Supporting Information:

Phenylalanine Mutation to Cyclohexylalanine Facilitates Triangular Trimer Formation by β-Hairpins Derived from Aβ

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Supporting Figures and Tables

Synthesis of peptides and cross-linked trimers

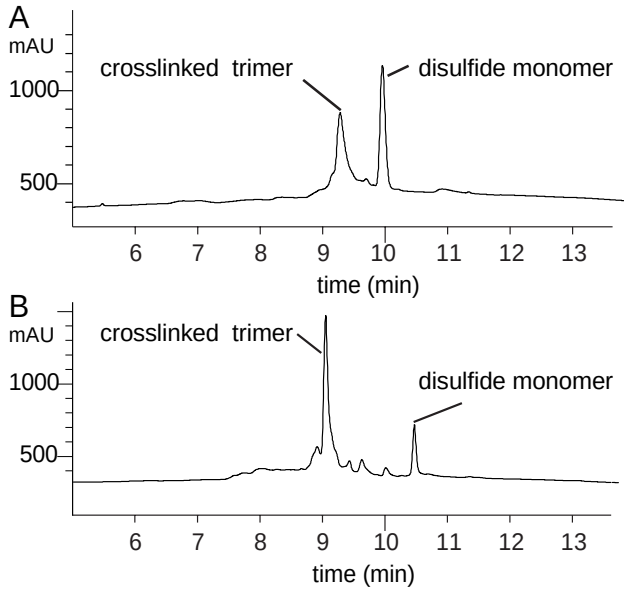


Figure S1. Oxidation of β-hairpin peptides. (A) HPLC chromatogram of DMSO oxidation of peptide **2**. (B) HPLC chromatogram of DMSO oxidation of peptide $\mathbf{2}_{\mathbf{F20Cha}}$. Analytical HPLC was performed on a C18 column with 5–100 % elution with acetonitrile over 20 minutes.

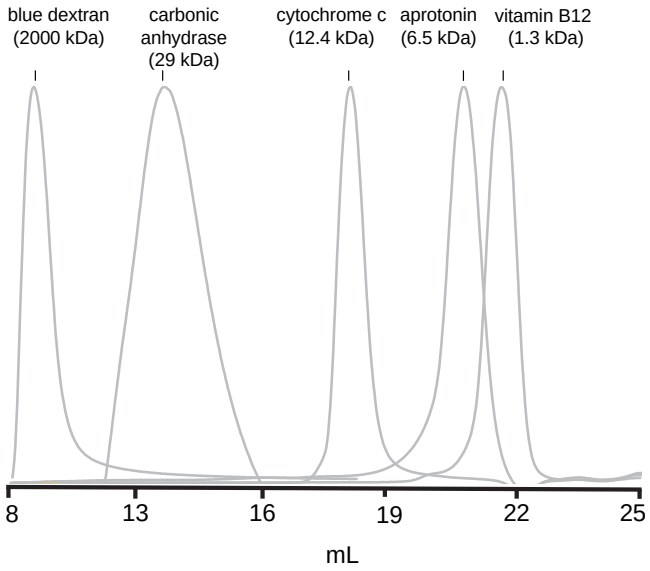


Figure S2. SEC chromatograms of size standards blue dextran, carbonic anhydrase, cytochrome C, aprotinin, and vitamin B12.

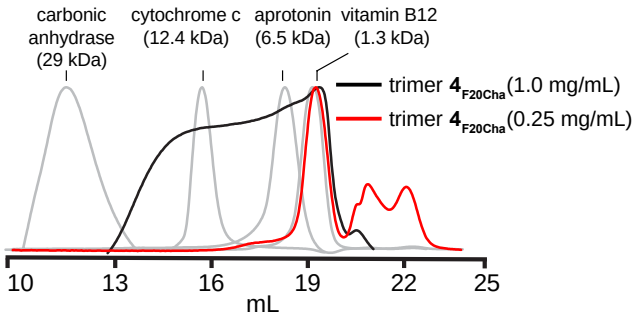


Figure S3. SEC chromatogram of trimer $\mathbf{4}_{\mathbf{F20Cha}}$ in glycine buffer pH 3.0 at 1.0 mg/mL (black) and 0.25 mg/mL (red).

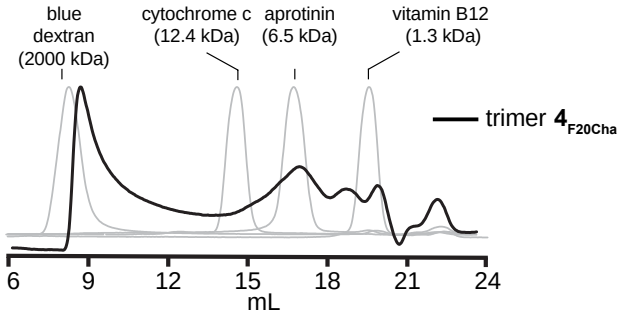


Figure S4. SEC chromatogram of trimer $\mathbf{4}_{\mathbf{F20Cha}}$ in TBS buffer pH 7.4.

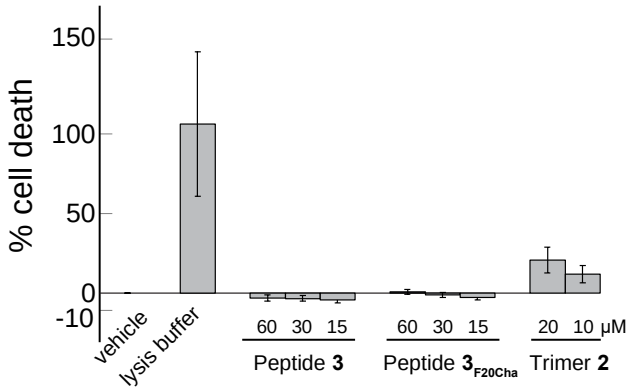


Figure S5. Cytotoxicity of peptides **3** and $\mathbf{3}_{\mathbf{F20Cha}}$ and trimer **2** as assessed by LDH release assay in SHSY-5Y cells. Data represents mean of six replicate wells, with the error bars corresponding to the standard deviation.

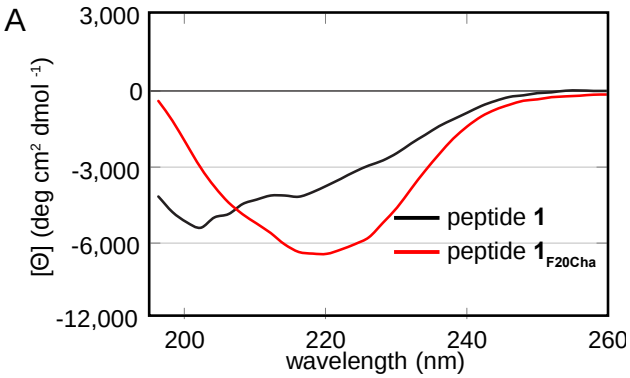


Figure S6. Circular dichroism spectra. Spectra were acquired at 25 μM peptides **1** and **1**$_{\mathbf{F20Cha}}$ in 10 mM sodium phosphate buffer at pH 7.4.

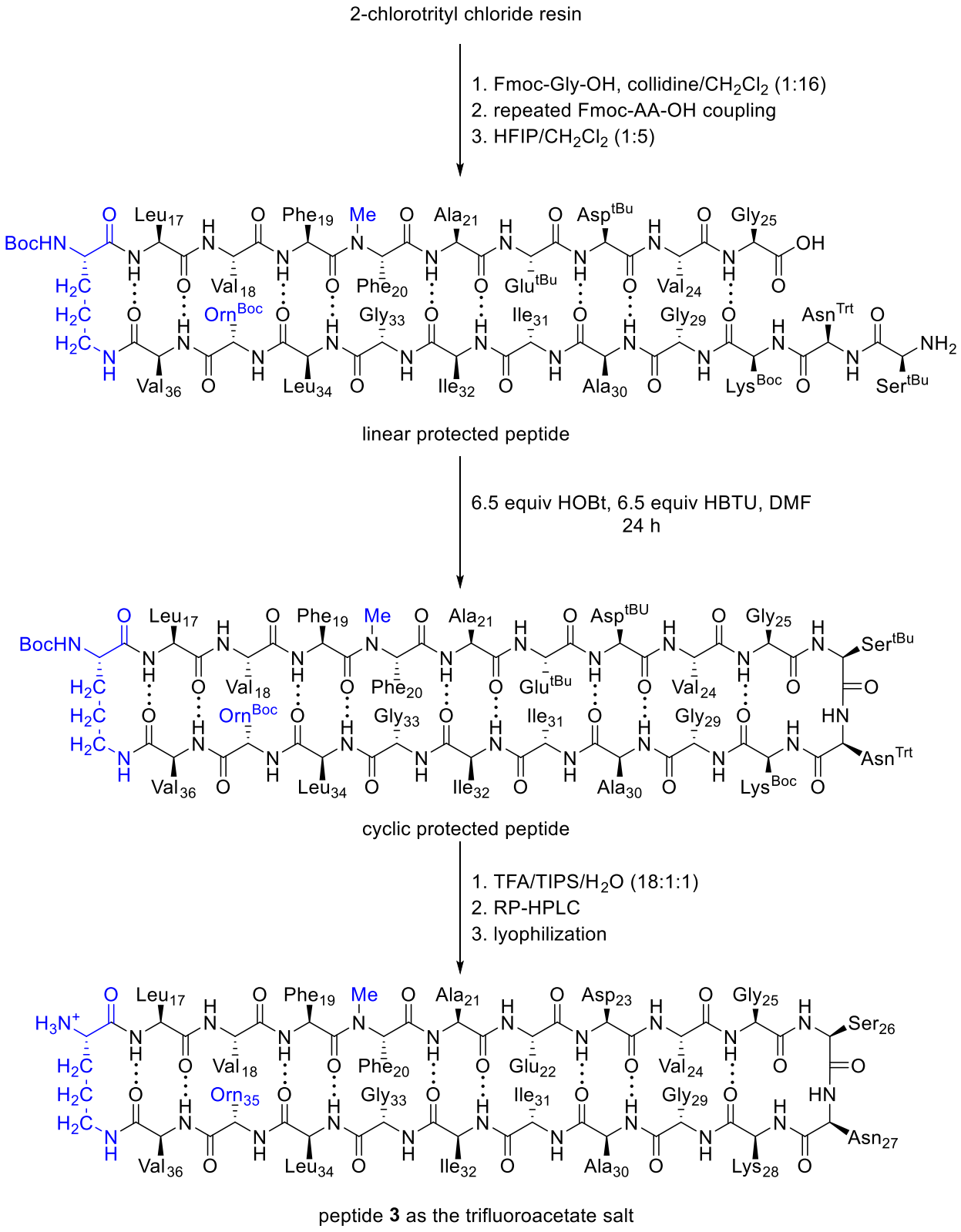
Syntheses of peptides and cross-linked trimers

General Information

All chemicals were used as received except where noted otherwise. Methylene chloride (CH_2Cl_2) was passed through alumina under argon in a solvent purification system prior to use. Anhydrous, amine free dimethylformamide (DMF) was purchased from Alfa Aesar. All reactions were performed at ambient temperature (ca. 20ºC), unless otherwise noted. Analytical reverse-phase HPLC was performed on an Agilent 1200 equipped with an Aeris PEPTIDE 2.6 μm XB-C18 column (Phenomonex). Preparative reverse-phase HPLC was performed on a Rainin Dynamax equipped with a ZORBAX SB-C18 column (Agilent). HPLC grade acetonitrile and 18 MΩ deionized water, each containing 0.1% trifluoroacetic acid (TFA), were used for analytical and preparative reverse-phase HPLC. Mass spectrometry was performed on a Waters Xevo G2-XS QTof mass spectrometer. All peptides were prepared and used as the trifluoroacetate salts and were assumed to have one trifluoroacetate ion per ammonium group present in each peptide.

*Synthesis of peptides **1**, **2**, and **2**$_{F20Cha}$*

Peptides **1**, **2**, and **2**$_{F20Cha}$ were synthesized using protocols that our laboratory has previously described.[S1, S2, S3]


2-chlorotrityl chloride resin
1. Fmoc-Gly-OH, collidine/CH2Cl2 (1:16)
2. repeated Fmoc-AA-OH coupling
3. HFIP/CH2Cl2 (1:5)
linear protected peptide
6.5 equiv HOBt, 6.5 equiv HBTU, DMF
24 h
cyclic protected peptide
1. TFA/TIPS/H2O (18:1:1)
2. RP-HPLC
3. lyophilization
peptide 3 as the trifluoroacetate salt

Scheme 1

*Synthesis of peptides **3**, **3**$_{F20Cha}$, **4**, and **4**$_{F20Cha}$.*

The syntheses of peptides **3**, **3**$_{F20Cha}$, **4**, and **4**$_{F20Cha}$ are modified from procedures that our laboratory previously described.[S1, S2] The Scheme **S1** summarizes the synthesis and purification of these peptides.

a. *Loading the resin.* The syntheses of peptides **3**, **3**$_{F20Cha}$, **4**, and **4**$_{F20Cha}$ start with loading Gly_{25} onto the resin. 2-Chlorotrityl chloride resin (300 mg, 1.6 mmol/g) was transferred to a Bio-Rad Poly-Pep chromatography column (10 mL). The resin was swelled with CH_2Cl_2 (5 mL) and the suspension was agitated for 30 minutes. The solvent was drained and a solution of Fmoc-Gly-OH (0.50 equiv, 47 mg, 0.15 mmol) in 6 % (v/v) 2,4,6-collidine in dry CH_2Cl_2 (5 mL) was added to the resin and the suspension was gently agitated for 12 hours. The solution was then drained and a solution of CH_2Cl_2/MeOH/*N*,*N*-diisopropylethylamine (DIPEA) (17:2:1, 8 mL) was added to the resin and agitated for 1 hour to cap the unreacted 2-chlorotrityl chloride sites of the resin. After an hour, the capping solution was drained and the resin was washed with DMF (2x).

b. *Peptide coupling*. The Fmoc-Gly-2-chlorotrityl resin was then transferred to a 250 mL ChemGlass peptide synthesis vessel connected to a nitrogen/vacuum Schlenk line. The linear peptide was then synthesized from the *C*-terminus to the *N*-terminus in consecutive cycles of amino acid coupling. Each cycle of amino acid coupling consists of i. Fmoc-deprotection with a solution of 20% (v/v) piperidine in DMF (5 mL) for 5 minutes, ii. washing with DMF (5 mL, 3x), iii. coupling of amino acid (0.75 mmol, 5 equiv) in the presence of HCTU (0.75 mmol, 5 equiv) dissolved in 20 % (v/v) 2,4,6-collidine in DMF (4 mL) for 20 minutes, iv. washing with DMF (5 mL, 3x). We used a special coupling procedure for the phenylalanine residues that follow the *N*-methylphenylalanine in peptides **3** and **4** or the *N*-methylcyclohexylalanine in peptides **3**$_{F20Cha}$ and **4**$_{F20Cha}$: The phenylalanine was coupled twice (0.75 mmol, 5 equiv, each time) and allowed to react

for 1 hour each time in the presence of HATU (5 equiv) and HOAt (5 equiv) dissolved in 20 % (v/v) 2,4,6-collidine in DMF (4 mL). After the last amino acid was coupled, the terminal Fmoc protecting group was cleaved using a solution of 20% (v/v) piperidine in DMF (5 mL, 5 minutes). The resin was then transferred to a Bio-Rad Poly-Pep chromatography column (10 mL).

c. *Cleavage of the peptide from the resin*. The resin was washed with CH_2Cl_2 (3x). To cleave the linear-protected peptide from the resin, a solution of 20% (v/v) 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in CH_2Cl_2 (7 mL) was added to the resin and the suspension was agitated for 1 hour. After an hour, the HFIP solution was filtered through the Bio-Rad column and the filtrate was collected in a 250 mL rounded-bottom flask. The resin was treated with a fresh solution of 20% (v/v) HFIP in CH_2Cl_2 (7 mL) and the suspension was agitated for 20 minutes. After 20 minutes, the solution was filtered. The combined filtrates were concentrated by rotary evaporation to yield the linear protected peptide.

d. *Cyclization of the linear peptide*. The linear protected peptide was dissolved in dry DMF (125 mL). HOBt (150 mg, 1.0 mmol, 6.5 equiv) and HBTU (375 mg, 1.0 mmol, 6.5 equiv) were added to the solution. 4-Methylmorpholine (0.33 mL, 3 mmol, 20 equiv) was added and the solution was stirred under nitrogen for 24–36 hours. The solution was then concentrated using rotary evaporation to afford the cyclic protected peptide.

e. *Global deprotection of the cyclic protected peptide*. A solution of TFA/triisopropylsilane (TIPS)/water (18:1:1, 36 mL) was added to the cyclic protected peptide. The solution was stirred under nitrogen for 1.5 hours to remove acid-labile side chain protecting groups of the cyclic protected peptide and yield the crude cyclic deprotected peptide.

f. *Ether precipitation of the cyclic deprotected peptide*. Diethyl ether precipitation was performed to separate the crude cyclic deprotected peptide from the global deprotection

solution. Diethyl ether was chilled on ice for an hour. The global deprotection mixture was split into six 15-mL conical tubes (ca. 6 mL each) and mixed with cold diethyl ether (ca. 6 mL each). The mixtures of global deprotection solution and diethyl ether were incubated on ice for 5 minutes and then centrifuged at 3300 rpm (1380 x G) for 5 minutes. The crude cyclic deprotected peptide forms a pellet upon centrifugation. The supernatant in each conical tube was discarded and the pellet was resuspended in cold diethyl ether (ca. 6 mL each). The suspensions were incubated on ice for 5 minutes and then centrifuged at 3300 rpm (1380 x G) for 5 minutes. The supernatant in each conical tube was discarded and the pellet was resuspended in cold diethyl ether. The suspensions were poured into a 250 mL rounded-bottom flask. The mixture was concentrated using rotary evaporation. The crude cyclic deprotected peptide was immediately subjected to reverse-phase HPLC purification.

g. *Reverse-phase HPLC purification*. The crude cyclic deprotected peptide was dissolved in 20% (v/v) ACN in water (10 mL) and filtered through a 0.2 µm PVDF syringe filter. The filtrate was then purified using RP-HPLC (20–50% ACN over 60 minutes). The pure fractions were combined, concentrated by rotary evaporation, and then lyophilized. Typical procedures yield ca. 40–55 mg of peptide as the TFA salt.

Syntheses of cross-linked trimers ***2***, $\mathbf{2}_{\mathbf{F20Cha}}$, *and* $\mathbf{4}_{\mathbf{F20Cha}}$

Cross-linked trimers **2** and $\mathbf{2}_{\mathbf{F20Cha}}$ were prepared by oxidizing peptides **2** and $\mathbf{2}_{\mathbf{F20Cha}}$ using procedures that our laboratory has previously described.[S2,S3] Cross-linked trimer $\mathbf{4}_{\mathbf{F20Cha}}$ was prepared by oxidizing peptide $\mathbf{4}_{\mathbf{F20Cha}}$ (6 mM) in 20% aqueous DMSO in the presence of triethylamine (60 mM) as follows: the dry lyophilized peptide $\mathbf{4}_{\mathbf{F20Cha}}$ was weighed and then transferred to a scintillation vial (20 mL) and dissolved in an appropriate volume of 20% (v/v)

aqueous dimethyl sulfoxide (DMSO) to make a 6 mM solution of the peptide. An appropriate volume of triethylamine was added to the solution to make a 60 mM solution of triethylamine. The reaction mixture was incubated at ambient temperature (ca. 20ºC) for two days. After incubation, the solution of the peptide was filtered through a 0.2 µm PVDF syringe filter, and the filtrate was injected into the RP-HPLC (20–50% ACN over 60 minutes) to purify cross-linked trimer **4$_{F20Cha}$**. It is necessary to heat the HPLC column to 60ºC during the purification of the cross-linked trimer.[S4] In a typical procedure, oxidation of 32 mg of peptide **4$_{F20Cha}$** (as the TFA salt) afforded ca. 7 mg (22%) of trimer **4$_{F20Cha}$** (as the TFA salt) after HPLC purification and lyophilization.

X-ray Crystallography

Crystallization of peptides **3** and **3$_{F20Cha}$** and trimer **4$_{F20Cha}$** was attempted using protocols that our laboratory has previously published.[S2, S5] Stock solutions of peptides **3** and **3$_{F20Cha}$** and trimer **4$_{F20Cha}$** (10 mg/mL and 20 mg/mL) were prepared by dissolving 1.0 mg of dry lyophilized peptide in 100 µL or 50 µL of filtered 18 MΩ deionized water (NanoPure). The stock solutions were screened in 96-well plates using two kits from Hampton Research (Crystal Screen and Index). For each crystallization condition, three 150 nL hanging drops were prepared with well solution in 100:50, 75:75, and 50:100 ratio using a TTP LabTech Mosquito pipetting robot. For wells that gave promising crystals, the crystallization conditions were further optimized in a 4x6 matrix using a Hampton VDX 24-well plate by varying the pH and the concentration of cryoprotectant. For each of the crystallization conditions, three 2- or 3-µL hanging drops were prepared by mixing the peptide and crystallization solutions in 1:1, 1:2, and 2:1 ratios. Peptide **3** did not afford any crystals. In contrast, peptide **3$_{F20Cha}$** afforded crystals suitable for X-ray crystallography from a crystallization condition that was first identified on a 96-well plate and then optimized in 24-well

plates. Trimer $\mathbf{4}_{\mathbf{F20Cha}}$ did not grow crystals under the screening conditions, but afforded crystals suitable for X-ray crystallography in conditions similar to those we had previously published for trimer **2**.[S2]

Data collection and structure determination

Initial diffraction data for peptide $\mathbf{3}_{\mathbf{F20Cha}}$ 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. The X-ray crystallographic phases for peptide $\mathbf{3}_{\mathbf{F20Cha}}$ were determined using single-wavelength anomalous diffraction (SAD) phasing by soaking a single crystal in a 1:1 mixture of 1 M potassium iodide and well solution.[S6] Coordinates for the anomalous signal from the iodide ions were determined by HySS in the Phenix software suite 1.10.1. 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. Coordinates were refined with phenix.refine.

Diffraction data for peptide $\mathbf{3}_{\mathbf{F20Cha}}$ 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. The synchrotron diffraction data were scaled and merged using XDS. The electron density map was generated by molecular replacement using the coordinates from the structure of peptide $\mathbf{3}_{\mathbf{F20Cha}}$ generated by soaking in KI using Phaser in the Phenix software suite 1.10.1. Molecular manipulation of the model was performed with Coot. Coordinates were refined with phenix.refine.

Data collection and structure determination of trimer $\mathbf{4}_{F20Cha}$

Diffraction data for trimer $\mathbf{4}_{F20Cha}$ 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. The X-ray crystallographic phases for trimer $\mathbf{4}_{F20Cha}$ were determined by molecular replacement using trimer **2** as a search model in Phaser. Molecular manipulation of the model was performed with Coot. Coordinates were refined with phenix.refine.

Table S1. Crystallographic properties, data collection, and model refinement statistics for peptide **3**$_{\mathbf{F20Cha}}$ and trimer **4**$_{\mathbf{F20Cha}}$**.**

Peptide	peptide **3**$_{\mathbf{F20Cha}}$	trimer **4**$_{\mathbf{F20Cha}}$
PDB accession number	7JXN	7JXO
Wavelength (Å)	1.00	1.54
Resolution range (Å)	32.19 - 2.0 (2.072–2.0)	32.57 - 2.802 (2.902–2.802)
Space group	P 63	P 62 2 2
Unit cell (Å, °)	37.167 37.167 116.994 90 90 120	65.078 65.078 51.923 90 90 120
Total reflections	12449 (1258)	3614 (352)
Unique reflections	6204 (625)	1807 (176)
Multiplicity	19.4 (19.5)	18.2 (17.8)
Completeness (%)	99.90 (100.00)	99.78 (100.00)
Mean I/sigma(I)	33.10 (13.21)	7.75 (2.74)
Wilson B-factor	20.55	34.04
R-merge	0.01302 (0.0532)	0.06261 (0.2047)
R-meas	0.01841 (0.07524)	0.08854 (0.2896)
R-pim	0.01302 (0.0532)	0.06261 (0.2047)
CC1/2	1 (0.987)	0.992 (0.902)
CC*	1 (0.997)	0.998 (0.974)
Reflections used in refinement	6200 (629)	1806 (176)
Reflections used for R-free	616 (66)	179 (17)
R-work	0.2698 (0.3845)	0.2766 (0.3416)
R-free	0.3201 (0.3577)	0.3012 (0.3749)
CC(work)	0.886 (0.464)	0.869 (0.679)
CC(free)	0.816 (0.363)	0.856 (0.531)
Number of non-hydrogen atoms	676	469
macromolecules	504	375
ligands	115	84
solvent	57	10
Protein residues	84	63
RMS(bonds)	0.003	0.003
RMS(angles)	0.7	0.73
Ramachandran favored (%)	75	84.62
Ramachandran allowed (%)	19.23	15.38
Ramachandran outliers (%)	5.77	0
Rotamer outliers (%)	3.85	9.52
Clashscore	9.57	6
Average B-factor	24.36	42.75
macromolecules	23.39	44.99
ligands	28.51	34.09
solvent	24.51	31.15
Number of TLS groups	10	6

Biological and biophysical assays

SDS-PAGE

Tricine SDS-PAGE was performed as described previously (Salveson et al. *J. Am. Chem. Soc.* **2018**, 140, 11745–11754), with the exception that the gel was run at a 50 V, instead of 80 V.[S3] Silver-staining was used to visualize trimer bands in the SDS-PAGE gel as our laboratory reported previously.[S2,S7]

Circular dichroism spectroscopy

CD spectra were acquired on a JASCO-810 circular dichroism spectropolarimeter at ambient temperature (ca. 20ºC). This procedure is slightly modified from Salveson et al. *J. Am. Chem. Soc.* **2018**, 140, 5842–5852.[S5] Solutions of peptides and trimers were prepared gravimetrically, with molecular weights based on the trifluoroacetate salts, with one TFA per amino group. A 75 µM solution of each peptide and a 25 µM solution of each cross-linked trimer was prepared by diluting appropriate amounts of 10 mg/mL stock solutions of peptides and trimers in 10 mM sodium phosphate buffer at pH 7.4 or in 10 mM glycine buffer containing 50 mM NaCl at pH 3.0. Solutions were prepared in Eppendorf tubes (1.5 mL) and then transferred to a 1 mm quartz cuvette. Data were collected using 0.2 nm intervals from 260 nm to 195 nm and averaged over 5 accumulations. Data were not collected at wavelengths below 195 nm, to avoid voltages greater than 600 V in the photomultiplier tube. Data were graphed as mean residue ellipticity, [Θ], which is calculated as follows:

[Θ] = millidegrees / (path length (nm) x [peptide] (M) x number of residues)

Dynamic light Scattering.

