Supporting information for:

Expression of N-Terminal Cysteine Aβ$_{42}$ and Conjugation to Generate Fluorescent and Biotinylated Aβ$_{42}$

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Materials and Methods[1]

General Information

Chemicals and Supplies. All chemicals were used as received unless otherwise noted. Deionized water (18 MΩ) was obtained from a Thermo Fisher Scientific Barnstead Genpure Pro water purification system. The pET-Sac-Aβ(M1–42) plasmid was a gift from Dominic Walsh (Addgene plasmid # 71875). DNA sequences that encode Aβ(MC1–42) was purchased in 500 ng quantities from Genewiz. *NdeI* and *SacI* restriction enzymes, CutSmart buffer, and shrimp alkaline phosphatase (rSAP) were purchased from New England Biolabs (NEB). TOP10 Ca^{2+}-competent *E. coli* and BL21 DE3 PLysS Star Ca^{2+}-competent *E. coli*, T4 ligase, and ethidium bromide were purchased from Thermo Fisher Scientific. Zymo ZR plasmid miniprep kit was purchased from

Zymo Research. Zymoclean Gel DNA Recovery Kit was purchased from Zymo Research. Fisher BioReagents LB Broth was purchased from Thermo Fisher Scientific. Carbenicillin was purchased from Gold Biotechnology and added to culture media as a 1000X stock solution (50.0 mg/mL) in water. Chloramphenicol was purchased from RPI Research Products and added to culture media as a 1000X stock solution (34.0 mg/mL) in EtOH. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was purchased from Gold Biotechnology and added to the culture media as a 1000X stock solution (23.8 mg/mL) in water. Maleimide-6-carboxyfluorescein (FAM) and maleimide-5-tetramethylrhodamine (TAMRA) were purchased from Lumiprobe and maleimide-PEG_2-biotin was purchased from Thermo Fisher Scientific. DMSO was purchased from Thermo Fisher Scientific. Urea and sodium borate were purchased from Thermo Fisher Scientific. Boric acid was purchased from Sigma-Aldrich. Tris hydrochloride was purchased from Avantor. Ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma-Aldrich. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (ACN) were purchased from Thermo Fischer Scientific. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from Oakwood Chemical. Thioflavin T (ThT) was purchased from Sigma-Aldrich. Spectra multicolor low range protein ladder and 6X SDS-PAGE loading buffer were purchased from Thermo Fisher Scientific. Sodium carbonate (≥99.5%) was purchased from Sigma-Aldrich.[*] Ammonium persulfate, formaldehyde, and silver nitrate were purchased from Thermo Fischer Scientific. N,N,N',N'-tetramethylethylenediamine (TEMDA) was purchased from Alfa Aesar. Sodium thiosulfate was purchased from Avantor. Corning® 96-well half area black/clear flat bottom polystyrene nonbinding surface (NBS) microplates (product number: 3881) were purchased from Corning Incorporated.[†] 0.22 μm

[*] Use of high-purity sodium carbonate is crucial for successful silver staining.
[†] Microplates with a non-binding surface give reproducible ThT assay results.

Hydrophilic polyethersulfone (PES) syringe filters were purchased from Genesee Scientific (catalog number: 25-244). 0.20 μm Nylon syringe filters were purchased from Thermo Fisher Scientific (catalog number: 09-719-006). DMEM:F12 media were purchased from Thermo Fisher Scientific. Molecular biology grade agarose was purchased from Thermo Fisher Scientific. Difco Mueller Hinton broth was purchased from Becton Dickinson and Company.

Instrumentation. The concentrations of the DNA and peptide samples were measured using a Thermo Fisher Scientific NanoDrop One spectrophotometer. *E. coli* were incubated in a Thermo Fisher Scientific MaxQ Shaker 6000. *E. coli* were pelleted by centrifugation using Beckman Coulter Avanti J-E centrifuge with JA-10 and JA-18 rotors. *E. coli* were lysed using a QSonica Q500 ultrasonic homogenizer. Analytical reverse-phase HPLC was performed on an Agilent 1260 Infinity II instrument equipped with a Phenomonex bioZen Peptide XB-C18 LC column (4.6 x 150 mm, 2.6 μm particle size). Preparative reverse-phase HPLC was performed on a Rainin Dynamax instrument SD-200 equipped an Agilent ZORBAX 300SB-C8 semi-preparative column (9.4 x 250 mm, 5.0 μm particle size) with a ZORBAX 300SB-C3 preparative guard column (9.4 x 15 mm, 7.0 μm particle size). During preparative HPLC purification, the C8 column and the guard column were heated to 80 °C in a Sterlite plastic bin equipped with a Wancle Sous Vide immersion circulator. HPLC grade acetonitrile and deionized water (18 MΩ), each containing 0.1% trifluoroacetic acid (TFA), were used for analytical and preparative reverse-phase HPLC. MALDI-TOF mass spectrometry and tandem mass spectrometry (MS/MS) peptide sequencing were performed using an AB SCIEX TOF/TOF 5800 System. Rotary evaporation was conducted using a Büchi Rotavapor. Lyophilization was conducted using a Labconco FreeZone Freeze Dry System. Sonication of the peptide DMSO stock was performed using a SharperTek ultrasonic cleaner. pH of the solutions was measures using a Thermo Fisher Scientific AB150 Benchtop pH Meter and a

Fisherbrand™ accumet™ pH probe. Fluorescence suppression assays and ThT fluorescence assays were performed using a Thermo Fisher Scientific Varioskan Lux microplate reader. The absorbance of the ThT solution and peptide solutions were measured using a Thermo Fisher Scientific Multiskan Go microplate spectrophotometer. SDS-PAGE gels were visualized using a ChemiDoc Touch Imaging System. Mammalian cell fluorescence microscopy was performed using a Keyence BZ-X810 fluorescence microscope. Mammalian cell fluorescence microscopy was performed using a Zeiss LSM 780 confocal fluorescence microscope. TEM images were obtained using a JEOL JEM-2100F-cryo-TEM instrument.

Molecular Cloning

This section describes the preparation of the pET-Sac-Aβ$_{(MC1–42)}$ plasmid. We have now deposited the pET-Sac-Aβ$_{(MC1–42)}$ plasmid with Addgene to make it available to others.[2]

Isolation of pET-Sac-Aβ$_{(M1–42)}$ Plasmid. The pET-Sac-Aβ$_{(M1–42)}$ plasmid was purchased as a bacterial stab from Addgene (Addgene plasmid #71875)[3], which was made available as a gift from D. Walsh.[4] The bacterial stab was immediately streaked onto a LB agar-plate containing carbenicillin (50 mg/L). Colonies grew in < 24 h. Single colonies were picked and used to inoculate 5 mL of LB broth containing carbenicillin (50 mg/L). The cultures were shaken at 225 rpm overnight at 37 °C. To isolate the pET-Sac-Aβ(M1–42) plasmids, minipreps were performed using a Zymo ZR plasmid miniprep kit. The concentration of the plasmids was measured using a Thermo Scientific Nanodrop instrument.

Restriction Enzyme Digestion of pET-Sac-Aβ$_{(M1–42)}$ Plasmid. The pET-Sac-Aβ$_{(M1–42)}$ plasmid was digested using *SacI* and *NdeI* restriction enzymes to remove the Aβ$_{(M1–42)}$ sequence

and prepare vector backbones for the subsequent generation of Aβ(MC1–42) recombinant plasmid. Table S1 details the restriction reaction conditions. Reagents were added in the order they are listed.

Table S1. Restriction enzyme digestion of the pET- Sac Aβ(M1–42) plasmid

Reagents	Amount
pET-Sac Aβ(M1–42)	20 µL of 50 ng/µL plasmid solution (1.0 µg in total)
10X CutSmart buffer	5.0 µL
H_2O	23.0 µL
NdeI restriction enzyme	1.0 µL (1 U)
SacI-HF restriction enzyme	1.0 µL (1 U)
Total	50.0 µL
Time	1.0 h
Temperature	37.0 °C

Next, to prevent vector backbone self-ligation, the digested plasmid was treated with shrimp alkaline phosphatase (rSAP). Table S2 details the rSAP reaction conditions.

Table S2. rSAP treatment of the vector backbones

Reagents	Amount
Restriction enzyme digestion mixture	50.0 µL
rSAP	1.0 µL (1U)
Total	51.0 µL
Time	0.5 h
Temperature	37.0 °C
Heat inactivation	65.0 °C for 20 min

After the rSAP reaction and heat inactivation were complete, the reaction mixture was mixed with DNA loading buffer and loaded onto a 1% agarose gel containing ethidium bromide (5 µL per 100 mL gel). The agarose gel was run at 100 V for ca. 30 min. A UV box was used to visualize the digested pET-Sac vector (ca.4500 bp), which was excised from the gel using a razor blade. The digested pET-Sac vector was purified from the agarose gel using a Zymoclean Gel DNA Recovery Kit. The concentration of the vector after purification was measured using a

Thermo Scientific Nanodrop instrument. The purified digested pET-Sac linear vector was used in the subsequent ligation step.

Design of the DNA Sequence for Aβ(MC1–42). DNA fragment for Aβ(MC1–42) was ordered from Genewiz. Figure S1 shows the design of the DNA sequence for Aβ(MC1–42).

3' and 5' overhangs *Nde1* restriction site/start codon
stop codons *Sac1* restriction site

GAT ATA CAT ATG TGC GAC GCT GAA TTC CGT CAC GAC TCT GGT TAC GAA GTT CAC CAC CAG AAG CTG GTG TTC TTC GCT GAA GAC GTG GGT TCT AAC AAG GGT GCT ATC ATC GGT CTG ATG GTT GGT GGC GTT GTG ATC GCT TAA TAG GAG CTC GAT CCG

Figure S1. Design of the DNA sequence for Aβ(MC1–42).

Restriction Enzyme Digestion of Aβ(MC1–42) DNA Fragment. The Aβ(MC1–42) DNA fragment was digested using *SacI* and *NdeI* restriction enzymes to generate the insert DNA. Table S3 details the restriction reaction conditions. Reagents were added in the order they are listed.

Table S3. Restriction enzyme digestion of the Aβ(MC1–42) DNA fragment

Reagents	Amount
DNA sequence encoding mutation	20 µL of 5 ng/µL DNA solution (100.0 ng in total)
10X CutSmart buffer	2.5 µL
H_2O	1.5 µL
NdeI restriction enzyme	0.5 µL (0.5 U)
SacI-HF restriction enzyme	0.5 µL (0.5 U)
Total	25.0 µL
Time	1.0 h
Temperature	37.0 °C
Heat inactivation	65.0 °C for 20 min

T4 Ligation of the Aβ(MC1–42) DNA Fragment and the pET-Sac Vector. The Aβ(MC1–42) DNA fragment (insert) and the backbone vector were ligated together using T4 ligase. Table S4 details the T4 ligation reaction conditions. Reagents were added in the order they are listed.

Table S4. T4 ligation of the insert and the vector

Reagents	Amount	
	Insert:Vector = 0:1 (molar ratio) (negative control)	**Insert:Vector = 5:1 (molar ratio)**
Vector	6.2 μL of 9.7 ng/μL DNA solution (60.0 ng in total)	6.2 μL of 9.7 ng/μL DNA solution (60.0 ng in total)
Insert	---	2.5 μL of 4.0 ng/μL DNA solution (10.0 ng in total)
10X T4 DNA ligase reaction buffer	2.0 μL	2.0 μL
T4 DNA ligase	1.0 μL	1.0 μL
H_2O	10.8 μL	8.3 μL
Total	20.0 μL	20.0 μL
Ligation time	10 min	
Temp	22.0 °C (room temperature)	
Heat inactivation	65.0 °C for 10 min	

Aβ(MC1–42) Recombinant Plasmid Preparation. 2 μL of the ligation reaction mixture was then transformed into TOP10 Ca^{2+}-competent *E. coli* using the heat shock method. The cell cultures were spread on LB agar plates containing carbenicillin (50 mg/L). Single colonies were picked to inoculate 5 mL of overnight cultures in LB media with carbenicillin (50 mg/L). The plasmid was extracted from TOP10 cells using Zymo ZR plasmid miniprep kit. The concentration of the plasmid was measured through Thermo Scientific NanoDrop spectrophotometer. The DNA sequence of the Aβ(MC1–42) recombinant plasmid was verified by DNA sequencing. The Aβ(MC1–42) recombinant plasmid was deposited to Addgene.[2]

Representative Schedule for the Expression, Purification, and Labeling of Aβ(C1–42)

Table S5. Representative schedule for the expression, purification, and labeling of Aβ(C1–42)

Day	Time	Steps
Day 1	Evening	Starter culture
Day 2	Morning	Daytime culture
	Afternoon	IPTG induced expression of Aβ(C1–42)
	Evening	Cell pelleting
Day 3	Morning	Cell lysis and urea extraction to obtain peptide solution
	Afternoon	Prep-HPLC purification of the 1st batch of peptide solution
	Evening	Prep-HPLC purification of the 2nd batch of peptide solution
A total of ca. 14.0 mg* of pure Aβ(C1–42) is obtained (in two batches of combined HPLC fractions)		
Day 4	Morning	Labeling of the 1st batch of Aβ(C1–42); prep-HPLC purification
	Afternoon	Labeling of the 2nd batch of Aβ(C1–42); prep-HPLC purification
Day 4–5	Overnight	Lyophilization of purified labeled Aβ(C1–42)
A total of ca. 3.0 mg† of pure labeled Aβ(C1–42) is obtained		
Day 5	Afternoon	HFIP treatment of pure labeled Aβ(C1–42); lyophilization

Bacterial expression of Aβ(C1–42)

Transformation of Aβ(MC1–42) Plasmid. All liquid cultures were performed in culture media (LB broth containing 50 mg/L carbenicillin and 34 mg/L chloramphenicol). Aβ(MC1–42) plasmid was transformed into BL21 DE3 PLysS Star Ca^{2+}-competent *E. coli* through heat shock method. The cell cultures were spread on LB agar plates containing carbenicillin (50 mg/L) and chloramphenicol (34 mg/L). Single colonies were picked to inoculate 5 mL of culture media for overnight culture (ca. 14 h). A glycerol stock of BL21 DE3 PLysS Star Ca^{2+}-competent *E. coli*

* The yield of Aβ(C1–42) was determined spectrophotometrically.
† The yield of labeled Aβ(C1–42) was determined gravimetrically.

bearing the plasmid was made. The glycerol stock was stored at -80 °C and was used in all subsequent expression procedures.

