

**Electronic Supplementary Information (ESI)**

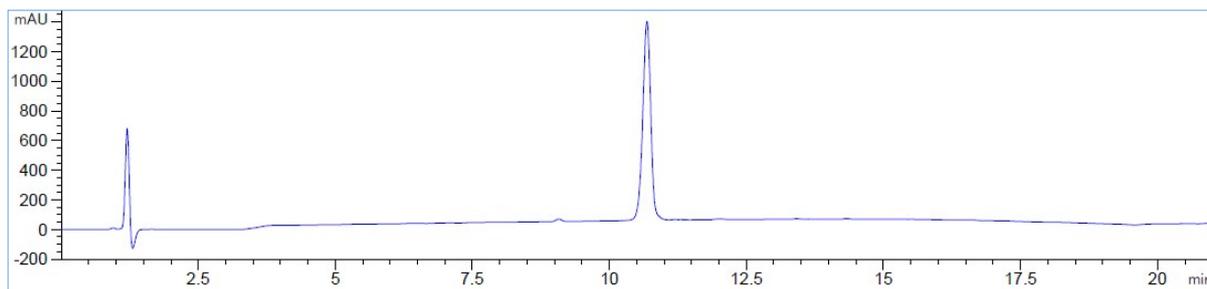
**Peptidomimetics Made by Tail-to-Side Chain One Component Peptide Stapling Inhibit Alzheimer's Amyloid- $\beta$  Fibrillogenesis**

**Sujan Kalita, Sourav Kalita, Ashim Paul, Amar Sarkar and Bhubaneswar Mandal\***

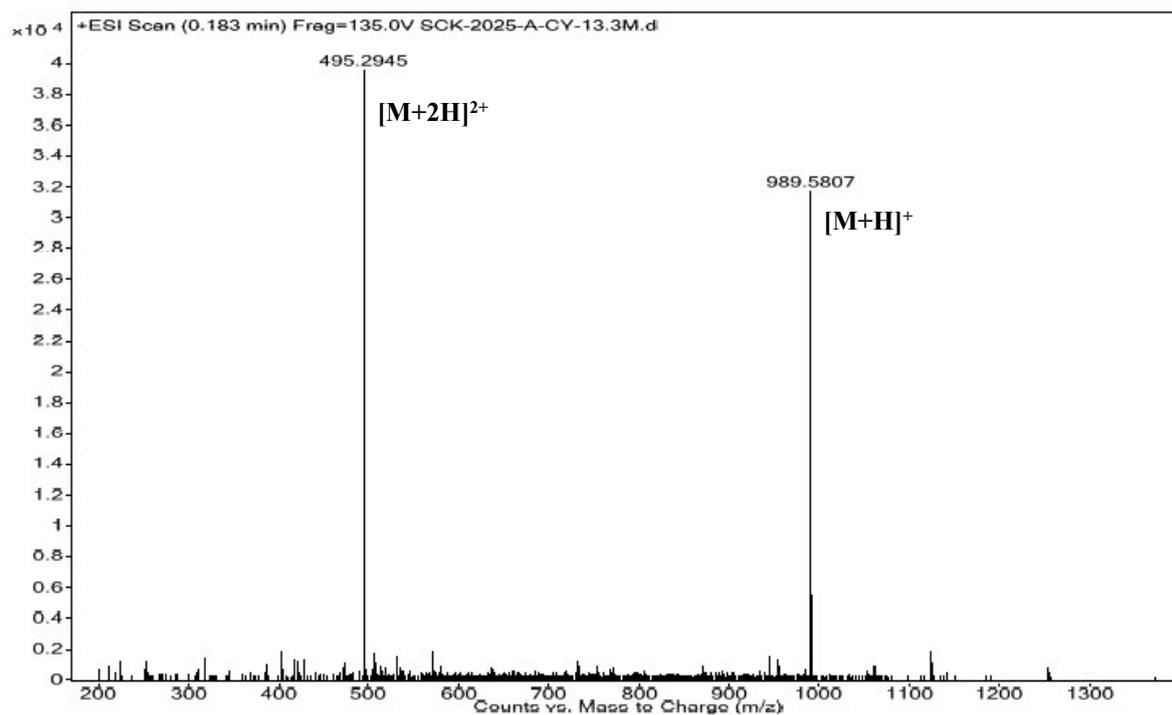
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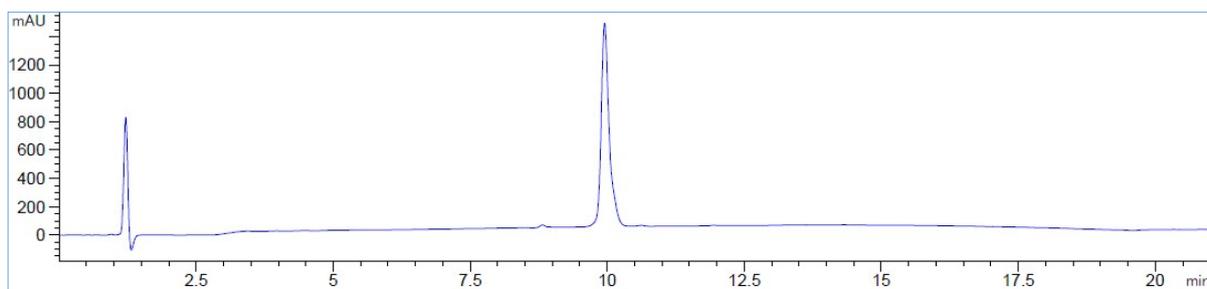
**Characterization data of the synthesized SPs by HPLC and High-Resolution Mass Spectrometry (HRMS):**



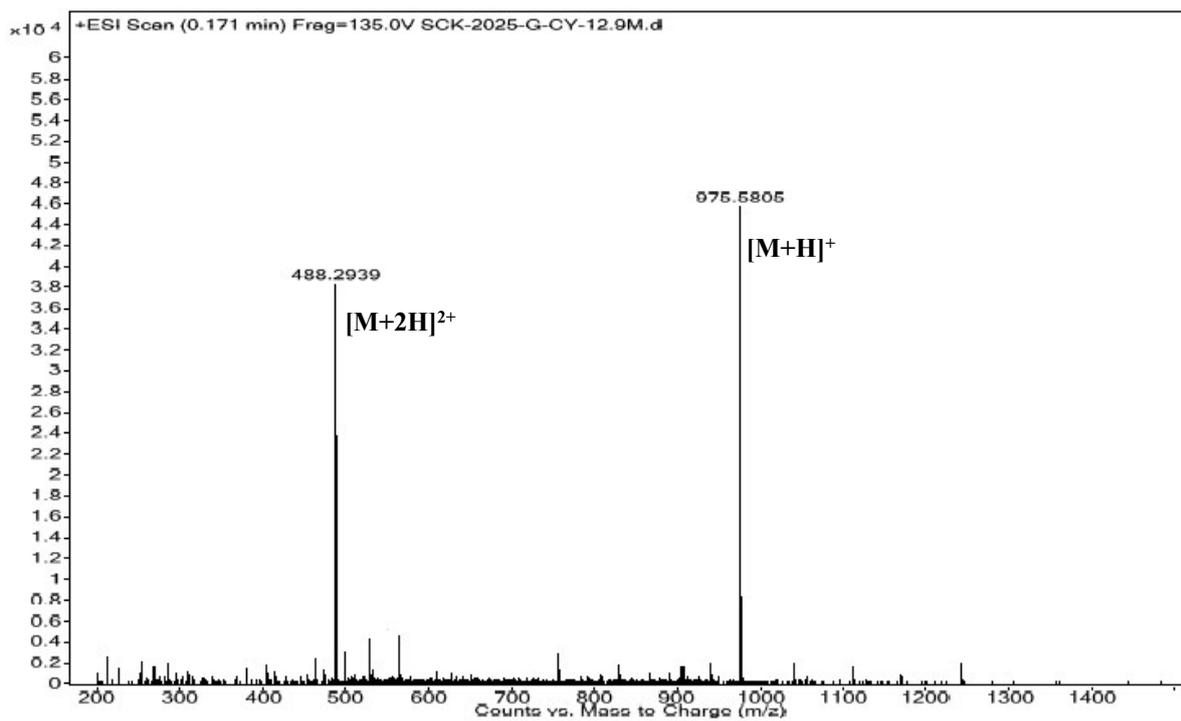
**Fig. S1.** HPLC profile of the purified peptide **SP1**. (% of purity = 98.93%)



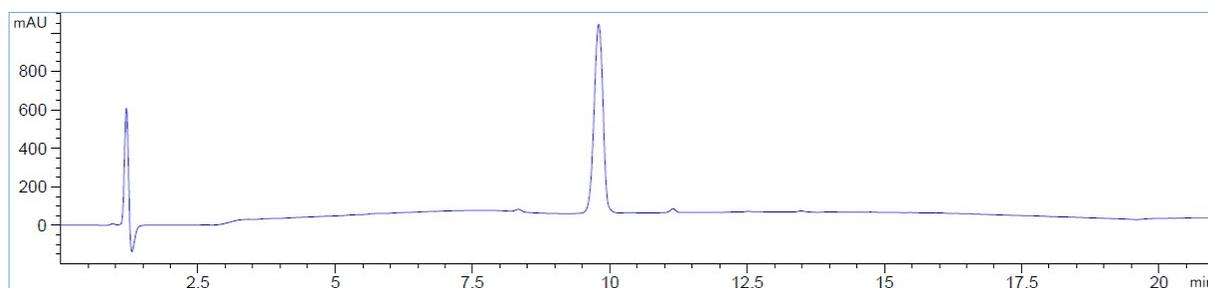
**Fig. S2.** ESI mass spectrum of peptide **SP1**. Calculated mass for  $C_{50}H_{76}N_{12}O_9$  is 989.5892  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 989.5807, and  $[M+2H]^{2+}$  is 495.2945.



