Probing Aromatic, Hydrophobic, and Steric Effects on the Self-Assembly of an Amyloid-β Fragment Peptide

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Figure S1. Reverse sedimentation data for solubilization of peptide monomers from resuspended fibrils derived from Aβ(16–22) variants indicating increase in monomer concentration over time.

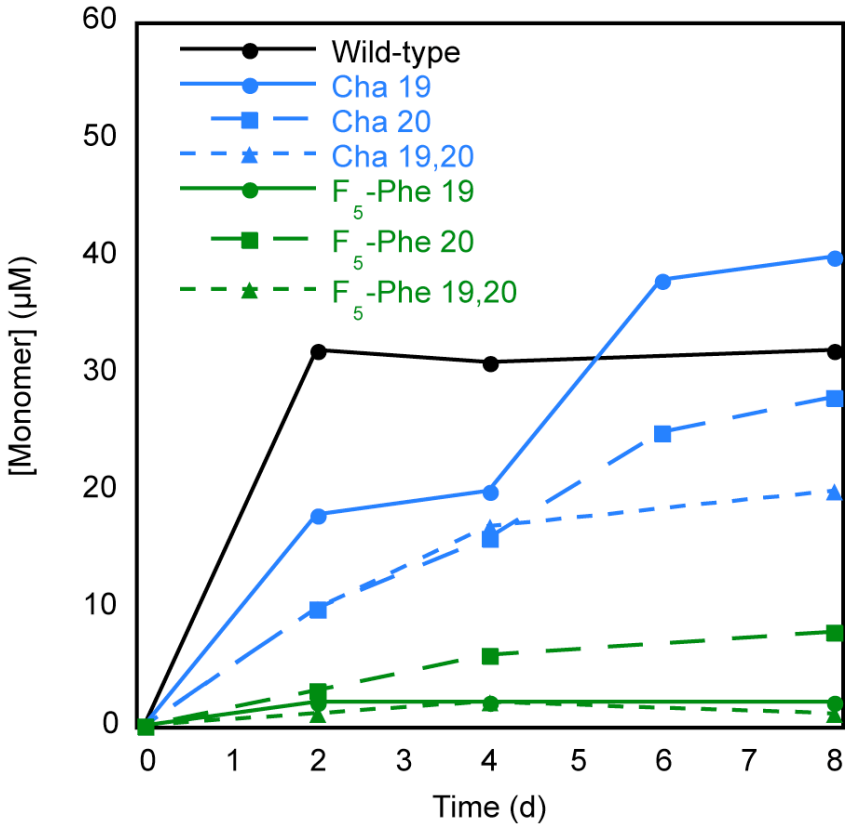


Figure S2. HPLC trace (215 nm) of Aβ(16–22) Wild-type.

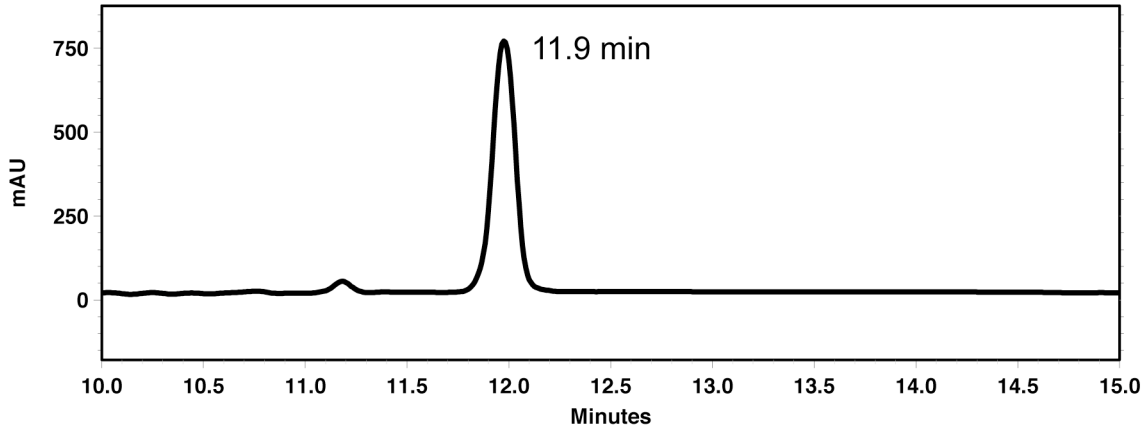


Figure S3. HPLC trace (215 nm) of Aβ(16–22) F_5-Phe 19.

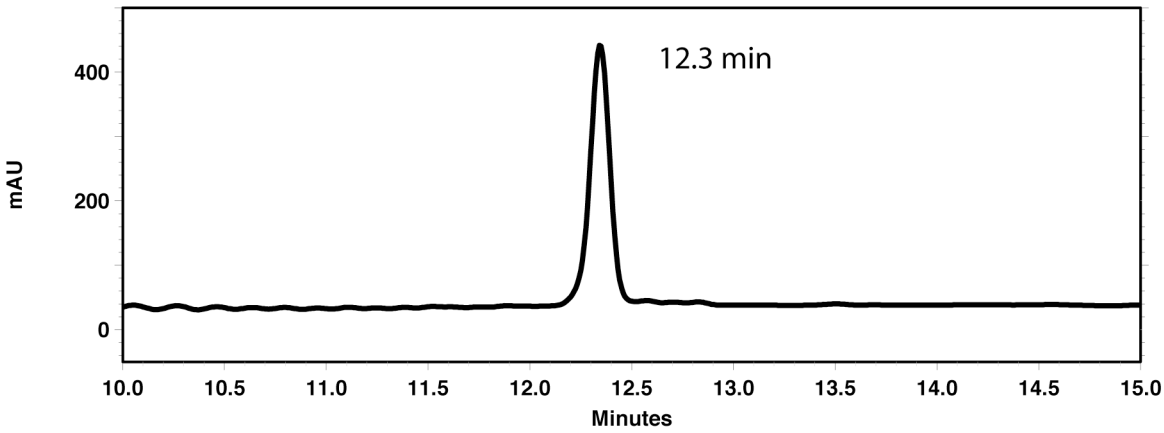


Figure S4. HPLC trace (215 nm) of Aβ(16–22) Cha 19.

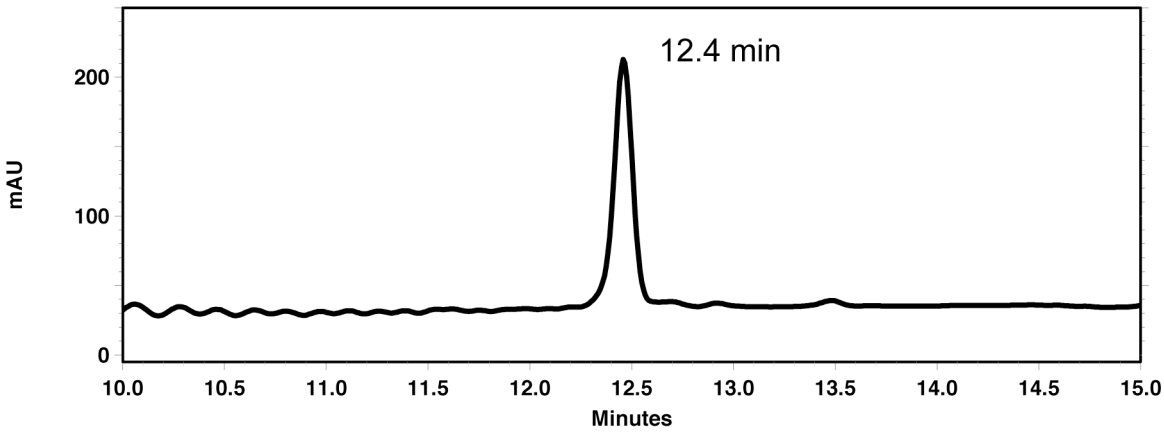


Figure S5. HPLC trace (215 nm) of Aβ(16–22) Ala 19.

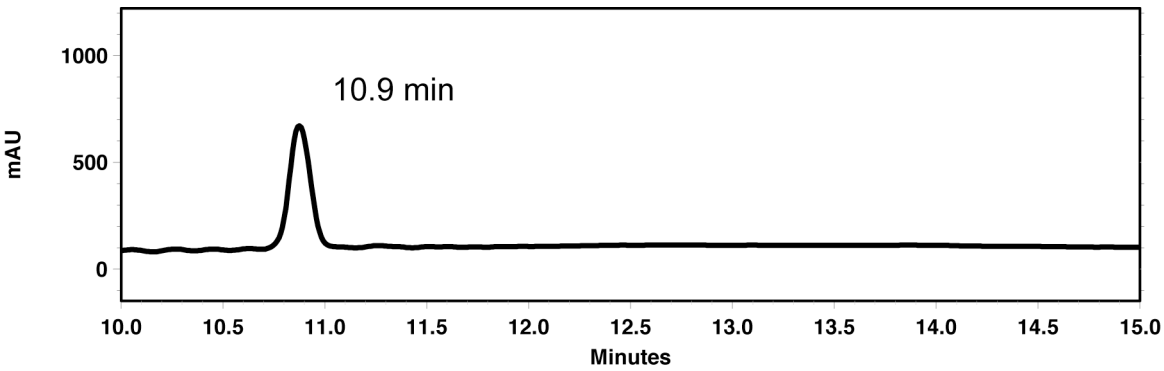


Figure S6. HPLC trace (215 nm) of Aβ(16–22) Tyr 19.

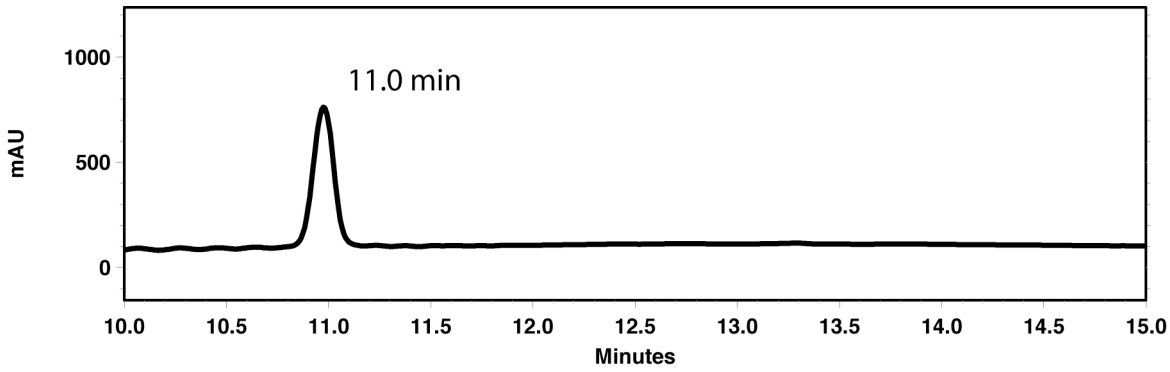


Figure S7. HPLC trace (215 nm) of Aβ(16–22) F_5-Phe 20.

