Supporting Information for:

Stabilization, Assembly, and Toxicity of Trimers Derived from Aβ

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Supporting Figures and Tables

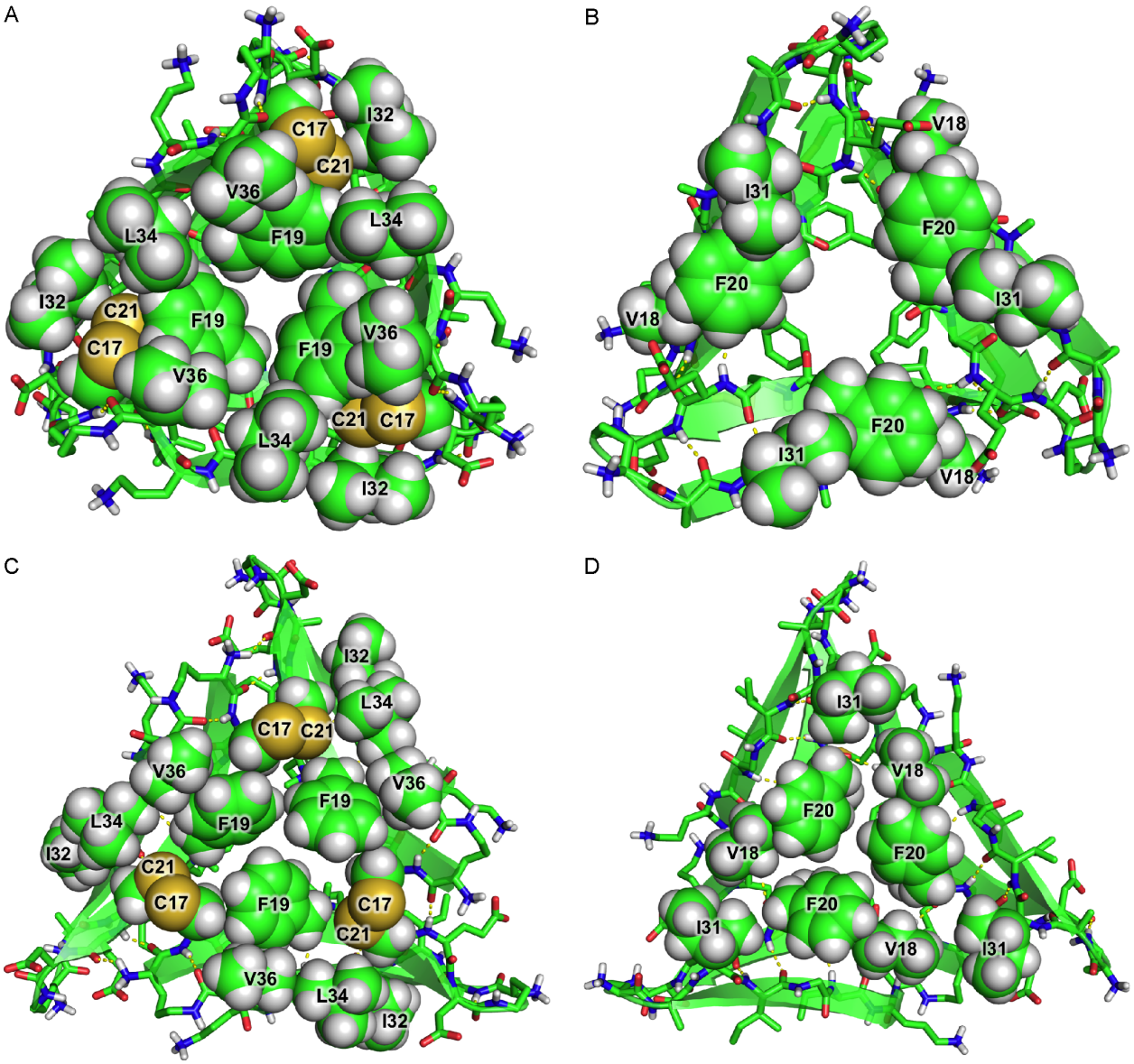


Figure S1. Hydrophobic surfaces of trimers **5** and **6** (PDB 5SUT and 5SUR). (A) The F_{19} face of trimer **5**. (B) The F_{20} face of trimer **5**. (C) The F_{19} face of trimer **6**. (D) The F_{20} face of trimer **6**.

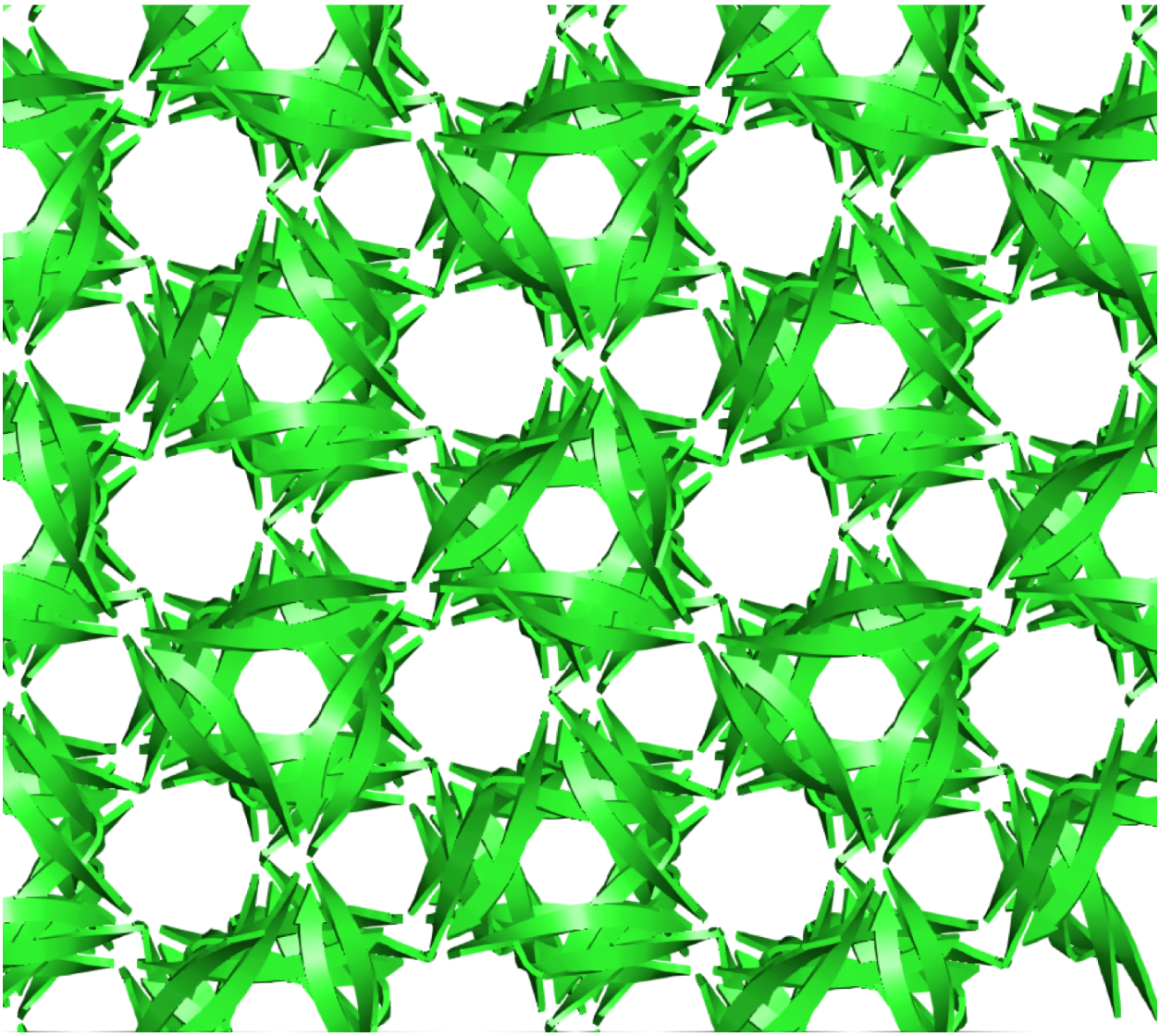


Figure S2. Hexagonal arrangement of the columns of hexamers formed by trimer **5** in the crystal lattice (PDB 5SUT).

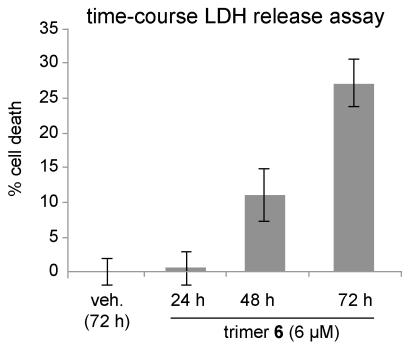


Figure S3. Time-course LDH release assay of trimer **6**. Data represent the mean of five replicate wells +/− s.d. Deionized water (vehicle, veh.) was used as a negative control.

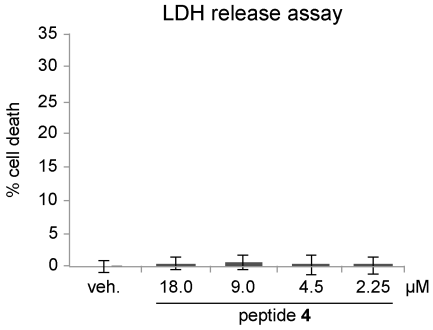


Figure S4. LDH release assay of peptide **4**. Data represent the mean of five replicate wells +/− s.d. Deionized water (vehicle, veh.) was used as a negative control.

Table S1. Crystallographic properties, crystallization conditions, and data collection and model refinement statistics for trimer **5**.

peptide	trimer **5** (synchrotron)	trimer **5** (X-ray diffractometer)
PDB ID	5SUT	5SUU
space group	*R*32	*R*32
a, *b*, *c* (Å)	49.59, 49.59, 64.18	50.23, 50.23, 64.80
α, β, λ (°)	90, 90, 120	90, 90, 120
peptides per asymmetric unit	2	2
crystallization conditions	0.1 M HEPES buffer at pH 7.3, 34% Jeffamine M-600 at pH 7.0	0.1 M HEPES buffer at pH 6.5, 32% Jeffamine M-600 at pH 7.0
wavelength (Å)	1.00	1.54
resolution (Å)	25.71–1.90 (1.97–1.902)	25.12–2.032 (2.104–2.032)
total reflections	5003 (502)	14674 (785)
unique reflections	2511 (255)	2115 (199)
multiplicity	2.0 (2.0)	6.9 (3.9)
completeness (%)	100 (100)	98.01 (90.87)
mean I/σ	32.71 (13.78)	33.18 (12.05)
Wilson B factor	21.97	19.35
R_{merge}	0.009174 (0.0249)	0.05975 (0.1306)
$R_{measure}$	0.01297 (0.03521)	0.06427
$CC_{1/2}$	1.000 (0.997)	0.999 (0.97)
CC^{*}	1.000 (0.999)	1.000 (0.992)
R_{work}	0.2053 (0.2177)	0.2163 (0.2512)
R_{free}	0.2160 (0.2297)	0.2398 (0.3544)
number of non-hydrogen atoms	260	262
RMS_{bonds}	0.027	0.007
RMS_{angles}	1.49	1.24
Ramachandran favored (%)	100	100
outliers (%)	0	0
clashscore	0	4.30
average B-factor	27.17	29.50
number of TLS groups	0	0
ligands/ions	Cl (1)	I (1), Cl (4)
water molecules	13	10

