**Electronic Supplementary Information (ESI)**

# Inhibition of Alzheimer's Amyloid-β Peptide Aggregation and its Disruption by a Conformationally Restricted α/β Hybrid Peptide

**Ashim Paul, Krishna Chaitanya Nadimpally, Tanmay Mondal, Kishore Thalluri, and Bhubaneswar Mandal***

Laboratory of Peptide and Amyloid Research, Department of Chemistry, Indian Institute of Technology Guwahati, Assam - 781039, India. Email: bmandal@iitg.ernet.in

**Table of contents:** Page no.

1. Reagents and synthetic methods 2-3

2. Instrumentations 3-6

3. Biophysical studies of the breaker peptides 6-8

4. Biophysical studies for the inhibition of amyloid formation of model aggregating peptide 8-9

5. Biophysical studies for the inhibition of amyloid formation of $A\beta_{1-40}$ peptide 9-14

6. Biophysical studies for the disruption of preformed amyloid fibrillar aggregates of $A\beta_{1-40}$ peptide 15-19

7. Characterization of prepared large unilamellar vesicles (LUVs) by transmission election microscope 20

8. Vesicle leakage study 20-21

9. Characterization data of peptides 1 to 5 21-25

10. References 25

## Reagents and synthetic methods

### Reagents and solvents

Rink amide MBHA resin was purchased from Fluka (Loading 1.1 mmol/g). BOP [(Benzotriazole-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate], PyBOP [(Benzotriazole-1-yl-oxy-tris-pyrolidine-phosphonium hexafluorophosphate], Diisopropyl ethylamine (DIPEA), Fmoc-Ant-OH (Fmoc-anthranilic acid) and 5(6)-Carboxyfluorescein were purchased from Sigma. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), Ganglioside GM1 were purchased from Avanti Polar Lipid, Inc. Dimethylformamide (DMF, extrapure grade), dichloromethane (extrapure grade) and acetonitrile of HPLC grade were obtained from Merck (India). Cholesterol (99 %), acetic anhydride (synthesis grade), N-methyl imidazole (extrapure), Trifluoroacetic acid (TFA) of extrapure grade were purchased from SRL (India). Milli-Q water at 18.2 Ω was used. All Fmoc amino acids and human Alzheimer's β-amyloid ($A\beta_{1\text{-}40}$) acid were purchased from GL Biochem (Shanghai) with following side chain protecting groups: *tert*-butyl for Asp and Ser.

### Peptide synthesis

All the described peptides were synthesized by standard Fmoc/$^{t}$Bu solid phase peptide synthesis method on MBHA-Rink amide resin (loading 1.1. mmol/g). The syntheses were performed manually on a Stuart blood tube rotator. The Resin was taken into a 5 ml frit-fitted plastic syringe and swollen in dichloromethane (DCM) for 2 h followed by DMF for 1 h. 2 equiv of Fmoc amino acids, 2.5 equiv of coupling reagent (BOP), and 5 equiv of base (DIPEA) were used. Each coupling steps are monitored by Kaiser's test and in cases of incomplete acylation, coupling cycles were repeated, followed by capping with acetic anhydride (2 equiv) and N-methyl imidazole (3 equiv). Fmoc deprotection was performed with 20% piperidine in DMF mixture for 21 min (7 min × 3). The final peptide was cleaved from the resin using a cleavage cocktail (80 % TFA, 15% DCM and 5% $H_2O$) for 3 h. After cleavage from the resin, the crude peptide was precipitated by cold diethyl ether followed by centrifugation to achieve crude solid peptide.

### Aβ Sample preparation.[1]

$A\beta_{1-40}$ was purchased from GL Biochem Shanghai, China and used directly without further purification. 1.2 mg of commercially available $A\beta_{1-40}$ was dissolved in 20 μL of TFA to

obtain disaggregated $A\beta_{1-40}$. TFA was evaporated using nitrogen gas. To remove TFA completely, HFIP was added and evaporated using nitrogen gas to obtain a disaggregated $A\beta_{1-40}$ material. This process was repeated twice. Into the disaggregated material 1.4 ml of PBS (50 mM, pH 7.4) was added followed by sonication and vortex to obtain transparent solution and the whole solution was divided into 7 equal portion followed by addition of 600 µL of PBS to each portion to obtained a final concentration of 50 µM.