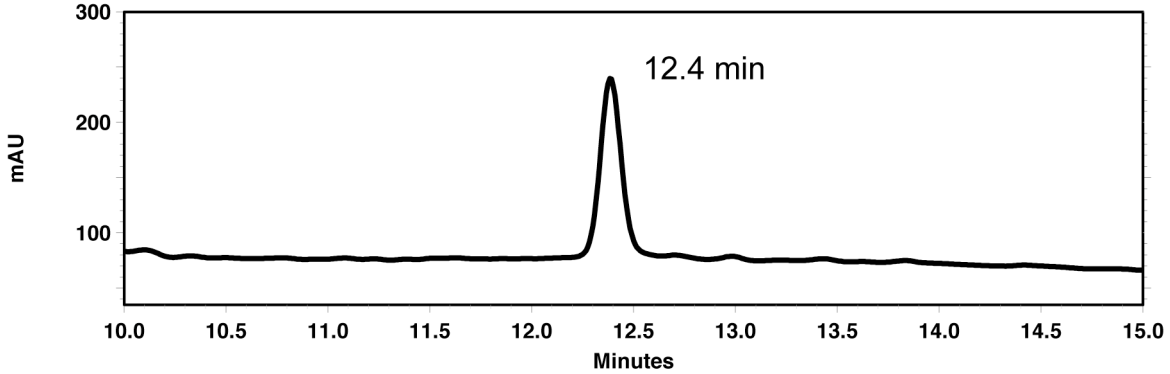


Figure S8. HPLC trace (215 nm) of Aβ(16–22) Cha 20.

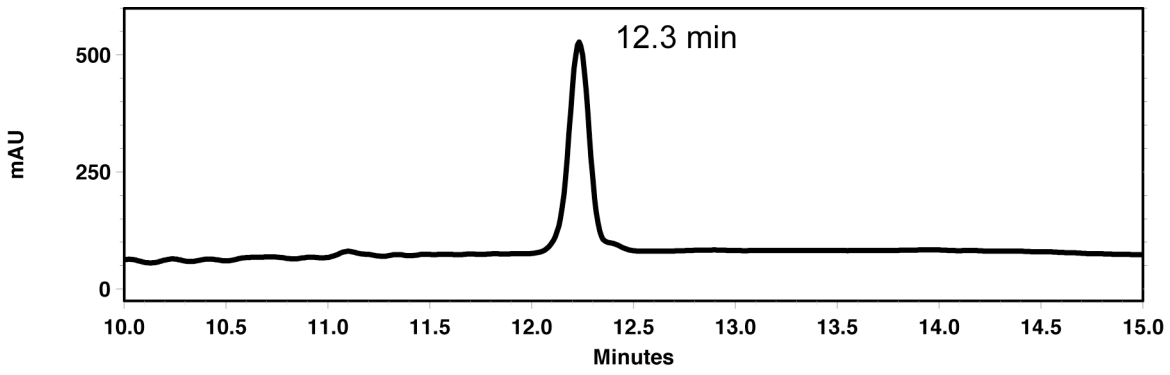


Figure S9. HPLC trace (215 nm) of Aβ(16–22) F_5-Phe 19,20.

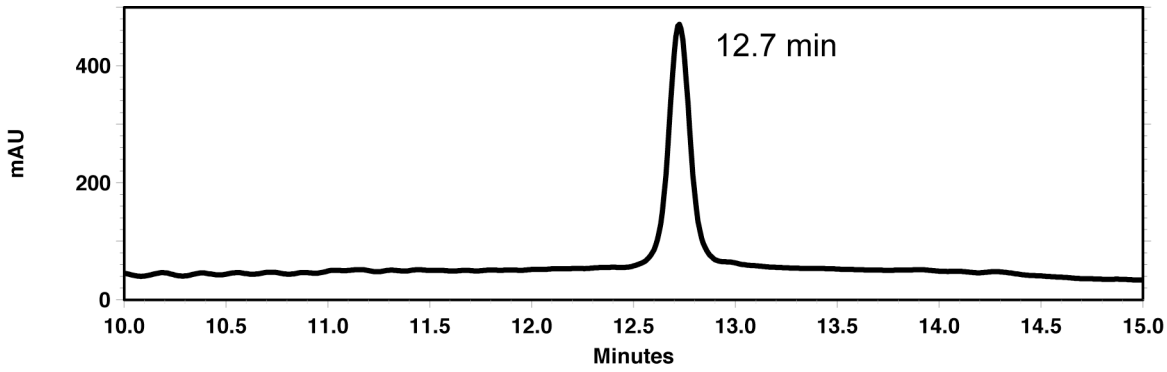


Figure S10. HPLC trace (215 nm) of Aβ(16–22) Cha 19,20.

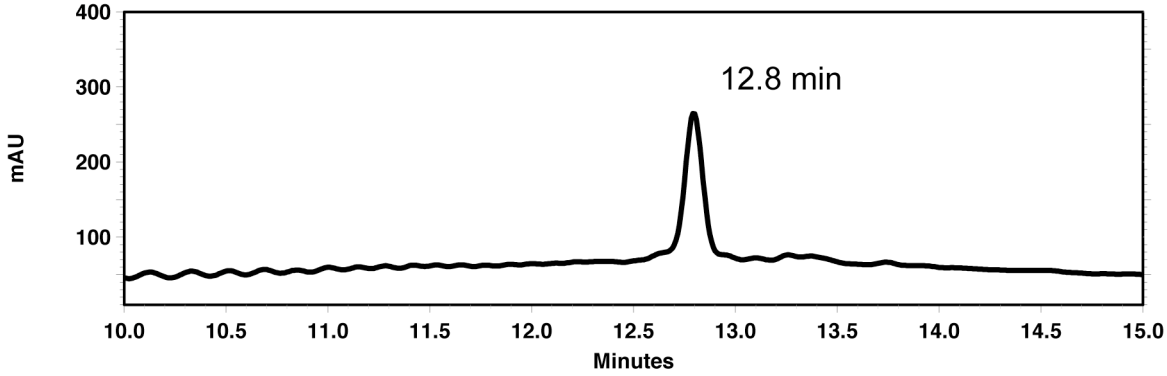


Table S1. Analytical HPLC conditions for peptides **1-9**.

Peptide	**Sequence**	**Retention time** (min)	**Gradient** (solution A: water/0.5% TFA; solution B: acetonitrile/0.5% TFA)
1	Ac-KLVFFAE-NH_2	11.9	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
2	Ac-KLVAFAE-NH_2	10.9	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
3	Ac-KLVAFAE-NH_2	11.0	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
4	Ac-KLV(F_5-Phe)FAE-NH_2	12.2	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
5	Ac-KLV(Cha)FAE-NH_2	12.4	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
6	Ac-KLVF(F_5-Phe)AE-NH_2	12.4	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
7	Ac-KLV(Cha)FAE-NH_2	12.3	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
8	Ac-KLV(F_5-Phe) (F_5-Phe)AE-NH_2	12.7	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes
9	Ac-KLV(Cha)(Cha)AE-NH_2	12.8	Isocratic 5% **B** over 5 minutes, increase 5-95% **B** over 10 minutes, maintain at 95% **B** over 5 minutes

Figure S11. HPLC trace for co-injection of peptides **1-5**.

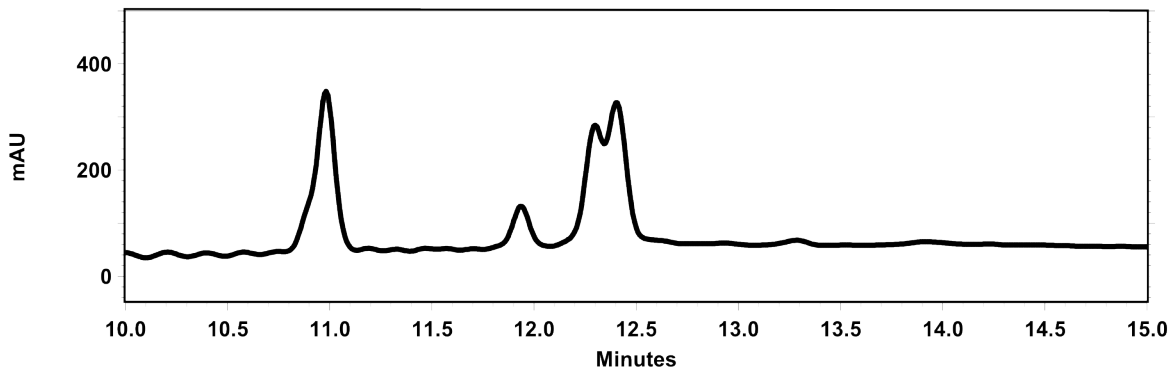


Table S2. MALDI mass spectra characterization of each peak from **Figure S10**.

Peak #	Retention time	Mass (observed)	Peptide
1	10.9-11	817.32, 910.01	Ala 19, Tyr 19
2	11.9	893.06	Wild-type
3	12.2	983.00	F_5-Phe 19
4	12.4	899.14	Cha 19

Figure S12. HPLC trace for co-injection of peptides **1** & **6-9.**

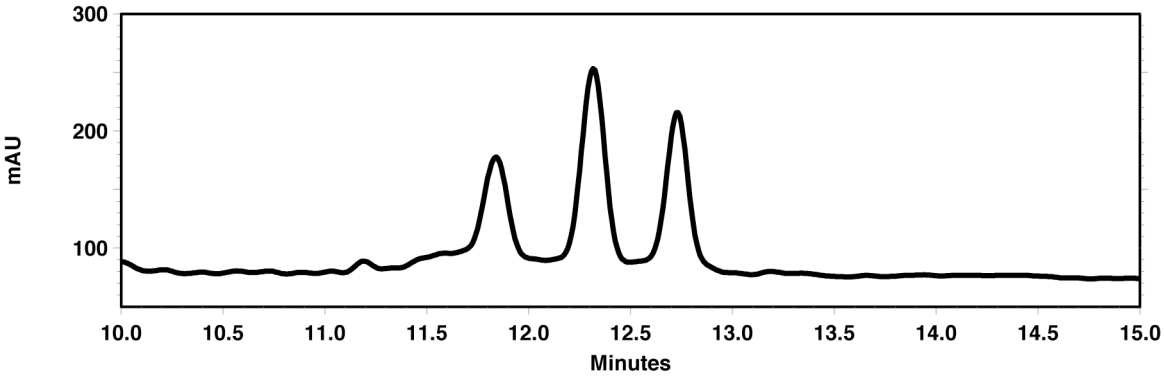


Table S3. MALDI mass spectra characterization of each peak from **Figure S11**.