Table S2. Crystallographic properties, crystallization conditions, and data collection and model refinement statistics for trimer **6**.

peptide	trimer **6** (synchrotron)	trimer **6** (X-ray diffractometer)
PDB ID	5SUR	5SUS
space group	$P6_322$	$P6_322$
a, *b*, *c* (Å)	57.40, 57.40, 94.14	57.08, 57.08, 93.68
α, β, λ (°)	90, 90, 120	90, 90, 120
peptides per asymmetric unit	4	4
crystallization conditions	0.1 M Tris buffer at pH 7.83, 0.2 M $MgCl_2$, 3.32 M 1,6-hexanediol	0.1 M Tris buffer at pH 7.70, 0.2 M $MgCl_2$, 3.45 M 1,6-hexanediol
wavelength (Å)	0.97	1.54
resolution (Å)	30.35–1.80 (1.866–1.801)	26.4–2.349 (2.433–2.349)
total reflections	17658 (1680)	8286 (792)
unique reflections	8947 (854)	4143 (395)
multiplicity	2.0 (2.0)	98.6 (77.9)
completeness (%)	99 (100)	100 (100)
mean I/σ	23.12 (1.13)	57.82 (13.40)
Wilson B factor	40.24	38.15
R_{merge}	0.01053 (0.6542)	0.089 (0.546)
$R_{measure}$	0.0149 (0.9252)	0.01254 (0.05735)
$CC_{1/2}$	0.972 (0.581)	0.945 (0.812)
CC^*	0.946 (0.692)	0.900 (0.279)
R_{work}	0.2114 (0.3543)	0.2241 (0.2355)
R_{free}	0.2523 (0.3648)	0.2823 (0.3445)
number of non-hydrogen atoms	564	521
RMS_{bonds}	0.011	0.011
RMS_{angles}	1.06	0.98
Ramachandran favored (%)	100	100
outliers (%)	0	0
clashscore	2.72	5.62
average B-factor	58.89	52.12
number of TLS groups	4	4
ligands/ions	Na (2), Cl (2), 1,6-hexanediol (4)	Na (1), Cl (1)
water molecules	35	27

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 phenylalanine that followed the *N*-methylphenylalanine in peptides **2** and **4**: The 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.

*Synthesis of trimers **5** and **6**.*

Trimers **5** and **6** were synthesized by oxidizing peptides **3** and **4** in 20% aqueous DMSO.[3,4] A 6 mM solution of either lyophilized peptide **3** or **4** was prepared gravimetrically by

dissolving the peptide in an appropriate amount of 20% (v/v) aqueous DMSO prepared with deionized water. The reaction was carried out in a capped 25-mL glass scintillation vial with rocking at room temperature for 48–72 h. Next, the reaction mixture was diluted to a concentration of 300-μM peptide and transferred to a 500-mL round-bottomed flask. The solution was stirred with a magnetic stir bar for an additional 48 h. After 48 h, the reaction mixture was concentrated to ≤ 5 mL by rotary evaporation and immediately subjected to RP-HPLC purification (gradient elution with 20-50% CH_3CN over 60 min). Pure fractions were concentrated by rotary evaporation and lyophilized. Typical syntheses yielded ~15 mg trimer **5** and ~20 mg of trimer **6** from a 0.1 mmol scale synthesis of peptides **3** and **4**.

*Crystallization procedure for trimer **5***.

Trimer **5** afforded crystals in the same conditions that afforded crystals of peptides **1** and **2**: 0.1 M HEPES buffer and Jeffamine M-600. We further optimized these conditions according to the procedures detailed in the supporting information of Spencer *et al*. to yield crystals of trimer **5** suitable for X-ray crystallography. The optimized conditions consist of 0.1 M HEPES at pH 7.3 with 34% Jeffamine M-600.

Crystallization conditions for trimer **5** 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 Jeffamine M-600 concentration in each column in increments of 2% (26%, 28%, 30%, 32%, 34%, 36%). The first well in the 4x6 matrix was prepared by combined 100 μL of 1 M HEPES buffer at pH 6.5, 520 μL of 50% (v/v) aqueous Jeffamine M-600, and 380 μL of deionized water. The other wells were prepared in analogous fashion, by combining 100

μL of HEPES buffer of varying pH and 50% (v/v) aqueous Jeffamine M-600 in varying amounts, and deionized 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 trimer **5** (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 trimer **5** suitable for X-ray diffraction grew in ~2 days. 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 trimer **5** are summarized in Table S1.

*Crystallization procedure for trimer **6**.*

Initial crystallization conditions for trimer **6** 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 trimer **6** (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 trimer **6** grew in ~48 h in a solution of 0.1 M Tris buffer at pH 7.0 with 0.2 M $MgCl_2$ and 3.5 M 1,6-hexanediol.

Crystallization conditions for trimer **6** were optimized using a 4x6 matrix Hampton VDX 24-well plate. The Tris 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 1,6-hexanediol concentration in each column in increments of 0.2 M (3.0 M, 3.2 M, 3.4 M, 3.6 M, 3.8 M, 4.0 M). The first well in the 4x6 matrix was prepared by combined 100 μL of 1 M Tris buffer at pH 6.5, 100 μL of 2 M $MgCl_2$, 600 μL of 5 M 1,6-hexanediol, and 200 μL of deionized water. The other wells were prepared in analogous fashion, by combining 100 μL of Tris buffer of varying pH, 100 μL of 2 M $MgCl_2$, 5 M 1,6-hexanediol in varying amounts, and deionized 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 trimer **6** (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 trimer **6** suitable for X-ray diffraction grew in ~5 days. Crystallization conditions were further optimized using smaller variations in Tris buffer pH (in increments of 0.25 pH units) and 1,6-hexanediol concentrations (in increments of 0.1 M). 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 trimer **6** are summarized in Table S2.

*X-ray crystallographic data collection, data processing, and structure determination for trimers **5** and **6**.*

Diffraction data for trimers **5** and **6** 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.[5] Coordinates were refined with phenix.refine.

Diffraction data for trimer **5** were also collected at the Advanced Light Source at Lawrence Berkeley National Laboratory with a synchrotron source at 1.00-Å wavelength to achieve higher resolution. Data for trimer **5** suitable for refinement at 2.03 Å were obtained from the diffractometer; data for trimer **5** suitable for refinement at 1.90 Å were obtained from the synchrotron. Diffraction data were scaled and merged using XDS.[6] The electron density map was generated by molecular replacement using the coordinates from the structure of trimer **5** generated by soaking in KI using Phaser in the Phenix software suite 1.10.1.[7] Molecular manipulation of the model was performed with Coot. Coordinates were refined with phenix.refine.

Diffraction data for trimer **6** were also collected at the Stanford Synchrotron Radiation Lightsource (SSRL) with a synchrotron source at 0.97-Å wavelength.[8] Diffraction data were scaled and merged using XDS.[6] The electron density map was generated by molecular replacement using the coordinates from the structure of trimer **6** generated by S-SAD using Phaser in the Phenix software suite 1.10.1.[7] Molecular manipulation of the model was performed with Coot. Coordinates were refined with phenix.refine.

Preparation of $A\beta_{42}$ oligomers

Recombinantly expressed $A\beta_{42}$ was purchased as the NH_4OH treated salt from rPeptide (catalog# A-1167-2). $A\beta_{42}$ oligomers were prepared according to the procedure developed by Teplow and coworkers.[9,10] A 1.0-mg aliquot of $A\beta_{42}$ received from rPeptide was dissolved in 1.0 mL of 2 mM NaOH, and sonicated in an ultrasonic water bath for 1 min. The pH of the $A\beta_{42}$ solution after NaOH addition was ~10.5. Next, the $A\beta_{42}$ solution was aliquotted into 0.0055-μmol aliquots in 0.5-mL microcentrifuge tubes. A hole in the top of each microcentrifuge tube was created by pushing a 22-gauge needle through the top of the tube. The samples were then frozen at -80 °C for ~5 hours, and lyophilized overnight. After lyophilization, the $A\beta_{42}$ aliquots were stored in a desiccator at -20 °C until use.

For LDH and caspase-3 activation assays a 0.0055-μmol aliquot of $A\beta_{42}$ was dissolved in 11 μL of 20 mM HEPES buffer at pH 7.4, and then immediately diluted with 99 μL of phenol red free, serum free DMEM:F12 (1:1) media to create a 50 μM $A\beta_{42}$ working solution. The 50 μM $A\beta_{42}$ working solution was serially diluted with DMEM:F12 (1:1) media to create 25- and 12.5-μM working solutions.

For SDS-PAGE a 0.0055-μmol aliquot of $A\beta_{42}$ was dissolved in 11 μL of 20 mM HEPES buffer at pH 7.4, and then immediately diluted with 11 μL of 2X SDS-PAGE loading buffer (100 mM Tris buffer at pH 6.8, 20% (v/v) glycerol, and 4% w/v SDS).

LDH release and caspase-3 activation assays.

The toxicity of peptides **1** and **2** and trimers **5** and **6** toward SH-SY5Y cells was assessed by LDH release and caspase-3 activation assays. Cells were incubated in the presence or absence of equivalent concentrations of peptides **1** and **2** or trimers **5** and **6** for 72 h in 96-well plates. The

LDH release assay was performed using the Pierce LDH Cytotoxicity Assay Kit from Thermo Scientific. The caspase-3 assay was performed using the Roche APO-One Homogeneous Caspase-3/7 Assay. 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** and **2** and trimers **5** and **6**.* 10-mg/mL stock solutions of peptides **1** and **2** and trimers **5** and **6** 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 180-µM working solutions of peptides **1** and **2** or 60-µM working solutions of trimers **5** and **6**. [These solutions contain equivalent concentrations of peptide.] The 180-µM working solutions of peptides **1** and **2** were serially diluted with deionized water to create 90-µM working solutions of peptides **1** and **2**. The 60-µM working solutions of trimers **5** and **6** were serially diluted with deionized water to create 30-, 15-, and 7.5-µM working solutions of trimers **5** and **6**.

b. *Preparation of SH-SY5Y cells for LDH release and caspase-3 activation assays.* SH-SY5Y cells were plated in a 96-well plate at 30,000 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** and **2** and trimers **5** and **6**.* 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 peptide **1** or **2** or trimer **5** or **6** was added to each well, for well concentrations of 18 μM and 9 μM for peptides **1** and **2**, 6 μM and 3 μM for trimer **5**, and 6 μM, 3 μM, 1.5 μM, and 0.75 μM for trimer **6**. 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, or staurosporine for the caspase-3 activation 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 minutes. 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, then 100 μL of stop solution was added to each well. 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:

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

e. *Caspase-3 activation assay.* After 67 hours, 10 μL of 150 μM or 50 μM staurosporine was added to the remaining five wells, and the cells were incubated for an additional 5 h. Next, the compound-containing media was removed and replaced with 25 μL of fresh serum-free DMEM/F12 media. A 25-μL aliquot of caspase-3 substrate, prepared according to manufacturer's protocol, was then added to each well. The plate was sealed with a clear adhesive plate sealer, and fluorescence was monitored over 18 h while shaking at 250 rpm using a Gemini

XPS fluorescence plate reader (excitation at 499 nm, emission at 521 nm). Data from the 18 h time point were processed by subtracting the relative fluorescence unit (RFU) values of the vehicle control wells from the RFU values of wells treated with peptides **1** and **2** and trimers **5** and **6**.

