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Supporting information for

Stabilizing of Amyloid-β Peptide by the N-terminus Capture is Capable of Preventing and Eliminating Amyloid-β Oligomers

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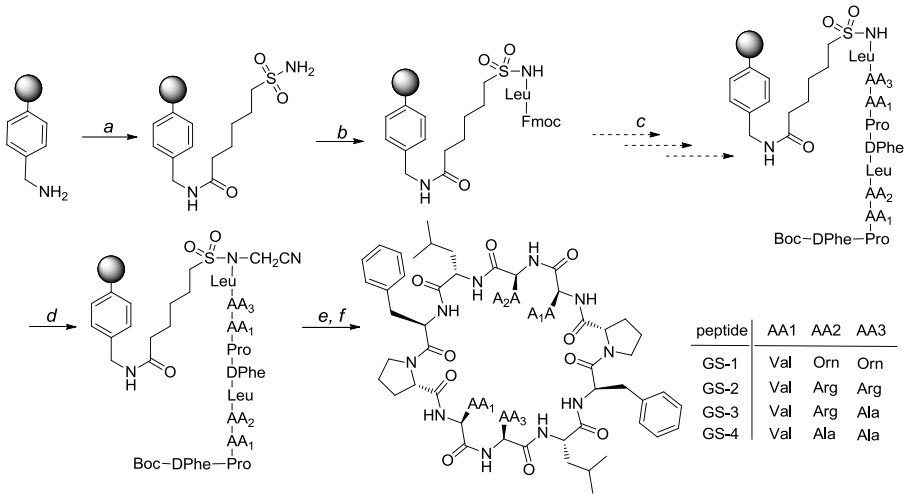
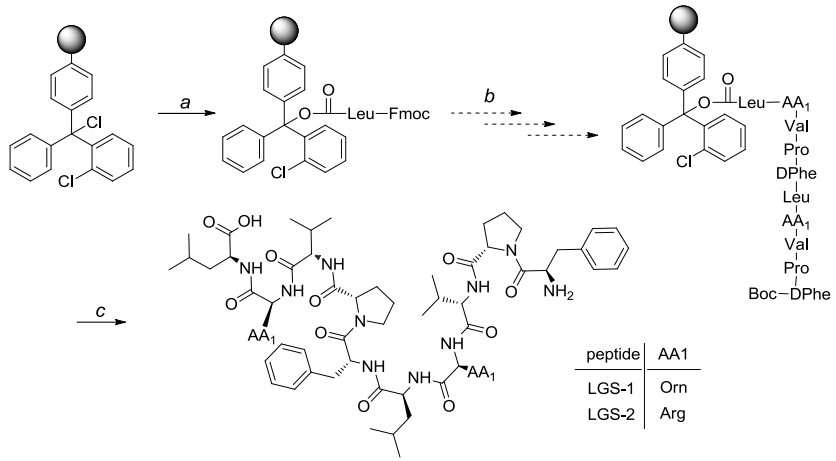
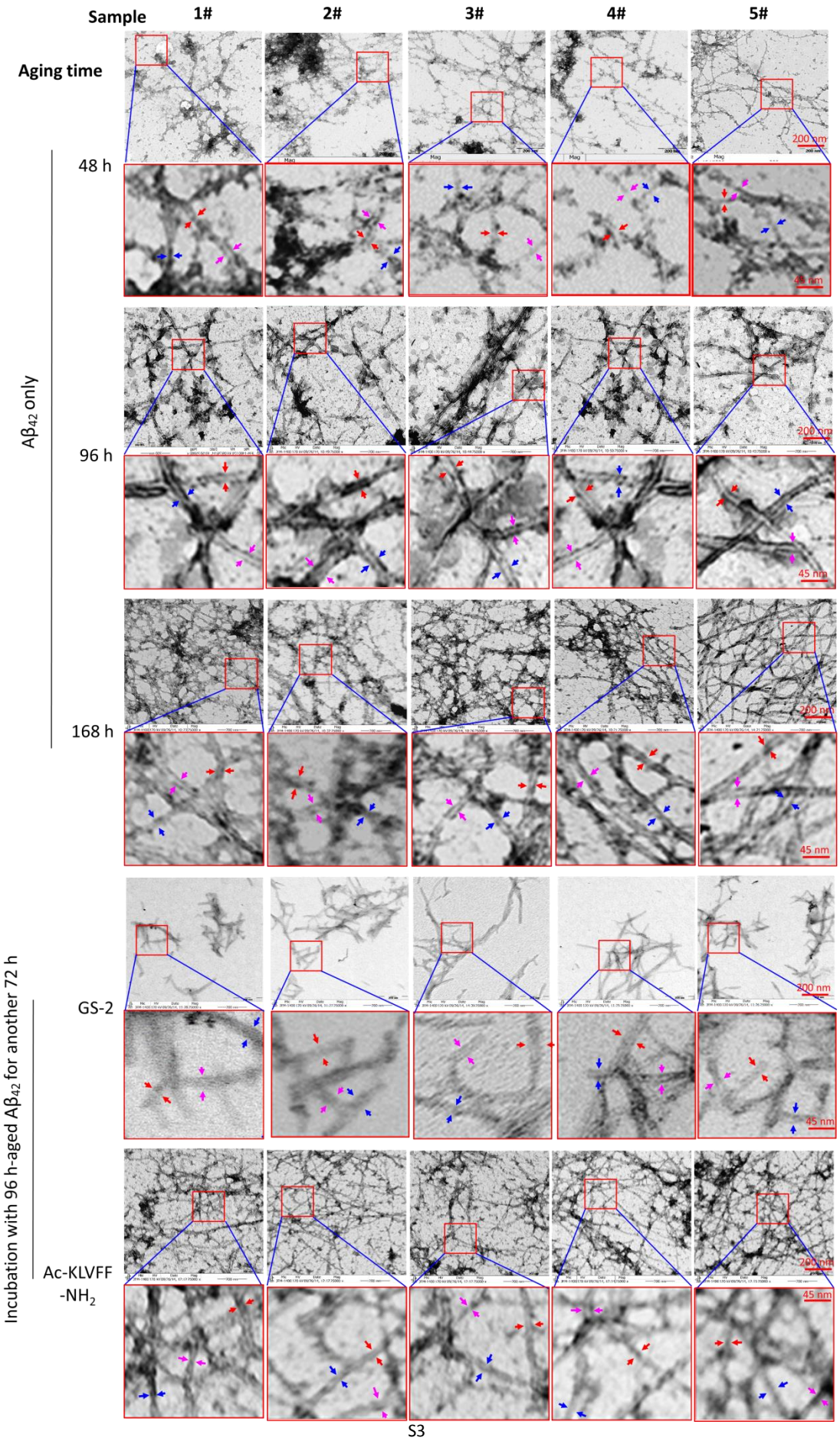
1. Supplementary Results