Expression of $A\beta_{(C1–42)}$. For a typical expression procedure, a starter culture was made by inoculating culture media with an aliquot of the glycerol stock. The next day, all 5 mL of the overnight culture were used to inoculate 1 L of culture media. After inoculation, the culture was shaken at 225 rpm at 37 ˚C (for ca. 3 hours and 50 minutes) until the cell density reached an OD_{600} of ca. 0.45. Protein expression was then induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM, and the cells were shaken at 225 rpm at 37 °C for 4 more hours with IPTG. The cells were then harvested by centrifugation at 4000 rpm using a JA-10 rotor (2800 x g) at 4 ˚C for 25 minutes, and the cell pellets were then stored at -80 °C.

Purification of the $A\beta_{(C1–42)}$ Peptide

Cell Lysis. To lyse the cells, the cell pellet was resuspended in 20 mL of buffer A (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and sonicated for 2 minutes on ice (50% duty cycle) until the lysate appeared homogenous. The lysate was then centrifuged for 25 minutes at 16000 rpm using a JA-18 rotor (38000 x g) at 4˚C. The supernatant was removed, and the pellet was resuspended in buffer A, sonicated and centrifuged as described above. The sonication and centrifugation steps were repeated two additional times. After the third supernatant was removed, the remaining pellet (inclusion bodies) was resuspended in 15 mL of freshly prepared buffer B (8 M urea, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0), and was sonicated as described above, until the peptide solution became clear.

Sample Filtering and Analytical HPLC. The peptide solution (ca. 15 mL) was then diluted with 10 mL of buffer A and filtered through a 0.22 µm hydrophilic PES syringe filter from Genesee Scientific (catalog number: 25-244) or a 0.22 µm hydrophilic PVDF syringe filter from Thermo Fisher Scientific (catalog number: 09-719-006).* Successful expression of the $A\beta_{(C1–42)}$ peptide was confirmed by analytical reverse-phase HPLC as follows: A 20-µL sample of the above solution was injected onto an Agilent 1260 Infinity II instrument equipped with a Phenomonex bioZen Peptide XB-C18 LC column (4.6 x 150 mm, 2.6 µm particle size). HPLC grade acetonitrile (ACN) and 18 MΩ deionized water, each containing 0.1% trifluoroacetic acid, were used as the mobile phase. The sample was eluted at 1.0 mL/min with a 5–67% acetonitrile gradient over 15 minutes, at 60 °C. The absorbance was taken at 214 nm. A typical analytical HPLC trace of the successfully expressed $A\beta_{(C1–42)}$ shows four major peaks, with the first peak (ca. 10.4 min) corresponding to the $A\beta_{(C1–42)}$ monomer (Figure S2).

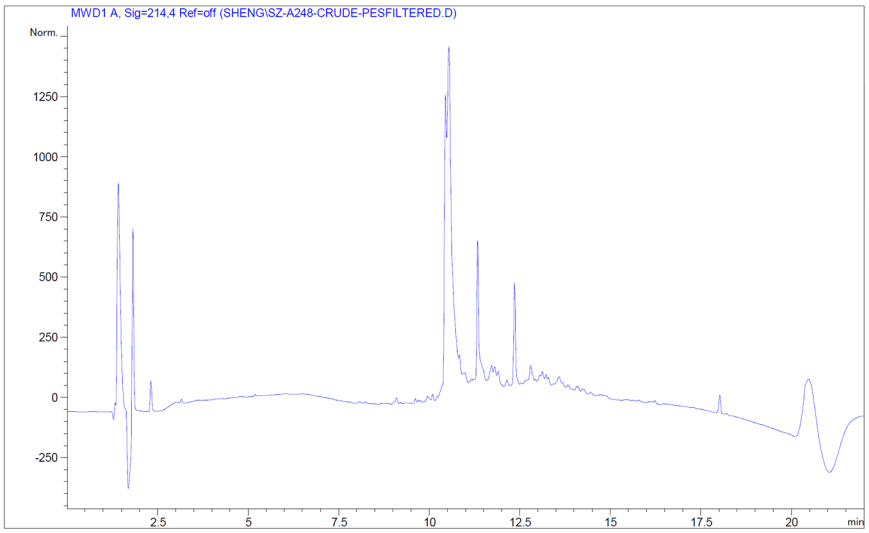


Figure S2. Representative HPLC trace of filtered, unpurified $A\beta_{(C1–42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm.

* Use of appropriate filter materials is essential for minimizing loss of peptide by adsorption during filtration.

Preparative HPLC Purification of Aβ(C1–42). The filtered peptide solution was then purified by preparative reverse-phase HPLC equipped with an Agilent ZORBAX 300SB-C8 semi-preparative column (9.4 x 250 mm) with a ZORBAX 300SB-C3 preparative guard column (9.4 x 15 mm). The C8 column and the guard column were heated to 80 °C in water bath using a Sterlite plastic bin equipped with a Wancle Sous Vide immersion circulator.* HPLC grade acetonitrile (ACN) and 18 MΩ deionized water, each containing 0.1% trifluoroacetic acid, were used as the mobile phase at a flow-rate of 5 mL/min. The filtered peptide solution (ca. 24 mL) was split into two ca. 12 mL batches and purified in two separate runs, with three injections in each run.† In each run, the peptide was loaded onto the column while flowing 20% ACN through the column. The Aβ(C1–42) peptide was then eluted using the following solvent gradient (Table S6). The absorbance was taken at 214 nm. Fractions containing the monomeric Aβ(C1–42) generally eluted from 33% to 35% ACN.

Table S6. HPLC solvent gradient for the purification of Aβ(C1–42)

% H_2O	% ACN	Elapsed Time
80	20	0 min
80	20	15 min
40	65	60 min
5	95	61 min
5	95	80 min

Column Maintenance. After the peptide was collected, the column was washed by injecting 5 mL of filtered buffer B (8 M urea, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0) while flushing at 95% ACN for 15 minutes. This cleaning procedure ensures elution of the peptide that is retained in the

* Any water heater large enough to submerge a HPLC column should be sufficient.
† It may be possible to purify the peptide in a single batch if a larger diameter column is used (e.g., 21.4 x 250 mm).

column and avoids problems of cross-contamination between runs. If an increase in column back-pressure is seen after several purifications, back-flushing the semi-preparative HPLC column using the following cleaning protocol will solve the problem (Table S7).

Table S7. Prep-HPLC column cleaning protocol

Solvent	Flow rate	Washing Time
100% ACN	5 mL/min	10 min
100% isopropanol	2 mL/min	15 min
100% hexanes	2 mL/min	20 min
100% isopropanol	2 mL/min	15 min
100% ACN	5 mL/min	10 min

Characterization of Purified Aβ(C1–42). The purity of each fraction was assessed using analytical reverse-phase HPLC. A 20-μL sample was injected onto the analytical HPLC. The sample was eluted at 1.0 mL/min with a 5–67% acetonitrile gradient over 15 min, at 60 °C. The absorbance was taken at 214 nm. Pure fractions were combined and the purity of the combined fractions were checked using analytical HPLC. The purity of Aβ(C1–42) was determined to be 97.7% by integration of the analytical HPLC trace at 214 nm (Figure S7). The yield of Aβ(C1–42) was determined to be ca. 14.0 mg per liter of bacterial culture, on the basis of the spectrophotometrically determined concentration of Aβ(C1–42) in the combined pure HPLC fractions of each batch and the total volume of the two batches of the combined pure HPLC fractions. The details of spectrophotometric determination of the concentration of Aβ(C1–42) in the combined pure HPLC fractions can be found in the "Labeling of Aβ(C1–42)" section of the Supporting Information. The composition of the combined peptides was assessed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and tandem mass

spectrometry (MS/MS) (Figure S8 and S9). The details of running MALDI-MS and MS/MS can be found in the "Mass Spectrometry" section of the Supporting Information.

Storage of the Combined Pure HPLC Fractions of Aβ(C1–42). The combined pure HPLC fractions of Aβ(C1–42) can be directly used for subsequent labeling, or stored at -80 °C for at least three weeks without forming detectable aggregates. It is recommended to combine and freeze (at -80 °C or with dry ice) the purified fractions within 5 hours after purification to avoid oxidation of methionine.

Labeling of Aβ(C1–42)

The labeling was performed for the first batch of combined pure HPLC fractions of Aβ(C1–42)., and then repeated for the second batch of Aβ(C1–42). A representative protocol to label one batch of combined pure HPLC fractions of Aβ(C1–42) is provided below.*

Stoichiometry Calculation. The combined pure HPLC fractions of Aβ(C1–42) was removed from the freezer or dry ice and allowed to thaw (ca. 20 min). The concentration of Aβ(C1–42) in the combined HPLC fractions was determined by UV absorbance at 280 nm using an estimated extinction coefficient (ε) for tyrosine of 1490 $M^{-1}cm^{-1}$. A 35% ACN solution (acetonitrile 35%, water 64.9% and trifluoroacetic acid 0.1%) was used as blank solution for the UV absorbance measurement.† The amount of the maleimide-based labeling reagent needed was then calculated based on a 2:1 molar ratio of the reagent to the Aβ(C1–42) peptide. A typical reagent table for labeling

* It may be possible to label and purify the peptide in a single batch if a larger diameter column is used (e.g., 21.4 x 250 mm).

† The typical volume of the combined pure HPLC fractions from purifying a 12-mL batch of peptide solution is ca. 10 mL. The typical UV absorbance of the combined pure HPLC fractions at 280 nm is ca. 0.2 and the typical peptide concentration in the combined pure HPLC fractions is ca. 140 μM.

$A\beta_{(C1–42)}$ with maleimide-5-tetramethylrhodamine (TAMRA) is provided here (Table S8). Labeling of $A\beta_{(C1–42)}$ with maleimide-6-carboxyfluorescein (FAM) or maleimide-PEG_2-biotin was performed in a similar manner.

Table S8. Reagent table for labeling $A\beta_{(C1–42)}$ with maleimide-5-TAMRA

Reagent	Combined HPLC fractions of $A\beta_{(C1–42)}$	Maleimide-5-TAMRA
Concentration	141.9 μM	---
volume	8.0 mL	---
Mass	---	1.26 mg dissolved in 126 μL DMSO
Molecular weight	---	552.6 g/mol
Moles	1.14 μmol	2.28 μmol
Molar equivalent	1.0	2.0

Sodium Borate Buffer Preparation. A 750 mM sodium borate buffer solution (pH 9.0) was made by dissolving 1.54 g of sodium borate decahydrate ($Na_2B_4O_7•10H_2O$) and 0.25 g of boric acid (H_3BO_3) in 22 mL of deionized water (18 MΩ), followed by adding more deionized water (18 MΩ) until the total volume was 27 mL (Table S9). The final pH of the 750 mM sodium borate buffer solution made through this recipe is typically 9.0. It is not necessary to further adjust the pH using acid or base.

Table S9. Recipe of making 750 mM sodium borate buffer (pH 9.0)

Reagent	Molecular weight	Mass/Volume
Sodium borate decahydrate ($Na_2B_4O_7•10H_2O$)	381.43 g/mol	1.54 g
Boric acid (H_3BO_3)	61.84 g/mol	0.25 g
Deionized water (18 MΩ)	---	Add 22 mL to dissolve $Na_2B_4O_7•10H_2O$ and H_3BO_3, then fill up to a total volume of 27 mL

Titration. A 1.0 mL portion of the combined HPLC fractions of $A\beta_{(C1–42)}$ was then set aside on ice and titrated with the 750 mM sodium borate buffer in increments of 20 μL without stirring

or shaking. The pH of the solution was checked with a pH meter after the addition of each aliquot. After a pH of 9.0 was reached, one additional 20 µL increment of sodium borate buffer was added. The titration results were recorded in a table and the titrated 1.0 mL portion of the combined HPLC fractions of $A\beta_{(C1–42)}$ was discarded. A typical titration table is provided below (Table S10).

Table S10. Titration of a 1.0 mL portion of the combined HPLC fractions of $A\beta_{(C1–42)}$

Total volume of 750 mM sodium borate buffer (pH 9.0) added	pH
0	2.3
20 µL	2.4
40 µL	6.0
60 µL	8.3
80 µL	8.8
100 µL	8.9
120 µL	9.0
140 µL	9.1

Basification. The volume of sodium borate buffer needed to basify the remainder of the combined HPLC fractions to pH 9.0 was calculated from the total volume of the sodium borate buffer used to titrate the 1.0 mL portion of the combined HPLC fractions of $A\beta_{(C1–42)}$. The appropriate volume of the sodium borate buffer was added in a single portion to the remainder of the combined HPLC fractions on ice without stirring or shaking (Table S11). The pH of the basified solution was checked with a pH meter. The basified solution was also checked with analytical HPLC to make sure $A\beta_{(C1–42)}$ stayed in mostly monomeric form after basification (Figure S3).*

* If the basification process is not properly performed, a substantial (> 20%) oligomer peak will appear after the monomer peak (at ca. 11.1 and 10.3 min, respectively). The labeling will still work, but the yield of labeled Aβ will be compromised.