## Instrumentations

### Liquid chromatography:

Crude peptides were dissolved in $CH_3CN/H_2O$ and purified by RP-HPLC (Waters 600E) using a C18-µ Bondapak column at a flow rate of 5 mL/ min. Binary solvent system were used, solvent A (0.1 % TFA in $H_2O$) and solvent B (0.1 % TFA in CH3CN). A Waters 2489 UV detector was used with an option of dual detection at 214 and 254 nm. A total run time of 20 min. was used and gradient used for purification was 5−100 % $CH_3CN$ for 18 min followed by 100% $CH_3CN$ till 20 min.

Purity of the peptides were confirmed using Waters 600E Analytical HPLC system, Waters C8 analytical column at a flow rate of 1 ml/min, linear gradient of 5-100% $CH_3CN$ over 18 minutes in a total run time of 20 min. Dual wavelength was selected at 214 nm and 254 nm. The O to N acyl migration (for peptide **5**, Table 1) was monitored by Waters UPLC-MS system (ESI) using solvent A (0.1% TFA in $H_2O$) and solvent B (0.1% TFA in $CH_3CN$) on C18 column with a flow rate of 0.25 ml/min. Dual wavelength were selected at 214 nm and 254 nm. Linear gradient of 5 to 95 % $CH_3CN$ was used in a total run time of 8 min.

### Mass spectrometry:

Mass of the peptide samples were analyzed on Agilent-Q-TOF 6500 instrument, in ESI positive mode, equipped with Mass hunter work station software. The O to N acyl migration was monitored by Waters UPLC-MS (ESI +ve mode) Micromass Q-TOF equipped with Masslynx software.

**Thioflavin T Fluorescence Assay:**[2]

Commercially available Thioflavin T (ThT) was purchased from Sigma Aldrich and a stock solution of concentration 50 μM in PBS (50 mM, pH 7.4) was prepared and stored the solution at 4 °C with proper protection with dark cover to prevent degradation from light. Purified solid peptide samples (amyloidogenic peptide alone and in some cases mixed with breaker peptide) were dissolved in PBS (50 mM, pH 7.4) to obtain a stock solution of 500 μM (50 μM in the case of $A\beta_{1-40}$ peptide) and incubated at 37 °C on a water bath.

To perform the fluorescence study, 40 μL of peptide sample was taken out from the stock solution and was mixed with 200 μL of thioflavin T solution (50 μM); final volume was made up to 400 μL with PBS. For a desired stock solution three different replicate solutions were prepared. For ThT fluorescence assay, emission was measured at 490 nm and excitation at 435 nm, using a slit of 3 nm on a Fluoromax-4, Horiba instrument. From the machine text file was taken and graph was plotted using OriginPro 8 software. For each data point 3 different sets of replicate solutions were scanned separately and average was taken with observed standard deviation.

**Transmission Electron Microscopy (TEM):**[3]

10 μL aliquot from the stock peptide solution after incubation of 5 days (7 days in case of $A\beta_{1-40}$) was added over the dark side of carbon coated copper grid and allowed to float for 1 min. Then 2% uranyl acetate solution (10 μL) was added on the same grid and was allowed to float for another 1 min. The excess solution was removed using blotting paper. The sample was dried at room temperature and was kept in desiccator before taking TEM analysis on JEOL (Model: JEM 2100) instrument at 200 kV.

**Congo-Red Stained Birefringence:**

Preparation of Congo red solution:[4] Commercially available (purchased from Sigma) Congo red was dissolved in 80 % aqueous ethanol to prepare a saturated solution. Then saturated solution of sodium chloride was added into the saturated Congo red solution and filtered to obtain required Congo red solution for analysis.

After 5 days of incubation (7 days in case of $A\beta_{1-40}$) of the stock, a 20 µL aliquot of the required peptide solution was placed over a glass slide followed by 40 µL of the saturated Congo red solution. The excess solution was removed using a blotting paper, the sample was dried at room temperature, and was kept in desiccator before birefringence analysis under a Leica ICC50 HD polarizable microscope.

**Circular dichroism (CD):[5]**

Purified solid peptide samples (amyloidogenic peptide alone and in some cases mixed with breaker peptide) were dissolved in PBS (50 mM, pH 7.4) to obtain a stock solution of 500 µM (50 µM in the case of $A\beta_{1-40}$ peptide) and incubated at 37 °C on a water bath. To perform the CD study, stock solution was diluted from respective buffer solutions to obtain final concentration of 100 µM ($A\beta_{1-40}$ peptide solution was used without further dilution). 400 µL of the sample was taken in a cuvette bandwidth of 1 mm. Three measurements were accumulated. Spectra were recorded from 190 nm to 260 nm on a JASCO J-815 instrument.

