Supporting Information

Stabilizing the monomeric amyloid-β peptide by Tyrocidine A prevents and reverses the amyloideogenesis without the accumulation of oligomers

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Contents

1. Supplementary Results

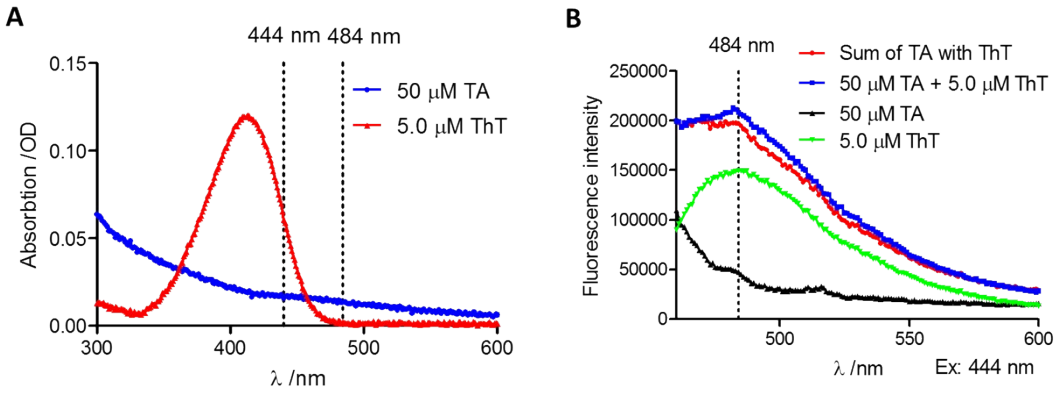


Figure S1. ThT (10 mM in pH 7.4 PBS) and TA (20 mM in ethylene glycol) were diluted with phosphate buffer saline (50 mM, pH 7.4) for the indication concentration. *A*, The absorbtion of UV spectra (300-600 nm) for TA (50 μM) and ThT (5.0 μM) recorded by ultraviolet spectrophotometer (UV 2450). *B*, The fluorescence spectra (Ex: 444 nm) of TA (50 μM), ThT (5.0 μM) and the mix solution of TA and ThT recorded by Spectrofluorometer (Fluoromax-4). The sum of TA with ThT is the addition of fluorescence intensity of TA and ThT respectively.

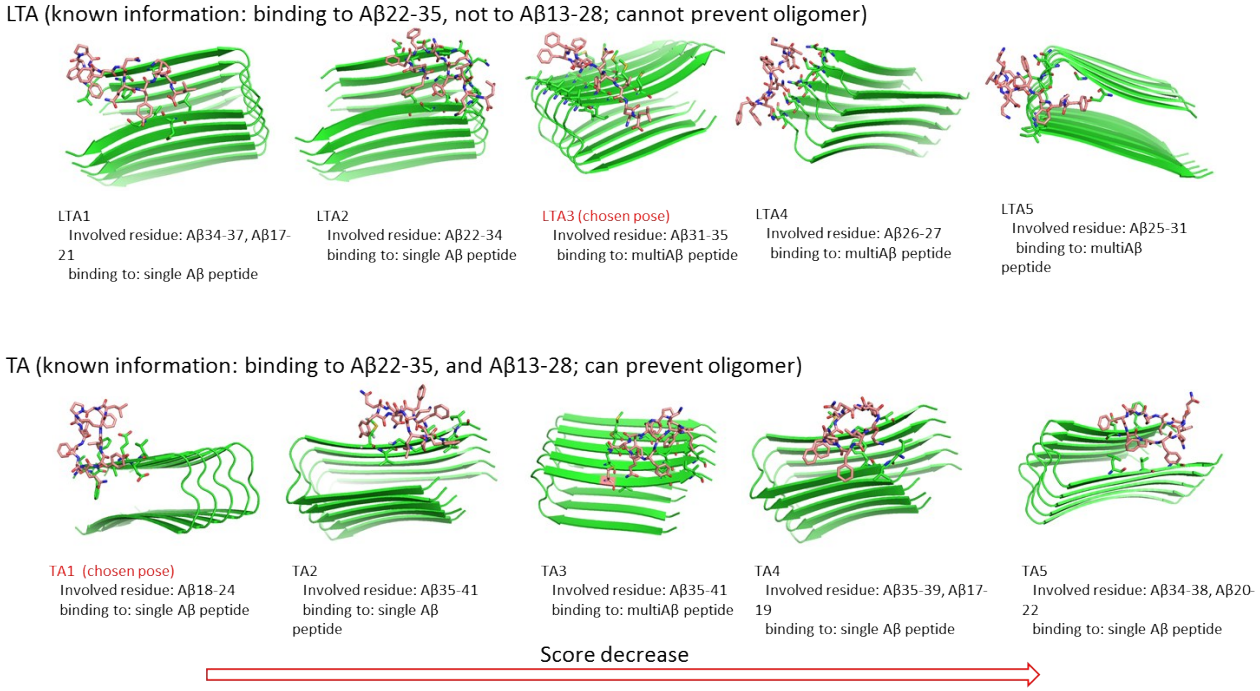


Figure S2. Rational decision based on the scores combined with obtained data. The top 5 high-scored poses of TA and LTA along with their binding features were shown.

2. Supplementary Methods

2.1 General

All commercial reagents and solvents were purchased from Sigma-Aldrich and were used without further purification or distillation. Resins, protected amino acids, and coupling regents were purchased from GL Biochem (Shanghai, China). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Burker BioSpin Ultrashield 600 NMR system. The Purities of peptides used for biological evaluation (>95%) were determined on a DIONEX Ultimate 3000 HPLC system (Chromeleon SR9 Build 2673): column, SHISEIDO C18，5 μm，4.6 × 250 mm, detection by UV (214 nm). Separation conditions were: For TA, 1.0 mL min^{-1} flow rate, a linear gradient of 20 to 80% A in 30 min, 80 to 100% A in another 5 min, washed with 100% A for 5 min, and then calibrated at 20% A for 10 min; For LTA, 1.0 mL min^{-1} flow rate, a linear gradient of 5 to 95% A in 25 min, 95 to 100% A in another 5 min, washed with 100% A for 5 min, and then calibrated at 5% A for 10 min. Solution A: 0.1% TFA in acetonitrile. Unless otherwise indicated, analytical stock solutions (20 mM) of TA and LTA were prepared in DMSO and diluted to the indication concentration, and the final concentration of DMSO is adjusted to the same concentration (0.125%) during assay. Unless otherwise indicated, analytical stock solutions (10 mM) of ThT were prepared in phosphate buffer saline (50 mM, pH 7.4).

