Supporting Information for:

X-ray Crystallographic Structures of a Trimer, Dodecamer, and Annular Pore Formed by an $A\beta_{17–36}$ β-Hairpin

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Supplemental Figures and Table

Materials and Methods

Supplemental Figures and Table

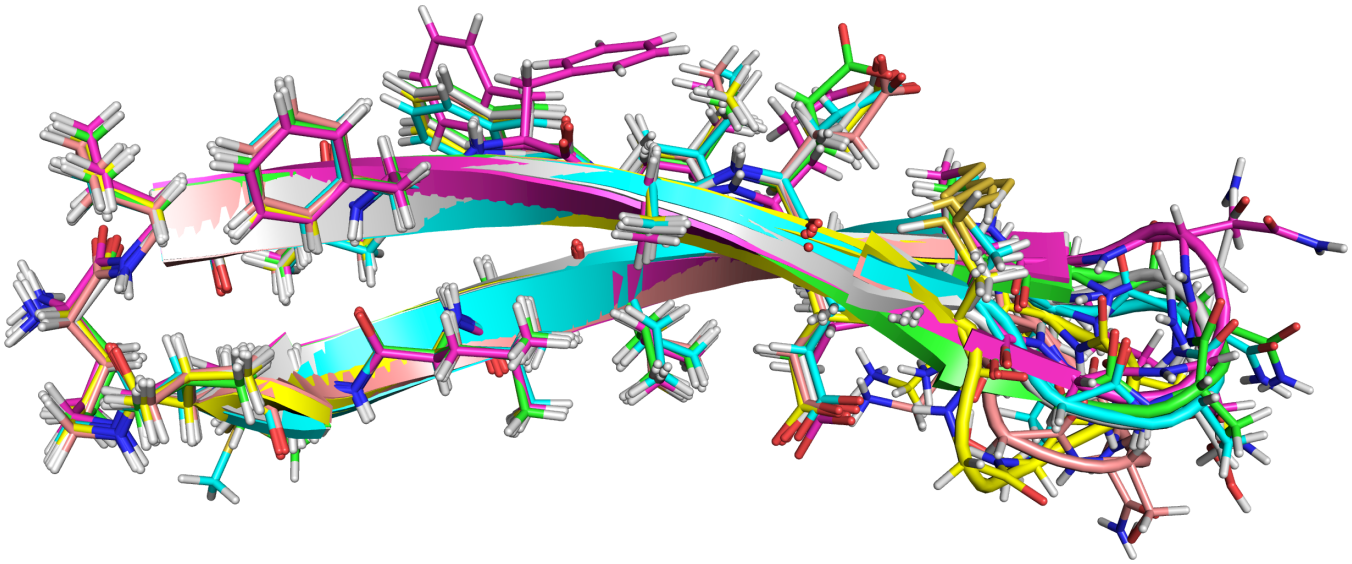


Figure S1. Alignment of the six monomers in the asymmetric unit of peptide **2**.

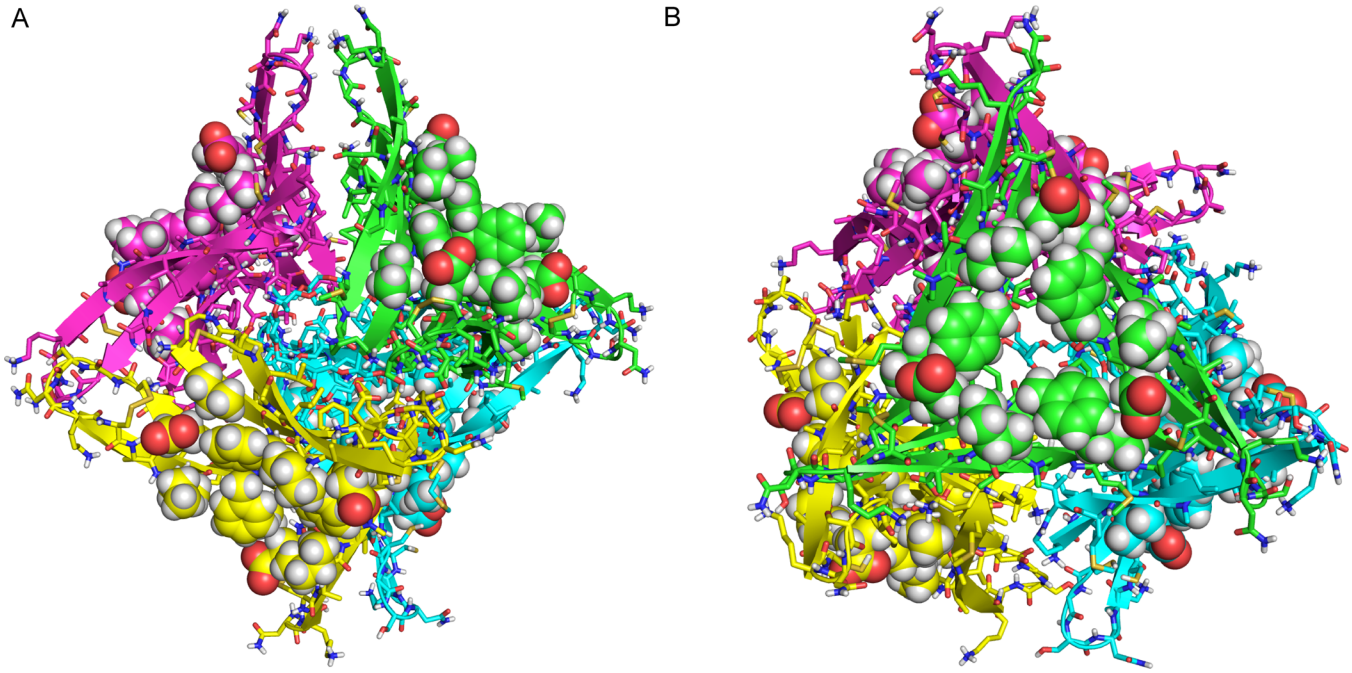


Figure S2. The dodecamer formed by peptide **2** displays four F_{20} faces. (A) Octahedral shape of the dodecamer. The residues of the four F_{20} faces of trimers that comprise the dodecamer are shown as spheres. (B) Tetrahedral arrangement of trimers that comprise the dodecamer. The residues of the four F_{20} faces of trimers that comprise the dodecamer are shown as spheres.

