Electronic Supplementary Information (ESI)

Copper Chelating Cyclic Peptidomimetic Inhibits Aβ Fibrillogenesis

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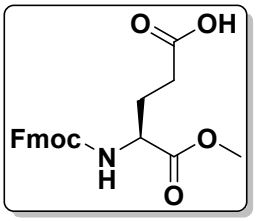

Structure of product **1**.

Characterisation data of Product 1 by HRMS and NMR:

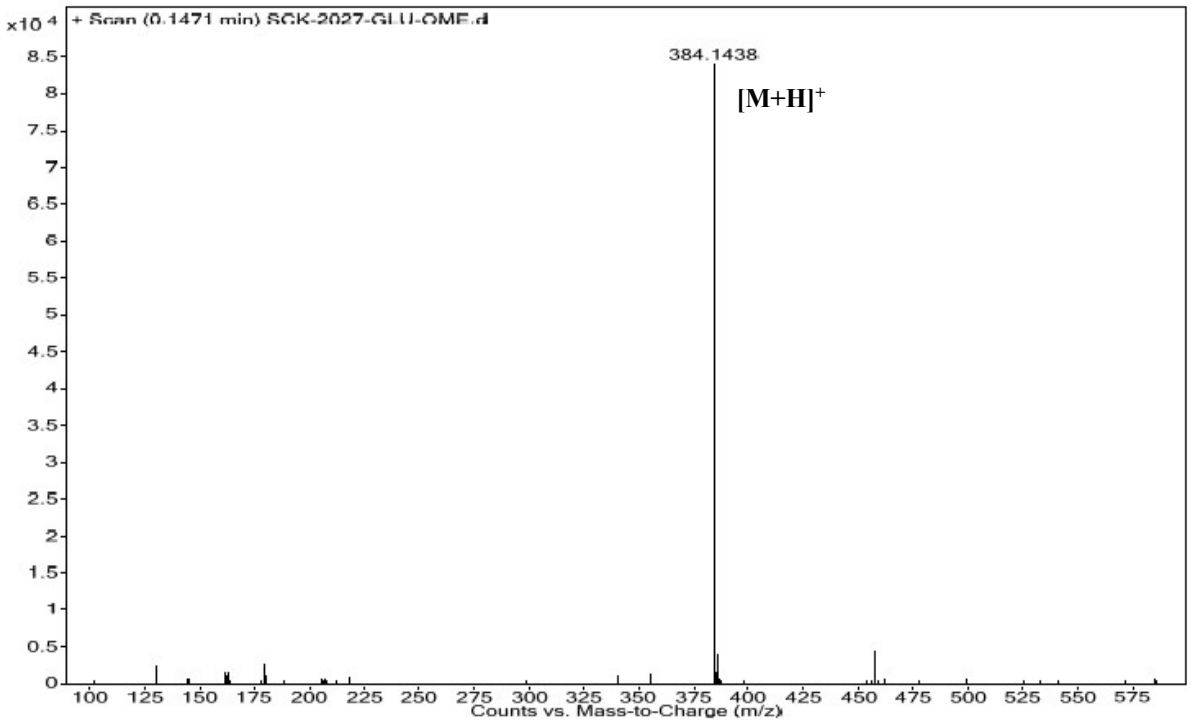

Fig. S1. ESI mass spectrum of product **1**. Calculated mass for $C_{21}H_{21}NO_6$ is 384.1402 $[M+H]^+$, observed mass of $[M+H]^+$ is 384.1438.

^{1}H and ^{13}C NMR Characterisation Data of the Synthesized Product 1:

Yield: 600 mg (78%); white solid; R_f = 0.50 (EtOAc:Hexane, 2.0:8.0); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.766-7.747 (d, *J* = 7.6 Hz, 2H), 7.598-7.581 (d, *J* = 6.8 Hz, 2H), 7.414-7.376 (t, *J* = 7.6 Hz, 2H), 7.330-7.293 (t, *J* = 7.2 Hz, 2H), 5.499 (br, 1H) , 4.455-4.397 (m, 3H), 4.226-4.192 (t, *J* = 6.8 Hz, 1H), 3.752 (s, 3H), 2.509-2.365 (m, 2H), 2.262-2.175 (m, 1H), 2.010-1.919 (m, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 172.5, 156.2, 143.8, 141.4, 127.9, 127.2, 125.2, 120.2 67.2, 53.3, 52.8, 47.3, 30.0, 27.5.

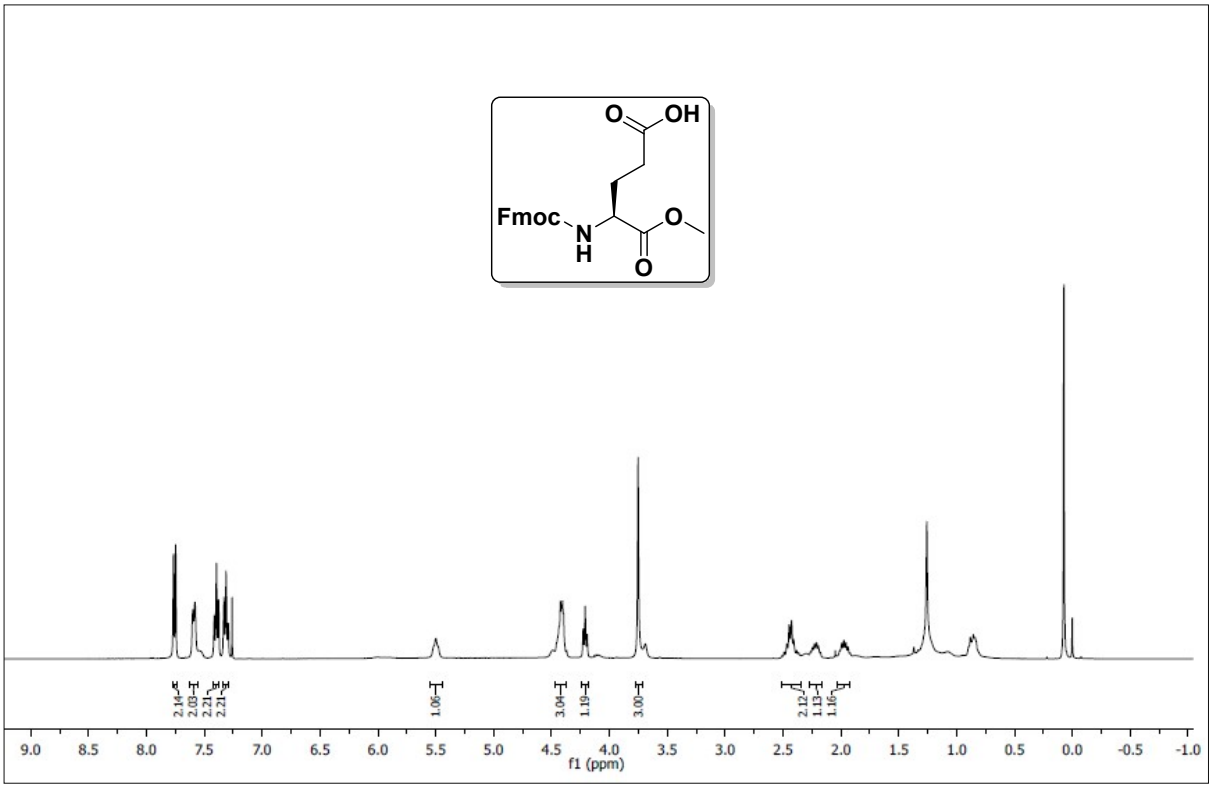

Fig. S2. ^{1}H-NMR spectra of purified product **1**.