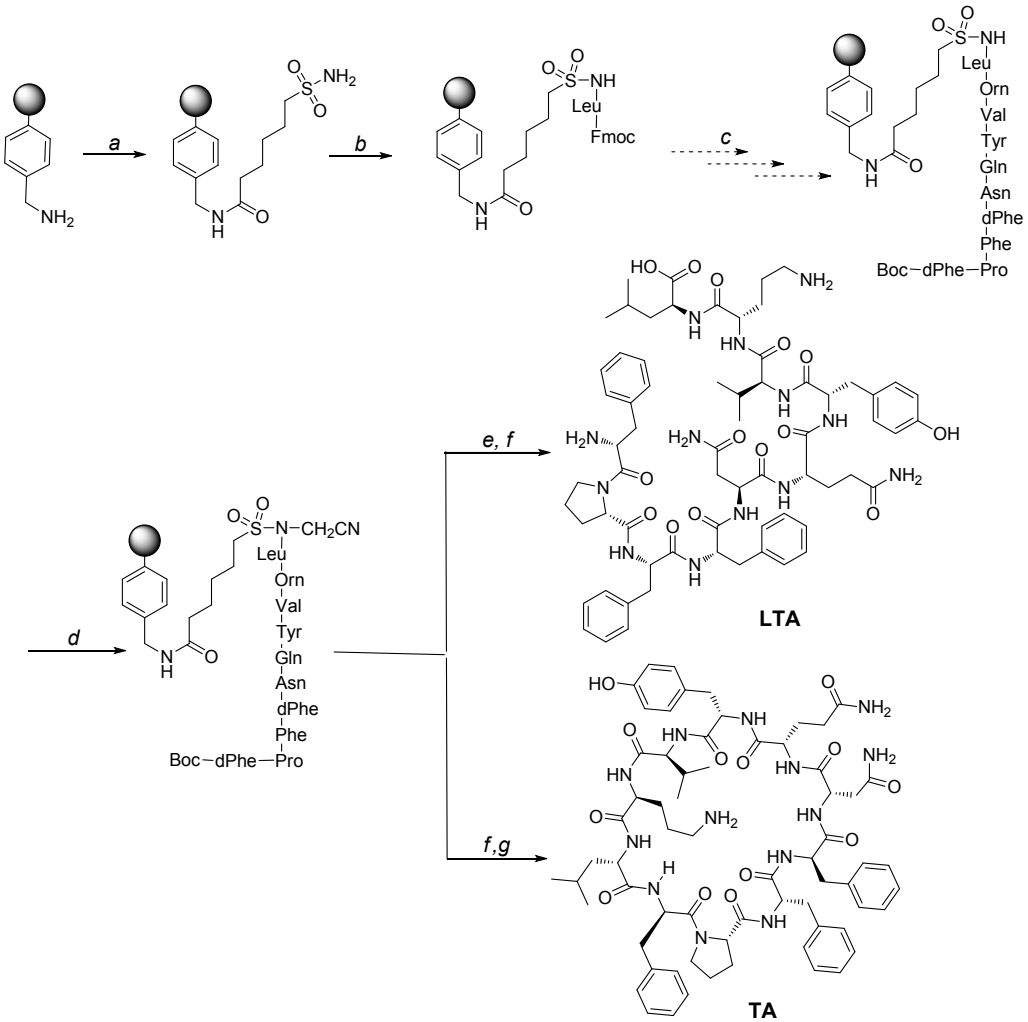
2.2 Synthesis of peptides

2.2.1 Solid-phase synthesis of Linear Tyrocidine A (LTA) and Tyrocidine A (TA)

LTA and TA was synthesized using standard Fmoc solid phase synthesis upon safety catch resin, and a spontaneous cyclization approach was adopted to produce the final products (Scheme S1).

Preparation of sulfonamide resin: Prior to the first coupling step, aminomethyl Resin (AM Resin, loading value 1.26, 200 mg, 1.0 *eq.*) was swollen in DMF for 30 min. The resin was added to a 25 mL round-bottle flask with N, N-diisopropylcarbodiimide (DIC, 120 mg, 3.0 *eq*.), N-hydroxybenzotriazole (HOBt, 127 mg, 3.0 eq), 6-sulfamoylhexanoic acid (183 mg, 3.0 *eq*.) and DMF (5.0 mL). The reaction was gently agitated for 3 h, and washed with DMF (5 × 5.0 mL). The coupling step was repeated once and washed with DMF (5 × 5.0 mL).

Attachment of the first amino acid: The synthetic sulfonamide resin was added to a 25 mL round-bottle flask. A solution of Fmoc-Leu-OH (553 mg, 5.0 *eq.*), N,N-Diisopropylethylamine (DIEA, 404 mg, 10 *eq.*) and $CHCl_3$ (5.0 mL) was added to the resin and stirred at room temperature under N_2 protection for 20 min, and then cooled to -20°C. The PyBOP (814 mg, 5.0 *eq.*) was added, and then the mixture was stirred at -20°C for 12 h. The solution was drained and washed with $CHCl_3$ (5 × 5.0 mL) and DMF (5 × 5.0 mL). The resin loading value was determined as 0.8 mmol/g by UV analysis of Fmoc cleavage product. A mixture of acetic anhydride/pyridine (1:1, 5.0 mL) was added to the resin for 2 h to cap the unreacted resin, and then wash with DMF (5 × 5.0 mL).