Dynamic light scattering was measured using a Malvern Zetasizer ZS Nano DLS at ambient temperature (ca. 20ºC). Solutions of trimers (25 µM) were prepared in a similar fashion to the circular dichroism experiments and transferred to 1 cm disposable plastic cuvettes. Data were collected in 10 seconds time intervals and averaged over 3 measurements. The scattering was measured with a 173º backscattering angle. Dynamic light scattering was also measured after centrifugation of the solutions. In these experiments, the solutions were centrifuged at 14000 rpm (17000 x G) for 2 minutes and then the supernatant was transferred to a plastic cuvette for DLS measurement. The optical density (OD_{214}) of each sample was measured before and after centrifugation using a Thermo Scientific NanoDrop One microvolume UV-Vis spectrophotometer to monitor the loss of aggregates formed in buffered solutions of the cross-linked trimers (Table S2).

Table S2. The optical density (OD_{214}) of trimer aliquots before and after centrifugation.

Aliquot	OD_{214} before centrifugation (AU)	OD_{214} after centrifugation (AU)
Trimer **4**$_{\mathbf{F20Cha}}$ (25 µM) in glycine buffer	1.52	1.56
Trimer **2** (25 µM) in glycine buffer	1.32	1.28
Trimer **4**$_{\mathbf{F20Cha}}$ (25 µM) in phosphate buffer	1.66	0.34
Trimer **2** (25 µM) in phosphate buffer	2.64	1.64

Size-exclusion Chromatography

All the SEC experiments were performed in 10 mM glycine containing 50 mM NaCl at pH 3.0 at ambient temperature (ca. 20ºC) on a GE Superdex 75 10/300 GL column. A 1.0 mg/mL

solution of each cross-linked trimer was prepared by diluting 80 µL of 10 mg/mL stock solutions of each trimer in 720 µL of the glycine buffer in an Eppendorf tube (1.5 mL). We found that 1.0 mg/mL solutions of peptides **1**, **3**, and $\mathbf{3_{F20Cha}}$ were too concentrated and resulted in saturation of the detector (> 2.5 AU), thus 0.25 mg/mL solutions of peptides **1**, **3**, and $\mathbf{3_{F20Cha}}$ were used for SEC experiments. The solutions were centrifuged at 14000 rpm (17000 x G) for 2 minutes. After centrifugation, the supernatant of each peptide aliquot was injected to the column and run using a flow rate of 0.5 mL/min. Chromatograms were recorded at 214 nm and normalized to the maximum absorbance of the run. Size standards blue dextran, carbonic anhydrase, cytochrome C, aprotinin, and vitamin B12 were run in a similar fashion. The optical density (OD_{214}) of each sample was measured before and after centrifugation using a Thermo Scientific NanoDrop One microvolume UV-Vis spectrophotometer to monitor the loss of aggregates formed in buffered solutions of the peptides and cross-linked trimers (Table **S3**).

Table S3. The optical density of aliquots of peptides and cross-linked trimers in glycine buffer before and after centrifugation.

Aliquot	OD_{214} before centrifugation (AU)	OD_{214} after centrifugation (AU)
Peptide **1** (0.25 mg/mL)	2.99	3.02
Peptide **3** (0.25 mg/mL)	2.64	3.52
Peptide $\mathbf{3_{F20Cha}}$ (0.25 mg/mL)	2.04	2.54
Trimer **2** (1.0 mg/mL)	11.07	12.36
Trimer $\mathbf{2_{F20Cha}}$ (1.0 mg/mL)	10.67	10.26
Trimer $\mathbf{4_{F20Cha}}$ (1.0 mg/mL)	12.28	12.22

LDH release assay

LDH release assay was performed on SHSY-5Y neuroblastoma cell-line and cell death was quantified using procedures that our laboratory previously described (Kreutzer et al. *J. Am. Chem. Soc*. **2017**, 139, 966–975).[S2]

References and Notes

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S2. Kreutzer, A. G.; Yoo, S.; Spencer, R. K.; Nowick, J. S. Stabilization, assembly, and toxicity of trimers derived from Aβ. *J. Am. Chem. Soc*. **2017**, 139, 966–975.

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S5. Salveson, P. J.; Haerianardakani, S.; Thuy-Boun, A.; Kreutzer, A. G.; Nowick, J. S. Controlling the oligomerization state of Aβ-derived peptides with light. *J. Am. Chem. Soc*. **2018**, 140, 5842–5852.

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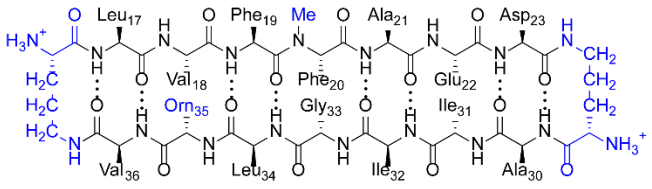
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Me
Ala21
Asp23
Val18
Phe20
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Orn35
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Ile31
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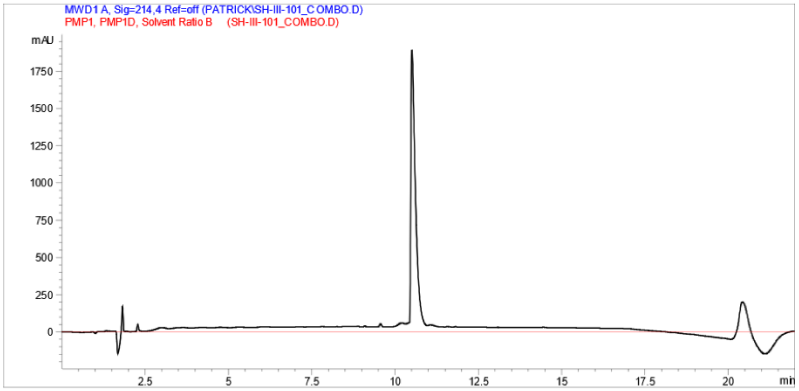
Leu34
Ile32
Ala30

peptide **1**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20
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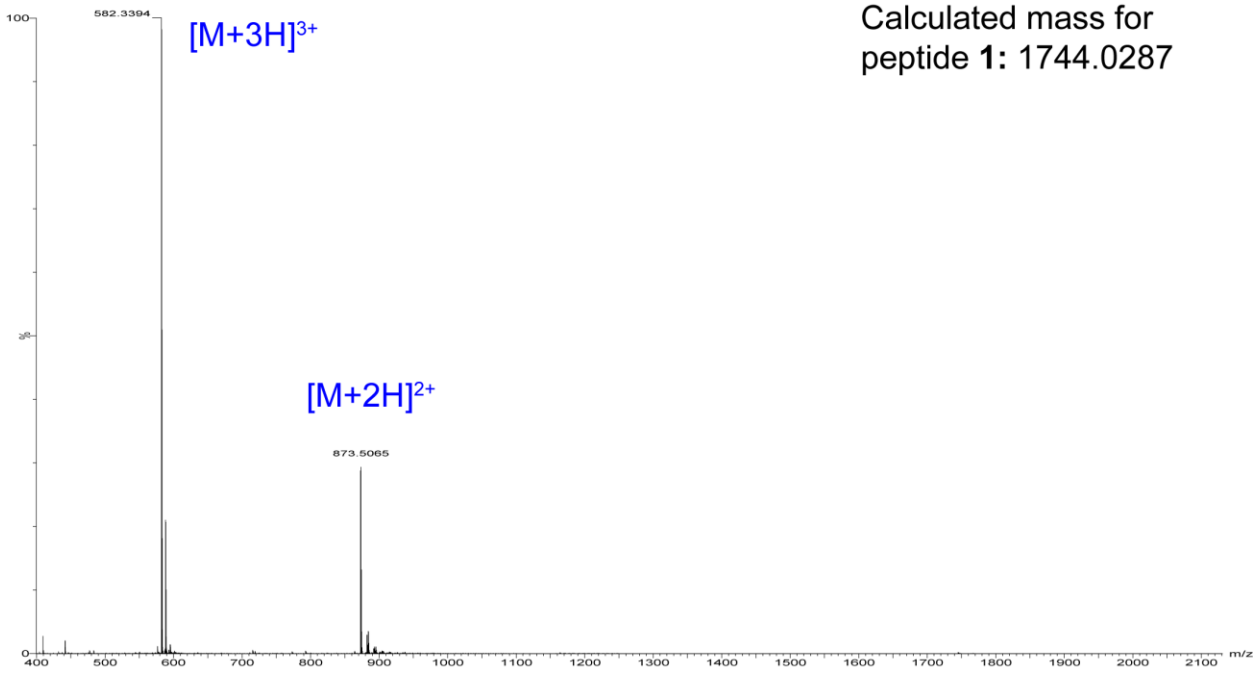
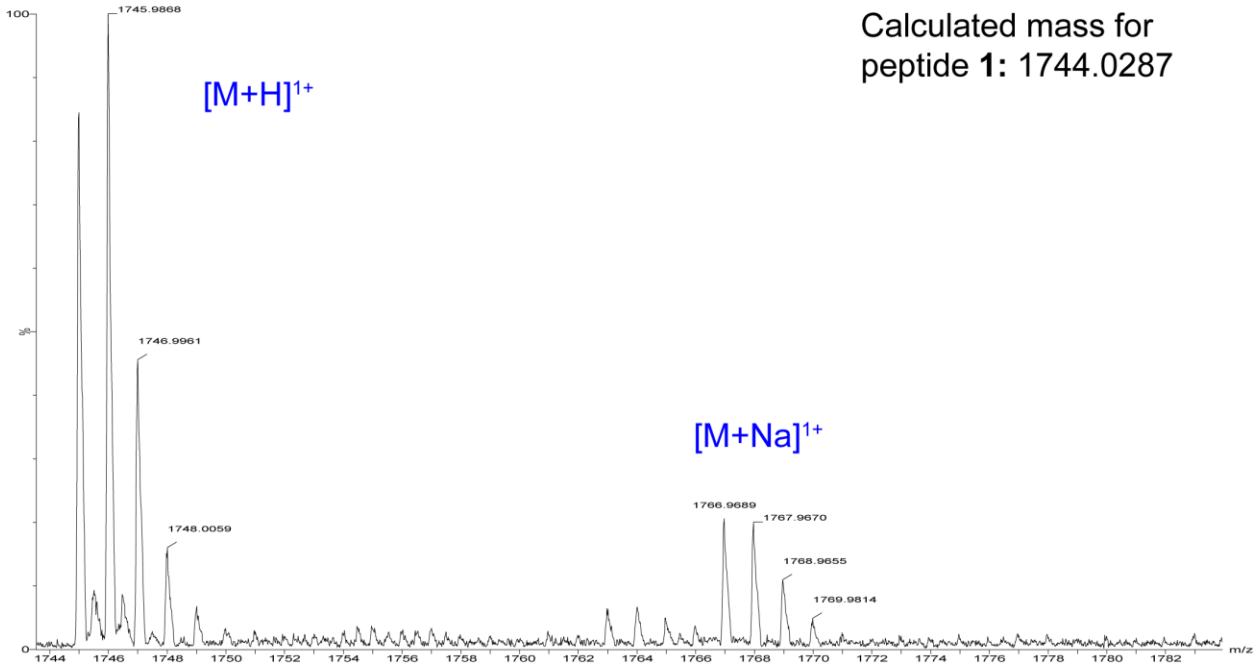
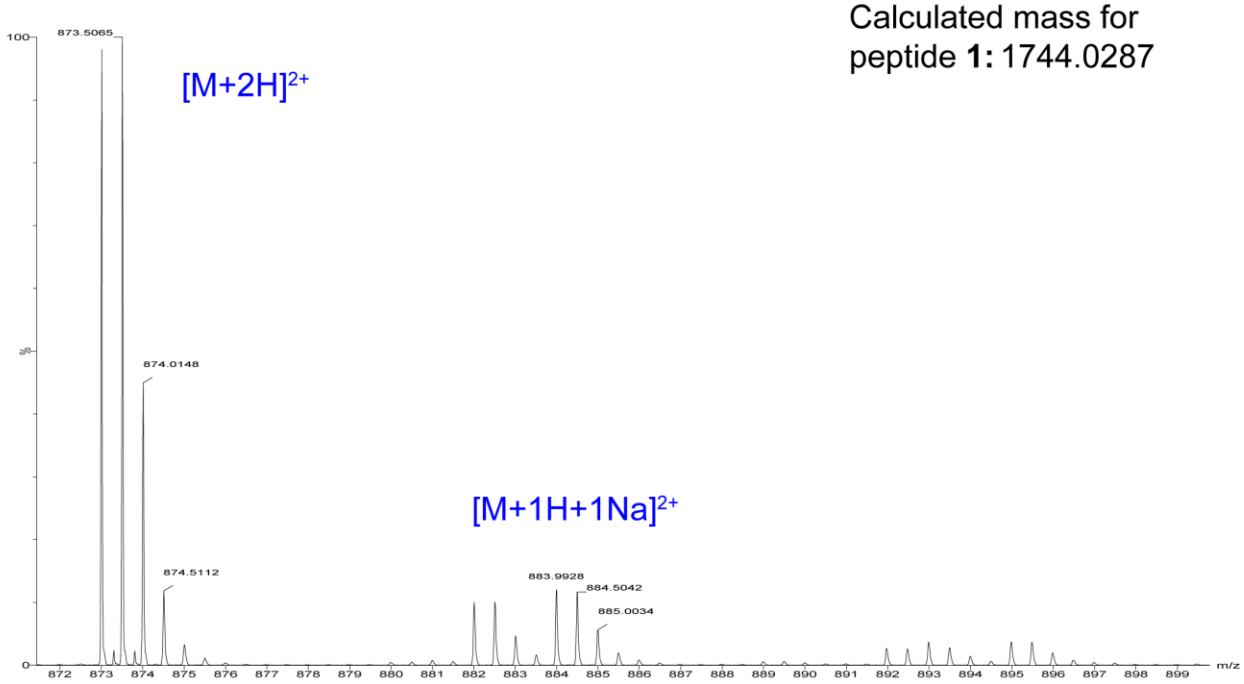


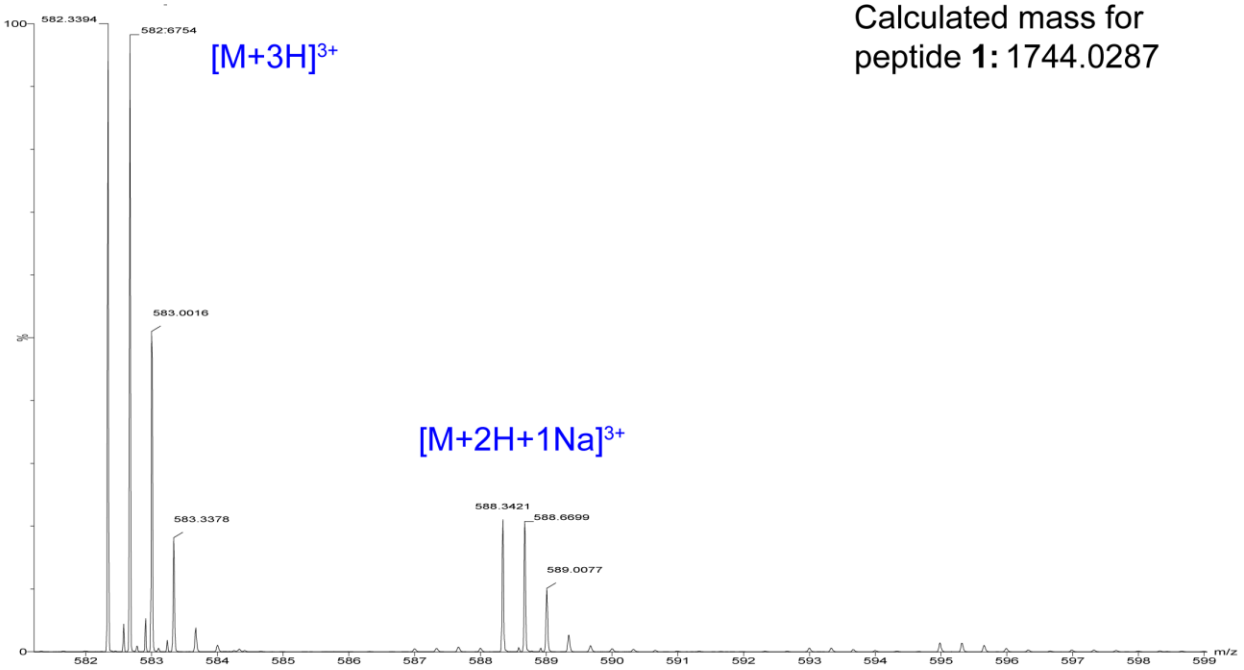
Calculated mass for peptide **1:** 1744.0287



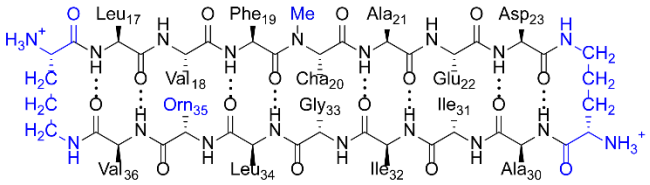
Calculated mass for peptide **1:** 1744.0287



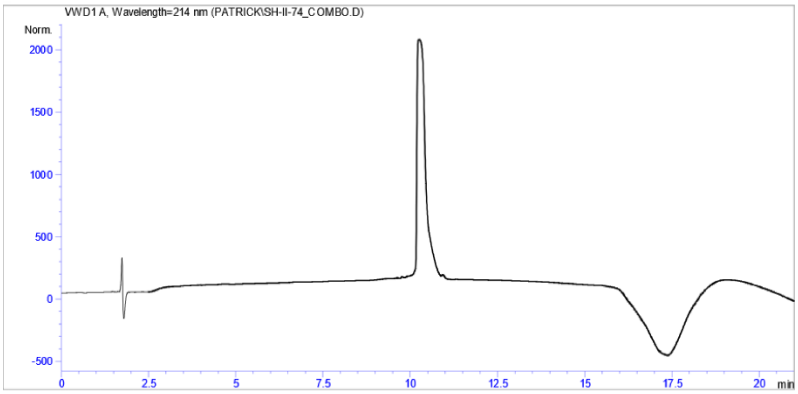
Calculated mass for peptide **1:** 1744.0287