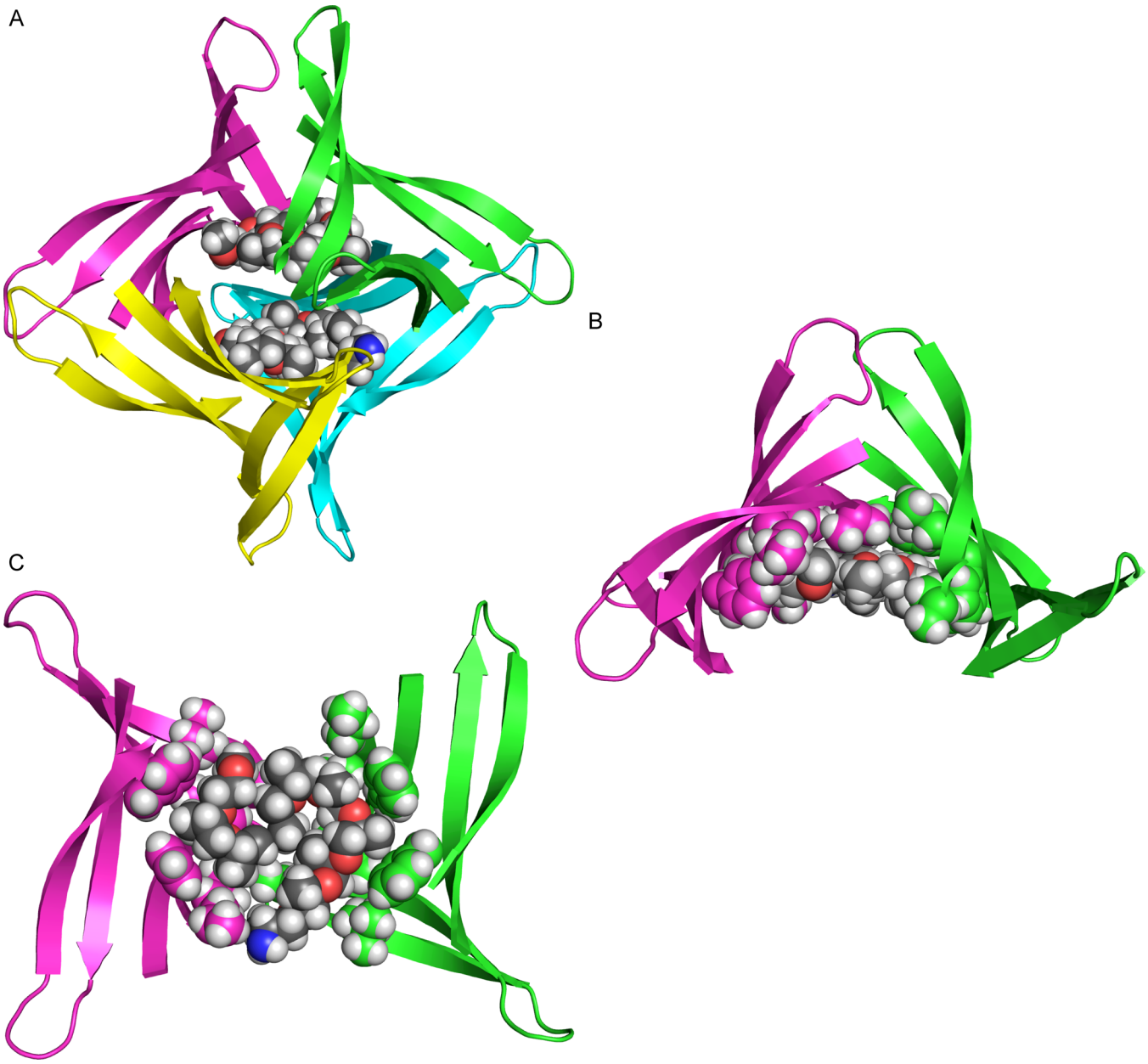


Figure S3. Jeffamine M-600 occupies the central cavity of the dodecamer. (A) The dodecamer formed by peptide **2** (cartoons) showing two molecules—one per asymmetric unit—of Jeffamine M-600 (grey) occupying the central cavity of the dodecamer. (B) View of two trimer subunits (magenta and green) from inside the cavity of the dodecamer. In panels B and C Jeffamine M-600 (grey) and key side chains are shown as spheres to illustrate the hydrophobic packing that occurs between Jeffamine M-600 and the side chains that line the central cavity. (C) Side view of two trimer subunits.

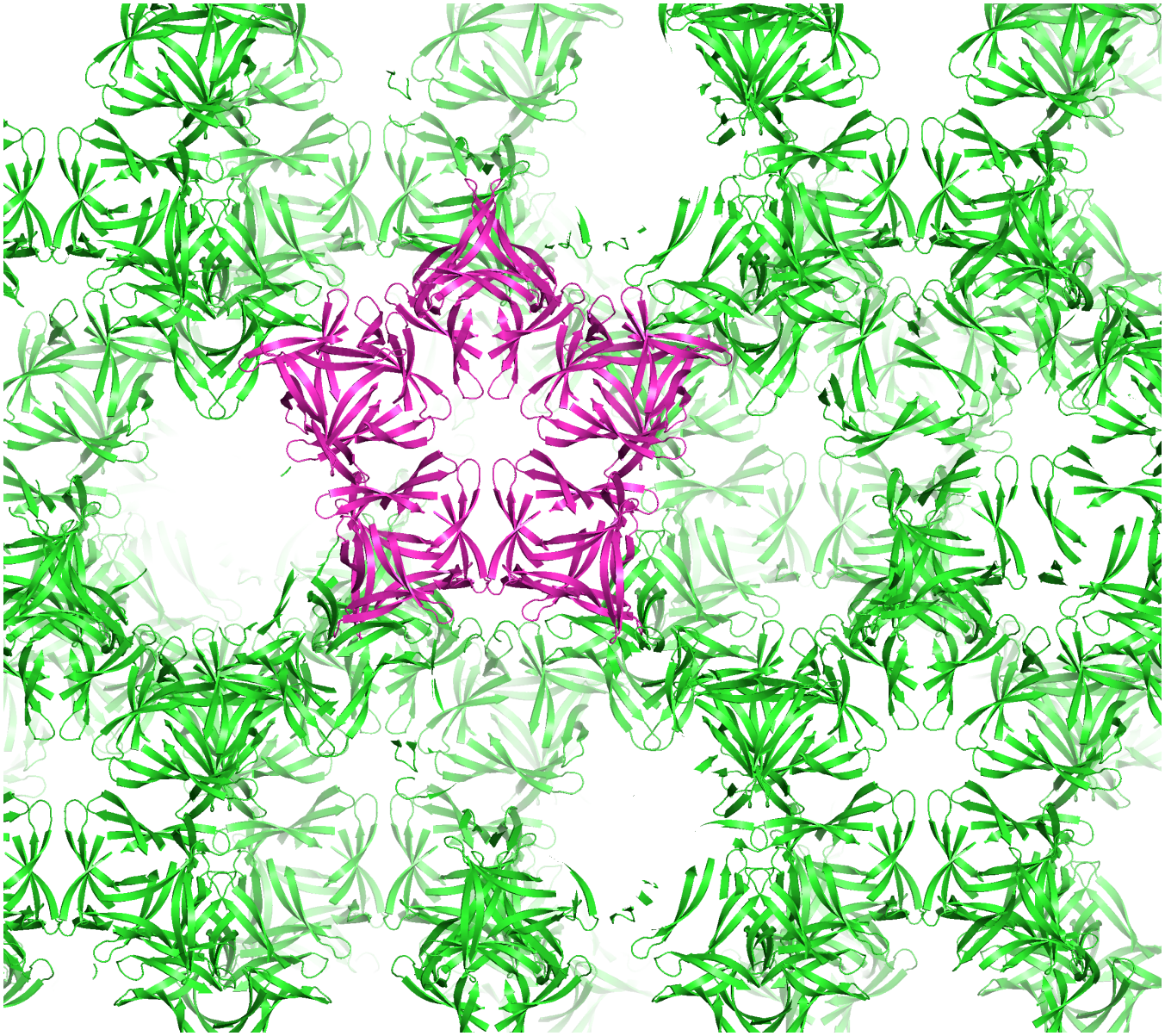


Figure S4. View of the annular pore formed by peptide **2** within the crystal lattice.

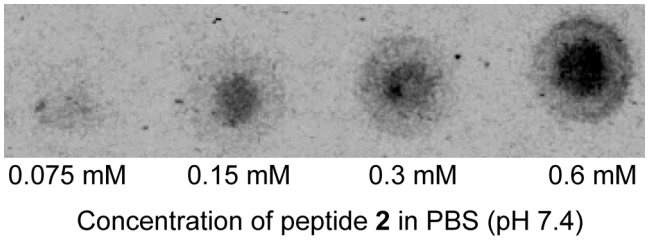


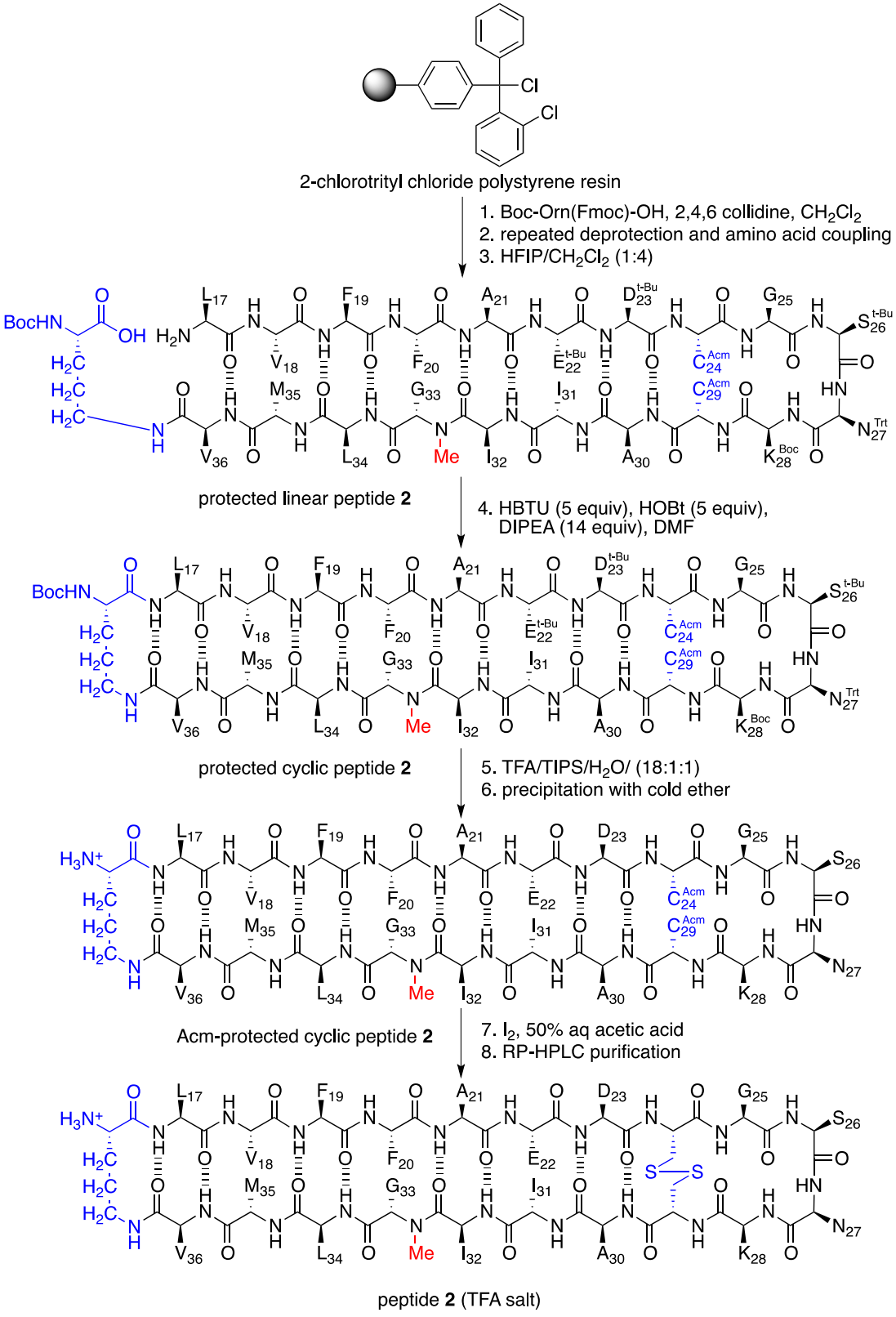
Figure S5. Dot blot showing reactivity of peptide **2** with the A11 antibody.