Table S11. Basification of the remainder of the combined HPLC fractions of Aβ(C1–42)

Total volume of 750 mM sodium borate buffer (pH 9.0) added	pH
0	2.4
1120 µL	8.9

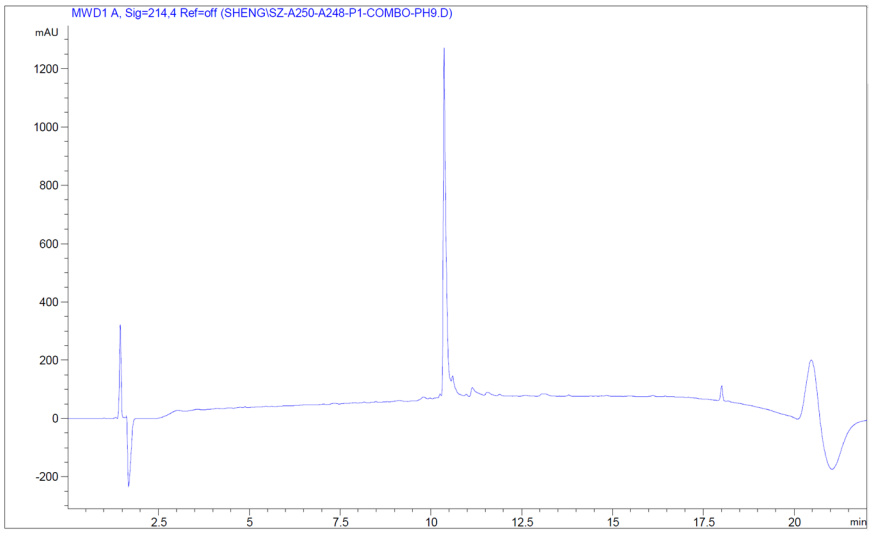


Figure S3. Representative HPLC trace of basified combined pure fractions of Aβ(C1–42). HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm.

Labeling. After basification, the remainder of the combined HPLC fractions of Aβ(C1–42) was removed from the ice bath. The calculated amount the maleimide reagent (Table S8) was dissolved in DMSO to give a 10 mg/mL stock solution. The maleimide reagent stock solution was slowly added to the remainder of the combined HPLC fractions of Aβ(C1–42) with gentle swirling. The reaction mixture* was then allowed to stand without stirring or shaking at room temperature for 15 minutes. If maleimide-5-TAMRA or maleimide-6-FAM was used as the labeling reagent, the reaction mixture was also protected from light.

* The composition of the reaction mixture: ca. 30.6% v/v acetonitrile, 0.1% v/v trifluoroacetic acid, 1.4% v/v DMSO, 67.9% v/v water, 92 mM sodium borate/boric acid, 250 µM maleimide reagent, and 125 µM Aβ(C1–42).

Purification of Labeled Aβ$_{(C1–42)}$

Sample Filtering and Analytical HPLC. After the reaction had proceeded for 15 minutes, the reaction mixture was filtered through a 0.22 μm hydrophilic PES syringe filter from Genesee Scientific (catalog number: 25-244). A PVDF syringe filter should not be used to filter the reaction mixture as it will lead to a substantial loss of labeled peptide in the reaction mixture. Successful labeling of the Aβ$_{(C1–42)}$ peptide was confirmed by analytical reverse-phase HPLC as follows: A 20-μL sample of the above solution was injected onto an Agilent 1260 Infinity II instrument equipped with a Phenomonex bioZen Peptide XB-C18 LC column (4.6 x 150 mm, 2.6 μm particle size). HPLC grade acetonitrile (ACN) and 18 MΩ deionized water, each containing 0.1% trifluoroacetic acid, were used as the mobile phase. The sample was eluted at 1.0 mL/min with a 5–67% acetonitrile gradient over 15 minutes at 60 °C. If maleimide-5-TAMRA was used as the labeling reagent, the absorbance was taken at 214 nm and 541 nm. If maleimide-6-FAM was used as the labeling reagent, the absorbance was taken at 214 nm and 494 nm. If maleimide-PEG$_2$-biotin was used as the labeling reagent, the absorbance was taken at 214 nm. A typical analytical HPLC trace of the successfully labeled Aβ$_{(C1–42)}$ shows a sharp peak corresponds to monomeric labeled Aβ$_{(C1–42)}$ at ca. 10.2 minutes, with a slight retention time change compare to the unlabeled Aβ$_{(C1–42)}$ (Figure S4, S5, and S6) (Table S12).

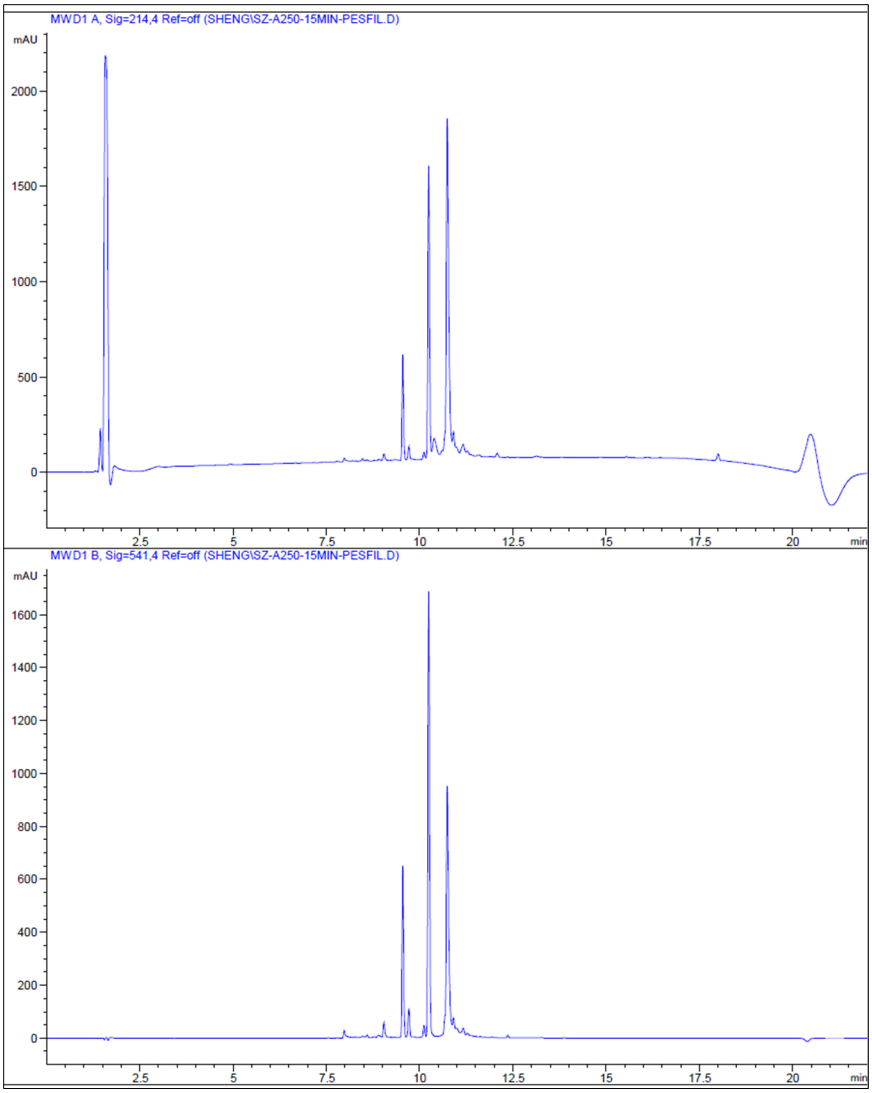


Figure S4. Representative analytical HPLC trace of filtered, unpurified TAMRA-labeled Aβ$_{(C1–42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm (top) and 541 nm (bottom).

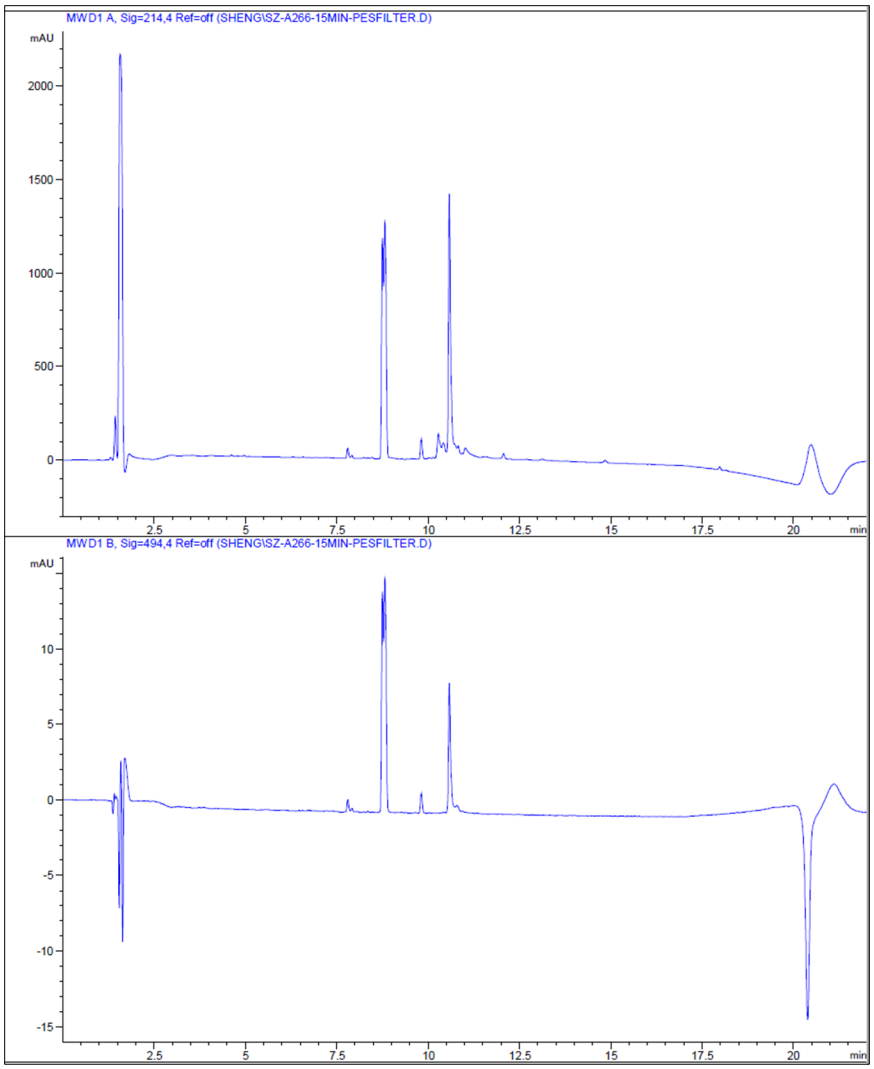


Figure S5. Representative analytical HPLC traces of filtered, unpurified FAM-labeled $A\beta_{(C1–42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm (top) and 494 nm (bottom).

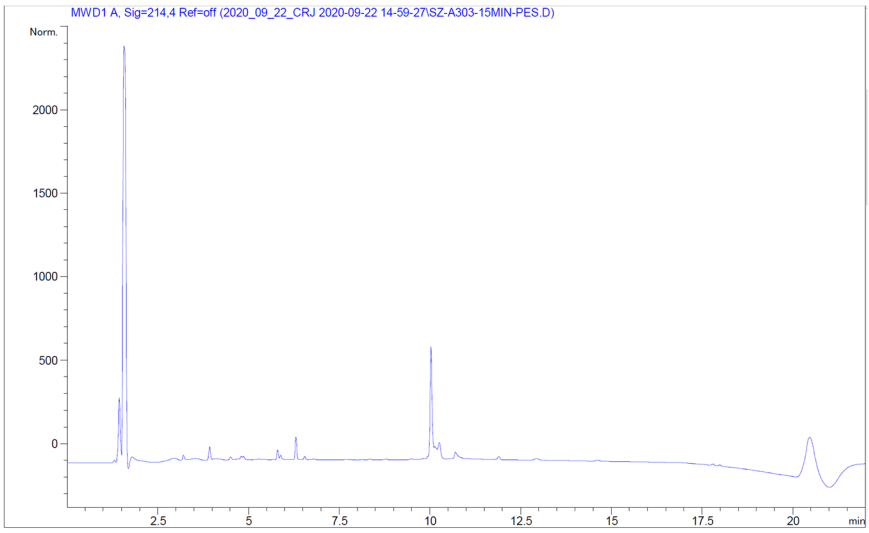


Figure S6. Representative analytical HPLC trace of filtered, unpurified biotin-labeled Aβ(C1–42). The absorbance was taken at 214 nm. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm.

Table S12. Analytical HPLC retention time

Peptide	Analytical HPLC retention time
Unlabeled Aβ(C1–42)	10.4 min
TAMRA-Aβ(C1–42)	10.7 min
FAM-Aβ(C1–42)	10.6 min
Biotin-Aβ(C1–42)	10.0 min

Preparative HPLC Purification of Labeled Aβ(C1–42). The reaction mixture was then purified by preparative reverse-phase HPLC equipped with an Agilent ZORBAX 300SB-C8 semi-preparative column (9.4 x 250 mm) with a ZORBAX 300SB-C3 preparative guard column (9.4 x 15 mm).* The C8 column and the guard column were heated to 80 °C in water bath using a Sterlite plastic bin equipped with a Wancle Sous Vide immersion circulator.† HPLC grade acetonitrile

* It may be possible to label and purify the peptide in a single batch if a larger diameter column is used (e.g., 21.4 x 250 mm).
† Any water heater large enough to submerge a HPLC column should be sufficient.