Supplementary Scheme S1: General synthetic approach for Gramicidin S and its analogues. Reagents and conditions: *a*, 6-sulfamoylhexanoic acid, DIC/HOBt, 3h; *b*, Fmoc-Leu-OH, PyBOP/DIPEA, -20^{0}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, DIPEA/NMP, dark, 24 h; *e,* CF_3COOH:phenol:i-Pr_3SiH:H_2O = 88:5:5:2, 3 h; *f,* DIPEA/THF 6h.



Supplementary Scheme S2: General synthetic approach for the linear peptide LGS-2. Reagents and conditions: *a*, Fmoc-Leu-OH, DIPEA/CH_2Cl_2, 3 h; *b*, 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; *c,*CF_3COOH:phenol:i-Pr_3SiH:H2O = 88:5:5:2, 3 h.


Sample
1#
2#
3#
4#
5#
Aging time
48 h
96 h
168 h
Aβ42 only
GS-2
Ac-KLVFF
-NH2
Incubation with 96 h-aged Aβ42 for another 72 h
200 nm
45 nm

S3

Figure S1: The diameter change of $A\beta_{42}$ (20 μM) fibrils incubated with or without 5.0 *eq* GS-2 or Ac-KLVFF-NH_2 was determined by TEM analysis. For each sample, representative five pictures were obtained from different visual fields. In each picture, the parent diameter and size distribution of $A\beta_{42}$ fibrils were analyzed by measurement of at least 3 fibrils as shown in the higher magnification. Scale bars, 200 nm and 45 nm.