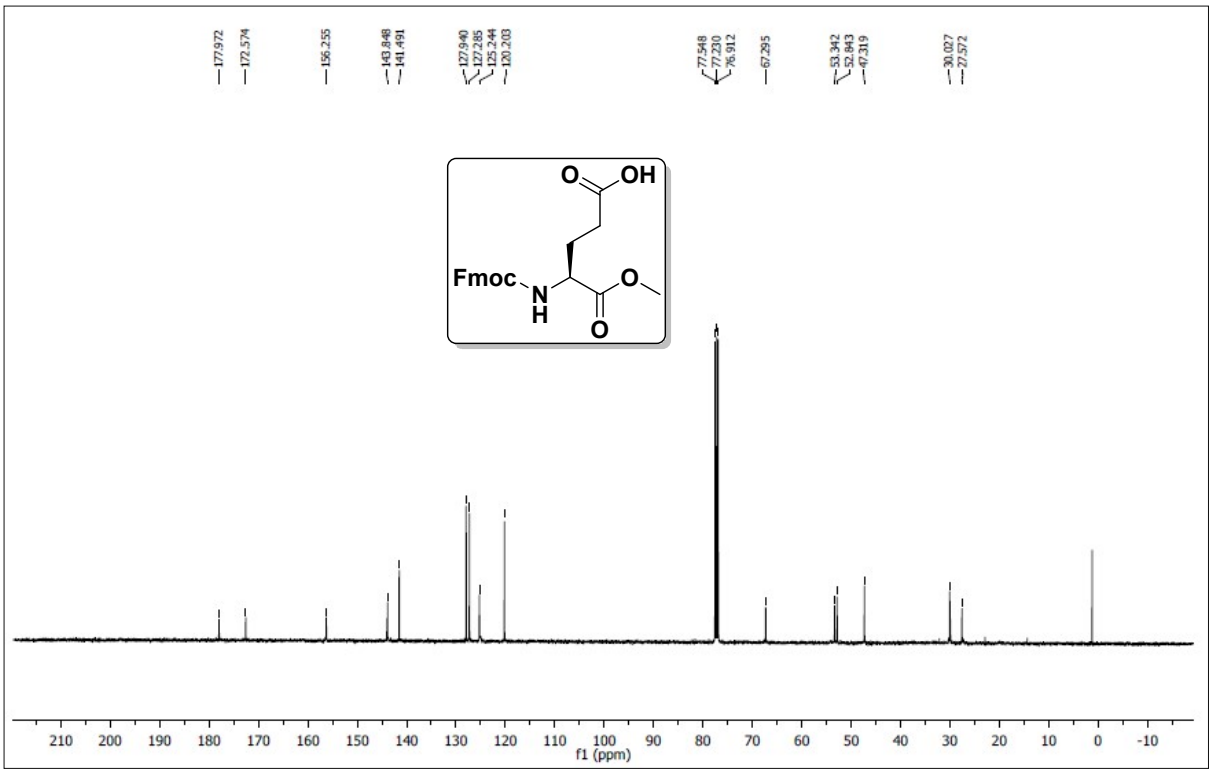

Fig. S3. ^{13}C-NMR spectra of purified product **1**.

Chemical Structures of Fluorophore Attached Peptides:

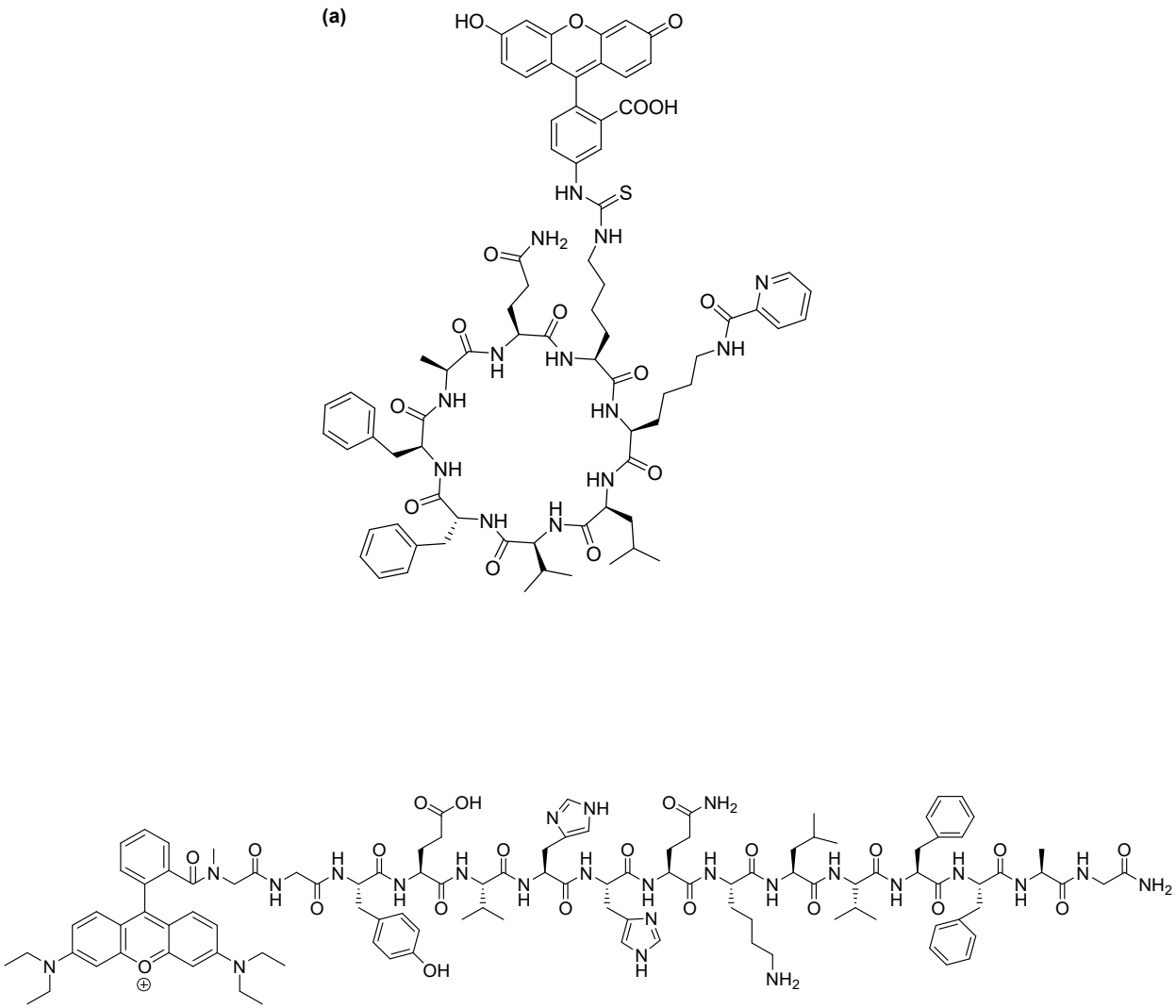

Fig. S4. Chemical structure of (a) **PA^{19}fCP-Fl** and (b) **MAβ-RhB.**

Characterisation Data of the Synthesized Peptides by HPLC and High Resolution Mass Spectrometry (HRMS):

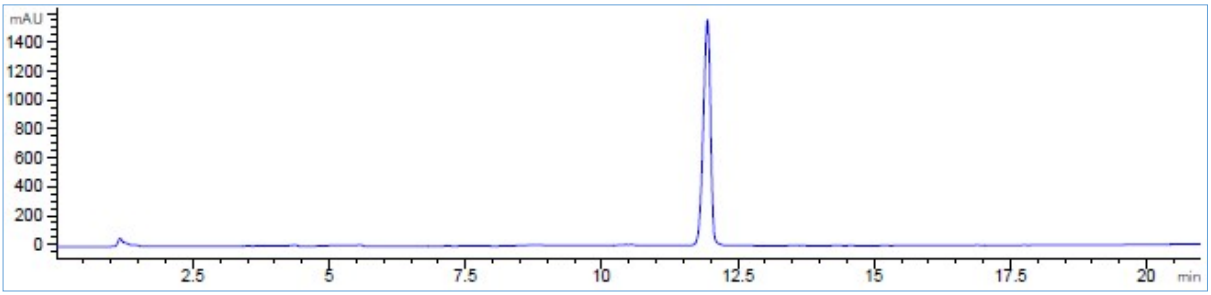

Fig. S5. HPLC profile of purified peptide **PA^{19}fCP**.