(ACN) and 18 MΩ deionized water, each containing 0.1% trifluoroacetic acid, were used as the mobile phase at a flow rate of 5 mL/min. The filtered reaction mixture was loaded with two 4-mL injections onto the column while flowing 20% ACN through the column. The labeled-$A\beta_{(C1–42)}$ peptide was then purified using the following solvent gradient (Table S13). The absorbance was taken at 214 nm. Fractions containing the monomeric labeled-$A\beta_{(C1–42)}$ generally eluted from 34% to 38% ACN.*

Table S13. HPLC solvent gradient for the purification of labeled-$A\beta_{(C1–42)}$

% H_2O	% ACN	Elapsed Time
80	20	0 min
80	20	15 min
40	65	60 min
5	95	61 min
5	95	80 min

Characterization of Purified labeled $A\beta_{(C1–42)}$. The purity of each fraction was assessed using analytical reverse-phase HPLC. A 20-μL sample was injected onto the analytical HPLC. The sample was eluted at 1.0 mL/min with a 5–67% acetonitrile gradient over 15 min, at 60 °C. If maleimide-5-TAMRA was used as the labeling reagent, the absorbance was taken at 214 nm and 541 nm. If maleimide-6-FAM was used as the labeling reagent, the absorbance was taken at 214 nm and 494 nm. If maleimide-PEG_2-biotin was used as the labeling reagent, the absorbance was taken at 214 nm. Pure fractions were combined and the purity of the combined fractions were checked using analytical HPLC. The purities of labeled $A\beta_{(C1–42)}$ were determined by integration of the analytical HPLC trace at 214 nm (Figure S10, S12, and S14) (Table S14). The composition

* An increase in column back-pressure is rare after purifying the reaction mixture. If column maintenance is needed, please refer to the "Column Maintenance" procedures from the "Purification of the $A\beta_{(C1–42)}$ Peptide" section.

of the combined peptides was assessed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) (Figure S11, S13, and S15). The details of running MALDI-MS can be found in the "Mass Spectrometry" section of the Supporting Information.

Table S14. Typical purities of labeled-$A\beta_{(C1–42)}$ peptides

Labeled-$A\beta_{(C1–42)}$ peptides	Purity
TAMRA-$A\beta_{(C1–42)}$	94.7%
FAM-$A\beta_{(C1–42)}$	94.0%
Biotin-$A\beta_{(C1–42)}$	96.6%

Storage of the Purified Labeled $A\beta_{(C1–42)}$. The combined pure HPLC fractions of labeled-$A\beta_{(C1–42)}$ were concentrated by rotary evaporation to remove ACN,* and then frozen with dry ice. It is recommended to combine and freeze the purified fractions within 5 hours after purification to avoid oxidation of methionine. The frozen sample was then lyophilized. For TAMRA- and FAM-labeled $A\beta_{(C1–42)}$, the sample should be protected from light during lyophilization process.

Yields of Labeled $A\beta_{(C1–42)}$ Peptides. After the lyophilization was completed, the lyophilized labeled Aβ from two batches of purifications are combined, and the yields of the labeled peptides were assessed gravimetrically. Table S15 shows the typical yields of labeled-$A\beta_{(C1–42)}$ peptides per liter of bacterial culture.

Table S15. Typical yields of labeled-$A\beta_{(C1–42)}$ peptides

Labeled-$A\beta_{(C1–42)}$ peptides	Yield (per liter of bacterial culture)
TAMRA-$A\beta_{(C1–42)}$	3.6 mg
FAM-$A\beta_{(C1–42)}$	2.6 mg
Biotin-$A\beta_{(C1–42)}$	3.0 mg

* The water bath for the rotary evaporator should not be heated.

HFIP Treatment of Labeled Aβ$_{(C1–42)}$ Peptides

The lyophilized labeled Aβ$_{(C1–42)}$ peptides were then treated with HFIP to provide aliquots of monomeric Aβ for subsequent biophysical and biological studies. Briefly, the lyophilized peptide was dissolved in HFIP to give a clear solution with 0.25 mg/mL concentration of peptide. The peptide solution was allowed to stand without stirring or shaking at room temperature for 3 hours. The solution was then placed on ice for an additional 30 minutes. For TAMRA-labeled Aβ$_{(C1–42)}$ and FAM-labeled Aβ$_{(C1–42)}$, the samples were protected from light. The Aβ$_{(M1–42)}$ peptide, which was prepared as a control, was treated in a similar fashion.*

The chilled peptide solution was then aliquoted into microcentrifuge tubes using a single-channel pipette.† We typically aliquot 0.02 µmol of peptide (ca. 0.1 mg) per tube, as shown in Table S16. The tubes were then frozen in dry ice and lyophilized to remove HFIP. The lyophilized aliquots were stored in a desiccator at -20 °C until needed for further use. For TAMRA-labeled Aβ$_{(C1–42)}$ and FAM-labeled Aβ$_{(C1–42)}$, the samples were protected from light.

Table S16. Volumes of HFIP peptide solution per aliquot

Peptide	Moles of peptide per tube[a]	Molecular weight[a]	Mass of peptide per tube	HFIP peptide solution concentration	Volume of HFIP peptide solution per tube
TAMRA-Aβ$_{(C1–42)}$	0.02 µmol	5170 g/mol	0.103 mg	0.25 mg/mL	412 µL
FAM-Aβ$_{(C1–42)}$		5116 g/mol	0.102 mg		408 µL
Biotin-Aβ$_{(C1–42)}$		5143 g/mol	0.103 mg		412 µL
Aβ$_{(M1–42)}$		4645 g/mol	0.093 mg		372 µL

[a] Molecular weight calculated for the free peptide. If trifluoroacetate counterions are present from HPLC purification, then the effective molecular weight of the peptide will be higher (by ca. 10-15%, depending on the number of trifluoroacetate counterions per peptide), and the number of µmol of peptide per tube will be slightly lower (by ca. 10-15%, depending on the number of trifluoroacetate counterions per peptide).

* Aβ$_{(M1–42)}$ is an expressed homologue with properties similar to native Aβ$_{(1–42)}$, which was used as an unlabeled Aβ control for subsequent biophysical and biological studies. Aβ$_{(M1–42)}$ was expressed, purified, and lyophilized following the protocol we published previously.[1]

† It is important to chill the HFIP solution to minimize evaporation and allow dispensing with a single-channel pipette. Alternatively, a positive displacement pipette may be used.

Mass Spectrometry

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and tandem mass spectrometry (MS/MS) were performed using an AB SCIEX TOF/TOF 5800 System. 0.5 μL of 2,5-dihydroxybenzoic acid (DHB) was dispensed onto a MALDI sample support, followed by the addition of 0.5 μL peptide sample. The mixture was allowed to air-dry. MALDI analyses were performed in positive reflector mode, collecting data with a molecular weight range of 1000–9000 Da. MS/MS analysis of the $A\beta_{(C1–42)}$ peptide was performed using CID fragmentation of the precursor mass 4617 ± 2 Da. The B&Y fragment ion was calculated with fragment ion calculators.

SDS-PAGE, Silver staining, and Fluorescence Imaging

Sample Preparation and SDS-PAGE. A 0.02 μmol aliquot of labeled $A\beta_{(C1–42)}$ or $A\beta_{(M1–42)}$ was dissolved in 20.0 μL of DMSO first, then diluted with 13.3 μL of 10 mM sodium phosphate buffer (pH 7.4) to give a 600 μM peptide stock solution. 15.0 μL of the 600 μM peptide stock solution was serial diluted with 15.0 μL of 10 mM sodium phosphate buffer (pH 7.4) to create 15.0 μL of peptide stock solutions with concentrations of 300 μM to 4.7 μM. The peptide stock solutions were then immediately diluted with 3.0 μL of 6X SDS-PAGE loading buffer to give 18.0 μL of peptide working solutions with concentrations from 500 μM to 3.9 μM. All 18.0 μL of each working solution was loaded on a 4% stacking polyacrylamide gel with a 16% running polyacrylamide gel. The gels were run at a constant 90 volts at room temperature for ca. 3 hours. For FAM-labeled $A\beta_{(C1–42)}$ and TAMRA-labeled $A\beta_{(C1–42)}$, the gels were protected from light.

Silver Staining. Silver staining method was used to visualize the SDS-PAGE gels for labeled $A\beta_{(C1–42)}$ and $A\beta_{(M1–42)}$. Briefly, the gel was first rocked in fixing solution (50% (v/v)

methanol and 5% (v/v) acetic acid in deionized water) for 20 minutes. Next, the fixing solution was discarded and the gel was rocked in 50% (v/v) aqueous methanol for 10 minutes. Next, the 50% methanol was discarded and the gel was rocked in deionized water for 10 minutes. Next, the water was discarded and the gel was rocked in 0.02% (w/v) sodium thiosulfate in deionized water for 1 minutes. The sodium thiosulfate was discarded and the gel was rinsed twice with deionized water for 1 minute (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 minutes. Next, the silver nitrate solution was discarded and the gel was rinsed with deionized water for 15 seconds (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 (ca. 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.

Fluorescence Imaging. Fluorescence imaging was also used to visualize the SDS-PAGE gels for TAMRA-labeled $A\beta_{(C1–42)}$ and FAM-labeled $A\beta_{(C1–42)}$. Before silver staining, the gels for TAMRA-labeled $A\beta_{(C1–42)}$ and FAM-labeled $A\beta_{(C1–42)}$ were imaged using a ChemiDoc Touch Imaging System. The gel for TAMRA-labeled $A\beta_{(C1–42)}$ was imaged with Krypton channel (excitation at ca. 530 nm, exposure time: 0.2 seconds). The gel for FAM-labeled $A\beta_{(C1–42)}$ was imaged with Fluorescein channel (excitation at ca. 490 nm, exposure time: 0.2 seconds).

Thioflavin T (ThT) Assay

ThT Solution Preparation. The ThT solution was freshly prepared and placed on ice before use. Briefly, a solution of ca. 40 μM ThT was prepared in a 1x PBS buffer (pH 7.4). The

* Use of high-purity (≥99.5%) sodium carbonate is crucial for successful silver staining.

concentration of ThT in the solution was determined by UV absorbance at 412 nm using an estimated extinction coefficient (ε) of 36000 $M^{-1}cm^{-1}$ and adjusted to 41.7 μM. The solution was filtered through a 0.22 μm nylon syringe filter. The ThT solution was stored on ice and protected from light.

Peptide Working Solution Preparation. A 0.02 μmol aliquot of biotin-labeled $A\beta_{(C1–42)}$ or $A\beta_{(M1–42)}$ was dissolved in 20 μL of DMSO first and sonicated for 30 s in a water bath, then diluted with 480 μL of ThT solution to give a working solution with final peptide concentration of 40 μM and ThT concentration of 40 μM. The peptide working solution was stored on ice and protected from light.

ThT Fluorescence Assay Setup. ThT fluorescence assays were conducted in Corning® 96-well half area black/clear flat bottom polystyrene nonbinding surface (NBS) microplates (product number: 3881). A 100-μL aliquot of the working solution was immediately transferred to each of five wells of 96-well plate, and the plate was kept at room temperature. ThT solution was transferred to each of five wells of 96-well plate as negative control. The 96-well plate was sealed with adhesive plate sealer. The 96-well plate was immediately inserted into a Thermo Fisher Scientific Varioskan Lux microplate reader incubated at 37 °C with or without shaking (240 rpm, low shaking force) and monitoring fluorescence (440 nm excitation, 485 nm emission, 5 nm bandwidth) over 6 hours using the bottom-read mode.

Fluorescence Suppression Assay

Peptide Working Solution Preparation. A 0.02 μmol aliquot of TAMRA-labeled $A\beta_{(C1–42)}$ or FAM-labeled $A\beta_{(C1–42)}$ was dissolved in 20 μL of DMSO first and sonicated for 30 s in a water

bath, then diluted with 480 μL of 1X PBS (pH 7.4) to give a working solution with final peptide concentration of 40 μM. The peptide working solution was stored on ice and protected from light.

Fluorescence Suppression Assay Setup. Fluorescence suppression assays were conducted in Corning® 96-well half area black/clear flat bottom polystyrene nonbinding surface (NBS) microplates (product number: 3881). A 100-μL aliquot of the working solution was immediately transferred to each of five wells of 96-well plate, and the plate was kept at room temperature. 1X PBS buffer was transferred to each of five wells of 96-well plate as negative control. The 96-well plate was sealed with adhesive plate sealer. The 96-well plate was immediately inserted into a Thermo Fisher Scientific Varioskan Lux microplate reader incubated at 37°C with or without shaking (240 rpm, low shaking force). The fluorescence was monitored with 541 nm excitation, 567 nm emission, 5 nm bandwidth for TAMRA-labeled $A\beta_{(C1–42)}$ and with 494 nm excitation, 520 nm emission, 5 nm bandwidth for FAM-labeled $A\beta_{(C1–42)}$ over 6 hours using the bottom-read mode.

Transmission Electron Microscopy (TEM)

Sample Preparation. A 0.02 μmol aliquot of labeled $A\beta_{(C1–42)}$ or $A\beta_{(M1–42)}$ was dissolved in 20 μL of DMSO first, then diluted with 480 μL of 1X PBS buffer (pH 7.4) to give a 40 μM peptide solution. The peptide solutions were then incubated at 37 °C for 24 hours, while shaking at 225 rpm.

TEM Imaging. TEM images of Aβ peptides were taken with a JEM-2100F transmission electron microscope (JEOL, Peabody, MA, USA) at 200 kV with an electron dose of ca. 15 e^-/A^2. The sample was cooled at liquid nitrogen temperature through the cryostage. The images were recorded on a Gatan OneView CCD (Gatan, Pleasanton, CA, USA), at magnification of 30000x

with a pixel size of 3.7 Angstrom at specimen space. The contrast and brightness of the images were adjusted as appropriate.