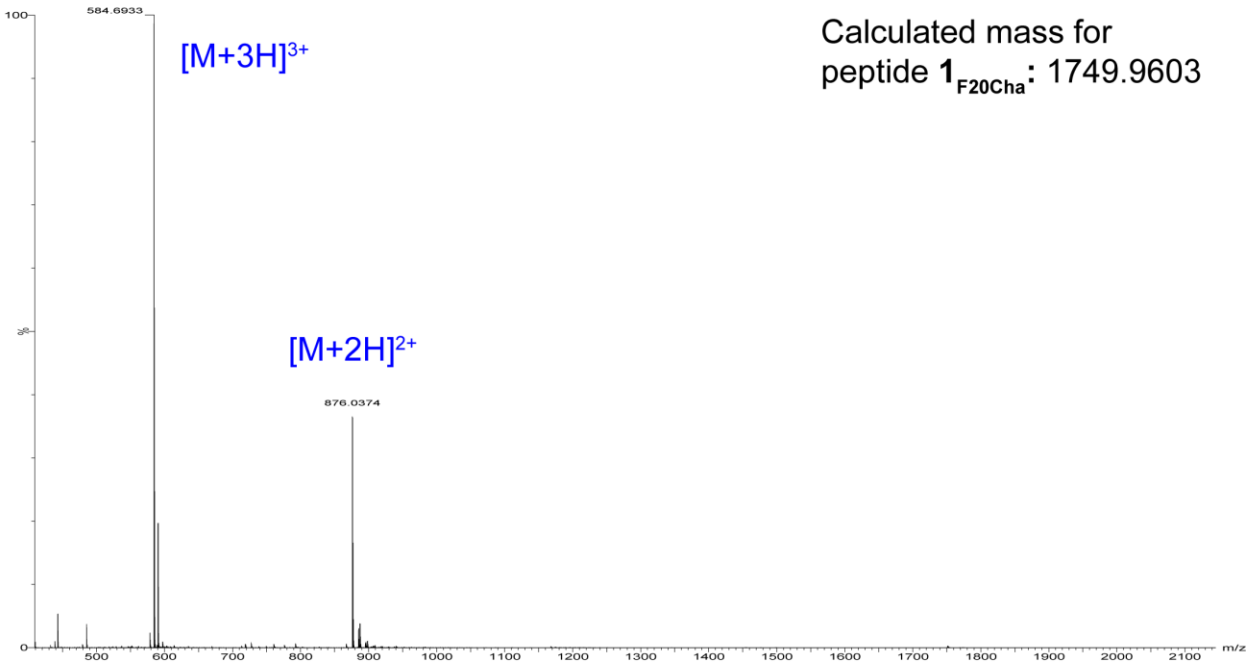


Calculated mass for peptide **1:** 1744.0287

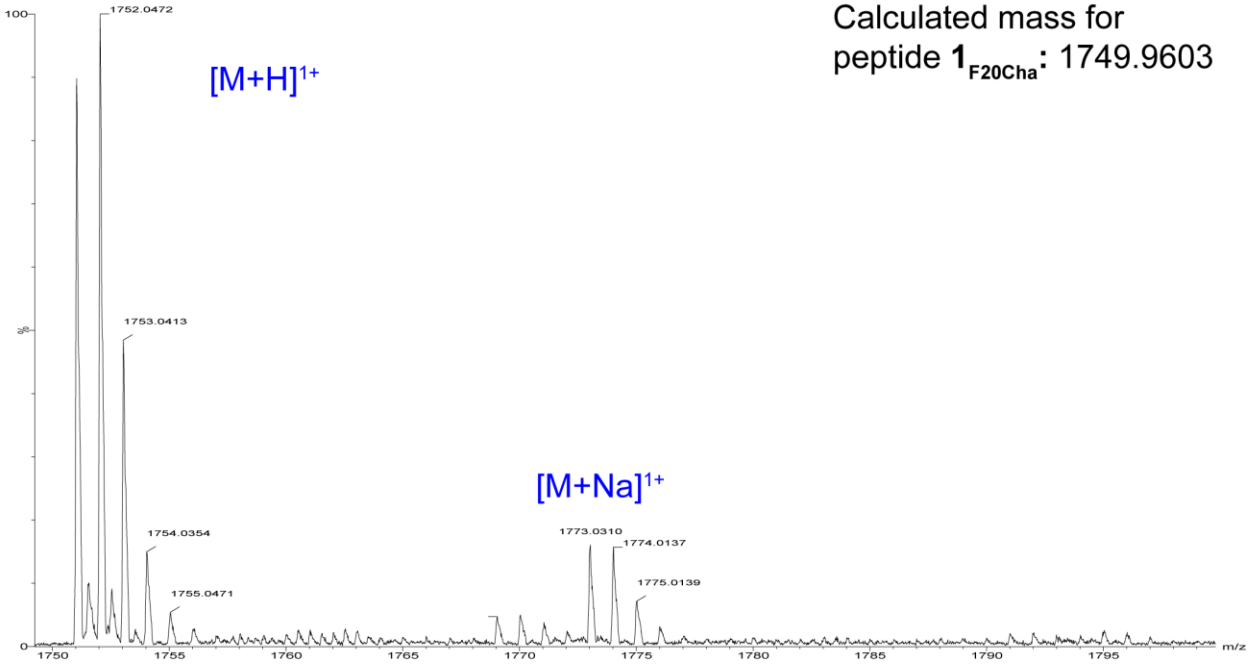


peptide **1**$_{\textbf{F20Cha}}$

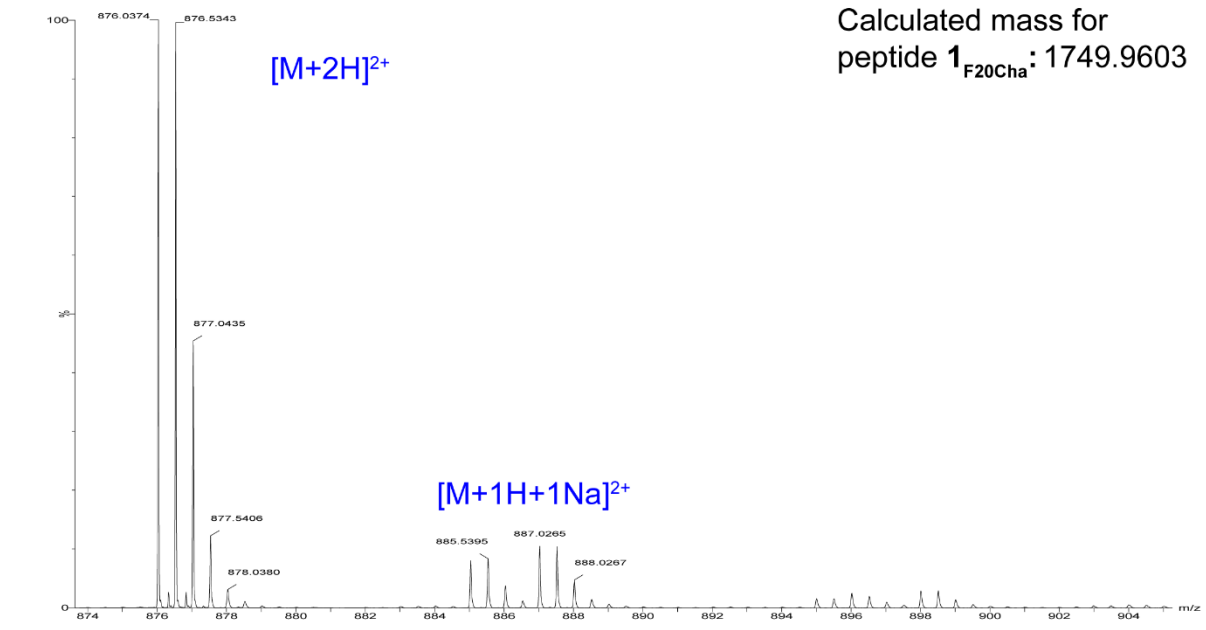




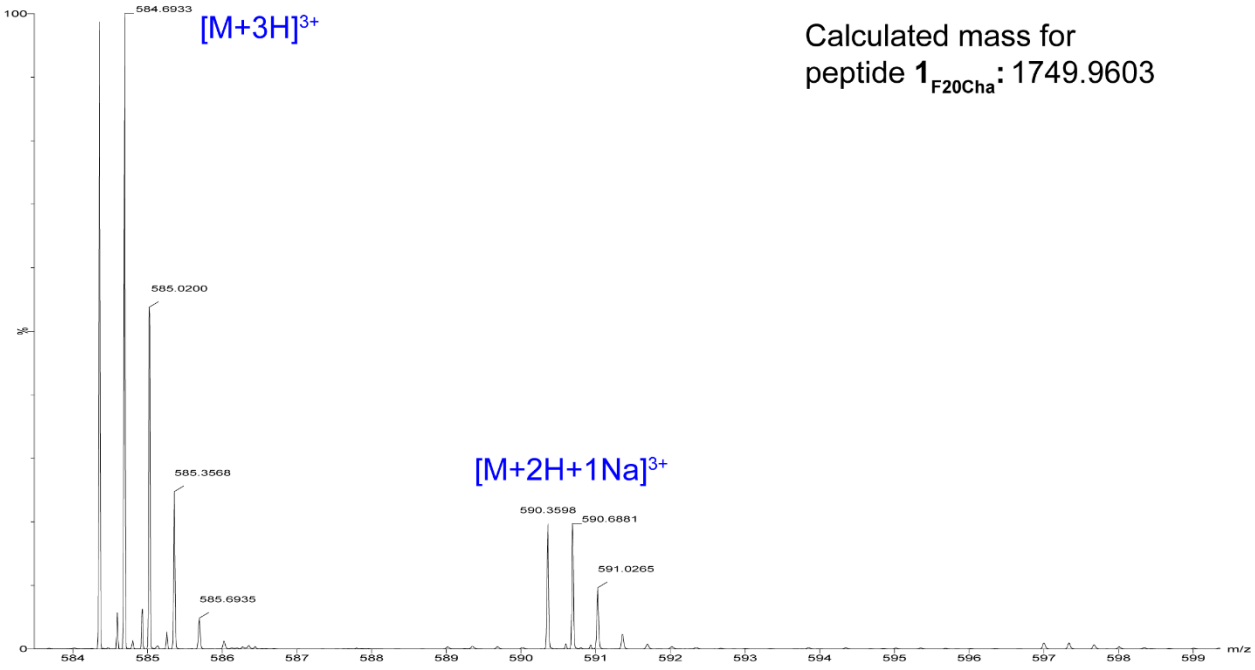
Calculated mass for peptide $\mathbf{1}_{\mathbf{F20Cha}}$: 1749.9603



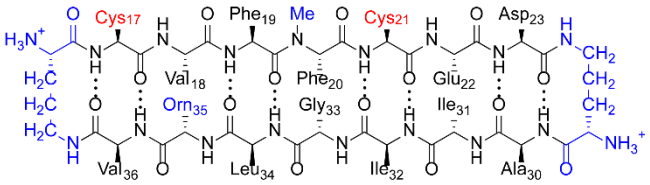
Calculated mass for peptide $\mathbf{1}_{\mathbf{F20Cha}}$: 1749.9603



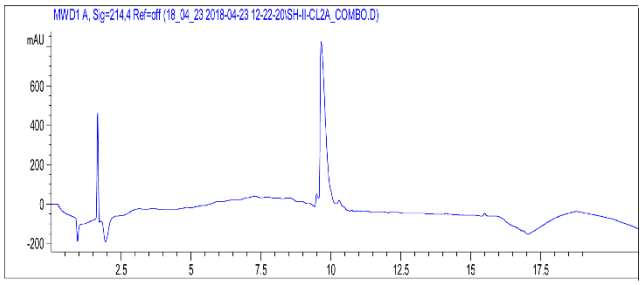
Calculated mass for peptide $\mathbf{1}_{\mathbf{F20Cha}}$: 1749.9603

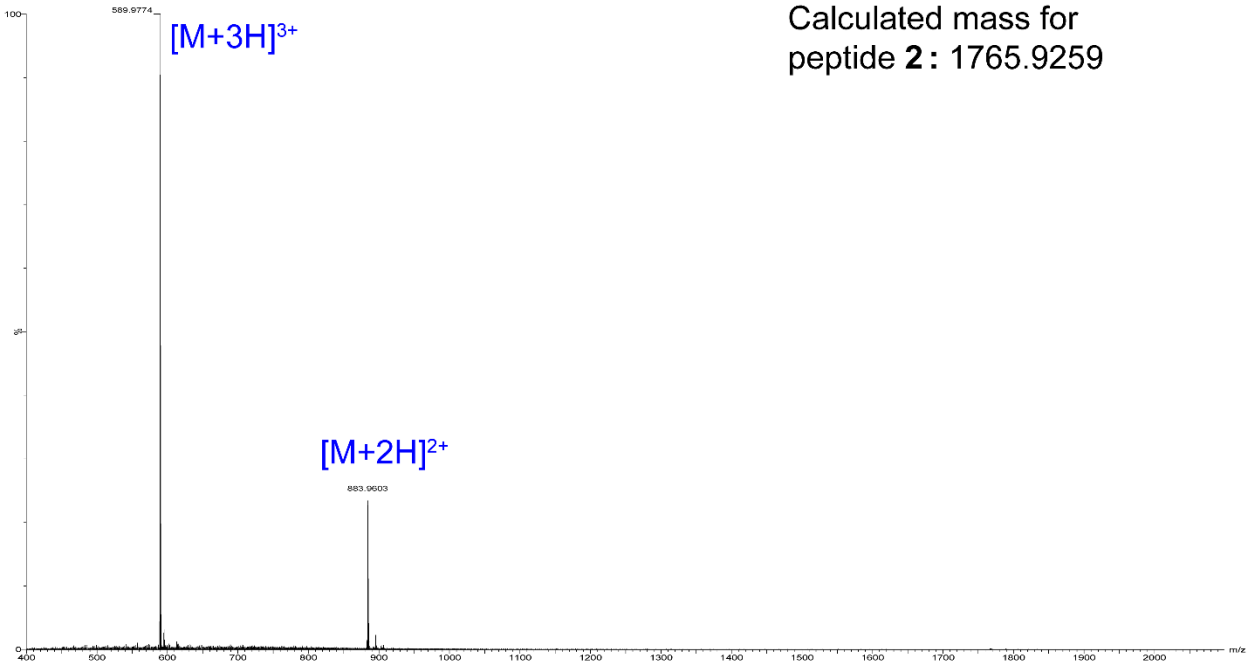


Calculated mass for peptide $\mathbf{1}_{\mathbf{F20Cha}}$: 1749.9603

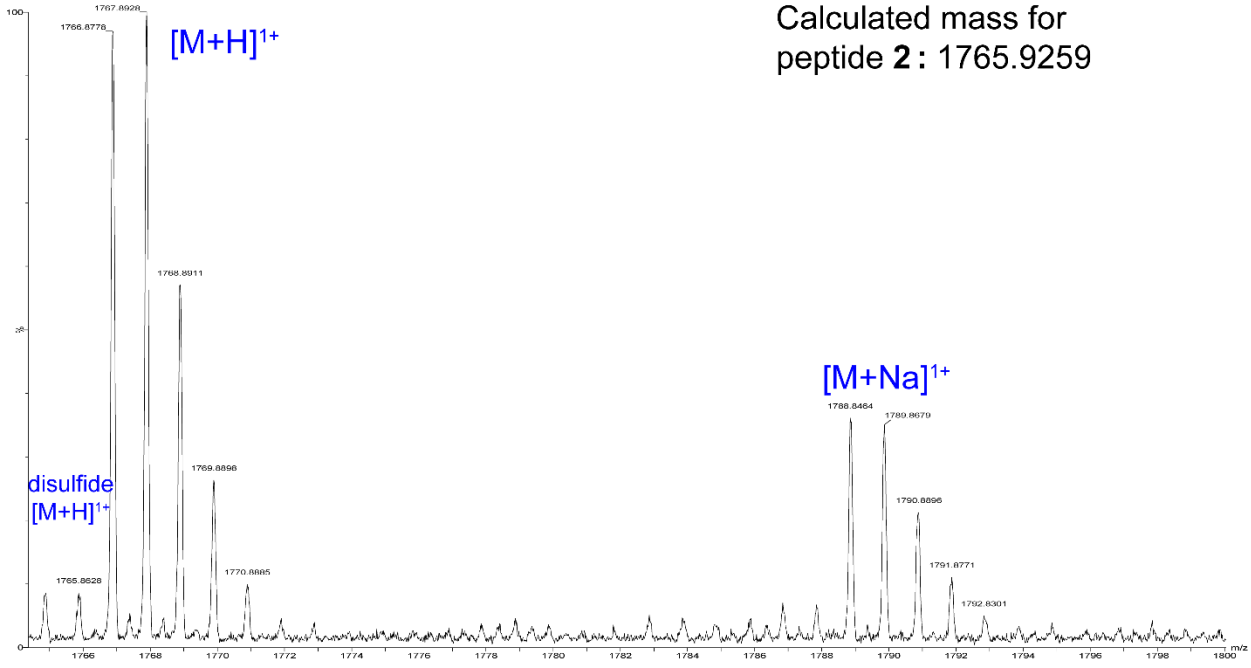

Cys17
Phe19
Me
Cys21
Asp23
Val18
Phe20
Glu22
Orn35
Gly33
Ile31
Val36
Leu34
Ile32
Ala30

peptide **2**

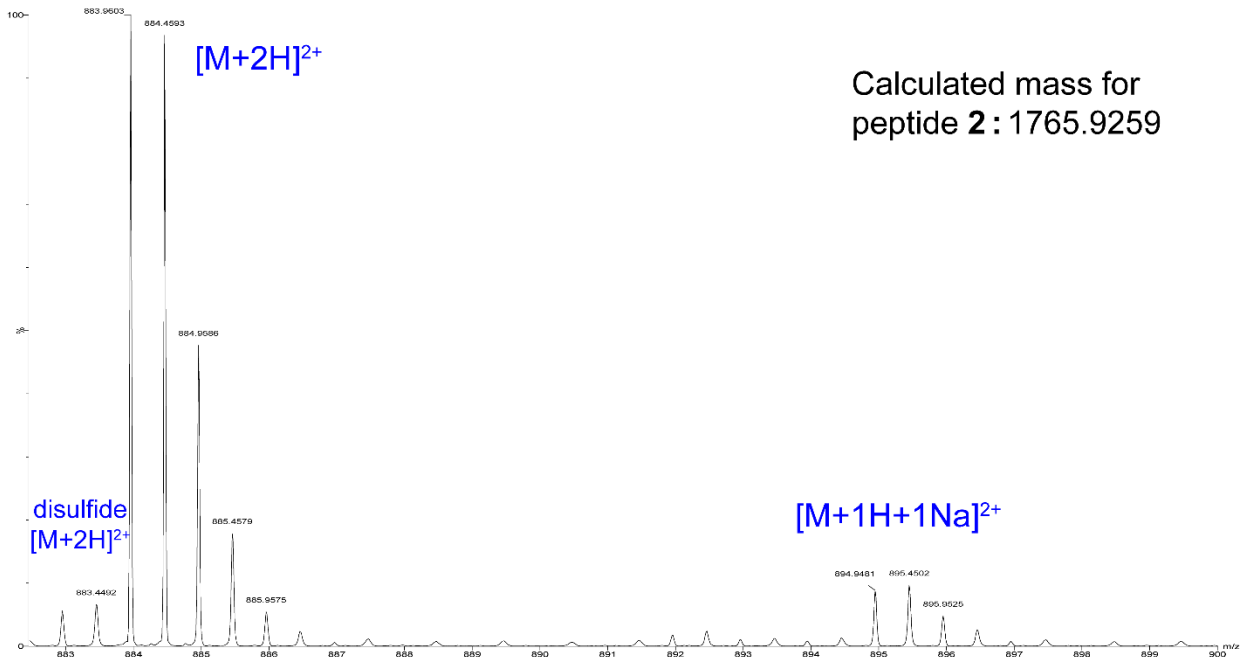

MWD1 A, Sig=214,4 Ref=off (18_04_23 2018-04-23 12-22-20\SH-II-CL2A_COMBO.D)
mAU
600
400
200
0
-200
2.5
5
7.5
10
12.5
15
17.5



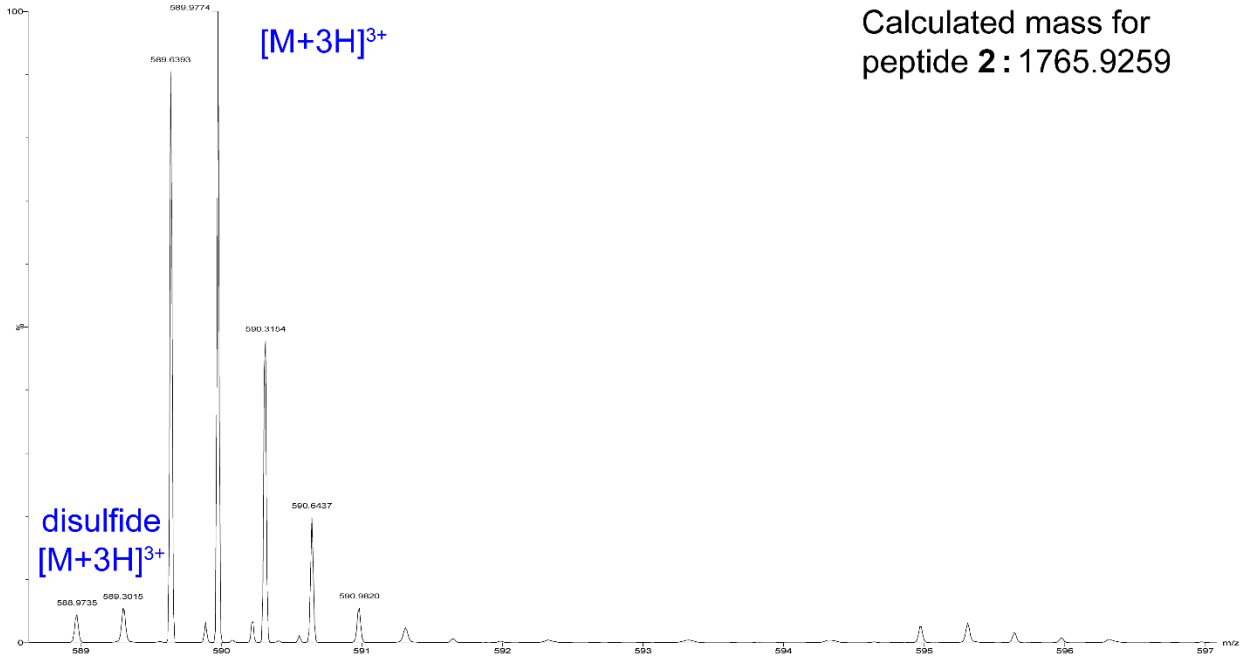
Calculated mass for peptide **2** : 1765.9259



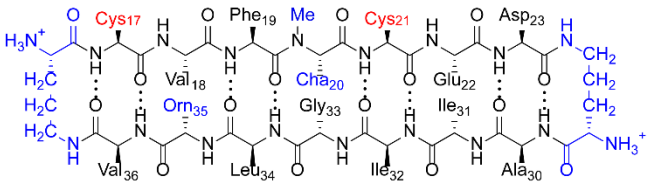
Calculated mass for peptide **2** : 1765.9259



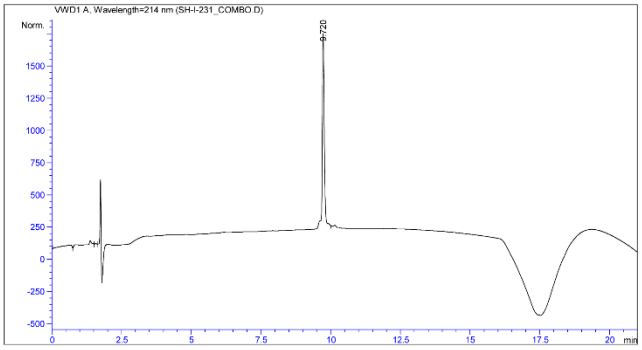
Calculated mass for peptide **2** : 1765.9259

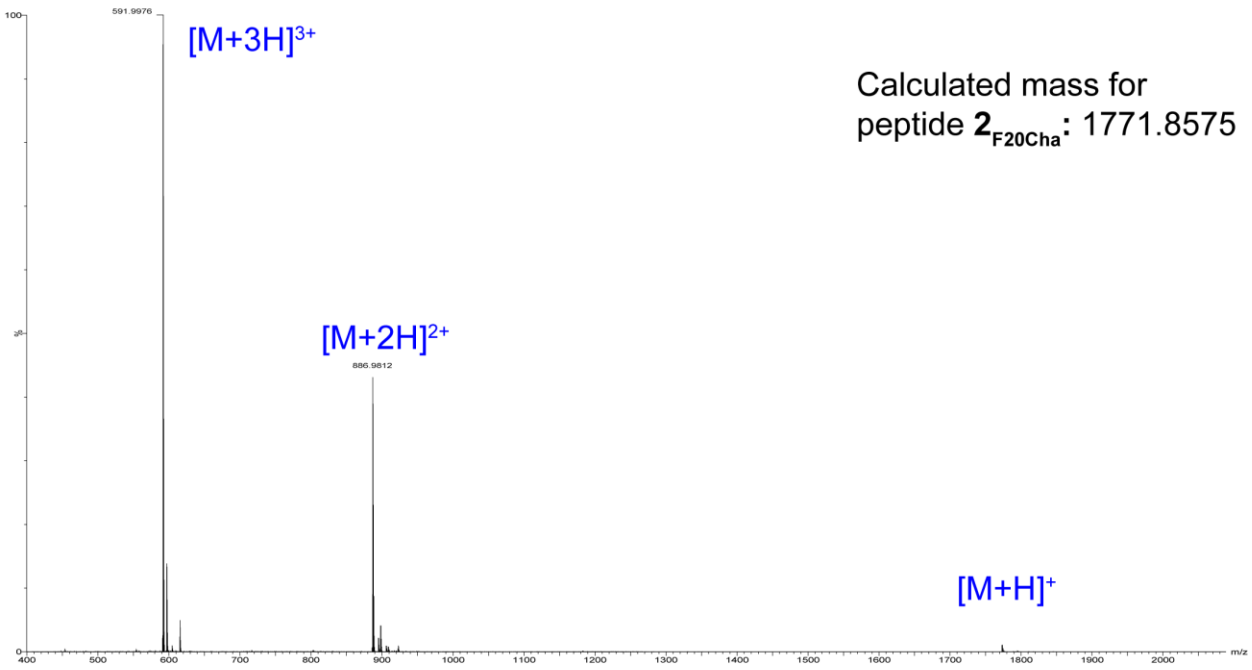


Calculated mass for peptide **2** : 1765.9259

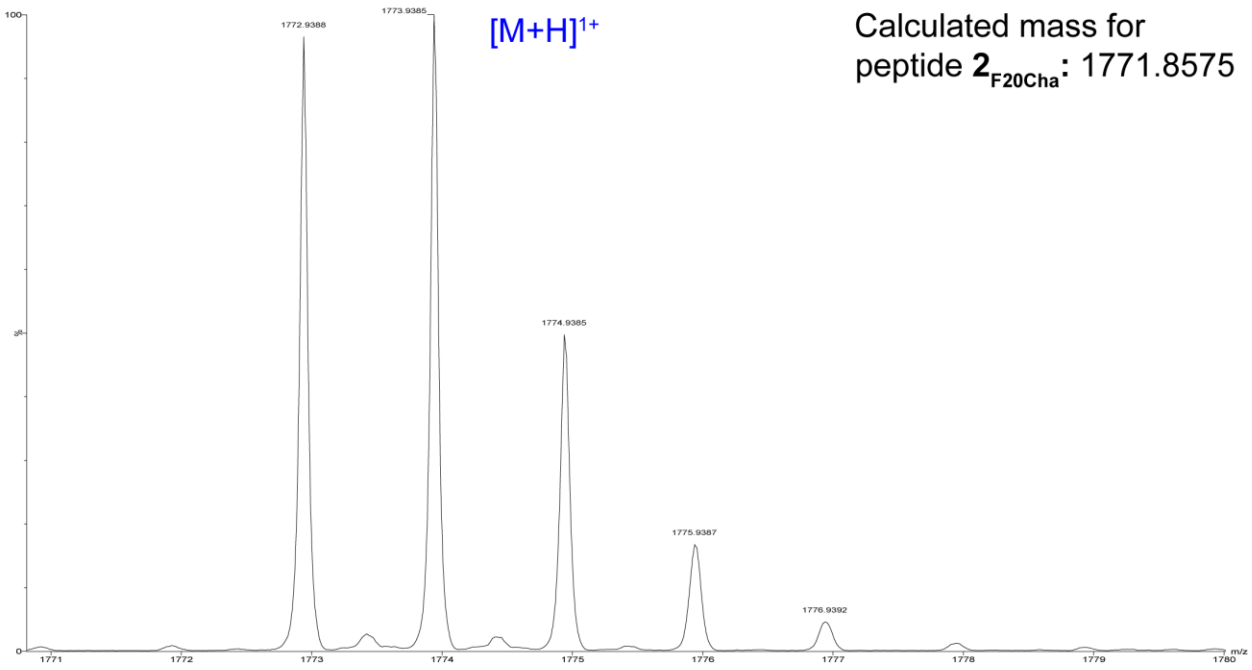


peptide **2**$_{\mathbf{F20Cha}}$

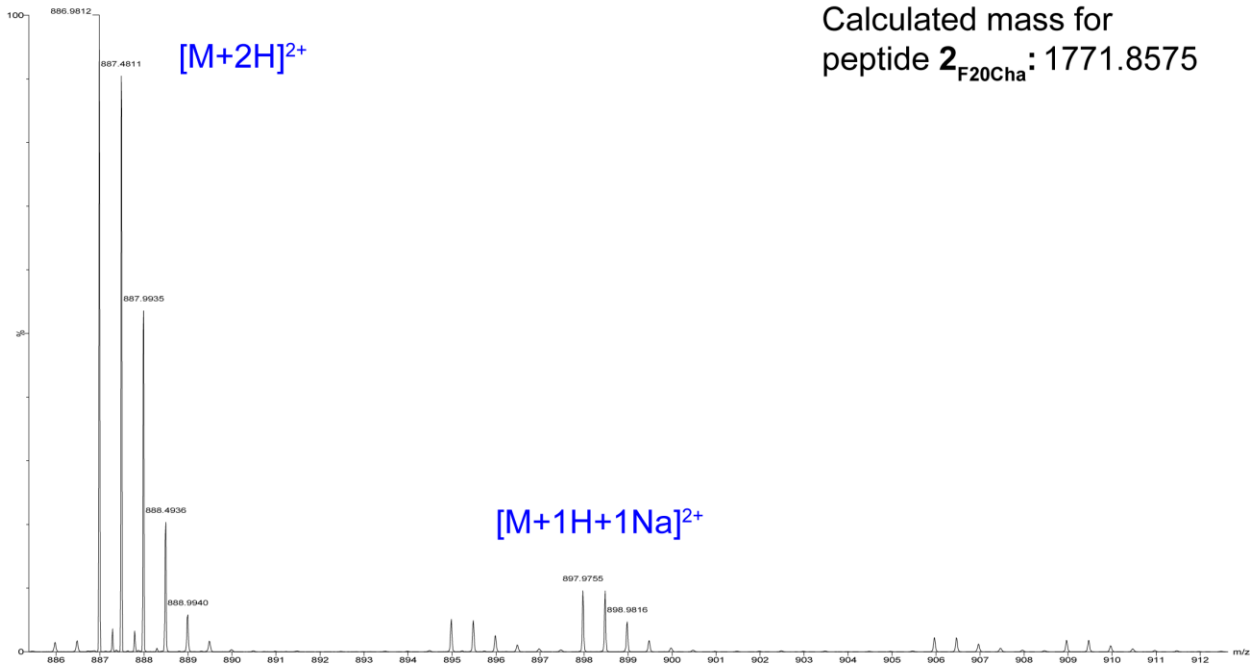




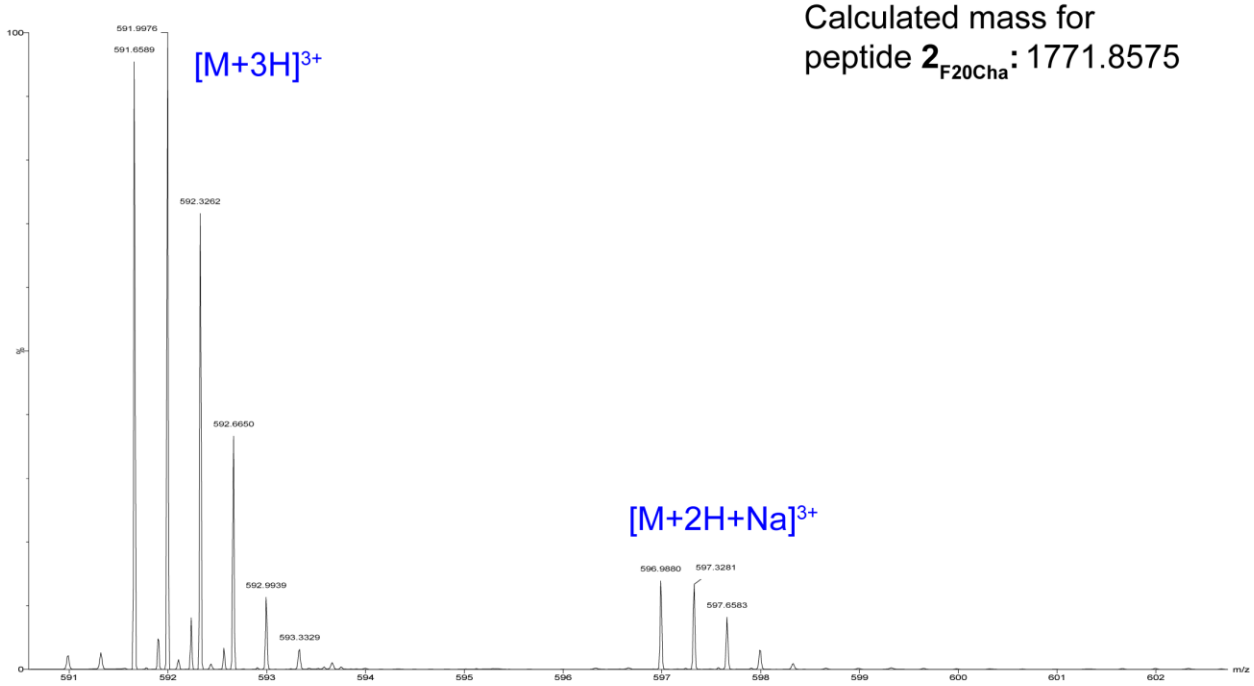
Calculated mass for peptide $\mathbf{2}_{\mathbf{F20Cha}}$: 1771.8575



