Supporting Information for:

A Hexamer of a Peptide Derived from $A\beta_{16–36}$

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Supporting Figures and Tables

Materials and Methods

Supporting Figures and Tables

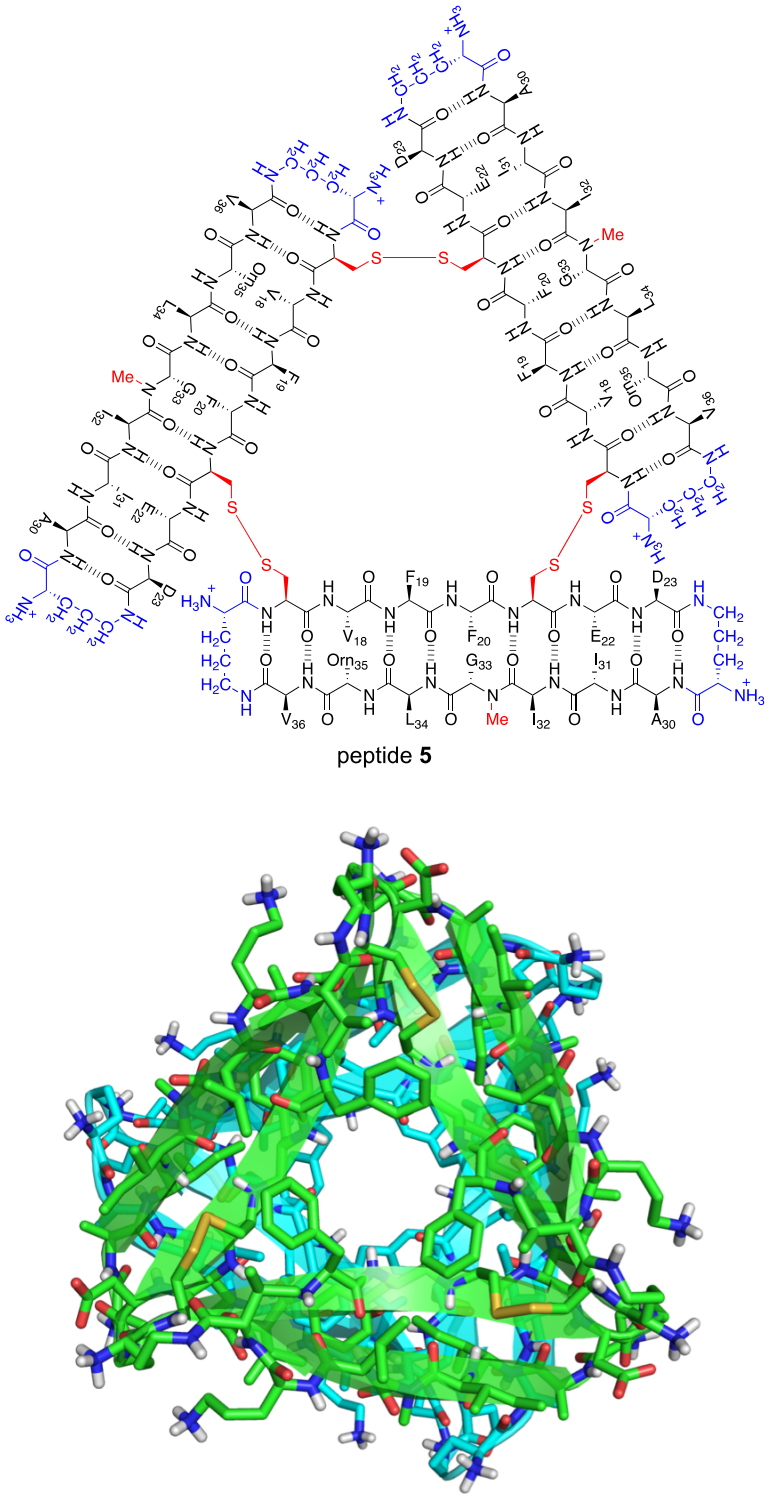
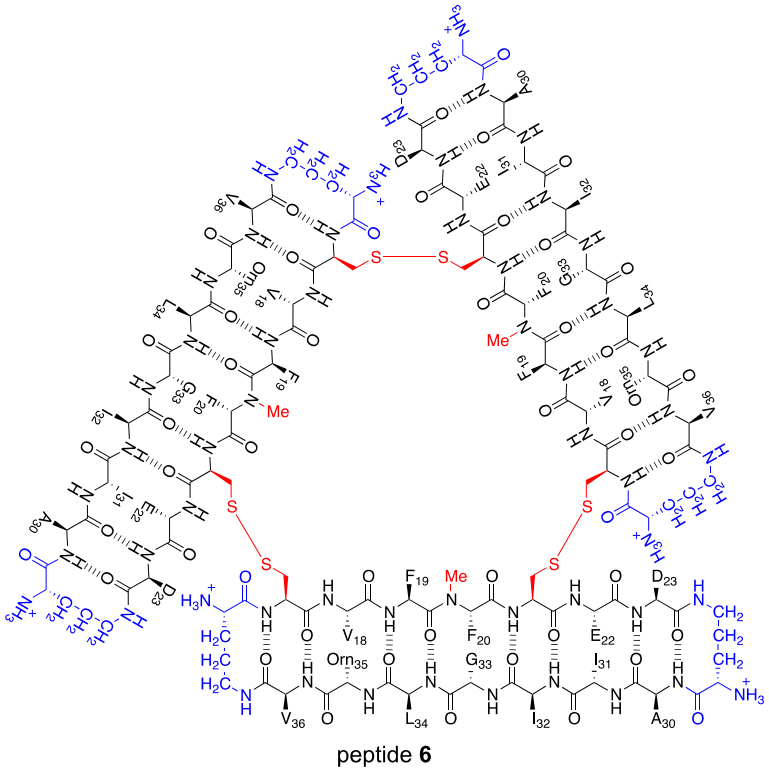


Figure S1. Chemical structure of covalent trimer peptide **5** (top). X-ray crystallographic structure of the hexamer formed by covalent trimer peptide **5** (PDB 5SUT, bottom).[4]



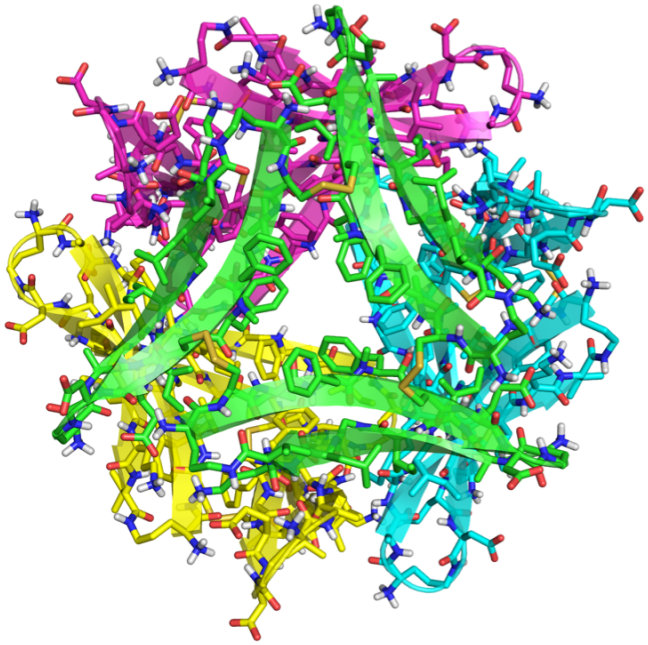


Figure S2. Chemical structure of covalent trimer peptide **6** (top). X-ray crystallographic structure of the dodecamer formed by covalent trimer peptide **6** (PDB 5SUR, bottom).[4]