Atomic Force Microscopy (AFM)

Sample Preparation. A 0.02 μmol aliquot of labeled $A\beta_{(C1–42)}$ or $A\beta_{(M1–42)}$ was dissolved in 20 μL of DMSO first, then diluted with 480 μL of 1X PBS buffer (pH 7.4) to give a 40 μM peptide solution. The peptide solutions were then incubated at 37 °C for 21 hours, while shaking at 225 rpm.

AFM Imaging. A drop of the peptide sample solution (3 μL) was deposited on freshly cleaved mica mounted on glass slides. After 5 minutes, the mica was gently rinsed once or twice with 50 μL aliquots of deionized water (18 MΩ) to remove salts. Excess liquid was blotted off by Whatman filter papers and the mica was left air-dry for an additional 5 minutes, and the samples were imaged immediately after the mica is dry. All AFM images of Aβ peptides were taken under ambient condition with an Anton Paar Tosca 400 AFM instrument. Scanning parameters were as follows: tapping mode, setpoint at 285.6 kHz which is 5% below cantilever resonance frequency, scan rate at 1.0 Hz.

Fluorescence Microscopy

SH-SY5Y Cell Preparation, Treatment, and Imaging. SH-SY5Y cells were plated in an Ibidi μ-Slide 8 Well Chamber Slide at 20,000 cells per well. Cells were incubated in 500 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 slide for 24 hours. Working solutions of FAM-Aβ and TAMRA-Aβ were

prepared immediately prior to cell treatment by adding 10 μL of 20 mM NaOH to one aliquot of 0.02 μmol lyophilized FAM-Aβ or TAMRA-Aβ. The resulting solution was diluted with 190 μL of media bringing this solution to a peptide concentration of 100 μM. An 80 μL aliquot of the 100 μM stock solution was added to 720 μL of warmed media for a final peptide concentration of 10 μM. After 24 hours, the media was removed from the wells and replaced with 200 μL of 1μg/mL Hoechst in phenol red free 1:1 DMEM:F12 media. After 30 minutes, the Hoechst-containing media was removed and replaced with 200 μL of 10 μM FAM-Aβ or TAMRA-Aβ. Control wells were treated with media containing no peptide. The slide was incubated in the microscope chamber for 0–48 hours and imaged for the duration using a Keyence BZ-X810 fluorescence microscope. Images were collected with a 60x oil immersion objective lens. Fluorescence micrographs of treated cells were recorded using the GFP filter cube [excitation wavelength = 470/40 nm (450–490 nm) and emission wavelength = 525/50 nm (500–550 nm)] for FAM-Aβ, and the TexasRed filter cube [excitation wavelength = 560/40 nm (540–580 nm) and emission wavelength = 630/75 nm (592.5–667.5 nm)] for TAMRA-Aβ. The image brightness of the FAM and TAMRA channels was adjusted using BZ-X810 Analyzer software.

RAW 264.7 Cell Preparation, Treatment, and Imaging. RAW 264.7 cells were plated in an Ibidi μ-Slide 8 Well Chamber Slide at 25,000 cells per well. Cells were incubated in 500 μL of DMEM media supplemented with 10% fetal bovine serum at 37 °C in a 5% CO_2 atmosphere and allowed to adhere to the bottom of the slide for 24 hours. Working solutions of FAM-Aβ and TAMRA-Aβ were prepared immediately prior to cell treatment by adding 10 μL of 20 mM NaOH to one aliquot of 0.02 μmol lyophilized FAM-Aβ or TAMRA-Aβ. This solution was diluted with 190 μL of media for a peptide concentration of 100 μM. An 80 μL aliquot of the 100 μM stock solution was added to 720 μL of warmed media for a final peptide concentration of 10 μM. After

24 hours, the media was removed from the wells and replaced with 200 μL of 1 μg/mL Hoechst in phenol red free 1:1 DMEM:F12 media. After 30 minutes, the Hoechst-containing media was removed and replaced with 200 μL of 10 μM FAM-Aβ or TAMRA-Aβ. Control wells were treated with media containing no peptide. The slide was incubated for 4 hours and imaged using a Keyence BZ-X810 fluorescence microscope. Images were collected with a 60x oil immersion objective lens. Fluorescence micrographs of treated cells were recorded using the GFP filter cube [excitation wavelength = 470/40 nm (450–490 nm) and emission wavelength = 525/50 nm (500–550 nm)] for FAM-Aβ, and the TexasRed filter cube [excitation wavelength = 560/40 nm (540–580 nm) and emission wavelength = 630/75 nm (592.5–667.5 nm)] for TAMRA-Aβ. The image brightness of the FAM and TAMRA channels was adjusted using BZ-X800 Analyzer software.

Bacteria Culturing, Treatment, and Imaging.[5] *B. subtilis* (ATCC 6051) and *E. coli* (ATCC 10798) were allowed to grow overnight (ca. 16 h) in Mueller-Hinton broth in a shaking incubator at 225 rpm and 37 °C. The following morning, the cultures were diluted 1:100 in the appropriate broth and were allowed to grow exponentially in a shaking incubator at 225 rpm and 37 °C. Once an OD_{600} of ca. 0.3 was achieved, 500 μL of bacteria was transferred to a sterile Eppendorf tube and the bacteria were centrifuged at 4000 rpm (1300 x G) for 5 min.

1 mg/mL DMSO stock solutions of FAM- and TAMRA-labeled Aβ were prepared by dissolving a 0.02 μmol aliquot of the lyophilized peptide (ca. 0.1 mg) in 100 μL of sterile DMSO in an autoclaved Eppendorf tube. Solutions of labeled Aβ were protected from excessive exposure to light in the imaging experiments by use of an unlit biosafety cabinet and minimizing exposure to room lights.

A 2% stock solution of agarose was prepared by adding 1.0 g of agarose into 50 mL of sodium phosphate buffer, autoclaving, and allowing the solution to cool until it completely

solidified. While the bacteria were growing in the shaking incubator, fresh 2% agarose beds were prepared to immobilize bacteria for fluorescence microscopy studies as follow: On a laboratory bench equipped with an alcohol burner (to help maintain sterility), microscope slides were gently warmed on a hot plate. While the slides were gently warming, the solidified 2% agarose solution was heated in a microwave oven until it became a homogenous liquid. Once the microscope slides were warm to the touch, a 75-µL aliquot of the molten 2% agarose solution was applied to each microscope slide, and a No. 1.5 coverslip was immediately applied gently to the drop of agarose. The assembly was allowed to set for at least 45 minutes before use.

While the bacteria were being centrifuged, 25 µg/mL solutions of FAM- and TAMRA-labeled Aβ were freshly prepared and then used immediately to stain the bacteria. The 25 µg/mL solutions were prepared by diluting 15 µL of each 1 mg/mL DMSO stock solution with 585 µL of sterile PBS. The diluted solutions were subsequently vortexed for 30 seconds and then used immediately to stain the bacteria.

After centrifuging the bacteria (see above), the supernatant was removed, the pellet was resuspended in 500 µL of 25 µg/mL of the probe solution, and the bacteria were incubated in a shaking incubator at 225 rpm, 37 °C for 2 h. The bacteria were centrifuged at 4000 rpm (1300 x G) for 5 min, and the supernatant was removed. The pellet was resuspended in 500 µL of sterile PBS, the suspension was centrifuged at 4000 rpm (1300 x G) for 5 min, and the supernatant was removed. This washing process was repeated two additional times. After the last wash, the cells were resuspended in 200−500 µL of sterile PBS.[*] On a sterile bench, the coverslip of each agarose

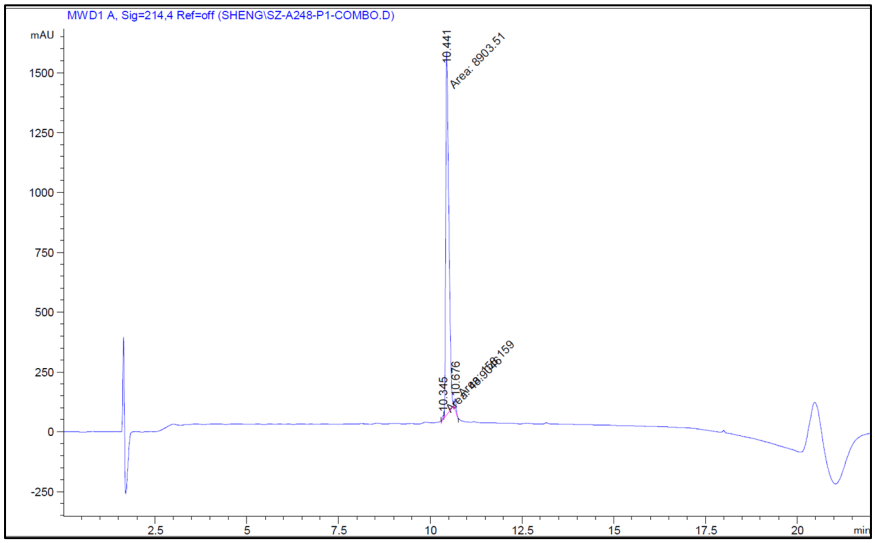
[*] The volume of phosphate buffer was selected based on the size of the pellet remaining after the washing steps.

bed was removed, and a 5-μL aliquot of the stained bacteria was applied to the coverslip. The coverslip was then sandwiched on top of the agarose bed.

The stained bacteria were immediately imaged on a Zeiss LSM 780 confocal fluorescence microscope. Images were collected with a 63x oil immersion objective lens, with additional optical zoom used as needed to provide detailed images. Fluorescence micrographs of bacteria treated with FAM-labeled Aβ were recorded with excitation at 488 nm and emission between 490–544 nm. Fluorescence micrographs of bacteria treated with TAMRA-labeled Aβ were recorded with excitation at 561 nm and emission between 568–639 nm. The image brightness of the FAM and TAMRA channels were adjusted linearly using Volocity 6.3 (Quorum Technologies), and a medium filter in the Volocity software was used to reduce noise in all channels.

Characterization Data and Additional Figures

Analytical HPLC Trace, MALDI Mass Spectrum, and MS/MS Spectrum of Aβ$_{(C1–42)}$



Signal 1: MWD1 A, Sig=214,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.345	MM	0.0500	48.90459	16.30959	0.5368
2	10.441	MM	0.0972	8903.51074	1526.55994	97.7272
3	10.676	MM	0.0565	158.15851	46.64415	1.7360
Totals :				9110.57384	1589.51367	

Figure S7. Representative analytical HPLC trace of Aβ$_{(C1–42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm. Purity: = 97.7%.

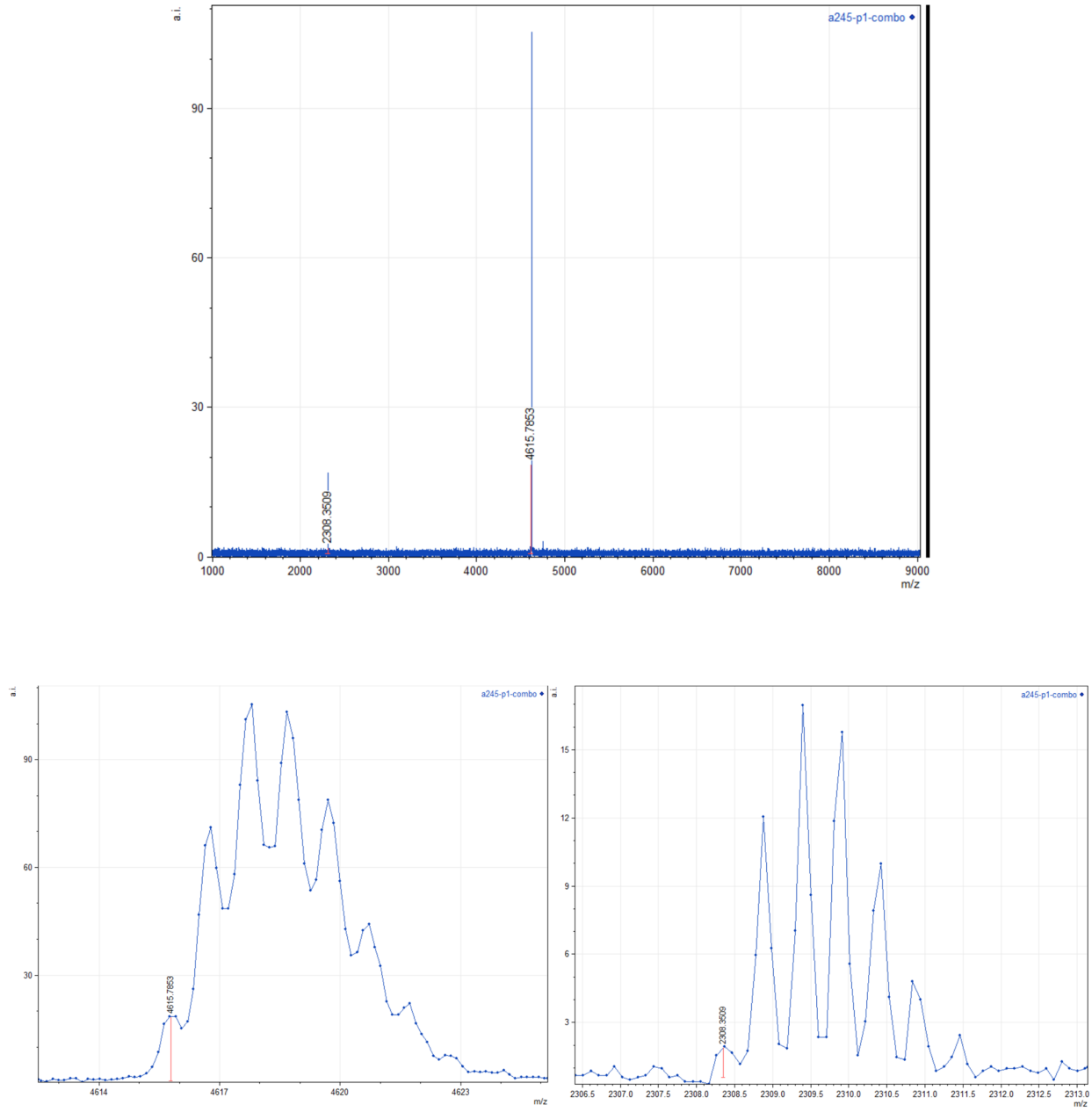
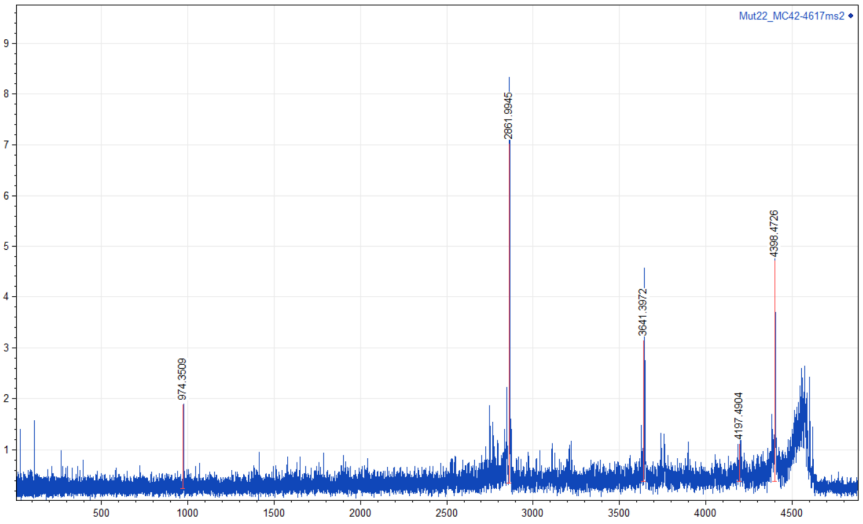