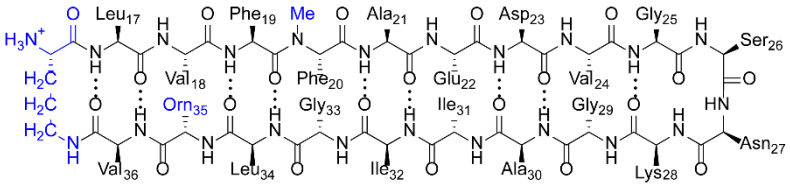
Calculated mass for peptide $\mathbf{2}_{\mathbf{F20Cha}}$: 1771.8575



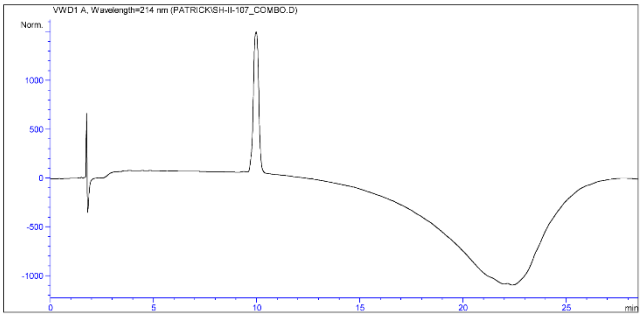
Calculated mass for peptide $\mathbf{2}_{\mathbf{F20Cha}}$: 1771.8575

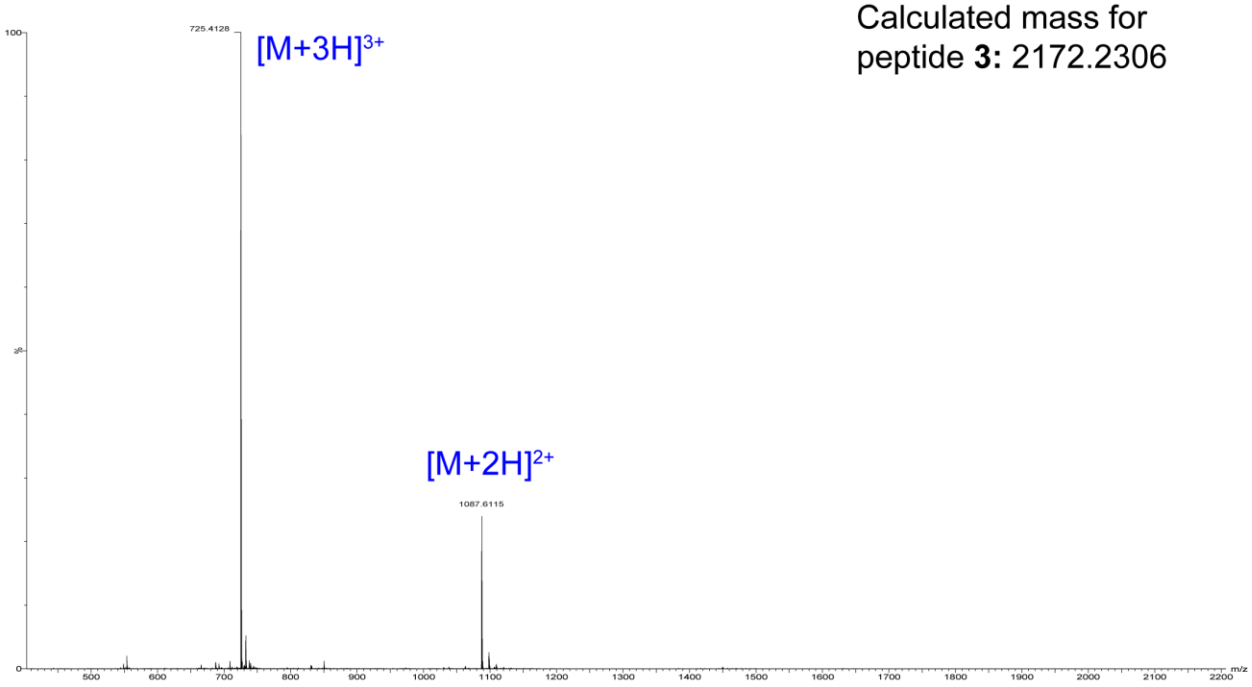


Calculated mass for peptide $\mathbf{2}_{\mathbf{F20Cha}}$: 1771.8575

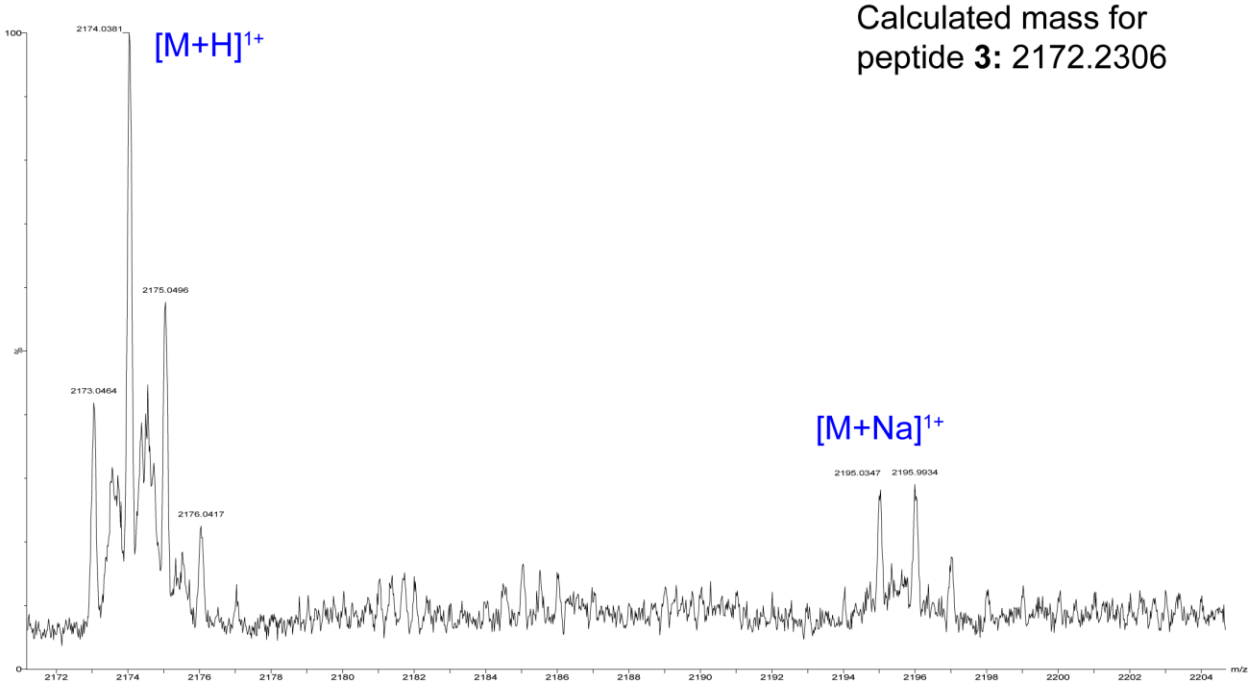

Leu17
Phe19
Me
Ala21
Asp23
Gly25
Ser26
Val18
Phe20
Glu22
Val24
Orn35
Gly33
Ile31
Gly29
Asn27
Val36
Leu34
Ile32
Ala30
Lys28

peptide **3**

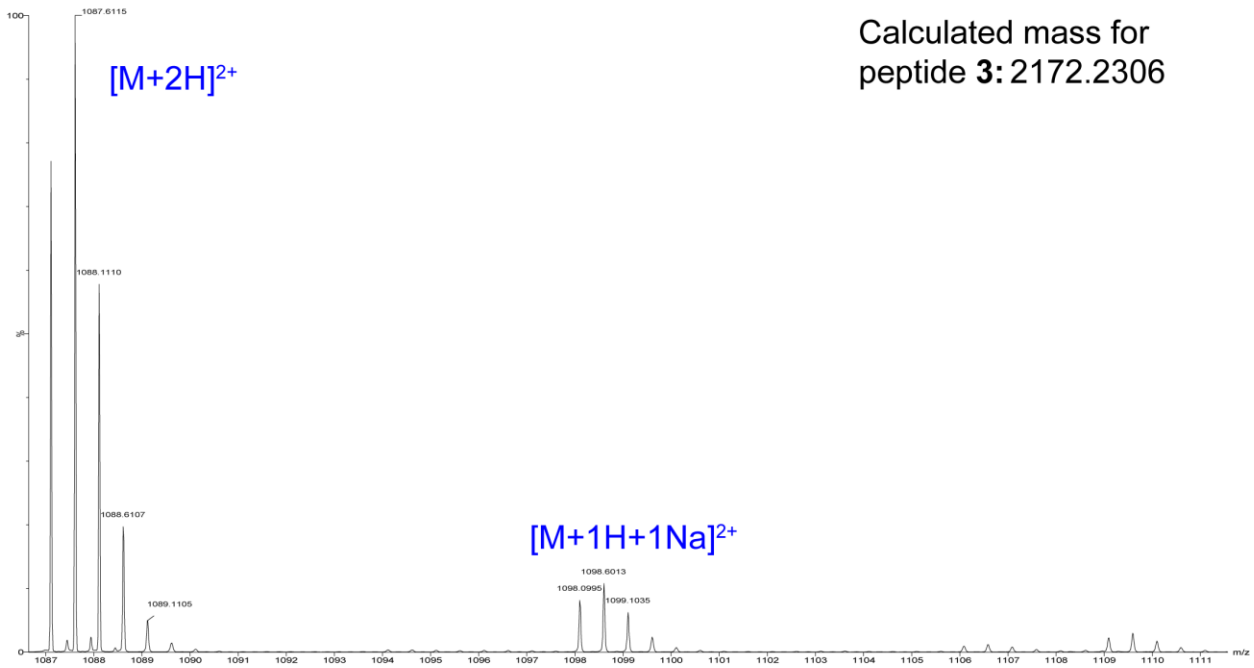

VWD1 A, Wavelength=214 nm (PATRICKSH-II-107_COMBO.D)
Norm.
1000
500
0
-500
-1000
0
5
10
15
20
25
min

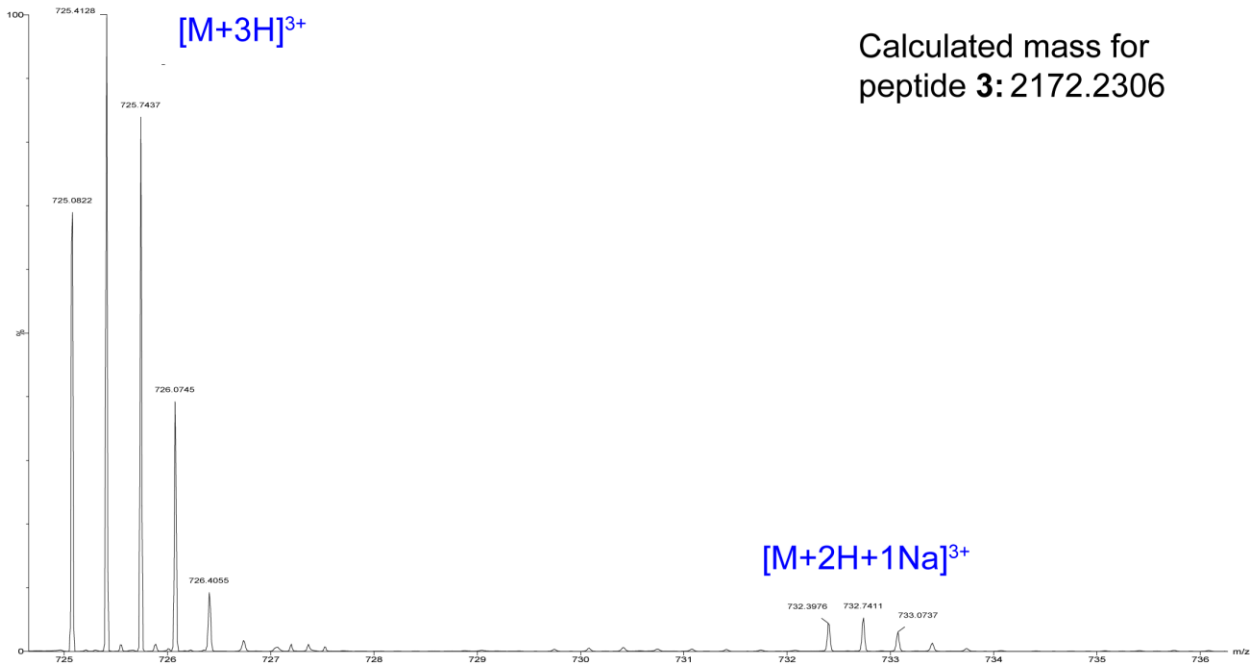


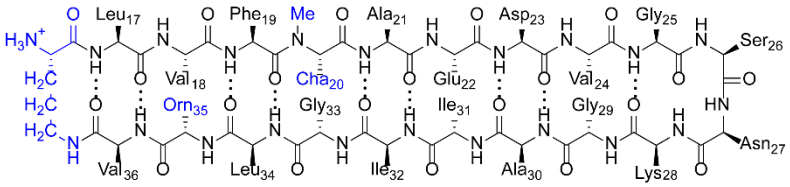
Calculated mass for peptide **3:** 2172.2306



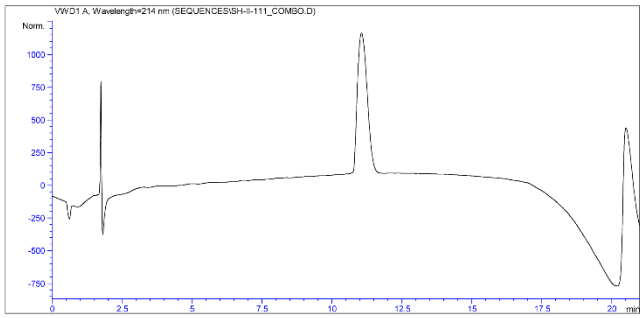
Calculated mass for peptide **3:** 2172.2306

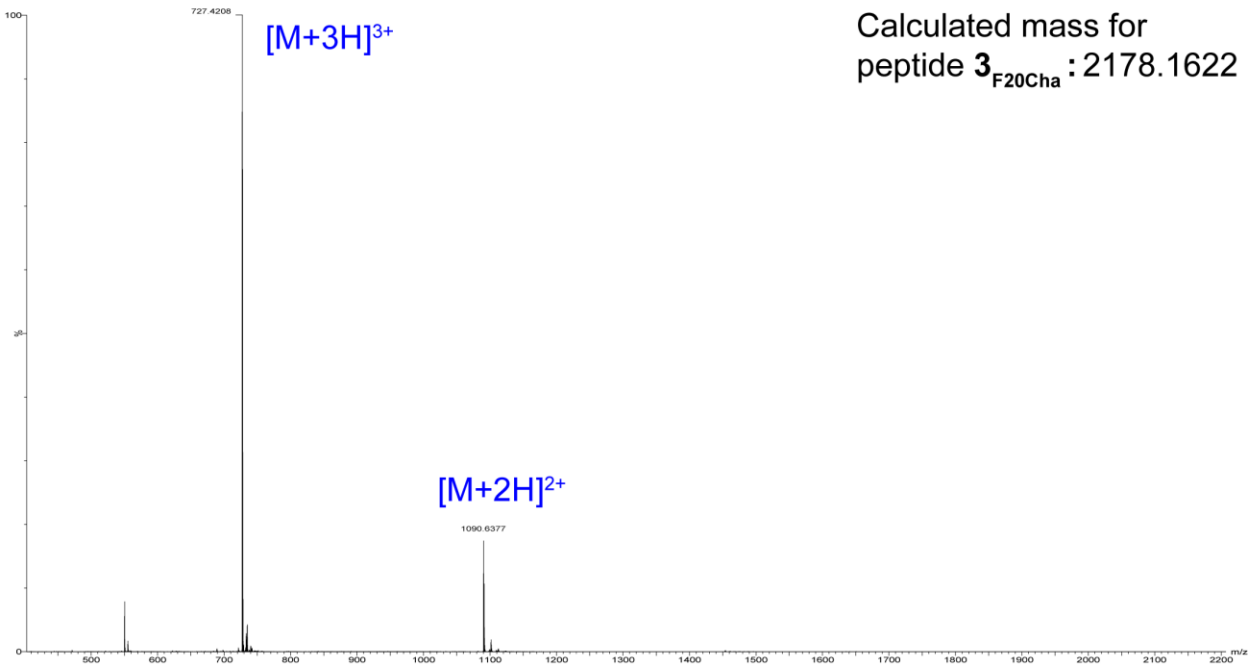

[M+2H]2+
Calculated mass for peptide 3: 2172.2306
1087.6115
1088.1110
1088.6107
1089.1105
[M+1H+1Na]2+
1098.6013
1098.0995
1099.1035
m/z


[M+3H]3+
Calculated mass for peptide 3: 2172.2306
725.4128
725.7437
725.0822
726.0745
726.4055
[M+2H+1Na]3+
732.3976
732.7411
733.0737
m/z