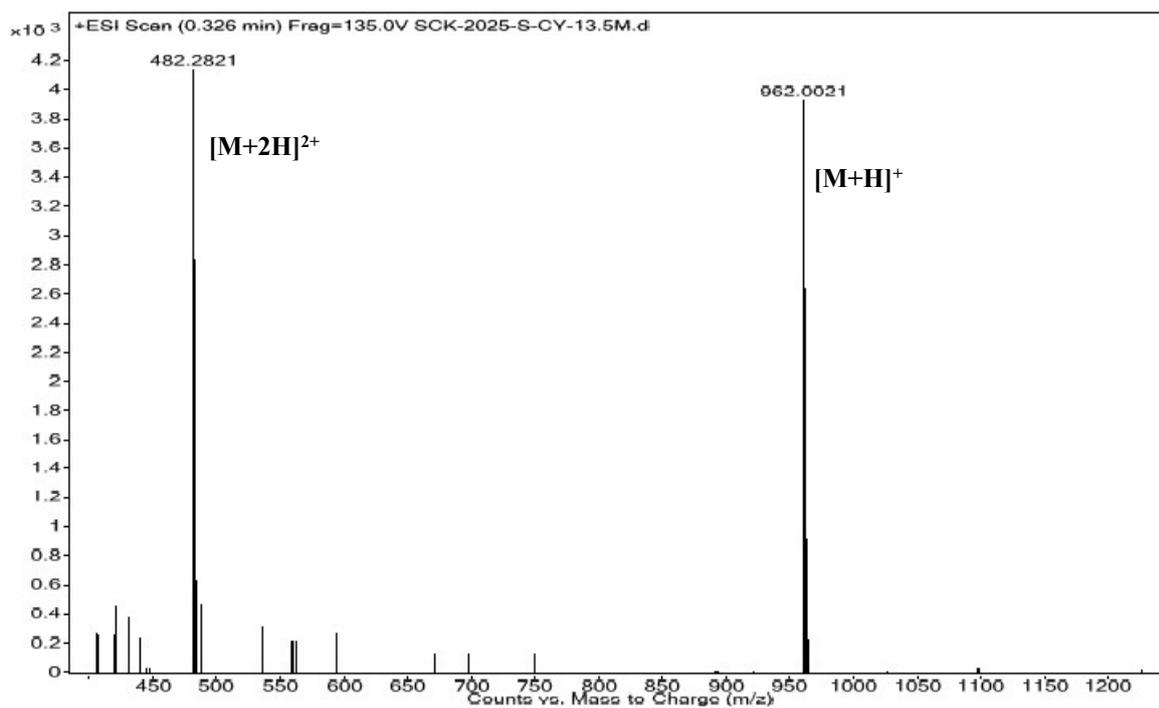
**Fig. S3.** HPLC profile of the purified peptide **SP2**. (% of purity = 98.94%)



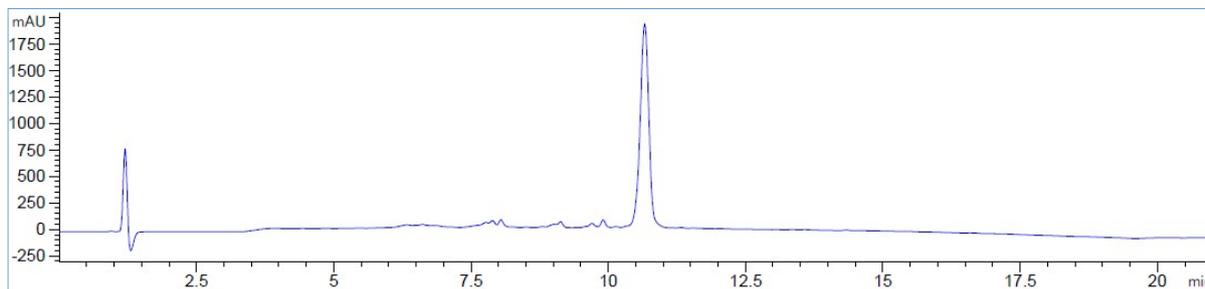
**Fig. S4.** ESI mass spectrum of peptide **SP2**. Calculated mass for  $C_{49}H_{74}N_{12}O_9$  is 975.5735  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 975.5805, and  $[M+2H]^{2+}$  is 488.2939.



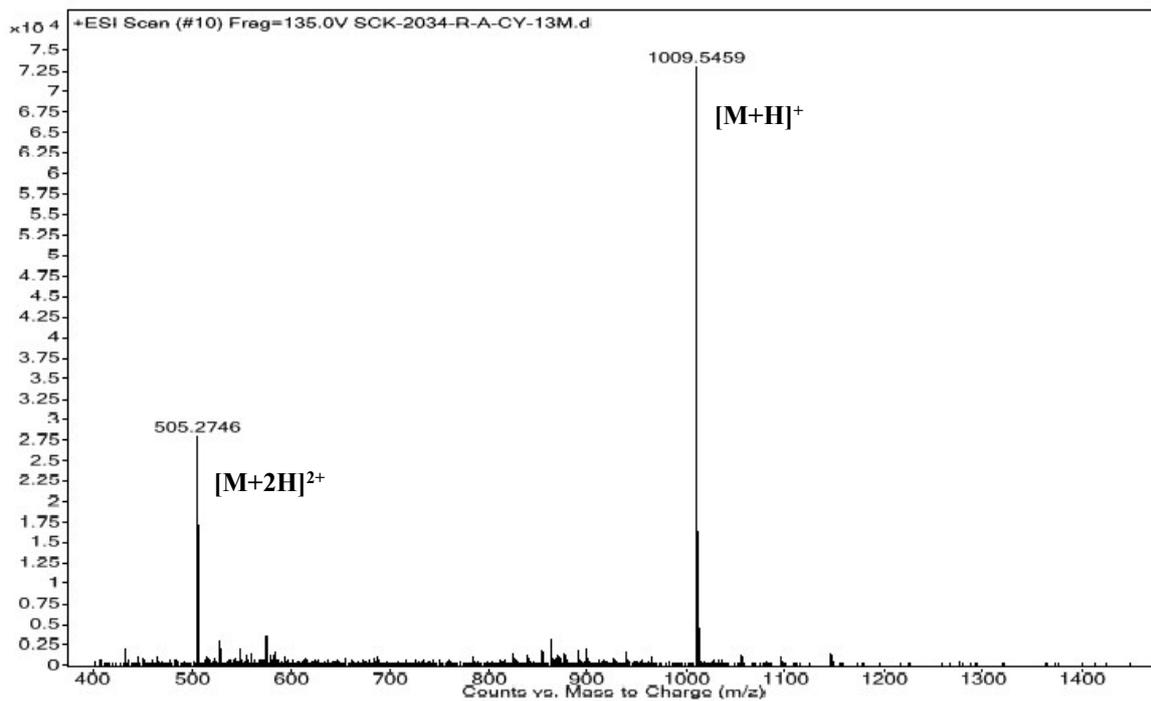
**Fig. S5.** HPLC profile of the purified peptide **SP3**. (% of purity = 97.87%)



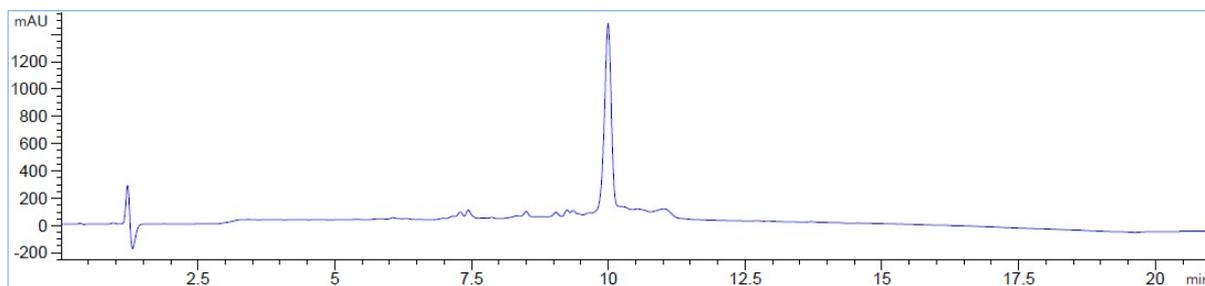
**Fig. S6.** ESI mass spectrum of peptide **SP3**. Calculated mass for  $C_{48}H_{72}N_{12}O_9$  is 962.0745  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 962.0021, and  $[M+2H]^{2+}$  is 482.2821.



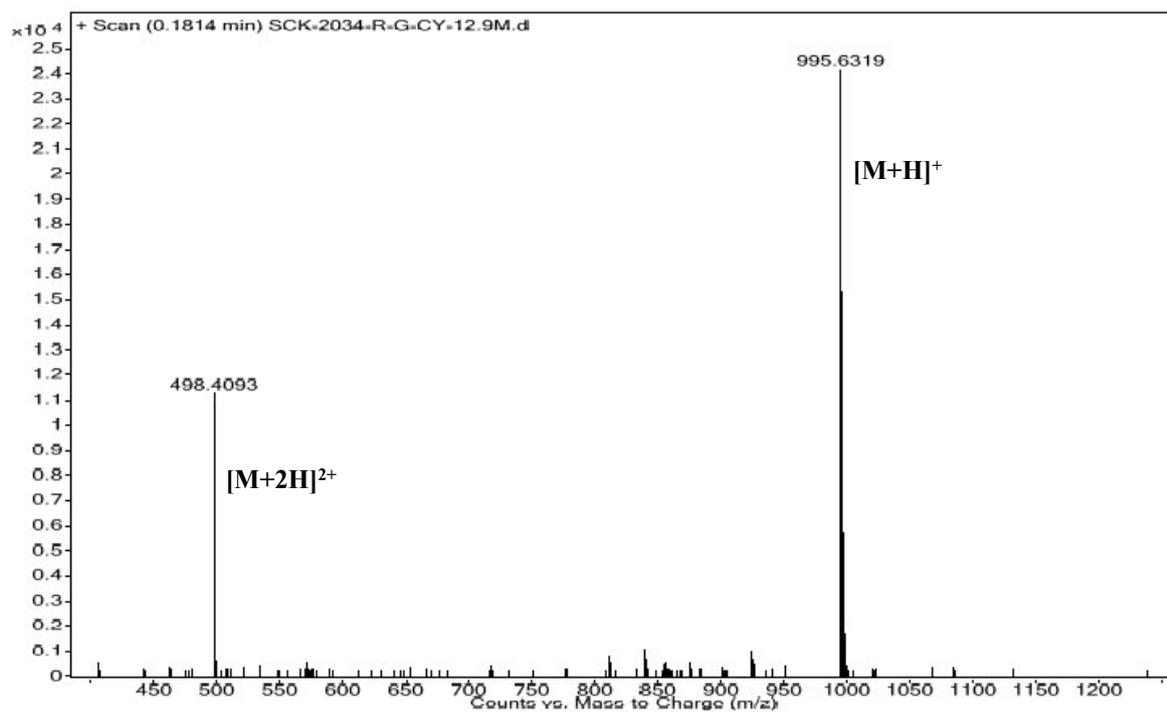
**Fig. S7.** HPLC profile of the purified peptide **SP4**. (% of purity = 95.71%)



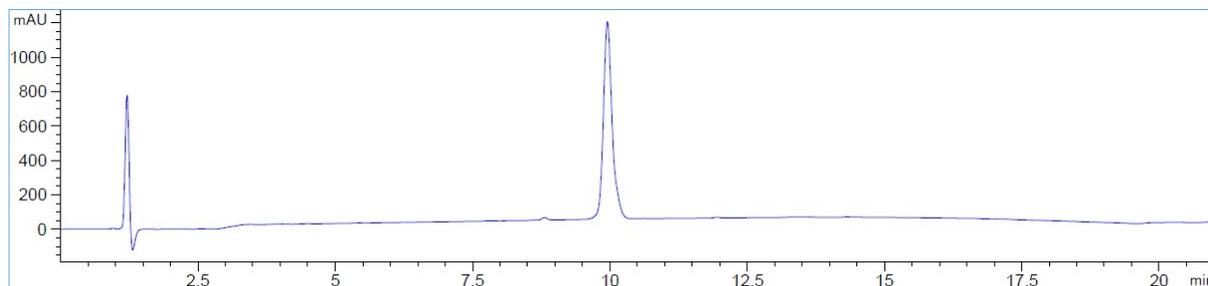
**Fig. S8.** ESI mass spectrum of peptide **SP4**. Calculated mass for  $C_{52}H_{72}N_{12}O_9$  is 1009.5579  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 1009.5459 and  $[M+2H]^{2+}$  is 505.2746.