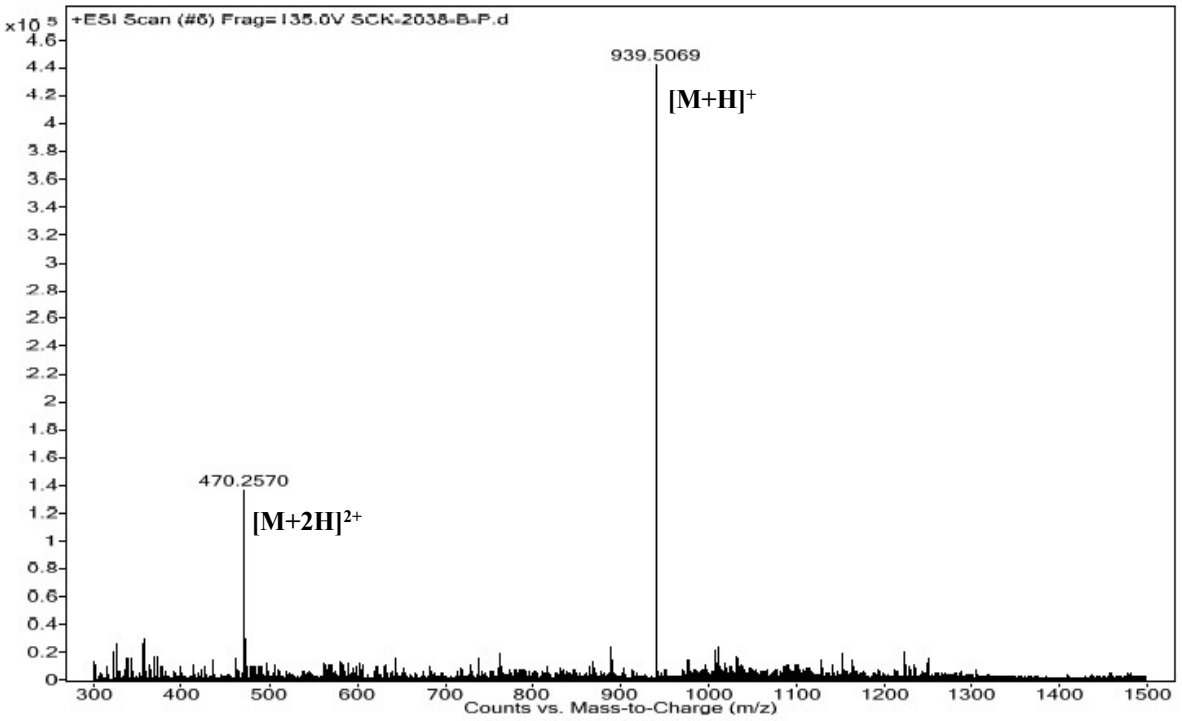

Fig. S6. ESI mass spectrum of purified peptide **PA^{19}fCP**. Calculated mass for $C_{49}H_{66}N_{10}O_9$ is 939.5048 $[M+H]^+$, observed mass of $[M+H]^+$ is 939.5069 and $[M+2H]^{2+}$ is 470.2570.

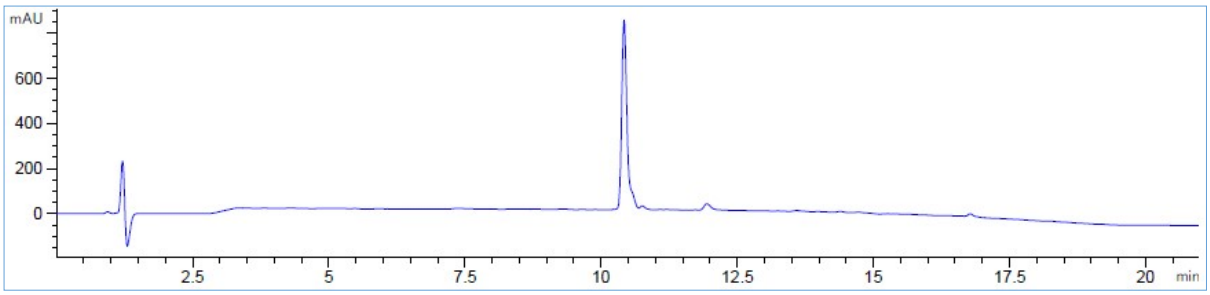

Fig. S7. HPLC profile of purified peptide **^{19}fCP**.

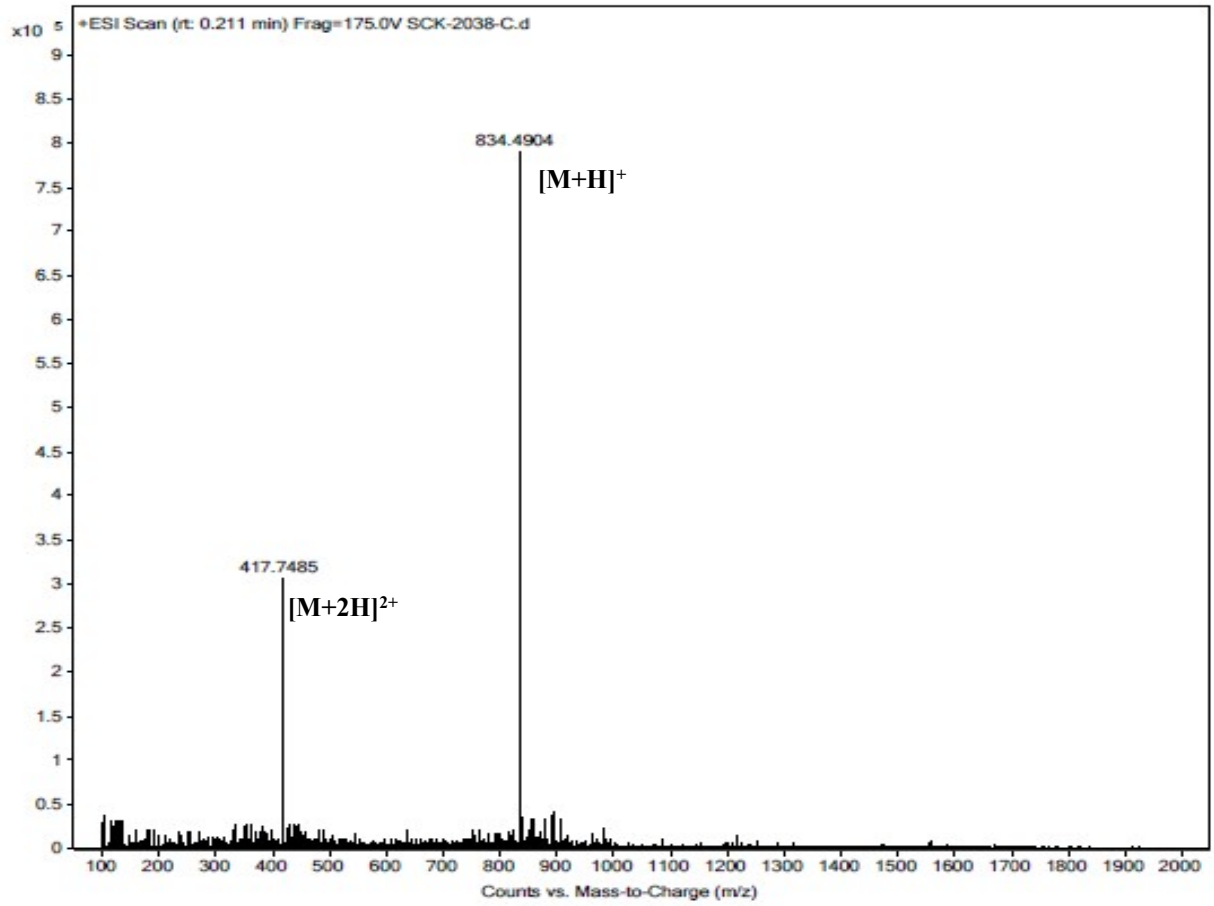

Fig. S8. ESI mass spectrum of purified peptide **^{19}fCP**. Calculated mass for $C_{43}H_{63}N_9O_8$ is 834.4833 $[M+H]^+$, observed mass of $[M+H]^+$ is 834.4904 and $[M+2H]^{2+}$ is 417.7485.

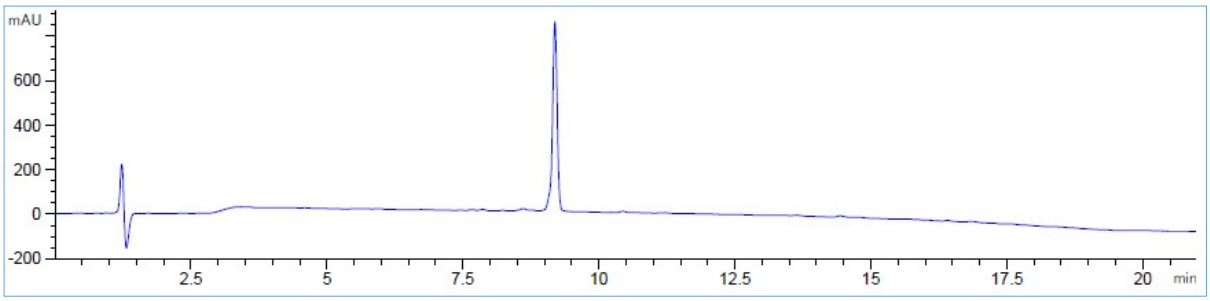

Fig. S9. HPLC profile of purified peptide **^{19}fLP**.