2. Supplementary Methods

2.1 General

All commercial reagents and solvents were purchased from vendors and used without further purification or distillation. Resins, protected amino acids, and coupling regents were purchased from GL Biochem(shanghai)Ltd. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Burker BioSpin Ultrashield 600 NMR system. The purity 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. Separation conditions were: For cyclic peptides, 1.0 mL min^{-1} flow rate, a linear gradient of 20% to 80% MeCN with 0.1% TFA in 30 min, 80% to 100% in another 5 min, washed with 100% for 5 min, and then calibrated at 20% for 10 min; For linear peptides, 1.0 mL min^{-1} flow rate, a linear gradient of 5% to 95% MeCN in 25 min, 95 to 100% in another 5 min, washed with 100% for 5 min, and then calibrated at 5% for 10 min. All the cyclic peptides and its linear peptides were dissolved in DMSO at 20 mM and stored at -20°C. $A\beta_{42}$ was purchased from Millipore (cat. No. AG970). Thioflavin-T, ammonium persulfate and Tris(2,2'-bipridyl)dichlororuthenium (II) were purchased from sigma. Antibody 6E10[1], antibody 1328 (monoclonal anti-β-Amyloid (13-28) antibody) and antibody 2235 (polyclonal anti-β-Amyloid (22-35) antibody were obtained from Covance (clone 6E10, cat. No. SIG-39320), sigma (clone BAM90.1, cat. No. A8978),[2] and sigma (clone polyclonal, cat. No. A3356)[3], respectively. ECL reagent kit was purchased from CWBIO (China).

2.2 Solid-phase synthesis of Gramicidin S and its analogues[4, 5]

All the linear the peptides were synthesized using standard Fmoc solid phase synthesis upon 2-Cl-Trityl resin. Cylcopeptides were synthesized using standard Fmoc solid phase synthesis upon safety catch resin and a spontaneous cyclization approach developed in our previous work[5-7] were adopted to produce the final products.

Preparation of sulfonamide resin. Prior to the first coupling step, aminomethyl Resin (AM Resin, loading 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 DIC (120 mg, 3.0 eq), HOBt (127 mg, 3.0 eq), 6-sulfamoylhexanoic acid (183 mg, 3.0 eq) and DMF (5 mL). The reaction was gently agitated for 3 h, and washed with DMF (5×5 mL). The coupling step was repeated once and washed with DMF (5×5 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 (553 mg, 5.0 eq), DIEA (404 mg, 10.0 eq) and $CHCl_3$ (5 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 mL) and DMF (5×5 mL). The resin loading was determined to be 0.83 by UV analysis of Fmoc cleavage product. A mixture of acetic anhydride/pyridine (1:1, 5 mL) was added to the resin for 2 h to cap the unreacted resin, and then wash with DMF (5×5 mL).

General methods for Fmoc-protection solid synthesis: In the Fmoc deprotection step, the solution of 20% piperidine was added to the above prepared resin (200 mg, loading: 0.8), agitated for 30 min by bubbling in N_2, and then washed with DMF (5×5 mL). Afterwards, in the coupling step, the solution of the Fmoc/Boc-protected amino acid (3.0 eq), DIC (3.0 eq) and HOBt (3.0 eq) was added and the mixture was gently agitated for 2h. After the resin was washed with DMF (5×5 mL), it was subjected to Kaiser test.

Activation of the carboxyl terminus. The resin was washed with NMP (5×5 mL) and then the a solution of NMP (5 mL), DIEA (15.0 eq) and iodoacetonitrile(10.0 eq) was added to the resin. The mixture stirred in a 25 mL round-bottle flask wrapped in aluminum foil for 24 h. Subsequently, the resin was washed sequentially with NMP (5×5 mL), DMF (5×5 mL) and CH_2Cl_2 (5×5 mL), and then dried in vacuo.

Removal of Boc and t-Bu protecting groups. 5 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 mL), MeOH (5×5 mL) and THF (5×5 mL).