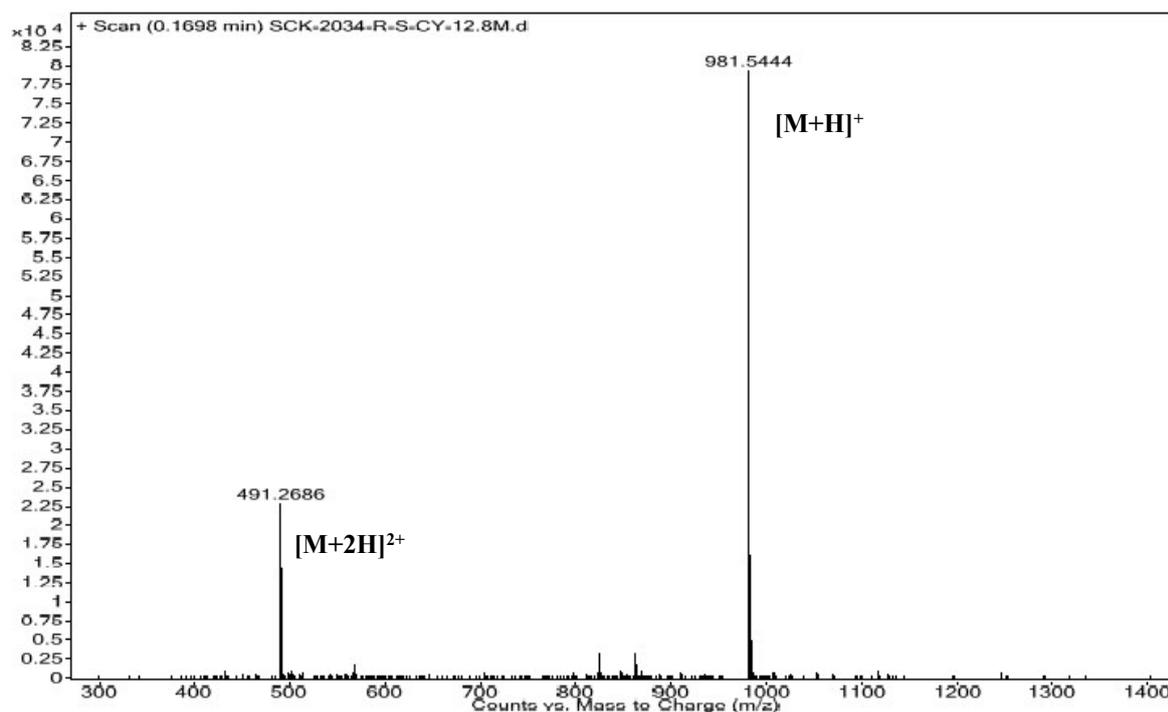
**Fig. S9.** HPLC profile of the purified peptide **SP5**. (% of purity = 94.40%)



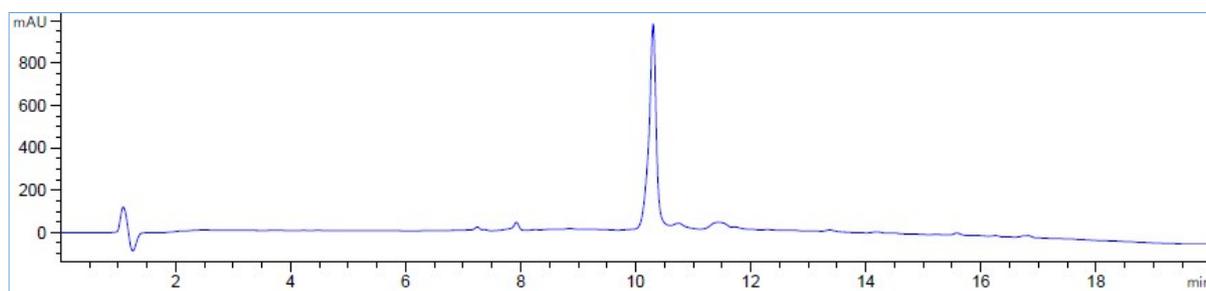
**Fig. S10.** ESI mass spectrum of peptide **SP5**. Calculated mass for  $C_{51}H_{70}N_{12}O_9$  is 995.5422  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 995.6319 and  $[M+2H]^{2+}$  is 498.4093.



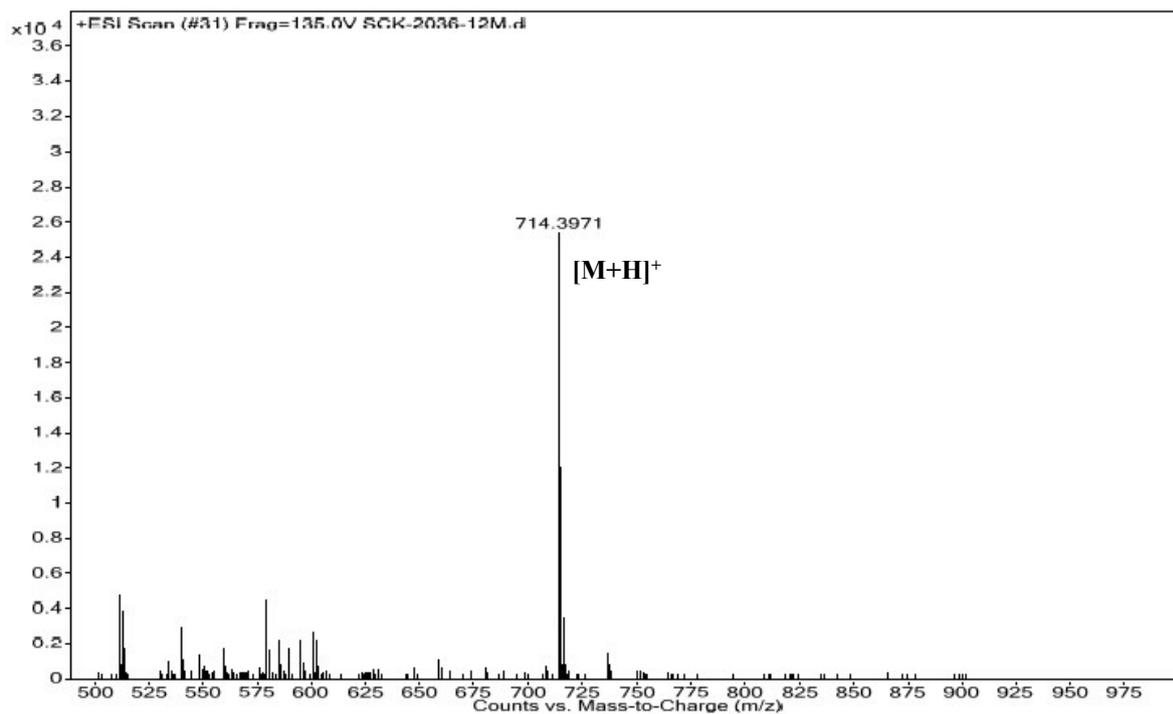
**Fig. S11.** HPLC profile of the purified peptide **SP6**. (% of purity = 98.72%)



**Fig. S12.** ESI mass spectrum of peptide **SP6**. Calculated mass for  $C_{50}H_{68}N_{12}O_9$  is 981.5266 [M+H]<sup>+</sup>, observed mass of [M+H]<sup>+</sup> is 981.5444 and [M+2H]<sup>2+</sup> is 491.2686.



**Fig. S13.** HPLC profile of the purified peptide **LP1**. (% of purity = 94.06%)



**Fig. S14.** ESI mass spectrum of peptide **LP1**. Calculated mass for  $C_{39}H_{51}N_7O_6$  is 714.3934  $[M+H]^+$ , observed mass of  $[M+H]^+$  is 714.3971.

## Reagents and Solvents

Rink Amide MBHA resin (loading 0.7 mmol/g), BOP [(Benzotriazole-1-yloxy) tris(dimethylamino) phosphonium hexafluorophosphate], all Fmoc-amino acids (with the following side-chains protecting groups: Boc for lysine and Pbf for arginine), N-methylated glycine and Amyloid-beta ( $A\beta_{1-40}$ ) were purchased from GL Biochem (Shanghai). Ethyl 2-cyano-2-(2-nitrobenzenesulfonyloxyimino) acetate (o-NosylOXY) was synthesized in laboratory. Diisopropylethylamine (DIPEA), adipic acid, glutaric anhydride, and succinic anhydride were purchased from Spectrochem. Pvt. Ltd. (India). Zinc bromide ( $ZnBr_2$ ) was purchased from Alfa-Aesar. Fmoc-Ant-OH (Fmoc-Anthranilic acid) and 5(6)-Carboxyfluorescein were purchased from Sigma. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and Ganglioside GM1 were purchased from Avanti Polar Lipid, Inc. Cholesterol (99%), trifluoroacetic acid (TFA) of extrapure grade, acetic anhydride (synthesis grade) and N-methyl-imidazole of extrapure grade were purchased from SPL (India). Acetonitrile of HPLC grade, dimethylformamide (DMF) of extrapure grade and dichloromethane of extrapure grade were purchased from Merck (India). Milli-Q water at  $18.2 \Omega$  was used.

## Synthetic Methods

All the peptides were synthesized manually by standard Fmoc/tBu solid phase peptide synthesis method using Rink Amide MBHA resin as the solid support on a Stuart blood tube rotator. The resin was taken inside a 5mL frit-fitted plastic syringe and swollen in DCM for 3h, followed by DMF for 1h. 2 equivalents of Fmoc-amino acids, 2.5 equivalents of the coupling reagent (o-Noslyoxy) and five equivalents of the base (DIPEA) were used for coupling. Each coupling was monitored by Kaiser's test. In case of incomplete coupling, the coupling cycle was repeated, followed by acetylation (capping) using  $Ac_2O/NMI$  (1:2) in

DCM for 1.5h. Fmoc deprotection was carried out using 20% piperidine in DMF for 21 mins (7min  $\times$  3). 10 eqs of adipic acid, three eqs of O-Noslyoxy as coupling reagent and 25 eqs of DIPEA as the base was used for coupling to N-methylated glycine. Similarly, for the other sequences, five eqs of glutaric anhydride/succinic anhydride, three eqs of O-Noslyoxy as coupling reagent and 12.5 eqs of DIPEA as the base was used for coupling to N-methylated glycine.