Peak #	Retention time	Mass (observed)	Peptide
1	11.9	894.52	Wild-type
2	12.4	900.58, 984.50	Cha 20, F_5-Phe 20
3	12.8	906.58, 1096.40	Cha 19,20, F_5-Phe 19,20

Figure S13. ESI mass spectrum of Aβ(16–22) Wild-type.

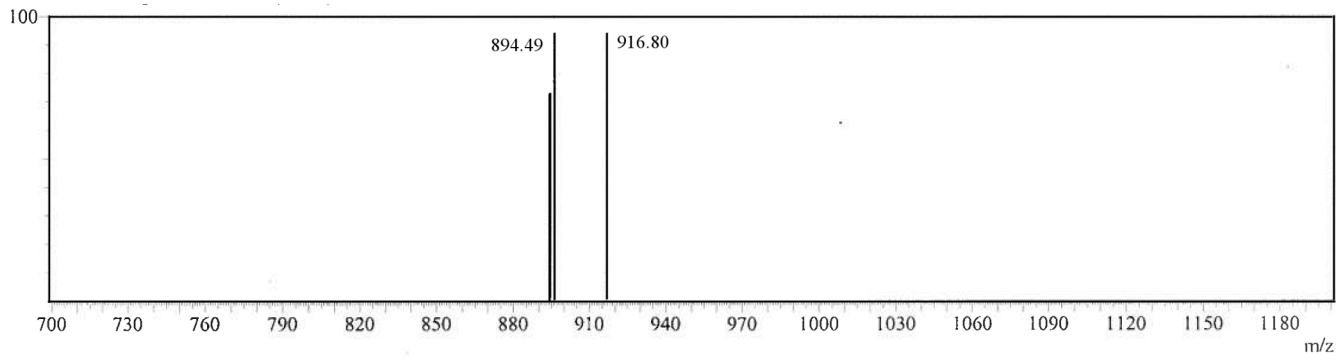


Figure S14. ESI mass spectrum of Aβ(16–22) F_5-Phe 19.

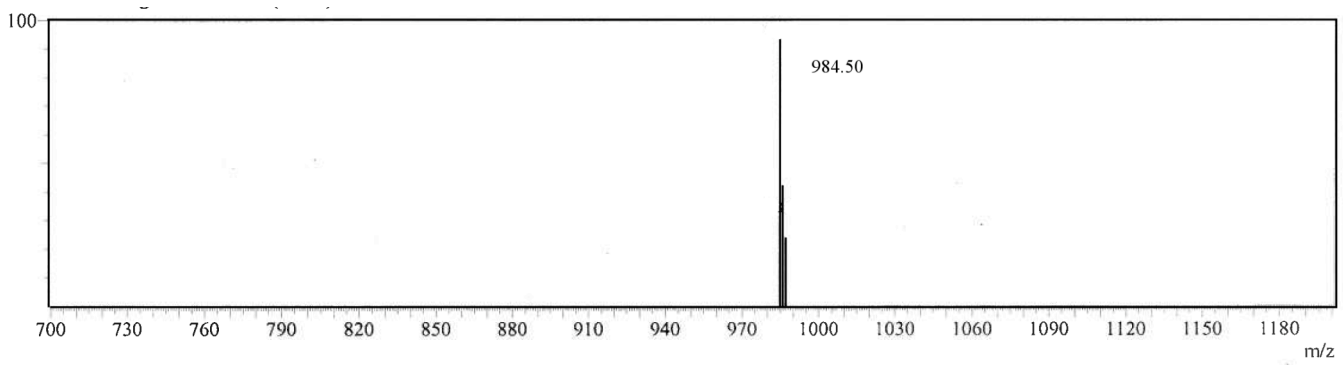


Figure S15. ESI mass spectrum of Aβ(16–22) Cha 19.

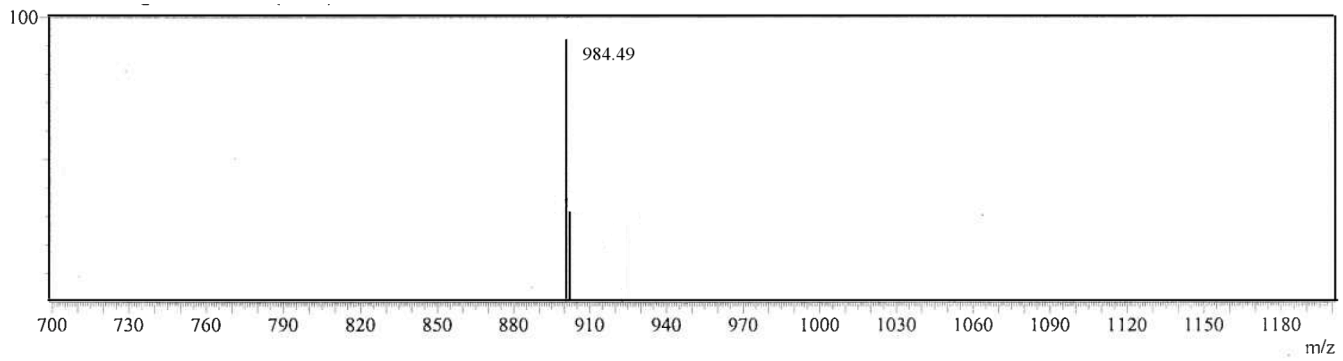


Figure S16. ESI mass spectrum of Aβ(16–22) Ala 19.

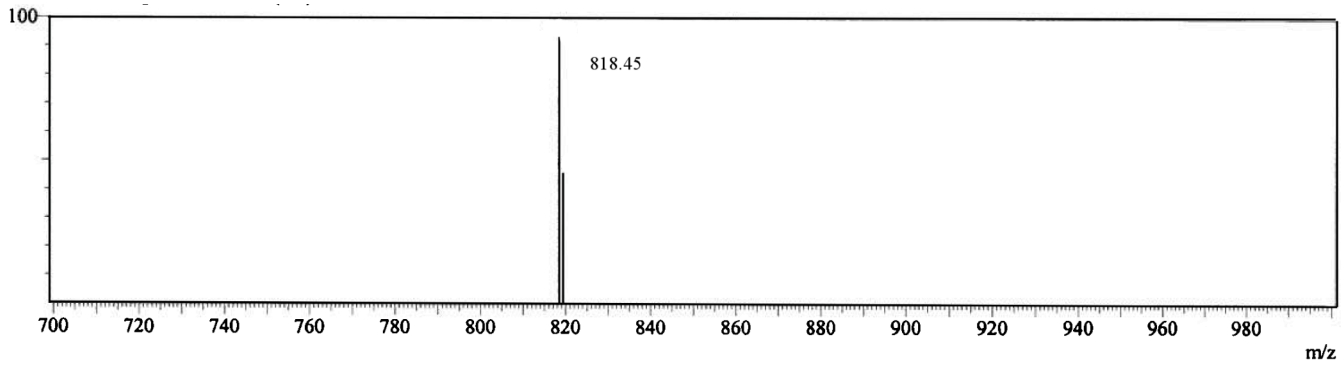


Figure S17. ESI mass spectrum of Aβ(16–22) Tyr 19.

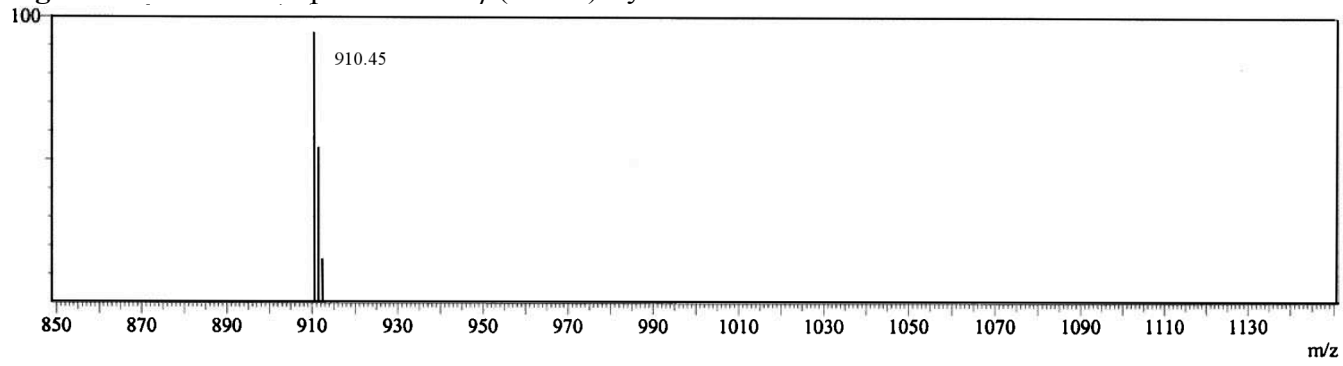


Figure S18. ESI mass spectrum of Aβ(16–22) Wild-type ([1-^{13}C] Leu17, Ala21).

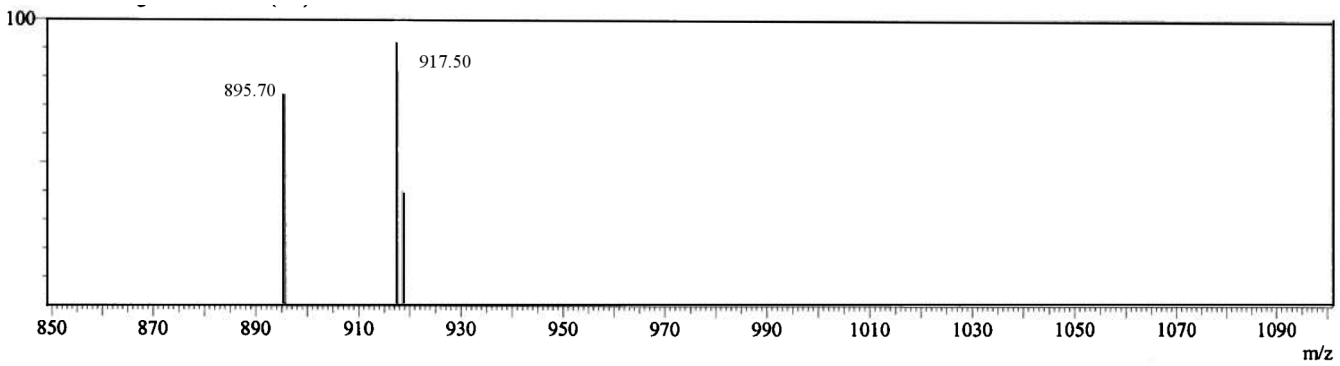


Figure S19. ESI mass spectrum of Aβ(16–22) F_5-Phe 19 ([1-^{13}C] Leu17, Ala21).