Table S1. Crystallographic properties, crystallization conditions, and data collection and model refinement statistics for peptides **2** and **4**.

peptide	peptide **2** (synchrotron)	peptide **2** (X-ray diffractometer)	peptide **4** (X-ray diffractometer)
PDB ID	5HOX	5HOY	5HOW
space group	$P6_122$	$P6_122$	$P6_122$
a, *b*, *c* (Å)	97.37, 97.37, 97.59	97.65, 97.65, 97.78	97.31, 97.31, 97.62
α, β, λ (°)	90, 90, 120	90, 90, 120	90, 90, 120
peptide per asymmetric unit	6	6	6
crystallization conditions	0.1 M HEPES, pH 7.1, 29% Jeffamine M-600	0.1 M HEPES, pH 7.1, 29% Jeffamine M-600	0.1 M HEPES, pH 6.4, 31% Jeffamine M-600
	Data collection		
wavelength (Å)	1.00	1.54	1.54
resolution (Å)	30.35–1.9 (1.968–1.900)	28.19–2.295 (2.377–2.295)	28.09–2.295 (2.377–2.295)
total reflections	44136 (4348)	25663 (2527)	25402 (2476)
unique reflections	22069 (2173)	12832 (1264)	12701 (1238)
multiplicity	2.0 (2.0)	2.0 (2.0)	2.0 (2.0)
completeness (%)	99 (100)	100 (100)	100 (99)
mean I/σ	13.82 (0.59)	11.44 (1.01)	15.73 (1.41)
Wilson B factor	46.44	55.58	49.69
R_{merge}	0.01344 (0.9116)	0.03762 (0.6526)	0.05629 (0.4378)
$R_{measure}$	0.019 (1.289)	0.05321 (0.9229)	0.07961 (0.6191)
$CC_{1/2}$	1 (0.597)	0.999 (0.275)	0.994 (0.528)
CC^*	1 (0.865)	1 (0.657)	0.999 (0.831)
	Refinement		
R_{work}	0.2199 (0.4081)	0.2446 (0.3921)	0.2415 (0.3656)
R_{free}	0.2505 (0.4048)	0.2755 (0.4228)	0.2700 (0.4170)
number of non-hydrogen atoms	1020	1011	973
RMS_{bonds}	0.027	0.021	0.025
RMS_{angles}	1.67	0.91	1.24
Ramachandran favored (%)	93	90	88
outliers (%)	0.97	0	0
clashscore	2.53	1.52	0.00
average B-factor	76.90	72.49	70.28
Number of TLS groups	15	13	17

Materials and Methods

Scheme S1. Synthesis of peptide **2**.



General information

All chemicals were used as received unless otherwise noted. Methylene chloride (CH_2Cl_2) was passed through alumina under nitrogen prior to use. Anhydrous, amine free dimethylformamide (DMF) was purchased from Alfa Aesar. Analytical reverse-phase HPLC was performed on an Agilent 1200 equipped with an Aeris PEPTIDE 2.6u XB-C18 column (Phenomonex). Semi-preparative reverse-phase HPLC was performed on a Beckman Gold Series P equipped with a Zorbax SB-C18 column (Agilent). HPLC grade acetonitrile and 18 MΩ H_2O, each containing 0.1% trifluoroacetic acid (TFA), were used for analytical and semi-preparative reverse-phase HPLC. All peptides were prepared and used as the trifluoroacetate salts, and were assumed to have one trifluoroacetic acid per ammonium group on each peptide.

*Synthesis of peptides **2**–**4***[1]

a. *Loading of the resin.* 2-Chlorotrityl chloride resin (300 mg, 1.2 mmol/g) was added to a Bio-Rad Poly-Prep chromatography column (10 mL, 0.8×4.0 cm). The resin was suspended in dry CH_2Cl_2 (10 mL) and allowed to swell for 30 min. The solution was drained from the resin and a solution of Boc-Orn(Fmoc)-OH (0.50 equiv, 82 mg, 0.18 mmol) in 6% (v/v) 2,4,6-collidine in dry CH_2Cl_2 (8 mL) was added immediately and the mixture was gently agitated for 12 h. The solution was then drained and a mixture of CH_2Cl_2/MeOH/*N*,*N*-diisopropylethylamine (DIPEA) (17:2:1, 10 mL) was added immediately. The mixture was gently agitated for 1 h to cap the unreacted 2-chlorotrityl chloride resin sites. The resin was then washed with dry CH_2Cl_2 (2x) and dried by passing nitrogen through the vessel. This procedure typically yields 0.12–0.15 mmol of loaded resin (0.4–0.5 mmol/g loading).

b. *Peptide coupling.* The 2-chlorotrityl-Orn(Fmoc)-Boc generated from the previous step was transferred to a microwave-assisted solid-phase peptide synthesizer reaction vessel and

submitted to cycles of automated peptide coupling with Fmoc-protected amino acid building blocks using a CEM Liberty 1 Automated Microwave Peptide Synthesizer. The linear peptide was synthesized from *C*-terminus to the *N*-terminus. Each coupling cycle consisted of i. Fmoc-deprotection with 20% (v/v) piperidine in DMF for 2 min. at 50 °C (2x), ii. washing with DMF (3x), iii. coupling of the amino acid (0.75 mmol, 5 equiv) in the presence of HCTU (0.675 mmol, 4.5 equiv) and 20% *N*-methylmorpholine (NMM) in DMF for 10 min. at 50 °C, iv. washing with DMF (3x). After coupling of the last amino acid, the terminal Fmoc group was removed with 20% (v/v) piperidine in DMF (10 min. 50 °C). The resin was transferred from the reaction vessel of the peptide synthesizer to a Bio-Rad Poly-Prep chromatography column.

c. *Cleavage of the peptide from the resin.* The linear peptide was cleaved from the resin by agitating the resin for 1 h with a solution of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in CH_2Cl_2. (1:4, 7 mL).[2] The suspension was filtered and the filtrate was collected in a 250 mL round-bottomed flask. The resin was washed with additional HFIP in CH_2Cl_2 (1:4, 7 mL) and then with CH_2Cl_2 (2×10 mL). The combined filtrates were concentrated by rotary evaporation to give a white solid. The white solid was further dried by vacuum pump to afford the crude protected linear peptide, which was macrolactamized without further purification.

d. *Macrolactamization of the linear peptide.* The crude protected linear peptide was dissolved in dry DMF (150 mL). HOBt (114 mg, 0.75 mmol, 5 equiv) and HBTU (317 mg, 0.75 mmol, 5 equiv) were added to the solution. DIPEA (0.33 mL, 1.8 mmol, 12 equiv) was added to the solution and the mixture was stirred under nitrogen for 24 h. The mixture was concentrated under reduced pressure to afford the crude protected cyclic peptide.

e. *Global deprotection of the acid-labile protecting groups.* The protected cyclic peptide was dissolved in TFA/triisopropylsilane (TIPS)/H_2O (18:1:1, 20 mL) in a 250 mL round-

bottomed flask equipped with a nitrogen-inlet adaptor. The solution was stirred for 1.5 h. The reaction mixture was then concentrated by rotary evaporation under reduced pressure to afford the crude cyclic peptide (peptide **3**), or the crude Acm-protected cyclic peptide (peptide **2** or peptide **4**) as a thin yellow film on the side of the round-bottomed flask. Peptide **3** was immediately subjected to purification by reverse-phase HPLC (RP-HPLC), as described below. Peptides **2** and **4** were precipitated with ice-cold ether and the Acm groups were removed to afford a disulfide linked cyclic peptide.