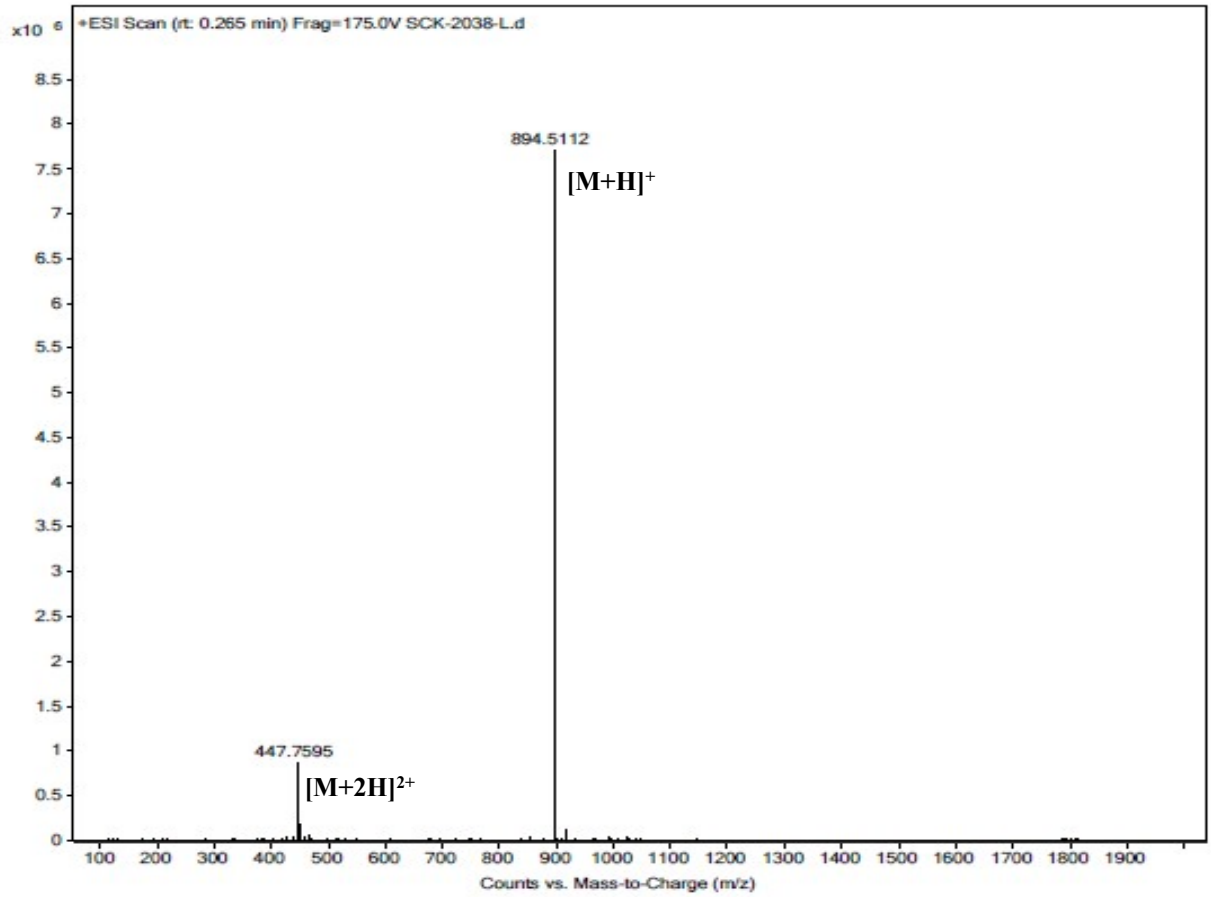

Fig. S10. ESI mass spectrum of purified peptide **^{19}fLP**. Calculated mass for $C_{45}H_{67}N_9O_{10}$ is 894.5044 $[M+H]^+$, observed mass of $[M+H]^+$ is 894.5112, and $[M+2H]^{2+}$ is 447.7595.

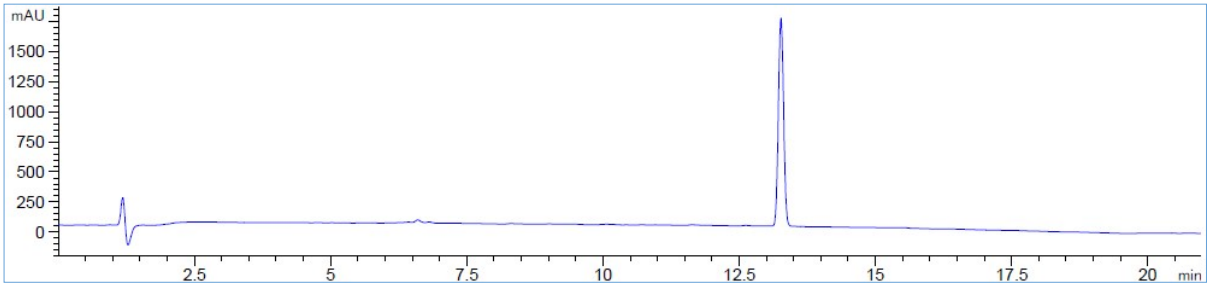

Fig. S11. HPLC profile of purified peptide **PA^{19}fCP-Fl**.

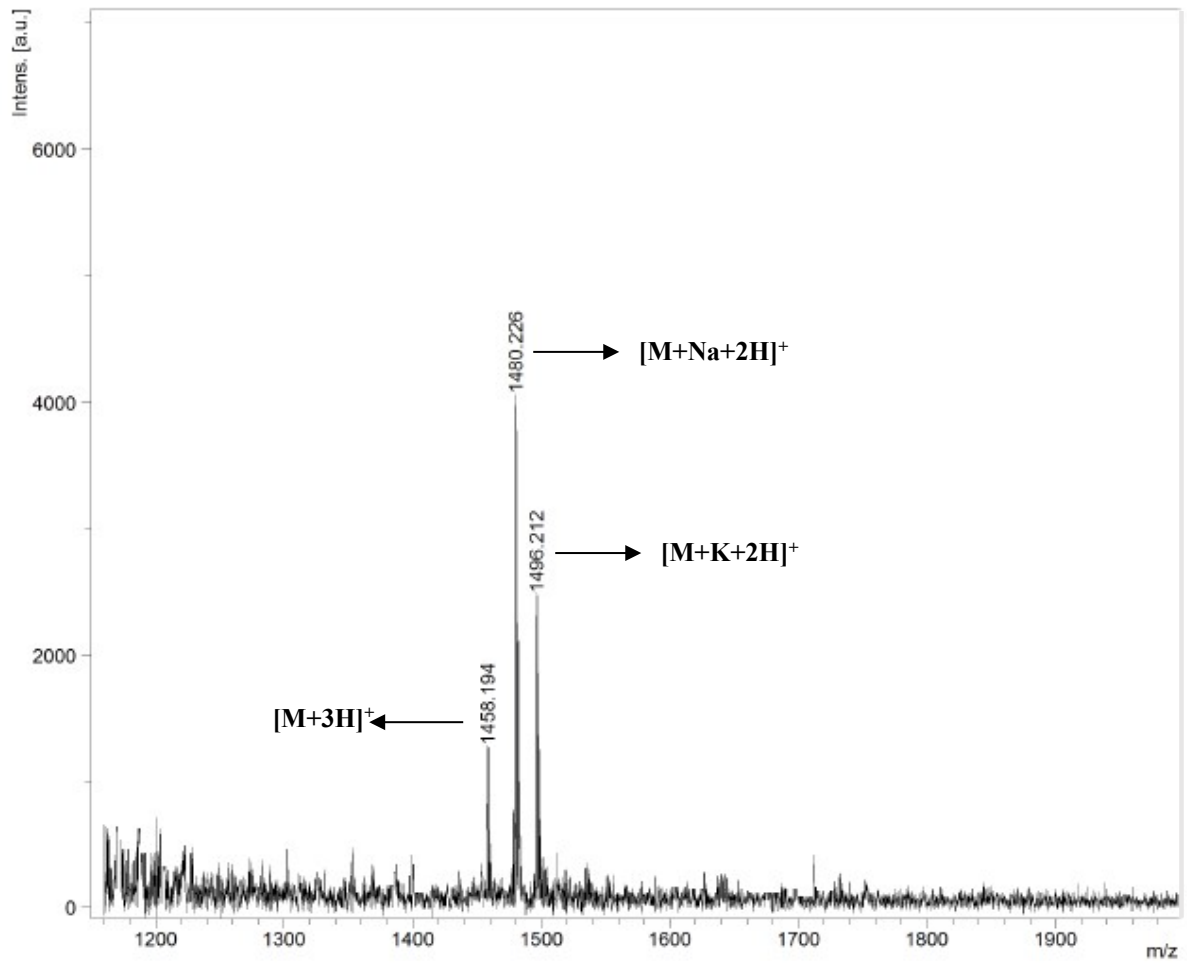

Fig. S12. MALDI-TOF mass spectra of **PA^{19}fCP-Fl**. Calculated mass for $C_{76}H_{89}N_{13}O_{15}S$ is 1456.6355 $[M+H]^+$, observed masses are 1458.194 $[M+3H]^+$, 1480.226 $[M+Na+2H]^+$ and 1496.212 $[M+K+2H]^+$.