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

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

**Fourier transformation infra red (FT-IR):[6]**

Peptide stock solution was prepared in a similar manner as described for CD sample preparation (above section). From the stock, an aliquot of 20 µL was taken out, mixed with KBr, and a pellet was prepared. The spectrum for each sample was recorded after required time interval (immediately after sample preparation, after 5 days or so). The background scan was subtracted from the sample scans and text files were plotted using OriginPro 8 software.

**Large Unilamellar Vesicles (LUVs) Preparation and Carboxyfluorescein Entrapment:[7]**

The large unilamellar vesicles (LUVs) were prepared using three different lipids, DPPC, Cholesterol and GM1 with 68:30:2 molar ratios. Prior to the vesicle preparation, all the lipids were taken in clean glass vial and solubilize to make 2 mM stock solution in chloroform and

methanol (2:1) and the solvents were evaporated to make lipid films using nitrogen gas. The glass vial containing lipid films were placed in a vacuum desiccator for over night to remove solvents completely. The lipid films were hydrated with 500 μL of carboxyfluorescein solution (200 μM) in 50 mM HEPES buffer of pH 7.4. Then, the solution was vortexed vigorously for 30 min to emulsify the lipid mixtures. Further, the glass vial was dipped into the liquid nitrogen for instant cooling and after 5 min the frozen solution was dipped into water bath at 50-60 °C for thawing.[8] This step was repeated five times. The excess dye was removed by ultracentrifugation at 20000 rpm and the supernatant dye solution was discarded and the lipid pellet was re-hydrated with 50 mM HEPES buffer. This step was repeated 2 more times and the final lipid pellet was collected followed by addition of 500 μL of HEPES buffer and vortexed to obtain homogenous suspension of 2 mM stock.[9] Finally the lipid solution was extruded through 0.45 μm polycarbonate membrane to obtain the dye loaded LUVs and the vesicle leakage study was performed on a Fluoromax-4, Horiba instrument. The formation of LUVs was confirmed by transmission electron microscopy.

## Biophysical studies of the breaker peptides

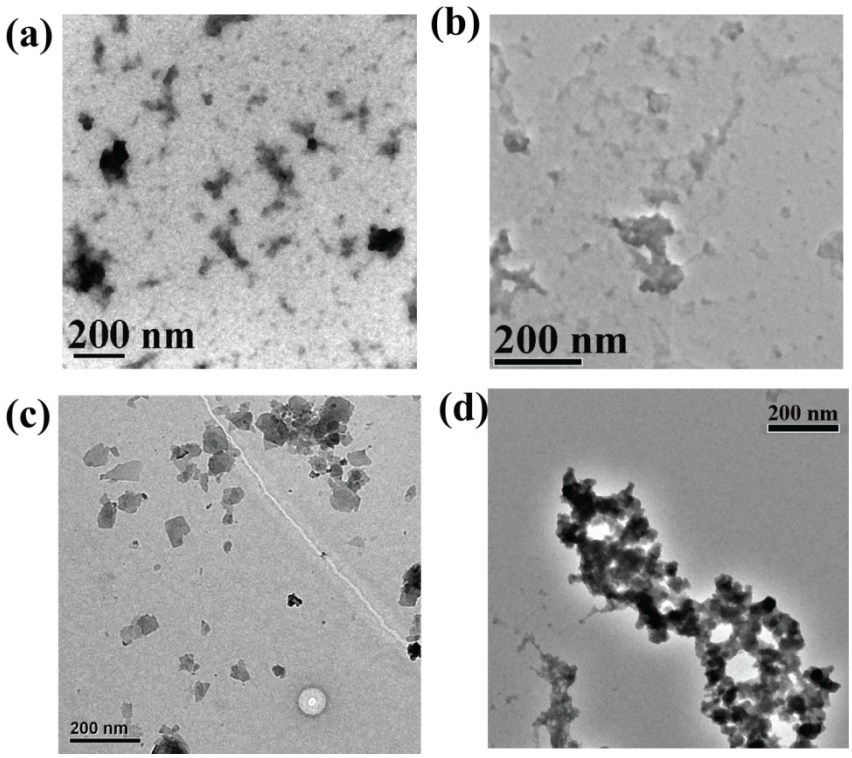


**Fig. S1.** TEM images of (a) peptide **1**, (b) peptide **2**, (c) peptide **3** and (d) peptide **4**. The peptide solutions were incubated in PBS of pH 7.4 at 37 °C. Scale bar is indicated as 200 nm.