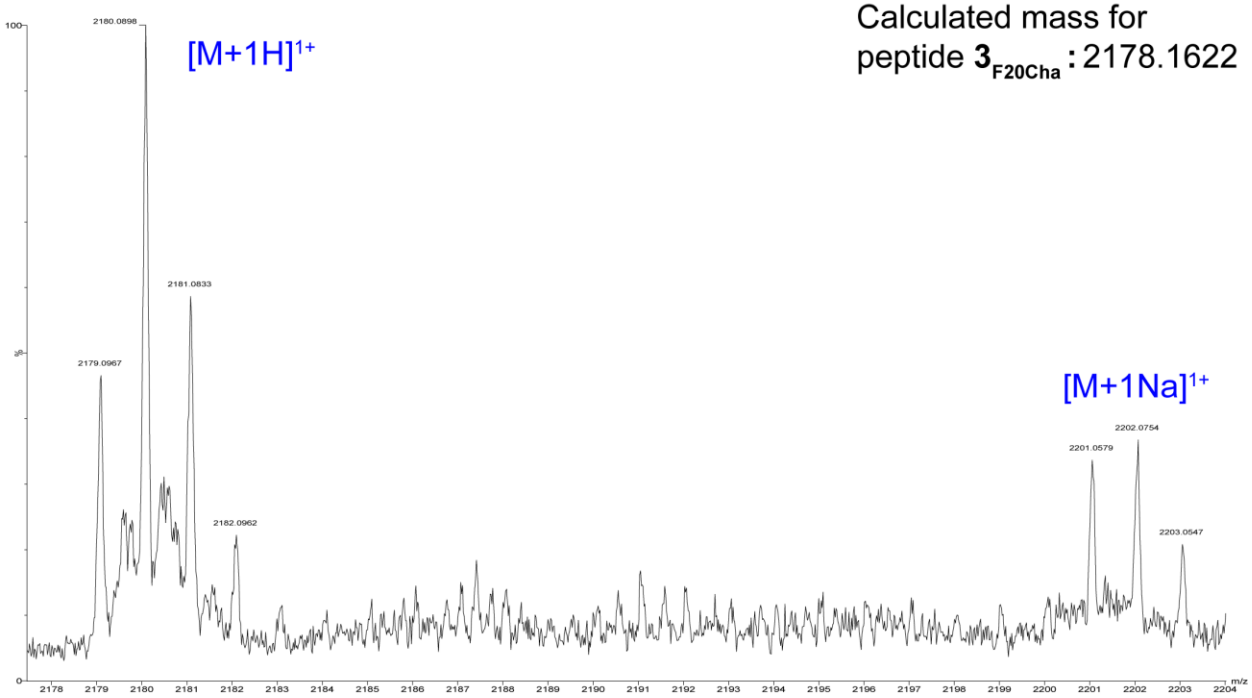


peptide **3**$_{\mathbf{F20Cha}}$

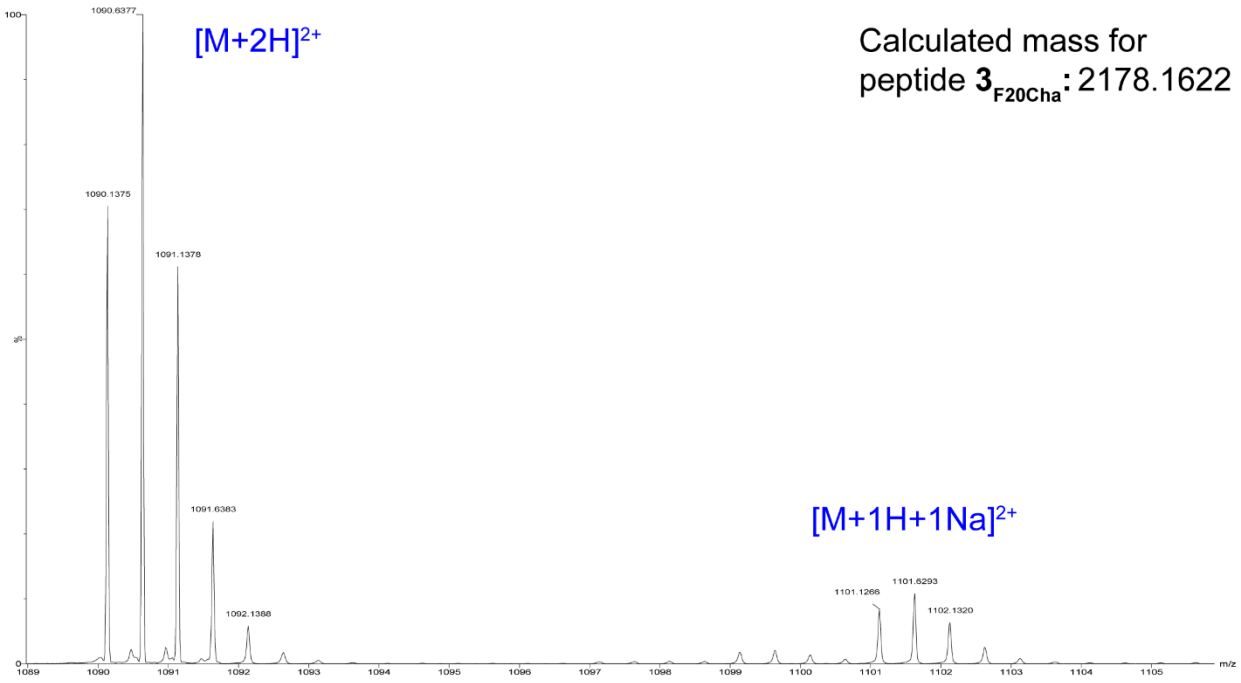


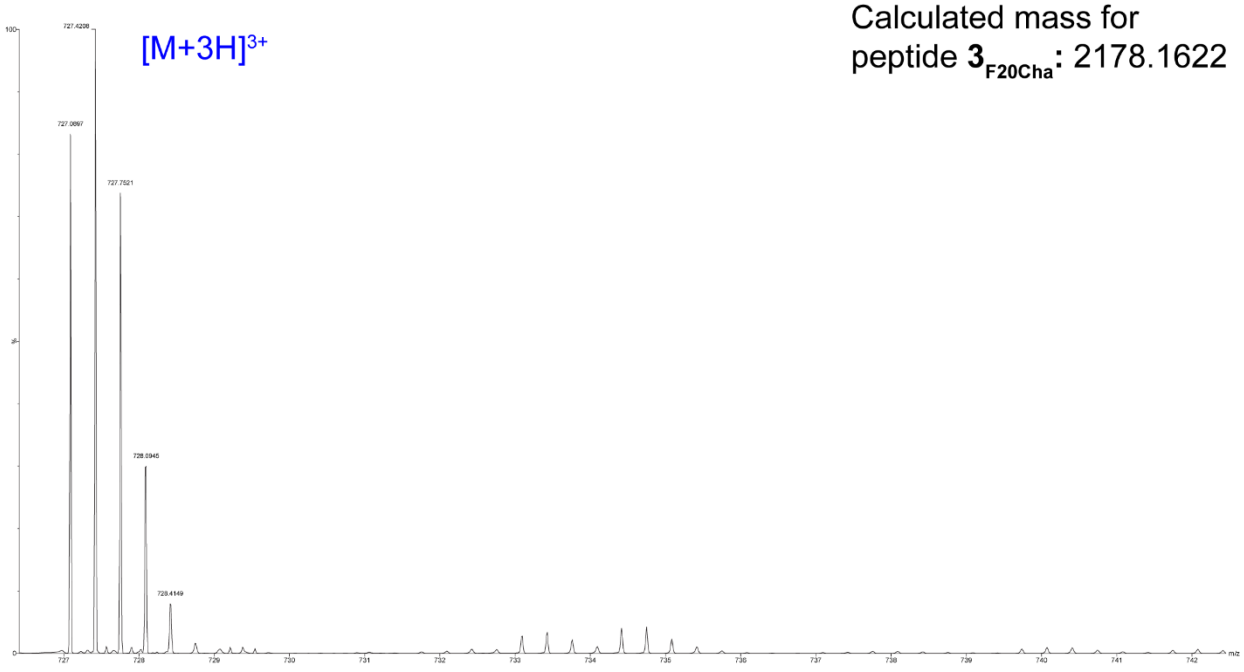


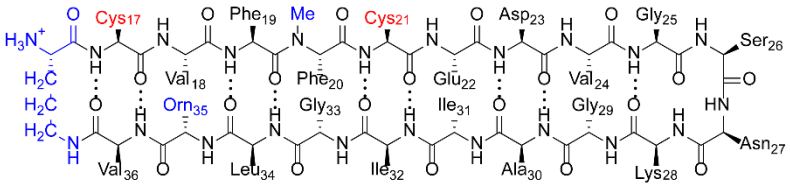
Calculated mass for peptide $\mathbf{3}_{\mathbf{F20Cha}}$: 2178.1622



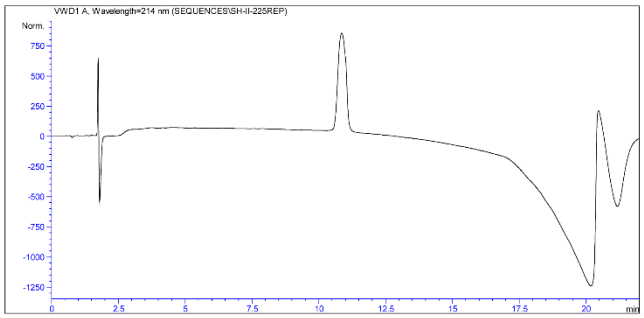
Calculated mass for peptide $\mathbf{3}_{\mathbf{F20Cha}}$: 2178.1622

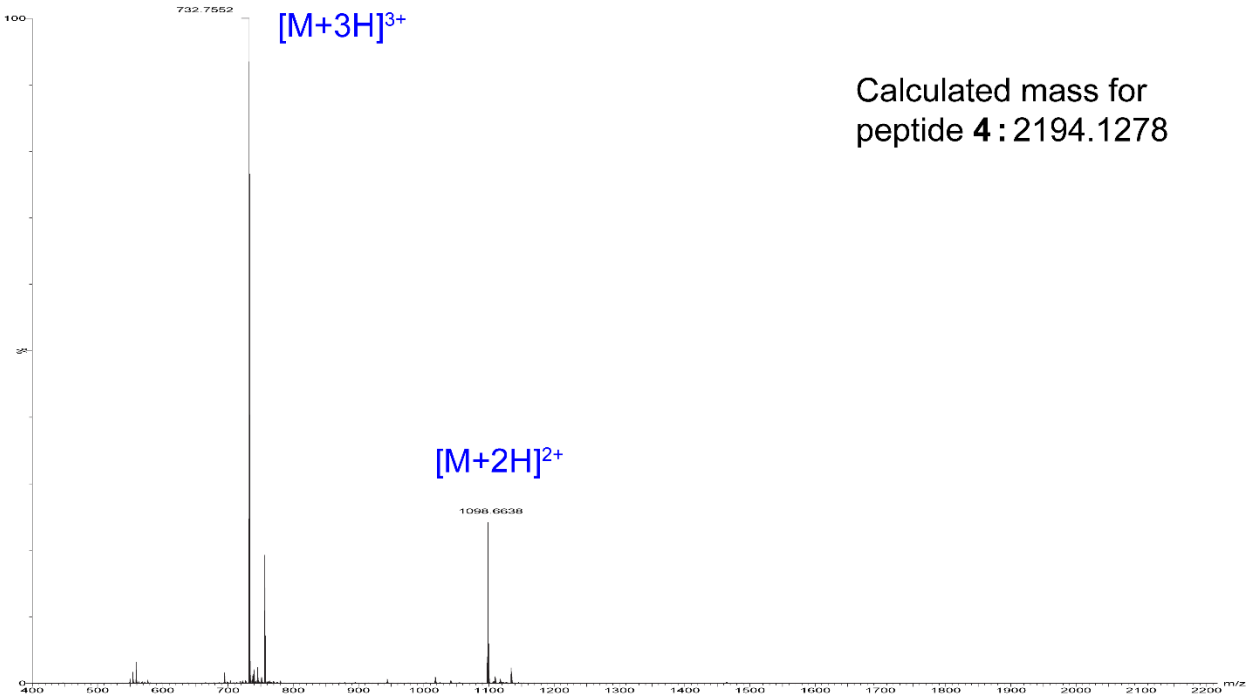

[M+2H]2+
Calculated mass for peptide 3F20Cha: 2178.1622
1090.6377
1090.1375
1091.1378
1091.6383
1092.1368
[M+1H+1Na]2+
1101.1266
1101.6293
1102.1320
%
m/z

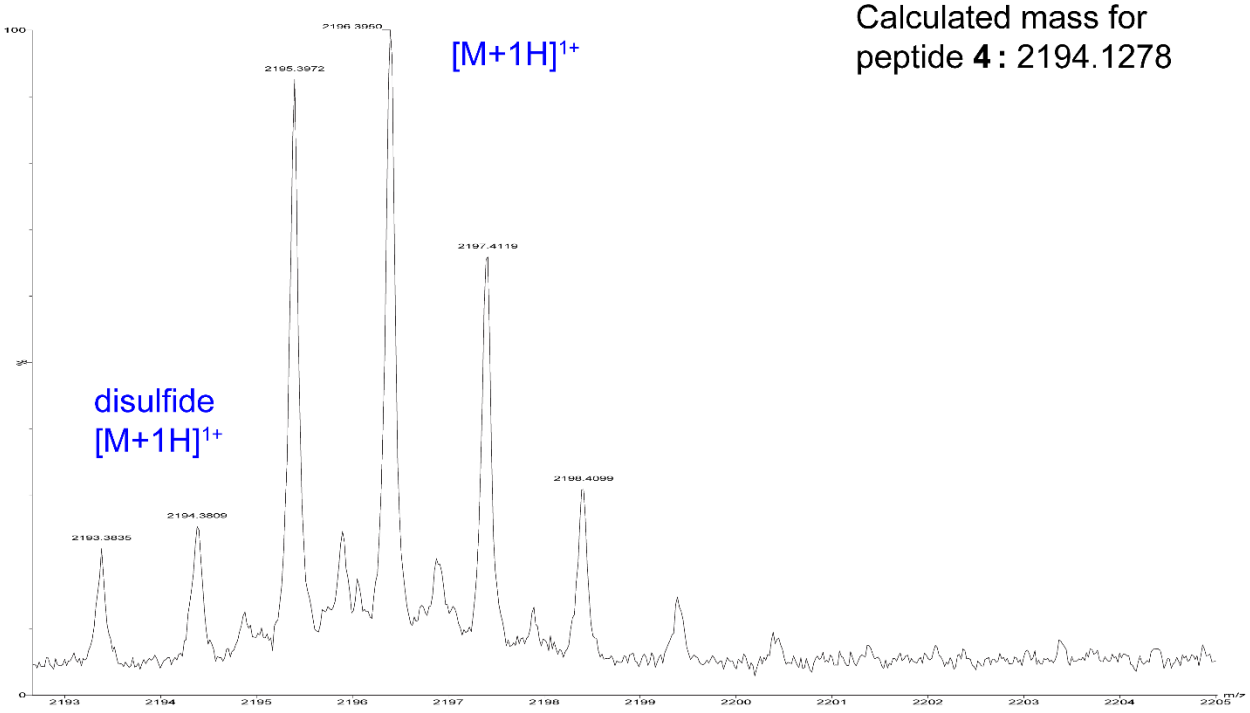

[M+3H]3+
Calculated mass for peptide 3F20Cha: 2178.1622
727.4208
727.0997
727.7521
728.0945
728.4149
%
m/z

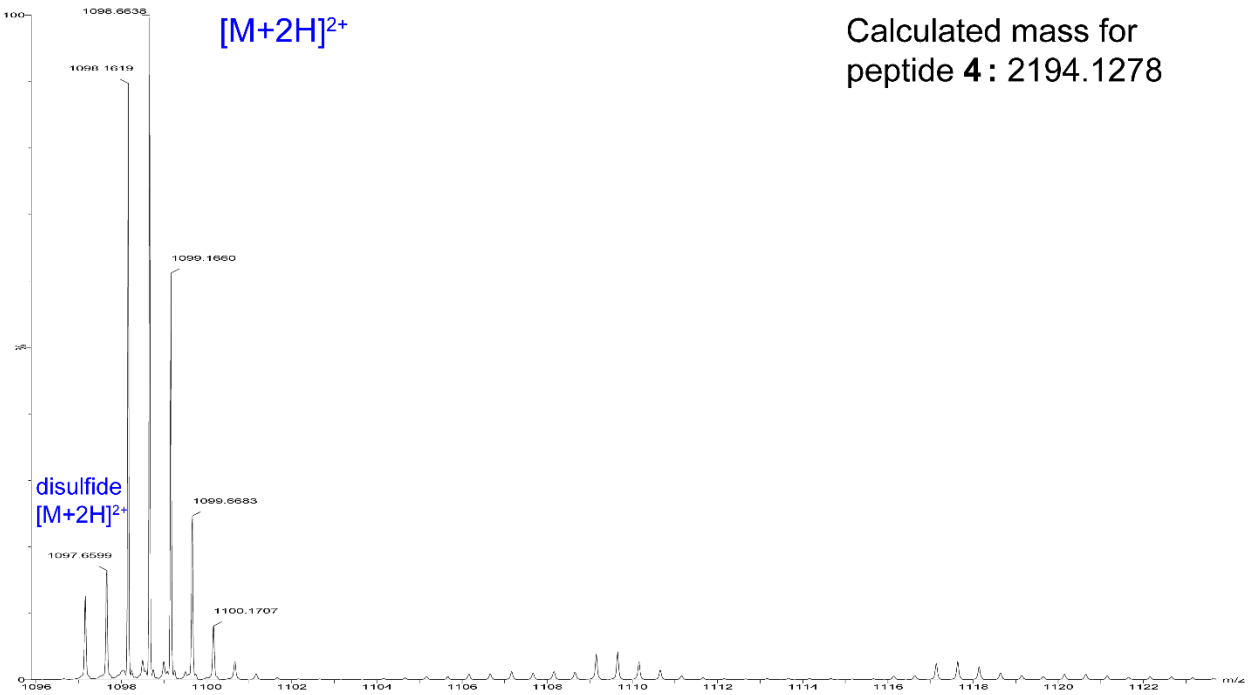

Cys17
Phe19
Me
Cys21
Asp23
Gly25
Ser26
Val18
Phe20
Glu22
Val24
Orn35
Gly33
Ile31
Gly29
Asn27
Val36
Leu34
Ile32
Ala30
Lys28

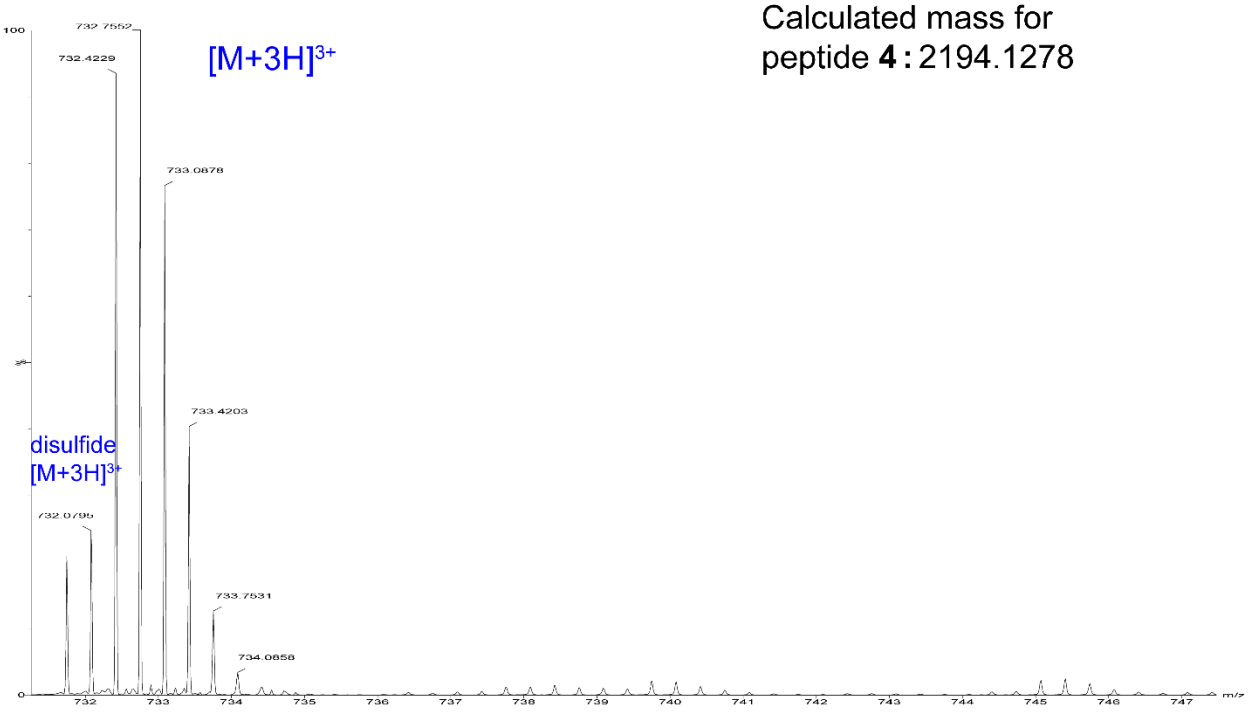
peptide **4**

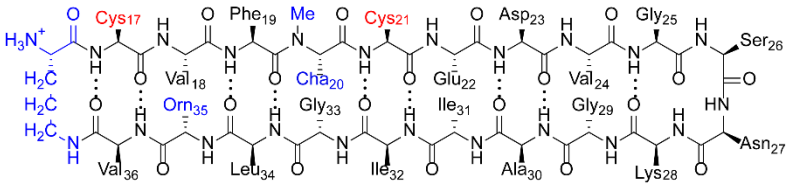

VWD1 A, Wavelength=214 nm (SEQUENCES\SH-II-225REP)
Norm.
750
500
250
0
-250
-500
-750
-1000
-1250
0
2.5
5
7.5
10
12.5
15
17.5
20
min


732.7552
[M+3H]3+
Calculated mass for
peptide 4 : 2194.1278
[M+2H]2+
1098.6638
100
%
0
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
m/z

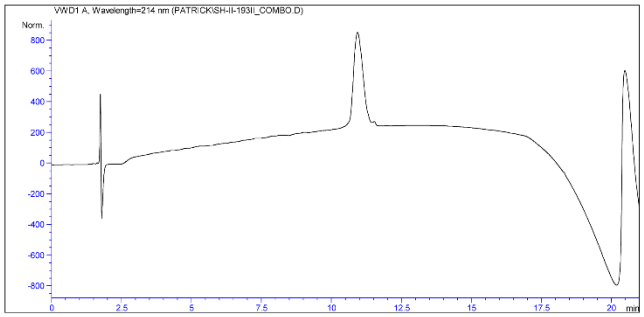

2196.3960
2195.3972
[M+1H]1+
Calculated mass for
peptide 4 : 2194.1278
2197.4119
disulfide
[M+1H]1+
2198.4099
2194.3809
2193.3835
100
%
0
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
m/z

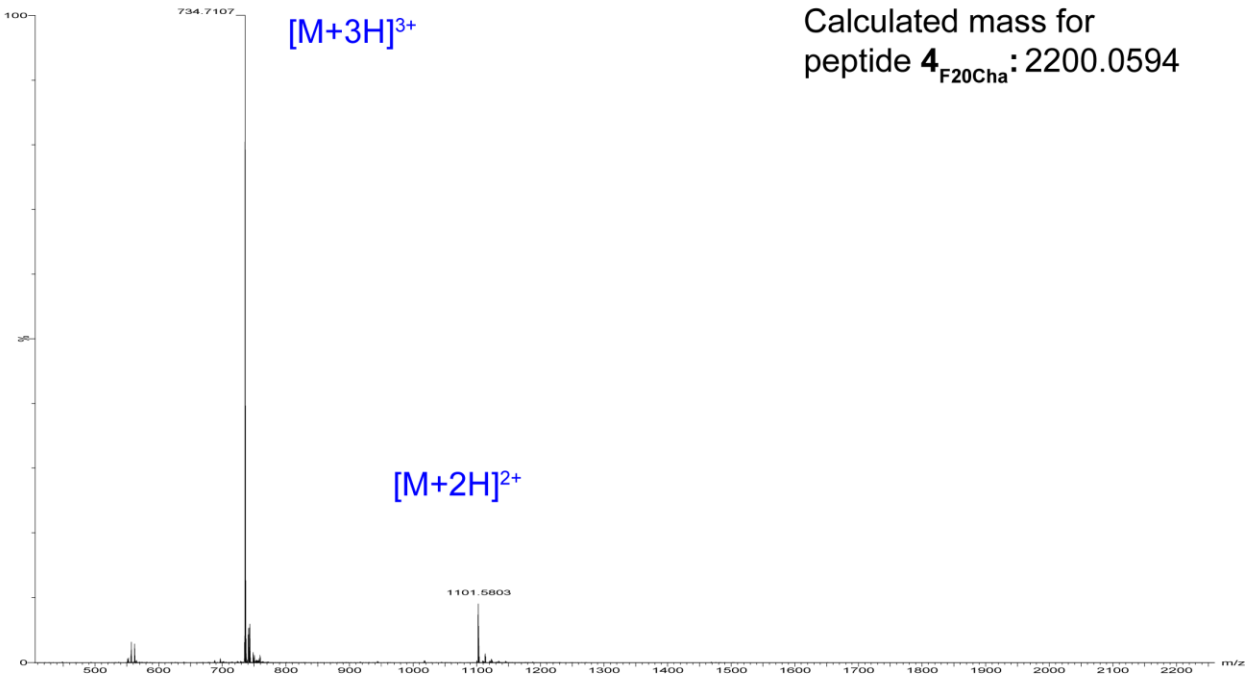

[M+2H]2+
Calculated mass for peptide 4 : 2194.1278
1098.6638
1098.1619
1099.1660
disulfide [M+2H]2+
1099.6683
1097.6599
1100.1707
100
%
0
1096
1098
1100
1102
1104
1106
1108
1110
1112
1114
1116
1118
1120
1122
m/z

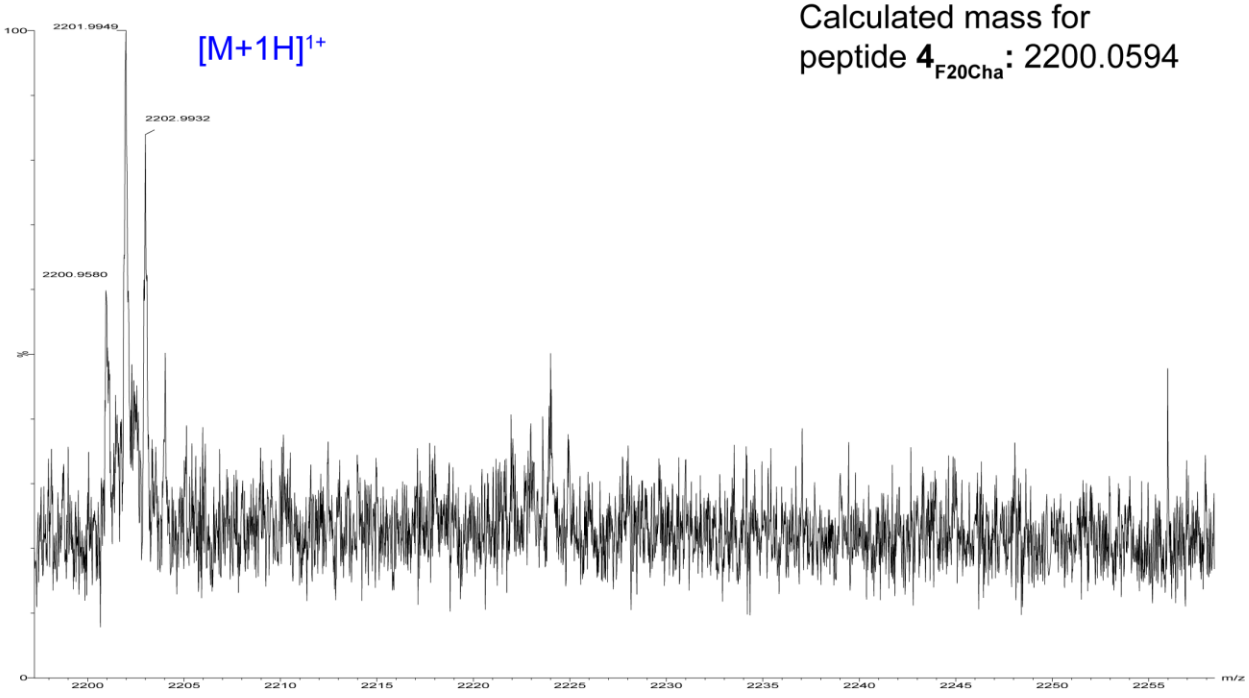

[M+3H]3+
Calculated mass for peptide 4 : 2194.1278
732.7552
732.4229
733.0878
733.4203
disulfide [M+3H]3+
732.0795
733.7531
734.0858
100
%
0
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
m/z