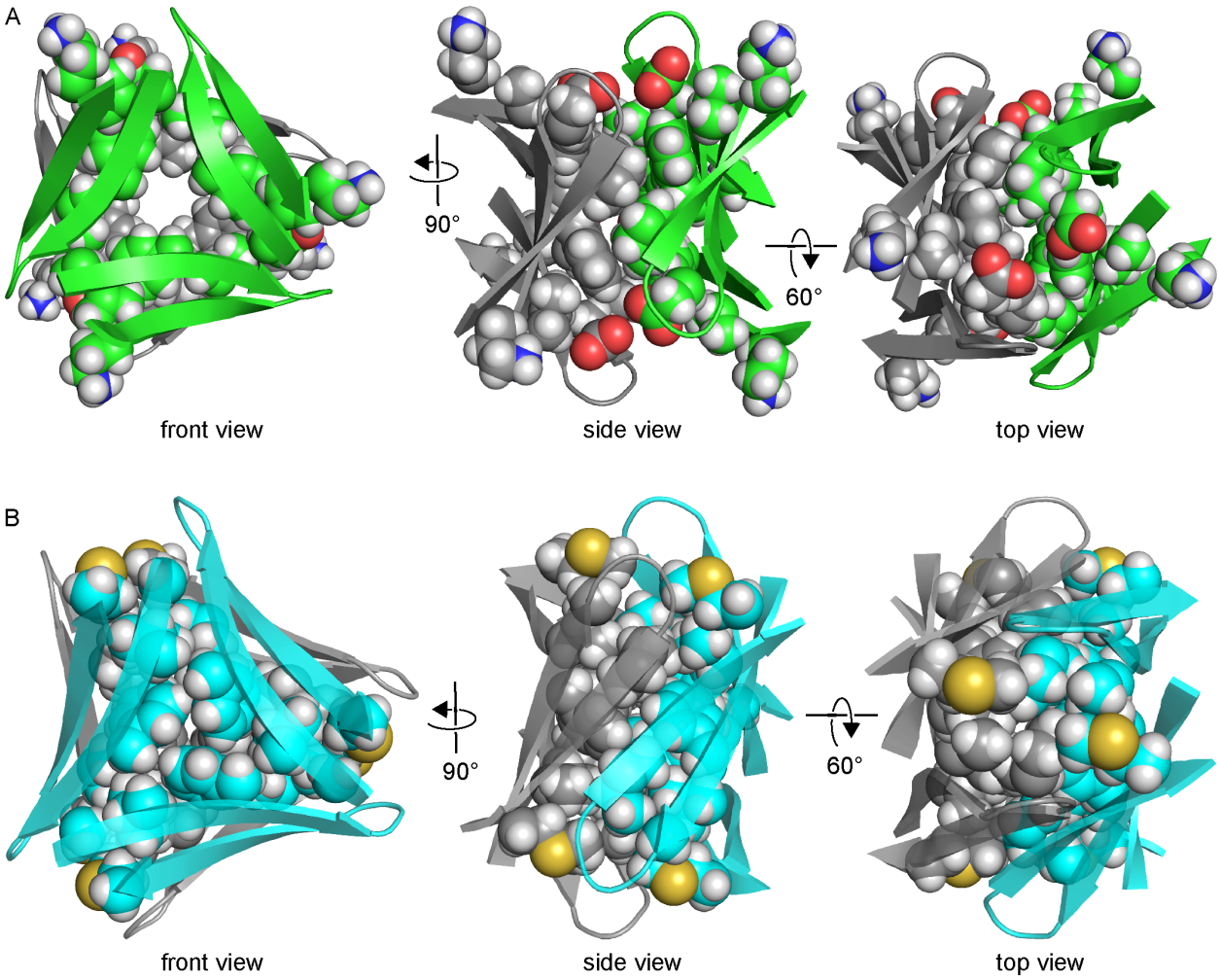


Figure S3. X-ray crystallographic structures of the hexamers formed by peptides **1** and **2**. The hexamers formed by peptide **1** (A) and peptide **2** (B) differ in the degree of their hydrogen bonding and hydrophobic packing. The buried surface area of the hexamer formed by peptide **1** is 3514 $Å^2$, whereas the buried surface area of the hexamer formed by peptide **2** is 5102 $Å^2$.

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

peptide	peptide **2** (synchrotron)	peptide **2** (X-ray diffractometer)
PDB ID	5W4H	5W4I
space group	*P*432	*P*432
a, *b*, *c* (Å)	67.53, 67.53, 67.53	67.74, 67.74, 67.74
α, β, λ (°)	90, 90, 90	90, 90, 90
peptides per asymmetric unit	3	3
crystallization conditions	0.1 M HEPES buffer (pH 7.0), 0.25 M magnesium chloride, 34% isopropanol	0.1 M HEPES sodium buffer (pH 7.5), 0.2 M sodium citrate, 22% isopropanol
wavelength (Å)	0.998	1.54
resolution (Å)	67.54–1.718 (1.78–1.718)	19.56–2.026 (2.098–2.026)
total reflections	12043 (1151)	7613 (727)
unique reflections	6026 (580)	3808 (365)
multiplicity	9.5 (6.3)	36.8 (17.2)
completeness (%)	99.34 (97.97)	99.9 (99.8)
mean I/σ	19.79 (1.99)	24.3 (2.1)
Wilson B factor	31.01	24.51
R_{merge}	0.009247 (0.2266)	0.03415 (0.2676)
$R_{measure}$	0.01308 (0.3205)	0.0483 (0.3784)
$CC_{1/2}$	1.000 (0.905)	0.999 (0.867)
CC^{*}	1.000 (0.975)	1.000 (0.964)
R_{work}	0.2033 (0.3037)	0.2201 (0.3162)
R_{free}	0.2358 (0.3823)	0.2628 (0.3903)
number of non-hydrogen atoms	408	415
RMS_{bonds}	0.015	0.010
RMS_{angles}	1.89	1.24
Ramachandran favored (%)	100	100
outliers (%)	0	0
clashscore	8.06	7.98
average B-factor	40.45	28.68
number of TLS groups	3	3
ligands/ions	N/A	I (5), Cl (3)
water molecules	33	32

Table S2. Crystallographic properties, crystallization conditions, and data collection and model refinement statistics for peptide **4**.

peptide	peptide **4**
PDB ID	5W4J
space group	$P22_12$
a, *b*, *c* (Å)	30.59, 46.94, 64.30
α, β, λ (°)	90, 90, 90
peptides per asymmetric unit	6
crystallization conditions	0.05 M HEPES buffer (pH 7.5), 0.2 M KCl, 37% pentaerythritol propoxylate (5/4 PO/OH)
wavelength (Å)	1.54
resolution (Å)	32.15–2.08 (2.154–2.08)
total reflections	11904 (1162)
unique reflections	5952 (581)
multiplicity	17.4 (14.5)
completeness (%)	99.82 (100)
mean I/σ	71.11 (28.06)
Wilson B factor	27.69
R_{merge}	0.007097 (0.01986)
$R_{measure}$	0.01004 (0.02809)
$CC_{1/2}$	1.00 (0.999)
CC^*	1.00 (1.00)
R_{work}	0.1969 (0.2194)
R_{free}	0.2410 (0.2892)
number of non-hydrogen atoms	789
RMS_{bonds}	0.011
RMS_{angles}	1.27
Ramachandran favored (%)	100
outliers (%)	0
clashscore	5.65
average B-factor	38.53
number of TLS groups	9
ligands/ions	N/A
water molecules	63

Materials and Methods[1]

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 *N*,*N*-dimethylformamide (DMF) was purchased from Alfa Aesar. Deionized water (18 MΩ) was obtained from a Barnstead NANOpure Diamond water purification system. Analytical reverse-phase HPLC was performed on an Agilent 1200 instrument equipped with a Phenomonex Aeris PEPTIDE 2.6u XB-C18 column. Preparative reverse-phase HPLC was performed on a Beckman Gold Series P instrument equipped with an Agilent Zorbax SB-C18 column. HPLC grade acetonitrile and deionized water, each containing 0.1% trifluoroacetic acid (TFA), were used for analytical and preparative reverse-phase HPLC. All peptides were prepared and used as the trifluoroacetate salts and were assumed to have one trifluoroacetic acid molecule per amine group on each peptide.

*Synthesis of peptides **1**–**4**.*

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). 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 suspension 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 Boc-Orn(Fmoc)-2-chlorotrityl resin 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 the *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% (v/v) *N*-methylmorpholine (NMM) in DMF for 10 min at 50 °C, iv. washing with DMF (3x). Special coupling conditions were used for the valine that followed the *N*-methylphenylalanine in peptides **1**–**3** and for the phenylalanine that followed the *N*-methylphenylalanine in peptide **4**: The valine or phenylalanine was double coupled (0.75 mmol, 5 equiv.) and allowed to react at ambient temperature for 1 h per coupling with HATU (5 equiv) and HOAt (5 equiv) in 20% (v/v) NMM in DMF. 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 cyclized without further purification.

d. *Cyclization 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 cyclic peptide.* 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 as a thin yellow film on the side of the round-bottomed flask. The crude cyclic peptide was immediately subjected to purification by reverse-phase HPLC (RP-HPLC), as described below.

f. *Reverse-phase HPLC purification.* 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). Pure fractions were concentrated by rotary evaporation and lyophilized. Typical syntheses yielded ~55 mg of the peptide as the TFA salt.

SDS-PAGE and silver staining.