The Boc protection of the side chain of lysine was deprotected using  $ZnBr_2$  (5 equivalents) in DCM for 24h. The stapling step was carried out using BOP as the coupling reagent and DIPEA as the base for 24h. After completion of synthesis, the final peptide was cleaved from the resin using 80% TFA, 15% DCM, and 5%  $H_2O$  for 3h. Thereafter, the crude peptide was obtained by precipitation in cold diethyl ether followed by centrifugation.

#### **A $\beta_{1-40}$ sample preparation<sup>1</sup>**

3.5 mg of commercially available A $\beta_{1-40}$  was taken and dissolved in 20  $\mu$ L of TFA to obtain the completely disaggregated peptide. TFA was evaporated by purging nitrogen gas. To ensure complete removal of TFA, 10  $\mu$ L of HFIP was added and then evaporated by purging nitrogen gas, and the process is repeated for three times. To this, 1 mL of PBS (50 mM, pH 7.4) containing 3% DMSO, was added followed by sonication and vortex to obtain a transparent solution, and then the solution is divided into 20 sets, with each set containing 50  $\mu$ L. For the native A $\beta_{1-40}$  only, the final volume was made up to 800  $\mu$ L to obtain a total concentration of 40  $\mu$ M.

## **Instrumentations**

### **High-Performance Liquid Chromatography (HPLC):**

The crude peptides were dissolved in CH<sub>3</sub>CN/H<sub>2</sub>O and purified using RP-HPLC (Thermo Scientific) using a C18- $\mu$  Bondapak column (dimensions 250  $\times$  10 mm, particle size 12  $\mu$ m, pore size 175 Å) at a flow rate of 4 mL/min. The binary solvent system was used as solvent A (0.1% TFA in H<sub>2</sub>O) and solvent B (0.1% TFA in CH<sub>3</sub>CN) for a total run time of 20 mins. Dual-wavelength of 214 nm and 254 nm were set at the UV detector. A linear gradient of 5-100% CH<sub>3</sub>CN for 18 mins followed by 100% CH<sub>3</sub>CN till 20 mins were used for purification. The purity of the peptides was further checked using RP-HPLC (Waters 600E Analytical System) Waters, C18 analytical column at a flow rate of 1 mL/min for a total run time of 20 mins using a linear gradient of 5-100% CH<sub>3</sub>CN for 18 mins, followed by 100% CH<sub>3</sub>CN till 20 mins, with the UV detector set at 214 nm and 254 nm.

### **Mass Spectrometry:**

The purified peptides were characterized by High-Resolution Mass Spectrometry (HRMS) on Agilent-Q-TOF 6500 instrument in ESI positive mode with the software Mass Hunter Work Station. [M+H]<sup>+</sup> peak was observed for the purified peptides.

### **MALDI-TOF Mass Spectrometry:**

During the enzymatic stability study, after precipitation of the serum, the supernatant was collected and analyzed by RP-HPLC. The fragments were collected and mixed with CHCA ( $\alpha$ -cyano-hydroxy-cinnamic acid) matrix in a 1:1 ratio and analyzed in Maldi-TOF spectrometer using Bruker Daltonics flexAnalysis.

### **Thioflavin T (ThT) fluorescence Assay:<sup>2</sup>**

Thioflavin T (ThT) was purchased from Sigma-Aldrich, and a stock solution of concentration 50  $\mu\text{M}$  in PBS (50 mM, pH 7.4) was prepared and stored at 4  $^{\circ}\text{C}$  with proper protection from light.  $\text{A}\beta_{1-40}$  and the purified peptide samples were dissolved in PBS (50 mM, pH 7.4) with 3% DMSO to obtain a stock solution of concentration 400  $\mu\text{M}$  for the peptide samples and 40  $\mu\text{M}$  for the  $\text{A}\beta_{1-40}$  solution. For the dose-dependent study, with the  $\text{A}\beta_{1-40}$  solutions, the different breaker peptide samples were then added in different molar ratios (1:0.5, 1:1 and 1:2 respectively) and the final volume was made up to 800  $\mu\text{L}$  with PBS.

To perform the fluorescence experiment, 40  $\mu\text{L}$  of the peptide solutions were taken, mixed with 160  $\mu\text{L}$  of PBS (50 mM, pH 7.4) and 200  $\mu\text{L}$  of the ThT solution was added to it. For the fluorescence assay, the excitation wavelength was set at 440 nm and the emission was measured at 485 nm with each scan being repeated thrice using slit 5 nm on a Fluoromax-4 Horiba Fluorospectrometer. The software data were copied and pasted on excel file. The excel file was taken and the graphs were plotted on OriginPro 8 software. For each data, three readings were recorded separately, and the average of three was plotted along y-axis and time (h) along the x-axis with observed standard deviation being set as y-error.

Finally, normalization was performed using the following equation:

$$\text{Relative \% of amyloid} = \frac{(\text{Observed fluorescence in the presence of SPs} - 1)}{(\text{Observed fluorescence in the absence of SPs} - 1)} \times 100\%$$

where 1 was considered as a normalization factor. Such normalization is required, as the intensity of fluorescence is expressed as  $F/F_0$ , where  $F$  is the fluorescence of the sample and  $F_0$  is the fluorescence of the reference. Thus, the minimum value of  $F/F_0$  becomes 1.

### **Transmission Electron Microscopy (TEM):<sup>3</sup>**

After 6 days of incubation of the stock peptide solutions, 10  $\mu$ L aliquot of each of them was added separately over the dark side of the carbon-coated copper grid and allowed to float for 2 mins. The excess solution was removed using a blotting paper. Thereafter, 10  $\mu$ L of 2% uranyl acetate solution was added to the same grid and allowed to float for another 2 mins, excess solution was removed using blotting paper. The samples were dried in an incubator overnight and kept in a desiccator. TEM images were captured on JEOL (Model: JEM-2100F Field Emission Electron Microscope) at 200 kV.

### **Congo-Red Stained Birefringence:<sup>4</sup>**

Congo-red was purchased from Sigma-Aldrich, and a saturated solution of it was prepared by dissolving it in 80% ethanol. The saturated solution was then filtered to obtain the Congo-red solution for analysis.

After 6 days of incubation of the stock peptide solutions, 10  $\mu$ L aliquot of each of them was placed over a cleaned glass slide and kept in an incubator overnight for drying. After that, 10  $\mu$ L of the saturated Congo-red solution was added over the samples and dried in an incubator. Birefringence analysis was carried out under a Leica ICC50 HD polarizable microscope.

### **Circular Dichroism (CD):<sup>5</sup>**

CD analysis was performed after six days of incubation of the stock peptide solutions. A $\beta$ <sub>1-40</sub> peptide solution (40  $\mu$ M) was used without further dilution. The rest of the stock peptide solutions with the breaker peptides were diluted with PBS (50 mM, pH 7.4) to obtain solutions of concentration 20  $\mu$ M, 40  $\mu$ M and 80  $\mu$ M for the solutions containing 0.5, 1 and 2 fold molar ratios respectively of the breaker peptides. A total volume of 400  $\mu$ L of the

peptide solutions was taken in a cuvette of bandwidth 1mm. Three scans were recorded for each sample starting from 190 nm to 260 nm on a JASCO J-815 spectrometer.

Observed ellipticity (mDeg) was converted to mean residue molar ellipticity using the following equation:

$$\theta \text{ (deg. cm}^2\text{. dmol}^{-1}\text{)} = \text{Ellipticity (mdeg). } 10^6 / (\text{Pathlength (mm). [Protein] } (\mu\text{M). N)$$

Where [Protein] is the concentration of the peptide samples (in  $\mu\text{M}$ ) and N represents the number of amide bonds in the peptide sequence.

#### **Fourier Transform Infrared (FT-IR):<sup>6</sup>**

FT-IR spectra were recorded after 6 days of incubation of the peptide solutions. From the stock solutions, 10  $\mu\text{L}$  aliquot was taken, mixed with KBr and pellet was prepared. For each sample, the spectrum was recorded, and the background scan was subtracted from the sample scan. The text files were taken and plotted in OriginPro 8 software.

#### **Dynamic Light Scattering (DLS):<sup>7</sup>**

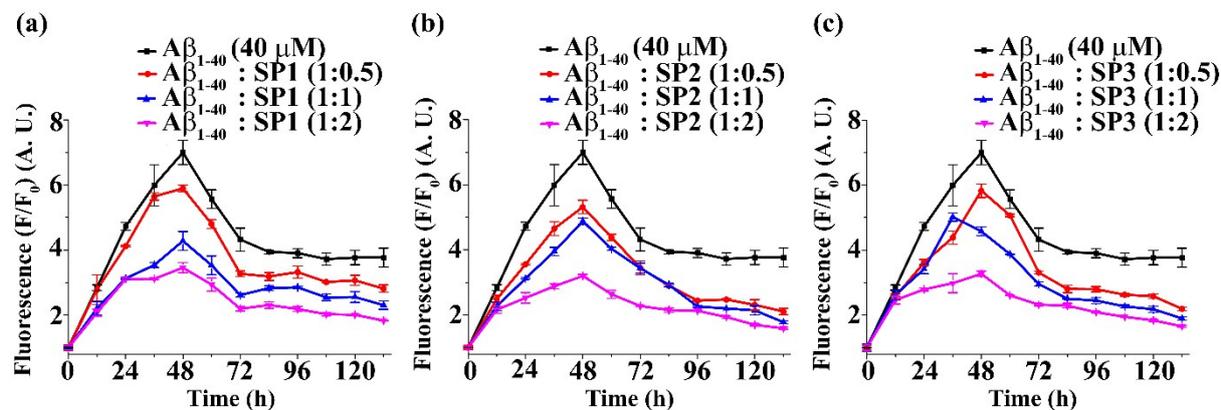
DLS measurements of  $\text{A}\beta_{1-40}$  alone (at different time intervals) as well as in the presence of 2 equivalents of the peptide samples were performed at 25 °C using a 633 nm He-Ne laser on a Zetasizer Nano-ZS90 (Malvern Instruments). A stock solution of  $\text{A}\beta_{1-40}$  (40  $\mu\text{M}$ ) was initially prepared in PBS (pH 7.4) with 3% DMSO and then 2 equivalents of the peptide solution were added to it. The solutions were incubated in PBS at 37 °C and 200  $\mu\text{L}$  of each of the samples ( $\text{A}\beta_{1-40}$  alone for studying the kinetics of amyloid fibrillation) were transferred to a cuvette for measurements at different time intervals. Prior to scanning, the system was equilibrated for 60 seconds. Each measurement thus obtained was an average of five scans, where intensity percentage and size distribution of the particles were recorded. The text values were

obtained directly from the Zetasizer software, then plotted in OriginPro 8 software, redrawn and finalized in Adobe Illustrator.