f. *Ether precipitation of peptides **2** and **4**.* The yellow film was dissolved in trifluoroacetic acid (TFA) (3 mL) and transferred in 0.5 mL aliquots to six different 15 mL polypropylene conical tubes. Ice-cold ether (14 mL) was added to each conical tube to precipitate the peptide. The peptide was pelleted by centrifugation and the supernatant was discarded [CAUTION: the conical tubes must be sealed tightly during centrifugation]. The peptide pellets were dissolved in acetonitrile (~10 mL per pellet), transferred to a 1 L round-bottomed flask, and the solution was concentrated by rotary evaporation under reduced pressure to afford a white solid. The white solid was further dried by vacuum pump to afford the crude Acm-protected cyclic peptide. The Acm groups were removed and the disulfide linkage was formed without further purification.

g. *Acm deprotection and disulfide linkage formation.* The 1 L flask containing the Acm-protected peptide was charged with 250 mL of 50% (v/v) aq acetic acid and swirled to dissolve the peptide. A 25 mL portion of 1.0 M HCl and a 5 mL portion of a solution of 0.10 M I_2 in glacial acetic acid was added, and the flask was fitted with a nitrogen-inlet adaptor. The solution was stirred magnetically for 2 h. The progress of the reaction was monitored by RP-HPLC and electrospray ionization mass spectrometry (ESI-MS). The reaction mixture was then

concentrated by rotary evaporation under reduced pressure to afford the crude disulfide-linked peptide as a brown solid. The brown solid was dissolved in TFA, and precipitated with ice-cold ether as described in the preceding section to afford the crude disulfide-linked peptide as an off-white solid.

h. *Reversed-phase HPLC purification of peptides **2**–**4**.* The peptide was dissolved in H_2O and acetonitrile (7:3, 10 mL), and the solution was filtered through a 0.2 μm syringe filter and purified by RP-HPLC (gradient elution with 20-50% CH_3CN over 50 min). The pure fractions were lyophilized to afford 15 mg of peptide **3**, 9 mg of peptide **4**, and 10 mg of peptide **2**.

*Crystallization procedure for peptides **2**–**4***[1]

Initial crystallization conditions were determined using the hanging-drop vapor-diffusion method. Crystallization conditions were screened for peptides **3** and **4** using three crystallization kits in a 96-well plate format (Hampton Index, PEG/Ion, and Crystal Screen). Three 150 nL hanging drops that differed in the ratio of peptide to well solution were made per condition in each 96-well plate for a total of 864 experiments. Hanging drops were made by combining an appropriate volume of peptide **3** (10 mg/mL in 18 MΩ water) or peptide **4** (10 mg/mL in 18 MΩ water) with an appropriate volume of well solution to create three 150 nL hanging drops with 1:1, 1:2, and 2:1 peptide:well solution. The hanging drops were made using a TTP LabTech Mosquito nanodisperse instrument. Peptide **3** did not grow crystals in any of the 864 conditions screened. Crystals of peptide **4** grew rapidly (~72 h) in a solution of 0.1 M HEPES buffer at pH 7.0 and Jeffamine M-600 at pH 7.0 (30% v/v).

We did not screen crystallization conditions for peptide **2**, as it afforded crystals suitable for X-ray diffraction in the same conditions, and with the same peptide concentration (10 mg/mL 18 MΩ water) as peptide **4**. Peptide **2** forms a cloudy suspension when a 10 mg/mL solution in 18 MΩ water is prepared. To better solvate peptide **2** for crystallographic experiments we sonicated the 10 mg/mL solution for ~2 h until it was less cloudy.

We optimized crystallization conditions for peptides **2** and **4** using a 4x6 matrix Hampton VDX 24-well plate. We varied the HEPES buffer pH in each row in increments of 0.5 pH units (6.5, 7.0, 7.5, and 8.0) and the pH 7.0 Jeffamine M-600 concentration in each column in increments of 2% (24%, 26%, 28%, 30%, 32%, and 34% v/v). For the first well in the 4x6 matrix we combined 100 μL of 1 M HEPES at pH 6.5, 480 μL of a 50% (v/v) solution of pH 7.0 Jeffamine M-600, and 420 μL of 18 MΩ water. [The 50% pH 7.0 Jeffamine M-600 solution was prepared by combining 200 mL of Jeffamine M-600 (pH 10) and 200 mL of 18 MΩ water, titrating with hydrochloric acid to pH 7.0, and filtering through a 0.2 μm syringe filter]. The other wells were prepared in analogous fashion, by combining 100 μL of HEPES buffer of varying pH, pH 7.0 Jeffamine M-600 in varying amounts, and 18 MΩ water for a total volume of 1 mL in each well.

Three hanging-drops were prepared per borosilicate glass slide by combining a solution of peptides **2** or **4** (1 μL, 10 mg/mL) and the well solution (1 μL) in a ratio of 1:1, 2:1, and 1:2. Slides were inverted and pressed firmly against the silicone grease surrounding each well. Crystals of peptides **2** and **4** grew in ~72 h. Crystallization conditions were further optimized using smaller variations in HEPES buffer pH (in increments of 0.25 pH units) and Jeffamine M-600 concentrations (in increments of 1%). Crystals were harvested with a nylon loop attached to

a copper or steel pin and flash frozen in liquid nitrogen prior to data collection. The optimized crystallization conditions for peptides **2** and **4** are summarized in Table S1.

*X-ray crystallographic data collection, data processing, and structure determination for peptides **2** and **4.***

Diffraction data for peptides **2** and **4** were collected on a Rigaku Micromax-007HF X-ray diffractometer with a rotating copper anode at 1.54 Å wavelength with 0.5° oscillation. Diffraction data were collected using CrystalClear. Diffraction data were scaled and merged using XDS.[4] Coordinates for the anomalous signals were determined by HySS in the Phenix software suite 1.10.1.[4] Electron density maps were generated using anomalous coordinates determined by HySS as initial positions in Autosol. Molecular manipulations of the models were performed with Coot. Coordinates were refined with phenix.refine.

Diffraction data for peptide **2** were also collected at Lawrence Berkeley National Laboratory (Berkeley, California) on synchrotron beamline 8.2.1 at 1.00 Å wavelength with 0.5° oscillation and a detector distance of 220 mm.[3] Diffraction data were scaled and merged using XDS.[4] Electron density maps were generated by isomorphous replacement of coordinates from peptide **4** using Phaser in software suite Phenix 1.10.1.[5] Molecular manipulations of the models were performed with Coot.[6] Coordinates were refined with phenix.refine.

*Dot blot analysis of peptide **2**.*

A 10 mg/mL (3.85 mM) stock solution of peptide **2** was prepared gravimetrically by dissolving 1.27 mg of peptide in 127 μL of 18 MΩ water. The stock solution was sonicated for at

least 2 h to further solvate the peptide. An aliquot of the stock solution was diluted with 18 MΩ water to make a 1.2 mM solution, which was serially diluted with 18 MΩ water to create 0.6 mM, 0.3mM, and 0.15 mM solutions of peptide **2**. A 5 μL aliquot of each solution from the serial dilution was combined with 5 μL a 2X solution of phosphate buffered saline (PBS) at pH 7.4 to create 0.6 mM, 0.3 mM, 0.15 mM, and 0.075 mM buffered solutions of peptide **2**. A 1 μL aliquot of each buffered solution of peptide **2** was spotted onto nitrocellulose membrane, and allowed to air dry (~5 min). The weight of peptide spotted onto the membrane from the 0.6 mM, 0.3 mM, 0.15 mM, and 0.075 mM solutions corresponds to 1.5 μg, 0.75 μg, 0.38 μg, and 0.19 μg, respectively. Non-reactive sites were blocked with 10% non-fat milk in low-Tween tris-buffered saline (TBS-lT: 20 mM Tris, 137 mM NaCl, 0.01% Tween 20, pH 7.6) for 1 h at room temperature with rocking. The membrane was washed with TBS-lT for 5 min (3X). The membrane was then incubated while rocking overnight at 4 °C in primary A11 antibody (200 μg/mL) in 5% milk in TBS-lT. The next day, the membrane was washed with TBS-lT for 5 min (3X). The membrane was then incubated while rocking with horseradish peroxidase conjugated goat anti-rabbit antibody (100 μg/mL) (Jackson ImmunoResearch catalog# 111-035-003) in 5% milk in TBS-lT for 1 h at room temperature. The membrane was then washed with TBS-lT for 5 min (3X). A 10 mL portion of chemiluminescence substrate (Thermo Scientific SuperSignal West Femto Maximum Sensitivity Substrate, product# 34095) was prepared according to manufacture's protocol and poured onto the membrane. The membrane was allowed to incubate in the chemiluminescence substrate for ~10 min before imaging. The blot was imaged using a standard SLR camera.[7]

References and Notes

[1] These procedures follow closely those that our laboratory has previously published, with the exception that we used a CEM Liberty 1 automated microwave peptide synthesizer instead of a Protein Technologies PS3 peptide synthesizer and that we used Acm-protected Cys to introduce disulfide linkages. The procedures in this section are adapted from and in some cases taken verbatim from Spencer, R. K.; Kreutzer, A. G.; Salveson, P. J.; Li, H.; Nowick, J. S. *J. Am. Chem. Soc.* **2015**, *137*, 6304–6311.

