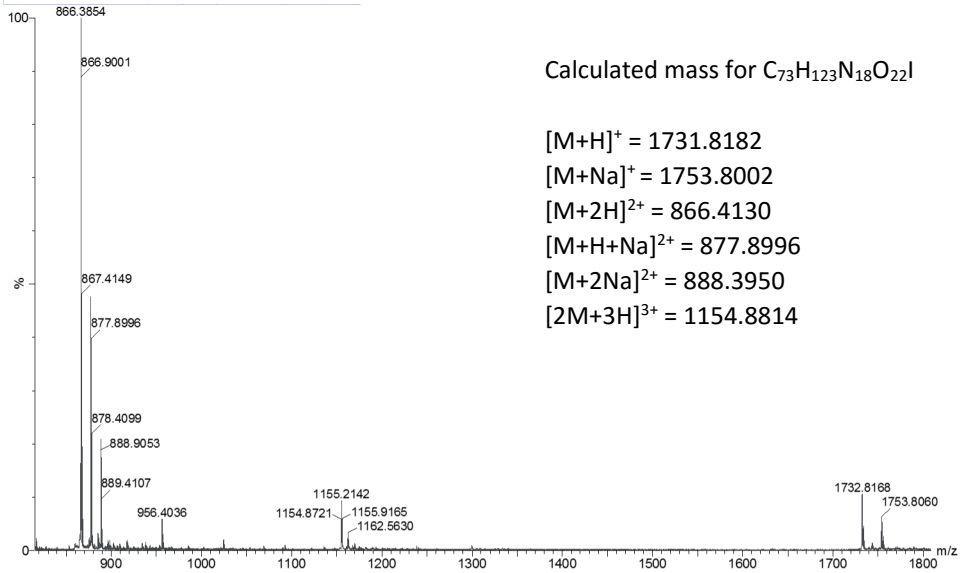


HPLC condition: 5-95% acetonitrile over 20 minutes



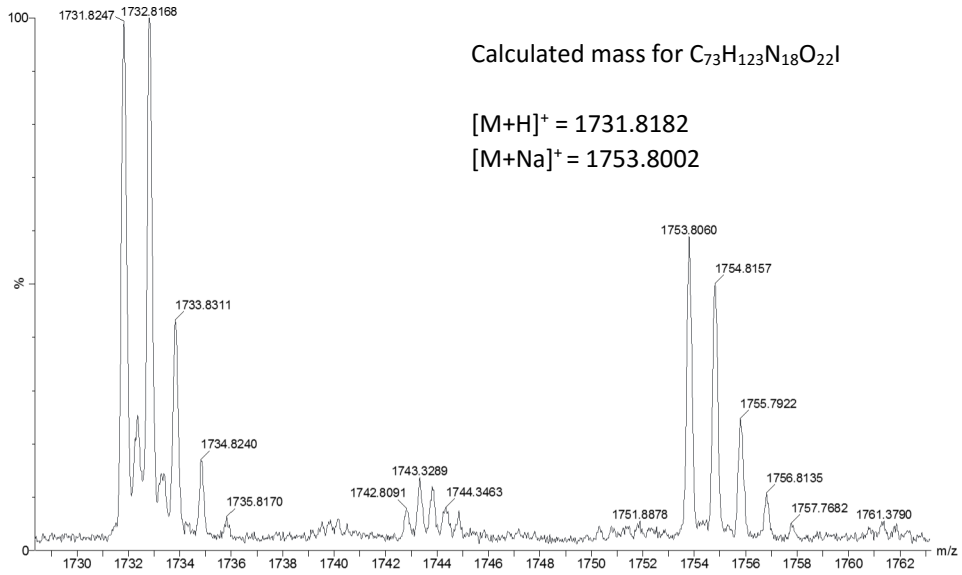
Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	4.699	MM	0.045	122.752	2.166	1.393
2	8.737	MM	0.071	8690.231	97.834	98.607



Calculated mass for $C_{73}H_{123}N_{18}O_{22}I$

$[M+H]^+$ = 1731.8182
$[M+Na]^+$ = 1753.8002
$[M+2H]^{2+}$ = 866.4130
$[M+H+Na]^{2+}$ = 877.8996
$[M+2Na]^{2+}$ = 888.3950
$[2M+3H]^{3+}$ = 1154.8814