*Dot blot analysis of peptides **1** and **2** and trimers **5** and **6**.*

10-mg/mL stock solutions of peptides **1** and **2** and trimers **5** and **6** were prepared gravimetrically by dissolving 1.0 mg of each compound in 100 µL of deionized water that was filtered through a 0.2 µm syringe filter. An aliquot of each stock solution was diluted with deionized water to make 3.0-mg/mL solutions. The 3.0-mg/mL solutions were then serially diluted with deionized water to create 1.5-, 0.75-, and 0.37-mg/mL solutions. A 5 µL aliquot of each solution from the serial dilution was combined with 5 µL of a 2X solution of phosphate buffered saline (PBS) at pH 7.4 to generate 1.5-, 0.75-, 0.37-, and 0.18-mg/mL buffered solutions of peptides **1** and **2** and trimers **5** and **6**. A 1.0-µL aliquot of each buffered solution was spotted onto a nitrocellulose membrane and allowed to air dry (~5 min). Non-reactive sites were blocked with 10% (w/v) non-fat powdered 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 then incubated while rocking overnight at 4 °C in primary A11 antibody (200 µg/mL) in 5% (w/v) non-fat powdered 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% (w/v) non-fat powdered 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 digital SLR camera.[11]

Size exclusion chromatography.

The oligomerization of peptides **1** and **2** and trimers **5** and **6** was studied by size-exclusion chromatography (SEC) at 4 °C in 50 mM sodium acetate/50 mM acetic acid buffer (sodium acetate buffer, pH 4.7) as follows: Each peptide or trimer was dissolved in deionized water to a concentration of 10 mg/mL. The peptide or trimer solutions were then diluted to 1 mg/mL by adding 80 μL of the 10-mg/mL solutions to 720 μL of sodium acetate buffer. The peptide or trimer solutions were 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 sodium acetate buffer 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.

SDS-PAGE and silver staining.

The oligomerization of peptides **1** and **2** and trimers **5** and **6** 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.[12]

Sample preparation. Each peptide or trimer 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 2.0-mg/mL solutions of peptides **1** and **2** and 0.12-mg/mL solutions of trimers **5**

and **6**. The 1.0-mg/mL solutions of peptides **1** and **2** and the 0.12-mg/mL solutions of trimers **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 1.0-mg/mL working solutions of peptides **1** and **2** and 0.06-mg/mL working solutions of trimers **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 60 volts at 4 °C.

Staining with silver nitrate was used to visualize peptides **1** and **2** and trimers **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**.[13] 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.

Circular dichroism spectroscopy.

A 0.30 mg/mL solution of either trimer **5** or **6** or peptide **1** or **2** was prepared by adding 15 μL of 10 mg/mL stock solutions in deionized water to 385 μL of 10 mM potassium phosphate buffer at pH 7.4. Each solution was transferred to a 1 mm quartz cuvette for data acquisition. CD spectra were acquired on a Jasco J-810 circular dichroism spectropolarimeter at room temperature. Data were collected using 0.2 nm intervals from 260 nm to 190 nm and averaged over five accumulations with smoothing.

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, and Spencer, R. K.; Li. H.; Nowick, J. S. *J. Am. Chem. Soc.* **2014**, *136*, 5595–5598.

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

3 Tam, J. P.; Wu, C. R.; Liu, W.; Zhang, J. W. *J. Am. Chem. Soc.* **1991**, *113*, 6657–6662.

4 Khakshoor, O.; Nowick, J. S. *Org. Lett.* **2009**, *11*, 3000-3003.

5 Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. *Acta. Cryst.* **2010**, *D66*, 486–501.

6 Kabsch, W. *Acta Cryst.* **2010**, *D66*, 125–132.

7 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.

8 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.

9 Teplow, D. B. *Methods Enzymol.* **2006**, *413*, 20–33.

10 Fezoui, Y.; Hartley, D. M.; Harper, J. D.; Khurana, R.; Walsh, D. M.; Condron, M. M.; Selkoe, D. J.; Lansbury, P. T. Jr.; Fink, A. L.; Teplow, D. B. *Amyloid*. **2000**, *7*, 166–178.

11 Khoury, M. K.; Parker, I.; Aswad, D.W. *Anal. Biochem.* **2010**, *397*, 129–131.

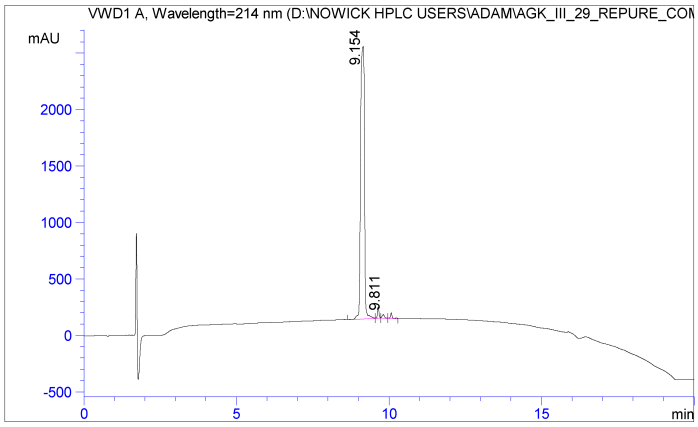
12 Schägger, H. *Nat. Protoc.* **2006**, *1*, 16–22.

13 Simpson, R. J. *CSH Protoc.* **2007**, doi: 10.1101/pdb.prot4727.

Characterization Data

*Characterization of peptide **1***

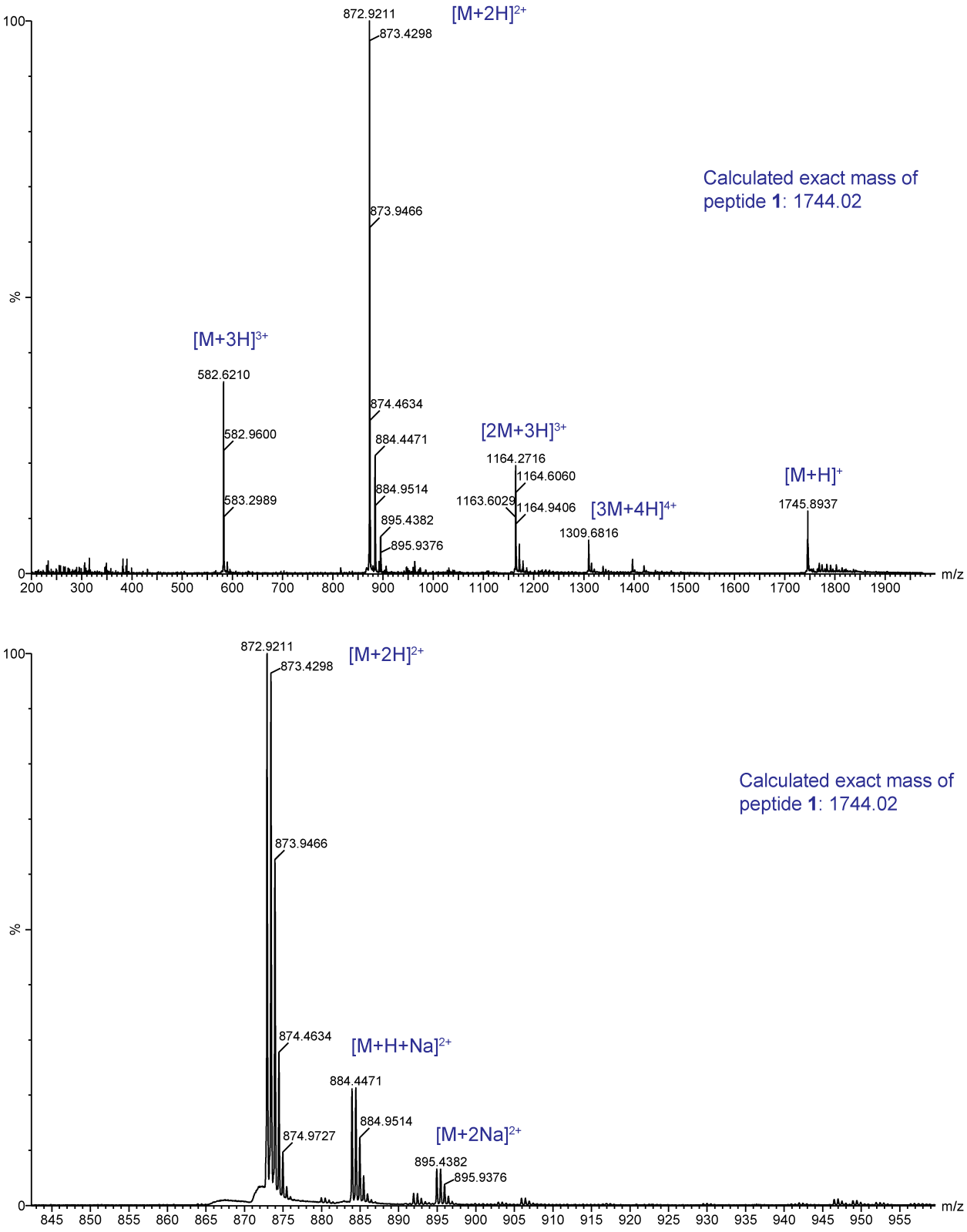
Analytical HPLC trace of peptide **1**.

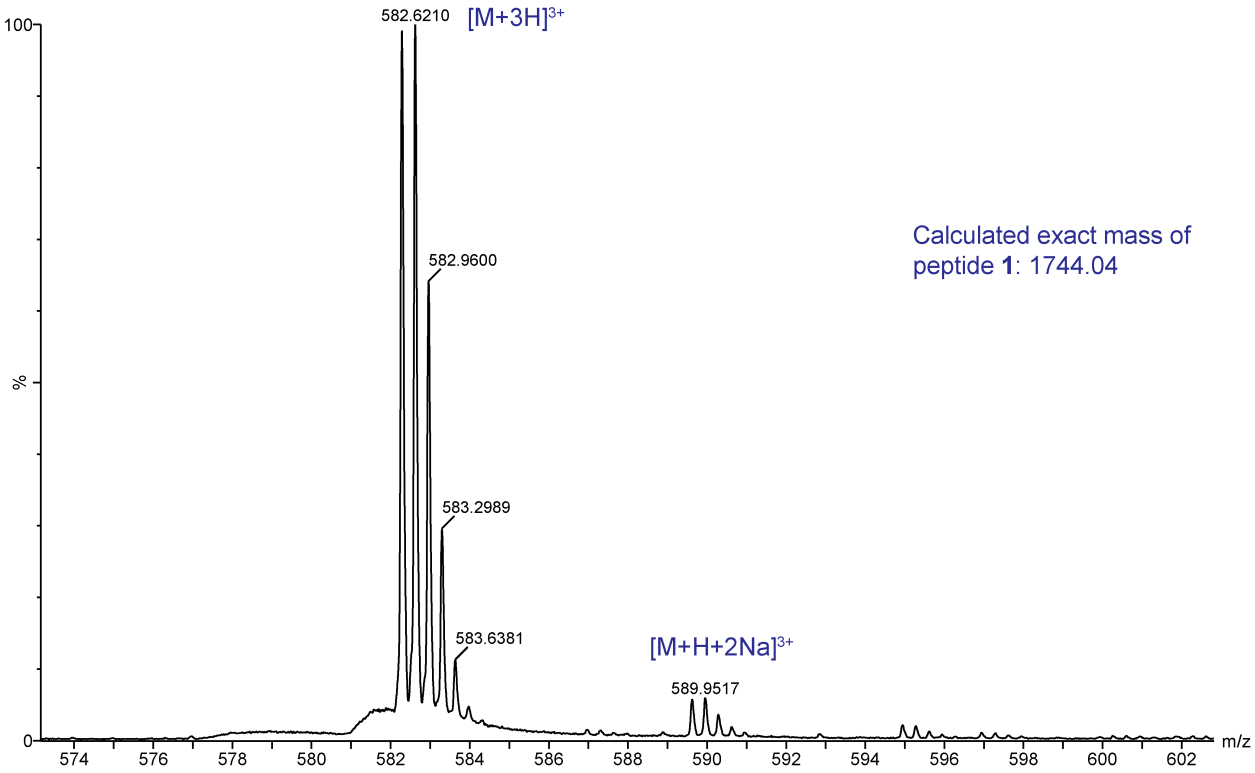


Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	9.154	MM	0.144	20963.105	92.861	96.669
2	9.649	MM	0.056	340.649	3.898	1.571
3	9.811	MM	0.088	165.284	1.202	0.762
4	10.078	MM	0.068	216.490	2.039	0.998