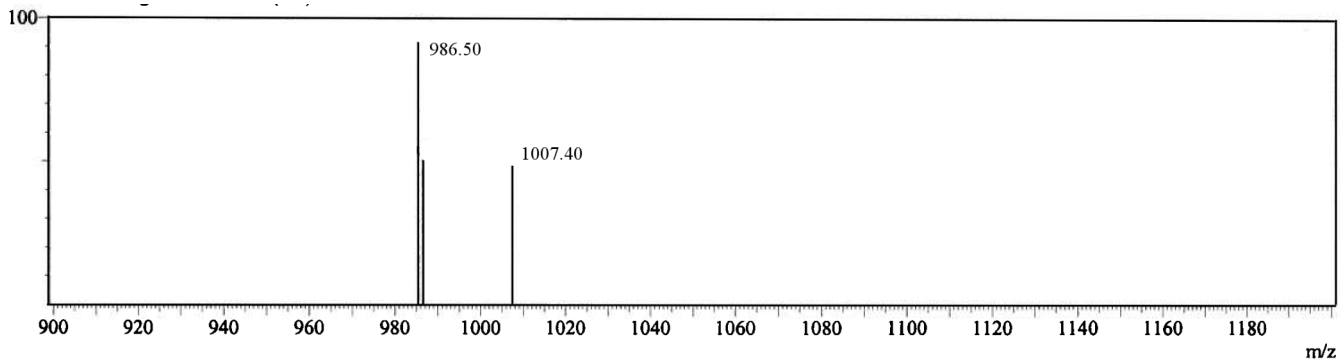


Figure S20. ESI mass spectrum of Aβ(16–22) Cha 19 ([1-^{13}C] Leu17, Ala21).

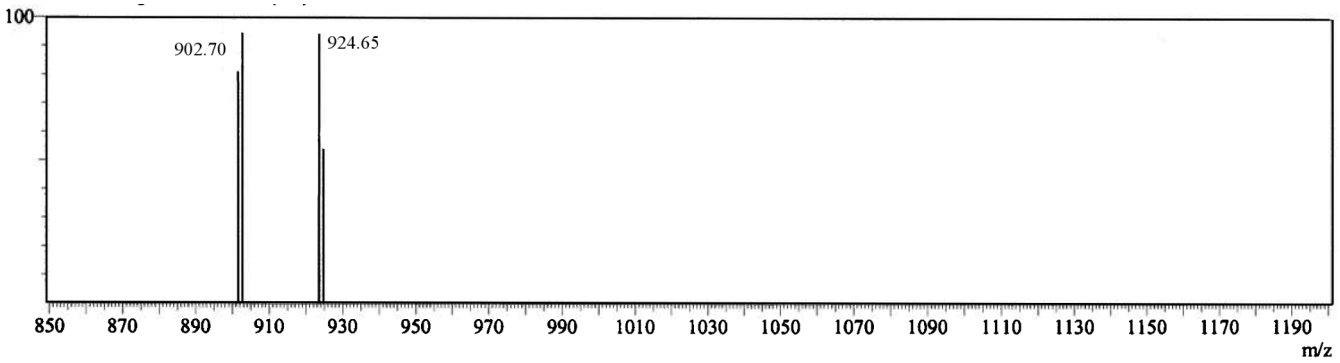


Figure S21. ESI mass spectrum of Aβ(16–22) Wild-type ([1-^{13}C] Leu17, Phe20).

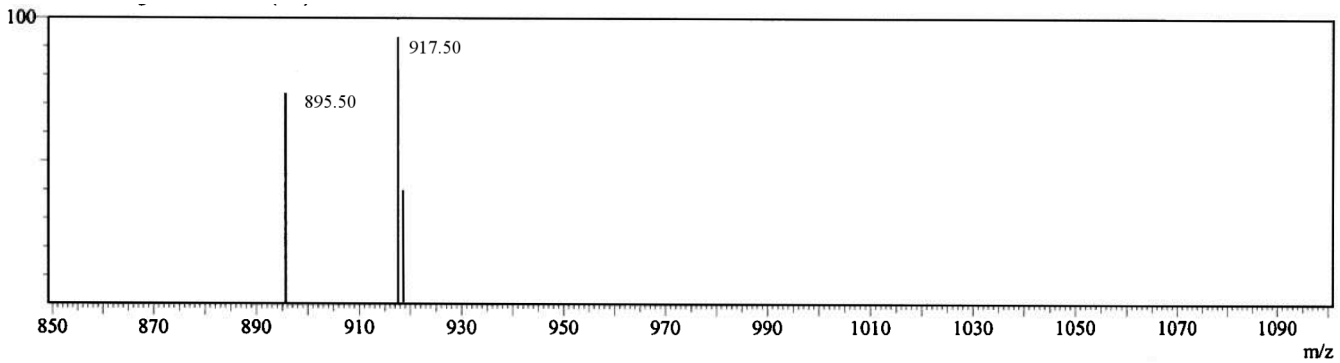


Figure S22. ESI mass spectrum of Aβ(16–22) F_5-Phe 19 ([1-^{13}C] Leu17, Phe20).

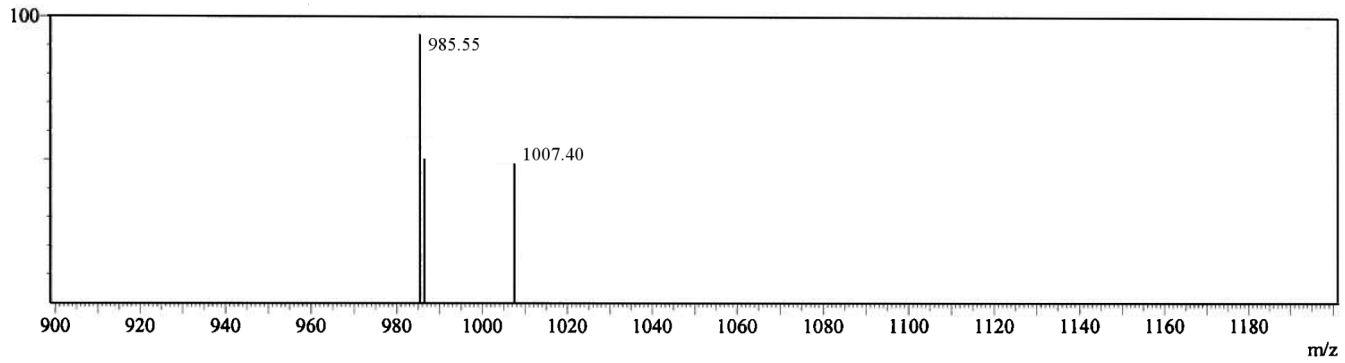


Figure S23. ESI mass spectrum of Aβ(16–22) Cha 19 ([1-^{13}C] Leu17, Phe20).

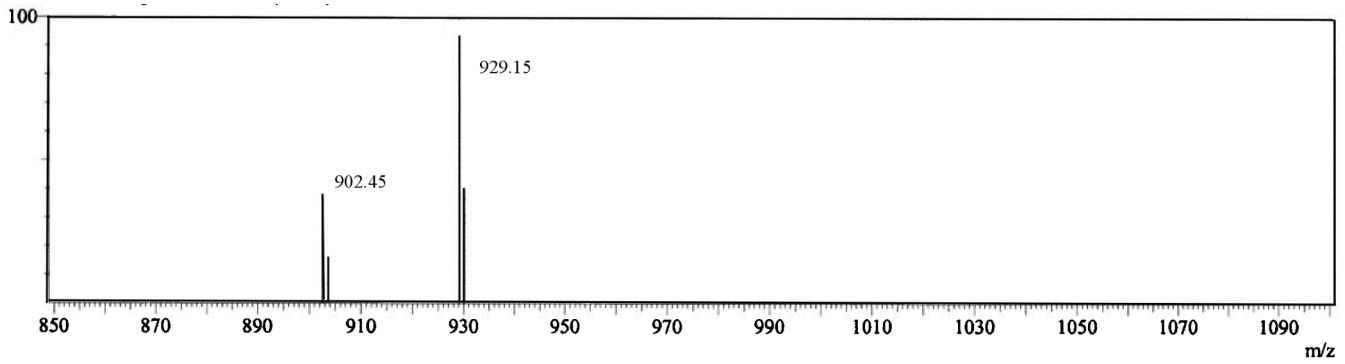


Figure S24. MALDI mass spectrum of Aβ(16–22) F_5-Phe 20.

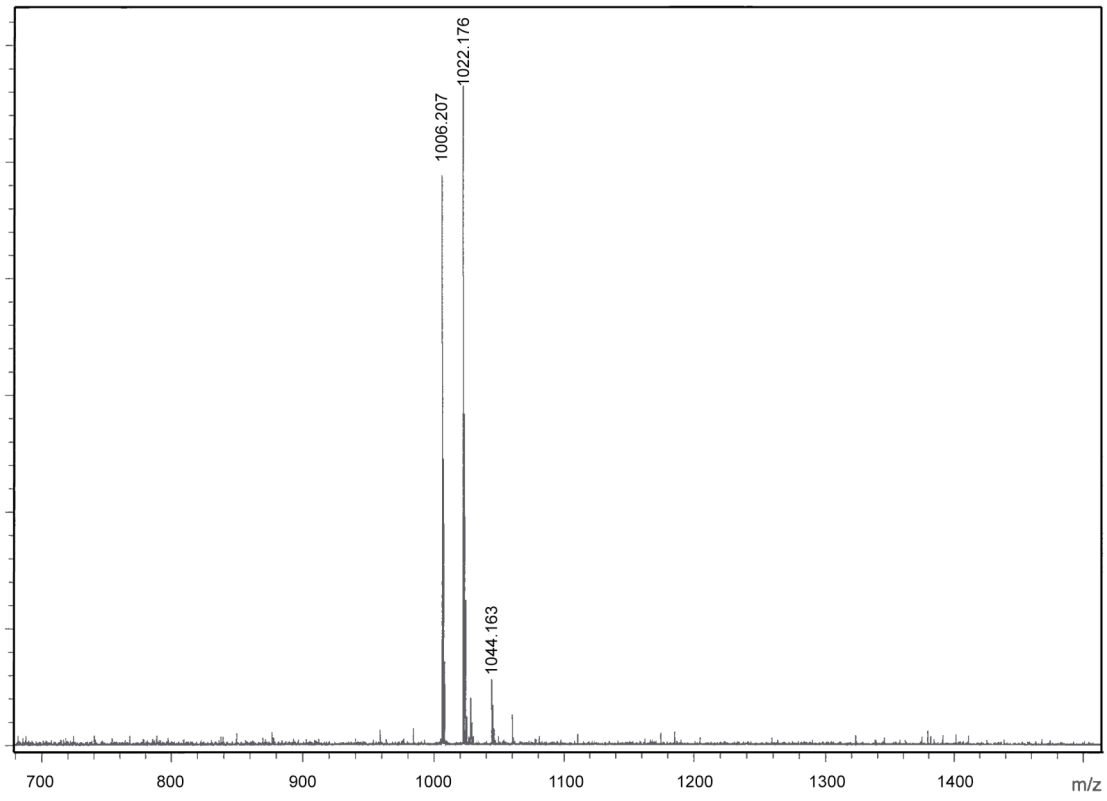


Figure S25. MALDI mass spectrum of Aβ(16–22) Cha 20.