Figure S8. Representative MALDI mass spectrum of Aβ(C1–42). Positive reflector mode; Matrix: 2,5-dihydroxybenzoic acid. Exact mass calculated for M^+: 4614.3; Exact mass calculated for $[M+H]^+$: 4615.3; Exact mass calculated for $[M+2H]^{2+}$: 2308.1. Observed $[M+H]^+$: 4615.8; Observed $[M+2H]^{2+}$: 2308.4.



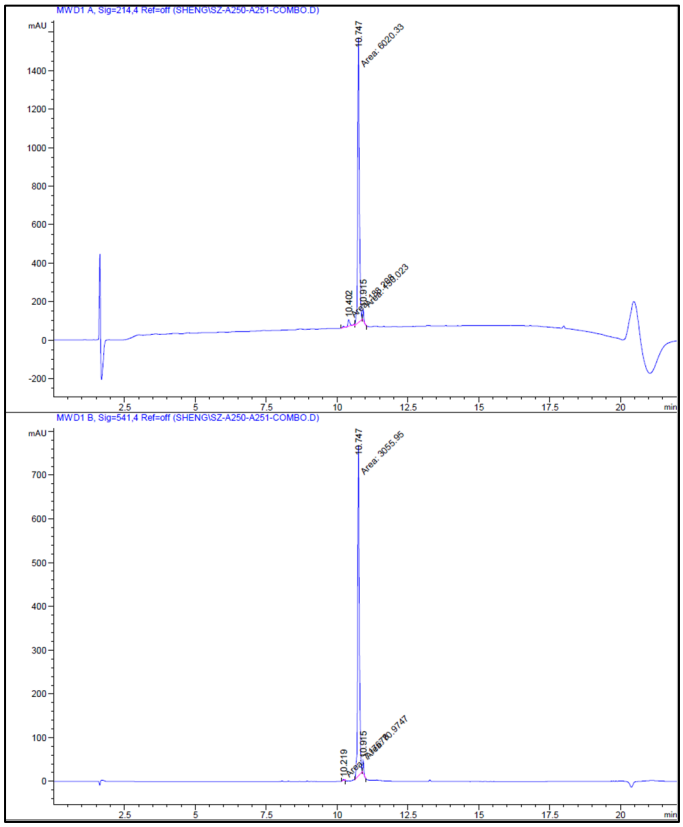
Sequence: **CDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA**, pI: **5.31109**

Fragment Ion Table, monoisotopic masses

Seq	#	B	Y	# (+1)
C	1	104.01651	4615.28610	43
D	2	219.04345	4512.27691	42
A	3	290.08056	4397.24997	41
E	4	419.12316	4326.21286	40
F	5	566.19157	4197.17027	39
R	6	722.29268	4050.10185	38
H	7	859.35159	3894.00074	37
D	8	974.37853	3756.94183	36
S	9	1061.41056	3641.91489	35
G	10	1118.43203	3554.88286	34
Y	11	1281.49535	3497.86139	33
E	12	1410.53795	3334.79807	32
V	13	1509.60636	3205.75547	31
H	14	1646.66527	3106.68706	30
H	15	1783.72418	2969.62815	29
Q	16	1911.78276	2832.56924	28
K	17	2039.87772	2704.51066	27
L	18	2152.96179	2576.41570	26
V	19	2252.03020	2463.33163	25
F	20	2399.09861	2364.26322	24
F	21	2546.16703	2217.19481	23
A	22	2617.20414	2070.12639	22
E	23	2746.24673	1999.08928	21
D	24	2861.27368	1870.04669	20
V	25	2960.34209	1755.01974	19
G	26	3017.36355	1655.95133	18
S	27	3104.39558	1598.92987	17
N	28	3218.43851	1511.89784	16
K	29	3346.53347	1397.85491	15
G	30	3403.55494	1269.75995	14
A	31	3474.59205	1212.73848	13
I	32	3587.67611	1141.70137	12
I	33	3700.76018	1028.61731	11
G	34	3757.78164	915.53324	10
L	35	3870.86570	858.51178	9
M	36	4001.90619	745.42772	8
V	37	4100.97460	614.38723	7
G	38	4157.99607	515.31882	6
G	39	4215.01753	458.29735	5
V	40	4314.08594	401.27589	4
V	41	4413.15436	302.20748	3
I	42	4526.23842	203.13906	2
A	43	4597.27554	90.05500	1

Figure S9. Representative MS/MS spectrum of Aβ(C1–42). MS/MS analysis was performed using CID fragmentation of the precursor mass 4617 ± 2 Da. The B&Y fragment ion was calculated with fragment ion calculators. The peptide sequence was determined to be CDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA.

Analytical HPLC Trace and MALDI Mass Spectrum of 5-TAMRA-Aβ(C1-42)



Signal 1: MWD1 A, Sig=214,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.402	MM	0.0923	188.20799	33.99847	2.9599
2	10.747	MM	0.0674	6020.33154	1488.95044	94.6807
3	10.915	MM	0.0440	150.02261	56.76593	2.3594
Totals :				6358.56215	1579.71484	

Signal 2: MWD1 B, Sig=541,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.219	MM	0.0480	7.17678	2.49154	0.2290
2	10.747	MM	0.0670	3055.95190	759.94574	97.5064
3	10.915	MM	0.0416	70.97472	28.44521	2.2646
Totals :				3134.10341	790.88250	

Figure S10. Representative analytical HPLC trace of 5-TAMRA-Aβ(C1-42). HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm (top) and 541 nm (bottom). Purity: = 94.7%.

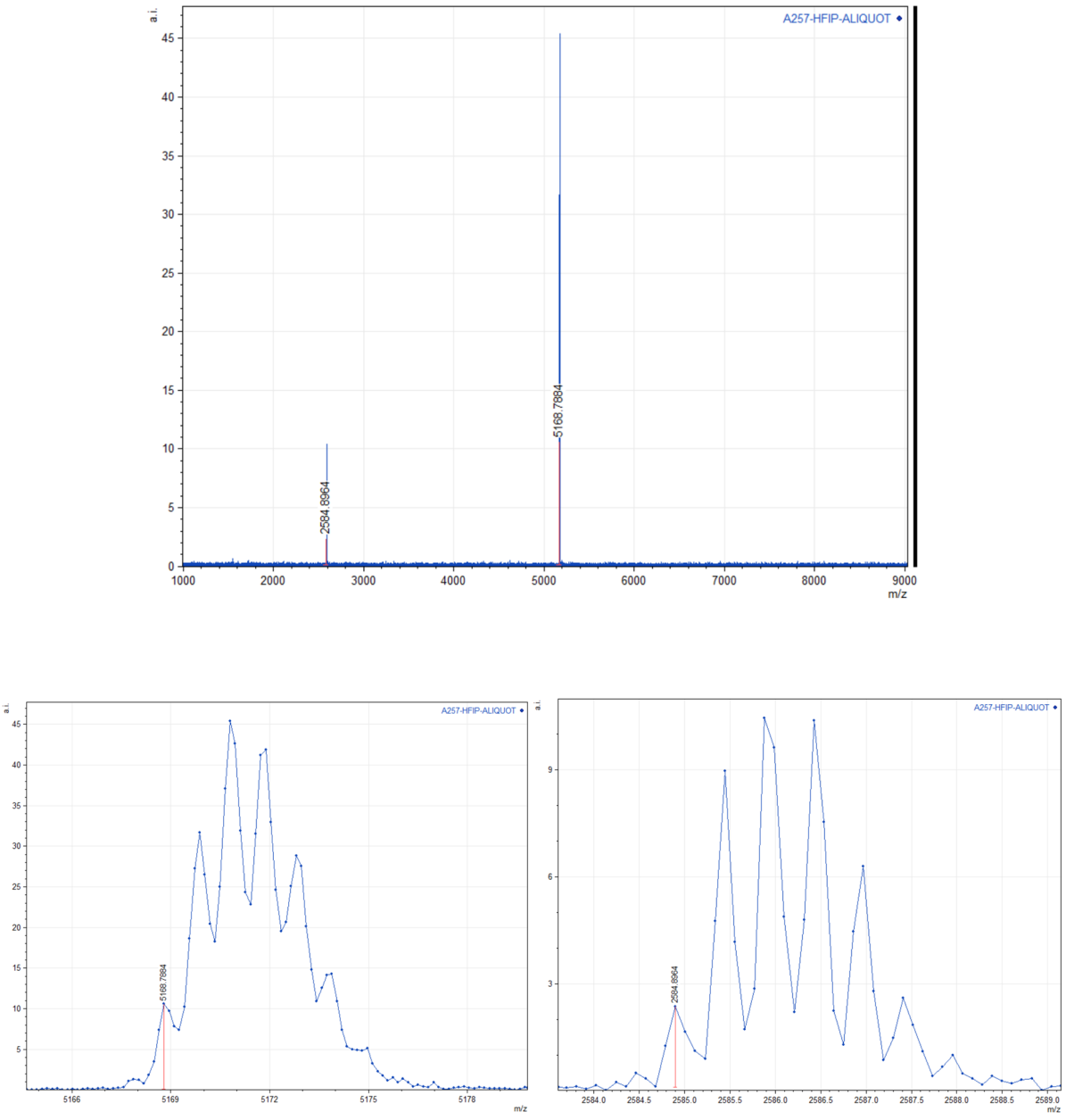
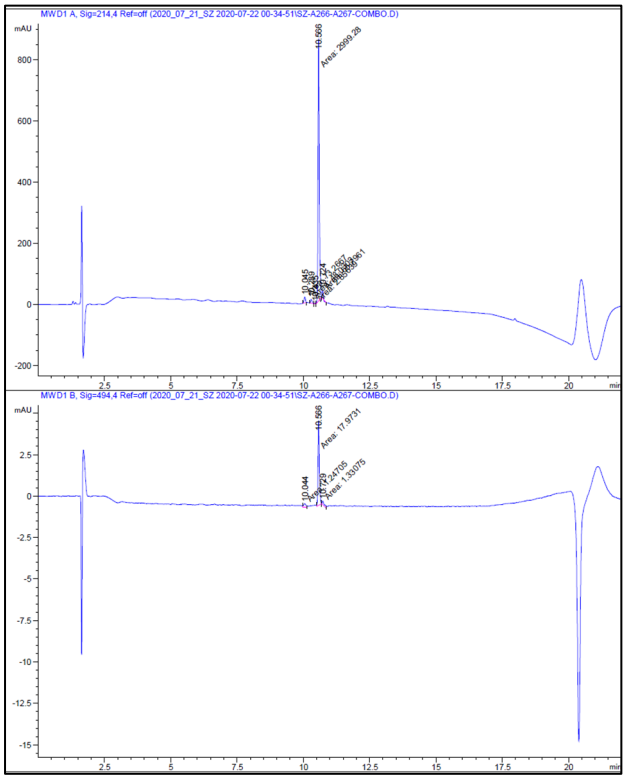


Figure S11. Representative MALDI mass spectrum of 5-TAMRA-Aβ(C1-42). Positive reflector mode; Matrix: 2,5-dihydroxybenzoic acid. Exact mass calculated for M^+: 5166.5; Exact mass calculated for $[M+H]^+$: 5167.5; Exact mass calculated for $[M+2H]^{2+}$: 2584.2. Observed $[M+H]^+$: 5168.8; Observed $[M+2H]^{2+}$: 2584.9.

Analytical HPLC Trace and MALDI Mass Spectrum of 6-FAM-Aβ$_{(C1-42)}$



Signal 1: MWD1 A, Sig=214,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.045	MM	0.0628	73.26669	19.44060	2.2973
2	10.289	MM	0.0602	48.03032	13.30617	1.5060
3	10.435	MM	0.0371	2.85639	1.28238	0.0896
4	10.566	MM	0.0577	2999.27979	866.74207	94.0440
5	10.724	MM	0.0422	65.79608	25.96672	2.0631
Totals :				3189.22927	926.73793	

Signal 2: MWD1 B, Sig=494,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.044	MM	0.1043	1.24705	1.99304e-1	6.0681
2	10.566	MM	0.0582	17.97314	5.15007	87.4565
3	10.729	MM	0.1004	1.33075	2.20823e-1	6.4754
Totals :				20.55094	5.57020	

Figure S12. Representative analytical HPLC trace of 6-FAM-Aβ$_{(C1-42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm (top) and 494 nm (bottom). Purity: = 94.0%.