The oligomerization of peptides **1**–**4** was studied by Tricine SDS-PAGE. Reagents and gels for Tricine SDS-PAGE were prepared according to recipes and procedures detailed in Schägger, H. *Nat. Protoc.* **2006**, *1*, 16–22.[3] The migration of peptides **1**–**4** was compared with a molecular weight protein ladder (Spectra™ Multicolor Low Range Protein Ladder, ThermoFisher Scientific, catalog #: 26628) and with previously reported peptides **5** and **6**.[4]

Sample preparation. Each peptide was dissolved in deionized water to a concentration of 10 mg/mL. Aliquots of the 10-mg/mL solutions were diluted with deionized water to create 0.30-mg/mL solutions of peptides **1**–**4** and 0.10-mg/mL solutions of peptides **5** and **6**. The 0.30-mg/mL solutions of peptides **1**–**4** and 0.10-mg/mL solutions of peptides **5** and **6** were further diluted with 2X SDS-PAGE loading buffer (100 mM Tris buffer at pH 6.8, 20% (v/v) glycerol, and 4% SDS) to create 0.15-mg/mL working solutions of peptides **1**–**4** and 0.05-mg/mL working solutions of peptides **5** and **6**. A 5.0-μL aliquot of each working solution was run on a 16% polyacrylamide gel with a 4% stacking polyacrylamide gel. The gels were run at a constant 80 volts.

Staining with silver nitrate was used to visualize peptides **1**–**4** and peptides **5** and **6** in the SDS-PAGE gel. Reagents for silver staining were prepared according to procedures detailed in Simpson, R. J. *CSH Protoc.* **2007**.[5] [The sodium thiosulfate solution, silver nitrate solution, and developing solution were prepared fresh each time silver staining was performed]. Briefly, the gel was removed from the casting glass and rocked in fixing solution (50% (v/v) methanol and 5% (v/v) acetic acid in deionized water) for 20 min. Next, the fixing solution was discarded and the gel was rocked in 50% (v/v) aqueous methanol for 10 min. Next, the 50% methanol was discarded and the gel was rocked in deionized water for 10 min. Next, the water was discarded

and the gel was rocked in 0.02% (w/v) sodium thiosulfate in deionized water for 1 min. The sodium thiosulfate was discarded and the gel was rinsed with deionized water for 1 min (2X). After the last rinse, the gel was submerged in chilled 0.1% (w/v) silver nitrate in deionized water and rocked at 4 °C for 20 min. Next, the silver nitrate solution was discarded and the gel was rinsed with deionized water for 1 min (2X). To develop the gel, the gel was incubated in developing solution (2% (w/v) sodium carbonate, 0.04% (w/v) formaldehyde until the desired intensity of staining was reached (~1–3 min). When the desired intensity of staining was reached, the development was stopped by discarding the developing solution and submerging the gel in 5% aqueous acetic acid.

Size exclusion chromatography.

The oligomerization of peptides **1**–**4** was studied by size exclusion chromatography (SEC) at 4 °C in TBS (50 mM Tris buffer (pH 7.5) and 100 mM NaCl) as follows: Each peptide was dissolved in deionized water to a concentration of 10 mg/mL. The peptide solutions were then diluted to 1 mg/mL by adding 80 μL of the 10-mg/mL solutions to 720 μL of TBS. The peptide solutions were centrifuged at 13,500 RPM for 30 seconds and then loaded onto a GE Superdex 75 10/300 GL column at 0.5 mL/min over 1 min. After loading, the samples were run with TBS at 1 mL/min Chromatograms were recorded at 214 nm and normalized to the highest absorbance value. Standards (cytochrome C, aprotinin, and vitamin B12) were run in the same fashion.

*Crystallization procedure for peptides **2** and **4**.*

Initial crystallization conditions for peptides **2** and **4** were determined using the hanging-drop vapor-diffusion method. Crystallization conditions were screened 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 **2** or **4** (10 mg/mL in deionized 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. Crystals of peptide **2** grew in ~72 h in a solution of 0.1 M HEPES buffer (pH 7.0), 0.25 M $MgCl_2$, and 30% isopropanol, as well as in a solution containing 0.1 M HEPES sodium buffer (pH 7.0), 0.2 M sodium citrate, and 30% isopropanol. Crystals of peptide **4** grew in ~72 h in a solution of 0.1 M HEPES buffer (pH 7.5), 0.2 M KCl, and 30% pentaerythritol propoxylate.

Crystallization conditions for peptides **2** and **4** were optimized using a 4x6 matrix Hampton VDX 24-well plate. The HEPES buffer pH was varied in each row in increments of 0.5 pH units (6.5, 7.0, 7.5, and 8.0) and the isopropanol or pentaerythritol propoxylate concentration in each column in increments of 2% (28%, 30%, 32%, 34%, 36%, 38%). The first well in the 4x6 matrix for peptide **2** was prepared by combined 100 μL of 1 M HEPES buffer at pH 6.5, 125 μL of 2 M $MgCl_2$, 280 μL of isopropanol, and 495 μL of deionized water. The other wells were prepared in analogous fashion, by combining 100 μL of HEPES buffer of varying pH, 125 μL of 2 M $MgCl_2$, isopropanol in varying amounts, and deionized water for a total volume of 1 mL in each well. Wells for peptide **4** were prepared in an analogous fashion.

Three hanging-drops were prepared per borosilicate glass slide by combining a solution of peptide **2** or peptide **4** (10 mg/mL in deionized water) and the well solution in the following amounts: 1 μL:1 μL, 2 μL:1 μL, and 1 μL:2 μL. Slides were inverted and pressed firmly against the silicone grease surrounding each well. Crystals of peptide **2** or of peptide **4** suitable for X-ray diffraction grew in ~3 days. Crystallization conditions were further optimized using smaller variations in HEPES buffer pH (in increments of 0.25 pH units) and isopropanol or pentaerythritol propoxylate (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 and Table S2.

*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.[6] Coordinates for the anomalous signals were determined by HySS in the Phenix software suite 1.10.1.[7] Electron density maps were generated using anomalous coordinates determined by HySS as initial positions in Autosol. Molecular manipulation of the model was performed with Coot.[6] Coordinates were refined with phenix.refine.

Diffraction data for peptide **2** were also collected at the Advanced Light Source at Lawrence Berkeley National Laboratory with a synchrotron source at 0.998-Å wavelength to achieve higher resolution. Data for peptide **2** suitable for refinement at 2.03 Å were obtained

from the diffractometer; data for peptide **2** suitable for refinement at 1.72 Å were obtained from the synchrotron. Data for peptide **4** suitable for refinement at 2.08 Å were obtained from the diffractometer. Diffraction data were scaled and merged using XDS.[7] The electron density map for peptide **2** was generated by molecular replacement using the coordinates from the structure of peptide **2** generated by soaking in KI using Phaser in the Phenix software suite 1.10.1.[8] The electron density map for peptide **4** was generated by sulfur single-wavelength anomalous diffraction (S-SAD) using the anomalous signal from the six sulfur atoms in methionine in the asymmetric unit using HySS in the Phenix software suite 1.10.1. Molecular manipulation of the peptide **2** and peptide **4** models was performed with Coot. Coordinates for peptide **2** and peptide **4** were refined with phenix.refine.

LDH release assays.

The toxicity of peptides **1**–**4** toward SH-SY5Y cells was assessed by LDH release assays. Cells were incubated in the presence or absence of equivalent concentrations of peptides **1**–**4** for 72 h in 96-well plates. The LDH release assay was performed using the Pierce LDH Cytotoxicity Assay Kit from Thermo Scientific. Experiments were performed in replicates of five, and an additional 10 wells were used for controls. Cells were cultured in the inner 60 wells (rows B–G, columns 2–11) of the 96-well plate. DMEM:F12 media (100 µL) was added to the outer wells (rows A and H and columns 1 and 12), in order to ensure the greatest reproducibility of data generated from the inner wells.

a. *Preparation of stock solutions of peptides **1**–**4***. 10-mg/mL stock solutions of peptides **1**–**4** were prepared gravimetrically by dissolving 1.0 mg of each compound in 100 µL of deionized water that was either filtered through a 0.2 µm syringe filter or autoclaved. The stock

solution was used to create 500-μM working solutions of peptides **1**–**4**. The 500-μM working solutions of peptides **1**–**4** was diluted with deionized water to create 250-μM working solutions of peptides **1**–**4**.

b. *Preparation of SH-SY5Y cells for LDH release assays.* SH-SY5Y cells were plated in a 96-well plate at 15,000 cells per well. Cells were incubated in 100 μL of a 1:1 mixture of DMEM:F12 media supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere and allowed to adhere to the bottom of the plate for 24 hours.

c. *Treatment of SH-SY5Y cells with peptides **1**–**4**.* After 24 hours, the culture media was removed and replaced with 90 μL of serum-free DMEM:F12 media. A 10-μL aliquot of the working solution of peptides **1**–**2** was added to each well, for well concentrations of 50 μM and 25 μM. Experiments were run in replicates of five. Five wells were used as controls and received 10-μL aliquots of deionized water (vehicle). Another five wells were left untreated, to be subsequently used as controls with lysis buffer for the LDH release assay. Cells were incubated at 37 °C in a 5% CO_2 atmosphere for 72 hours.

d. *LDH release assay.* After 72 hours, 10 μL of 10x lysis buffer—included with the assay kit—was added to the five untreated wells, and the cells were incubated for an additional 45 min. After 45 min, a 50-μL aliquot of the supernatant media from each well was transferred to a new 96-well plate and 50 μL of LDH substrate solution, prepared according to manufacturer's protocol, was added to each well. The treated plates were stored in the dark for 30 min. The absorbance of each well was measured at 490 and 680 nm (A_{490} and A_{680}). Data were processed by calculating the differential absorbance for each well ($A_{490}-A_{680}$) and comparing those values to those of the lysis buffer controls and the untreated controls:

$$\% \text{ cell death} = [(A_{490}-A_{680})_{\text{compound}} - (A_{490}-A_{680})_{\text{vehicle}}] / [(A_{490}-A_{680})_{\text{lysis}} - (A_{490}-A_{680})_{\text{vehicle}}]$$

Replica exchange molecular dynamics (REMD).