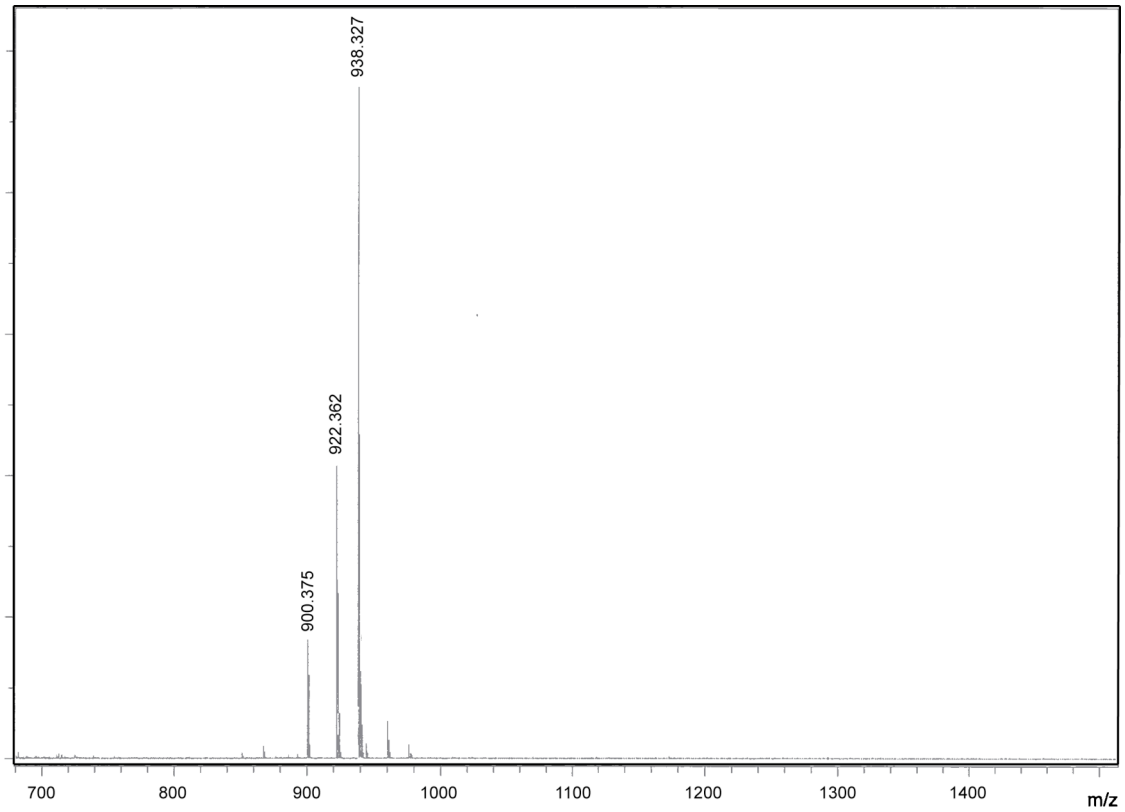


Figure S26. MALDI mass spectrum of Aβ(16–22) F_5-Phe 20 ([1-^{13}C] Lys16, Ala21).

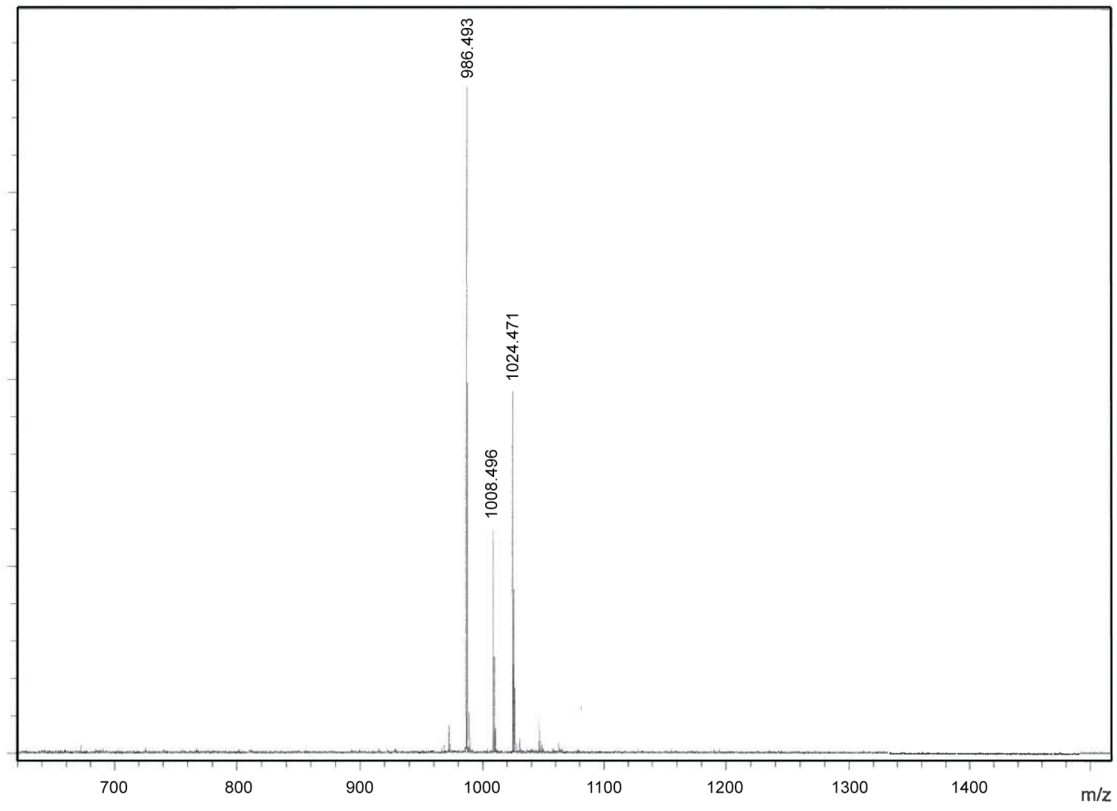


Figure S27. MALDI mass spectrum of Aβ(16–22) Cha 20 ([1-^{13}C] Lys16, Ala21).

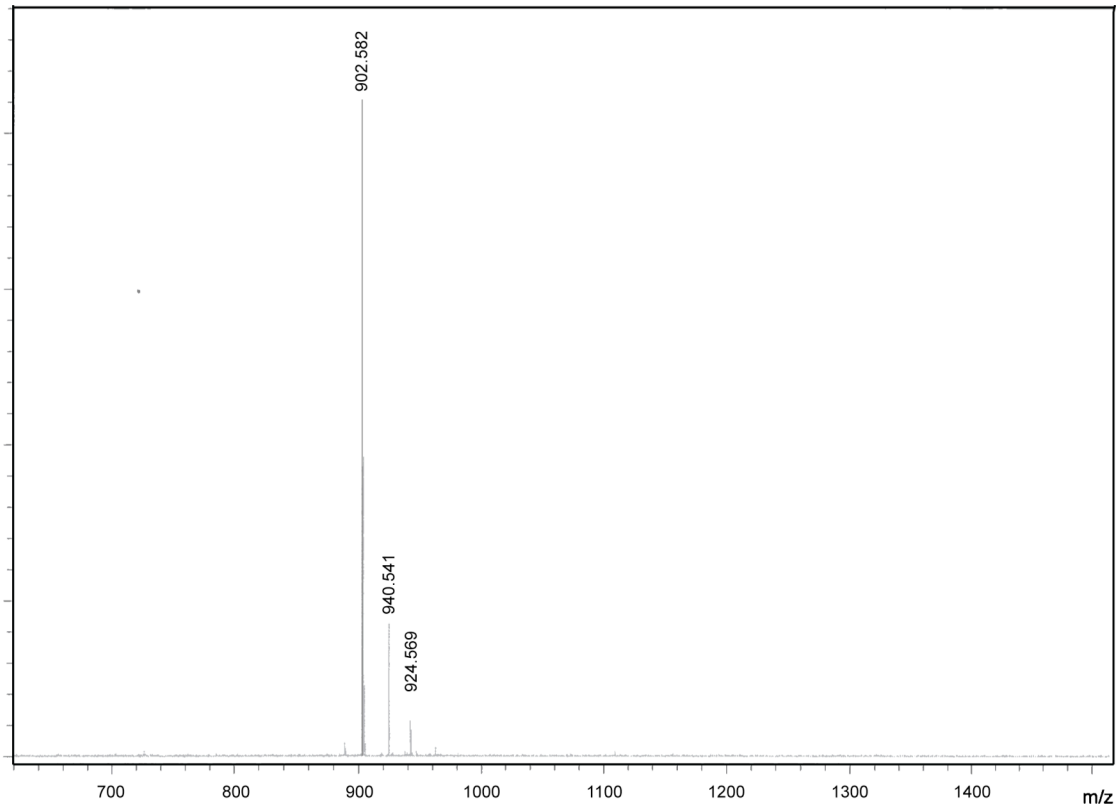


Figure S28. MALDI mass spectrum of Aβ(16–22) F_5-Phe 19,20.