On-resin cyclization of the linear peptide precursors. The above resin was immersed in 5 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 THF (5×5 mL). The aqueous and washing solution were combined and dried in vacuo to obtain white crude cyclic products. All the crude product were purified by reverse-phase HPLC (linear gradient of 50~70% MeCN with 0.1% TFA) yielding a white powder after lyophilization.

GS-1, cyclo-(DPhe-Pro-Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu): 55 mg, 30.3% yield as a white powder. HPLC purity 96.97%, t_R = 21.34 min. ^{1}H NMR (600 MHz, MeOD)： δ 8.95-7.70（signal of amide protons）， 7.36 – 7.32 (m, 4 H), 7.30 (t, *J* = 7.2 Hz, 2 H), 7.27 (d, *J* = 6.8 Hz, 4 H), 5.04 – 4.95 (m, 2 H), 4.74 – 4.65 (m, 2 H), 4.57 – 4.48 (m, 2 H), 4.37 (d, *J* = 6.4 Hz, 2 H), 4.18 (t, *J*= 8.8 Hz, 2 H), 3.81 – 3.72 (m, 2 H), 3.11 (dd, *J* = 12.5, 4.9 Hz, 2 H), 3.09 – 3.02 (m, 2 H), 2.97 (t, *J* = 11.9 Hz, 2 H), 2.93 – 2.85 (m, 2 H), 2.55 – 2.46 (m, 2 H), 2.34 – 2.24 (m, 2 H), 2.11 – 2.05 (m, 2 H), 2.05 – 1.98 (m, 2 H), 1.83 – 1.76 (m, 4 H), 1.76 – 1.67 (m, 4 H), 1.68 – 1.58 (m, 4 H), 1.58 – 1.50 (m, 4 H), 1.47 – 1.38 (m, 2 H), 0.98 (d, *J* = 6.7 Hz, 6 H), 0.94 – 0.88 (m, 18 H). ^{13}C NMR (151 MHz, MeOD) δ 173.63, 173.60, 173.47, 172.86, 172.54, 136.97, 130.47, 129.75, 128.55, 62.06, 60.48, 56.04, 55.94, 52.57, 51.55, 51.52, 47.99, 42.04, 40.38, 37.37, 37.31, 32.03, 30.86, 30.69, 25.73, 24.55, 24.51, 23.27, 23.10, 19.70, 19.51. HRMS: calcd for $C_{60}H_{92}N_{12}O_{10}$ $[M+2H]^{2+}$ m/z 571.3602, found, 571.3611.

GS-2, cyclo-(DPhe-Pro-Val-Arg-Leu-DPhe-Pro-Val-Arg-Leu): 60 mg, 30.7% yield as a white powder. HPLC purity 97.14%, t_R = 22.25 min. ^{1}H NMR (600 MHz, MeOD)： δ 8.95-7.68（signal of amide protons）， 7.33 (d, *J* = 7.5 Hz, 4 H), 7.32 – 7.28 (m, 2 H), 7.27 (d, *J* = 7.0 Hz, 4 H), 5.03 – 4.94 (m, 2 H), 4.68 – 4.58 (m, 2 H), 4.56 – 4.49 (m, 2 H), 4.42 (d, *J* = 6.6 Hz, 2 H), 4.21 (t, *J* = 8.8 Hz, 2 H), 3.82 – 3.73 (m, 2 H), 3.25 (t, *J* = 6.6 Hz, 4 H), 3.09 (dd, *J* = 12.6, 5.0 Hz, 2 H), 2.97 (t, *J* = 11.8 Hz, 2 H), 2.61 – 2.50 (m, 2 H), 2.34 – 2.22 (m, 2 H), 2.08 – 1.98 (m, 2 H), 1.90 – 1.79 (m, 2 H), 1.80 – 1.49 (m, 16 H), 1.49 – 1.38 (m, 2 H), 0.97 (d, *J* = 6.7 Hz, 6 H), 0.95 – 0.89 (m, 18 H). ^{13}C NMR (151 MHz,) δ 173.67, 173.55, 173.47, 172.95, 172.89, 172.81, 158.60, 137.07, 130.43, 129.74, 128.49, 61.93,

60.34, 60.29, 55.88, 53.31, 53.22, 51.82, 47.89, 42.22, 42.09, 37.32, 32.09, 30.96, 30.75, 26.22, 25.81, 24.49, 23.19, 23.16, 19.75, 19.35. HRMS: calcd for $C_{62}H_{96}N_{16}O_{10}$ $[M+2H]^{2+}$ m/z 613.3820, found, 613.3838.