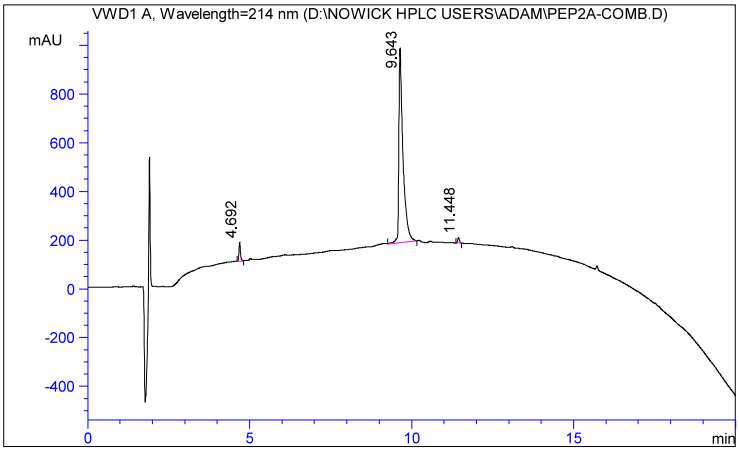
Mass spectrum and expansions of peptide **1**.




582.6210
[M+3H]3+
582.9600
583.2989
583.6381
[M+H+2Na]3+
589.9517
Calculated exact mass of peptide 1: 1744.04
100
%
0
574
576
578
580
582
584
586
588
590
592
594
596
598
600
602
m/z

*Characterization of peptide **2***

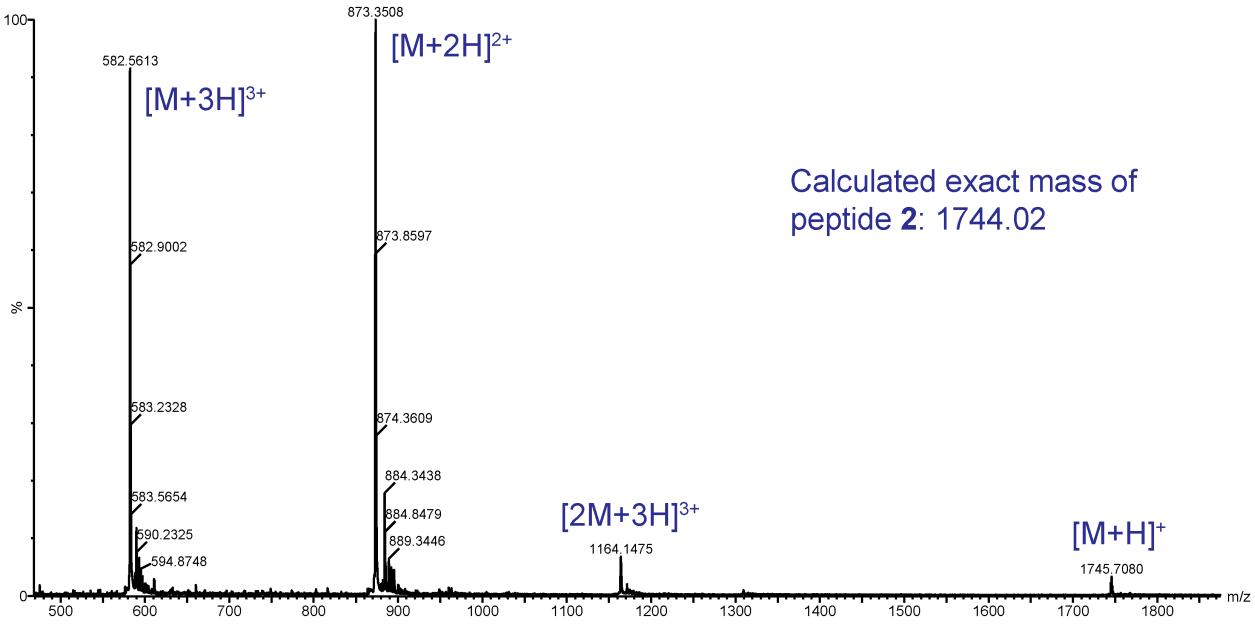
Analytical HPLC trace of peptide **2**.

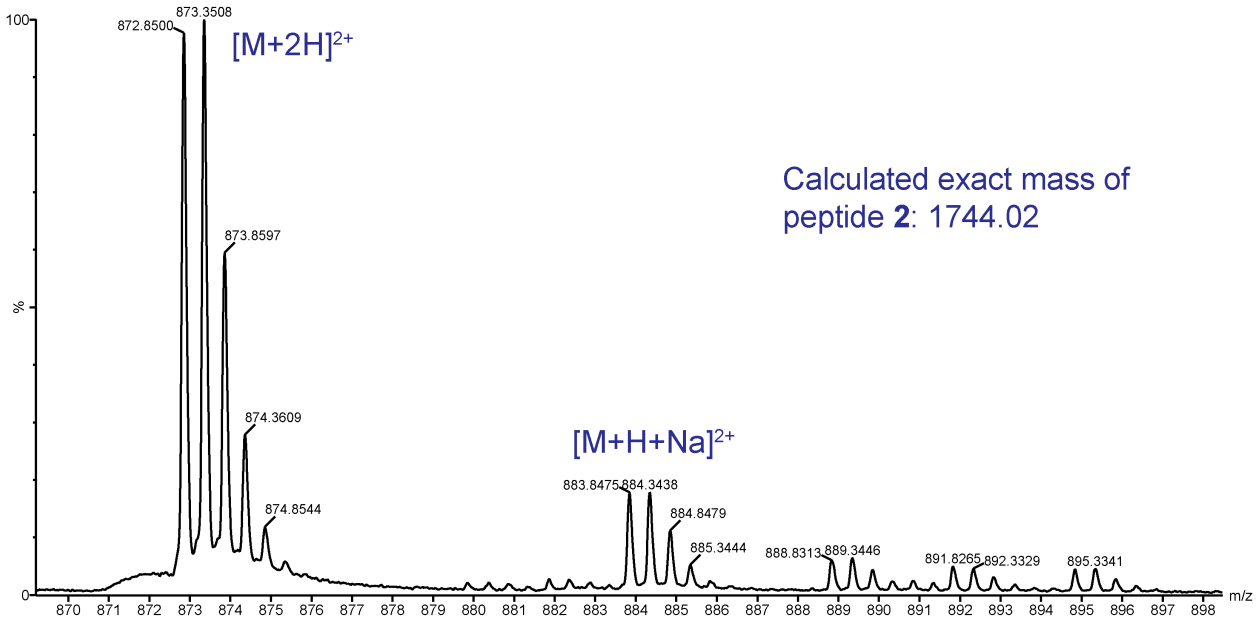


Signal 1:VWD1 A, Wavelength=214 nm

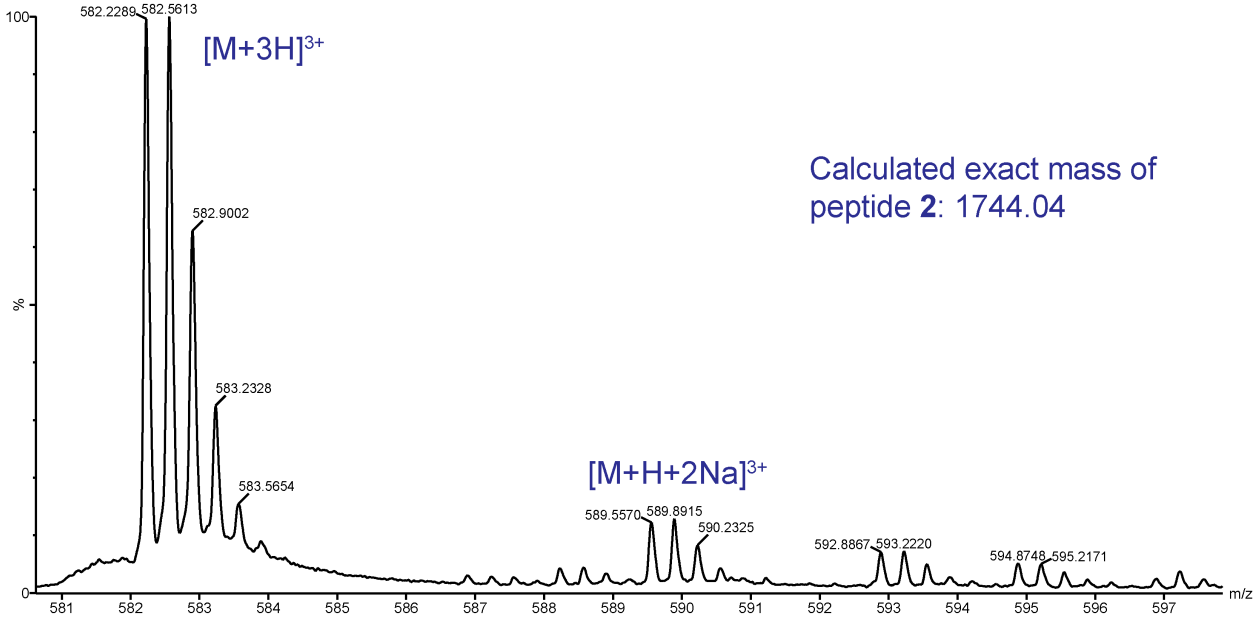
Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	4.692	MM	0.058	278.627	8.806	3.732
2	9.643	MM	0.148	7104.079	88.643	95.157
3	11.448	MM	0.060	82.934	2.551	1.111

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





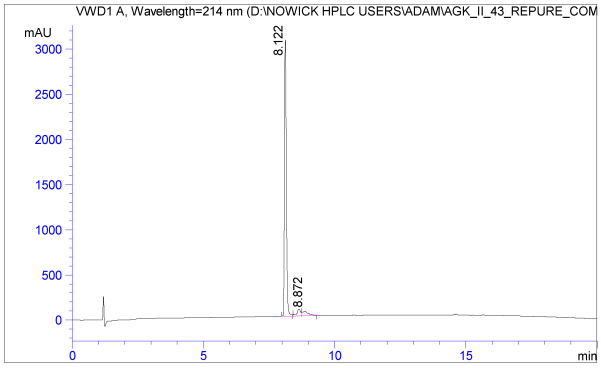
Calculated exact mass of peptide **2**: 1744.02