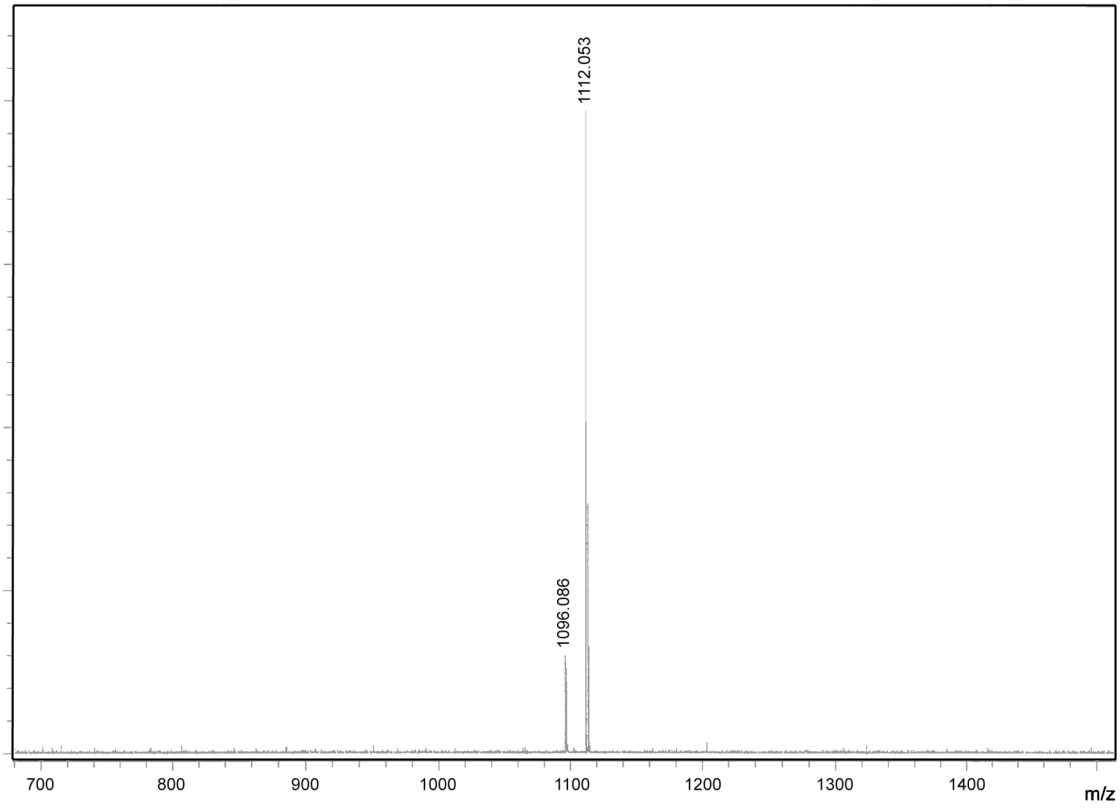


Figure S29. MALDI mass spectrum of Aβ(16–22) Cha 19,20.

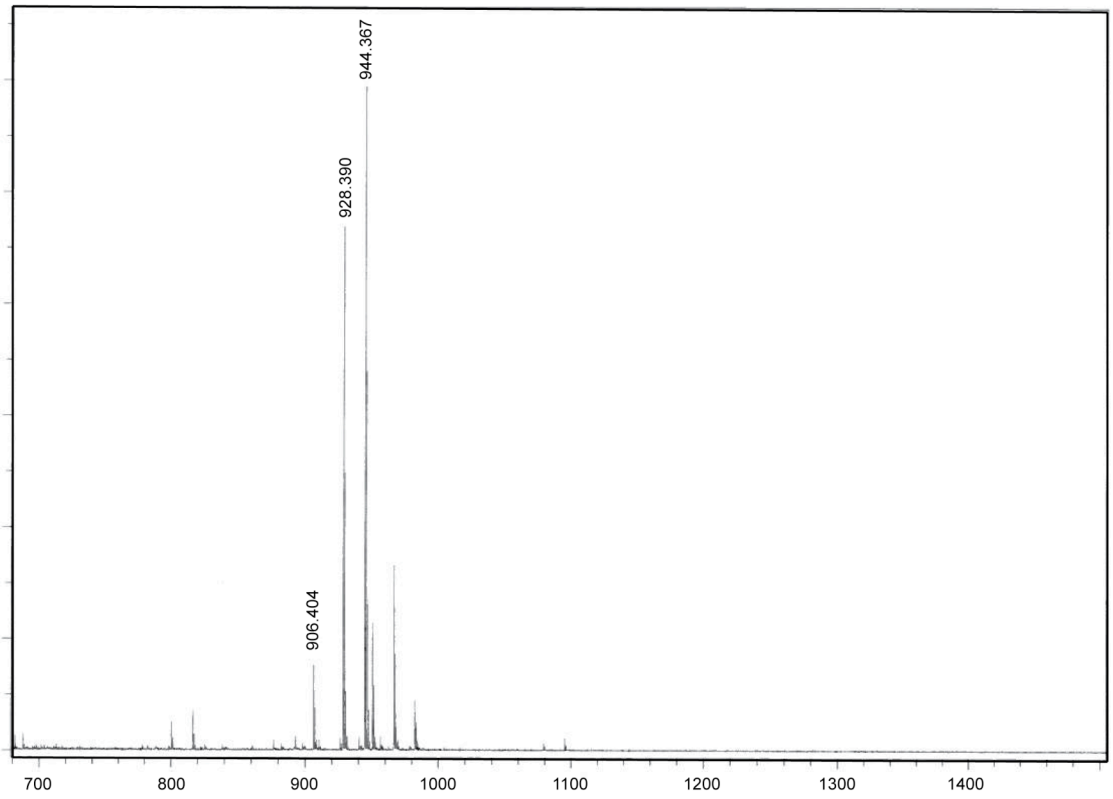


Figure S30. MALDI mass spectrum of Aβ(16–22) F_5-Phe 19,20 ([1-^{13}C] Lys16, Ala21).

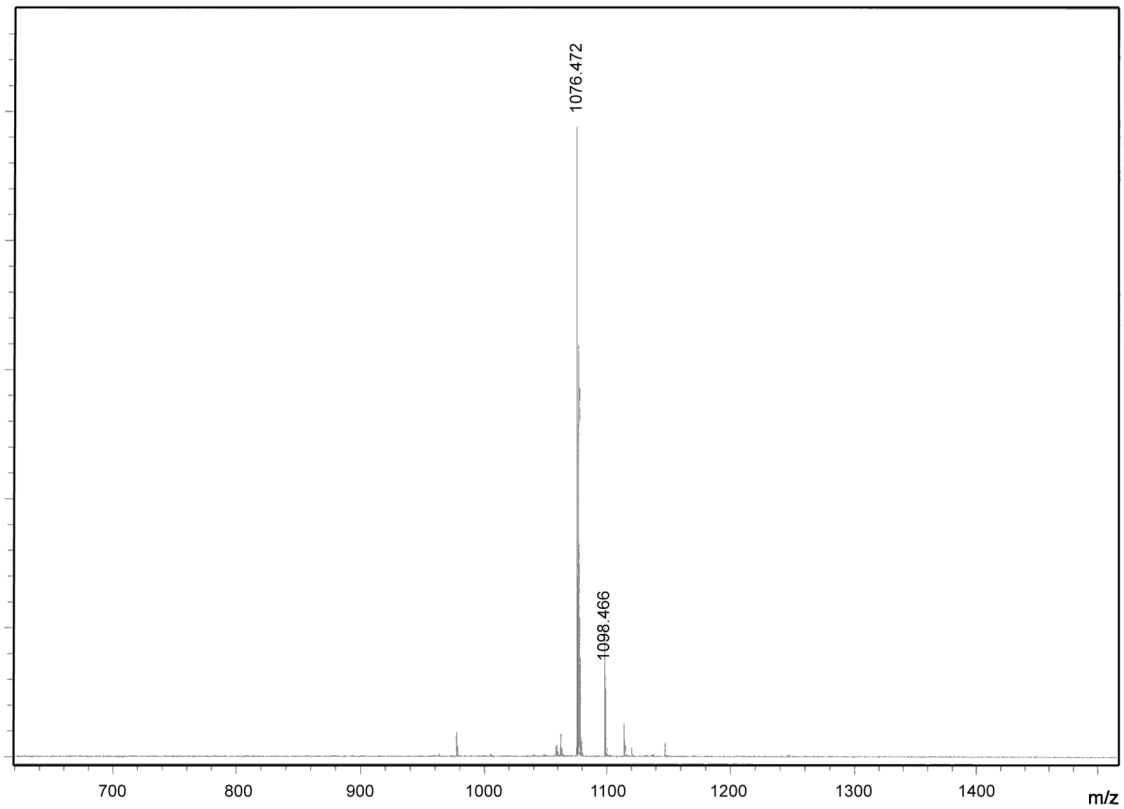


Figure S31. MALDI mass spectrum of Aβ(16–22) Cha 19,20 ([1-^{13}C] Lys16, Ala21).

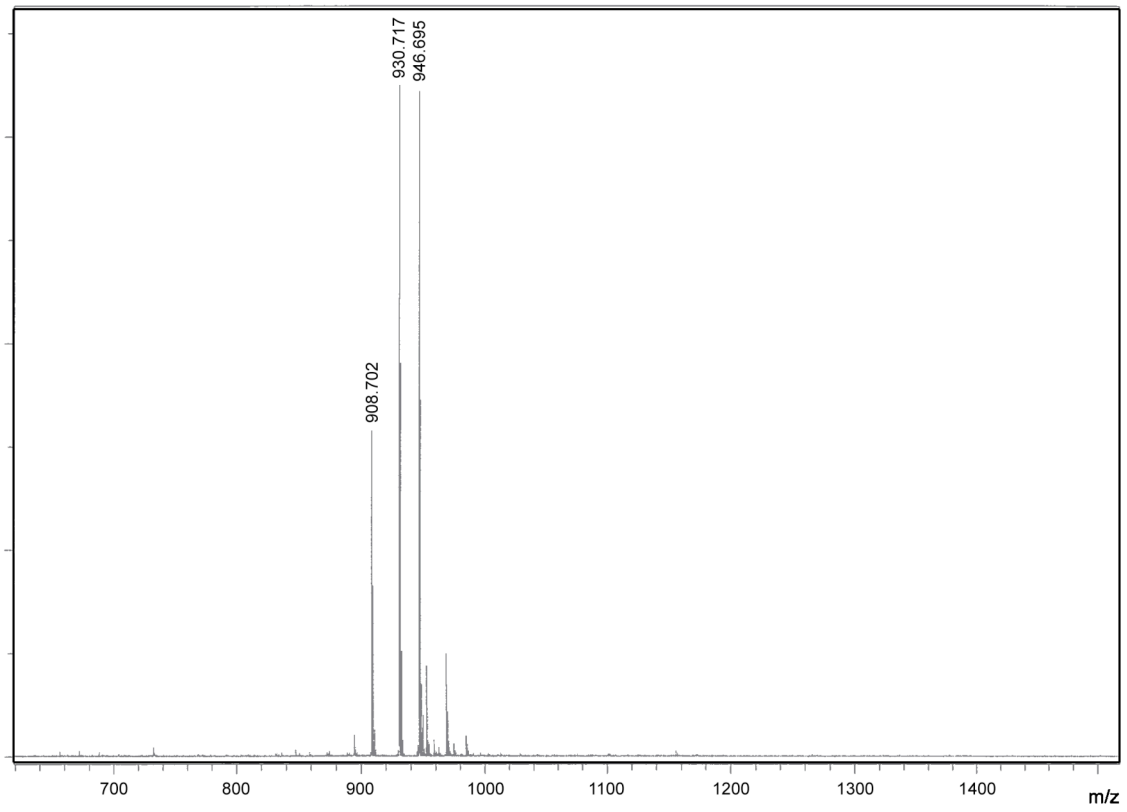


Table S4. Calculated and observed *m/z* for peptides **1–11**.