Scheme S1: General synthetic approach for LTA and TA. Reagents and conditions: *a*, 6-sulfamoylhexanoic acid, DIC/HOBt, 3 h; *b*, Fmoc-Leu-OH, PyBOP/DIEA, -20°C, 12 h; *c,* Standard Fmoc/tBu SPPS. Deprotection: 20% piperidine in DMF, 30 min; coupling: Fmoc-amino acid (Boc-dPhe-OH for the last residue), DIC, HOBt, 2 h; *d,* ICH_2CN, DIEA/NMP, dark, 24 h; *e*, 1N NaOH in 50% THF; *f,* CF_3COOH: phenol: *i*-Pr_3SiH: H_2O = 88:5:5:2, 3 h; *g,* DIEA/THF 6 h.

General methods for solid phase synthesis: Standard Fmoc chemistry was used in the solid phase synthesis. Leu, Orn, Val, Tyr, Gln, Asn, dPhe, Phe, and Pro were used with the following prective form respectively: Fmoc-Leu-OH, Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Tyr(*t*-Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-dPhe-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, while the last dPhe was used with Boc-dPhe-OH. In the coupling step, the solution of the Fmoc-protected amino acid (3.0 eq), DIC (3.0 eq) and HOBt (3.0 eq) was added and the mixture was gently agitated for 2 h. After the resin was washed with DMF (5 × 5.0 mL), it was subjected to Kaiser test. In the Fmoc deprotection step, the solution of 20% piperidine was added to the above prepared resin (200.0 mg, loading value: 0.8 mmol/g), agitated for 30 min by bubbling in N_2, and then washed with DMF (5 × 5.0 mL).

Activation of the carboxyl terminus: The resin was washed with N-Methyl pyrrolidone (NMP, 5 × 5.0 mL) and then the a solution of NMP (5.0 mL), DIEA (15 *eq.*) and iodoacetonitrile (10 *eq.*) was added to the resin. The mixture stirred in a 25.0 mL round-bottle flask wrapped in aluminum foil for

24 h. Subsequently, the resin was washed sequentially with NMP (5 × 5.0 mL), DMF (5 × 5.0 mL) and CH_2Cl_2 (5 × 5.0 mL), and then dried in vacuo.

Removal of protective groups: Freshly prepared 5.0 mL Reagent B (CF_3COOH: phenol: *i*-Pr_3SiH: H_2O = 88: 5: 5: 2) was added to the activated resin and agitated for 3 h. Afterwards, the resin was washed with DMF (5 × 5.0 mL), MeOH (5 × 5.0 mL) and THF (5 × 5.0 mL).

Cleavage and separation for LTA: LTA was cleaved from a half of the resin with 1N NaOH in 5.0 mL 50% THF for 12 h after the activation of the carboxyl terminus. The crude product was treated with Reagent B to remove the protective groups and then precipitated by cold ether, and finally purified by reverse-phase HPLC (linear gradient of 20~50% MeCN with 0.1% TFA) yielding a white powder after lyophilization.

On-resin cyclization for TA: The other resin was first treated with the Reagent B to remove all protective groups, then was immersed in 5.0 mL of 20% (v/v) DIEA in THF for 6 h at room temperature and then separated from the aqueous solution. The resin was washed with DMF (5 × 5.0 mL). The aqueous and washing solution were combined and dried in vacuo to obtain white crude cyclic product TA. All the crude product was purified by reverse-phase HPLC (linear gradient of 50~70% MeCN with 0.1% TFA) yielding a white powder after lyophilization.

LTA, DPhe-Pro-Phe-DPhe-Asn-Gln-Tyr-Val-Orn-Leu: 20.1% yield as a white powder. HPLC purity: 95.26%, T_R=14.39 min. ^{1}H NMR (600 MHz, MeOD): δ 8.39-7.80 (signal of amide protons) δ 7.43 – 7.38 (m, 2H), 7.38 – 7.34 (m, 1H), 7.35 – 7.30 (m, 2H), 7.30 – 7.24 (m, 4H), 7.24 – 7.20 (m, 3H), 7.17 (t, *J* = 8.2 Hz, 3H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 2H), 5.10 – 5.04 (m, 1H), 4.97 – 4.91 (m, 1H), 4.73 – 4.68 (m, 1H), 4.62 – 4.50 (m, 3H), 4.39 (dd, *J* = 9.4, 5.1 Hz, 1H), 4.30 (t, *J* = 8.6 Hz, 1H), 4.20 (dd, *J* = 9.1, 4.3 Hz, 1H), 4.11 – 4.06 (m, 1H), 3.61 – 3.54 (m, 1H), 3.32 – 3.24 (m, 2H), 3.23 – 3.17 (m, 1H), 3.17 – 3.10 (m, 2H), 3.08 – 3.01 (m, 3H), 3.01 – 2.96 (m, 2H), 2.95 – 2.90 (m, 1H), 2.90 – 2.82 (m, 1H), 2.68 – 2.59 (m, 1H), 2.19 – 2.06 (m, 2H), 2.01 – 1.89 (m, 2H), 1.90 – 1.79 (m, 3H), 1.79 – 1.69 (m, 3H), 1.68 – 1.53 (m, 3H), 1.53 – 1.44 (m, 1H), 1.44 – 1.36 (m, 2H), 1.05 (d, *J* = 3.9 Hz, 3H), 1.03 (d, *J* = 3.9 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 176.88, 173.76, 172.70, 172.55, 172.48, 172.38, 172.14, 171.94, 171.61, 171.08, 170.86, 170.62, 155.78, 137.67, 137.49, 135.52, 129.59, 129.29, 129.27, 128.75, 128.28, 127.99, 127.94, 127.80, 127.18, 126.43, 126.37, 114.87, 60.11, 58.23, 57.15, 55.36, 54.83, 53.90, 53.48, 51.32, 51.05, 49.66, 46.30, 41.77, 40.09, 39.01, 37.54, 36.96, 36.78, 35.22, 31.95, 31.47, 30.28, 28.40, 25.50, 24.99, 23.02, 22.55, 22.01, 21.62, 18.30, 17.85. HRMS: calcd for $C_{66}H_{89}N_{13}O_{14}$ $[M+2H]^{2+}$ m/z 644.8451, found, 644.8399.