Calculated exact mass of peptide **2**: 1744.04

*Characterization of peptide **3***

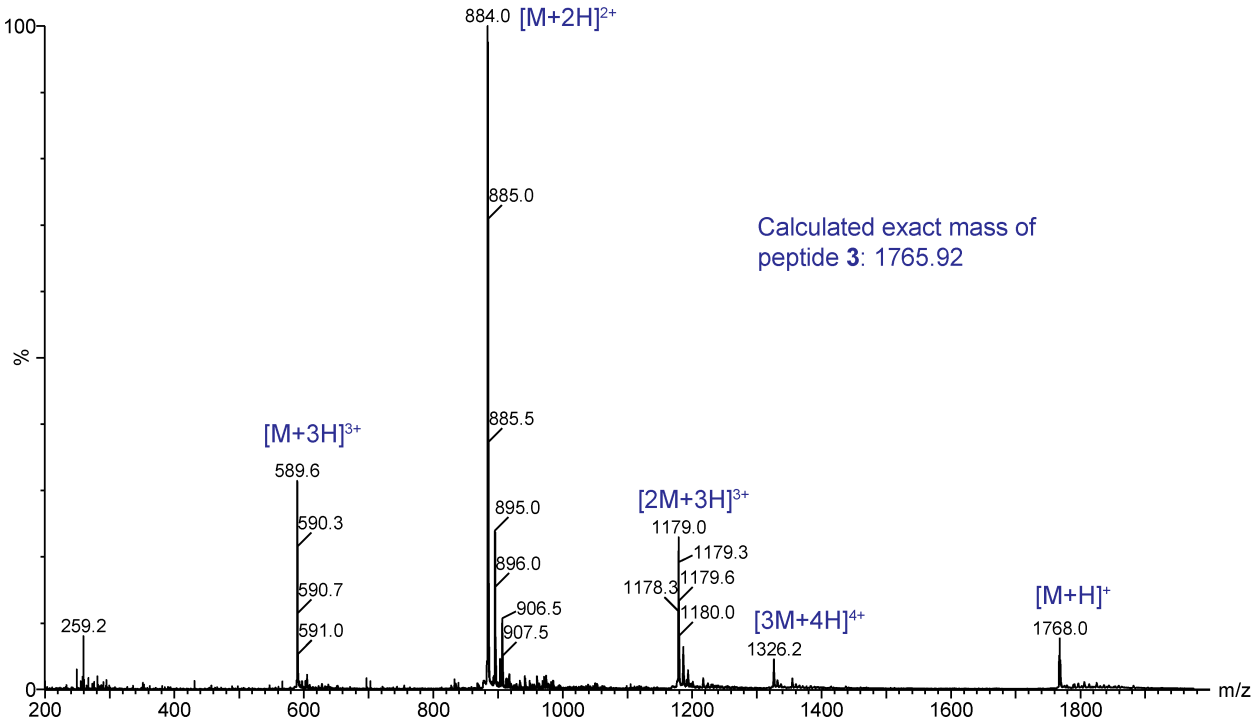
Analytical HPLC trace of peptide **3**.

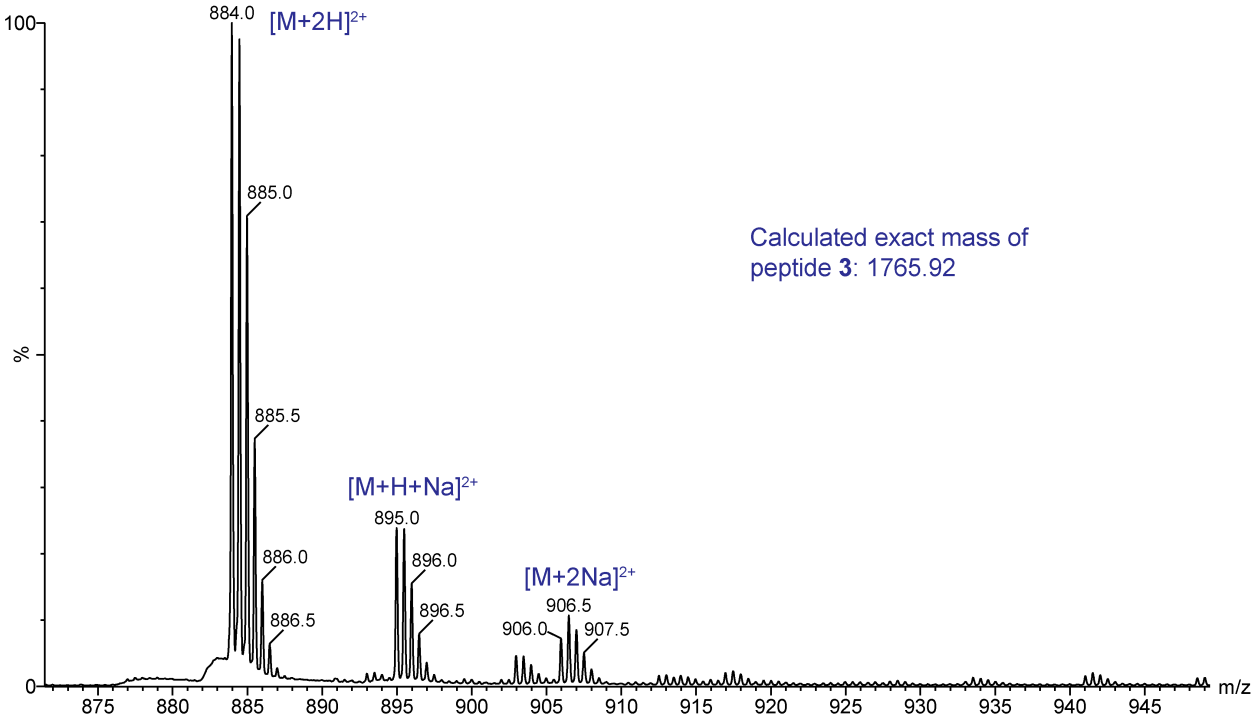


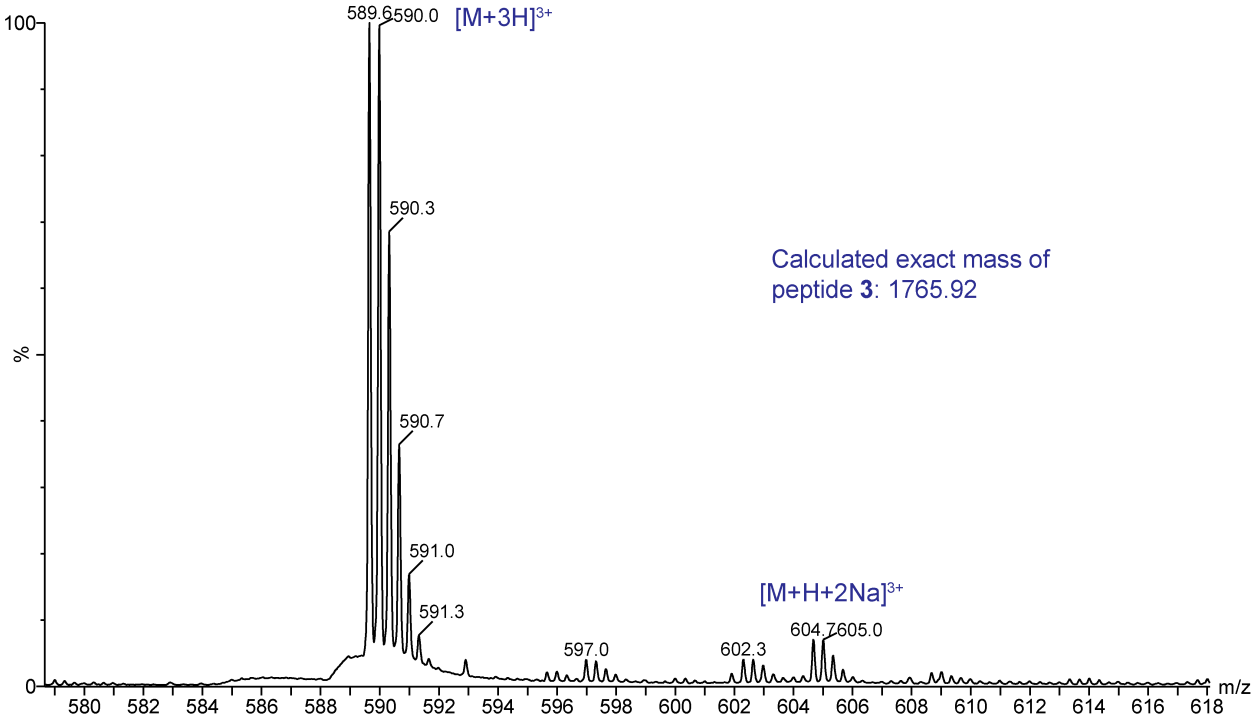
Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	8.122	BB	0.081	15238.801	96.038	90.206
2	8.641	BV	0.149	763.724	2.318	4.521
3	8.872	VB	0.218	890.721	1.645	5.273

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

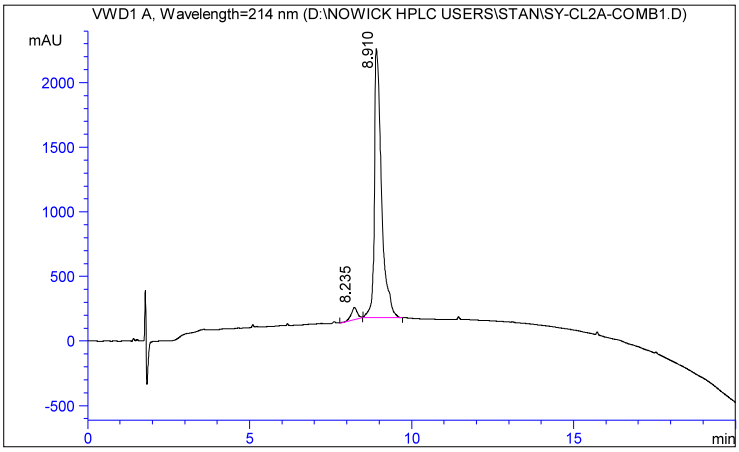



100
%
0
884.0
[M+2H]2+
885.0
885.5
886.0
886.5
[M+H+Na]2+
895.0
896.0
896.5
[M+2Na]2+
906.0
906.5
907.5
Calculated exact mass of
peptide 3: 1765.92
875 880 885 890 895 900 905 910 915 920 925 930 935 940 945
m/z


100
%
0
589.6
590.0
[M+3H]3+
590.3
590.7
591.0
591.3
597.0
602.3
604.7
605.0
[M+H+2Na]3+
Calculated exact mass of
peptide 3: 1765.92
580 582 584 586 588 590 592 594 596 598 600 602 604 606 608 610 612 614 616 618
m/z