Peptide	Sequence	Calculated	Observed
1	Ac-KLVFFAE-NH_2	894.50 (M^+H)	894.49
2	Ac-KLV(F_5-Phe)FAE-NH_2	984.45 (M^+H)	984.50
3	Ac-KLV(Cha)FAE-NH_2	900.55 (M^+H)	900.70
4	Ac-KLVAFAE-NH_2	818.46 (M^+H)	818.45
5	Ac-KLVYFAE-NH_2	910.49 (M^+H)	910.45
6	Ac-K*LVFF*AE-NH_2	895.60 (M^+)	895.70
7	Ac-K*LV(F_5-Phe)F*AE-NH_2	986.45 (M^+H)	986.50
8	Ac-K*LV(Cha)F*AE-NH_2	902.55 (M^+H)	902.70
9	Ac-K*LVF*FAE-NH_2	895.60 (M^+)	895.50
10	Ac-K*LV(F_5-Phe)*FAE-NH_2	985.45 (M^+)	985.55
11	Ac-K*LV(Cha)*FAE-NH_2	902.55 (M^+H)	902.45
12	Ac-KLVF(F_5-Phe)AE-NH_2	1006.44 (M^+H)	1006.21
13	Ac-KLVF(Cha)AE-NH_2	900.55 (M^+H)	900.38
14	Ac-*KLVF(F_5-Phe)*AE-NH_2	986.45 (M^+H)	986.49
15	Ac-*KLVF(Cha)*AE-NH_2	902.55 (M^+H)	902.58
16	Ac-KLV(F_5-Phe) (F_5-Phe)AE-NH_2	906.60 (M^+H)	906.40
17	Ac-KLV(Cha) (Cha)AE-NH_2	1096.39 (M^+Na)	1096.09
18	Ac-*KLV(F_5-Phe) (F_5-Phe)*AE-NH_2	908.60 (M^+H)	908.70
19	Ac-*KLV(Cha)(Cha)*AE-NH_2	1076.50 (M^+H)	1076.47

* Indicates [1-^{13}C] label.

Figure S32. HPLC concentration curve for Aβ(16–22) Wild-type.

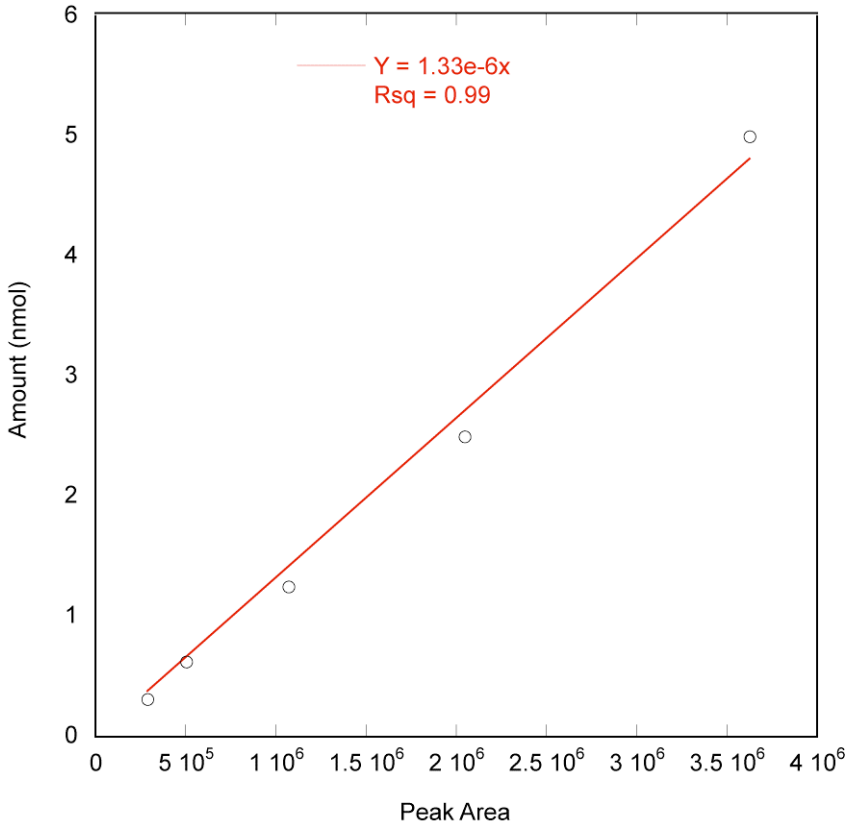


Figure S33. HPLC concentration curve Aβ(16–22) F_5-Phe 19 and F_5-Phe 20.

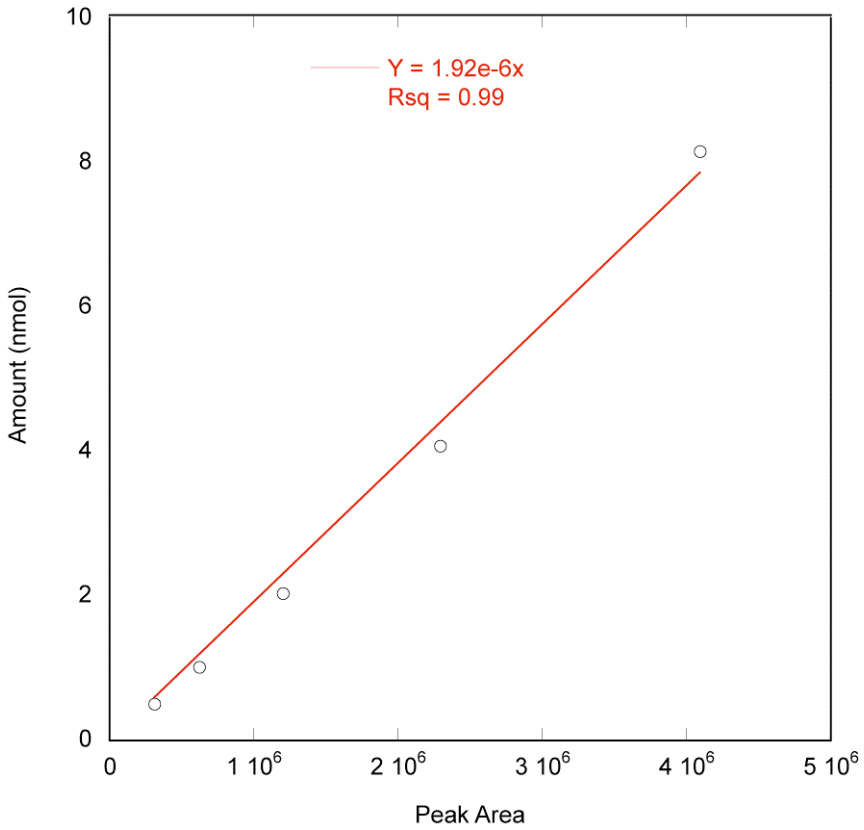


Figure S34. HPLC concentration curve Aβ(16–22) Cha 19 and Cha 20.

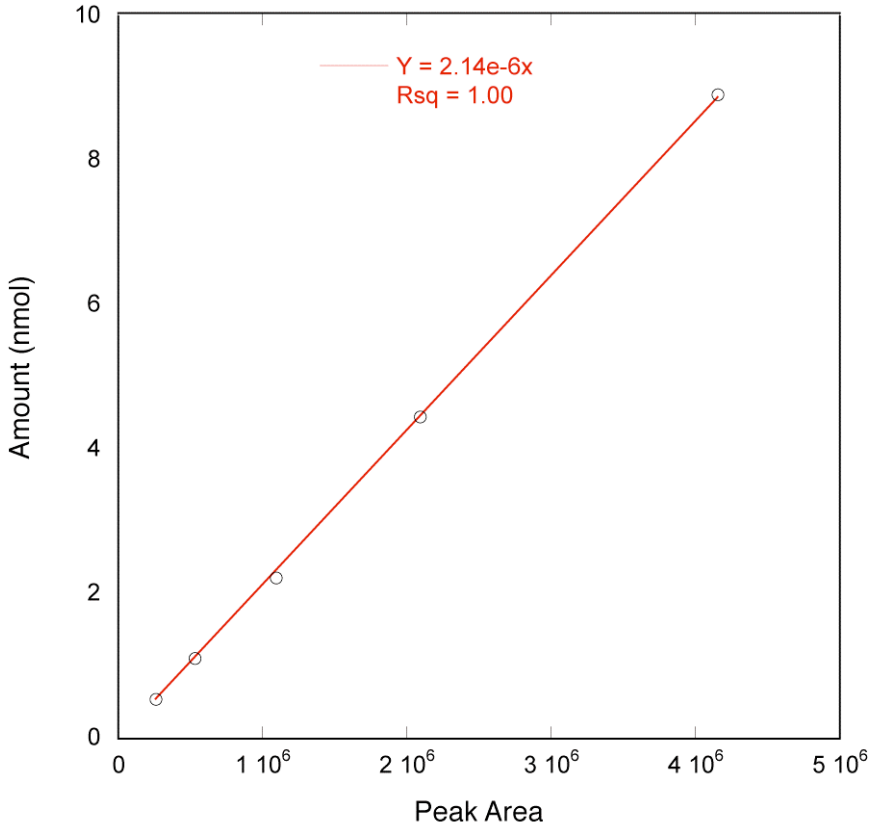


Figure S35. HPLC concentration curve Aβ(16–22) Ala 19.