TA, cyclo-(DPhe-Pro-Phe-DPhe-Asn-Gln-Tyr-Val-Orn-Leu): 12.9% yield, white powder. HPLC purity: 96.03%, T_R=22.08 min. ^{1}H NMR (600 MHz, MeOD): δ 9.45-7.5 (signal of amide protons) δ 7.35 (d, *J* = 7.4 Hz, 2H), 7.32 – 7.26 (m, 5H), 7.23 (m, 6H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.18 – 7.14 (m, 1H), 6.86 (d, *J* = 7.7 Hz, 2H), 6.49 (d, *J* = 7.6 Hz, 2H), 5.87 (t, *J* = 9.4 Hz, 1H), 5.51 (m, 1H), 4.95 (m, 2H), 4.70 (s, 1H), 4.67 – 4.61 (m, 1H), 4.61 – 4.55 (m, 1H), 4.51 (m, 1H), 4.16 (d, *J* = 8.1 Hz, 1H), 4.11 – 4.04 (m, 1H), 3.39 (t, *J* = 8.6 Hz, 1H), 3.35 (s, 1H), 3.30 (s, 1H), 3.25 (m, 1H), 3.21 (m2H), 3.16 – 3.06 (m, 2H), 3.04 – 2.95 (m, 1H), 2.91 (t, *J* = 13.0 Hz, 1H), 2.85 (m 1H), 2.44 (t, *J* = 13.2 Hz, 1H), 2.29 (m, 1H), 2.21 (m, 3H), 2.04 – 1.96 (m, 1H), 1.93 (m, 1H), 1.89 – 1.64 (m, 8H), 1.57 – 1.43 (m, 2H), 1.41 – 1.27 (m, 2H),

1.15 (m, 6 H), 1.11 (d, *J* = 6.4 Hz, 3 H), 1.08 (d, *J* = 6.3 Hz, 3 H). ^{13}C NMR (151 MHz, MeOD): δ 178.14, 175.95, 174.75, 174.13, 174.05, 173.89, 173.77, 173.52, 173.35, 173.32, 172.85, 172.61, 170.17, 157.29, 139.15, 138.33, 135.47, 131.19, 130.67, 130.52, 130.21, 130.19, 129.45, 129.39, 129.34, 129.14, 127.89, 127.63, 116.39, 62.65, 60.35, 60.26, 57.69, 57.66, 56.29, 55.98, 55.52, 54.78, 52.49, 52.12, 51.10, 41.73, 40.43, 40.23, 38.34, 38.08, 37.39, 36.89, 32.46, 31.77, 31.03, 30.32, 27.13, 25.95, 25.21, 24.45, 23.29, 22.19, 19.62, 19.39. HRMS: calcd for $C_{66}H_{87}N_{13}O_{13}$: $[M+2H]^{2+}$ m/z 635.8346, found, 635.8368.

2.3 ThioflavinT (ThT) aggregation assay

Monomeric Aβ preparation: Monomeric form of $A\beta_{42}$ was prepared by dissolving $A\beta_{42}$ (Millipore, AG970) in 1, 1, 1, 3, 3, 3-hexafluoroisopropanol (HFIP) (1.0 mg/mL) and incubating overnight. The solution was aliquoted, lyphylized and the resulting monomeric $A\beta_{42}$ was stored at -80°C. Before use, the monomeric $A\beta_{42}$ was dissolved in 1% $NH_3{\cdot}H_2O$ to 1.0 mg/mL, sonicated for 30 s to 1 min after it has gone into solution and then diluted into the appropriate concentration.

Inhibition: $A\beta_{42}$ and the tested peptide were diluted with phosphate buffer saline (PBS, 50 mM, PH 7.4). 20.0 μM $A\beta_{42}$ (5.0 μL, final volume) was incubated for 72 h at 37°C in the present or absent of the tested peptide (various of concentration). Blanks using phosphate buffer saline (PBS, 50 mM, PH 7.4) instead of $A\beta_{42}$ with or without the peptide were carried out. Following incubation, thioflavin-T (45 μL, 5.0 μM in 50 mM glycine-NaOH buffer, pH 8.5) was added and the fluorescence of amyloid-bound ThT was recorded 5 min later at 444 nm (λ_{ex}) and 484 nm (λ_{em}). Fluorescence measurements were recorded with an Infinite M1000 EVO758 plus 1 microplate reader. The fluorescence of buffer with tested peptide and ThT but without $A\beta_{42}$ was subtracted as background in data analysis.

Disaggregation: 20.0 μM $A\beta_{42}$ was aged in phosphate buffer saline (50 mM, PH 7.4) for 4 days at 37°C to afford the maximal ThT fluorescence. The aged $A\beta_{42}$ was incubated with or without the tested peptide for a further 72 h at 37°C. The extent of aggregation was determined by the fluorescence of amyloid-bound ThT assay, relative to $A\beta_{42}$ incubated without the tested peptide. The fluorescence from buffer with tested peptide and ThT but without $A\beta_{42}$ was subtracted as background in data analysis.

2.4 ThioflavinT (ThT) fluorescence kinetics assay

$A\beta_{42}$ (1 mg/mL) and the tested peptide were diluted with phosphate buffer saline (50 mM, PH 7.4) containing 5.0 μM ThT. $A\beta_{42}$ (10 μM, final concentration) was incubated for 40 h at 30°C in the present or absent of the tested peptide. All the samples (40 μL) were prepared over ice and added into a plate with 384 wells. The wells were prepared in triplicate. Fluorescence measurements were recorded with an Infinite M1000 EVO758 plus 1 microplate reader every 1 h, by using excitation and emission wavelengths of 444 and 484 nm, respectively. The fluorescence from buffer with tested peptide and ThT but without $A\beta_{42}$ was subtracted as background in data analysis.