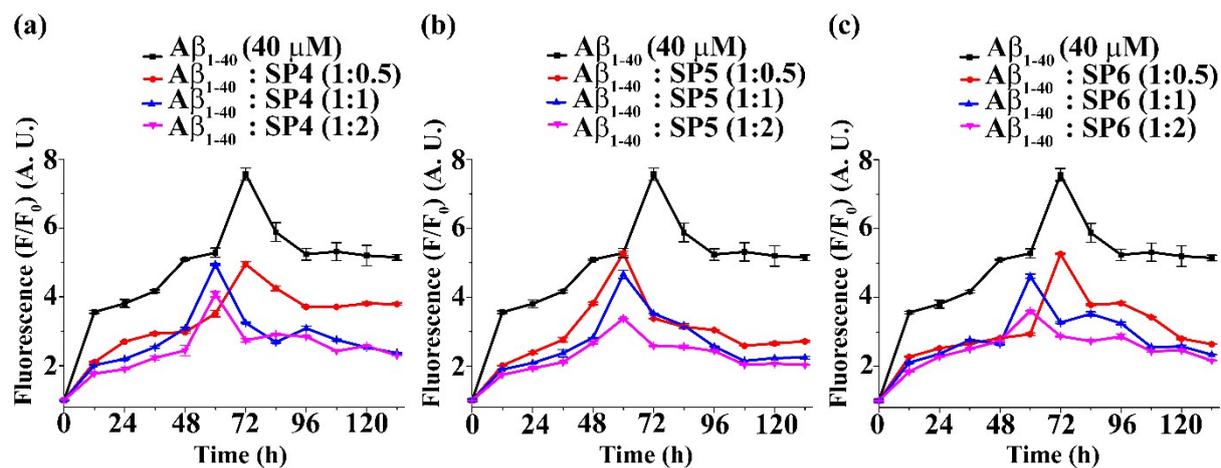
### **Large Unilamellar Vesicles (LUVs) Preparation:<sup>8</sup>**

For the LUV preparation, three different lipids were used, namely DPPC, Cholesterol, and GM1 with a molar ratio of 68:30:2. All the lipids were weighed in a cleaned Eppendorf and dissolved in chloroform and methanol ( $\text{CHCl}_3/\text{MeOH} = 2:1$ ) to prepare a two mM stock solution of the lipids. The solvents were evaporated and dried completely using nitrogen gas to form a thin lipid layer. Next, the carboxyfluorescein dye is weighed and dissolved in 500  $\mu\text{L}$  of PBS (50 mM, pH 7.4) to make a 200  $\mu\text{M}$  stock solution. The lipid film was hydrated in the prepared dye solution and then vortexed vigorously for 30 mins to emulsify the lipid mixtures. For complete entrapment of the dye, the Eppendorf was cooled by dipping it in liquid nitrogen. After that, the frozen solution was immediately dipped in a water bath at 60-70  $^\circ\text{C}$  for thawing,<sup>9</sup> and the process is repeated five times. The resulting solution was centrifuged at 15000 rpm, and the supernatant dye solution was discarded to remove the excess dye. The lipid pellet thus obtained was hydrated in 500  $\mu\text{L}$  of PBS buffer and vortexed to obtain a homogeneous suspension of a two mM stock solution of the lipids. The resulting solution was filtered to obtain the dye loaded LUVs, and the dye leakage assay was performed on a Fluoromax-4 Horiba Fluorospectrometer. The formation of LUVs was confirmed by Transmission Electron Microscopy (TEM).

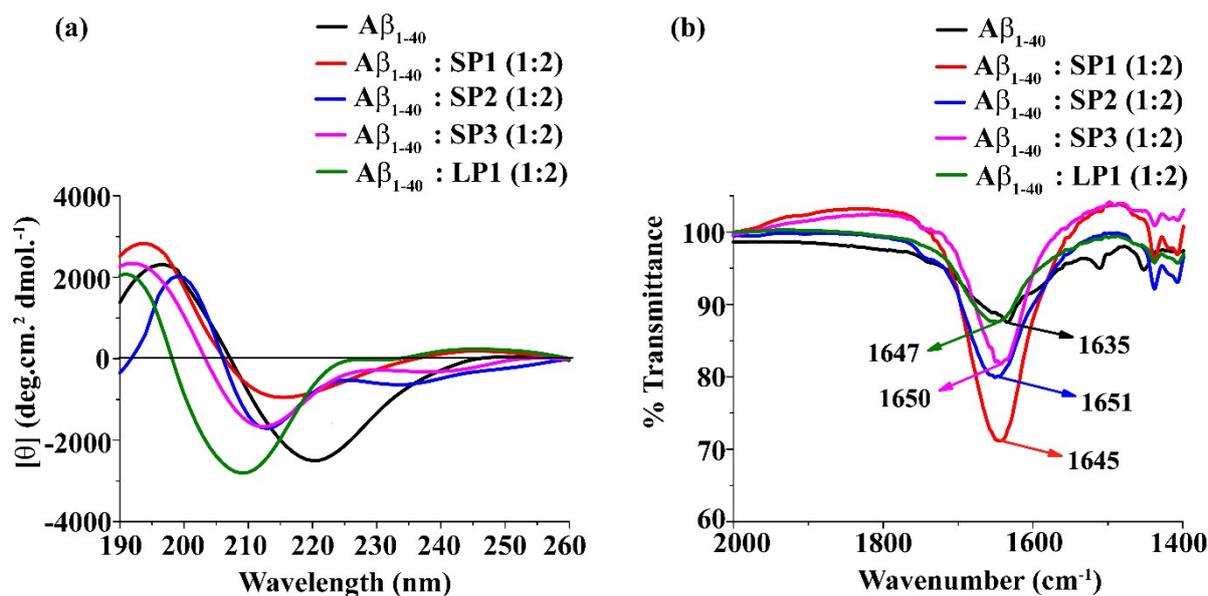
## Inhibition of Amyloid ( $A\beta_{1-40}$ ) Aggregation by Different Doses of SPs:



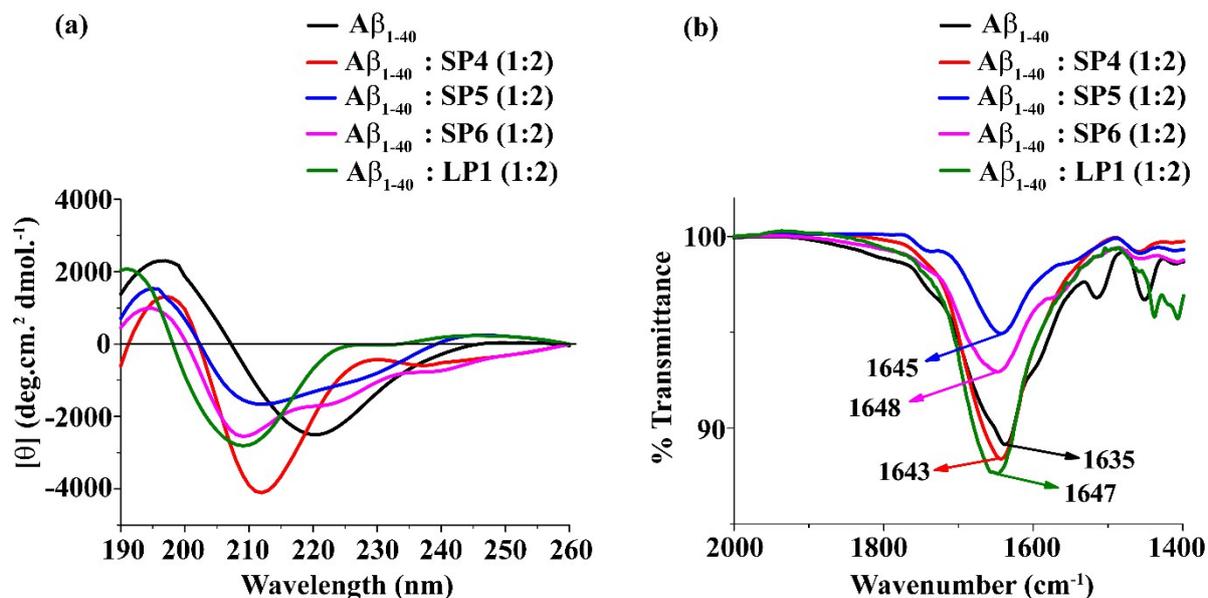
**Fig. S15.** Time-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu$ M) in the absence (black) and presence of -0.5, 1 and 2-fold molar excess of (a) SP1, (b) SP2 and (c) SP3. The peptide solutions were incubated in PBS at pH 7.4 and 37  $^{\circ}$ C.



**Fig. S16.** Time-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu$ M) in the absence (black) and presence of -0.5, 1 and 2-fold molar excess of (a) SP4, (b) SP5 and (c) SP6. The peptide solutions were incubated in PBS at pH 7.4 and 37  $^{\circ}$ C.

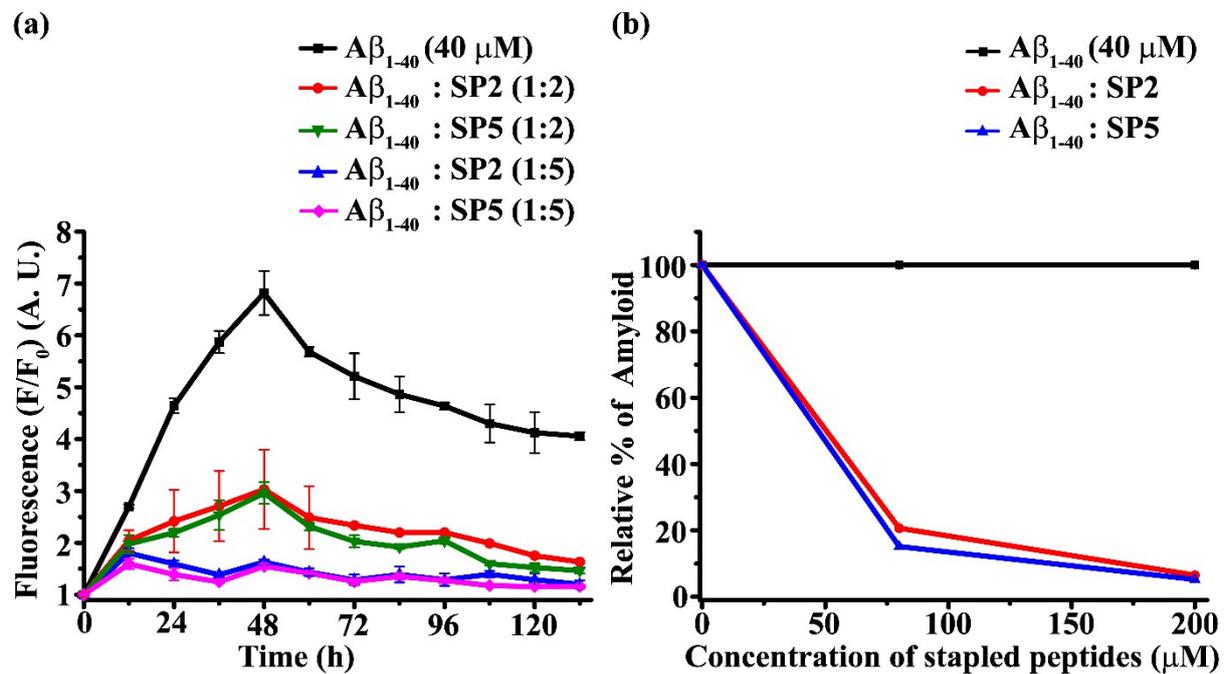


**Fig. S17.** (a) CD spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP1 (red), SP2 (blue), SP3 (magenta) and LP1 (olive). (b) FTIR spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP1 (red), SP2 (blue), SP3 (magenta) and LP1 (olive). The spectra were recorded after 6 days of incubation in PBS at pH 7.4 and 37 °C.