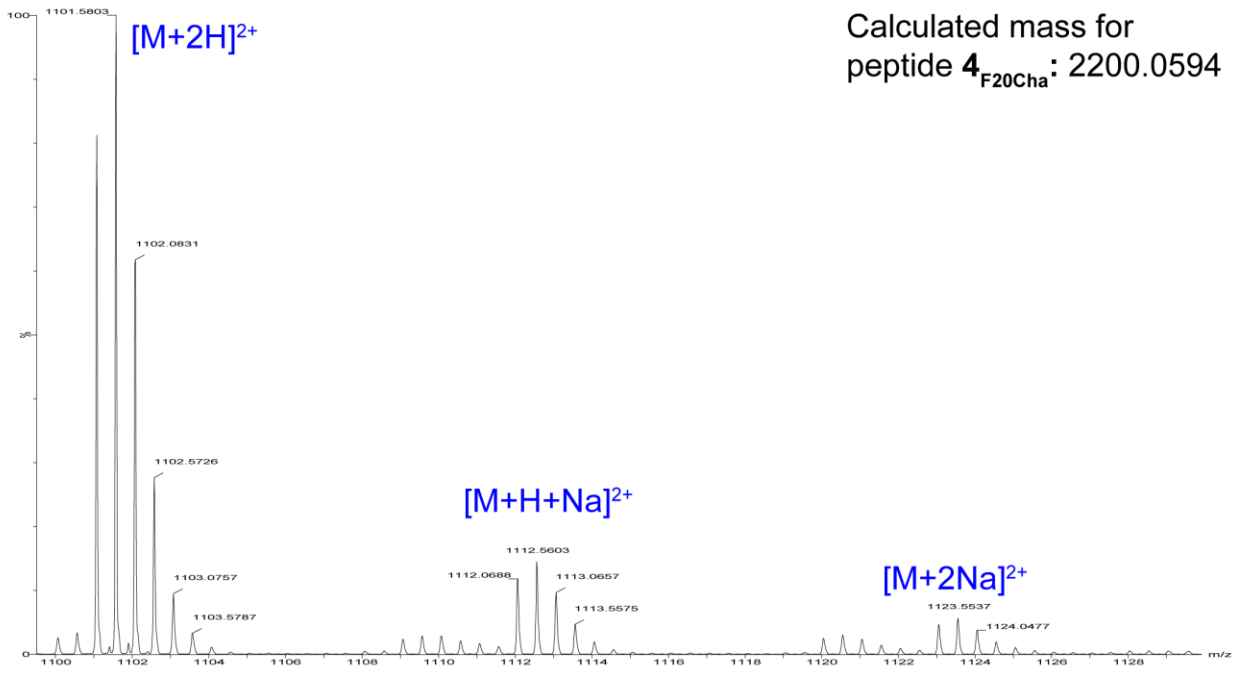


peptide **4**$_{\mathbf{F20Cha}}$

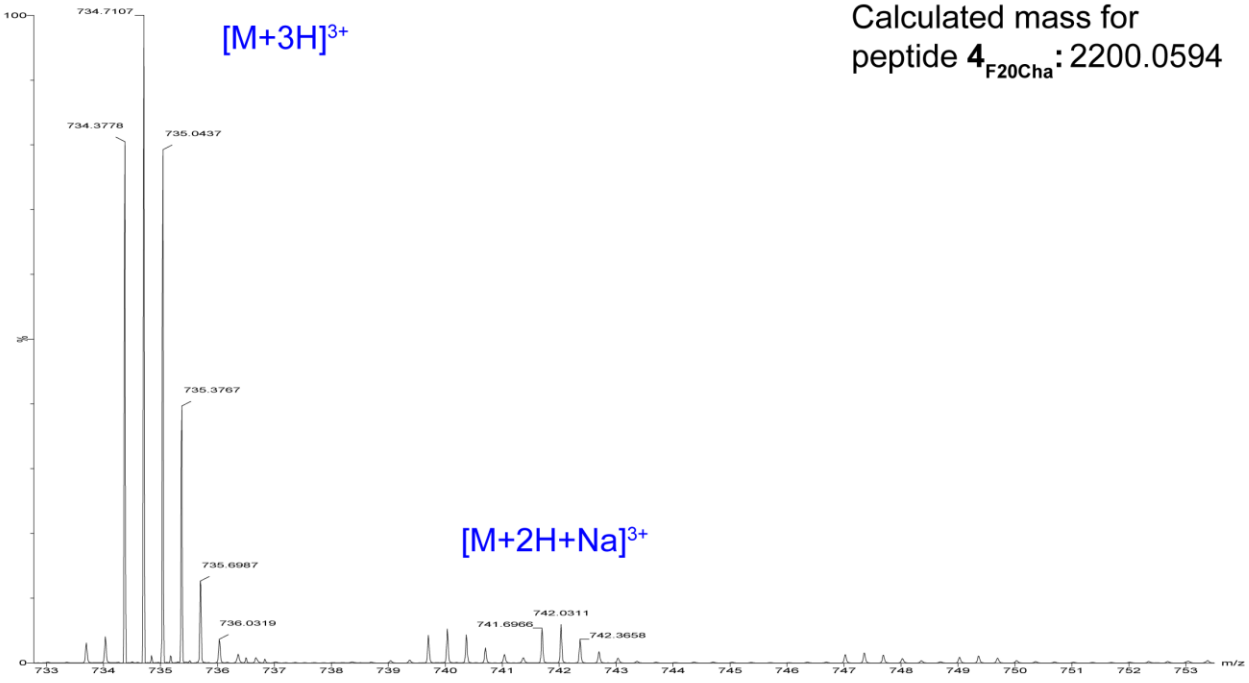



734.7107
[M+3H]3+
Calculated mass for
peptide 4F20Cha: 2200.0594
[M+2H]2+
1101.5803
100
%
0
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
m/z

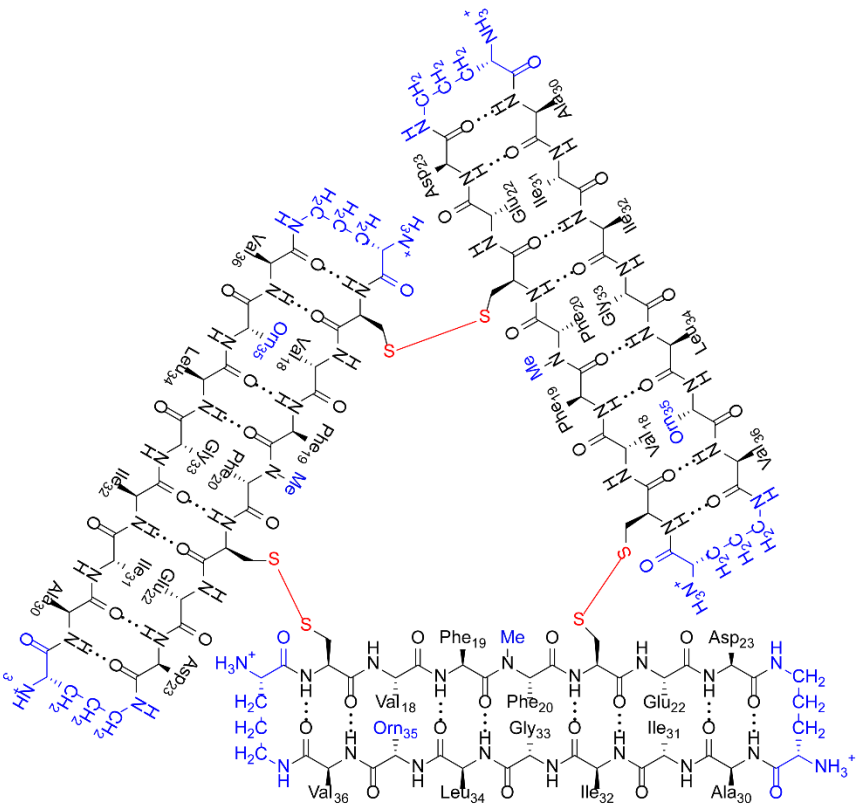

2201.9949
[M+1H]1+
Calculated mass for
peptide 4F20Cha: 2200.0594
2202.9932
2200.9580
100
%
0
2200
2205
2210
2215
2220
2225
2230
2235
2240
2245
2250
2255
m/z



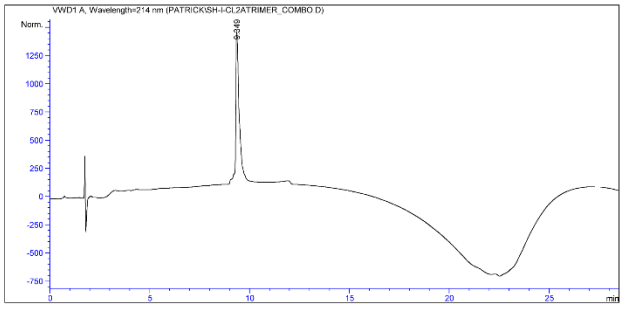
Calculated mass for peptide $\mathbf{4}_{\mathbf{F20Cha}}$: 2200.0594

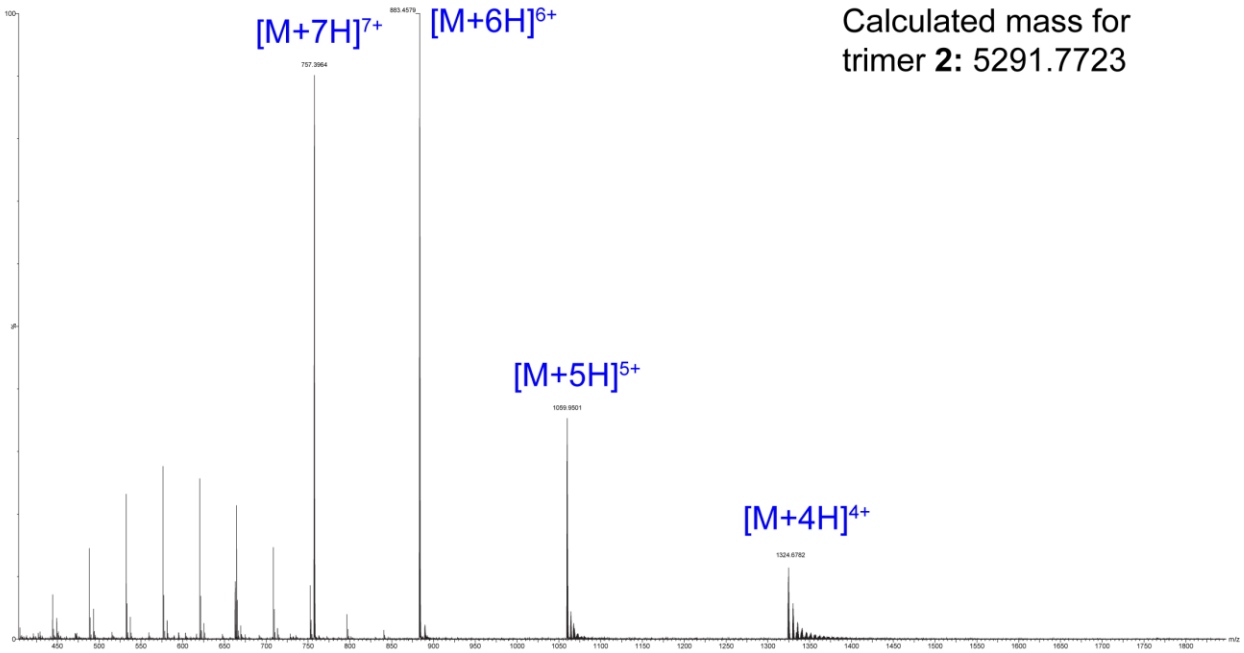


Calculated mass for peptide $\mathbf{4}_{\mathbf{F20Cha}}$: 2200.0594

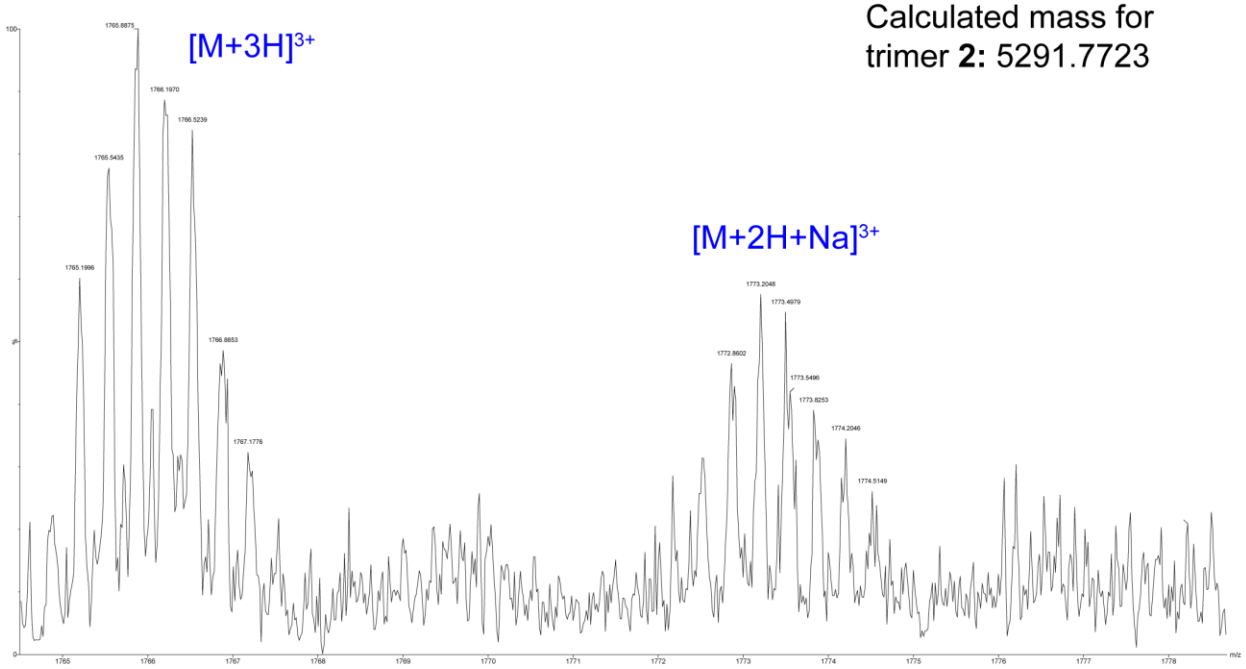

Phe19
Me
Asp23
Val18
Phe20
Glu22
Orn35
Gly33
Ile31
Val36
Leu34
Ile32
Ala30

trimer **2**

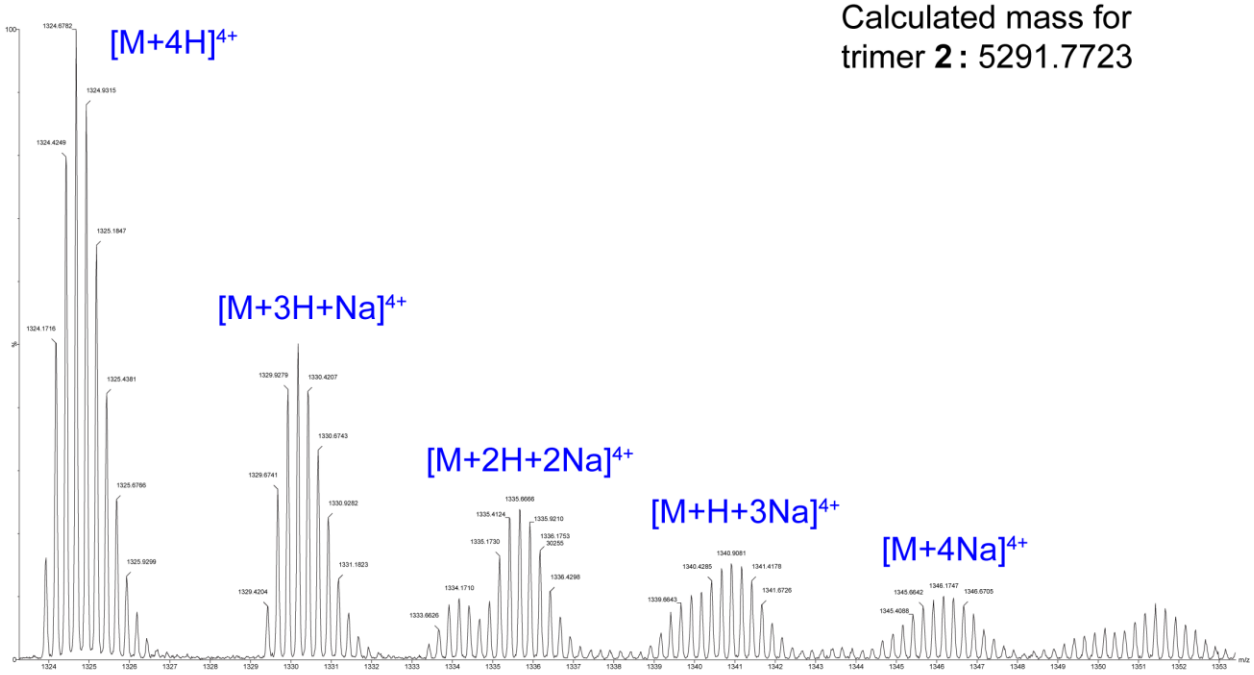

VWD1 A, Wavelength=214 nm (PATRICK\SH-I-CL2ATRIMER_COMBO.D)
Norm.
min



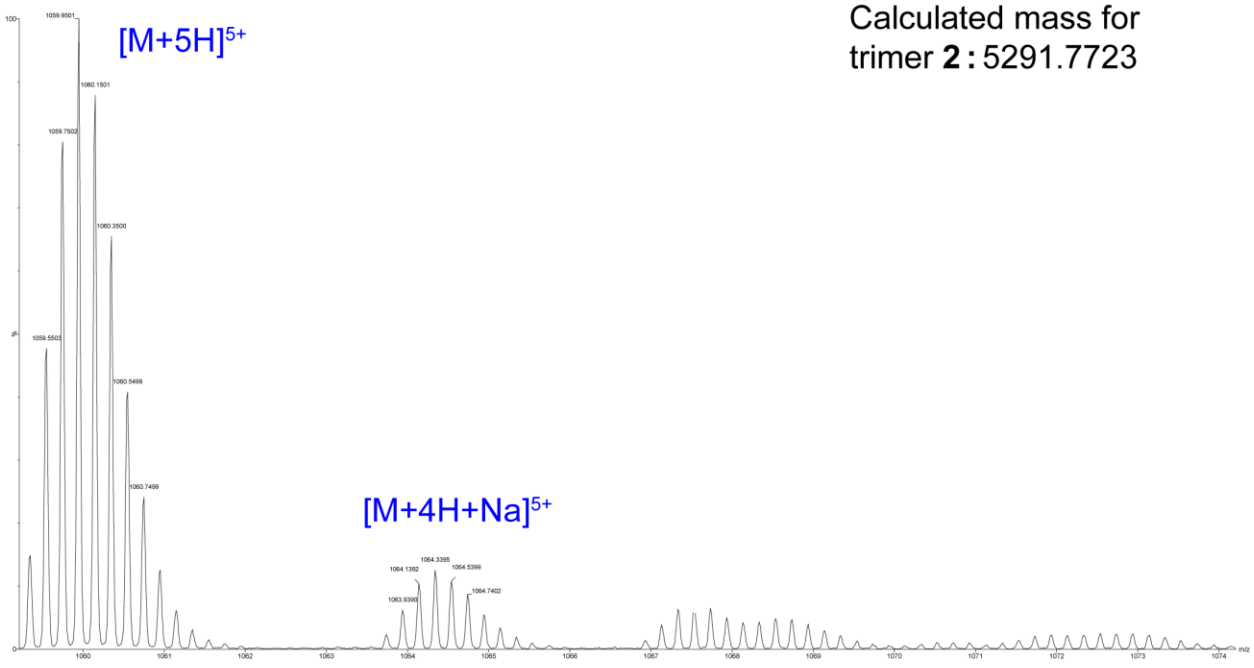
Calculated mass for trimer **2:** 5291.7723



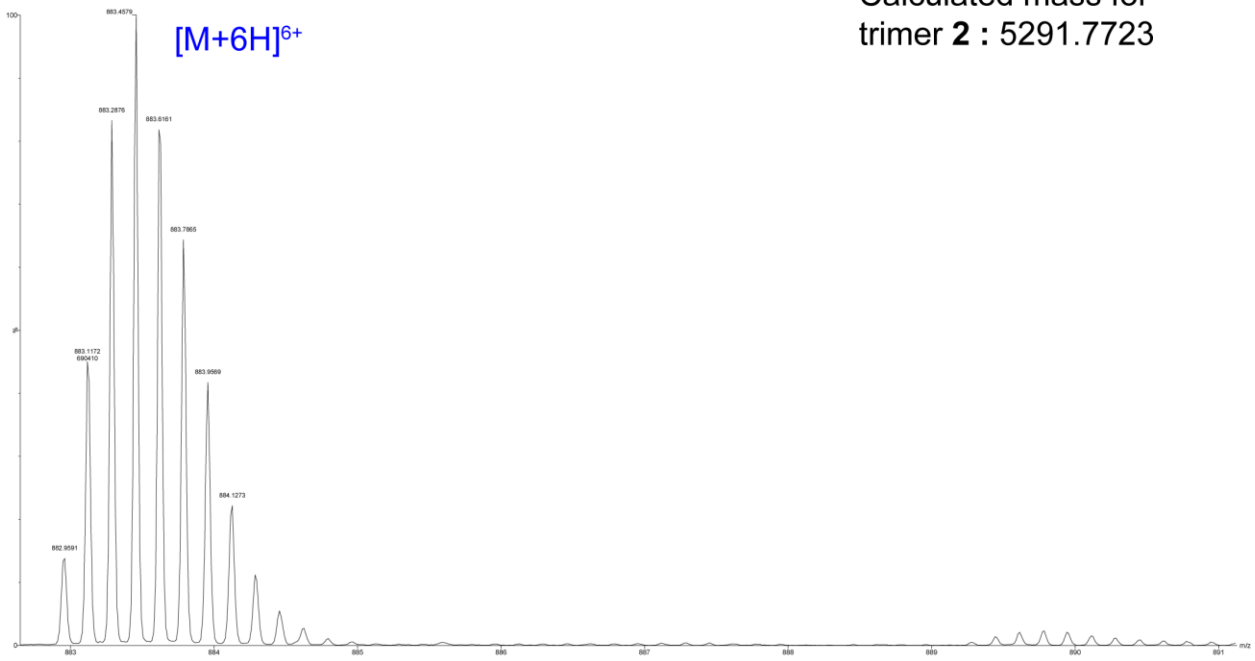
Calculated mass for trimer **2:** 5291.7723



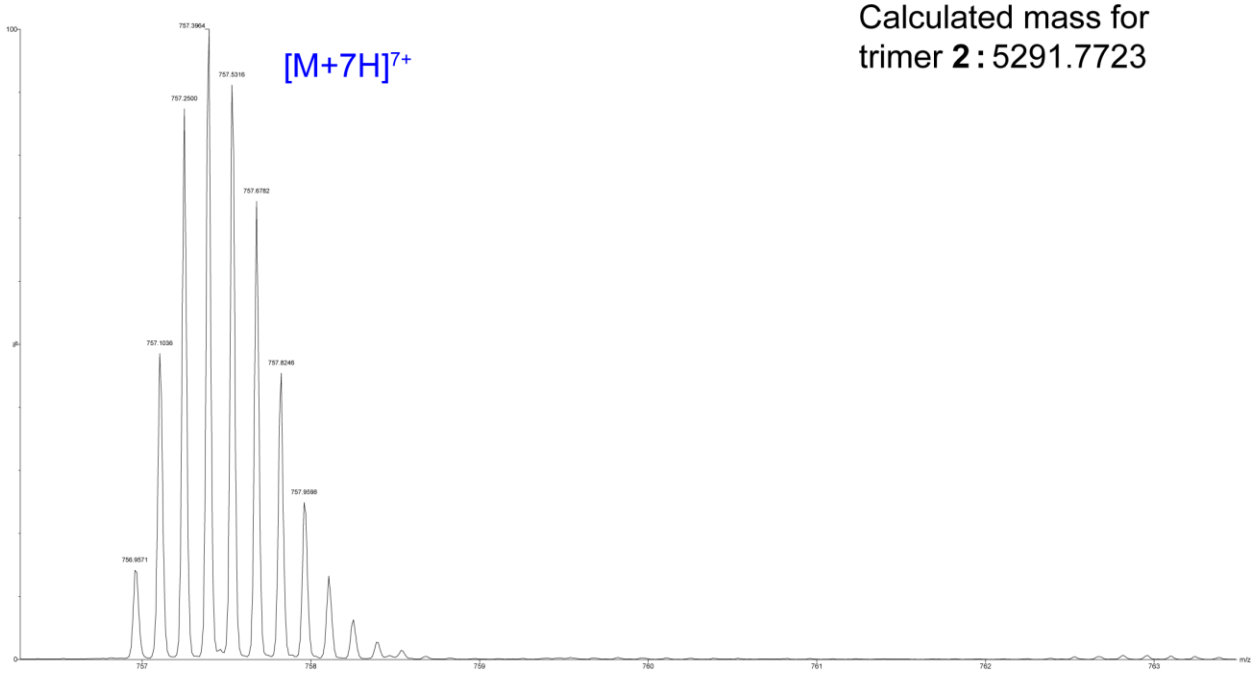
Calculated mass for trimer **2** : 5291.7723



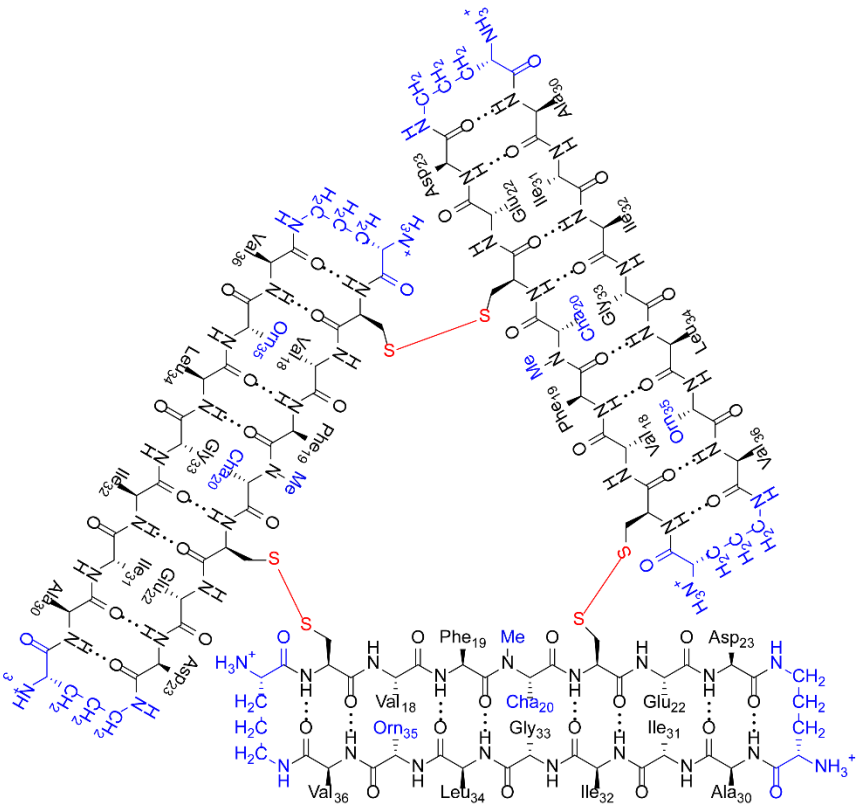
Calculated mass for trimer **2** : 5291.7723