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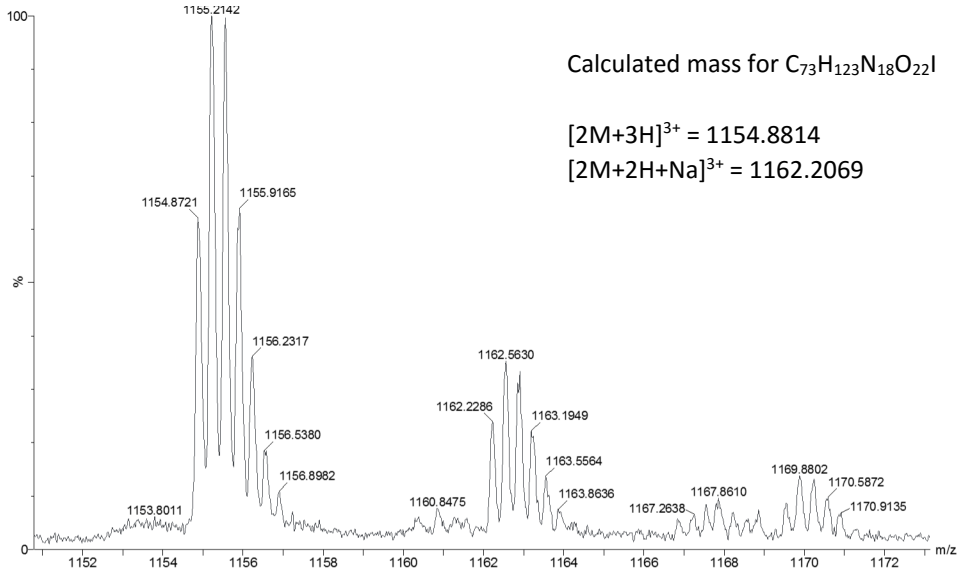
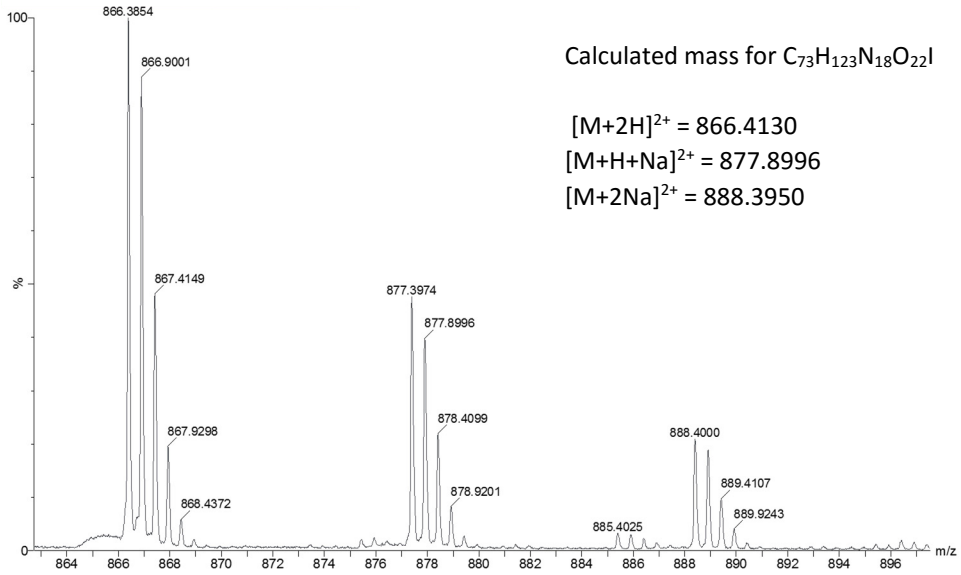