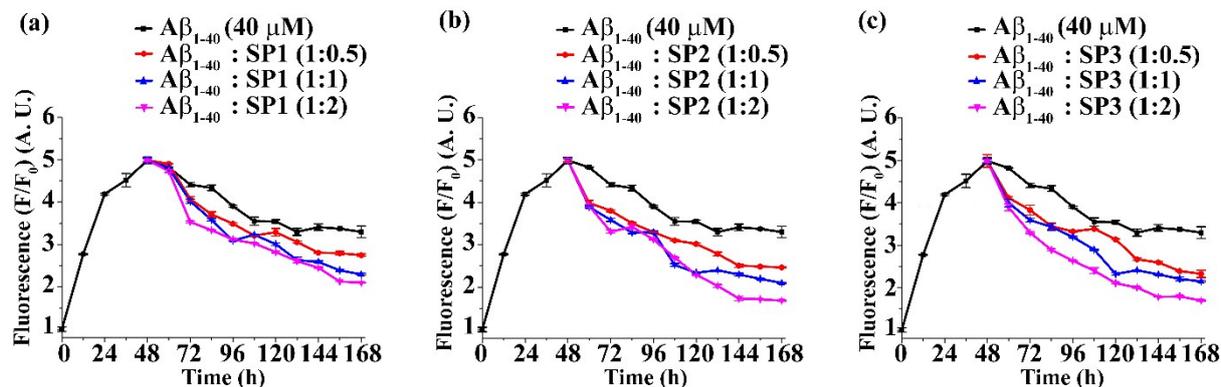
**Fig. S18.** (a) CD spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP4 (red), SP5 (blue), SP6 (magenta) and LP1 (olive). (b) FTIR spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP4 (red), SP5 (blue), SP6 (magenta) and LP1 (olive). The spectra were recorded after 6 days of incubation in PBS at pH 7.4 and 37 °C.

**Inhibition of Amyloid ( $A\beta_{1-40}$ ) Aggregation by High Doses of SP2 and SP5 (5-fold molar excess and its comparison with 2-fold molar excess):**

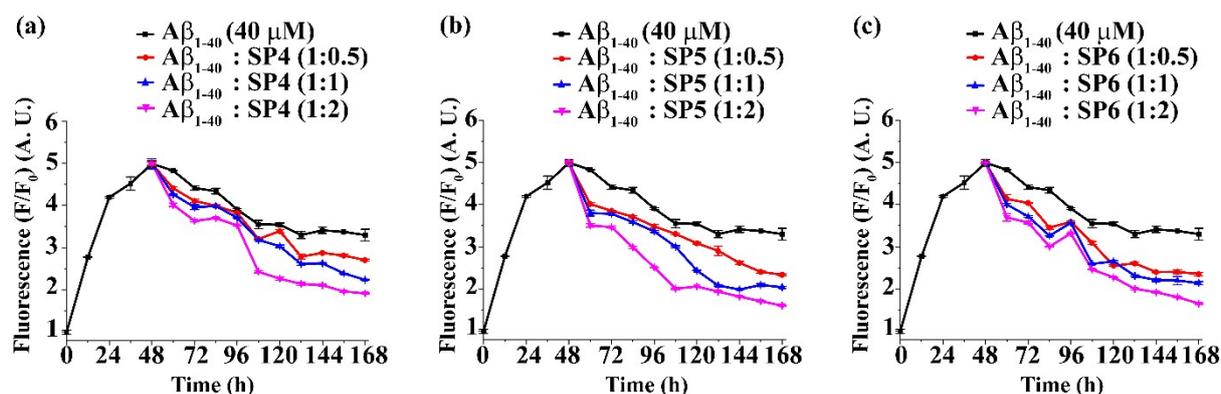


**Fig. S19.** Time-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) in the absence (black) and presence of 2-fold molar excess of SP2 (red) and SP5 (olive), 5-fold molar excess of SP2 (blue) and SP5 (magenta). (b) Dose-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) in the absence (black) and presence of 2 and 5-fold molar excesses of SP2 (red) and SP5 (blue). The peptide solutions were incubated in PBS at pH 7.4 and 37  $^{\circ}\text{C}$ .

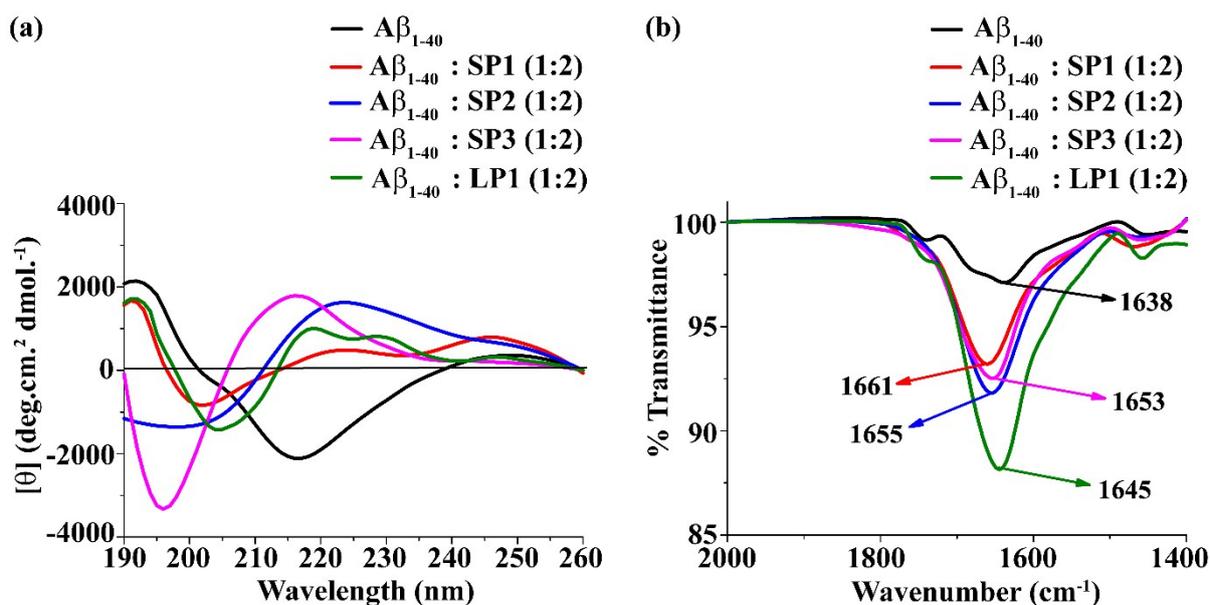
## Disruption of Preformed Amyloid ( $A\beta_{1-40}$ ) Fibrils by Different Doses of SPs:



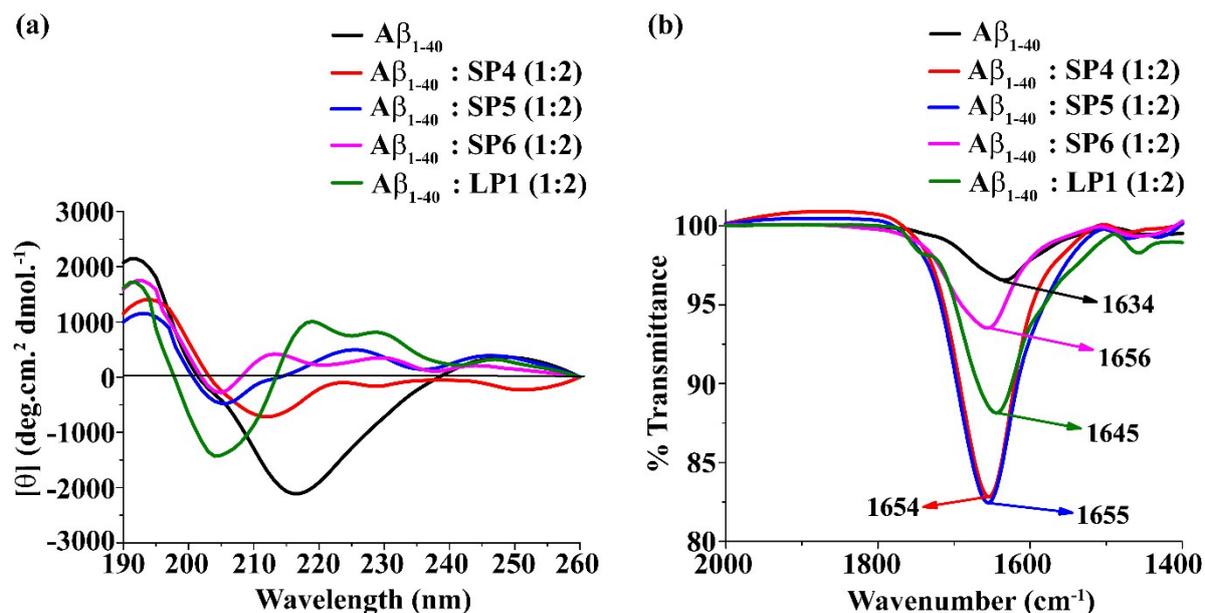
**Fig. S20.** Time-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu$ M) in the absence (black) and presence of -0.5, 1 and 2-fold molar excess of (a) SP1, (b) SP2 and (c) SP3. The peptide solutions were incubated in PBS at pH 7.4 and 37  $^{\circ}$ C.



**Fig. S21.** Time-dependent ThT fluorescence assay of  $A\beta_{1-40}$  (40  $\mu$ M) in the absence (black) and presence of -0.5, 1 and 2-fold molar excess of (a) SP4, (b) SP5 and (c) SP6. The peptide solutions were incubated in PBS at pH 7.4 and 37  $^{\circ}$ C.