[2] Bollhagen, R.; Schmiedberger, M.; Barlosb, K.; Grell, E. *J. Chem. Soc., Chem. Commun.*, **1994**, 2559–2560.

[3] The Berkeley Center for Structural Biology is supported in part by the National Institutes of Health, National Institute of General Medical Sciences, and the Howard Hughes Medical Institute. The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

[4] Kabsch, W. *Acta Cryst.*, **2010**, *D66*, 125-132.

[5] Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart., P. H. *Acta Cryst.*, **2010**, *D66*, 213-221.

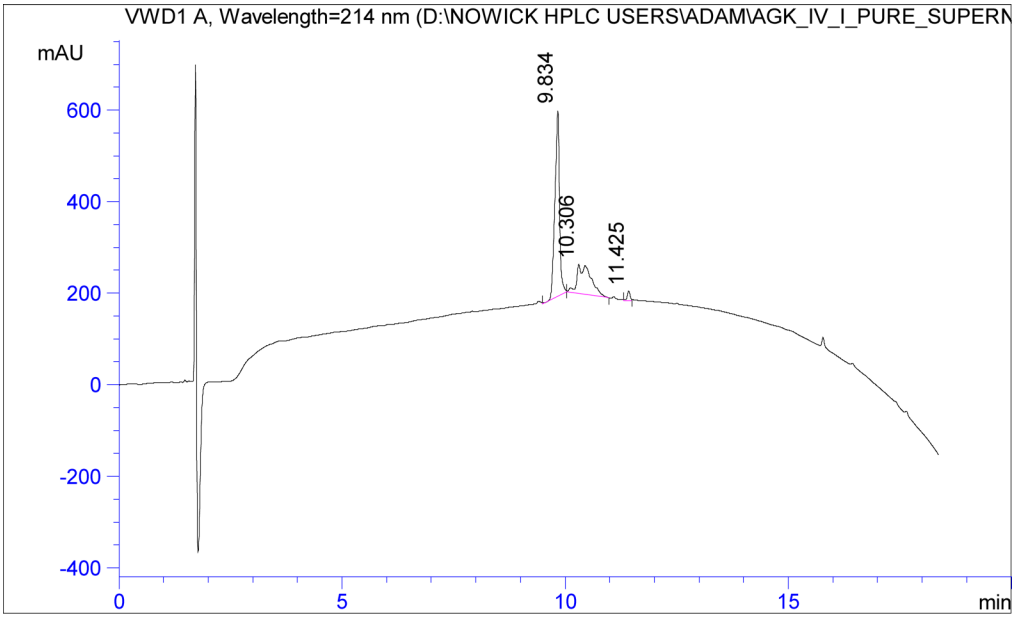
[6] Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. *Acta. Cryst.*, **2010**, *D66*, 486-501.

[7] Khoury, M. K.; Parker, I.; Aswad, D.W. *Anal. Biochem.* **2010**, *397*, 129-131

Characterization Data

Characterization of peptide ***2***

Analytical HPLC trace of peptide **2**.



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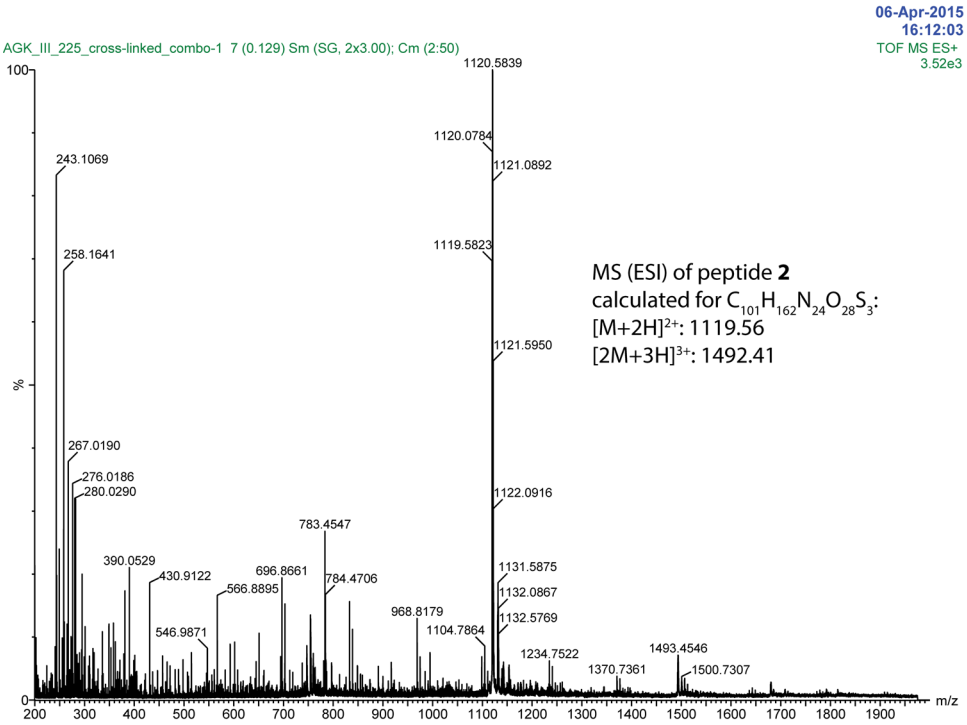
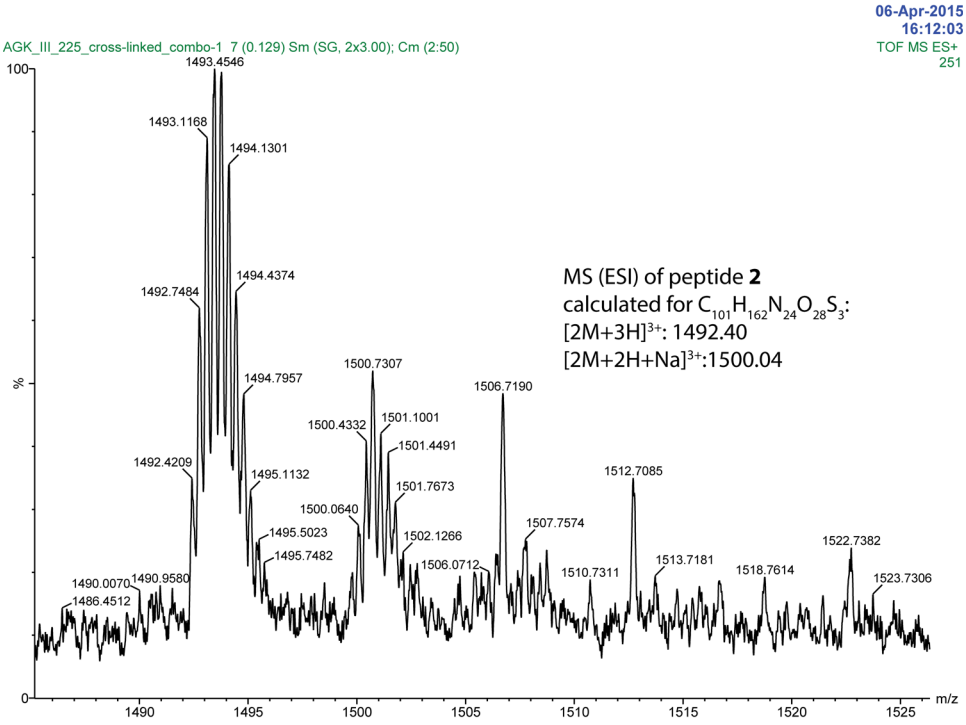
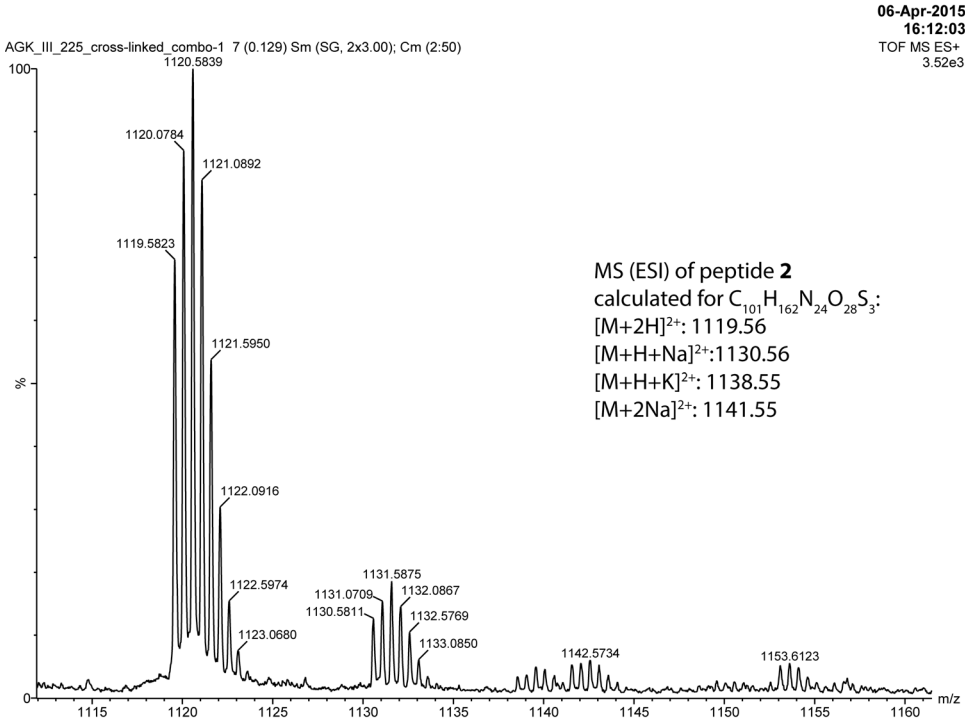
2	10.306	MM	0.362	1386.542	12.913	31.776
3	11.425	MM	0.075	97.364	4.386	2.231