*Characterization of peptide **4***

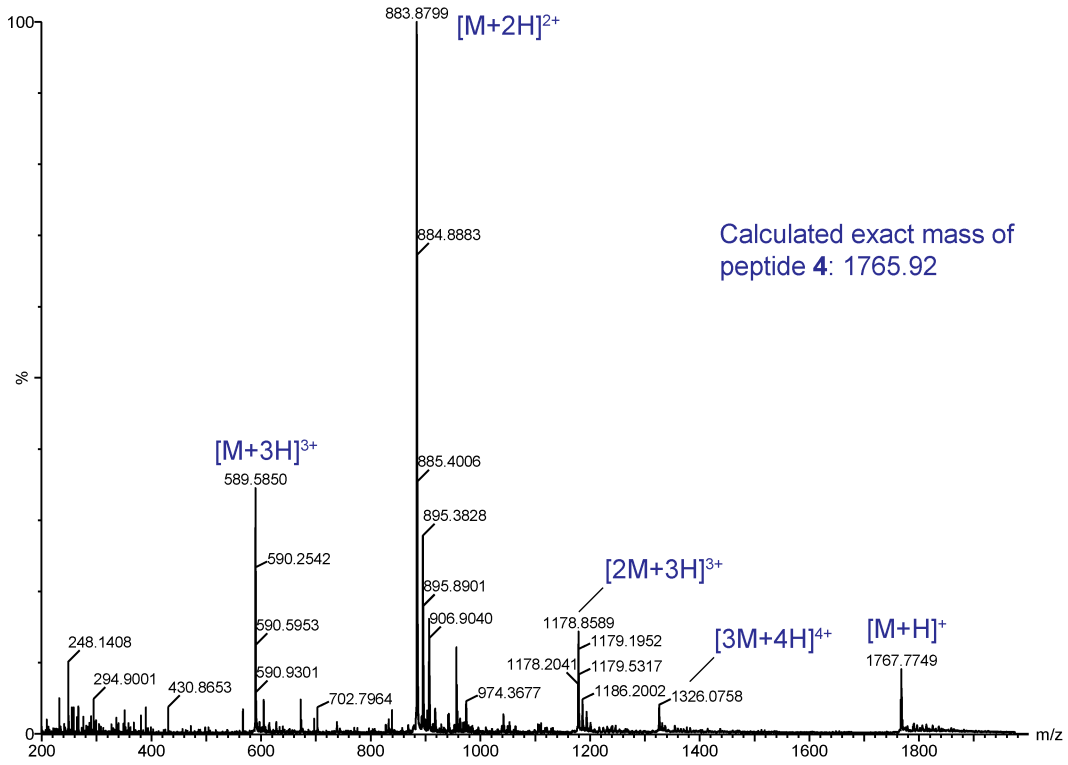
Analytical HPLC trace of peptide **4**.

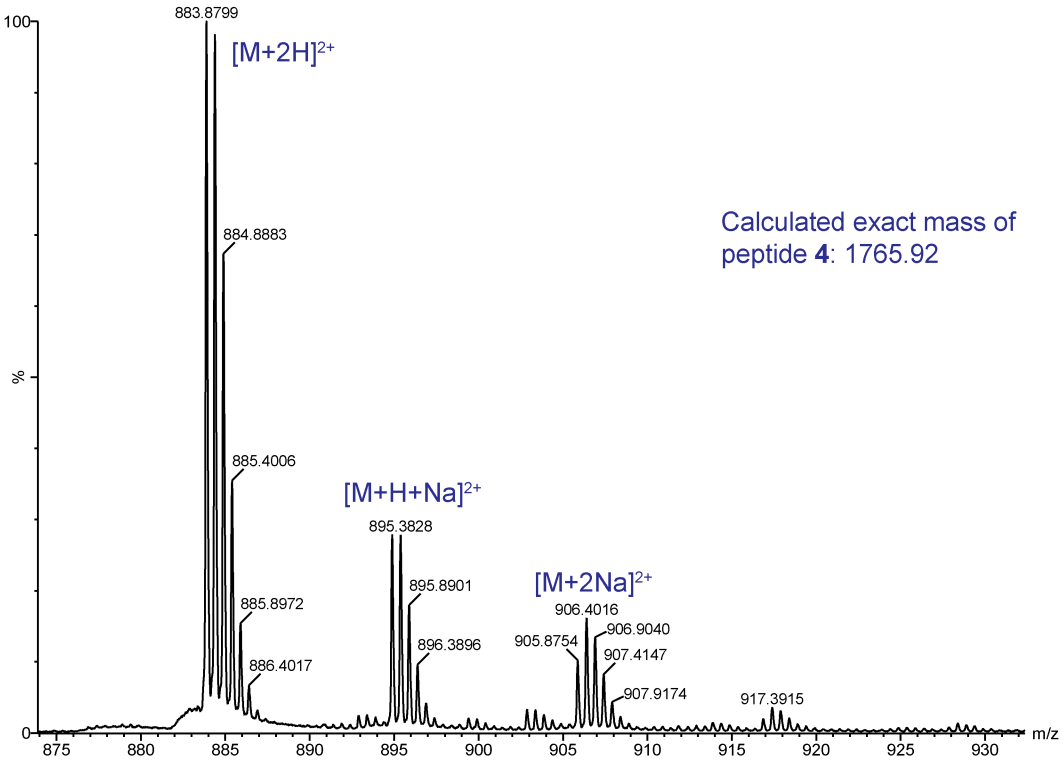


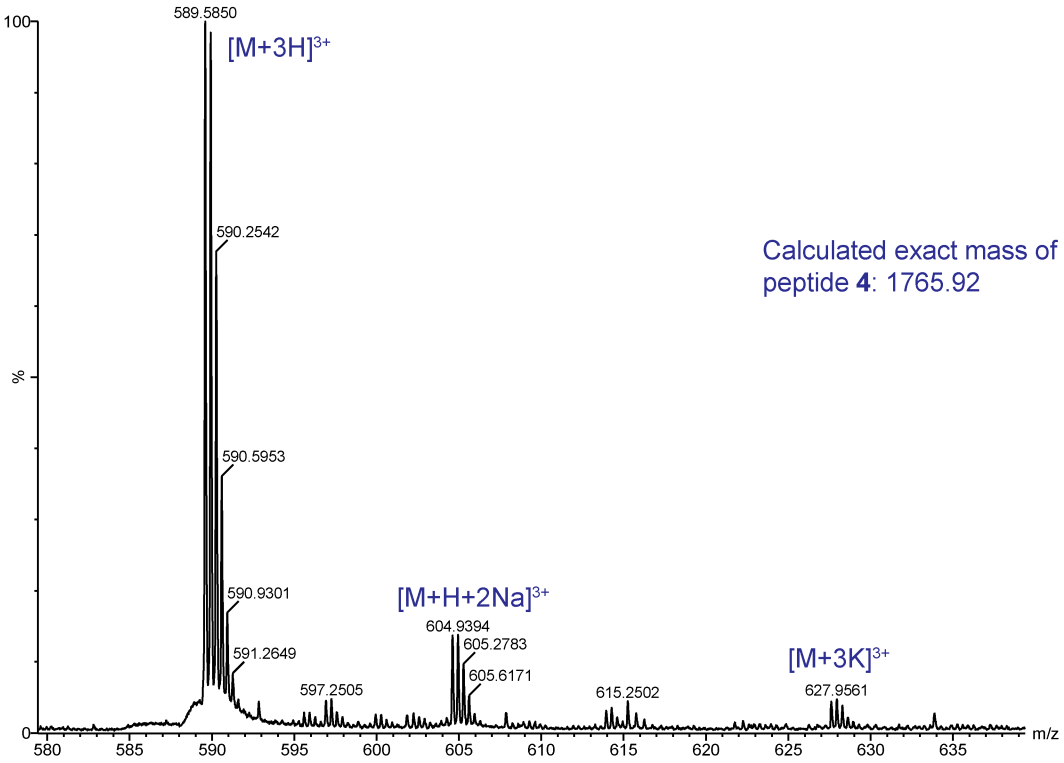
Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	8.235	MM	0.225	1285.120	4.362	3.856
2	8.910	MM	0.256	32039.193	95.638	96.144

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

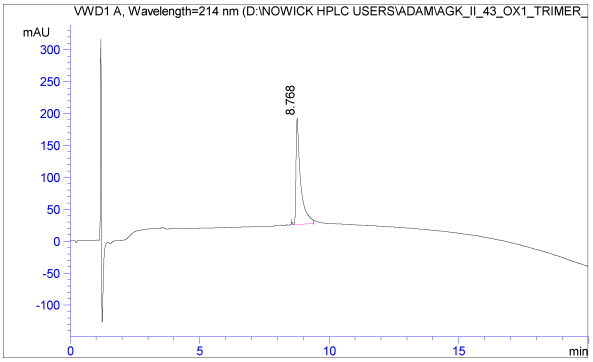





589.5850
[M+3H]3+
590.2542
590.5953
590.9301
591.2649
597.2505
[M+H+2Na]3+
604.9394
605.2783
605.6171
615.2502
[M+3K]3+
627.9561
100
%
0
580
585
590
595
600
605
610
615
620
625
630
635
m/z
Calculated exact mass of peptide 4: 1765.92

*Characterization of trimer **5***

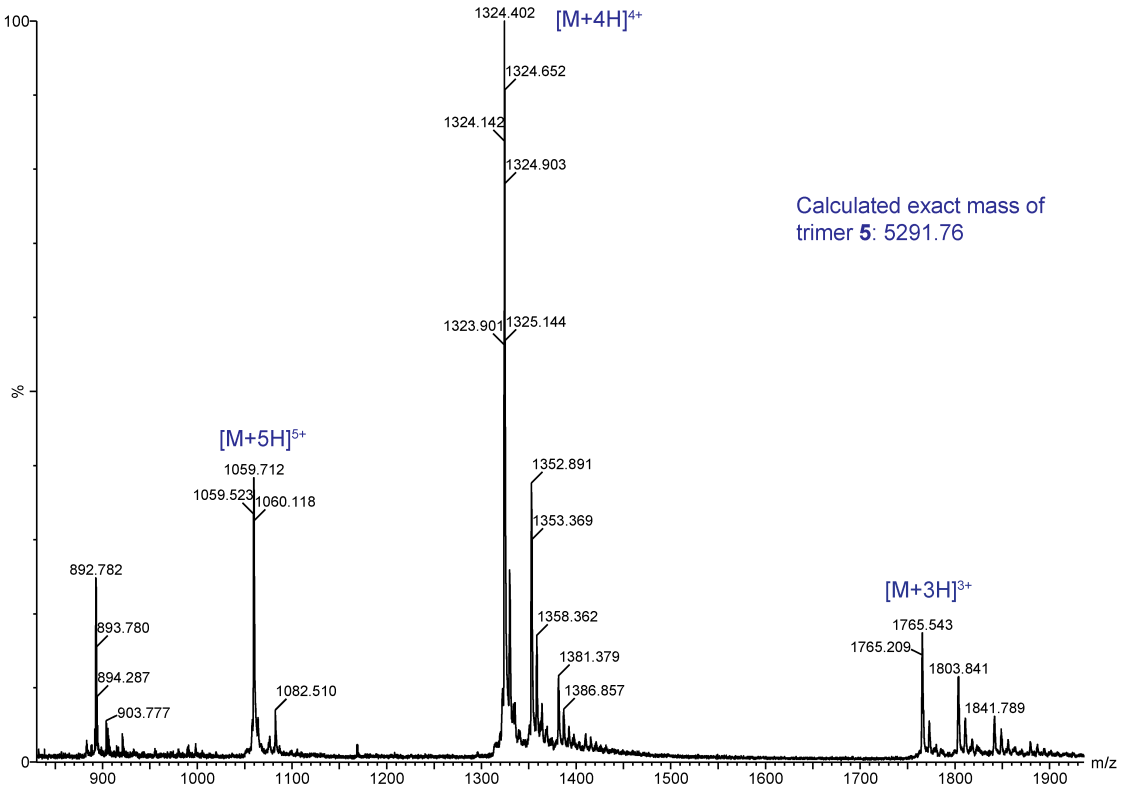
Analytical HPLC trace of trimer **5**.