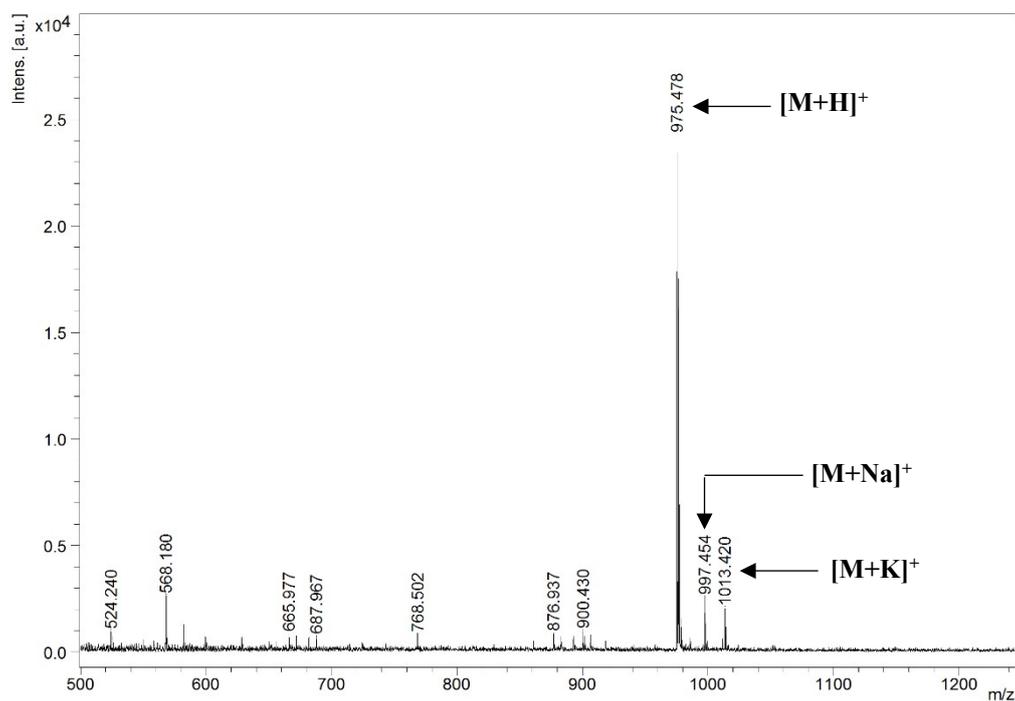


**Fig. S22.** (a) CD spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP1 (red), SP2 (blue), SP3 (magenta) and LP1 (olive). (b) FTIR spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP1 (red), SP2 (blue), SP3 (magenta) and LP1 (olive). The spectra were recorded after six days of incubation in PBS at pH 7.4 and 37 °C.

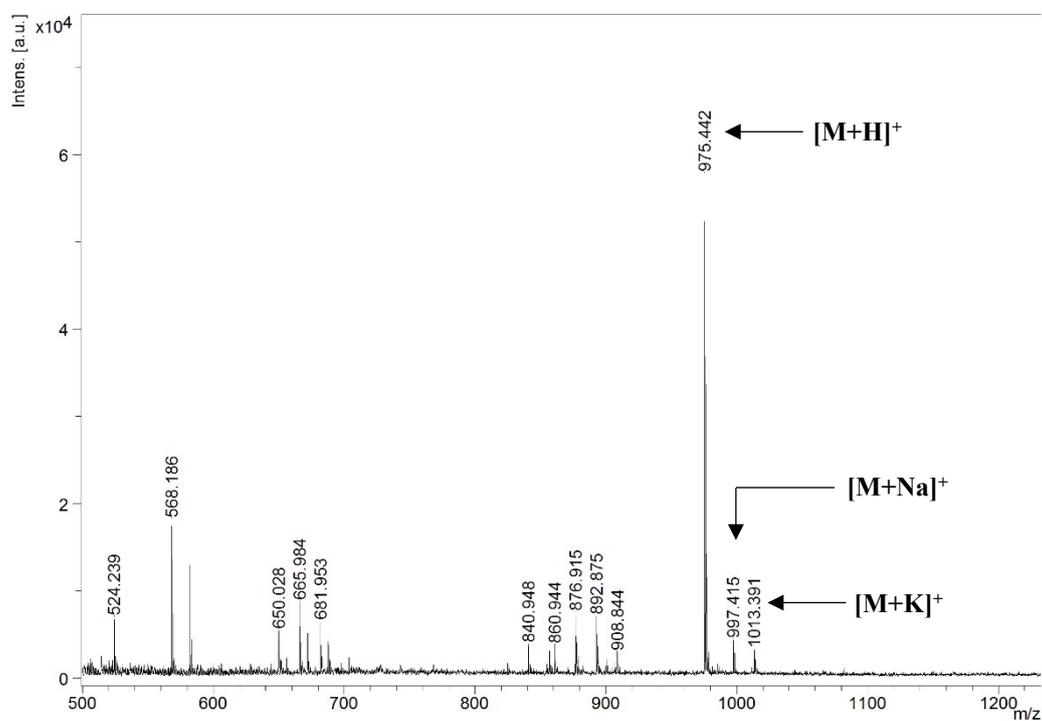


**Fig. S23.** (a) CD spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP4 (red), SP5 (blue), SP6 (magenta) and LP1 (olive). (b) FTIR spectra of  $A\beta_{1-40}$  (40  $\mu\text{M}$ ) alone (black) and in the presence of 2-fold molar excess of SP4 (red), SP5 (blue), SP6 (magenta) and LP1 (olive). The spectra were recorded after six days of incubation in PBS at pH 7.4 and 37 °C.

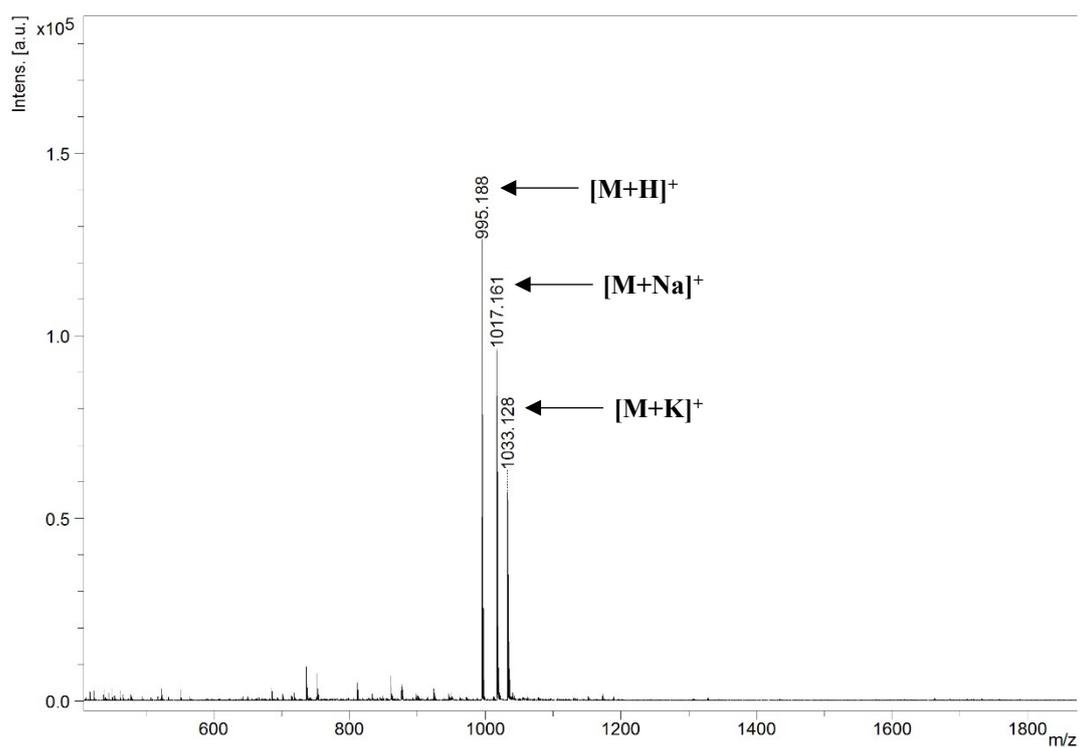
## Enzymatic Stability Study<sup>10, 11</sup>



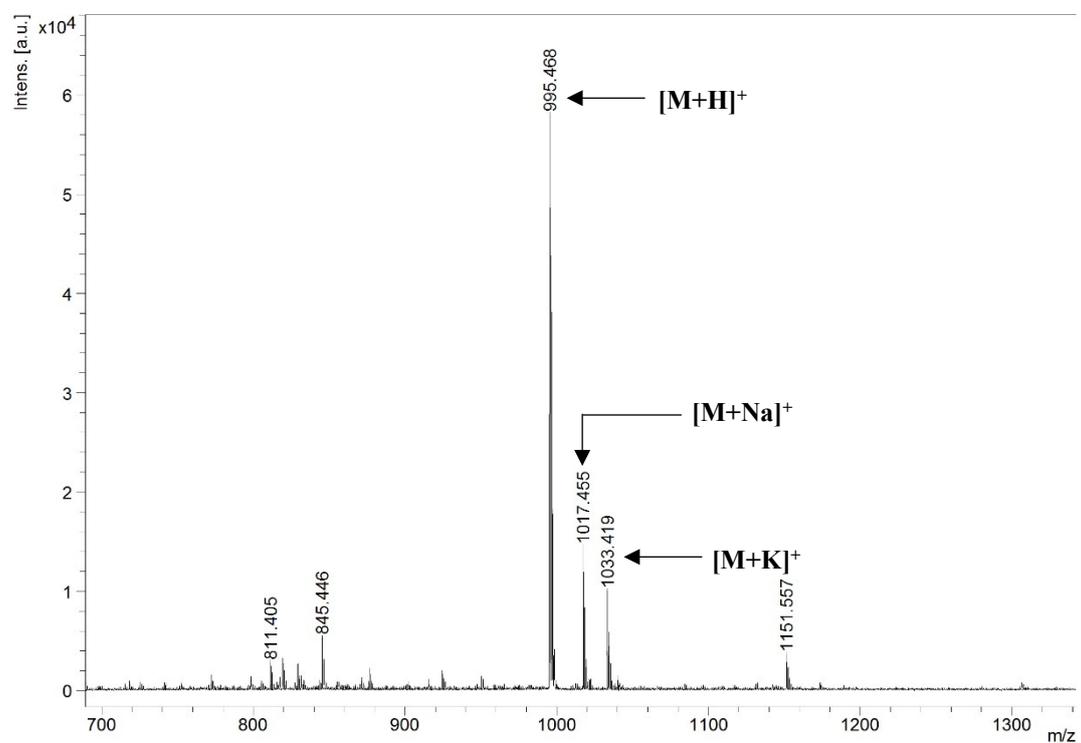
**Fig. S24.** MALDI-TOF mass spectra of **SP2** after addition of FBS serum at **0h**. Calculated mass for  $C_{49}H_{74}N_{12}O_9$  is 974.5702; observed masses are 975.478 [M+H]<sup>+</sup>, 997.454 [M+Na]<sup>+</sup> and 1013.420 [M+K]<sup>+</sup>.