A model of an $A\beta_{12-40}$ barrel-like hexamer was generated by replica-exchange molecular dynamics as follows: Starting coordinates for $A\beta_{12-40}$ were generated from the crystallographic coordinates of peptide **2**. Symmetry mates of peptide **2** were displayed in PyMOL. Six copies of peptide peptide **2** corresponding to the barrel-like hexamer were selected and saved to a new PDB file. The two delta-linked ornithine residues were deleted from each macrocycle. Glu_{22} and Ala_{30} were connected with seven alanine residues in PyMOL. Four alanine residues were added to the *N*-terminus of the β-hairpin, and four alanine residues were added to the *C*-terminus. These added residues were minimized in PyMOL using the clean function, ensuring that the crystallographic coordinates of $A\beta_{16-22}$ and $A\beta_{30-36}$ were not perturbed. After this minimization, each added alanine was mutated to its corresponding wild-type residue from Aβ. The mutated residues were again minimized in PyMOL using the clean function. Each *N*-Me-Phe_{19} was replaced with the wild-type Phe_{19}.

The autopsf plugin in VMD was used to prepare the required files for simulation. The coordinates for $A\beta_{16–22}$ and $A\beta_{30–36}$ were fixed throughout the simulation. REMD simulations were run in NAMD with the CHARMM22 force field and generalized Born implicit solvent (GBIS) on 32 replicas. The temperatures for these replicas varied between 300 and 800K. The simulation was performed for 8.5 ns. Representative coordinates were selected uniformly from the last 7.5 ns of the simulation.

References and Notes

1 These procedures follow closely those that our laboratory has previously published. The procedures in this section are adapted from and in some cases taken verbatim from Kreutzer, A. G.; Hamza, I. L.; Spencer, R. K.; Nowick J. S. *J. Am. Chem. Soc.* **2016**, *138*, 4634–4642, Spencer, R. K.; Kreutzer, A. G.; Salveson, P. J.; Li, H.; Nowick, J. S. *J. Am. Chem. Soc. 137*, **2015**, 6304–6311, Spencer, R. K.; Li. H.; Nowick, J. S. *J. Am. Chem. Soc.* **2014**, *136*, 5595–5598, and Kreutzer, A. G.; Yoo, S.; Spencer, R. K.; Nowick, J. S. *J. Am. Chem, Soc.* **2017**, *139*, 966–975.

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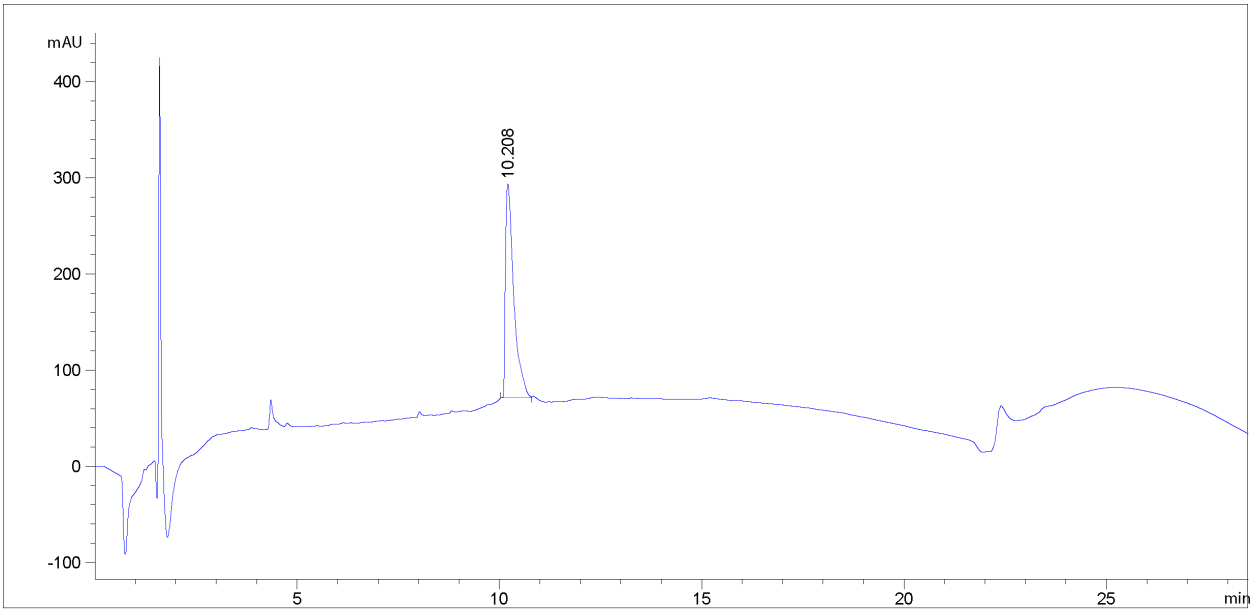
Characterization Data