GS-3, cyclo-(DPhe-Pro-Val-Ala-Leu-DPhe-Pro-Val-Arg-Leu): 65 mg, 35.7% yield as a white powder. HPLC purity 96.91%, t_R = 26.25 min. ^{1}H NMR (600 MHz, MeOD)：δ 8.85-7.40（signal of amide protons） 7.36 – 7.32 (m, 4 H), 7.31 – 7.29 (m, 2 H), 7.29 – 7.25 (m, 4 H), 5.09 – 5.01 (m, 1 H), 5.01 – 4.94 (m, 1 H), 4.66 – 4.56 (m, 2 H), 4.56 – 4.47 (m, 2 H), 4.40 (t, *J* = 6.9 Hz, 2 H), 4.20 (t, *J* = 8.8 Hz, 1 H), 4.13 (t, *J* = 9.1 Hz, 1 H), 3.78 (t, *J* = 9.1 Hz, 1 H), 3.75 – 3.70 (m, 1 H), 3.32 – 3.25 (m, 1 H), 3.25 – 3.18 (m, 1 H), 3.12 – 3.05 (m, 2 H), 3.03 – 2.92 (m, 2 H), 2.59 – 2.45 (m, 2 H), 2.36 – 2.20 (m, 2 H), 2.08 – 1.98 (m, 2 H), 1.94 – 1.82 (m, 1 H), 1.79 – 1.51 (m, 13 H), 1.50 – 1.40 (m, 2 H), 1.35 (d, *J* = 6.9 Hz, 3 H), 0.97 (d, *J* = 3.2 Hz, 3 H), 0.96 (d, *J* = 3.2 Hz, 3 H), 0.94 – 0.89 (m, 18 H). ^{13}C NMR (151 MHz, MeOD) δ 174.19, 173.79, 173.65, 173.58, 173.56, 173.36, 173.28, 173.22, 173.06, 172.35, 158.48, 137.21, 137.14, 130.50, 130.46, 129.73, 129.71, 128.48, 128.46, 61.95, 61.83, 60.61, 60.35, 55.94, 55.86, 53.12, 51.93, 51.85, 49.59, 47.94, 47.73, 42.08, 42.05, 41.78, 37.40, 37.34, 32.12, 31.72, 30.74, 30.61, 26.10, 25.90, 25.82, 24.54, 24.49, 23.26, 23.17, 23.08, 19.75, 19.69, 19.58, 19.50, 19.35. HRMS: calcd for $C_{59}H_{89}N_{13}O_{10}$ $[M+2H]^{2+}$ m/z 570.8500, found,570.8491

GS-4, cyclo-(DPhe-Pro-Val-Ala-Leu-DPhe-Pro-Val-Ala-Leu): 80 mg, 47.6% yield as a white powder. HPLC purity 95.37%, t_R = 30.54 min. ^{1}H NMR (600 MHz, MeOD) δ 8.72-7.46（signal of amide protons） 7.32 – 7.27 (m, 4 H), 7.27 – 7.25 (m, 2 H), 7.25 – 7.22 (m, 4 H), 4.94 – 4.88 (m, 2 H), 4.61 – 4.54 (m, 2 H), 4.50 (dd, *J* = 10.8, 5.5 Hz, 2 H), 4.39 (d, *J* = 7.6 Hz, 2 H), 4.23 (t, *J* = 8.8 Hz, 2 H), 3.74 – 3.67 (m, 2 H), 3.06 – 3.00 (m, 2 H), 3.00 – 2.92 (m, 2 H), 2.56 – 2.47 (m, 2 H), 2.27 – 2.13 (m, 2 H), 2.05 – 1.97 (m, 2 H), 1.71 – 1.50 (m, 10 H), 1.49 – 1.42 (m, 2 H), 1.36 (d, *J* = 7.0 Hz, 6 H), 0.93 – 0.86 (m, 24 H). ^{13}C NMR (151 MHz, MeOD) δ 174.50, 174.42, 173.81, 173.78, 173.18, 173.12, 171.94, 171.93, 137.27, 130.50, 129.67, 128.37, 61.80, 61.78, 59.88, 59.79, 55.63, 51.84, 51.74, 49.74, 49.70, 47.74, 41.77, 41.74, 37.46, 32.31, 32.28, 30.54, 25.87, 24.51, 23.21, 19.74, 19.29, 19.24. HRMS: calcd for $C_{56}H_{82}N_{10}O_{10}$$[M+Na]^+$ m/z 1077.6108, found,1077.6057.