Mass spectra of peptide **2.**






06-Apr-2015
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1131.5875
1131.0709
1132.0867
1130.5811
1132.5769
1133.0850
1142.5734
1153.6123
%
m/z
MS (ESI) of peptide 2
calculated for C101H162N24O28S3:
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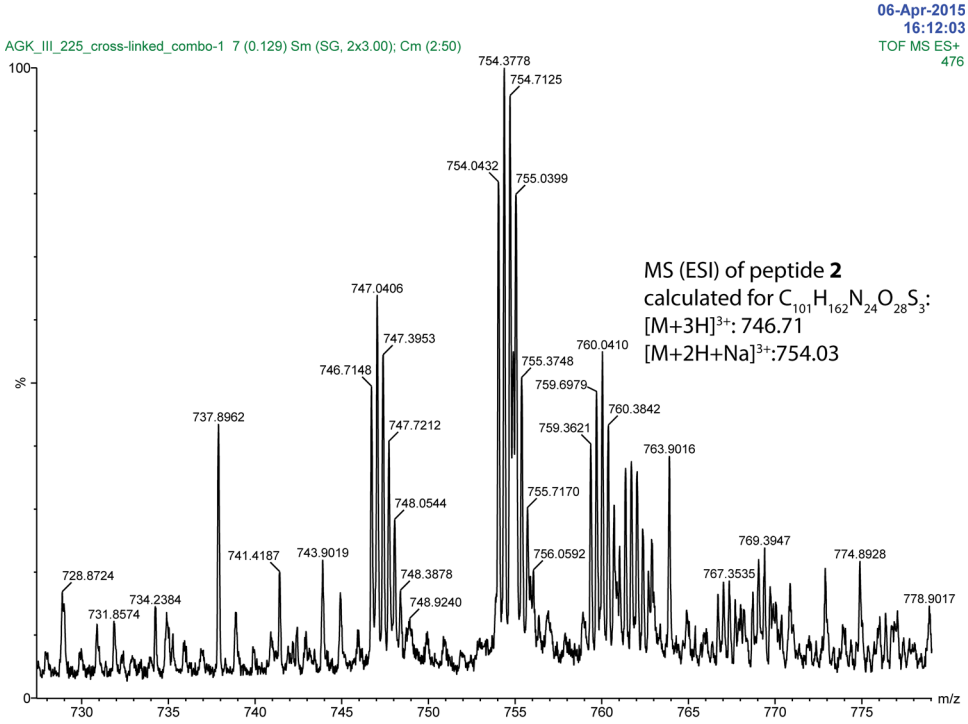
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759.3621
763.9016
755.7170
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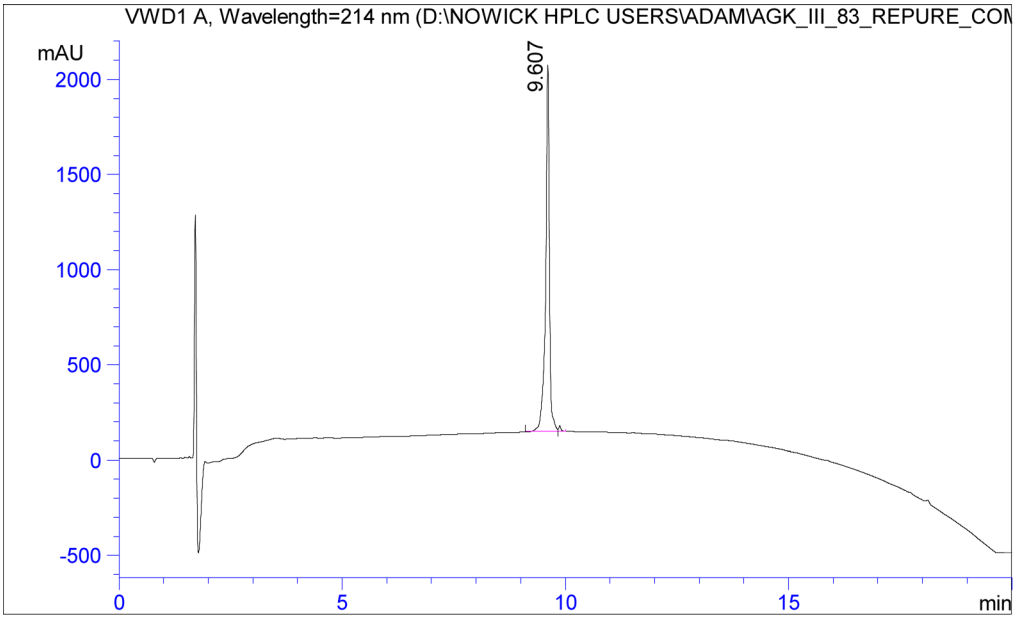
769.3947
774.8928
741.4187
743.9019
756.0592
767.3535
728.8724
748.3878
778.9017
734.2384
748.9240
731.8574
%
m/z
MS (ESI) of peptide 2
calculated for C101H162N24O28S3:
[M+3H]3+: 746.71
[M+2H+Na]3+:754.03

*Characterization of peptide **3***

Analytical HPLC trace of peptide **3**.



Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
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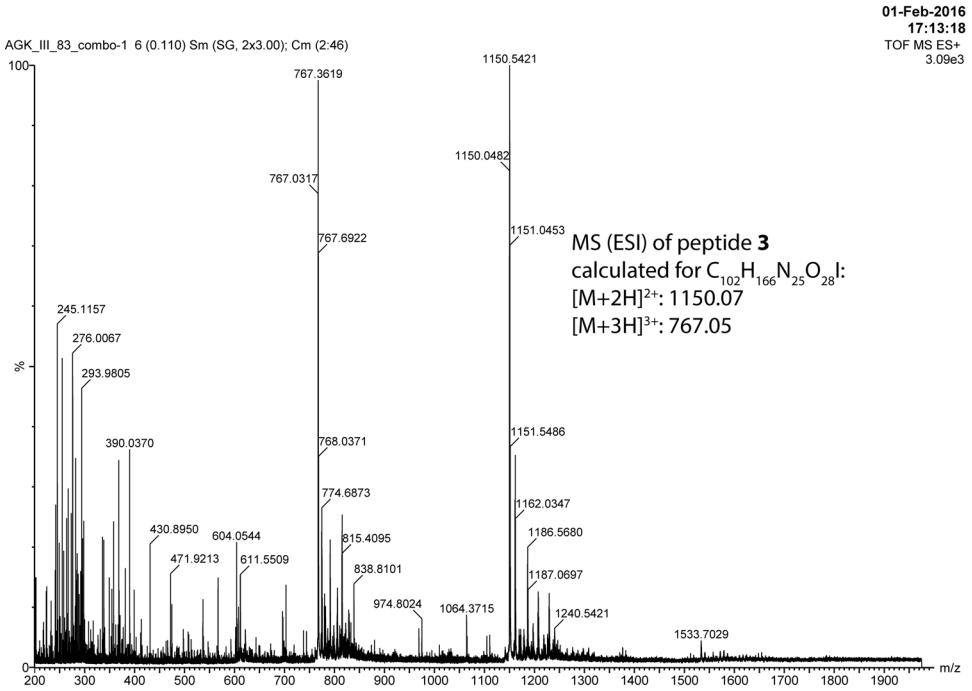
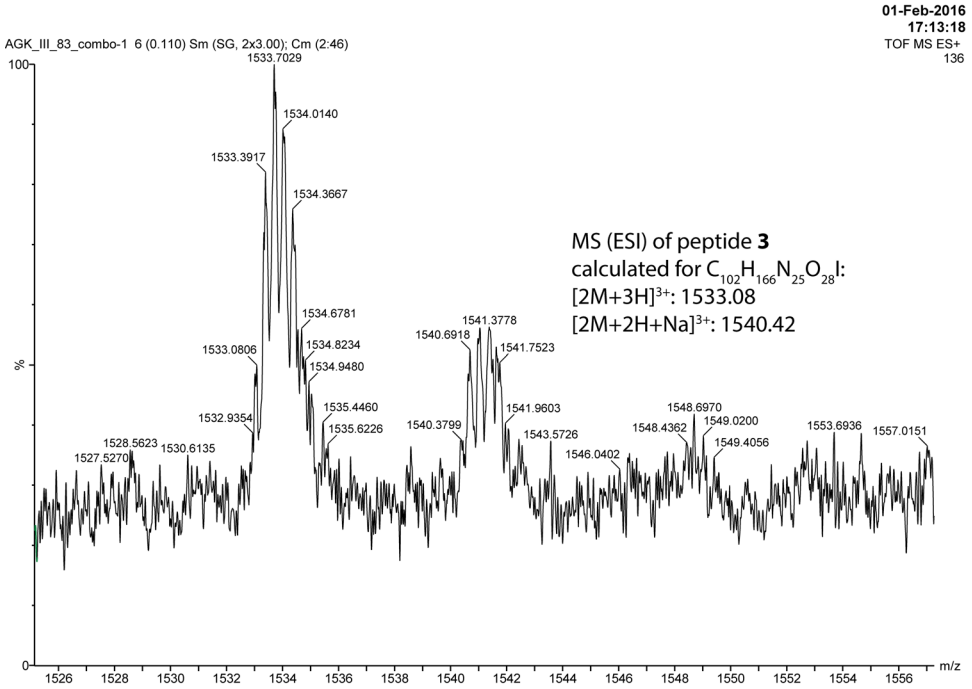
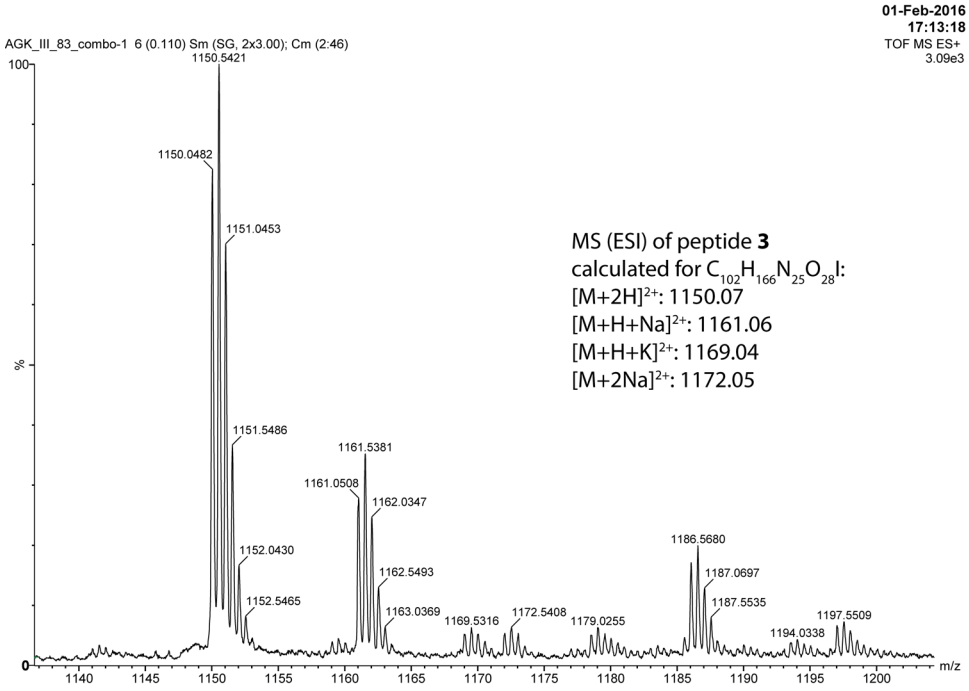
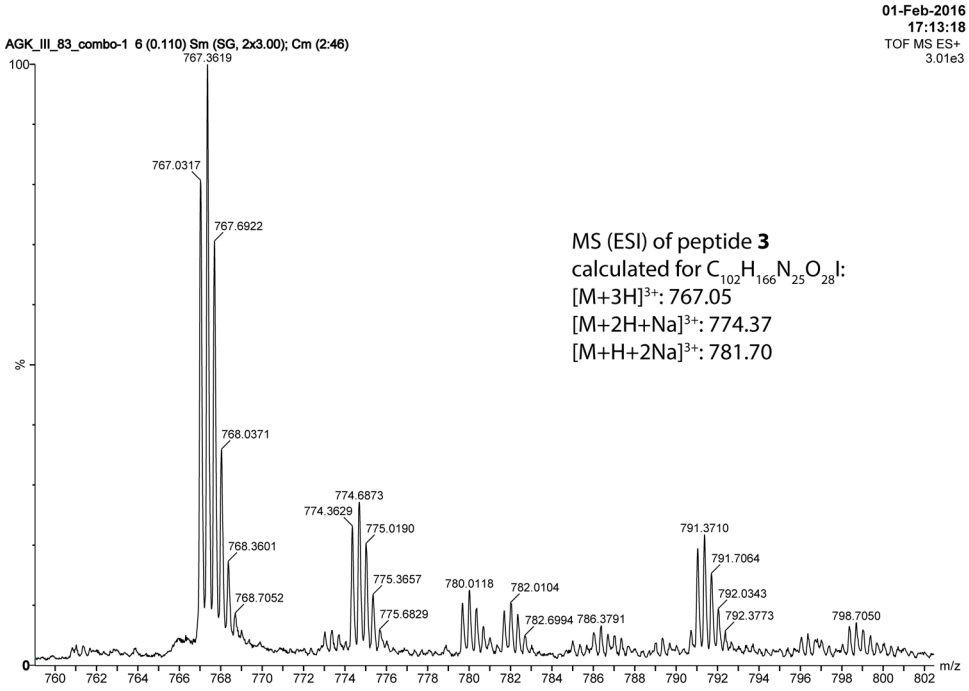
Mass spectra of peptide **3.**







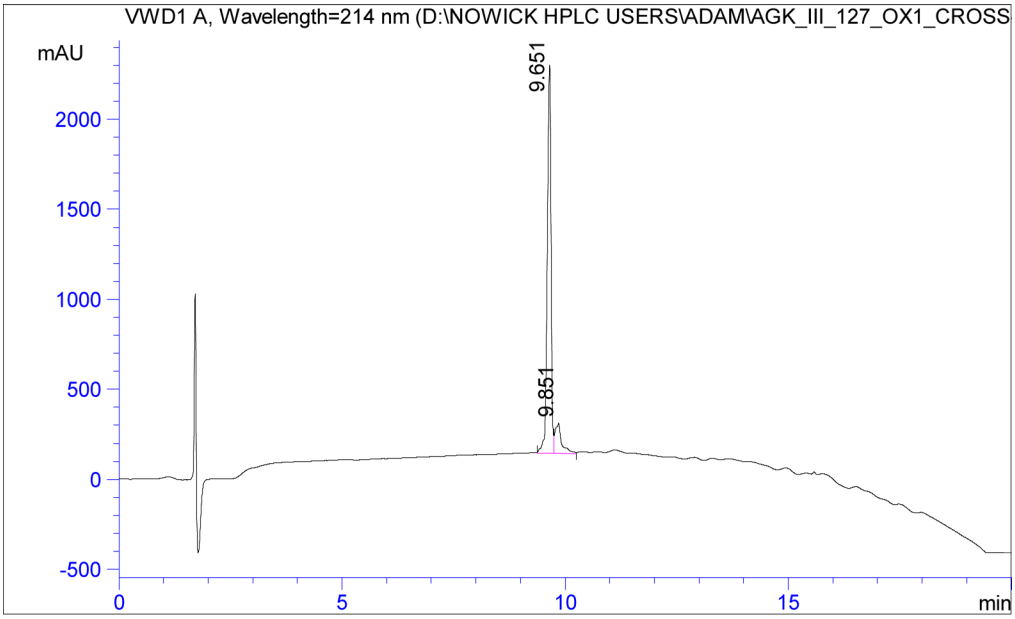
MS (ESI) of peptide **3**
calculated for $C_{102}H_{166}N_{25}O_{28}I$:
$[M+2H]^{2+}$: 1150.07
$[M+H+Na]^{2+}$: 1161.06
$[M+H+K]^{2+}$: 1169.04
$[M+2Na]^{2+}$: 1172.05



MS (ESI) of peptide **3**
calculated for $C_{102}H_{166}N_{25}O_{28}I$:
$[M+3H]^{3+}$: 767.05
$[M+2H+Na]^{3+}$: 774.37
$[M+H+2Na]^{3+}$: 781.70