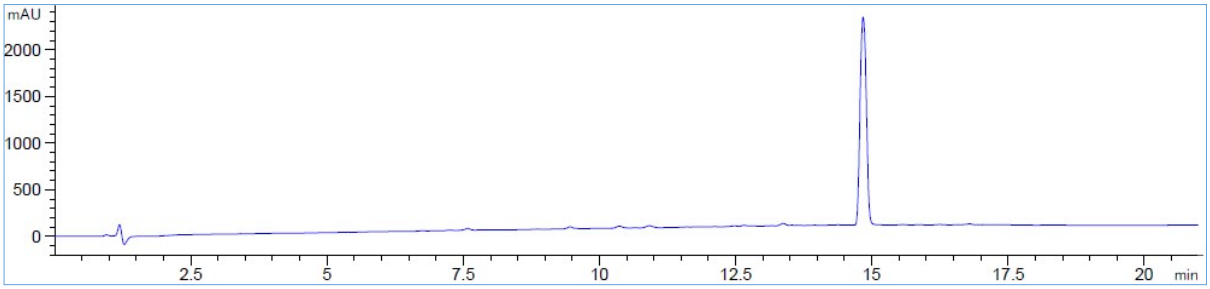

Fig. S13. HPLC profile of purified peptide **MAβ-RhB**.

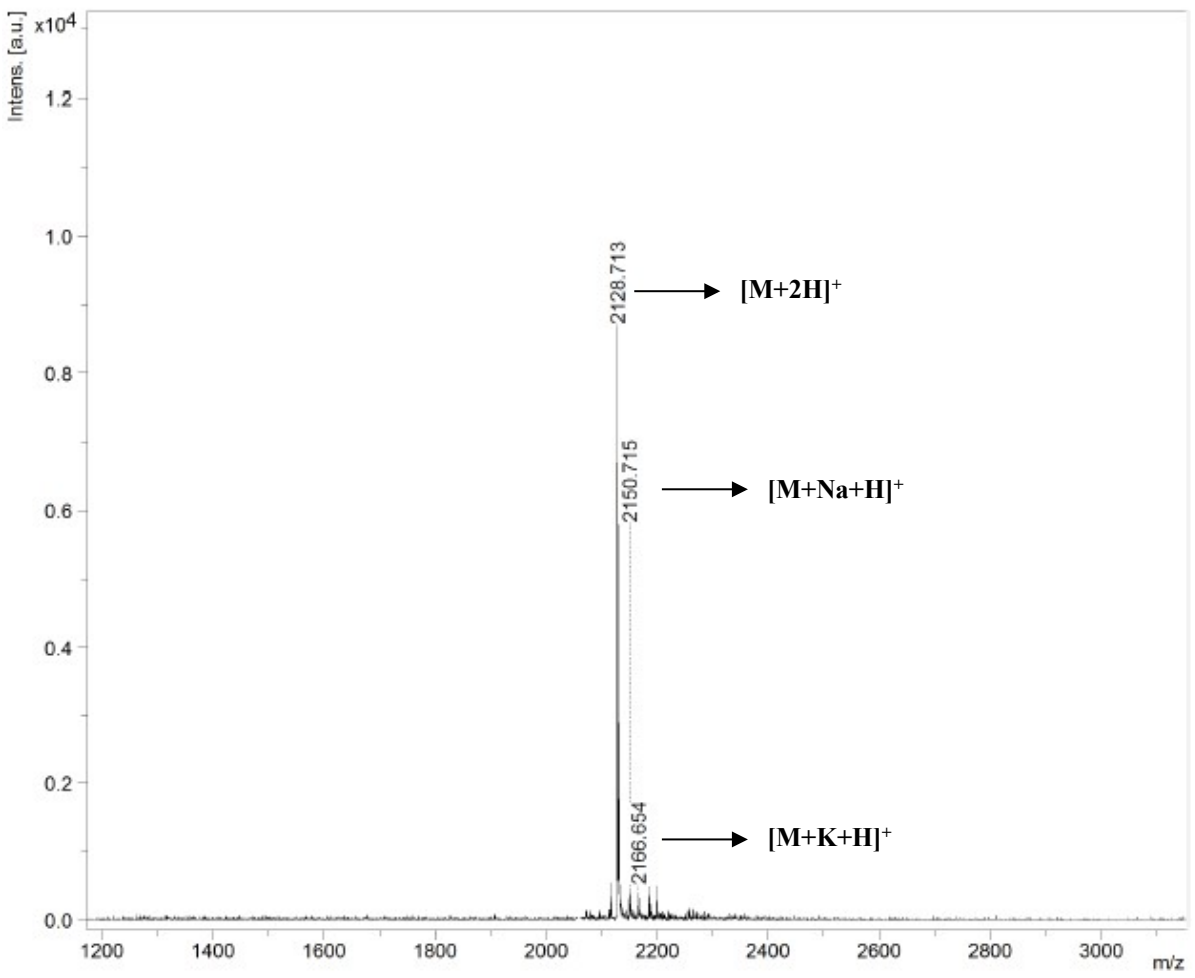

Fig. S14. MALDI-TOF mass spectra of **MAβ-RhB**. Calculated mass for $C_{109}H_{145}N_{24}O_{21}$ is 2127.1044 $[M+H]^+$, observed masses are 2128.713 $[M+2H]^+$, 2150.715 $[M+Na+H]^+$ and 2166.654 $[M+K+H]^+$.

Reagents and Solvents:

Amyloid-beta ($A\beta_{1-40}$) peptide, Rink Amide MBHA resin (loading 1.1 mmol/g) and all Fmoc-protected amino acids (with the following side-chains protecting groups: Boc for lysine and OtBu for glutamic acid) were purchased from GL Biochem (Shanghai, China). Diisopropylethylamine (DIPEA) was purchased from Spectrochem. Pvt. Ltd. (India). Zinc bromide ($ZnBr_2$) was purchased from Alfa-Aesar and picolinic acid was purchased from Sigma Aldrich. Trifluoroacetic acid (TFA) of extrapure grade, acetic anhydride (synthesis grade) and N-methyl-imidazole of extrapure grade were purchased from SPL (India). Hexafluoroisopropanol (HFIP), Thioflavin T (ThT) and Congo–red dyes were purchased from Sigma Aldrich. Acetonitrile of HPLC grade, dimethylformamide (DMF) of extrapure grade and dichloromethane of extrapure grade were purchased from Merck (India). Ethyl 2-cyano-2-(2-nitrobenzenesulfonyloxyimino) acetate (o-NosylOXY) was synthesized in our laboratory starting from Ethyl-hydroxyiminocyanoacetate (Oxyma) and 2-nitrobenzenesulphonyl chloride.

Synthetic Methods:

Amyloid-beta ($A\beta_{1-40}$) peptide was used as the aggregating peptide in all the experiments. All the peptide syntheses were carried out manually inside a frit-fitted plastic syringe by standard Fmoc/tBu solid-phase peptide synthesis (SPPS) on a Stuart tube rotator using Rink Amide MBHA resin as the solid support. The resin was first swollen in DCM for 3h and then in DMF for 1h. Fmoc deprotection in each step was performed using 20% piperidine in DMF for 21 mins. Peptide coupling was carried out using 2 equivalents of Fmoc-protected amino acids, 2.5 equivalents of *o*-NosylOXY as coupling reagent, and 5 equivalents of DIPEA as the base. Each and every coupling was monitored by performing Kaiser's test. Incomplete peptide coupling was confirmed from the bluish-purple colour in the test, indicating the presence of a free $–NH_2$

group. In such cases, the respective coupling was repeated, followed by acetylation (capping) using Ac_2O/NMI (1:2) in DCM for 1.5h.

Prior to the synthesis of the cyclic peptides, Fmoc-Glu(OtBu)-OH was converted into its methyl ester derivative using methyl iodide (MeI) and DIPEA in acetonitrile (ACN). The acid (1 eq) was dissolved in ACN, followed by the addition of DIPEA (2 eq). The solution was stirred for 2-3 mins for thorough mixing, followed by the addition of MeI (5 eq). The reaction mixture was stirred for 12-15 h at room temperature. After completion of the reaction, acetonitrile was evaporated completely, and the reaction mixture was diluted with ethyl acetate; the organic layer was washed thoroughly with 5% citric acid and 5% $NaHCO_3$ solution, dried by anhydrous Na_2SO_4 and then filtered off; the solvent was evaporated to obtain the methyl ester product. The product thus obtained was dissolved in 80% TFA solution in DCM, and the reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, TFA was evaporated completely by purging nitrogen gas and then by rotary evaporator. The reaction mixture was washed thoroughly with DCM several times to ensure complete removal of TFA, followed by the addition of hexane to obtain the solid product (Fmoc-Glu-OMe). The solvent was decanted off, and the solid product was dried with a rotary vacuum evaporator. The product was purified by silica gel column chromatography, yielding a colorless amorphous solid (yield = 600 mg, 78%). The purified product (**1**) was characterised by mass spectrometry (Fig. **S1**), ^{1}H-NMR (Fig. **S2**) and ^{13}C-NMR (Fig. **S3**). The solid-phase peptide synthesis was carried out first by anchoring Fmoc-Glu-OMe to Rink Amide resin through its side chain and the peptide sequence, maintaining sequence homology with the recognizing motif of $A\beta_{1\text{-}40}$ peptide, was continued to obtain a full sequence of Fmoc-KLVfFAE-COOMe, using *o*-NosylOXY as the coupling reagent and DIPEA as the base. The only D-amino acid incorporated into the sequence of all L-amino acids was D-Phenylalanine. The Boc-protection in the side chain of Fmoc-Lys(Boc)-OH was deprotected using $ZnBr_2$ in DCM for 24h and thereafter, picolinic acid (3