2.3 Fmoc-protection Solid-phase synthesis of the linear peptides

Prior to the first coupling step, 2-Chlorotrityl chloride resin (300 mg, loading 0.9, 1.0 eq) was swollen in CH_2Cl_2 for 30 min. The resin was added to a 25mL round-bottle flask with a solution of Fmoc-protected amino acid (3.0 eq), DIEA (3.3 eq) and CH_2Cl_2 (5 mL). After the reaction was gently agitated for 0.5 h, DIEA (6.6 eq) was added to the reaction. After the mixture was gently agitated for 2.5 h, the solution was drained and washed with CH_2Cl_2 (5×5 mL). The unreacted site on the resin were capped by a mixture of CH_2Cl_2/MeOH/DIEA (8:1.5:0.5, 5 mL) for 15 min. After that, the resin was washed with DMF (5×5 mL). Following Fmoc deprotection using 20% piperidine in DMF for 30 min, chain elongation was performed with the solution of the Fmoc/Boc-protected amino acid (3.0 eq), DIC (3.0 eq) and HOBt (3.0 eq) for 2 h. Each deprotecting and coupling step was followed by washing the resin with DMF (5×5 mL). The completeness of each coupling step was checked with Kaiser test. After completion of the synthesis, the linear peptide was cleaved from the resin with 5 mL Reagent B (CF_3COOH : phenol : i-Pr_3SiH : H_2O = 88 : 5 : 5 : 2) for 3 h. The crude product was then precipitated by cold ether and purified by reverse-phase HPLC (linear gradient of 20~50% MeCN with 0.1% TFA) yielding a white powder after lyophilization.

LGS-1, DPhe-Pro-Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu: 188 mg, 52% yield as a white powder, HPLC purity 98.04%, t_R = 14.21 min. HRMS: calcd for $C_{60}H_{94}N_{12}O_{11}$ $[M+2H]^{2+}$ m/z 580.3655, found, 580.3657

LGS-2, DPhe-Pro-Val-Arg-Leu-DPhe-Pro-Val-Arg-Leu: 176 mg, 45% yield as a white powder, HPLC purity 98.52%, t_R = 14.57 min. HRMS: calcd for $C_{62}H_{98}N_{16}O_{11}$ $[M+2H]^{2+}$ m/z 622.3873, found, 622.3879

2.4 ThioflavinT (ThT) Aggregation Assay[8]

Monomeric $A\beta_{42}$ 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 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% $NH_3 \bullet H_2O$, 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). $A\beta_{42}$ (20 μM) was incubated for 72 h at 37°C in the present or absent of the tested peptide (20 μM). Blanks using phosphate buffer saline (PBS, 50 mM, PH 7.4) instead of $A\beta_{42}$ with or without the peptide (20 μM) were carried out. Following incubation, ThT (45 μL, 5 μM in 50 mM glycine-NaOH buffer, pH 8.5) was added into 5 μL of incubated mixture, and the fluorescence of amyloid-bound ThT was recorded with an Infinite M1000 EVO758 plus 1 microplate reader at 444 nm (λ_{ex}) and 484 nm (λ_{em}). Each sample was examined in triplicate. The percentage of sample on aggregation was calculated with the following equation: $(1\text{-}IF_c/IF_b)\times100$. IF_c and IF_b are the fluorescence intensities obtained for $A\beta_{42}$ in the presence and absence of the peptide after subtracting the background respectively.