Calculated mass for $C_{73}H_{123}N_{18}O_{22}I$

$[2M+3H]^{3+}$ = 1154.8814
$[2M+2H+Na]^{3+}$ = 1162.2069



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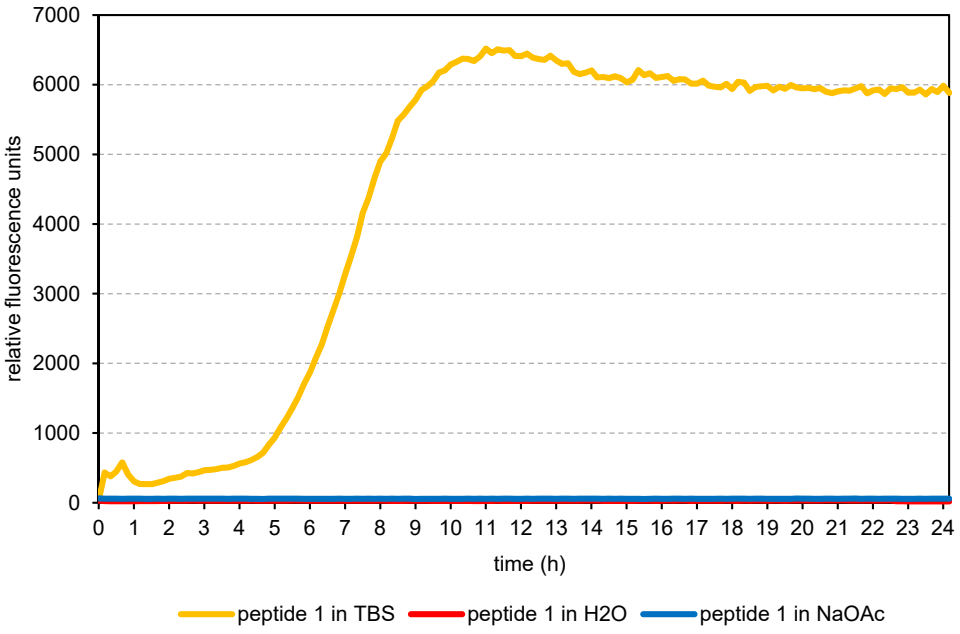
Thioflavin T fluorescence assays of peptide 1

Thioflavin T (ThT) fluorescence assays were used to monitor the aggregation of peptide **1**. Peptide **1** was suspended in aqueous solutions of H_2O, 100 mM NaOAc at pH 5.3, and 1X Tris buffered saline (TBS) in the presence of ThT. ThT fluorescence was monitored over 24 hours at 37°C using a fluorescence plate reader. The peptide did aggregate in TBS, indicated by elevated fluorescence, but did not aggregate in H_2O or in 100 mM NaOAc, which is similar to the crystallization condition. In TBS, the ThT-active species started to form after 4 hours of incubation and plateaued after 9 hours. These data show that peptide **1** can aggregate to form ThT-active species in aqueous solution, which might resemble the extended β-sheets observed in the X-ray crystallographic structure.

The ThT fluorescence assays were conducted in a 96-well plate with shaking in a Gemini XPS fluorescence plate reader (Molecular Devices, 442 nm excitation, 482 nm emission) at 37 °C. For each well, the mixture of 60 μM peptide **1** and 100 μM ThT was subjected to the equal volume of H_2O, 2X TBS, and 200 mM pH 5.3 NaOAc to prepare the solution of 30 μM peptide, 50 μM ThT in H_2O, 1X TBS (50 μM Tris, 100 mM NaCl, pH 7.5), or 100 mM NaOAc. Five replicate runs were performed to ensure reproducibility.



Circular dichroism of peptide 1

Circular dichroism (CD) spectroscopy of peptide **1** was performed in TBS to further study the aggregation. Peptide **1** was incubated at 37 °C and several CD spectra were acquired over the course of 25 hours. At 0 hours, the peptide exhibited mostly random coil and little β-sheet structure. The peptide developed more β-sheet structure over time, where the spectra show a maximum near 200 nm and a minimum near 220 nm. After 25 hours, the peptide exhibited a characteristic β-sheet maximum at 197 nm and minimum at 222 nm. These spectra corroborate the ThT fluorescence assay and further suggests that peptide **1** forms extended β-sheets in aqueous solution.

The CD spectra were recorded on a 30 μM solution of peptide **1** in TBS. A stock solution was prepared by combining 60 μM of the peptide in 18 MΩ deionized water and 2X TBS (100 μM Tris, 200 mM NaCl, pH 7.5) in 1:1 ratio. The stock solution was shaken in an incubator at 37 °C and aliquots were removed for time-point measurements. CD spectra were acquired on a Jasco J-810 circular dichroism spectropolarimeter at ambient temperature (20 °C) using 1 mm quartz cuvette. Data were collected from 260 nm to 190 nm and averaged over 5 accumulations. Spectra were smoothened using Savitsky-Golay smoothing. Data are graphed as mean residue ellipticity, [Θ], which is calculated as follows:

$$[\Theta] = \text{millidegrees} / (\text{path length(mm)} \times [\text{peptide}]\ (\text{M}) \times \text{number of residues})$$