2.5 Photo-induced cross-linking of unmodified proteins (PICUP)-Western blot assay

Samples prepared for PICUP was similar to ThT assays. For $A\beta_{42}$ aggregation inhibition: 25 μM $A\beta_{42}$ was incubated for different time at 37°C in the present or absent of the tested peptide. For $A\beta_{42}$ fibrils disaggregation: 25 μM $A\beta_{42}$ was aged in phosphate buffer saline (50 mM, PH 7.4) for 4 days at 37°C. The aged $A\beta_{42}$ was incubated with or without the tested peptide for a further 72 h at 37°C. After each time interval, ammonium persulfate (APS, 1.0 μL, 40 mM), Tris (2,2'-bipridyl)dichlororuthenium (II) (sigma, 1.0 μL, 2.0 mM) were added to the above prepared sample (18 μL) in a clear PCR tube. The mixture was then irradiated for 1 s using a 200 W lamp position 10 cm from the bottom of the PCR tube. The cross-linking reaction is quenched immediately by adding 5.0 μL 5× loading buffer containing 5% β-mercaptoethanol. Samples were analyzed using Tricine-SDS-PAGE gels and visualized by western blot using monoclonal anti-β-Amyloid 1328 antibody (1:1000 dilution ratio) as primary antibody.

2.6 Transmission electron microscopy (TEM)

The sample preparation was the same as ThT assays. Samples (5.0 μL) were spotted onto a carbon-coated copper grid for 2 min. Excess samples were removed using filter paper and then each grid was stained with Phosphotungstic acid solution (2%, 5.0 μL) for 2 min. After getting rid of the excess staining solution with filter paper, samples were analyzed by a transmission electron microscope JEM 1400 (JEOL).

2.7 Dot blot assay

5.0 μM $A\beta_{42}$ was incubated for different time with or without different concentration of peptide in phosphate buffer saline (50 mM, PH 7.4) at 37°C. After incubated, the samples (10 μL) were spotted onto nitrocellulose membranes and dried at room temperature. The membranes were then blocked for 2 h with 5% nonfat milk in TBST (10 mM Tris buffered saline and 0.01% Tween 20). After washing, the membranes were incubated at 4°C overnight with primary antibody. The membranes were washed with TBST and then incubated for 2 h with appropriate horseradish peroxidase (HRP) conjugated secondary antibodies. After washing with TBST, the blots were developed using ECL reagent kit.

2.8 Circular dichroism (CD) Spectroscopy

Analytical stock solutions (20.0 mM) of TA and LTA were prepared in THF and diluted to 50 μM in H_2O or THF/ H_2O mixtures [1:1 (v/v)]. For time-course experiments, analytical stock solutions (20 mM) of TA were prepared in ethylene glycol and diluted to 50 μM in 50 mM phosphate buffer saline (pH 7.4, excluding NaCl and KCl). The monomeric $A\beta_{42}$ was dissolved in ethylene glycol to 20 mg/mL, and diluted to 10 μM in 50 mM phosphate buffer saline (pH 7.4, excluding NaCl and KCl). The $A\beta_{42}$ was incubated with or without TA for different time at 37°C.CD measurements were performed on a Chirascan CD spectrophotometer (Applied Photophysics, UK). Measurements were carried out at room temperature in a 1 mm optical path length cell without dilution, and the spectra recorded over a wavelength range of 190–260 nm at a 1-nm bandwidth, 1-nm step size, and 0.5-s per point.

The spectra of the blank solutions were autosubtracted. Final analysis of the data was conducted with Chirascan.

2.9 Molecular docking

The structures of TA and LTA were prepared by MOE 2009.10. The structure of $A\beta_{17-42}$ (PDB code: 2BEG) was chosen to model the β-sheet. The terminal amino acids of $A\beta_{17-42}$ were protonated. The poses were generated by Triangle Matcher to align ligand triplets of atoms on triplets of alpha spheres. The first scoring was set default as London dG method. The refinement scoring method of the docking poses was the same as the previous one. The final figures were drawn by PyMol.

2.10 Surface Plasmon Resonance (SPR)

NeutrAvidin-coated GLH sensor chips in combination with a ProteOn XPR36 protein interaction system (Bio-Rad Laboratories, Hercules, CA) were used. $A\beta_{42}$ monomer (50 μg/mL) was immobilized (~1000 RU) in flow cells by direct coupling. The analytes were diluted with running buffer (PBS containing 0.01% Tween 20) to six concentrations (0, 1.25, 2.50, 5.00, 10.0, 20.0 μM), and then injected simultaneously at a flow rate of 25 μL/min for 4 min for associating, followed by 6 min of disassociation at 25°C. The GLH chip was regenerated with a short injection of 0.42% H_3PO_4 between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams. The data were analyzed with ProteOnmanager software, choosing suitable model to obtain the equilibrium dissociating constants (K_D).