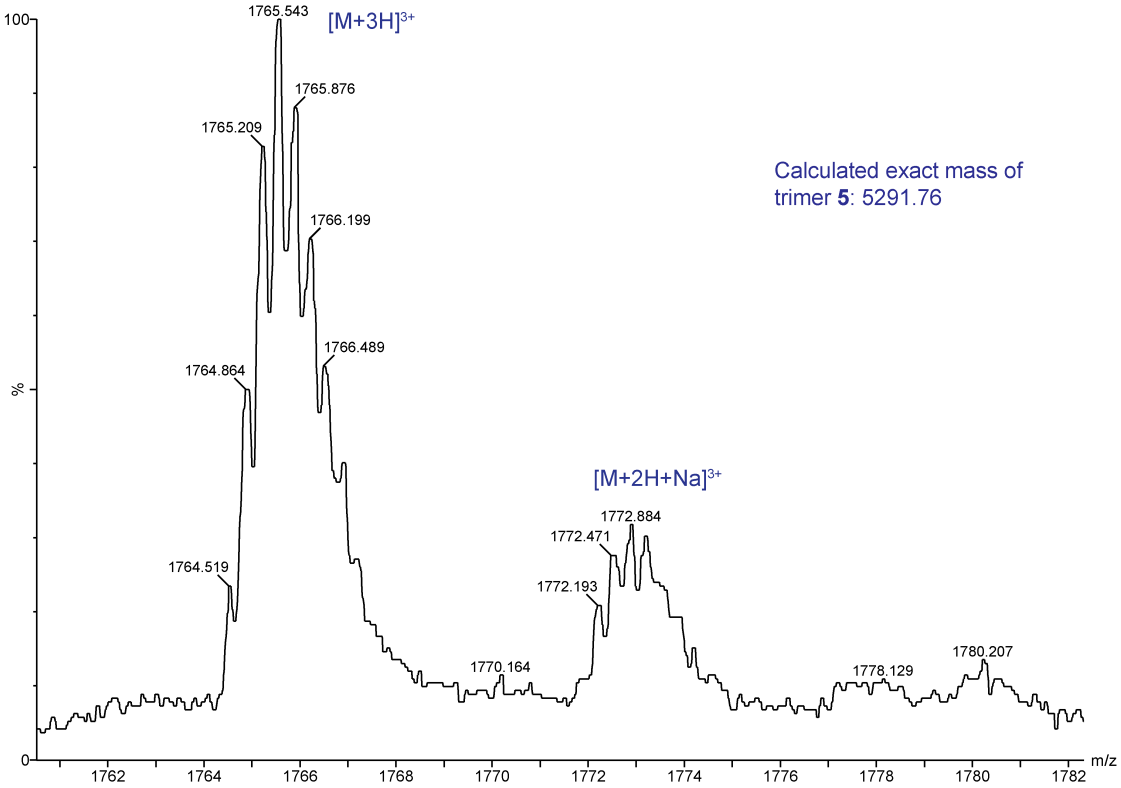


Signal 1:VWD1 A, Wavelength=214 nm

Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	8.768	BB	0.174	2057.338	100.000	100.000

Mass spectrum and expansions of trimer **5**.




100
%
0
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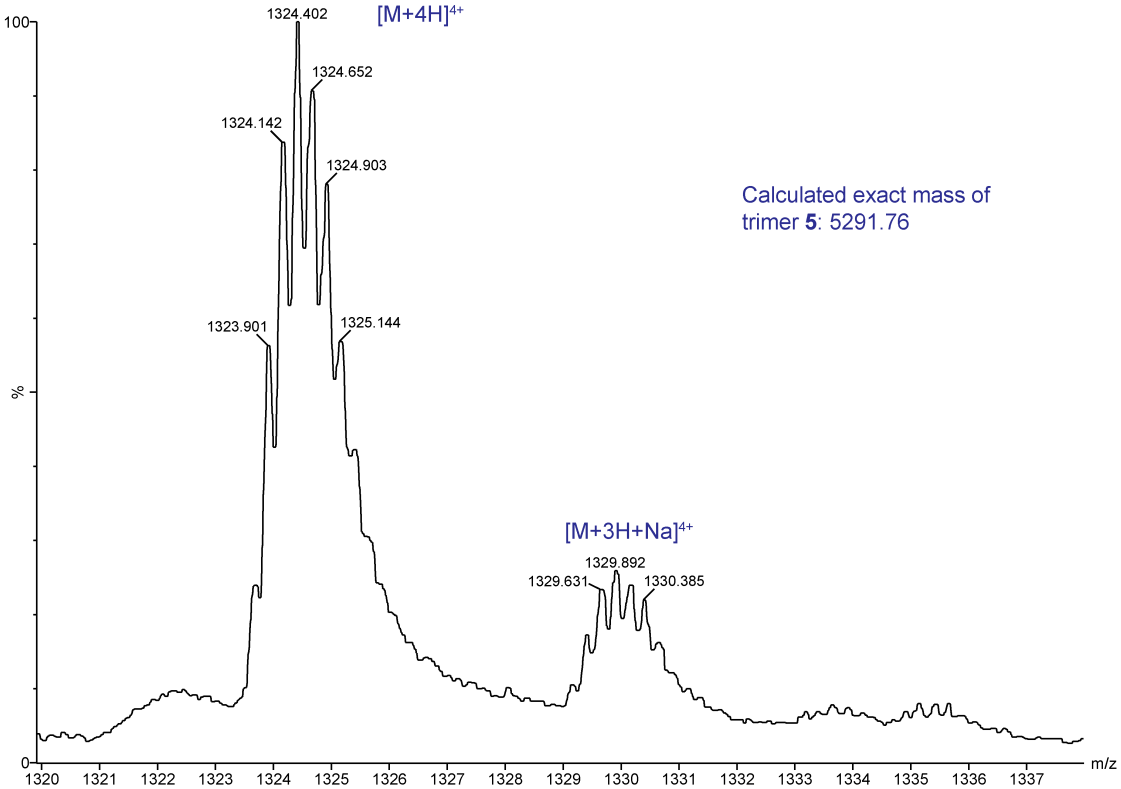
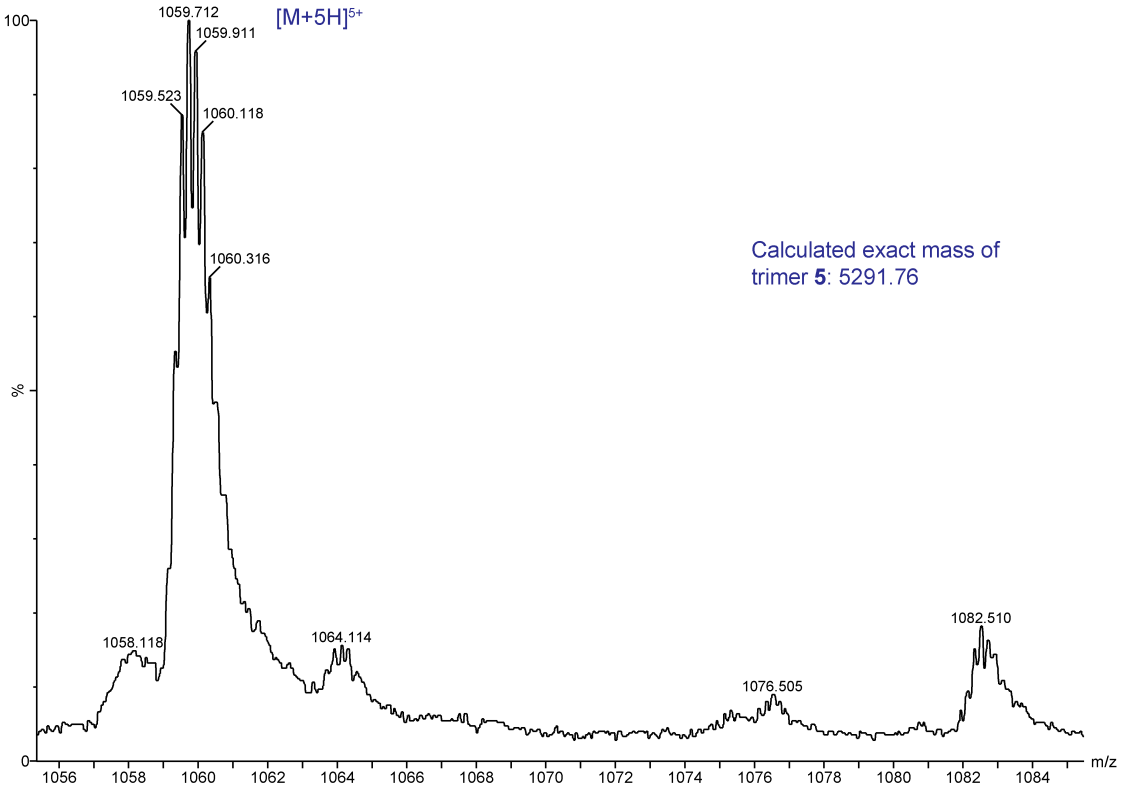
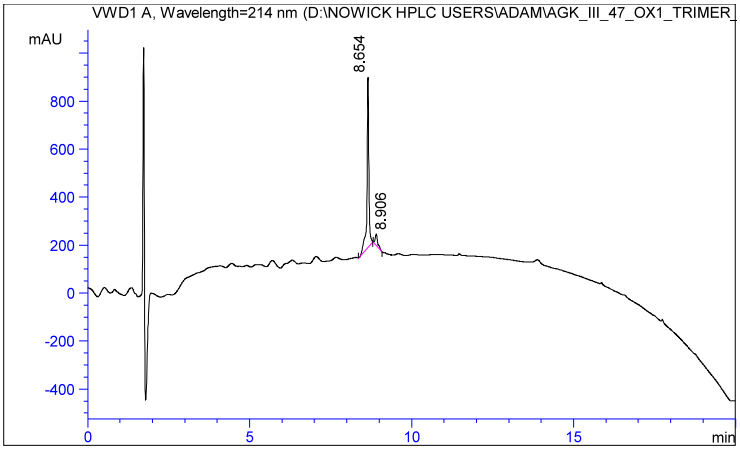
[M+3H]3+
1765.876
1765.209
1766.199
1766.489
1764.864
1764.519
[M+2H+Na]3+
1772.884
1772.471
1772.193
1770.164
1778.129
1780.207
m/z
1762
1764
1766
1768
1770
1772
1774
1776
1778
1780
1782
Calculated exact mass of
trimer 5: 5291.76


100
%
0
1324.402
[M+4H]4+
1324.652
1324.142
1324.903
1323.901
1325.144
[M+3H+Na]4+
1329.892
1329.631
1330.385
m/z
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
Calculated exact mass of
trimer 5: 5291.76


[M+5H]5+
1059.712
1059.911
1059.523
1060.118
1060.316
1058.118
1064.114
1076.505
1082.510
Calculated exact mass of
trimer 5: 5291.76
100
%
0
m/z
1056
1058
1060
1062
1064
1066
1068
1070
1072
1074
1076
1078
1080
1082
1084