eq) was coupled to it using *o*-NosylOXY (3.5 eq) and DIPEA (7 eq) for 12h. Methyl ester at the C-terminus was cleaved using 5 eq of LiOH in THF/H_2O for 4h, followed by Fmoc-cleavage at the N-terminus using 20% piperidine in DMF. After that, head-to-tail cyclisation was carried out using 3.5 eq of BOP as the coupling reagent and 7 eq of DIPEA as the base for 24h. For proper comparison, maintaining the same peptide sequence, a linear peptide was also synthesized and used as a control. After completing the synthesis, the head-to-tail cyclic peptide and the linear peptide were cleaved from the resin using 80% TFA, 15% DCM and 5% H_2O for 3h. After cleavage from the amide resin, glutamic acid was converted to glutamine at the C-terminus. The crude peptides were precipitated in cold diethyl ether, obtained by centrifugation, and then characterised by mass spectrometry and HPLC. The crude peptides were purified using semi-preparative RP-HPLC with a C18-μ Bondapak column at a flow rate of 5ml/min using ACN and H_2O as the binary solvent system.

Sample Preparation:

$A\beta_{1-40}$ sample preparation[1]

2.8 mg of commercially available $A\beta_{1-40}$ was taken as the aggregating peptide. To obtain completely disaggregated peptide, 20 μL of TFA was added to $A\beta_{1-40}$. TFA was then evaporated by purging nitrogen gas until and unless a thin film of $A\beta_{1-40}$ was formed. To ensure complete removal of TFA, 10 μL of HFIP was added and then evaporated by purging nitrogen gas. The process is repeated for three times. To this, 1 mL of PBS (50 mM, pH 7.4) 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 μL. For the native $A\beta_{1-40}$ only, the final volume was made up to 800 μL to obtain a total concentration of 40 μM.

Instrumentation:

High-Performance Liquid Chromatography (HPLC):

All the crude peptides were purified using RP-HPLC (Thermo Scientific) using a C18-μ Bondapak column (dimensions 250 × 10 mm, particle size 12 μm, pore size 175 Å) at a flow rate of 5 mL/min by dissolving in CH_3CN/H_2O solvents. The binary solvent system was used as solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in CH_3CN) for a total run time of 30 mins. Dual-wavelength of 214 nm and 254 nm were set at the UV detector. A linear gradient of 5-100% CH_3CN for 18 mins followed by 100% CH_3CN till 30 mins were used for purification. The purity of the peptides was further checked using RP-HPLC (Agilent Analytical System) C18 analytical column at a flow rate of 1 mL/min for a total run time of 21 mins using a linear gradient of 5-100% CH_3CN for 18 mins, followed by 100% CH_3CN till 21 mins, with the UV detector set at 214 nm and 254 nm.

Mass Spectrometry:

The purified peptides and product **1** 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. $[M+H]^+$ and $[M+2H]^{2+}$ peaks were observed for each of the purified peptides.

MALDI-TOF Mass Spectrometry:

After purification by RP-HPLC, the fluorophore attached peptides were characterized by MALDI-TOF mass spectrometry. The desired fragments were collected and mixed with CHCA (α-cyano-hydroxy-cinnamic acid) matrix in a 1:1 ratio and analyzed in Maldi-TOF spectrometer using Bruker Daltonics FlexControl software.

Nuclear Magnetic Resonance (NMR) Spectroscopy:

After purification by column chromatography, product **1** was dried under reduced pressure using Buchi rotary evaporator and then characterised by NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 MHz NMR spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.

Thioflavin T (ThT) fluorescence Assay:[2, 3, 4]

All samples were prepared in phosphate-buffered saline, PBS (50 mM, pH 7.4). A stock solution of Thioflavin T (ThT) of concentration 50 μM was prepared in PBS and stored at 4 °C with proper protection from light. $A\beta_{1-40}$ and the purified peptide samples were dissolved in PBS to obtain a stock solution of concentration 400 μM for the peptide samples and 40 μM for the $A\beta_{1-40}$ solution. For the dose-dependent study, to 50 μL of $A\beta_{1-40}$ solutions, the different peptide solutions (**PA^{19}fCP**, **^{19}fCP** and **^{19}fLP**) were then added in different molar ratios (1:0.5, 1:1 and 1:2) to each of the solutions and the final volume was made up to 800 μL with PBS. For the inhibition of metal-induced $A\beta_{1-40}$ aggregation experiment, stock solutions of concentration 5 mM for each of $CuSO_4$, $FeSO_4$ and $ZnCl_2$, 40 μM for $A\beta_{1-40}$ and 80 μM for **PA^{19}fCP** were prepared. 5 μL from each of the respective metal stock solutions were taken and diluted to obtain a final concentration of 40 μM. In one set of solutions, the metal solutions were added to the $A\beta_{1-40}$ solution maintaining a 1:1 molar ratio and in another set, to the mixture of metal and $A\beta_{1-40}$ solutions (1:1), the peptide solution was added in a 2-fold molar ratio. All the samples were incubated at 37 °C for 36h and thereafter, metal-mediated aggregation of $A\beta_{1-40}$ and the metal chelation efficiency of **PA^{19}fCP** was studied by ThT fluorescence assay.

To perform the fluorescence experiment, 40 μL of the peptide solutions were taken, mixed with 160 μL of PBS and 200 μL 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 in a 1 mL quartz cuvette 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. The relative percentage of amyloid was calculated using the following equation-

$$Relative\ \%\ of\ amyloid\ = \frac{(Observed\ fluorescence\ of\ the\ peptide\ sample - 1)}{(Observed\ fluorescence\ of\ the\ A\beta 1-40\ sample - 1)} \times 100\%$$

where 1 was considered as a normalization factor.

For FRET study, ~20 μM solutions of the fluorophoric peptides (**PA^{19}fCP-Fl**, **MAβ-RhB** and **PA^{19}fCP-Fl + MAβ-RhB**, in a 1:1 molar ratio) were initially prepared in PBS (50 mM, pH 7.4) and all the three samples were kept in incubation at 37 °C for 24h. Thereafter, fluorescence emissions were measured separately for all the peptide samples in a 1 mL quartz cuvette using slit 2 nm on a Fluoromax-4 Horiba Fluorospectrometer. For peptides **PA^{19}fCP-Fl** (FRET donor) and **MAβ-RhB** (FRET acceptor), the excitation wavelengths were set at 492 nm and 567 nm respectively, and the emissions were measured at 518 nm and 601 nm respectively. On the other hand, for the peptide containing both the donor and acceptor (**PA^{19}fCP-Fl + MAβ-RhB**), the excitation wavelength was set at 492 nm (absorption λ_{max} of the donor peptide, **PA^{19}fCP-Fl**) and emissions were measured at 518 nm and 604 nm.

Transmission Electron Microscopy (TEM):[5]

After 6 days of incubation of the stock peptide solutions, 10 μL 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 μL 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:[6]

A saturated solution of Congo-red 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 μL aliquot of each of them was placed over a cleaned glass slide and kept in an incubator overnight for drying. Thereafter, 10 μL 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):[7]

CD analysis was performed after six days of incubation of the stock peptide solutions. $A\beta_{1-40}$ peptide solution (40 μM) 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 μM, 40 μM and 80 μM for the solutions containing 0.5, 1 and 2 fold molar ratios respectively of the breaker peptides. A total volume of 400 μL of the peptide solutions was taken in a cuvette of bandwidth 1 mm. 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:

θ (deg. cm^2. $dmol^{-1}$) = Ellipticity (mdeg). 10^6/ (Pathlength (mm). [Protein] (μM). N)

Where [Protein] is the concentration of the peptide samples (in μM) and N represents the number of amide bonds in the peptide sequence.

Fourier Transform Infrared (FT-IR):[8]

FT-IR spectra were recorded on a Perkin Elmer FT-IR spectrometer after 6 days of incubation of the peptide solutions. From the stock solutions, 10 μL 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.

UV-visible study:[3]

All the UV-visible studies were performed using Perkin Elmer Lambda 35 UV/VIS Spectrometer. A stock solution of 100 μM of **PA^{19}fCP** was initially prepared in methanol and then 200 μL of it was taken and diluted to 200 μL of solvent to obtain a concentration of 50 μM for **PA^{19}fCP**. Stock solutions of 100 μM of each of $CuSO_4$, $FeSO_4$ and $ZnCl_2$ were prepared in methanol and then 200 μL of each of these solutions were taken and diluted to 200 μL of solvent to obtain a concentration of 50 μM for each of these metal salts solutions. The peptide solution was added to $CuSO_4$, $FeSO_4$ and $ZnCl_2$ salt solutions separately both in a 1:1 and 2:1 molar ratios. For proper mixing, all the samples were thoroughly sonicated and vortexed. The absorption spectra of **PA^{19}fCP** alone as well as in the presence of 1:1 and 2:1 molar ratios of $CuSO_4$, $FeSO_4$ and $ZnCl_2$ were recorded at room temperature in a 1 mL quartz cuvette.