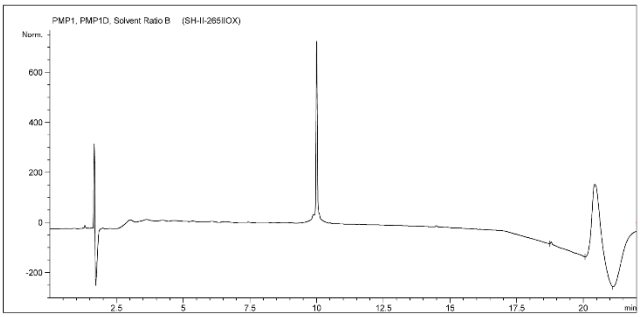
Calculated mass for trimer **2** : 5291.7723

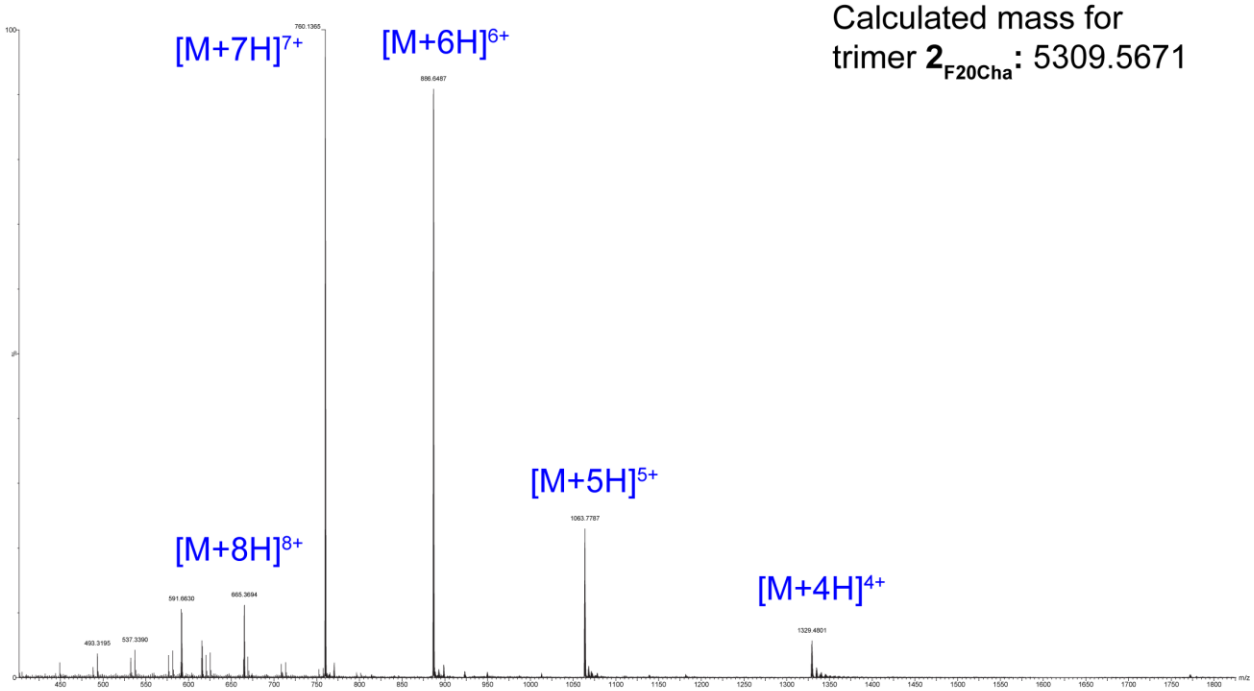


Calculated mass for trimer **2** : 5291.7723

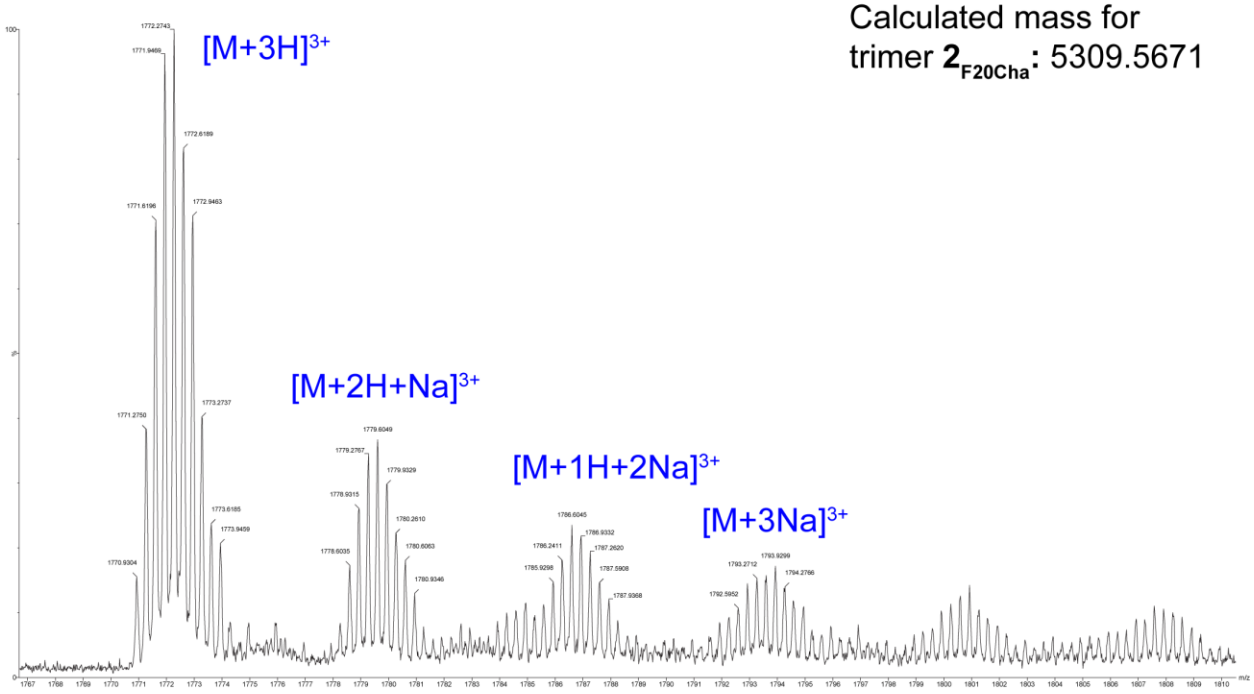


trimer **2**$_{\mathbf{F20Cha}}$

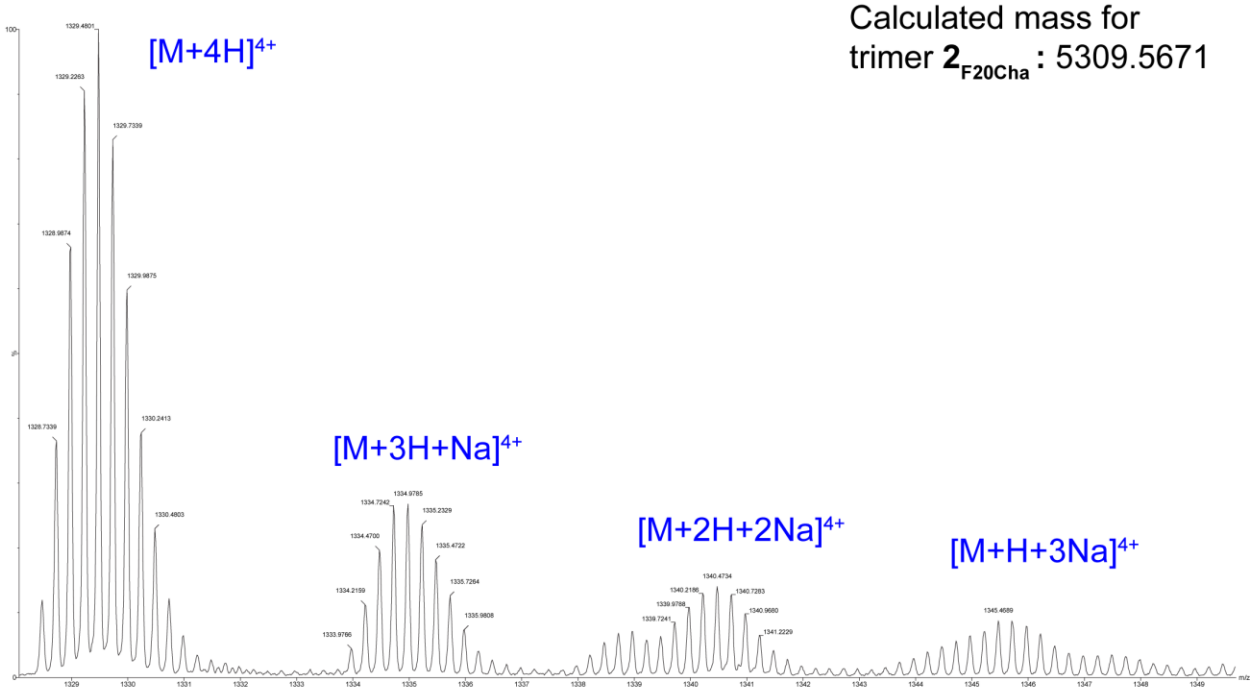




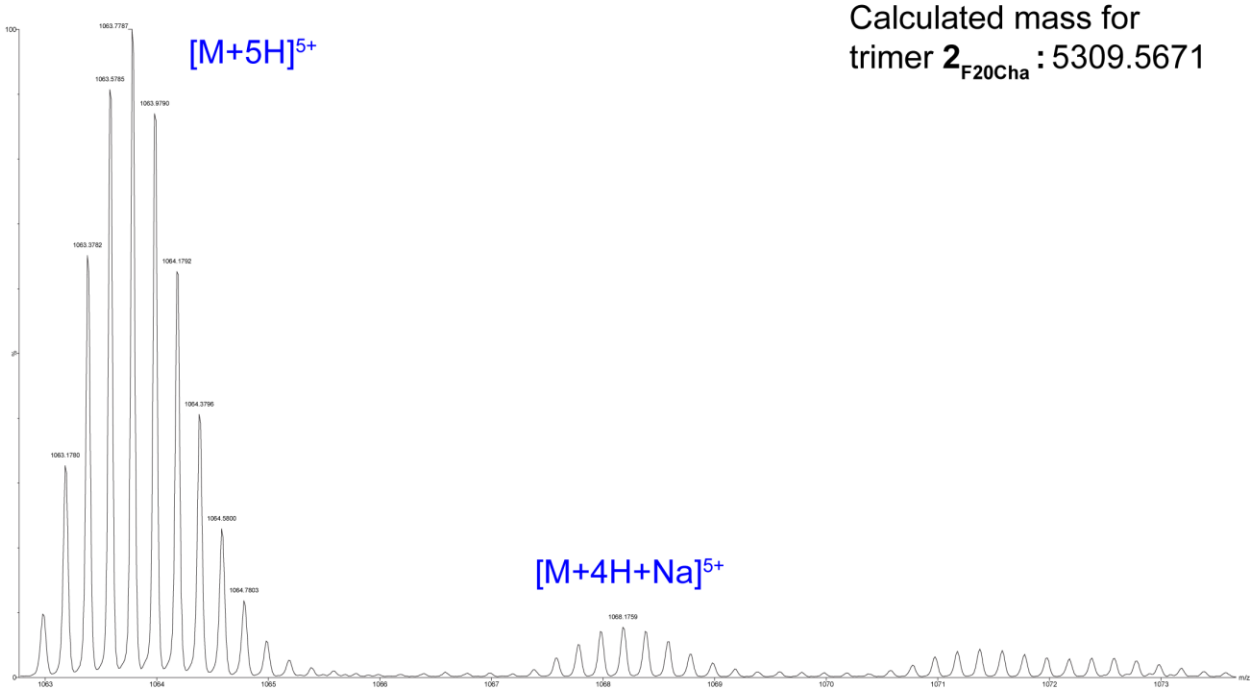
Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671



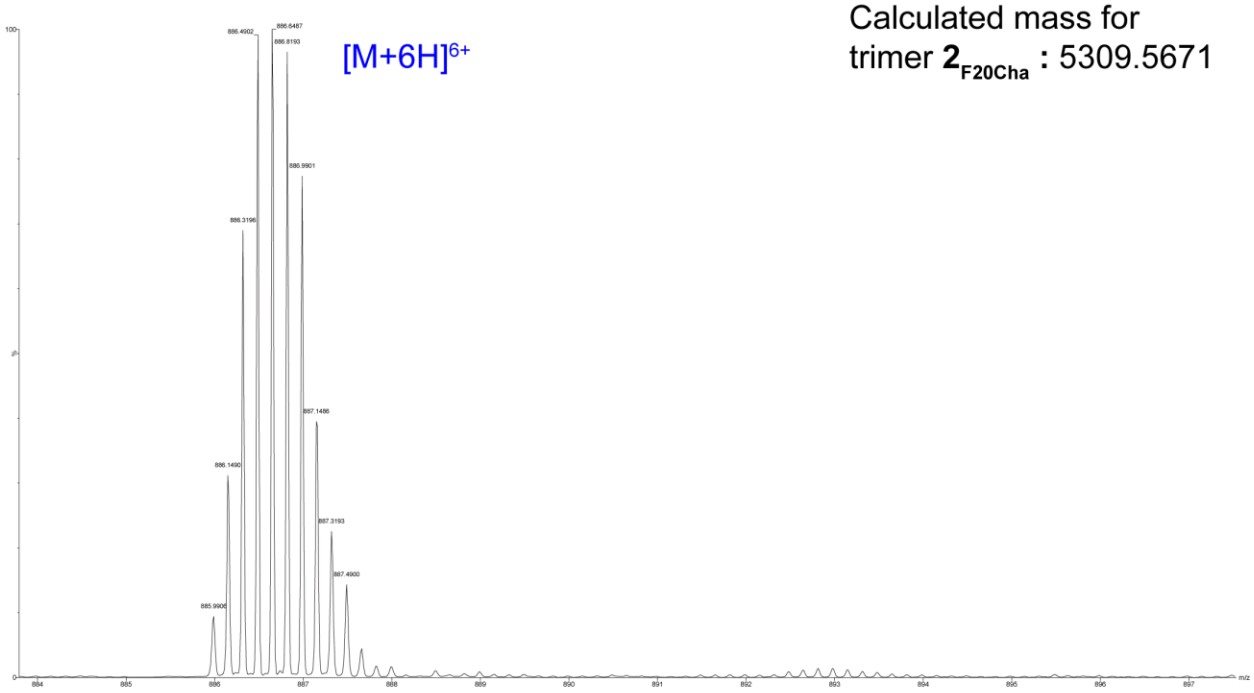
Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671



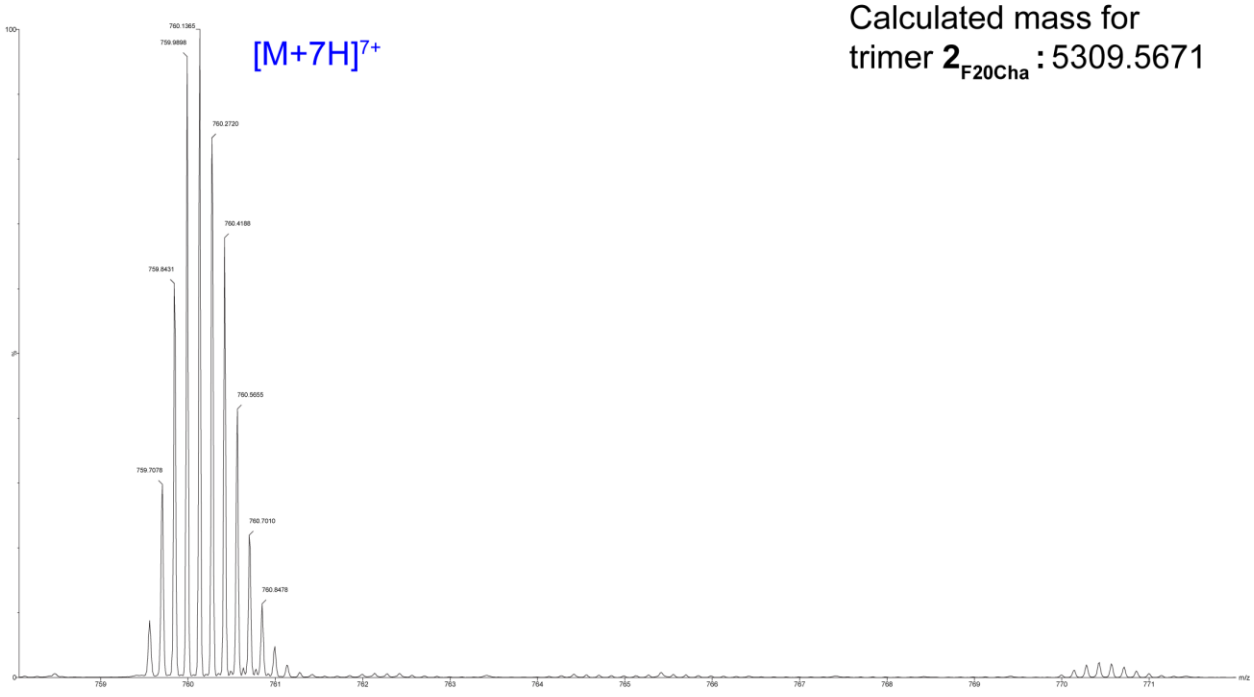
Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671



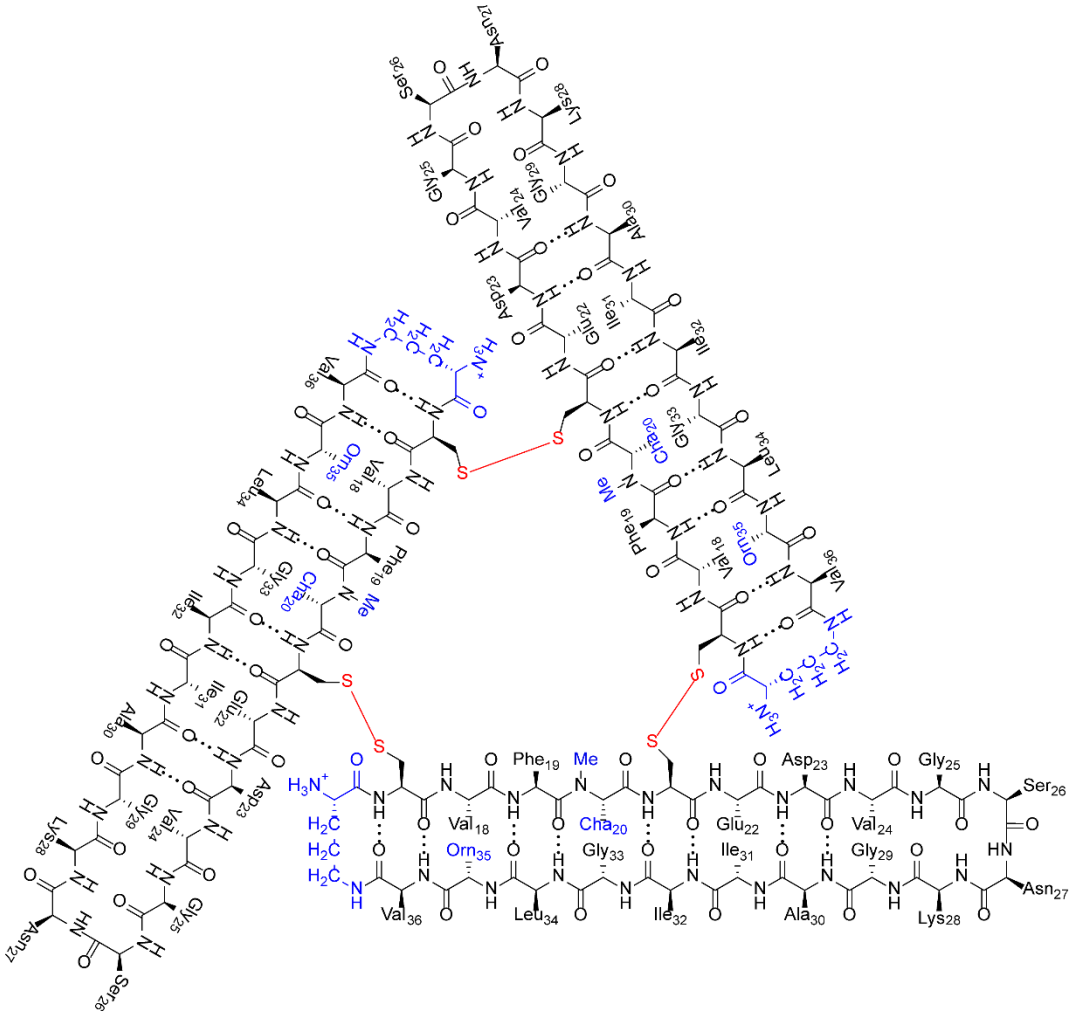
Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671



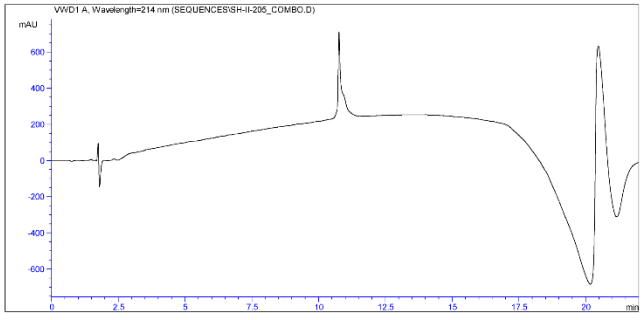
Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671