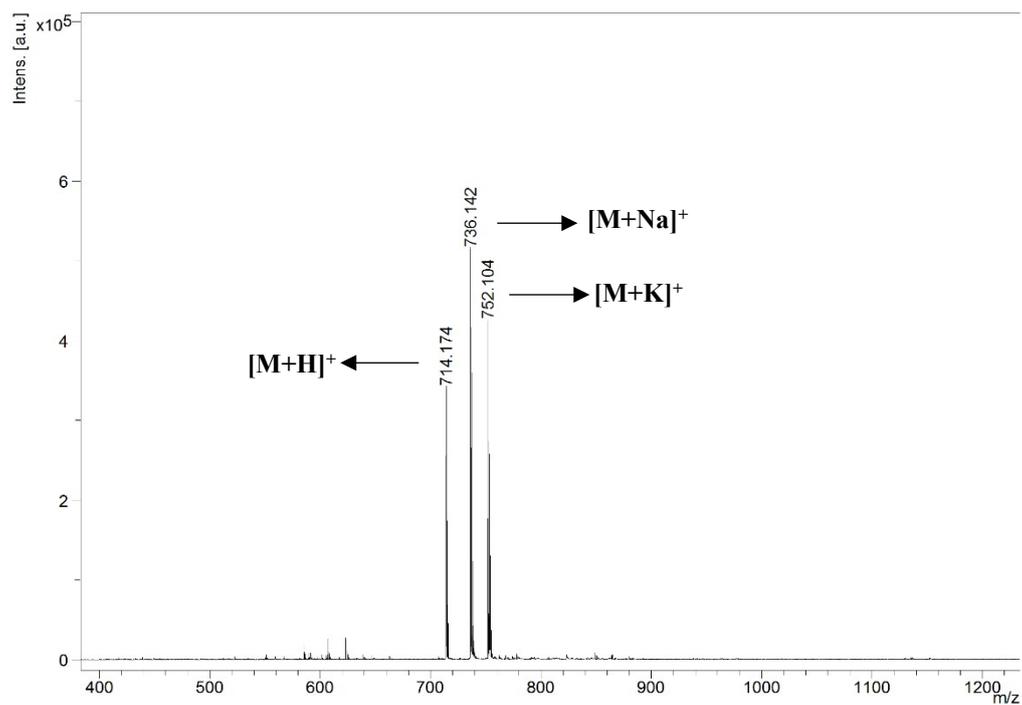
**Fig. S25.** MALDI-TOF mass spectra of **SP2** after addition of FBS serum at **25h**. Calculated mass for  $C_{49}H_{74}N_{12}O_9$  is 974.5702, observed masses are 975.442 [M+H]<sup>+</sup>, 997.415 [M+Na]<sup>+</sup> and 1013.391 [M+K]<sup>+</sup>.



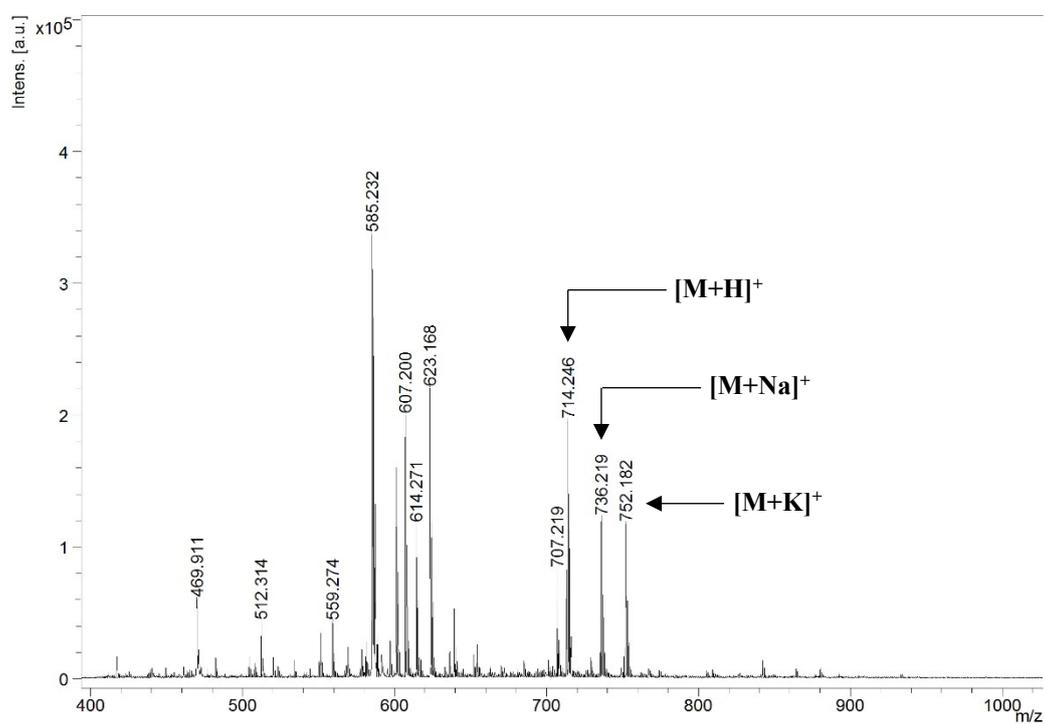
**Fig. S26.** MALDI-TOF mass spectra of **SP5** after addition of FBS serum at **0h**. Calculated mass for  $C_{51}H_{70}N_{12}O_9$  is 994.5389, observed masses are 995.188 [M+H]<sup>+</sup>, 1017.161 [M+Na]<sup>+</sup> and 1033.128 [M+K]<sup>+</sup>.



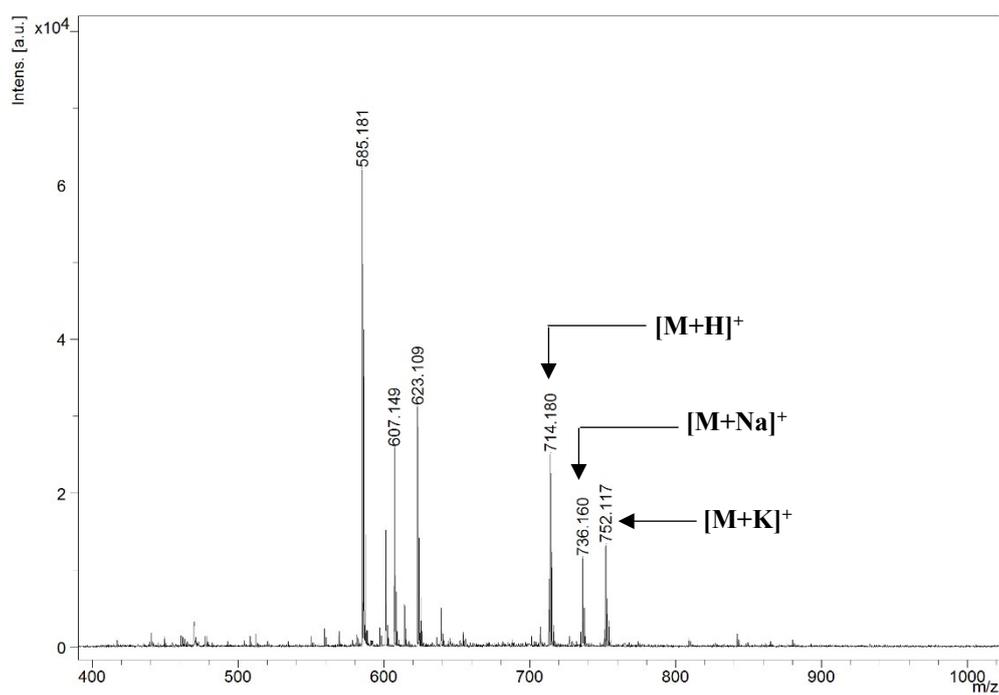
**Fig. S27.** MALDI-TOF mass spectra of **SP5** after addition of FBS serum at **25h**. Calculated mass for  $C_{51}H_{70}N_{12}O_9$  is 994.5389, observed masses are 995.468 [M+H]<sup>+</sup>, 1017.455 [M+Na]<sup>+</sup> and 1033.419 [M+K]<sup>+</sup>.



**Fig. S28.** MALDI-TOF mass spectra of LP1 after addition of FBS serum at 0h. Calculated mass for  $C_{39}H_{51}N_7O_6$  is 713.3901, observed masses are 714.174 [M+H]<sup>+</sup>, 736.142 [M+Na]<sup>+</sup> and 752.104 [M+K]<sup>+</sup>.

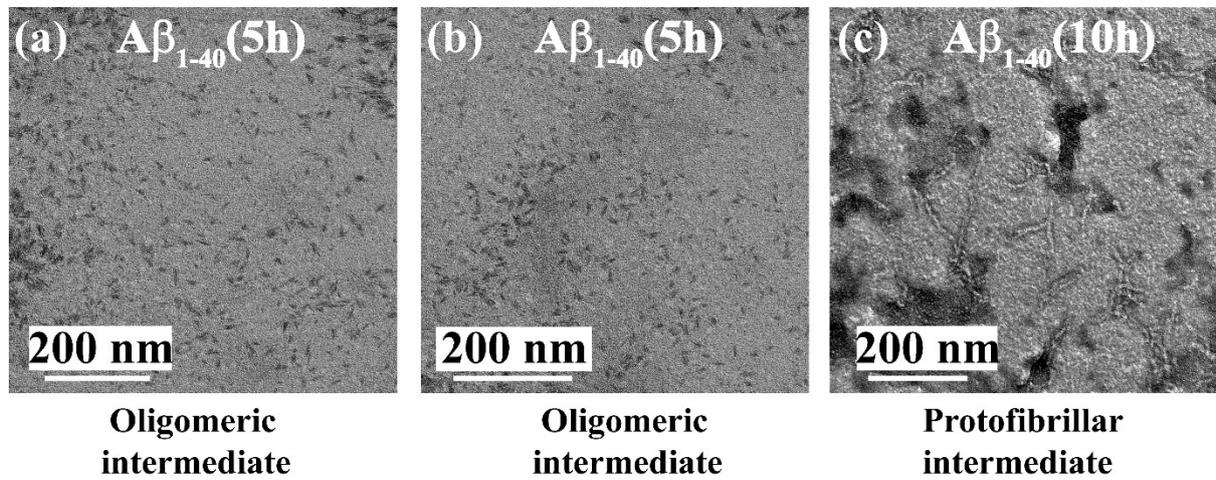


**Fig. S29.** MALDI-TOF mass spectra of LP1 after addition of FBS serum at 10h. Calculated mass for  $C_{39}H_{51}N_7O_6$  is 713.3901, observed masses are 714.246 [M+H]<sup>+</sup>, 736.219 [M+Na]<sup>+</sup> and 752.182 [M+K]<sup>+</sup>.



**Fig. S30.** MALDI-TOF mass spectra of **LP1** after addition of FBS serum at **25h**. Calculated mass for  $C_{39}H_{51}N_7O_6$  is 713.3901, observed masses are 714.180  $[M+H]^+$ , 736.160  $[M+Na]^+$  and 752.117  $[M+K]^+$ .

**TEM Images of Oligomeric and Protofibrillar Intermediates:<sup>12,13</sup>**



**Fig. S31.** TEM images of oligomeric intermediate species of Aβ<sub>1-40</sub> formed after 5h of incubation [(a) and (b)] and protofibrillar intermediate amyloid species formed after 10h of incubation (c). The scale bar is indicated as 200 nm.

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