*Characterization of peptide **1***

Analytical HPLC trace of peptide **1**.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
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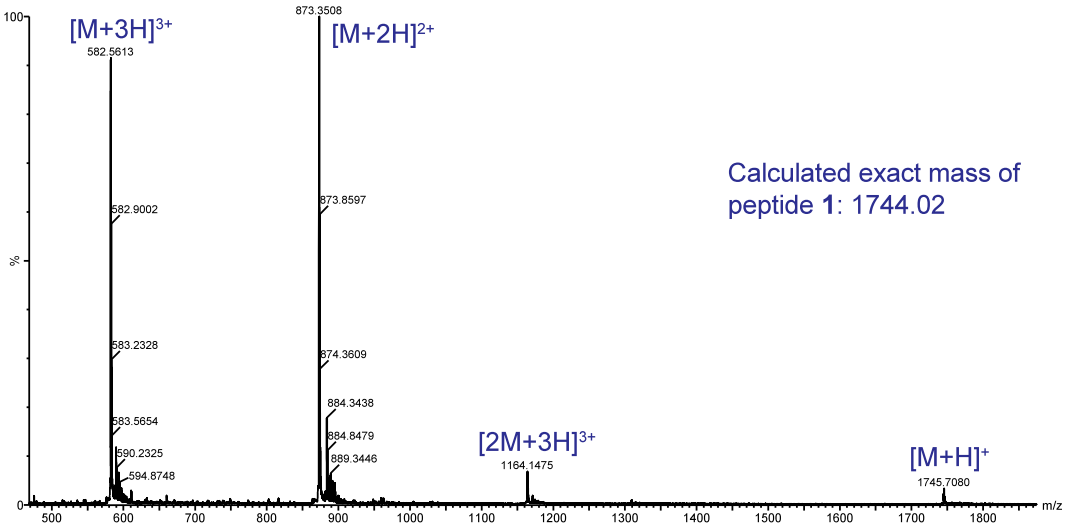
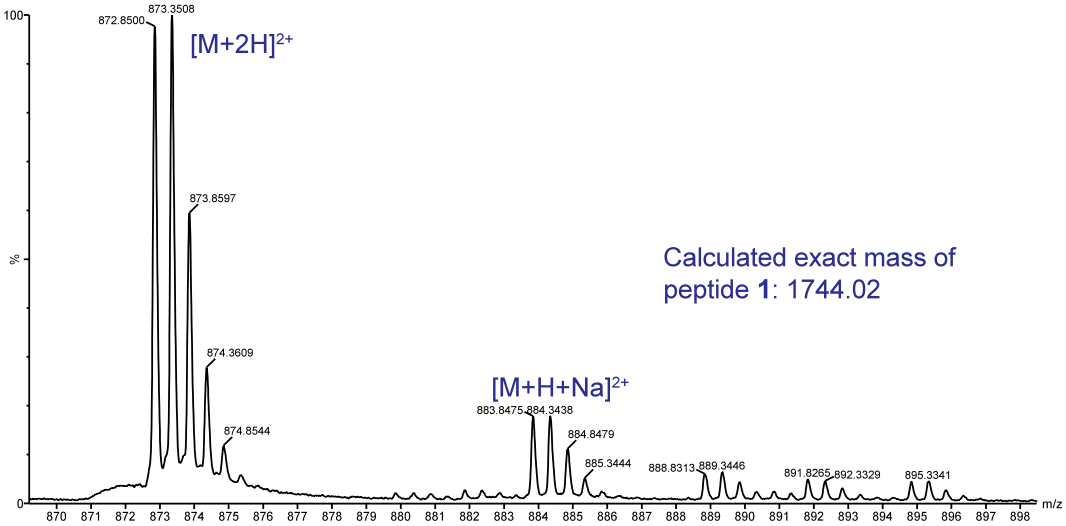
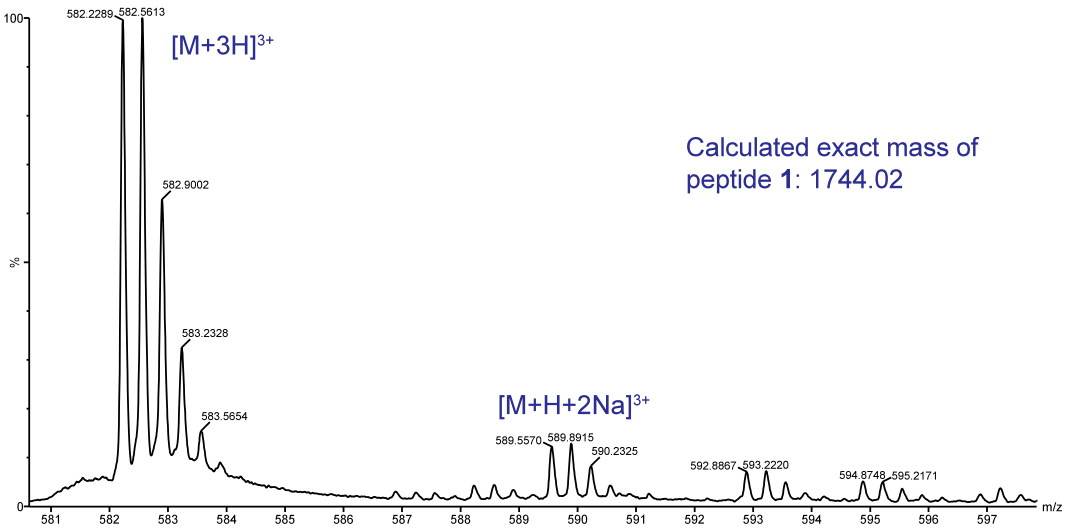
High-resolution mass spectrometry of peptide **1**.

HRMS (ESI/MeOH) *m/z* calcd for $C_{85}H_{140}N_{19}O_{21}S$ $[M + H]^+$ 1745.0365, found 1745.0377

Mass spectrum and expansions of peptide **1**.



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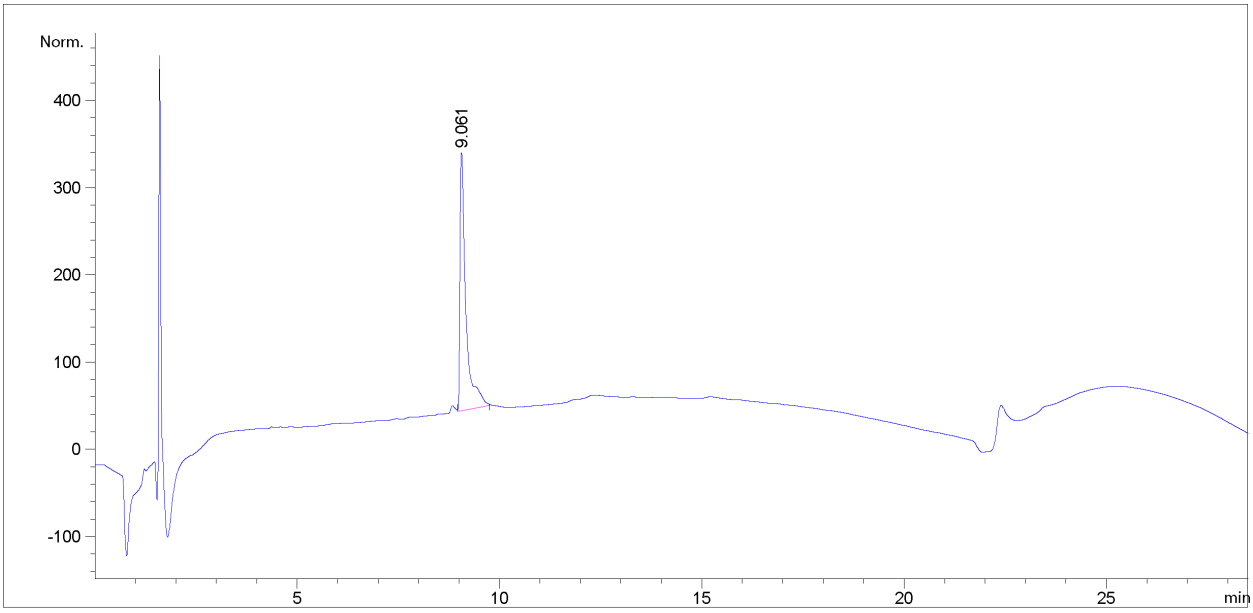
582.5613
[M+3H]3+
582.9002
583.2328
583.5654
[M+H+2Na]3+
589.5570
589.8915
590.2325
592.8867
593.2220
594.8748
595.2171
Calculated exact mass of peptide 1: 1744.02
100
%
0
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
m/z

*Characterization of peptide **2***

Analytical HPLC trace of peptide **2**.