Disaggregation*:* $A\beta_{42}$ (20 μM) was aged in phosphate buffer saline (PBS, 50 mM, PH 7.4) for 4 days to afford the maximal ThT fluorescence. The aged $A\beta_{42}$ was incubated with or without the tested peptide for a further 72 h. 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 ThT fluorescence values of aged $A\beta_{42}$ were referred to 100% ThT fluorescence.

2.5 ThioflavinT (ThT) fluorescence kinetics assay[9]

$A\beta_{42}$ and the tested peptide were diluted with phosphate buffer saline (PBS, 10 mM, PH 7.4) containing ThT (5 μM). $A\beta_{42}$ (40 μL, 10 μM) was incubated for 7 d h at 15°C in the present or absent of the tested peptide. All the samples were prepared over ice and added into a plate with 384 wells. The wells were prepared in quadruplicate. Fluorescence measurements were recorded with an Infinite M1000 EVO758 plus 1 microplate reader every 6 h, by using excitation and emission wavelengths of 444 and 484 nm, respectively. The fluorescence intensities were calculated by subtracting the background.

2.6 Photo-Induced Cross-Linking of Unmodified Proteins (PICUP)[10]

Samples prepared for PICUP was similar to that for aggregation assay.

For $A\beta_{42}$ aggregation inhibition: $A\beta_{42}$ (18 μL, 25 μM) was incubated for different time at 37°C in the

present or absent of various concentrations of tested peptides.

For $A\beta_{42}$ fibrils disaggregation: $A\beta_{42}$ was aged in phosphate buffer saline (PBS, 50 mM, PH 7.4) for 4 days. The aged $A\beta_{42}$ (18 μL, 25 μM) was incubated with or without the equivoluminal tested peptide for a further 72 h.

After each time interval, ammonium persulfate (APS, 1.0 μL, 40 mM), Tris (2,2'-bipridyl)dichlororuthenium (II) (1.0 μL, 2 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 5μL 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 (13-28) antibody (1:1000 dilution ratio) as primary antibody.

2.7 Transmission Electron Microscopy (TEM)[8]

The sample preparation was the same as ThT assays. Samples (5 μL) were spotted onto a carbon-coated copper/rhodium grid for 2 min. Excess samples were removed using filter paper and then each grid was stained with uranyl acetate (1%, 5 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). At least five pictures were obtained from each sample. The apparent diameter and size distribution of $A\beta_{42}$ fibrils were analyzed by manual measurement of >3 fibrils in each picture.

2.8 Dot Blot Assay[1]

$A\beta_{42}$ films (5 μM) was incubated for different time with or without different concentration of peptides in phosphate buffer saline (PBS, 50 mM, PH 7.4). After incubated, the samples (10 μL) were diluted with 90 μL PBS, spotted onto nitrocellulose membranes, and dried at room temperature. Then the membranes were blocked for 2 h with 5% nonfat milk in TBST (10 mM Tris buffered saline and 0.01% Tween 20). After washing membranes were incubated at 4°C overnight with 6E10 (Covance, 1:1000 dilution ratio), monoclonal anti-β-Amyloid (13-28) antibody (1:1000 dilution ratio), polyclonal anti-β-Amyloid (22-35) antibody (1:2000 dilution ratio), anti-β-Amyloid

fiber antibody (OC), or anti-β-Amyloid oligomer antibody (A11) in primary antibody dilution buffer (Beyotime). The membranes were washed with TBST and then incubated for 2 h with appropriate horseradish peroxidase (HRP) conjugated secondary antibodies. After washed with TBST, the blots were developed using ECL reagent kit.

2.9 Surface Plasmon Resonance (SPR)[11, 12]

For SPR experiments NeutrAvidin-coated GLH sensor chips in combination with a ProteOn XPR36 protein interaction system (Bio-Rad Laboratories, Hercules, CA) were used.