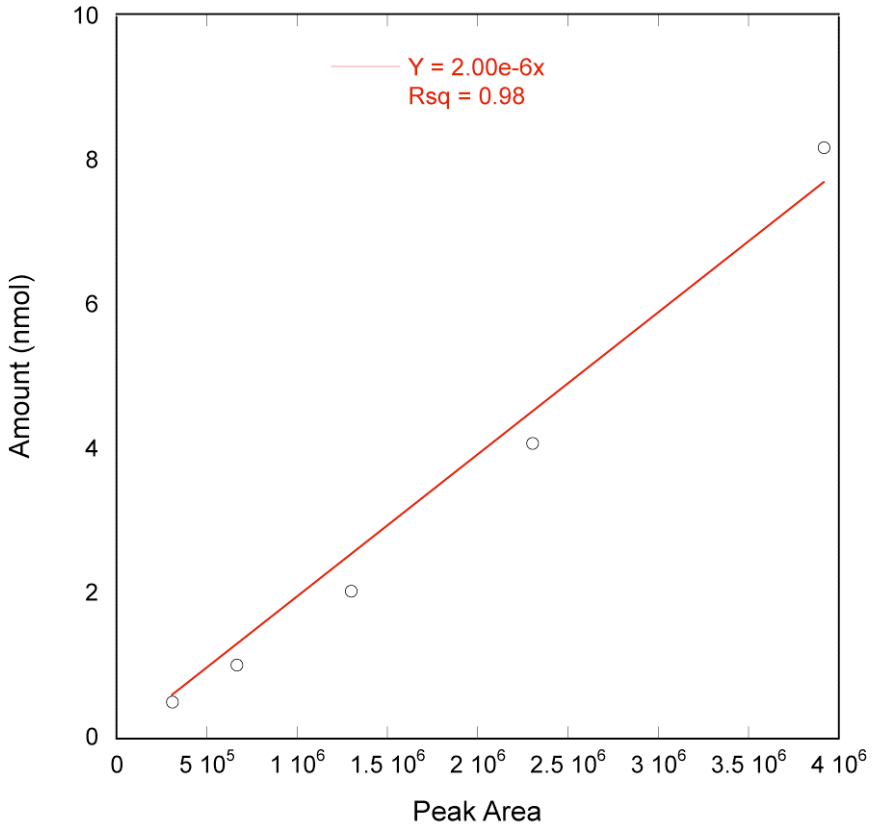


Figure S36. HPLC concentration curve Aβ(16–22) Tyr 19.

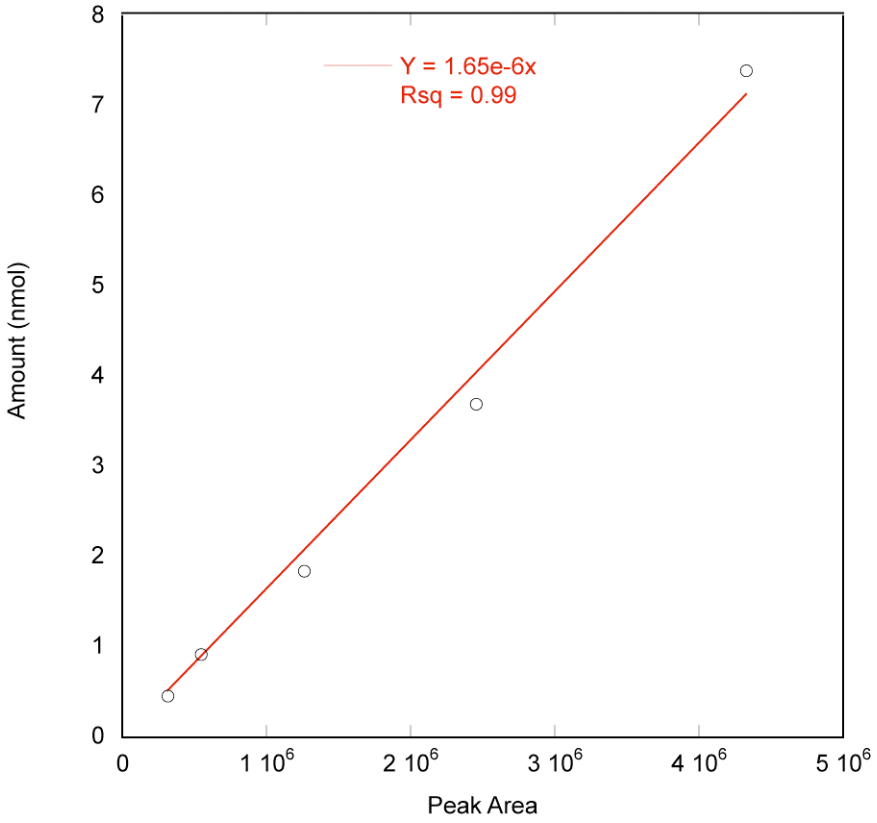


Figure S37. HPLC concentration curve Aβ(16–22) F_5-Phe 19,20.

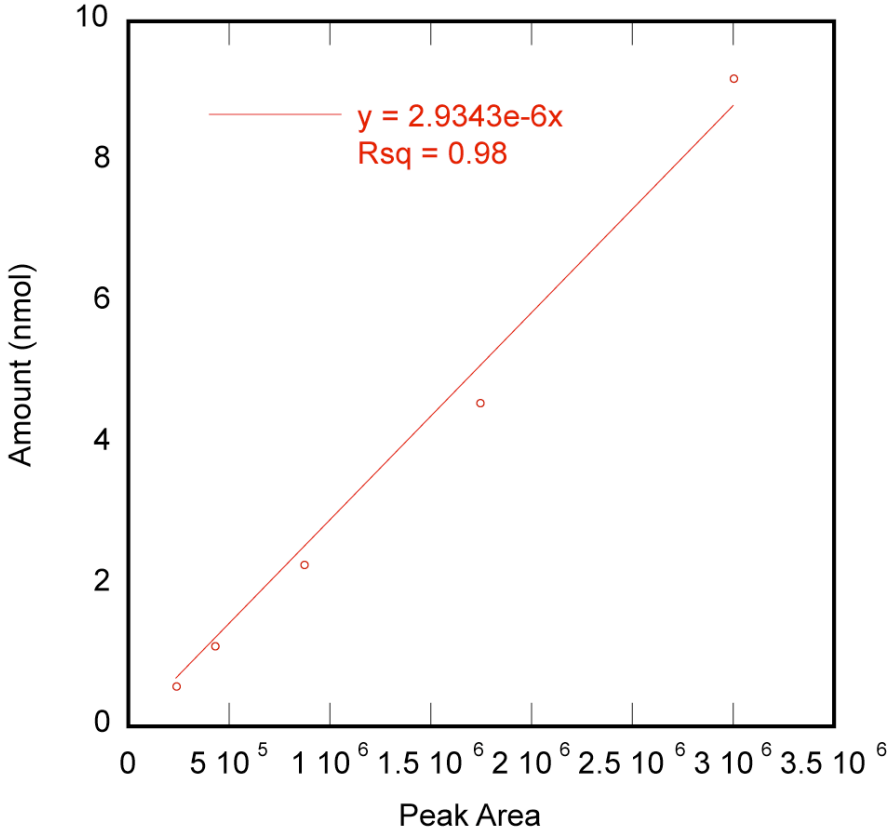
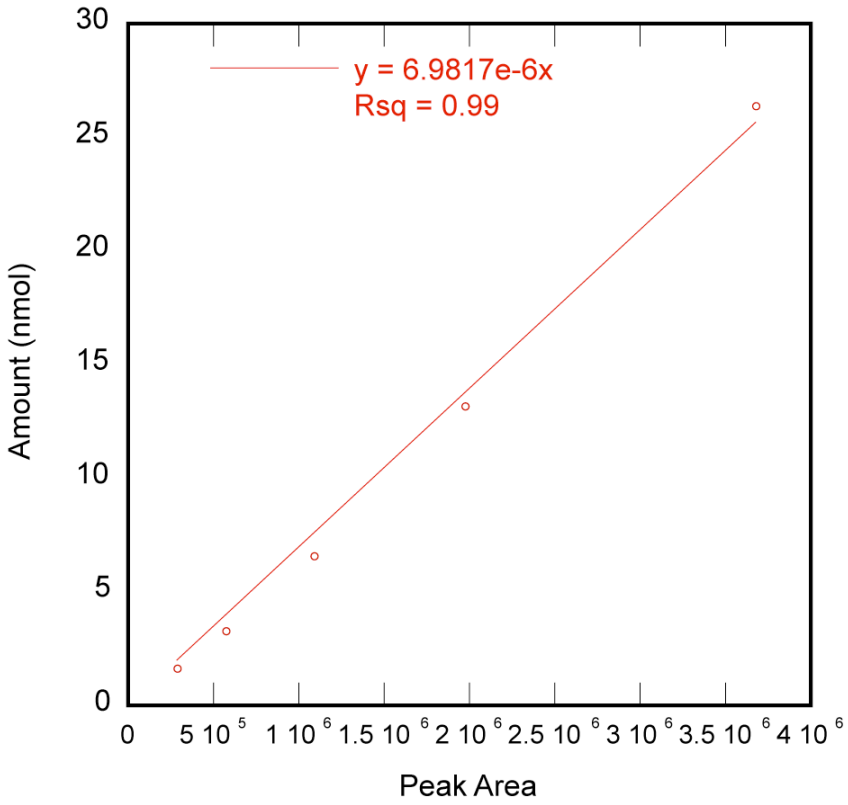


Figure S38. HPLC concentration curve Aβ(16–22) Cha 19,20.



Preparation of HPLC concentration curves

Concentration curves were produced using a protocol adopted from Wetzel *et al*. [O'Nuallain, B.; Thakur, A.M.; Williams, A. D.; Bhattacharyya, A. M.; Chen, S.; Thiagarajan, G.; Wetzel, R. (2006) *Methods Enzymol*. *413*, 34–74]. The curves are instrument specific and are only valid for the exact instrument and column combination used here (Shimadzu HPLC system: SPD-20A UV-vis detector, SIL-10ADVP auto-injector, LC-10ATVP solvent delivery modules; Column: Waters X-Bridge BEH300 Prep C18 10mm).

Freshly dissociated peptide was dissolved in DMSO. Serial dilutions of this DMSO stock were subsequently prepared injected in triplicate on an HPLC column monitored by UV at 215 nm. Each injection was followed by a blank (neat DMSO) to insure there was no carry-over from previous injections. The peak areas (based on UV absorbance) were plotted against the amount of peptide injected, providing linear concentration curves. At higher concentrations these curves diverge from linearity, and in these cases, lower concentrations are necessary to insure accurate determination of concentration. At least solutions corresponding to distinct points on the concentration curve were then submitted for amino acid analysis (American International Biotechnology Services, Richmod, VA) to determine the precise amount of peptide in each solution. These values and the known injection volumes were correlated to the concentration curves, allowing precise determination of peptide quantity as a function of UV absorbance in HPLC eluent. Peptide concentrations from HPLC sedimentation assays were determined using these concentration curves.