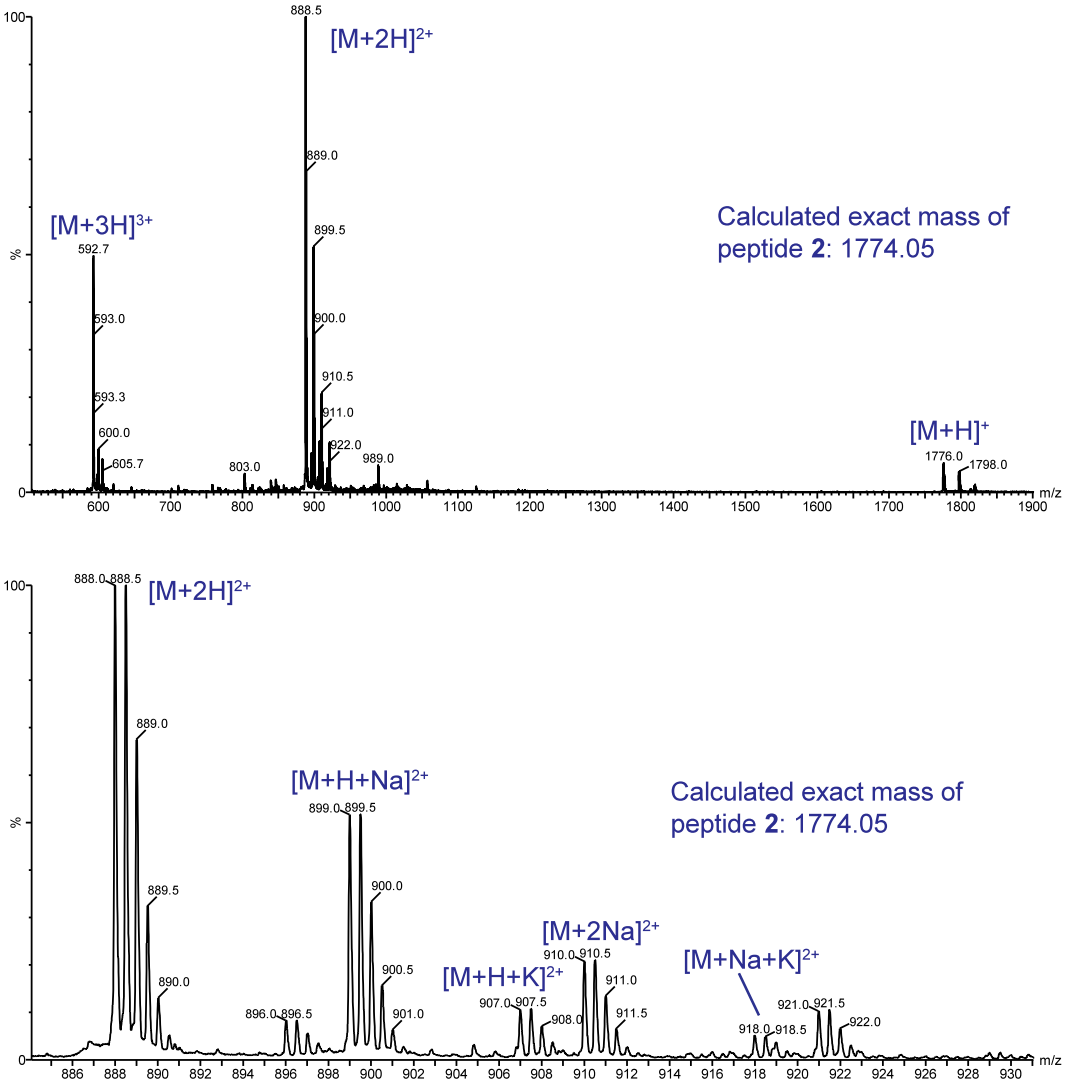


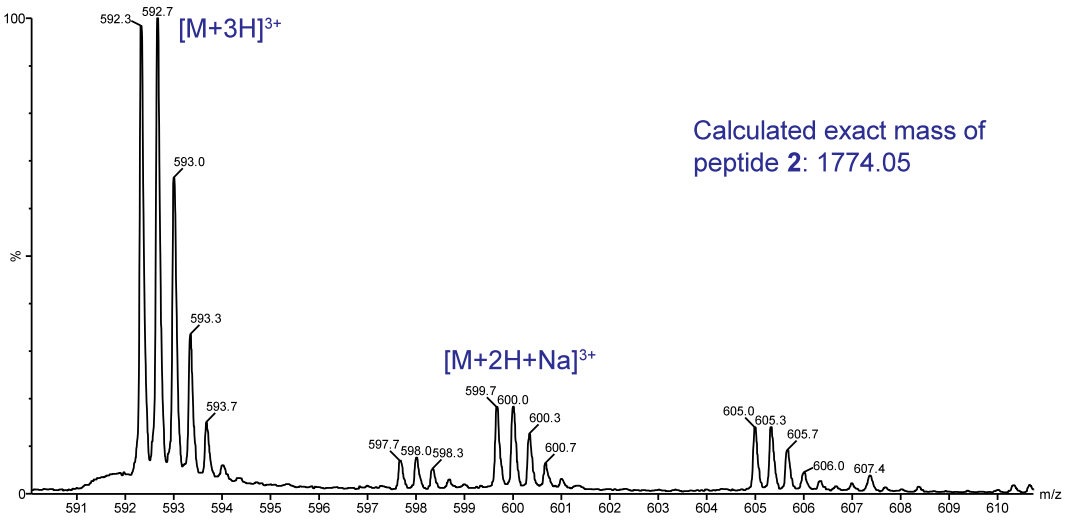
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.061	MM	0.1679	2686.53735	266.66986	100.0000

High-resolution mass spectrometry of peptide **2**.

HRMS (ESI/MeOH) *m/z* calcd for $C_{87}H_{144}N_{19}O_{18}S$ $[M + H]^+$ 1775.0657, found 1775.0638

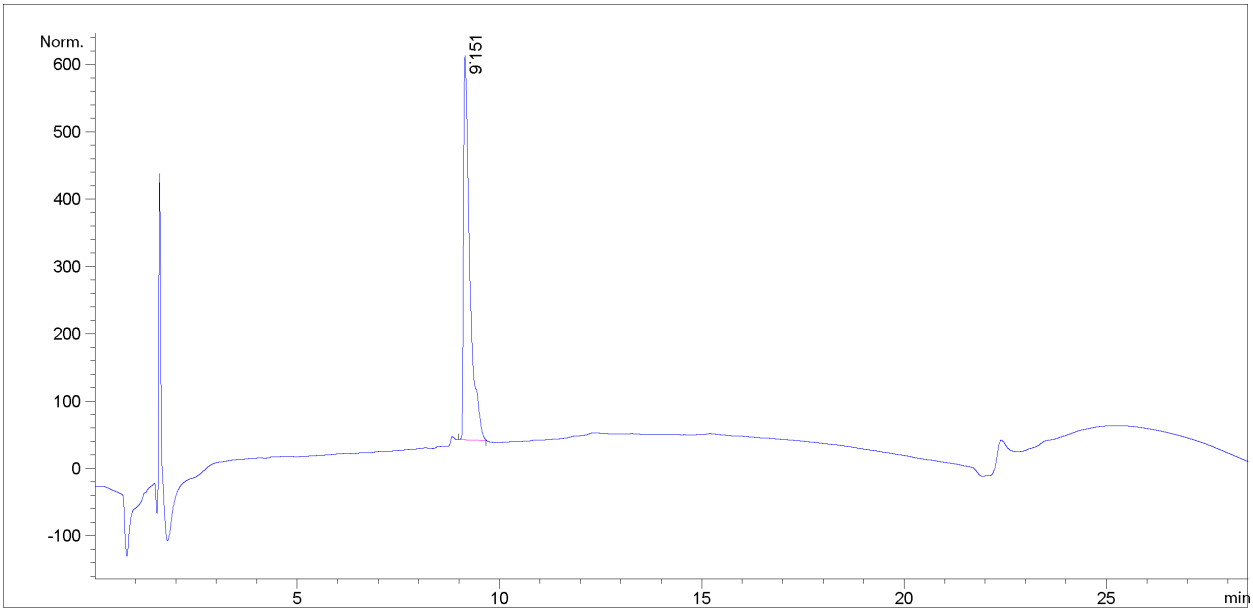
Mass spectrum and expansions of peptide **2**.




[M+3H]3+
[M+2H+Na]3+
Calculated exact mass of
peptide 2: 1774.05
100
%
0
592.3
592.7
593.0
593.3
593.7
597.7
598.0
598.3
599.7
600.0
600.3
600.7
605.0
605.3
605.7
606.0
607.4
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
m/z

*Characterization of peptide **3***

Analytical HPLC trace of peptide **3**.