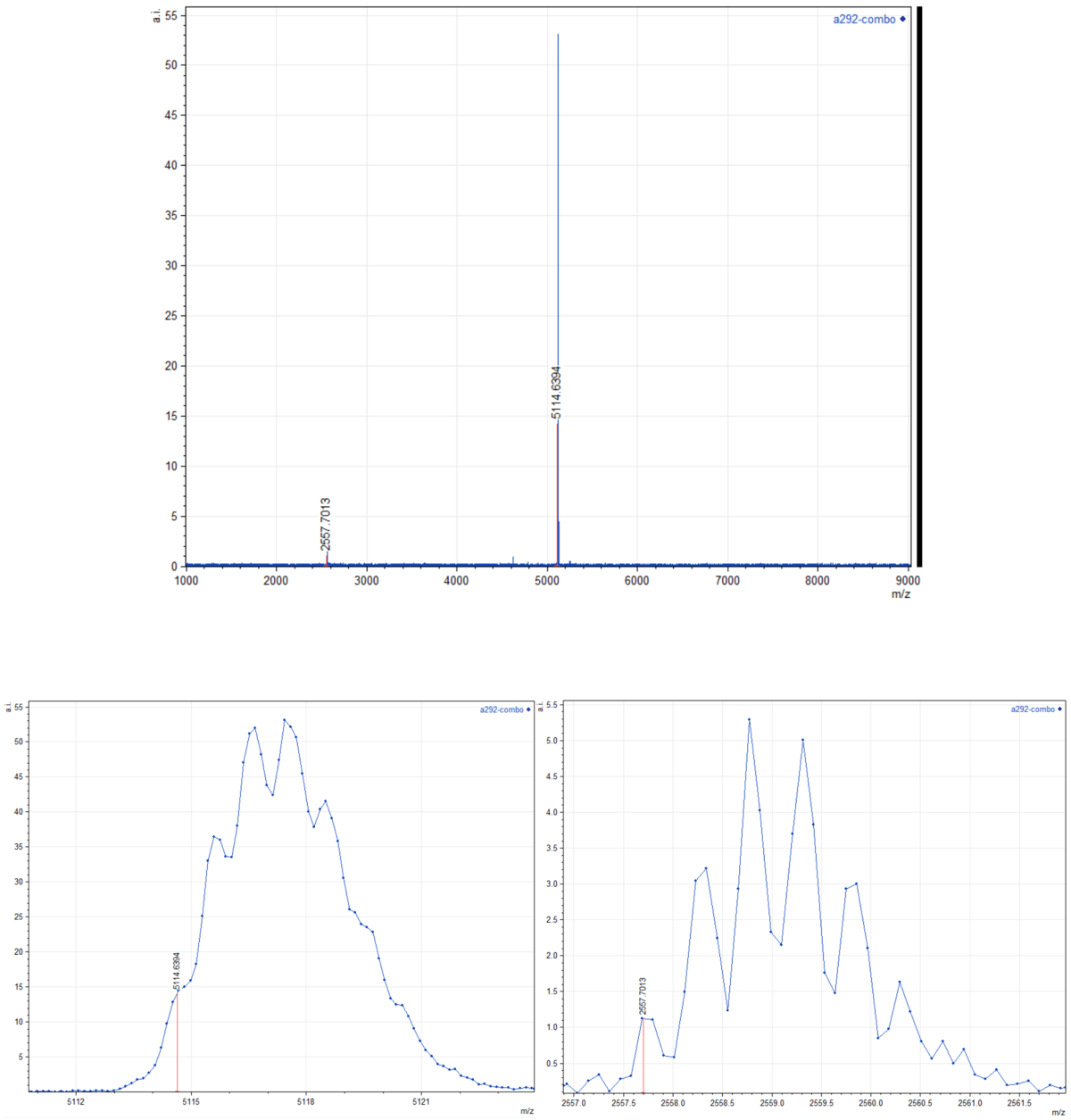
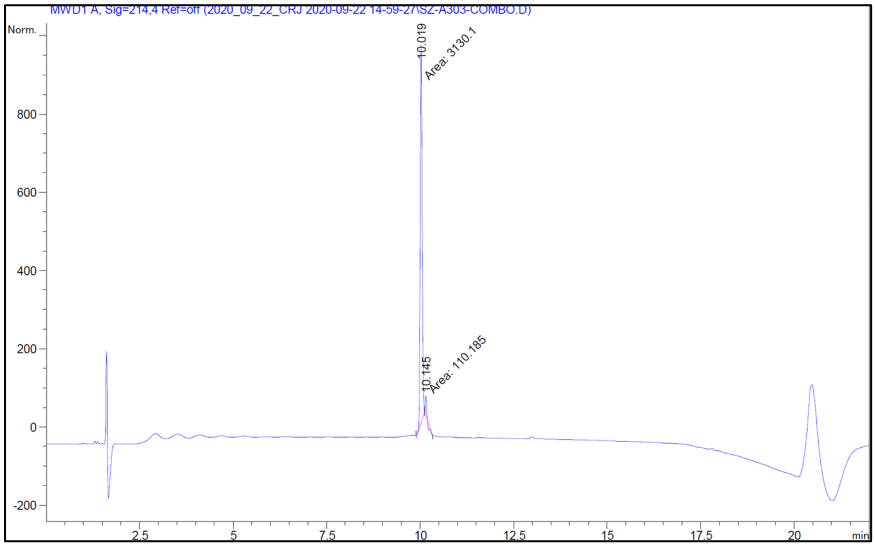


Figure S13. Representative MALDI mass spectrum of 6-FAM-Aβ(C1-42). Positive reflector mode; Matrix: 2,5-dihydroxybenzoic acid. Exact mass calculated for M^+: 5112.4; Exact mass calculated for $[M+H]^+$: 5113.4; Exact mass calculated for $[M+2H]^{2+}$: 2557.2. Observed $[M+H]^+$: 5114.6; Observed $[M+2H]^{2+}$: 2557.7.

Analytical HPLC Trace and MALDI Mass Spectrum of PEG_2-Biotin-$A\beta_{(C1-42)}$



Signal 1: MWD1 A, Sig=214,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.019	MM	0.0595	3130.10132	876.57098	96.5995
2	10.145	MM	0.0403	110.18530	45.62219	3.4005
Totals :				3240.28662	922.19318	

Figure S14. Representative analytical HPLC trace of PEG_2-biotin-$A\beta_{(C1–42)}$. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes, and absorbance was monitored at 214 nm. Purity: = 96.6%.

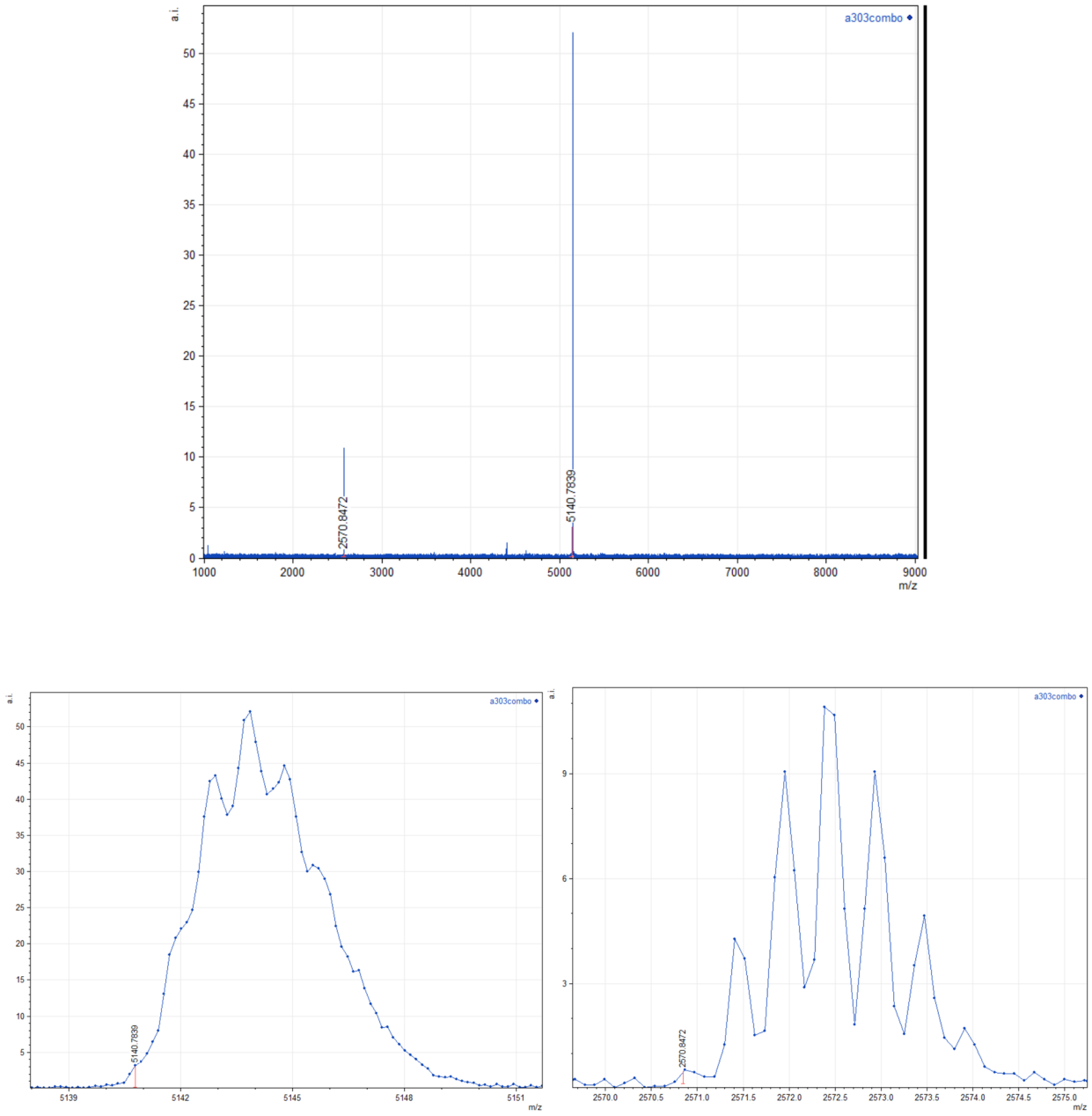


Figure S15. Representative MALDI mass spectrum of PEG_2-biotin-$A\beta_{(C1-42)}$. Positive reflector mode; Matrix: 2,5-dihydroxybenzoic acid. Exact mass calculated for M^+: 5139.5; Exact mass calculated for $[M+H]^+$: 5140.5; Exact mass calculated for $[M+2H]^{2+}$: 2570.8. Observed $[M+H]^+$: 5140.8; Observed $[M+2H]^{2+}$: 2570.8.

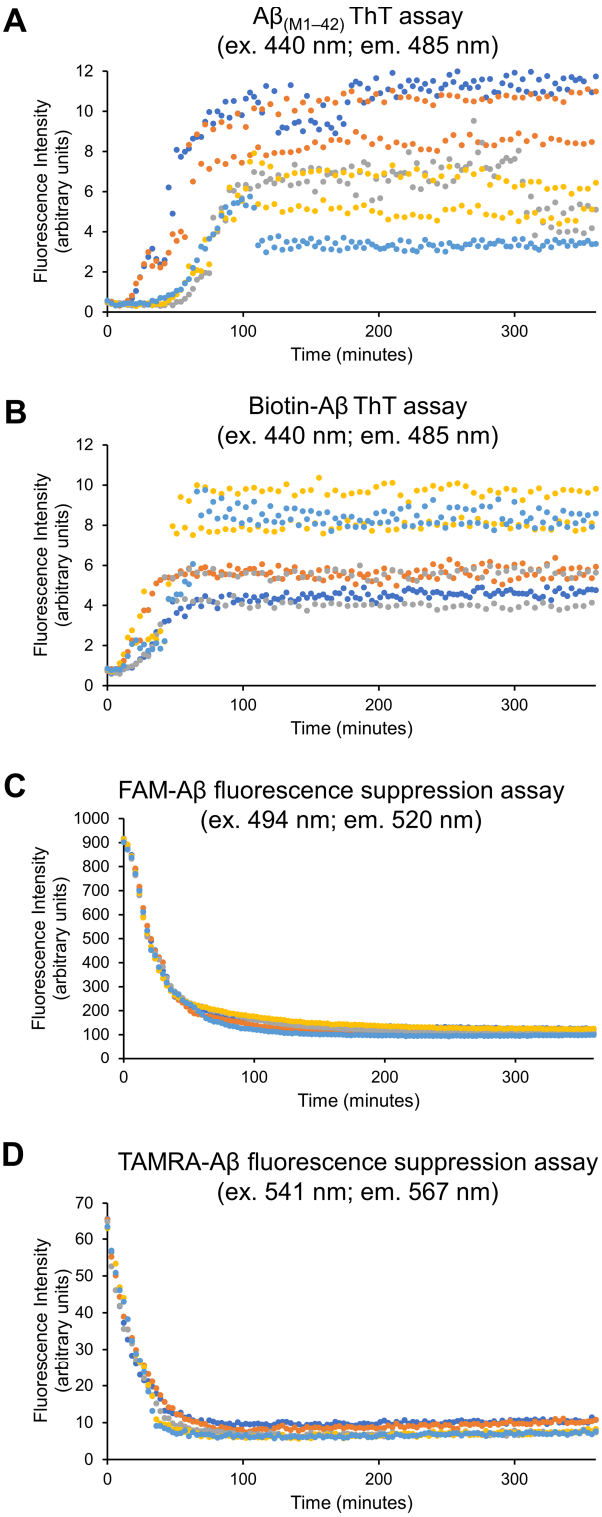


Figure S16. ThT fluorescence assays and fluorescence suppression assays of labeled Aβ(C1–42) and Aβ(M1–42). (A and B) ThT fluorescence assays of Aβ(M1–42) and biotin-labeled Aβ (40 μM peptide, 40 μM ThT, 37 °C, 240 rpm shaking, 6 hours). (C and D) Fluorescence suppression assays of FAM-labeled Aβ and TAMRA-labeled Aβ (40 μM peptide, 37 °C, 240 rpm shaking, 6 hours). These assays were performed in five replicates (light blue, dark blue, orange, yellow, and gray).