3. Characterization data of LTA and TA

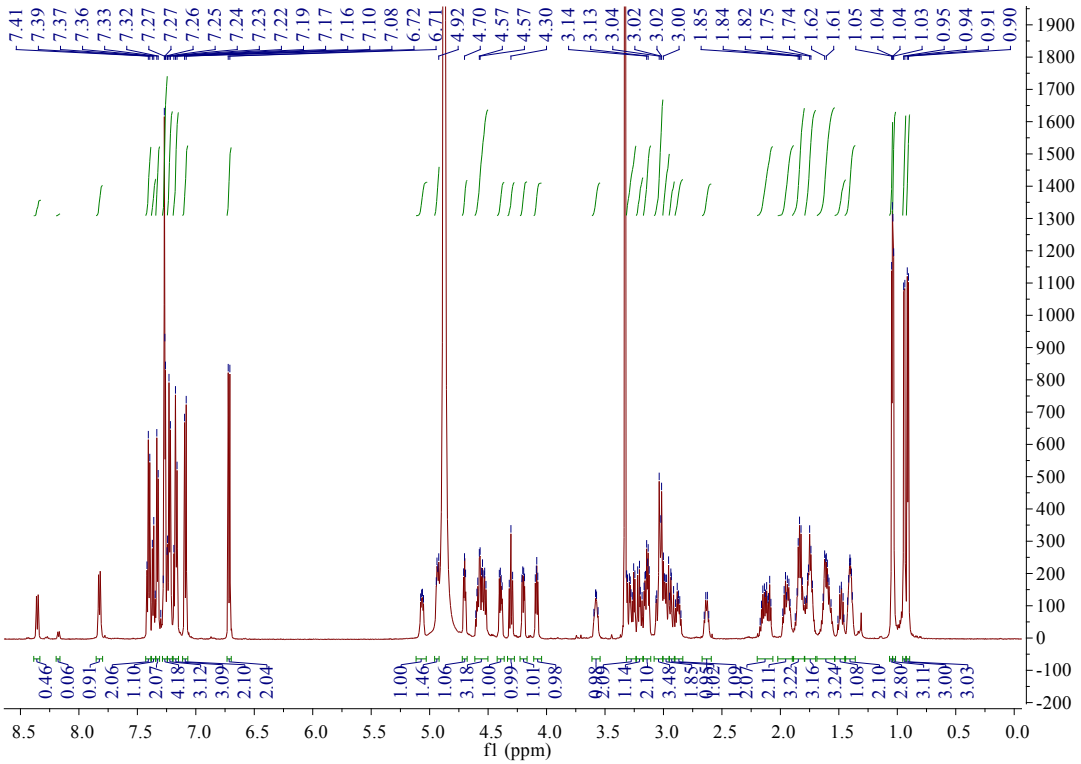


Figure S3 ^{1}H-NMR spectrum of LTA

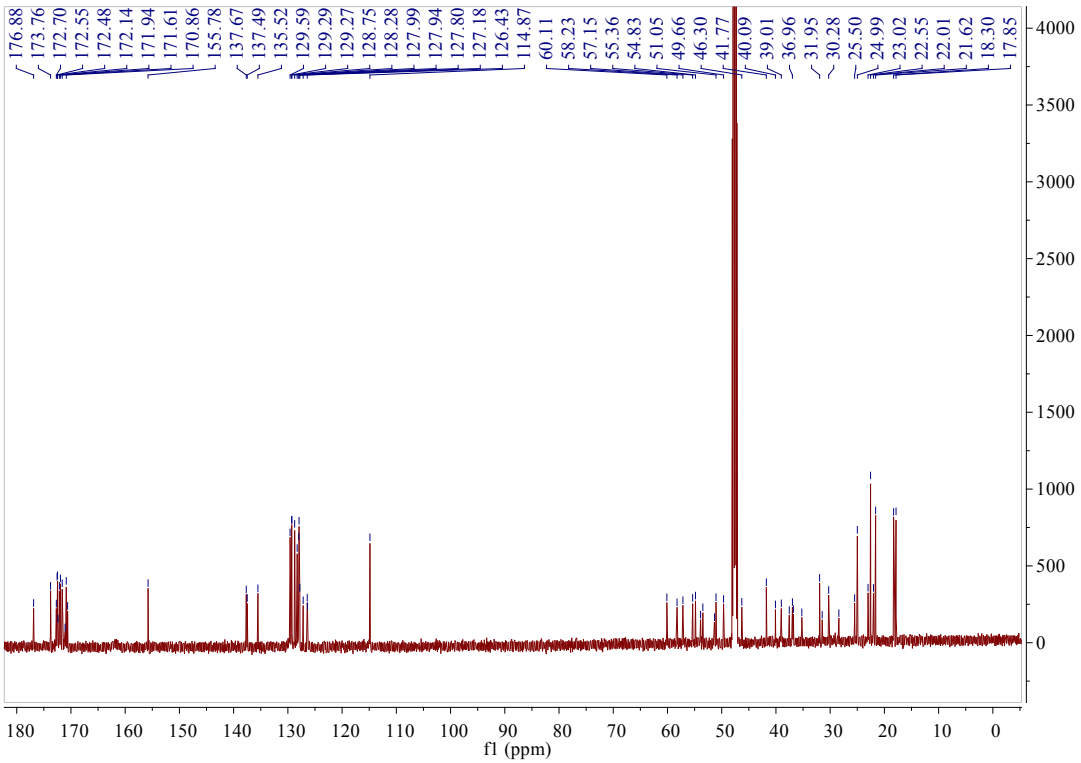