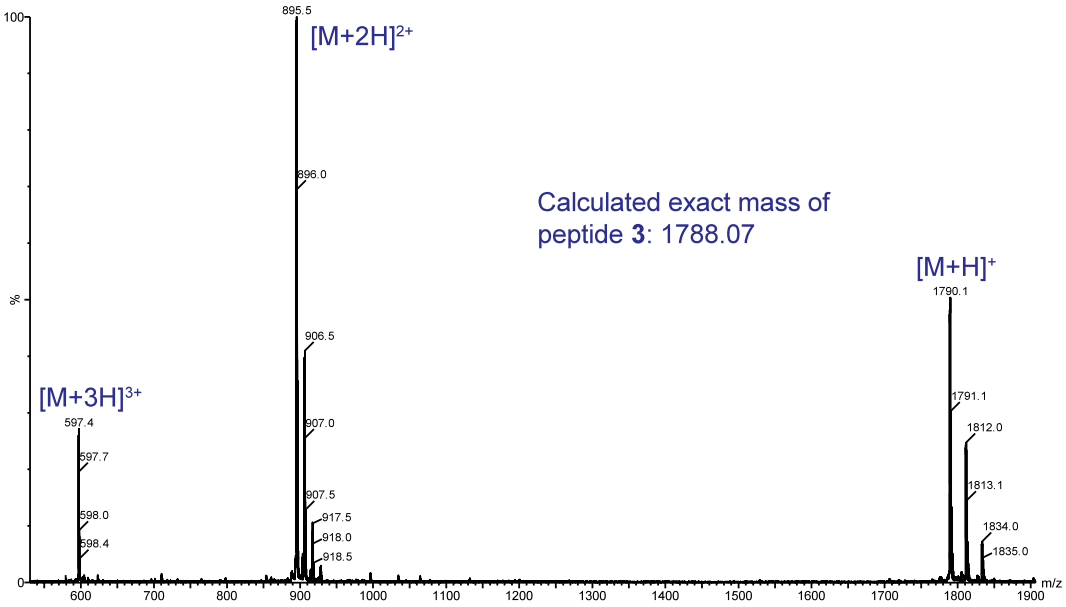


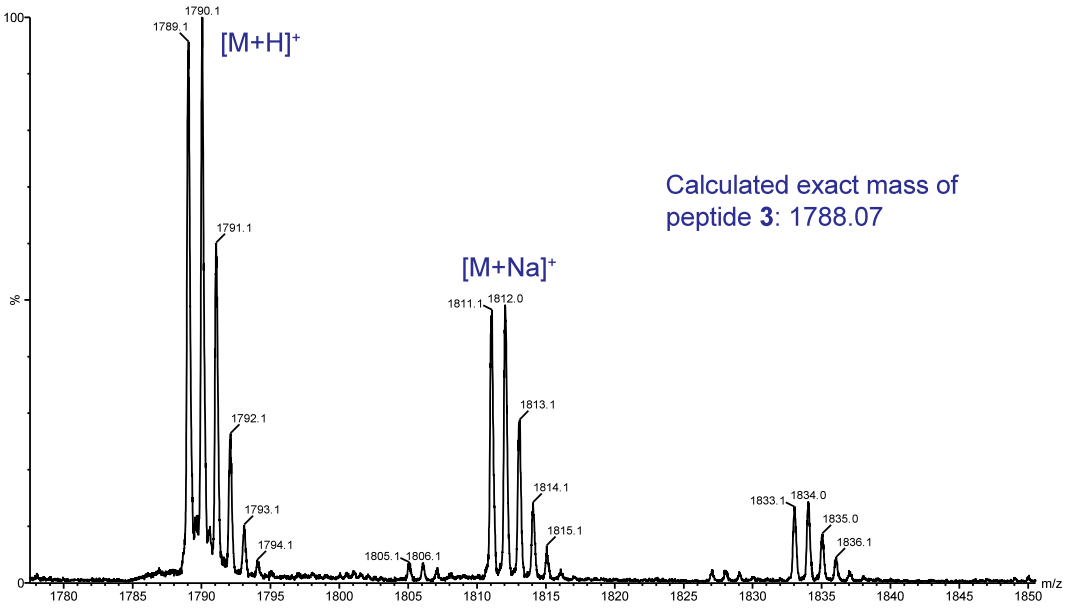
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.151	MM	0.1788	5522.78467	514.87726	100.0000

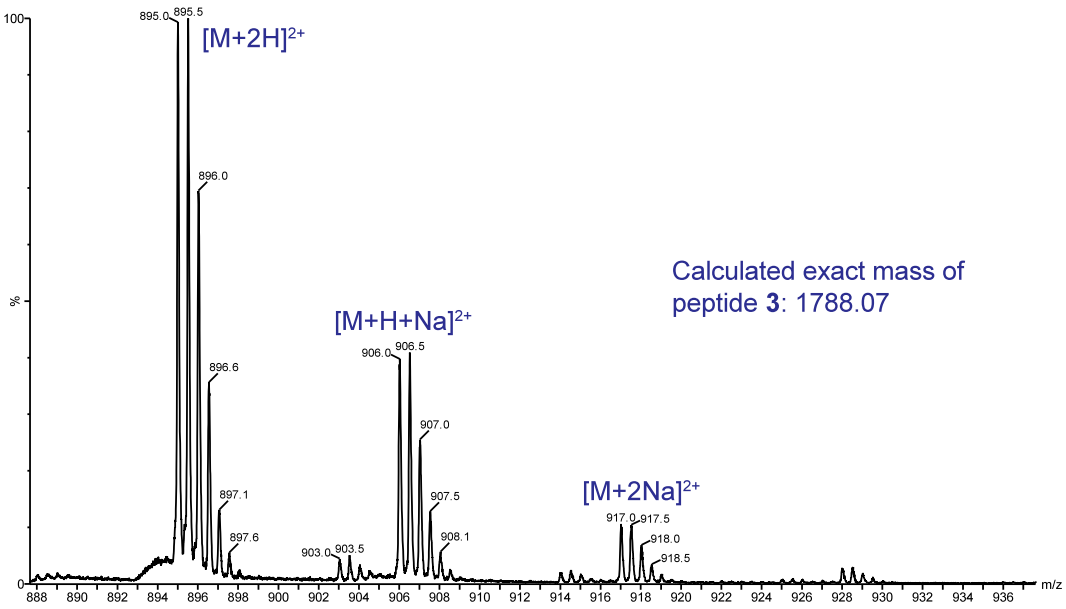
High-resolution mass spectrometry of peptide **3**.

HRMS (ESI/MeOH) *m/z* calcd for $C_{88}H_{146}N_{19}O_{18}S$ $[M + H]^+$ 1789.0814, found 1789.0822

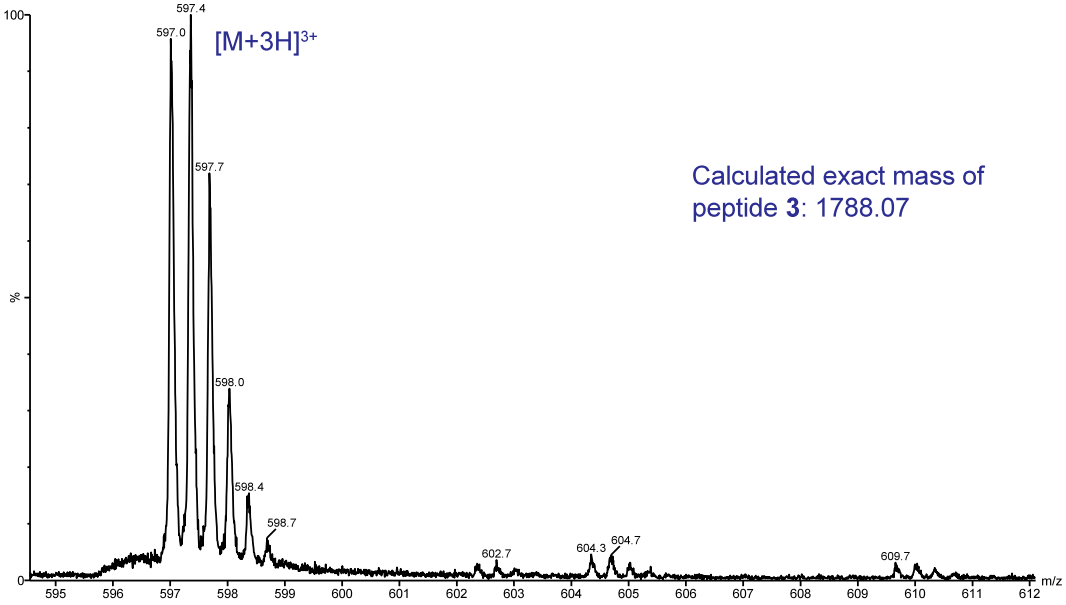
Mass spectrum and expansions of peptide **3**.







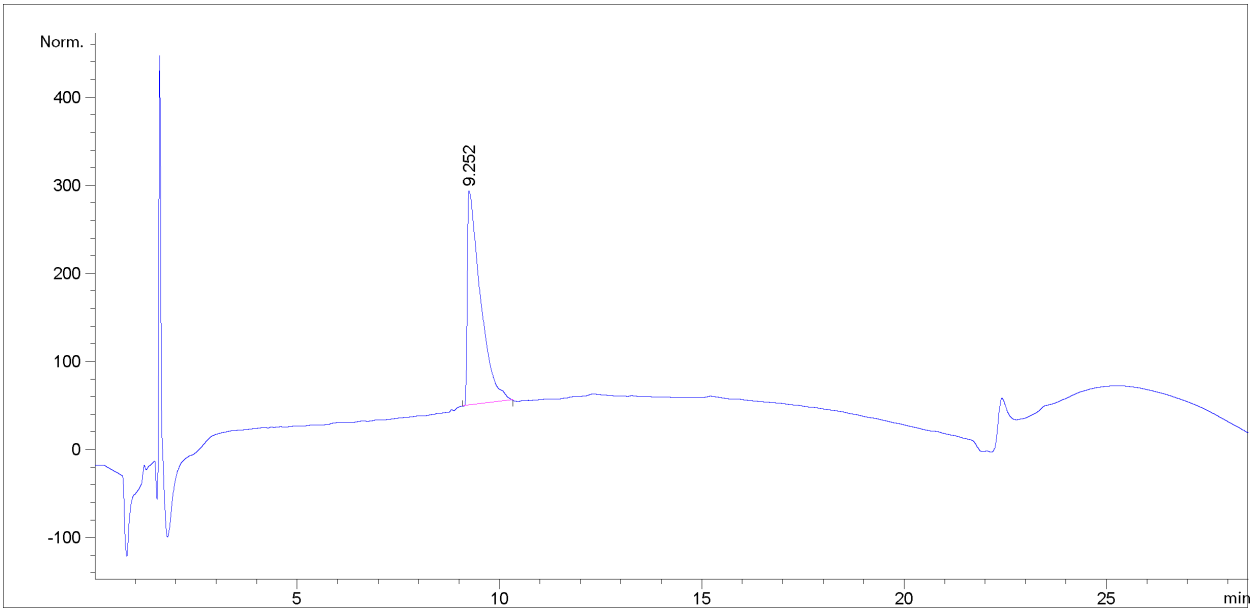
Calculated exact mass of peptide **3**: 1788.07



Calculated exact mass of peptide **3**: 1788.07

*Characterization of peptide **4***

Analytical HPLC trace of peptide **4**.