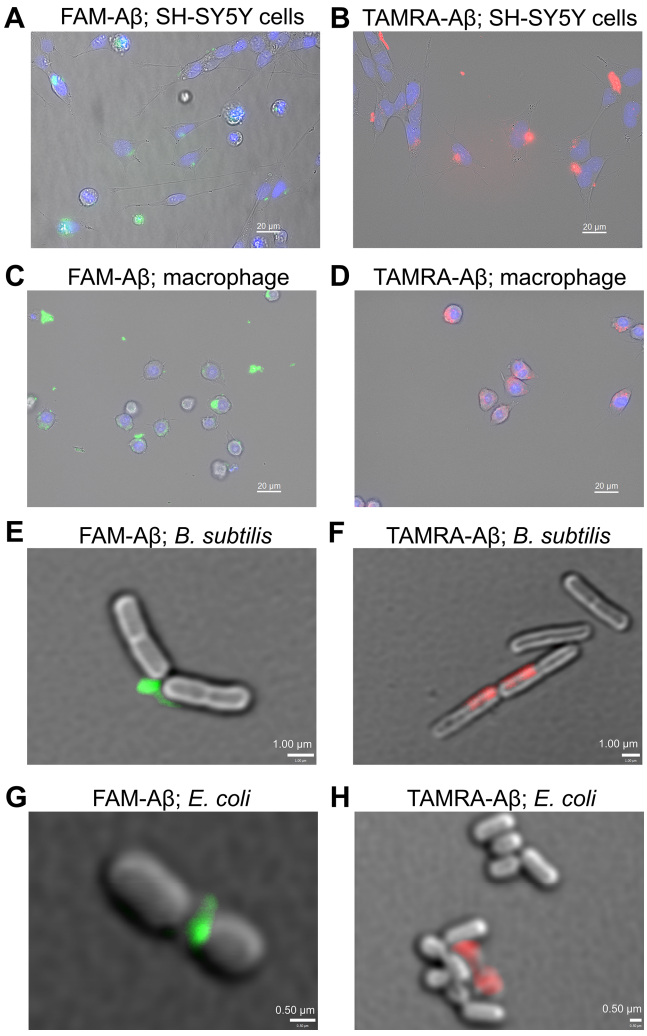


Figure S17. Fluorescence micrographs of labeled Aβ with mammalian cells (low magnification views). (A and B) SH-SY5Y neuroblastoma cells treated with FAM- and TAMRA-labeled Aβ (10 μM peptides, 37 °C, 48 and 3 hours, respectively). Nuclei are shown in blue through DAPI staining. (C and D) RAW 264.7 macrophage cells treated with FAM- and TAMRA-labeled Aβ (10 μM peptides, 37 °C, 4 hours).

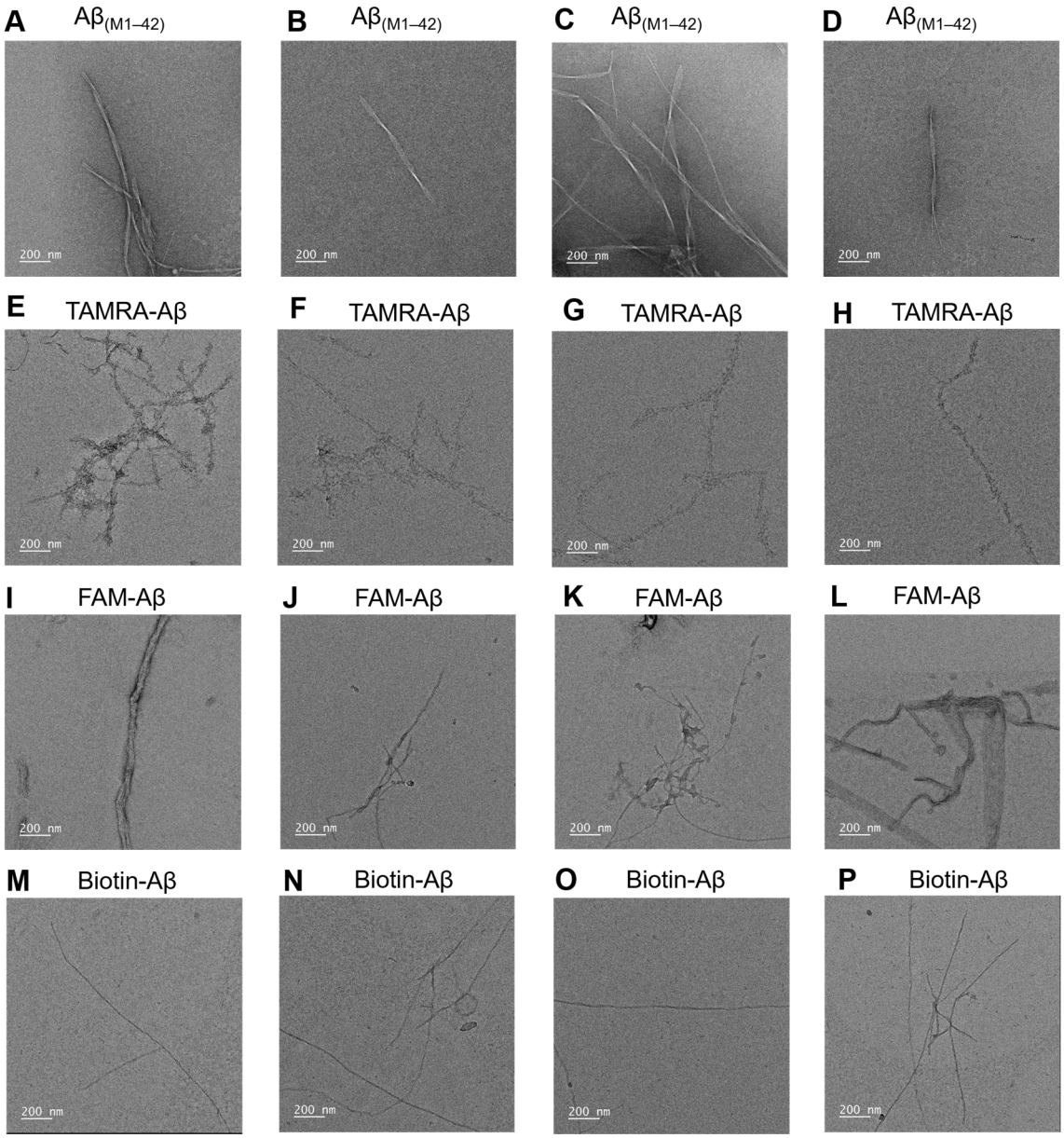


Figure S18. Transmission electron micrographs of the fibrils formed by Aβ peptides: (A–D) $Aβ_{(M1–42)}$; (E–H) TAMRA-labeled Aβ; (I–L) FAM-labeled Aβ; (M–P) Biotin-labeled Aβ. Fibrils were formed by incubating 40 μM of each peptide in PBS buffer (pH 7.4, 37 °C, 1 day, 225 rpm shaking).

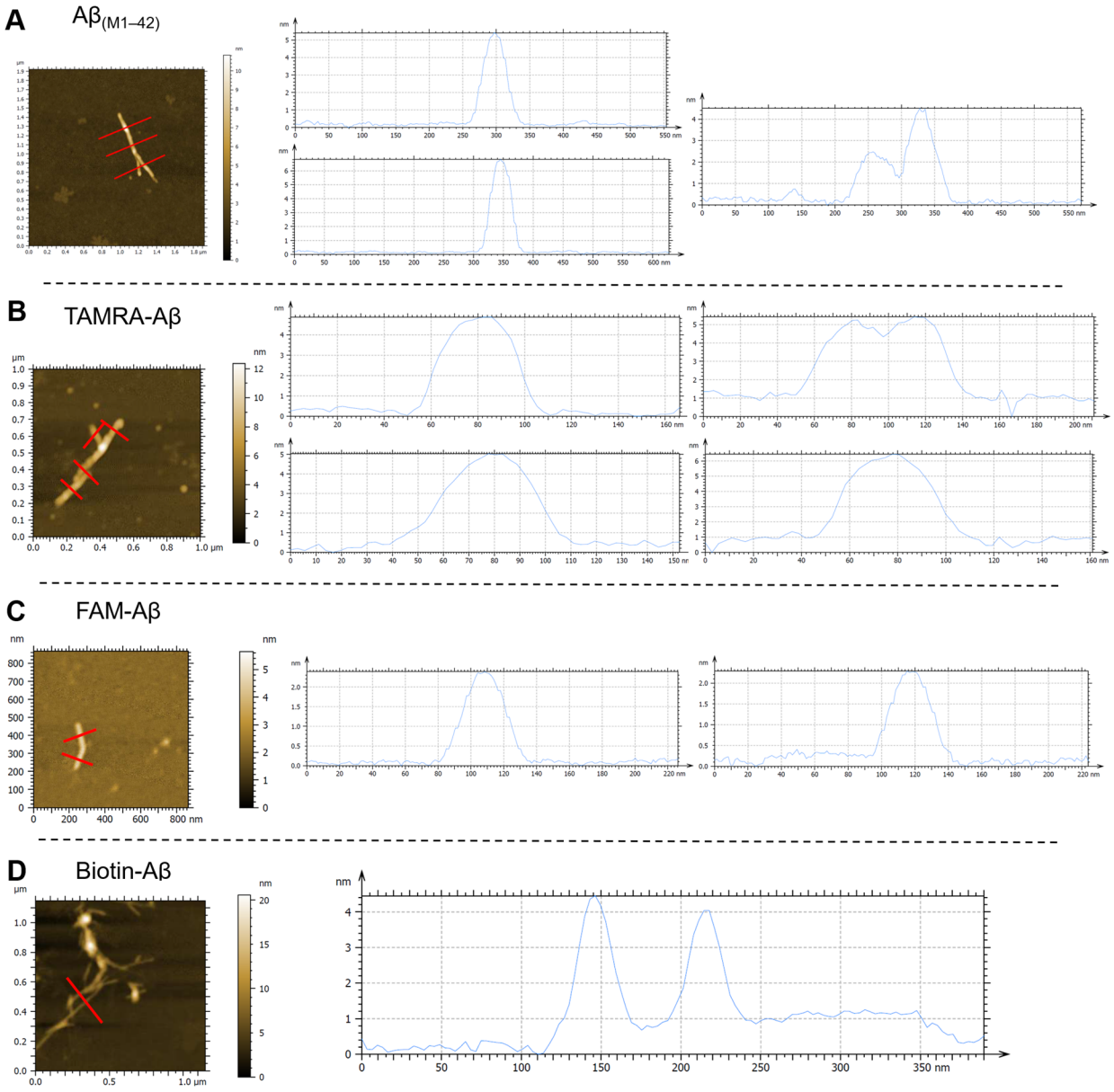


Figure S19. Atomic force micrographs of the fibrils formed by Aβ peptides: (A) $Aβ_{(M1–42)}$ AFM image and fibril height measurement data, fibril height ca. 4.5 nm; (B) TAMRA-labeled Aβ AFM image and fibril height measurement data, fibril height ca. 5.2 nm; (C) FAM-labeled Aβ AFM image and fibril height measurement data, fibril height ca. 2.3 nm; (D) Biotin-labeled Aβ AFM image and fibril height measurement data, fibril height ca. 4.1 nm. Fibrils were formed by incubating 40 μM of each peptide in PBS buffer (pH 7.4, 37 °C, 1 day, 225 rpm shaking). The red bars indicate the locations where the heights of the fibrils were measured.

References and Notes

1. The molecular cloning, protein expression, protein purification, and SDS-PAGE procedures in this section are adapted from and in some cases taken verbatim from Yoo, S.; Zhang, S.; Kreutzer, A. G.; Nowick, J. S. An Efficient Method for the Expression and Purification of Aβ(M1-42). Biochemistry **2018**, *57* (26), 3861–3866. https://doi.org/10.1021/acs.biochem.8b00393.

2. Addgene, pET-Sac-Abeta(MC1-42), Plasmid #127151. https://www.addgene.org/127151/.

3. Addgene, pET-Sac-Abeta(M1-42), Plasmid #71875. https://www.addgene.org/71875/.

4. Walsh, D. M.; Thulin, E.; Minogue, A. M.; Gustavsson, N.; Pang, E.; Teplow, D. B.; Linse, S., A facile method for expression and purification of the Alzheimer's disease-associated amyloid beta-peptide. FEBS J. **2009**, *276* (5), 1266-81. https://doi.org/10.1111/j.1742-4658.2008.06862.x.

5. Bacteria imaging protocol was adapted from: Morris, M. A.; Malek, M.; Hashemian, M. H.; Nguyen, B. T.; Manuse, S.; Lewis, K.; Nowick, J. S. A Fluorescent Teixobactin Analogue. ACS Chem. Biol. **2020**, *15* (5), 1222–1231. https://doi.org/10.1021/acschembio.9b00908.