For FRET study, the absorption spectra of the fluorophoric peptides (**PA^{19}fCP-Fl** and **MAβ-RhB**) were recorded in a 1 mL quartz cuvette. From the UV-visible spectra, the λ_{max} values for **PA^{19}fCP-Fl** and **MAβ-RhB** were observed to be 492 nm and 567 nm respectively. The software data was transferred to an excel file and the graphs were plotted on OriginPro 8 software.

Isothermal Titration Calorimetry (ITC):[9]

ITC experiments were performed in a Microcal ITC 200 microcalorimeter in Tris-HCl buffer at pH 7.4 and 27 °C. A solution of 0.05 mM concentration of **PA^{19}fCP** and 1.5 mM each of $CuSO_4$, $FeSO_4$ and $ZnCl_2$ solutions were prepared. 40 μL of the metal solutions were placed at the syringe and 300 μL of the peptide solution was placed at the sample cell. Three independent titrations were carried out separately maintaining all the ITC run parameters and injection parameters constant. A total of 30 injections were carried out for all the experiments. For each injection, 1.3 μL of the metal solutions were added from the syringe into the sample cell at an interval of 150 secs with a stirring speed of 200 rpm. The thermodynamic parameters were obtained from fitting the experimental data into a two-site sequential binding model. Gibbs free energy change, ΔG can be calculated from the thermodynamic equation (1)-

$$\Delta G = \Delta H - T\Delta S = -RT\ln K_a \quad (1)$$

where ΔG, ΔH and ΔS are the changes in Gibbs free energy, enthalpy and entropy of binding, respectively, K_a is the association constant (binding constant), T is the absolute temperature and R = 1.98 cal mol^{-1} K^{-1}, is the universal gas constant.

Table 1: Thermodynamic parameters for binding of metal ions to **PA^{19}fCP** obtained by ITC:

M^{2+}	K (M^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})
Cu	$(1.08 \pm 1.1) \times 10^5$	–6.9	–3.6 ± 1.70	0.3
	$(4.07 \pm 5.4) \times 10^4$	–6.3	2.5 ± 1.76	0.8
Zn	$(5.13 \pm 1.2) \times 10^4$	–6.4	0.7 ± 55.0	0.6
	$(1.63 \pm 0.4) \times 10^3$	–4.4	0.4 ± 269	0.4
Fe	$(4.95 \pm 3.0) \times 10^4$	–6.4	0.3 ± 68.1	0.6
	$(6.15 \pm 2.7) \times 10^3$	–5.2	0.7 ± 249	0.5

Note: While calculating ΔG, the error in the values of K was not considered.

From the fitting of the experimental data in to sequential two sites binding model, it is evident of the fact that **PA** in **PA^{19}fCP** utilizes two binding sites for chelating metal ions.

Electron Spin Resonance (ESR):[10]

Copper binding to **PA** in **PA^{19}fCP** was further confirmed by ESR studies. ESR experiment was performed at room temperature on an ESR spectrometer (JEOL, model: JES-FA200). For performing the ESR experiment, 2.0 mg of **PA^{19}fCP** was dissolved in 100 μL of Tris-HCl buffer to obtain a concentration of 20 mM solution. Similarly, 2.5 mg of $CuSO_4$ was weighed and dissolved in 1 mL of Tris-HCl buffer to obtain a concentration of 10 mM solution. 50 μL of this $CuSO_4$ solution was diluted with 50 μL of buffer to obtain a $CuSO_4$ solution of 5 mM concentration. ESR spectrum of only $CuSO_4$ solution (5 mM) was first recorded. Thereafter, copper was added to the peptide solutions maintaining 1:1 and 1:2 molar ratios. The frequency of the microwave radiation applied was adjusted at 9443.56 MHz and the amplitude of the signal was 100. ESR signal was collected by recording the electron resonance intensity as a function of field strength. For each sample, three scans were taken and the final signal thus obtained was the average of three scans. The approximate 'g' values for all the solutions were

obtained from the ESR signal itself. The text values were collected and plotted in OrginPro 8 software taking intensity of the signal as y-axis and field (mT) as x-axis.

Tyrosine (TYR10) Fluorescence Assay:[11]

The intrinsic fluorescence of tyrosine (TYR10) (excitation: 285 nm and emission: 308 nm) of the $A\beta_{1-40}$ peptide gets quenched upon binding with Cu^{2+}.[12] Therefore, the fluorescence intensity at 308 nm was measured to investigate the binding of Cu^{2+} to $A\beta_{1-40}$ peptide. Treating the $A\beta_{1-40}$–Cu^{2+} complex with the chelating peptide **PA^{19}fCP** is expected to extract Cu^{2+} out from the complex and restore the fluorescence intensity of tyrosine at 308 nm. For this, a solution of $A\beta_{1-40}$ (40 μM) was prepared in PBS buffer (50 mM, pH 7.4) and fluorescence was measured at 308 nm. Thereafter, 40 μM of Cu^{2+} was added to the $A\beta_{1-40}$ solution with Aβ:Cu^{2+} maintaining a molar ratio of 1:1 and incubated for 1h at 37 °C. Thereafter, fluorescence was again measured at 308 nm to find a large decrement of fluorescence intensity. Finally, **PA^{19}fCP** (80 μM) was added to the $A\beta_{1-40}$–Cu^{2+} solution and after 1h of incubation, the fluorescence was further recorded to find the restoration of fluorescence at 308 nm. Tyrosine fluorescence was measured at 308 nm in a 1 mL quartz fluorescence cuvette using slit 5 nm on a Fluoromax-4 Horiba Fluorospectrometer and the emission graphs were normalized and represented as bar diagram.

Dynamic Light Scattering:[13]

DLS measurements of $A\beta_{1-40}$ alone (at 1h, 6h, 12h and 24h of incubation) as well as in the presence of 2 equivalents of **PA^{19}fCP** were performed at 25 °C using a 633 nm He-Ne laser on a Zetasizer Nano series Nano-ZS90 (Malvern Instruments). A stock solution of $A\beta_{1-40}$ (40 μM) was initially prepared in PBS (50 mM, pH 7.4) with 3% DMSO and then 2 equivalents of **PA^{19}fCP** was added to it and incubated at 37 °C. For DLS measurement, 200 μL of each of the samples ($A\beta_{1-40}$ and $A\beta_{1-40}$ + **PA^{19}fCP**) were transferred to a 1 mL cuvette at different time

intervals to monitor the amyloid aggregation and its inhibition. Prior to recording, the system was equilibrated for 120 seconds. Each measurement thus obtained was an average of five scans, where intensity percentage versus size distribution of the particles were recorded. The text values were obtained directly from the Zetasizer software, plotted in OriginPro 8 software, redrawn and finalized in Adobe Illustrator.

Large Unilamellar Vesicles (LUVs) Preparation:[14]

The LUV preparation involves three different lipids namely DPPC, Cholesterol and GM1 in a molar ratio of 68:30:2. Initially, a 2 mM stock solution of the lipids were prepared and for this, all the lipids were weighed together in a cleaned eppendorf and dissolved in chloroform and methanol ($CHCl_3$/MeOH = 2:1). The solvents were evaporated and dried completely using nitrogen gas to form a thin lipid layer. Thereafter, a 200 μM stock solution of the carboxyfluorescein dye is prepared in 500 μL of PBS (50 mM, pH 7.4). The lipid film was mixed with the prepared dye solution and then vortexed vigorously for 30 mins to emulsify the lipid mixtures. The eppendorf was cooled in liquid nitrogen and then, the frozen solution was immediately dipped in a water bath at 60-70 °C for thawing.[15] This process is repeated five times for complete entrapment of the dye. The resulting solution was centrifuged at 15000 rpm and the excess supernatant dye solution was discarded. The lipid pellet thus formed was dissolved in 500 μL of PBS buffer and vortexed to obtain a homogeneous suspension of a 2 mM stock solution of the lipids. Thereafter, 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). For the dye leakage experiment, all samples including the carboxyfluorescein entrapped untreated LUV solution were excited at 485 nm and fluorescence emission was measured at 516 nm using slit 5 nm. To obtain complete dye leakage (100% dye

release) from the vesicles, 10 μL of Triton X-100 was added at the end of the experiment and the total fluorescence was measured.