*Characterization of peptide **4***

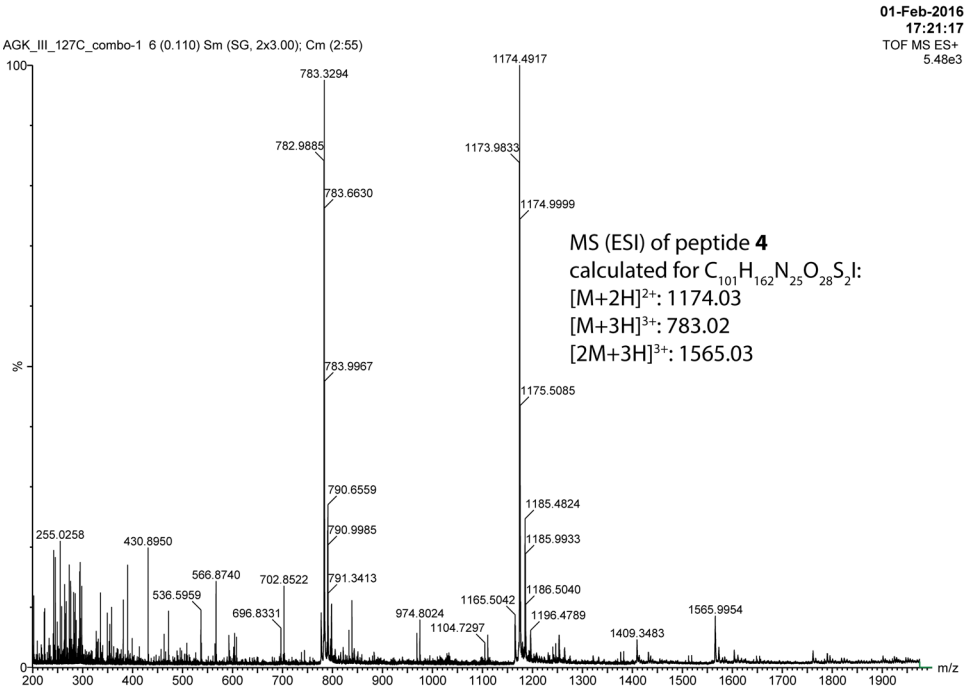
Analytical HPLC trace of peptide **4**.

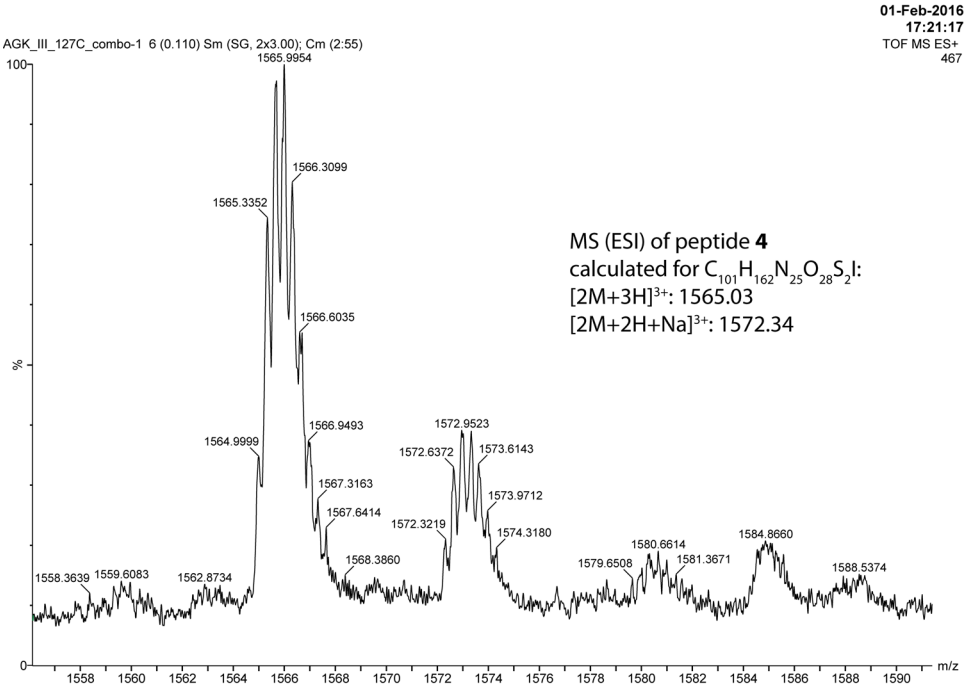


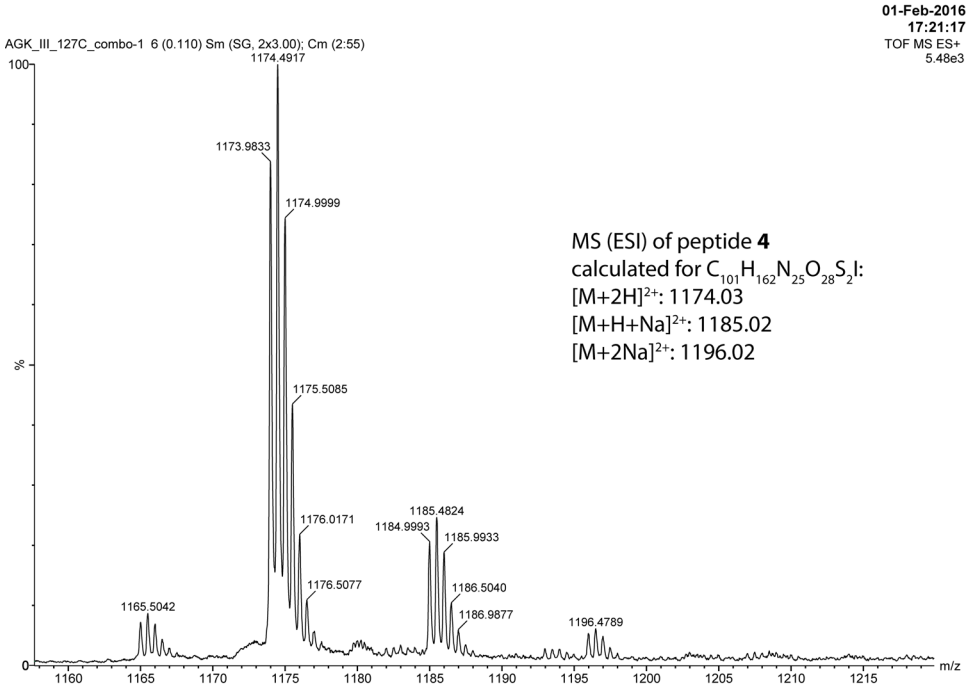
Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	9.651	BV	0.094	12783.123	92.780	87.948
2	9.851	VV	0.136	1751.695	7.220	12.052

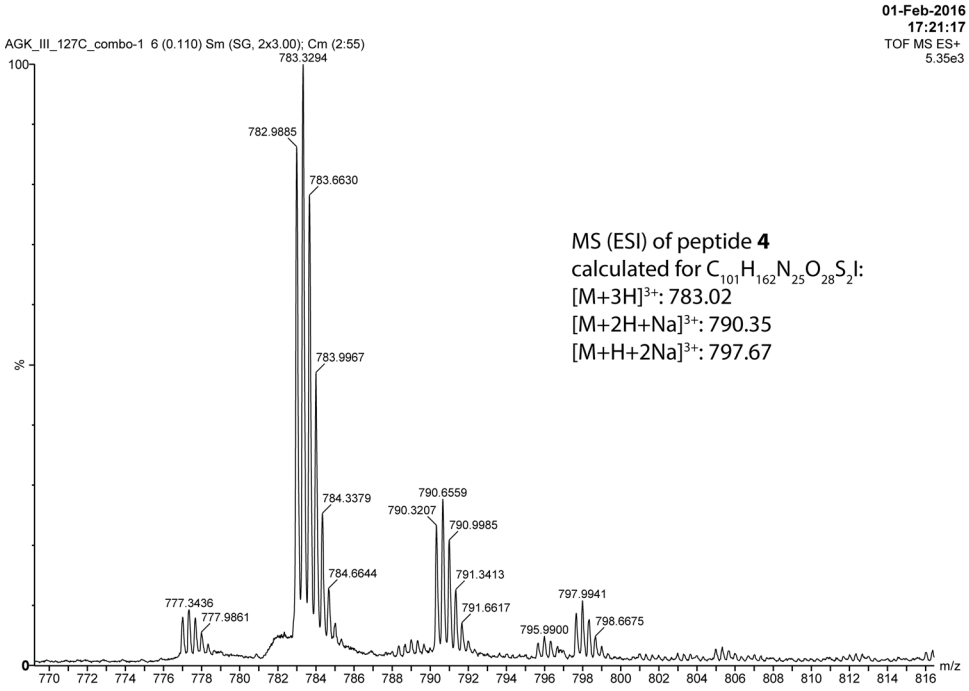
Mass spectra of peptide **4.**







MS (ESI) of peptide **4**
calculated for $C_{101}H_{162}N_{25}O_{28}S_2I$:
$[M+2H]^{2+}$: 1174.03
$[M+H+Na]^{2+}$: 1185.02
$[M+2Na]^{2+}$: 1196.02



MS (ESI) of peptide **4**
calculated for $C_{101}H_{162}N_{25}O_{28}S_2I$:
$[M+3H]^{3+}$: 783.02
$[M+2H+Na]^{3+}$: 790.35
$[M+H+2Na]^{3+}$: 797.67