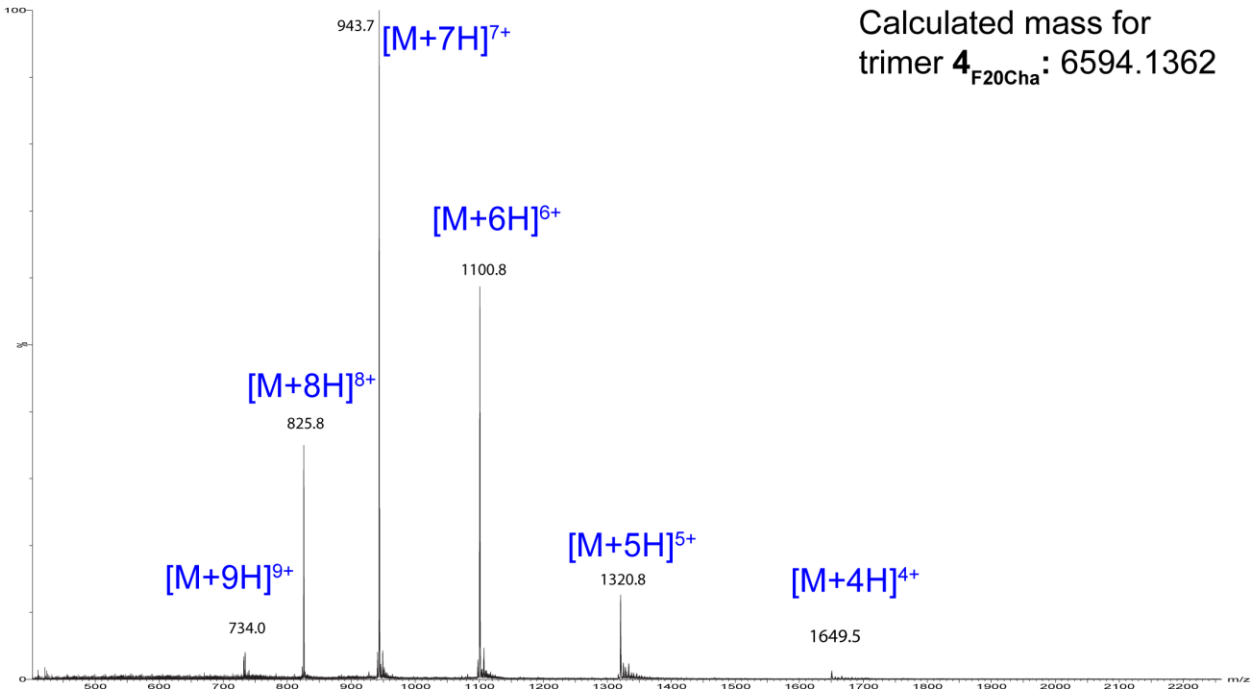


Calculated mass for trimer $\mathbf{2}_{\mathbf{F20Cha}}$: 5309.5671

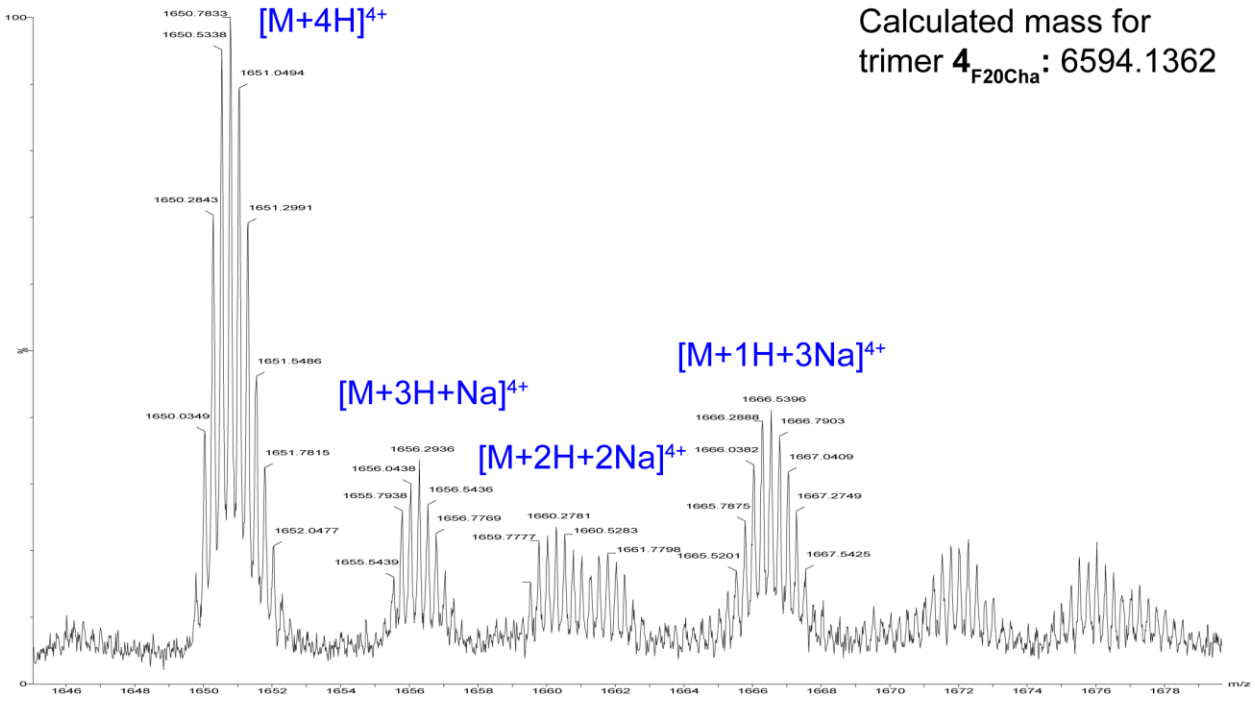


trimer **4**$_{\mathbf{F20Cha}}$

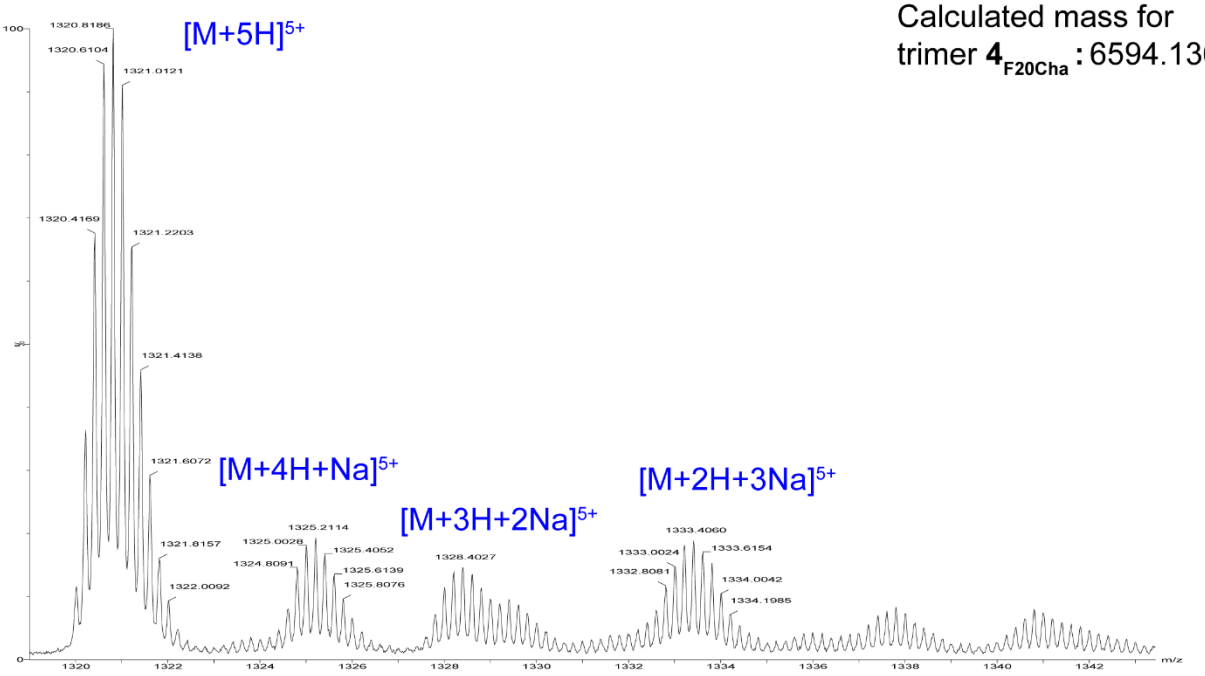




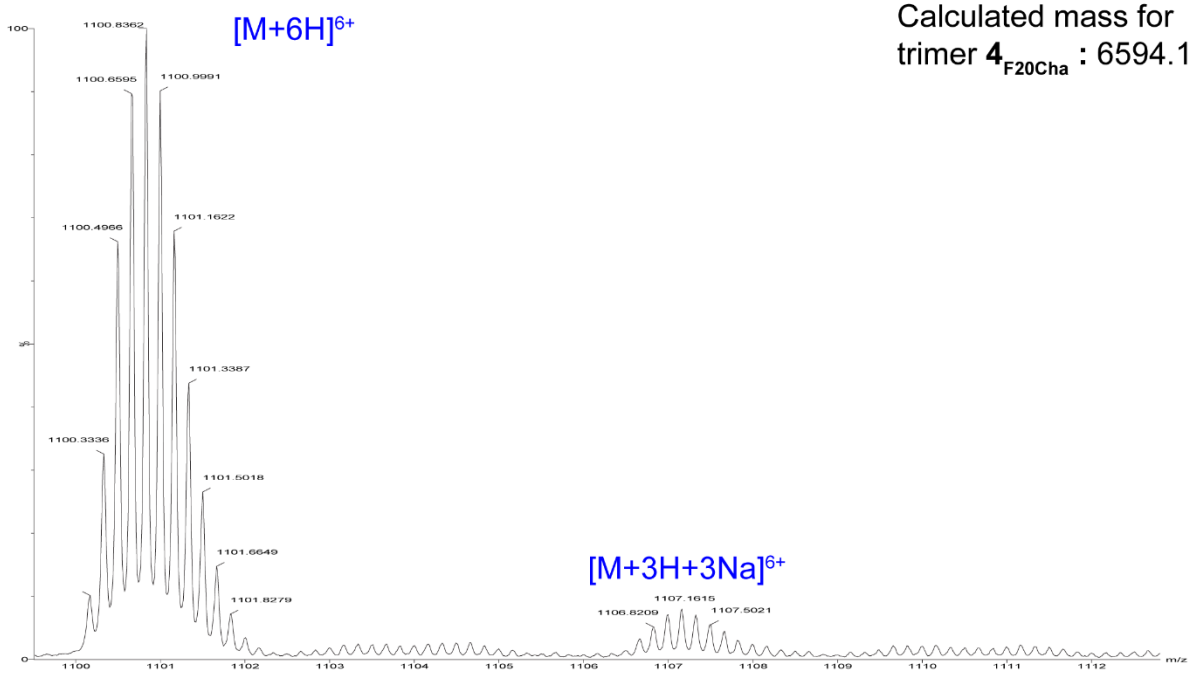
Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362



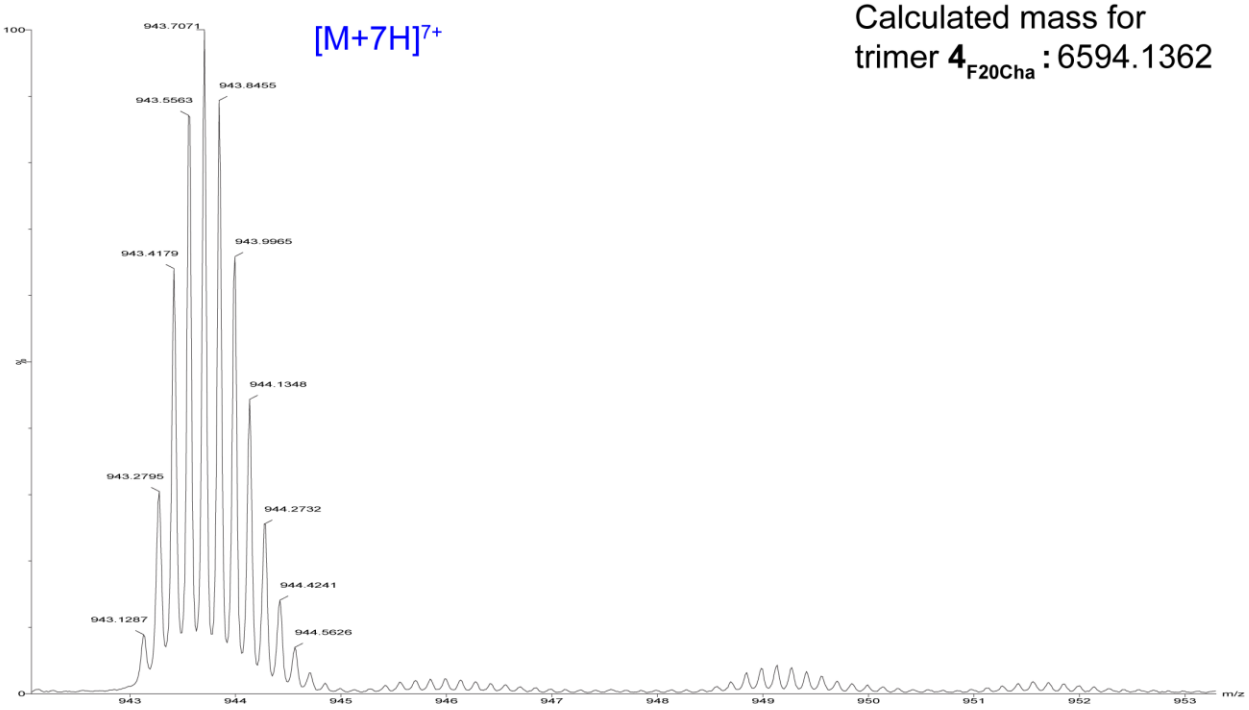
Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362



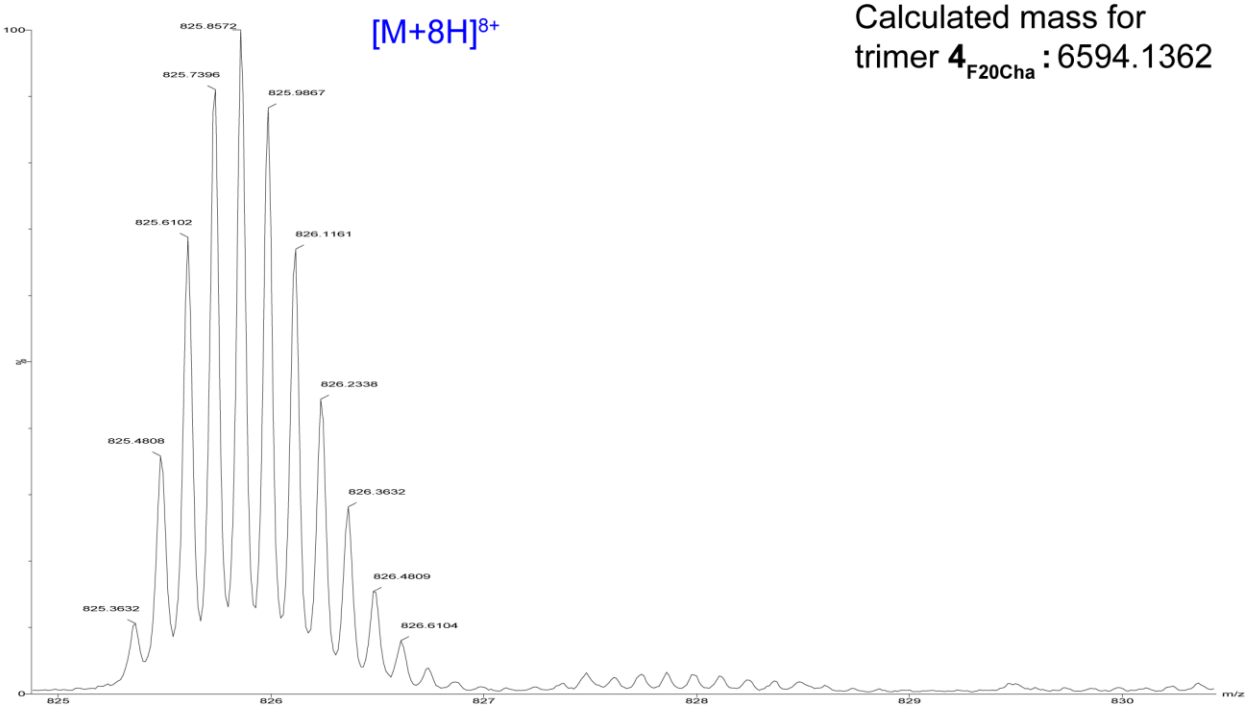
Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362



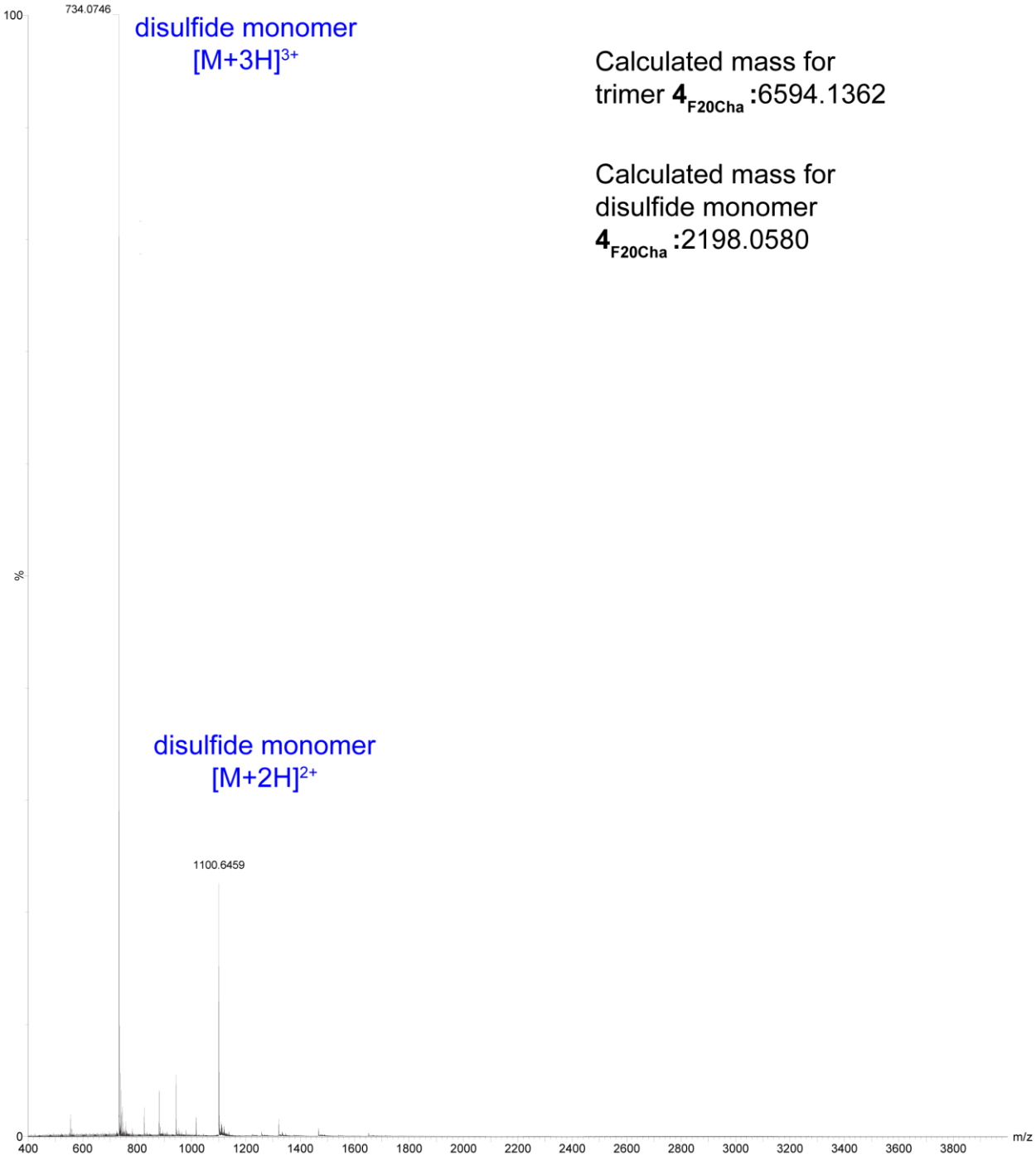
Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362

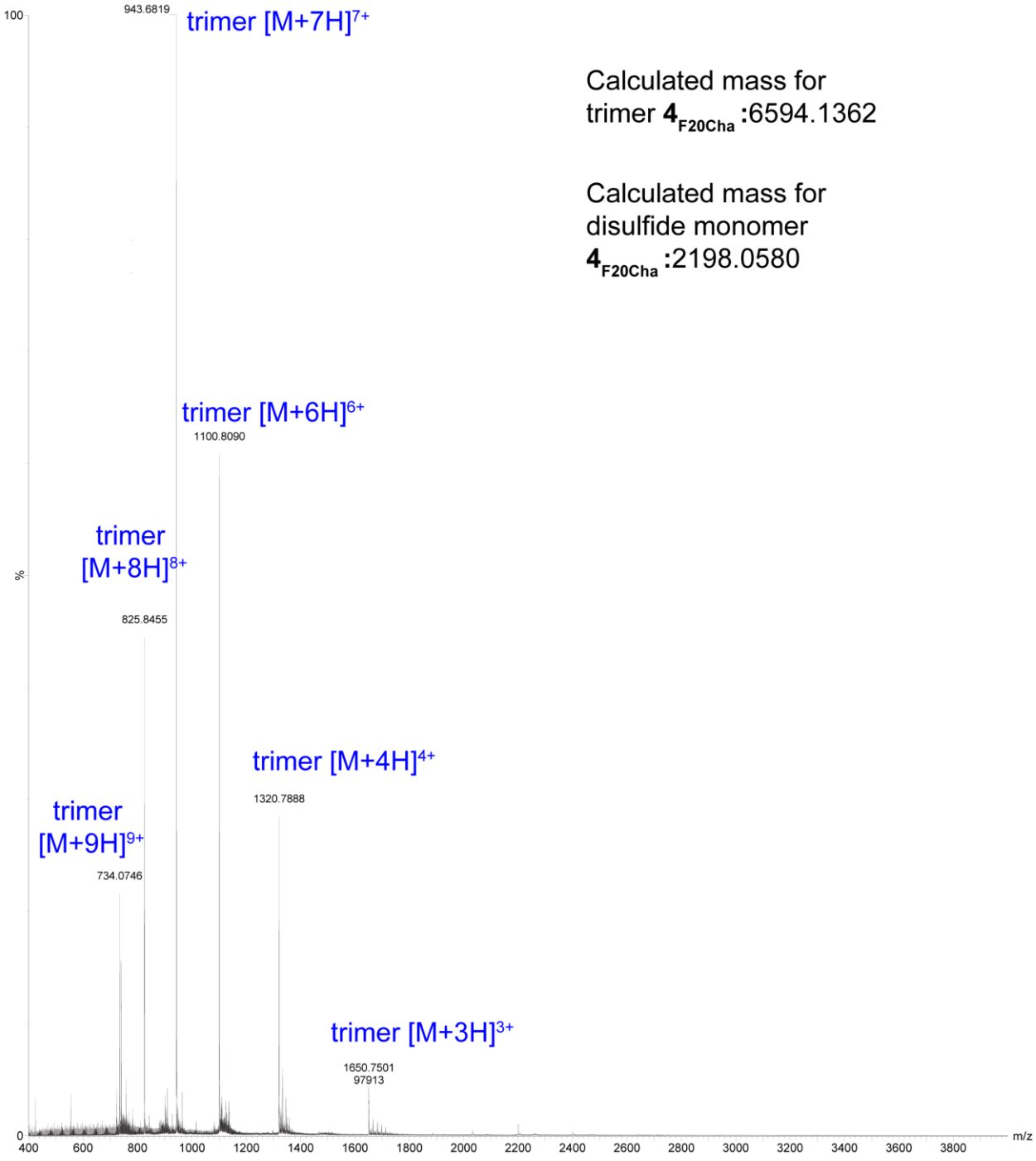


Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362



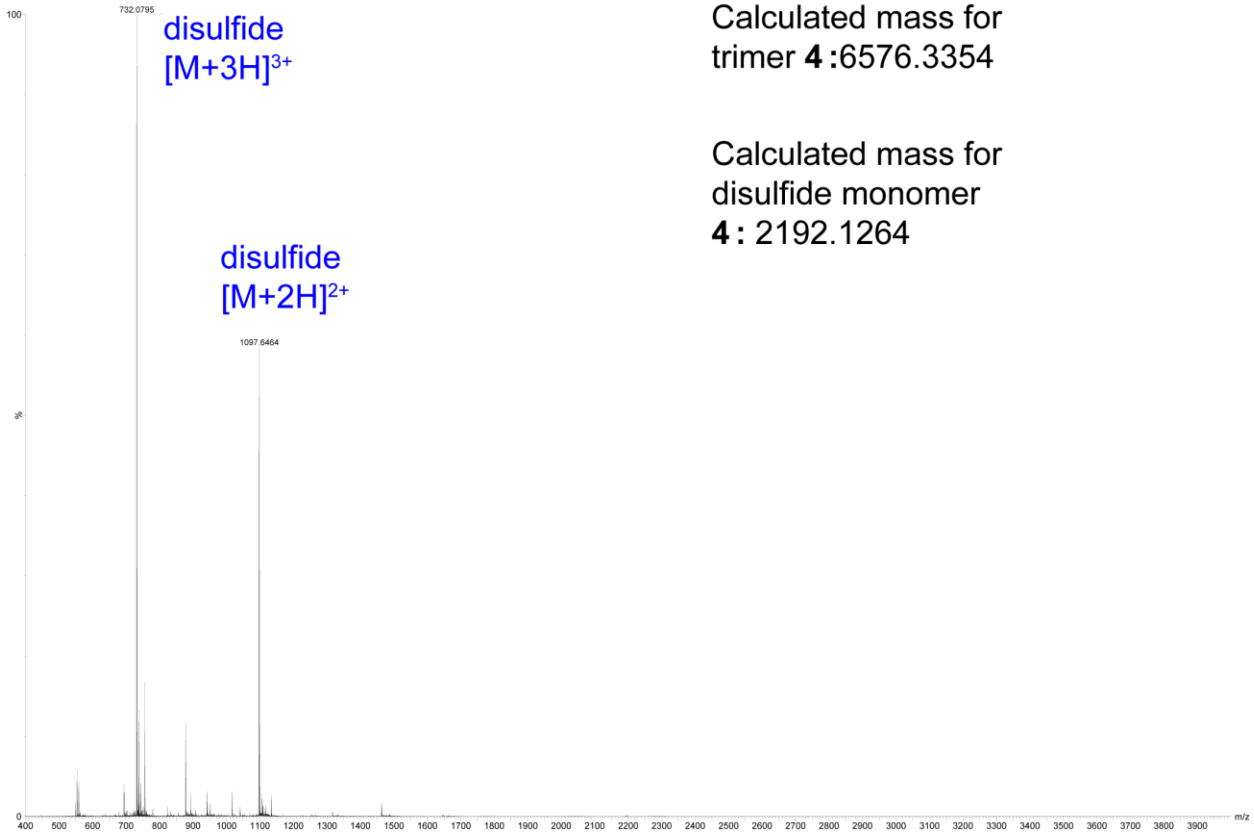
Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$: 6594.1362


734.0746
disulfide monomer
[M+3H]3+
Calculated mass for
trimer 4F20Cha :6594.1362
Calculated mass for
disulfide monomer
4F20Cha :2198.0580
disulfide monomer
[M+2H]2+
1100.6459
100
%
0
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
m/z



Calculated mass for trimer $\mathbf{4}_{\mathbf{F20Cha}}$:6594.1362

Calculated mass for disulfide monomer $\mathbf{4}_{\mathbf{F20Cha}}$:2198.0580



Calculated mass for trimer **4** :6576.3354

Calculated mass for disulfide monomer **4** : 2192.1264

Calculated mass for trimer **4** :6576.3354

Calculated mass for disulfide monomer **4** : 2192.1264