Figure S4 ^{13}C-NMR spectrum of LTA

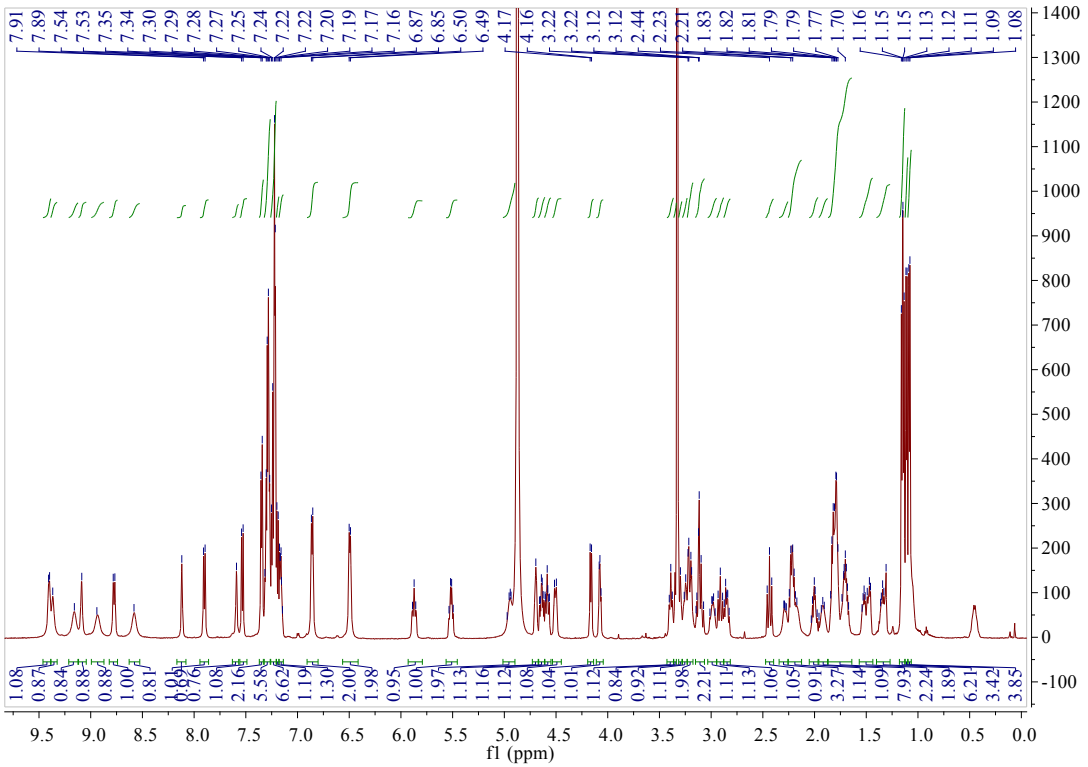


Figure S5 ^{1}H-NMR spectrum of TA

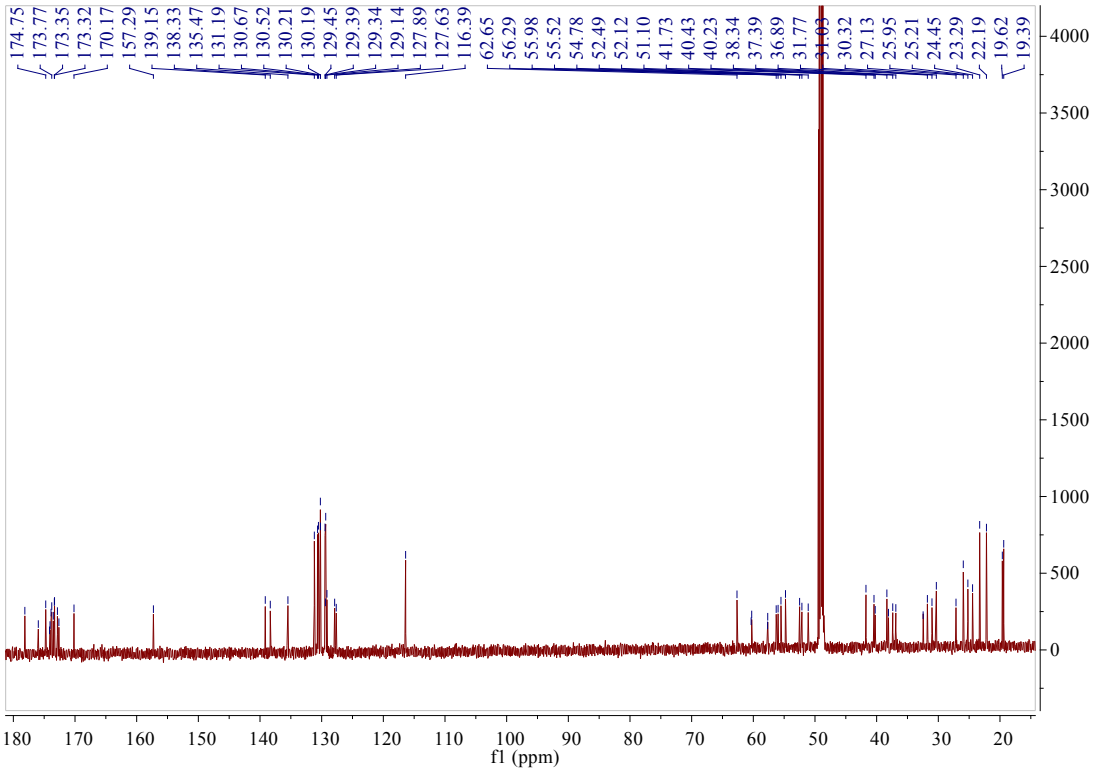