Binding selectivity towards $A\beta_{42}$: in a typical experiment, sequence-specific antibodies (6E10, 1328 and 2235 were immobilized (~12000 RU) by EDC/NHS according to the protocol in flow cells, with one flow cell used as a blank. Prior to the experiment, $A\beta_{42}$ (10 μM) was incubated at 37°C for 3 h in the absence or presence of the tested peptide. The sample was diluted 5-fold with running buffer, and injected over immobilized antibody at a flow rate of 30 μL/min for 6 min for associating, followed by 6 min of disassociation at 25°C. The GLH chip was regenerated with a short injection of 0.85% H_3PO_4 between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams. The observed signal enhancements in the case of antibodies 1328 and 2235 may be attributed to the increasedmolecular weight of the formed GS-2/Ab42.

Binding Affinity towards $A\beta_{42}$*:* $A\beta_{42}$ monomer (75 μg/mL) was immobilized (~4000 RU) in flow cells by direct coupling. The analytes were diluted with running buffer (PBS containing 0.01% Tween 20) to a series of concentrations (0, 0.31, 0.63, 1.25, 2.50, 5.00, 10.00 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 the nonspecific response observed in the reference surface. The data were analyzed with ProteOnmanager software, choosing suitable model to obtain the equilibrium dissociating constants (K_D).

2.10 Statistical Analysis

All values were expressed as mean ± SEM. Two-tailed Student's t tests and graphs were performed using GraphPad Prism v5.0c software. Significance is represented by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus control.

3. Supplementary References:

1. J. Bieschke, M. Herbst, T. Wiglenda, R. P. Friedrich, A. Boeddrich, F. Schiele, D. Kleckers, J. M. Lopez del Amo, B. A. Gruning, Q. Wang, M. R. Schmidt, R. Lurz, R. Anwyl, S. Schnoegl, M. Fandrich, R. F. Frank, B. Reif, S. Gunther, D. M. Walsh and E. E. Wanker, *Nature chemical biology*, 2012, **8**, 93-101.
2. J. Olah, O. Vincze, D. Virok, D. Simon, Z. Bozso, N. Tokesi, I. Horvath, E. Hlavanda, J. Kovacs, A. Magyar, M. Szucs, F. Orosz, B. Penke and J. Ovadi, *The journal of biological chemistry*, 2011, **286**, 34088-34100.
3. S. K. Singh, R. Gaur, A. Kumar, R. Fatima, L. Mishra and S. Srikrishna, *Neurotoxicity research*, 2014, **26**, 331-350.
4. C. Qin, X. Bu, X. Wu and Z. Guo, *Journal of combinatorial chemistry*, 2003, **5**, 353-355.
5. X. Bu, X. Wu, N. L. Ng, C. K. Mak, C. Qin and Z. Guo, *The journal of organic chemistry*, 2004, **69**, 2681-2685.
6. X. Wu, X. Bu, K. M. Wong, W. Yan and Z. Guo, *Organic letters*, 2003, **5**, 1749-1752.
7. C. Qin, X. Bu, X. Zhong, N. L. Ng and Z. Guo, *Journal of combinatorial chemistry*, 2004, **6**, 398-406.
8. Z. Wang, Y. Wang, B. Wang, W. Li, L. Huang and X. Li, *Journal of medicinal chemistry*, 2015, **58**, 8616-8637.
9. L. Pujadas, D. Rossi, R. Andres, C. M. Teixeira, B. Serra-Vidal, A. Parcerisas, R. Maldonado, E. Giralt, N. Carulla and E. Soriano, *Nature communications*, 2014, **5**, 3443.
10. M. Richman, S. Wilk, M. Chemerovski, S. K. Warmlander, A. Wahlstrom, A. Graslund and S. Rahimipour, *Journal of the American chemical society*, 2013, **135**, 3474-3484.
11. M. Stravalaci, A. Bastone, M. Beeg, A. Cagnotto, L. Colombo, G. Di Fede, F. Tagliavini, L. Cantu, E. Del Favero, M. Mazzanti, R. Chiesa, M. Salmona, L. Diomede and M. Gobbi, *The journal of biological chemistry*, 2012, **287**, 27796-27805.
12. B. R. Fluharty, E. Biasini, M. Stravalaci, A. Sclip, L. Diomede, C. Balducci, P. La Vitola, M. Messa, L. Colombo, G. Forloni, T. Borsello, M. Gobbi and D. A. Harris, *The journal of biological chemistry*, 2013, **288**, 7857-7866.