The percentage of dye leakage was calculated as[16]

$$\%\ Leakage = \frac{(Observed\ Fluorescence - Initial\ Fluorescence)}{(Total\ Fluorescence\ - Initial\ Fluorescence)} \times\ 100\%$$

FRET Calculations:

In a FRET pair, the distance between the donor and the acceptor at which energy transfer efficiency is 50% is referred to as the Förster radius (R_0).[17] The magnitude of R_0 depends on the overlap integral of the fluorescence spectrum of donor with the absorption spectrum of acceptor and their mutual molecular orientation as expressed by the following equation[18]-

$$R_0 = 0.2108\ [\kappa^2 \Phi_D n^{-4} \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\ \lambda^4 d\lambda]^{1/6} \quad (1)$$

Where, κ^2 (=2/3) is the orientation factor of transition dipole moment between donor (D, **PA[19]fCP-Fl**) and acceptor (A, **MAβ-RhB**); Φ_D (= 0.773) is the fluorescence quantum yield of the donor (**PA[19]fCP-Fl**) in the absence of the acceptor (**MAβ-RhB**); n (= 1.33) is the refractive index of the intervening medium; and $\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\ \lambda^4 d\lambda$ (= 5.59×10^{14}) is the spectral overlap integral of the fluorescence emission spectrum of the donor (**PA[19]fCP-Fl**) and the absorption spectrum of the acceptor (**MAβ-RhB**), where, F_D is the normalized emission spectrum of the donor and ε_A is the molar extinction coefficient (in $M^{-1}cm^{-1}$) of acceptor; and λ is the wavelength (in nm). The Förster radius (R_0) of FITC/Rhodamine B was calculated by substituting these values in equation (1) and found to be 44.80 Å.

The fluorescence quantum yield (Φ_D) of the donor (**PA^{19}fCP-Fl**) was determined using equation 2 where fluorescein was used as a reference with known quantum yield value of 0.90 in 0.1 N NaOH solution.[19] Initially, the UV-visible spectrum of fluorescein was recorded exhibiting absorbance ~0.1 and thereafter, the fluorescence emission of the same solution was measured with the excitation wavelength set at 490 nm (absorption λ_{max} of fluorescein).

$$\Phi_D = \Phi_R \frac{Fl_D^{Area}.\ Abs_R\ .\ n_D^2}{Fl_R^{Area}.\ Abs_D\ .\ n_R^2} \quad (2)$$

Where, Φ_R is the quantum yield of the standard reference, Fl_D^{Area} (donor) and Fl_R^{Area} (reference) are the integrated emission peak areas, Abs_D (donor) and Abs_R (reference) are the absorbance at the excitation wavelength, n_D (donor) and n_R (reference) are the refractive indices of the respective solutions.

Further, the efficiency of energy transfer (E)[20] between the donor (**PA^{19}fCP-Fl**) and the acceptor (**MAβ-RhB**) in the mixture (**PA^{19}fCP-Fl** + **MAβ-RhB**) was determined using equation (3) and found to be 99.97%.

$$E = 1 - \frac{F_{DA}}{F_D} \quad (3)$$

Where, F_{DA} is the fluorescence intensity of the donor in the presence of acceptor (**PA^{19}fCP-Fl** + **MAβ-RhB**) and F_D is the fluorescence intensity of the donor in the absence of the acceptor (**PA^{19}fCP-Fl**).

The actual distance (r) between the donor (**PA^{19}fCP-Fl**) and the acceptor (**MAβ-RhB**) in the mixture (**PA^{19}fCP-Fl** + **MAβ-RhB**) was determined using equation (4) and found to be 11.6 Å.[20]

$$r = R_0\{(1/E) - 1\}^{1/6} \quad (4)$$

Where, R_0 is the Förster radius between the donor and the acceptor and E is the efficiency of energy transfer between them.

Materials and methods for cell-based studies:

Toxicity Study:

The Neuro2a cells were seeded in 96-well plates to get 70% confluency after 12 h incubation at 37 °C. Different concentrations of **PA^{19}fCP-Fl** and **MAβ-RhB** starting from 200 μM to 3.1 μM were prepared in plain Dulbecco's modified Eagle's medium (DMEM). Cells were washed with phosphate-buffered saline (PBS) and prepared concentrations were added directly on top of cells. After 24 h, the MTT solution was prepared at 5 mg/mL in PBS and filtered through a 0.2 μm filter. Approx. 100 μL of 10% diluted MTT in plain DMEM was added in each well. Cells were incubated for 3 h at 37 °C with 5% CO_2, 95% air, and complete humidity. After 4 h, the MTT solution was removed and replaced with 100 μL of DMSO. The plate was further incubated for 15 min in the dark at room temperature. The optical density (OD) of the wells was determined using a Multiscan Go plate reader at a test wavelength of 570 nm and a reference wavelength of 630 nm.

Peptide Internalizations:

The Neuro2a cells were seeded in 35 mm cell culture dish. After 12 h, 2 ml of 100 μM of each **PA^{19}fCP-Fl** and **MAβ-RhB** were prepared in 2% FBS, DMEM. Seeded cells were washed with PBS and peptide dilutions were added on top of cells. After 24 h of incubation at 37 °C

with 5% CO_2, 95% air, and complete humidity cells were washed with PBS thrice and visualised in FLoid™ Cell Imaging Station. Images were captured in 20X magnification with green and red filter for **PA^{19}fCP-Fl** and **MAβ-RhB** repectively.

Co-localization study:

The Neuro2a cells were seeded on cover slips in 35 mm cell culture dish. After 12 h, 2 ml of 100 μM of both peptides in 2% FBS, DMEM were treated separately and in combination of ratio 1:1. After 24 h of incubation, cells were washed with PBS and fixed by 4% paraformaldehyde for 20 mins. Cells were rinsed with PBS and mounted on microscopy slides using Mowiol as mounting medium. Cell images were taken in 63X objective using a confocal microscope (Leica SP5, Germany). **PA^{19}fCP-Fl** with FITC tag was excited at 492 nm and the emission was detected at 518 nm and **MAβ-Rh** with rhodamine tag was excited at 567 nm and detection was from 601 to 604 nm. Co-localization of FRET pair were observed through merged images of individual channels.

Conformational transitions monitored by CD and FT-IR analyses during inhibition of amyloid ($A\beta_{1-40}$) aggregation:

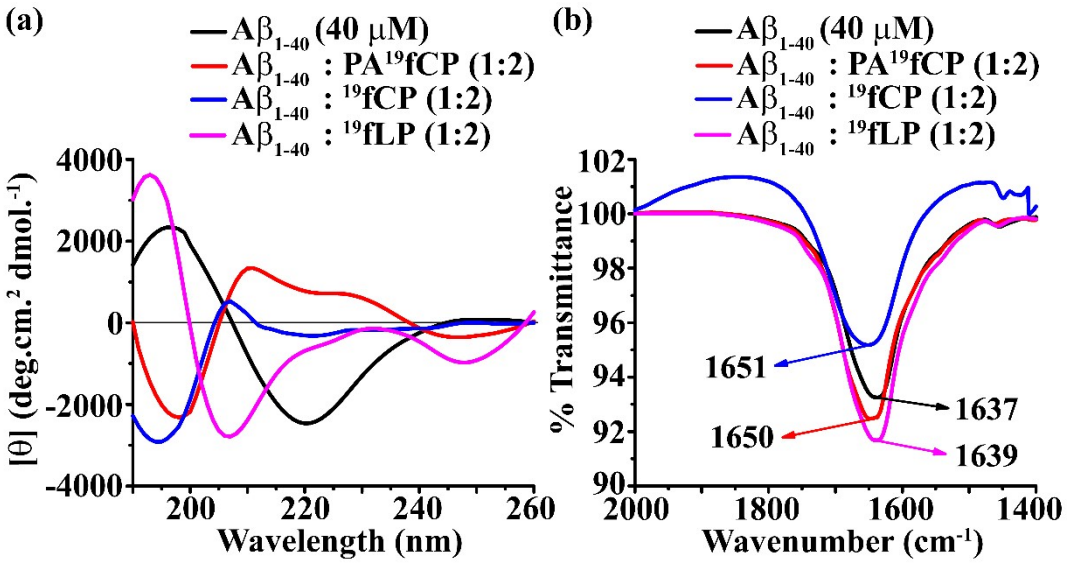

Fig. S15. (a) CD spectra of $A\beta_{1\text{-}40}$ (40 μM) alone (black) and in the presence of 2-fold molar excesses of **PA^{19}fCP** (red), **^{19}fCP** (blue) and **^{19}fLP** (magenta), (b) FT-IR spectra of $A\beta_{1\text{-}40}$ (40 μM) alone (black) and in the presence of 2-fold molar excesses of **PA^{19}fCP** (red), **^{19}fCP** (blue) and **^{19}fLP** (magenta). The spectra were recorded after 6 days of incubation of the peptide solutions in PBS at pH 7.4 and 37 °C.

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