Figure S6 ^{13}C-NMR spectrum of TA

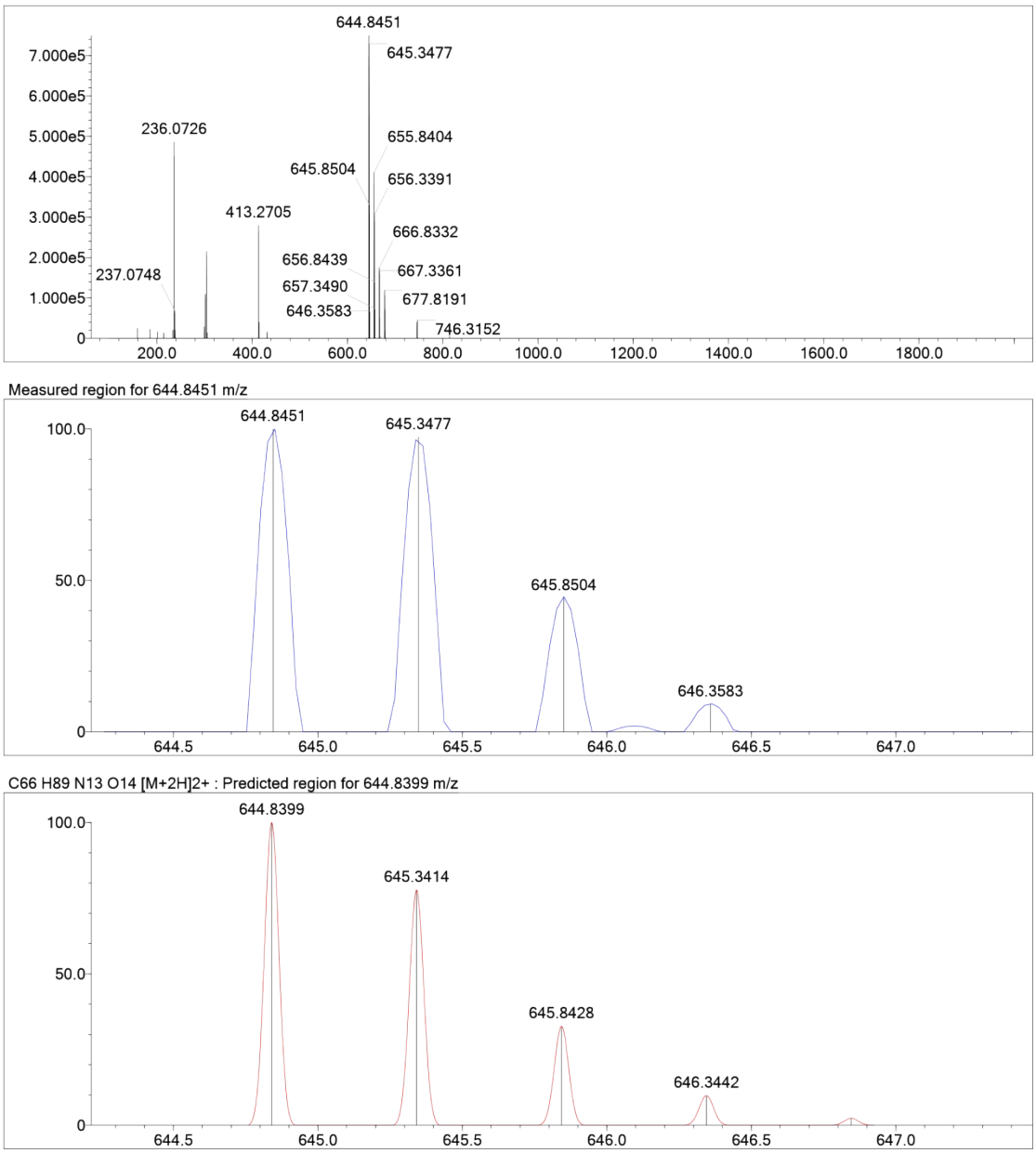


Figure S7 HRMS of LTA

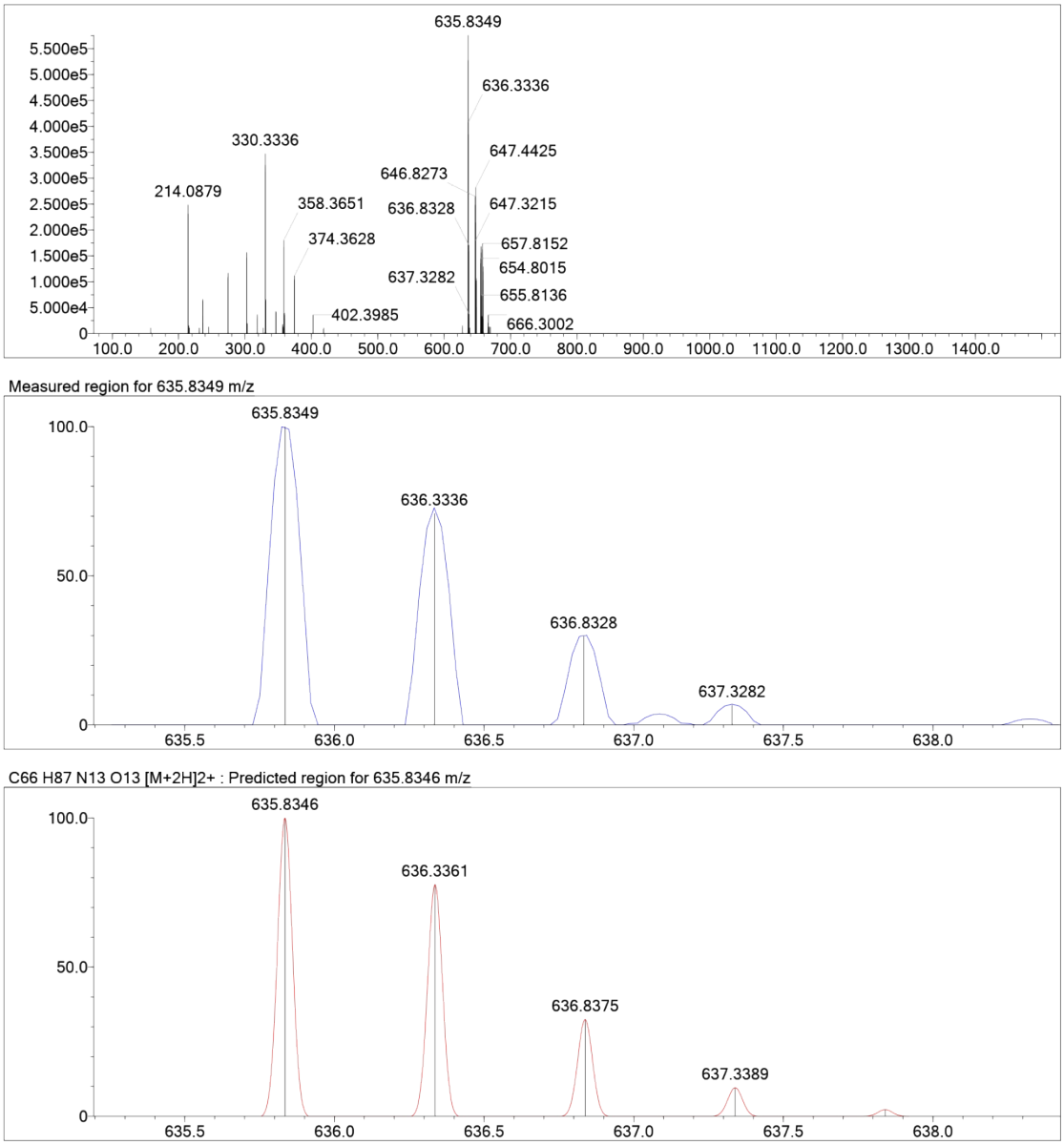


Figure S8 HRMS of TA