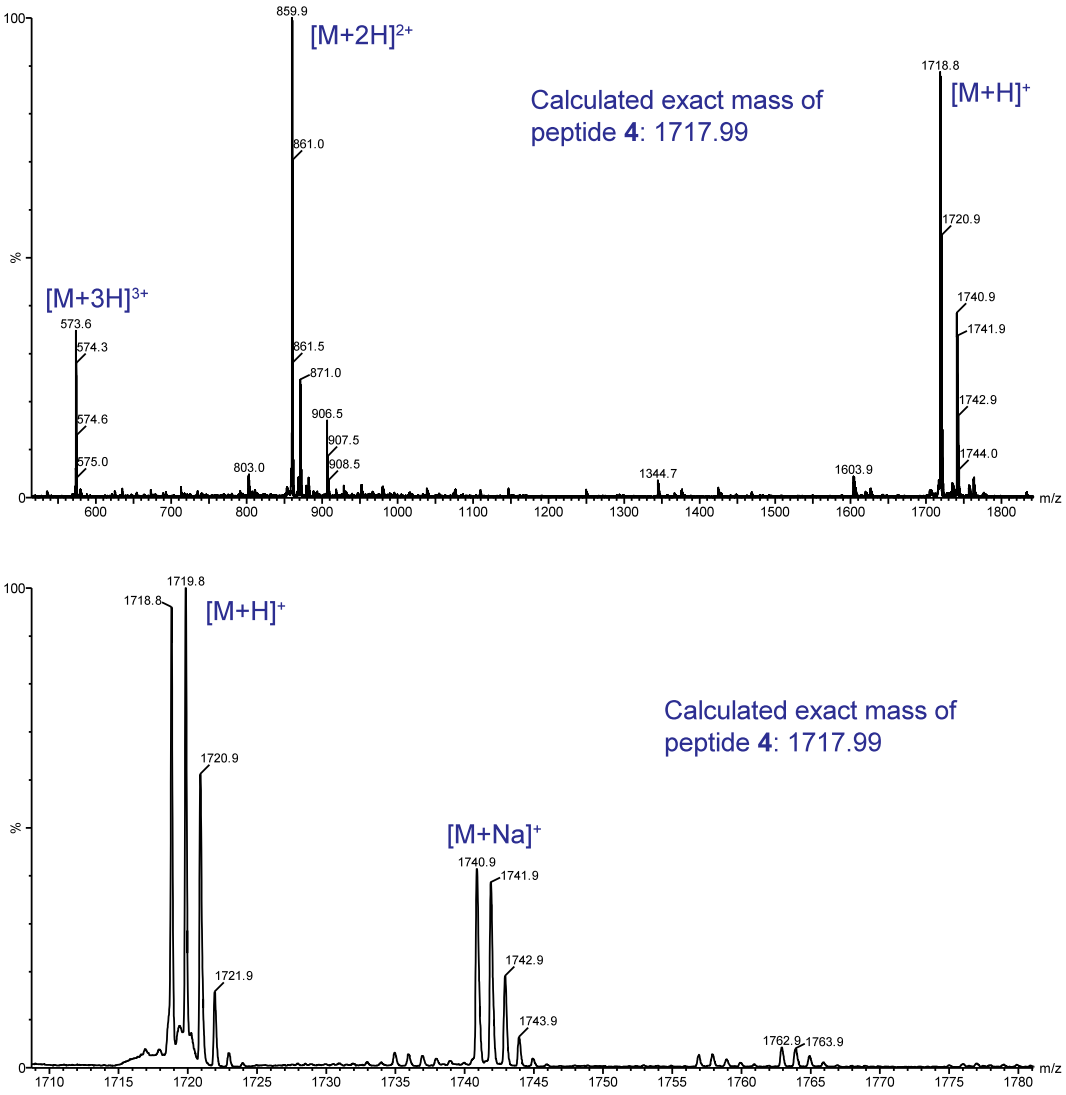


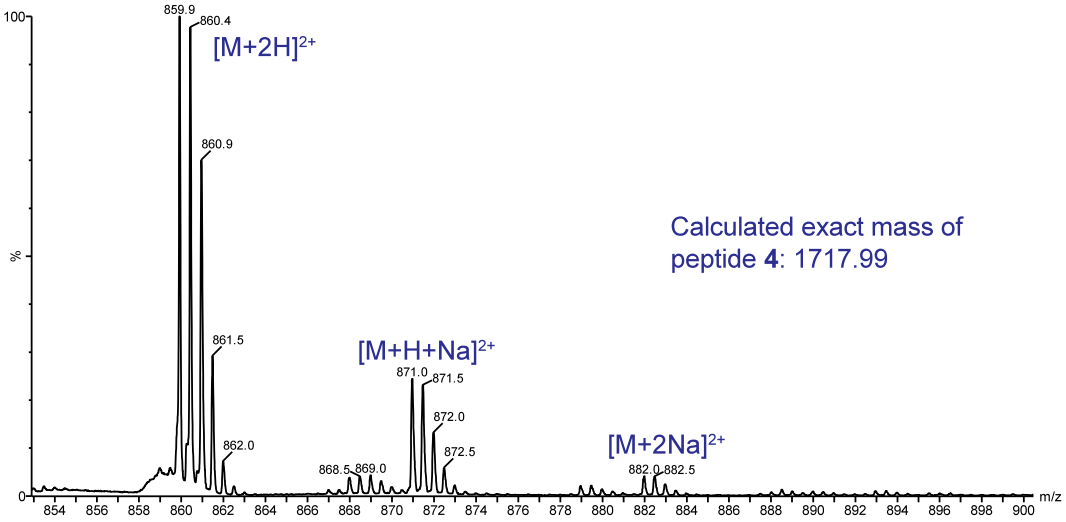
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.252	MM	0.3742	4923.85791	219.30423	100.0000

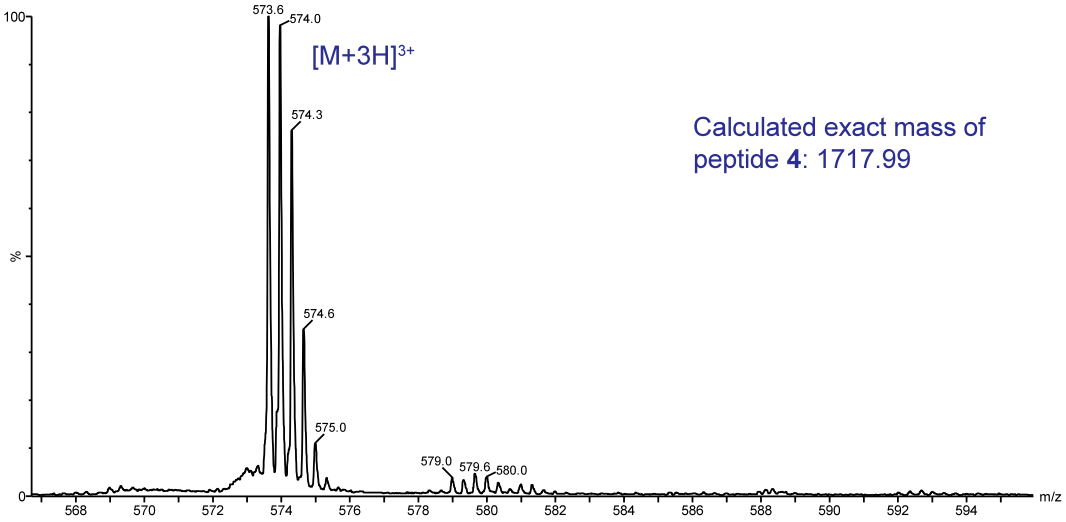
High-resolution mass spectrometry of peptide **4**.

HRMS (ESI/MeOH) *m/z* calcd for $C_{83}H_{136}N_{19}O_{18}S$ $[M + H]^+$ 1719.0031, found 1719.0042

Mass spectrum and expansions of peptide **4**.




100
%
0
859.9
860.4
[M+2H]2+
860.9
861.5
862.0
[M+H+Na]2+
871.0
871.5
872.0
872.5
868.5
869.0
[M+2Na]2+
882.0
882.5
854 856 858 860 862 864 866 868 870 872 874 876 878 880 882 884 886 888 890 892 894 896 898 900
m/z
Calculated exact mass of
peptide 4: 1717.99


100
%
0
573.6
574.0
[M+3H]3+
574.3
574.6
575.0
579.0
579.6
580.0
568 570 572 574 576 578 580 582 584 586 588 590 592 594
m/z
Calculated exact mass of
peptide 4: 1717.99