*Characterization of trimer **6***

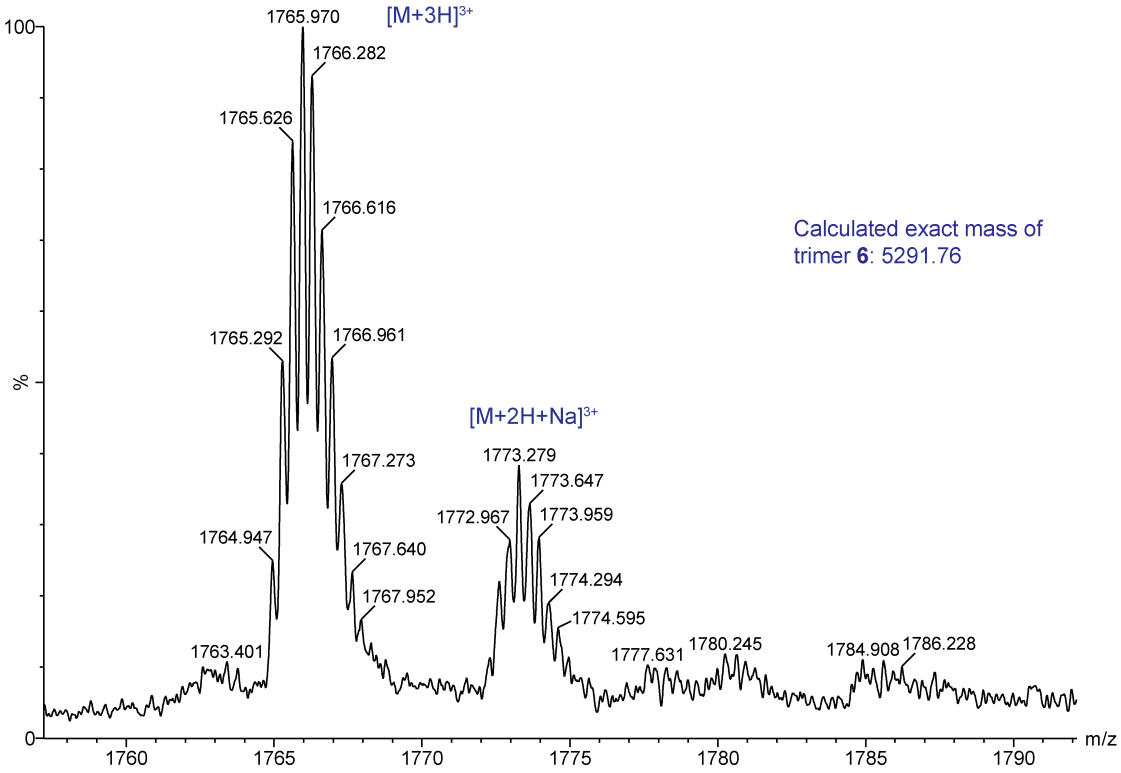
Analytical HPLC trace of trimer **6**.

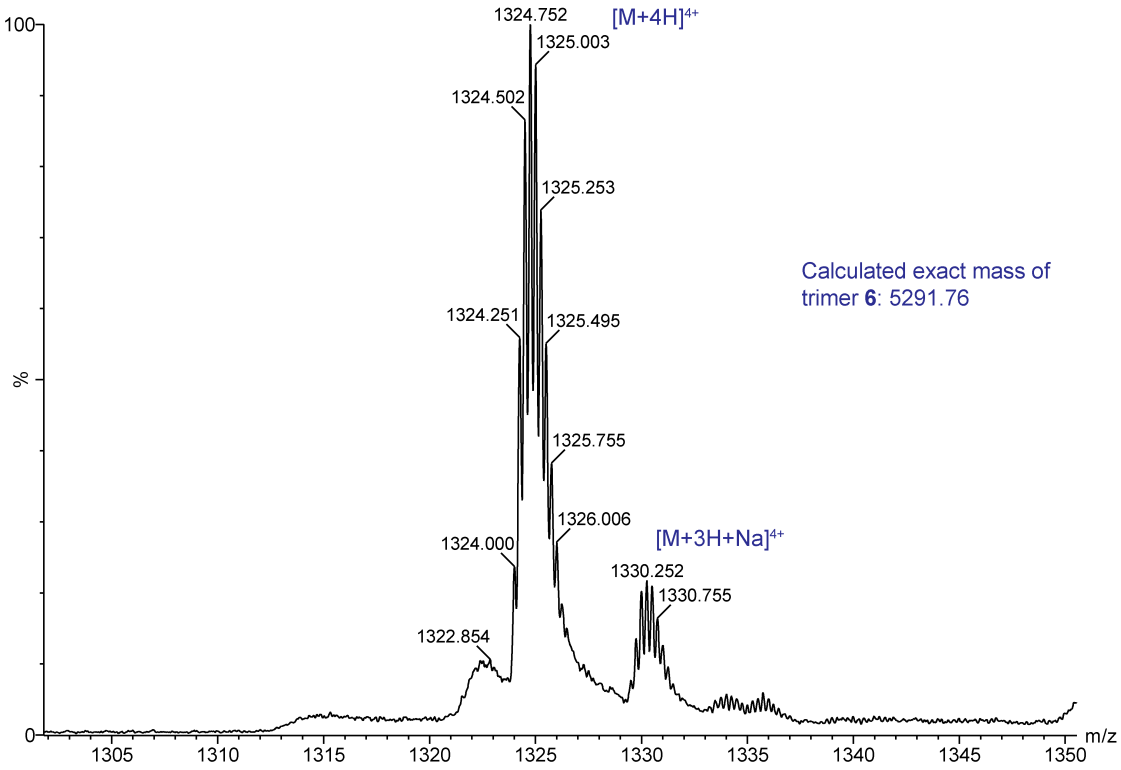


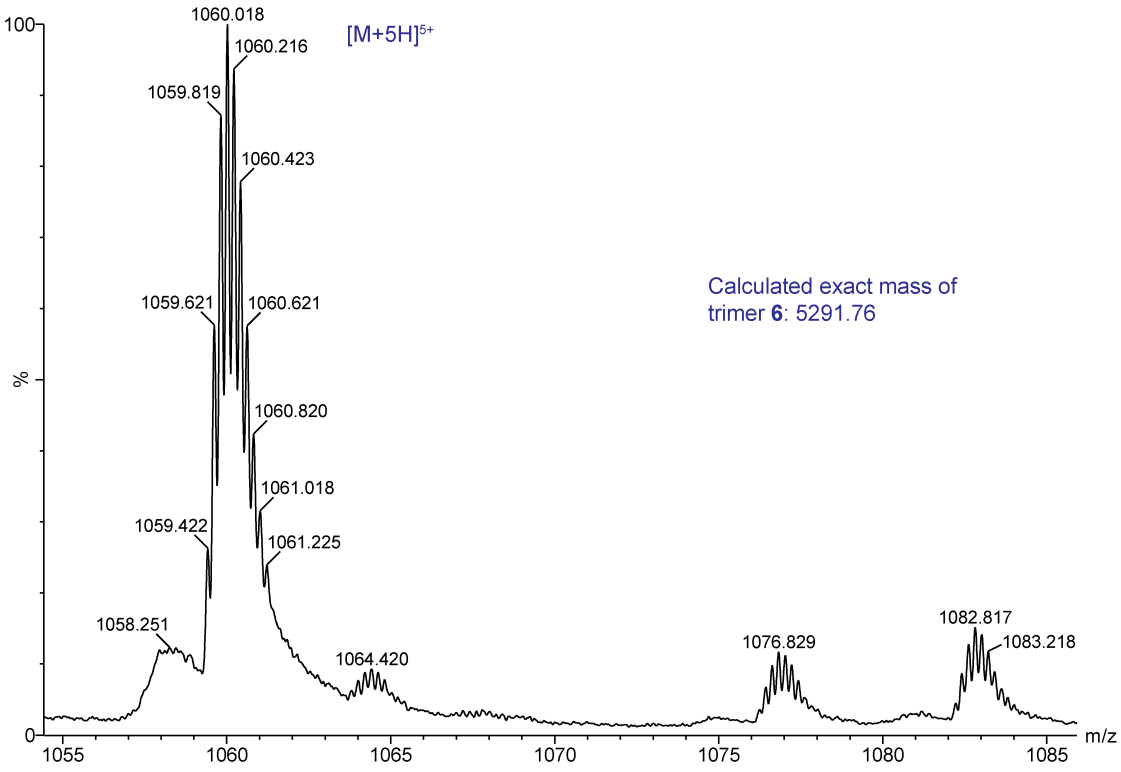
Signal 1:VWD1 A, Wavelength=214 nm

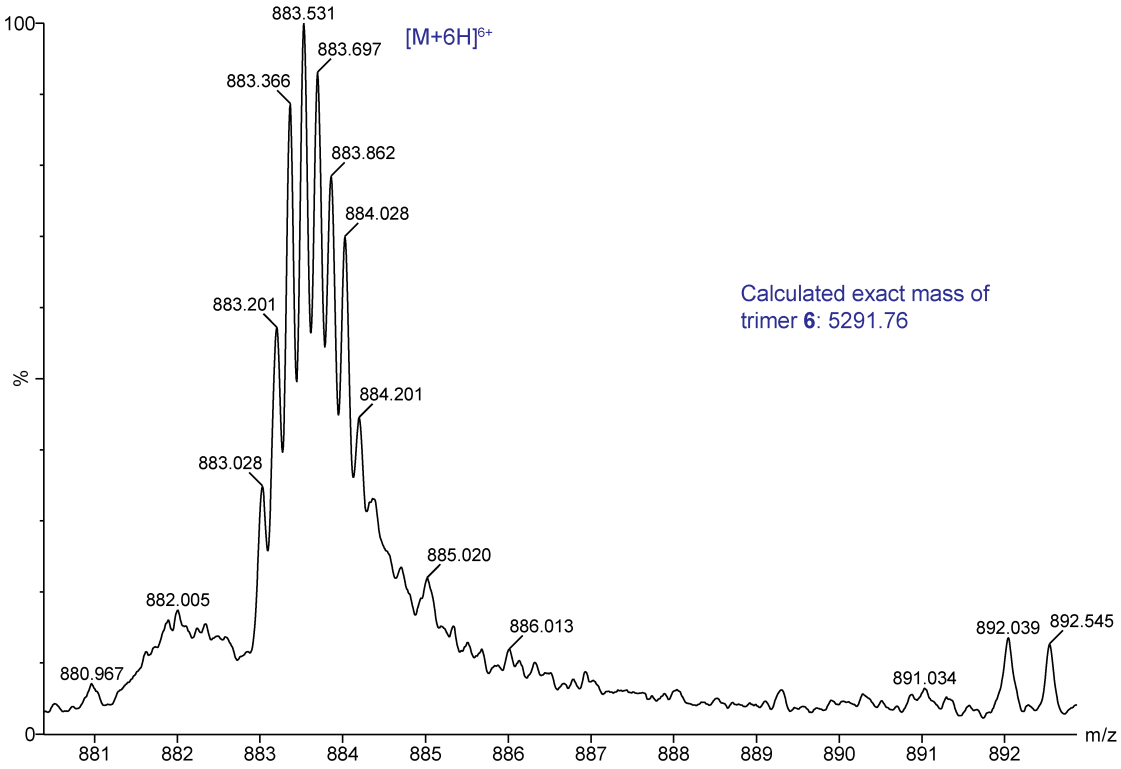
Peak #	RT [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	8.654	MM	0.068	2907.973	93.730	91.555
2	8.906	MM	0.094	268.220	6.270	8.445

Mass spectra expansions of trimer **6**.






100
%
0
1060.018
1060.216
1059.819
1060.423
1059.621
1060.621
1060.820
1061.018
1059.422
1061.225
1058.251
1064.420
1076.829
1082.817
1083.218
[M+5H]5+
Calculated exact mass of
trimer 6: 5291.76
1055
1060
1065
1070
1075
1080
1085
m/z


100
%
0
883.531
883.697
883.366
883.862
884.028
883.201
884.201
883.028
885.020
882.005
886.013
880.967
891.034
892.039
892.545
[M+6H]6+
Calculated exact mass of
trimer 6: 5291.76
881
882
883
884
885
886
887
888
889
890
891
892
m/z