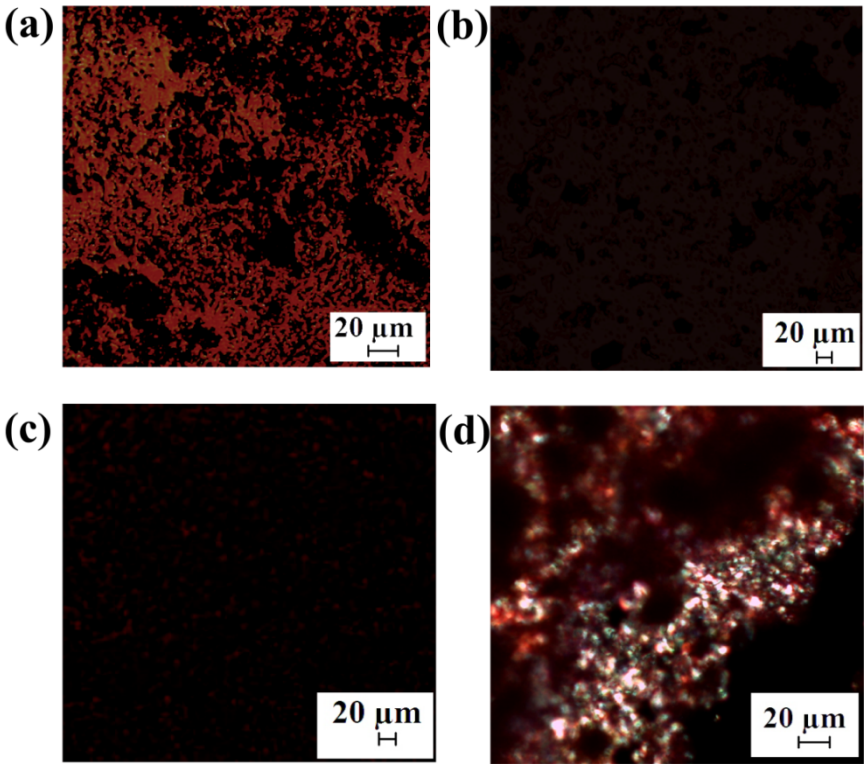


**Fig. S2.** Congo red stained birefringence images of (a) peptide **1**, (b) peptide **2**, (c) peptide **3** and (d) peptide **4**. Scale bar is indicated as 20 µm.

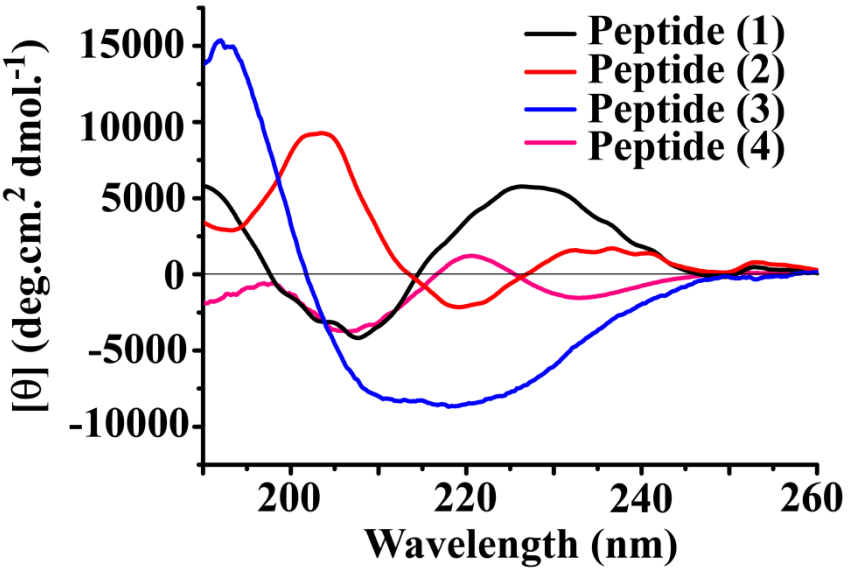


**Fig. S3.** CD spectra of breaker peptide **1** (black), peptide **2** (red) peptide **3** (blue) and peptide **4** (magenta). The concentration of the peptide solution in the cuvette was 100 µM (concentration of the stock solution was 500 µM).

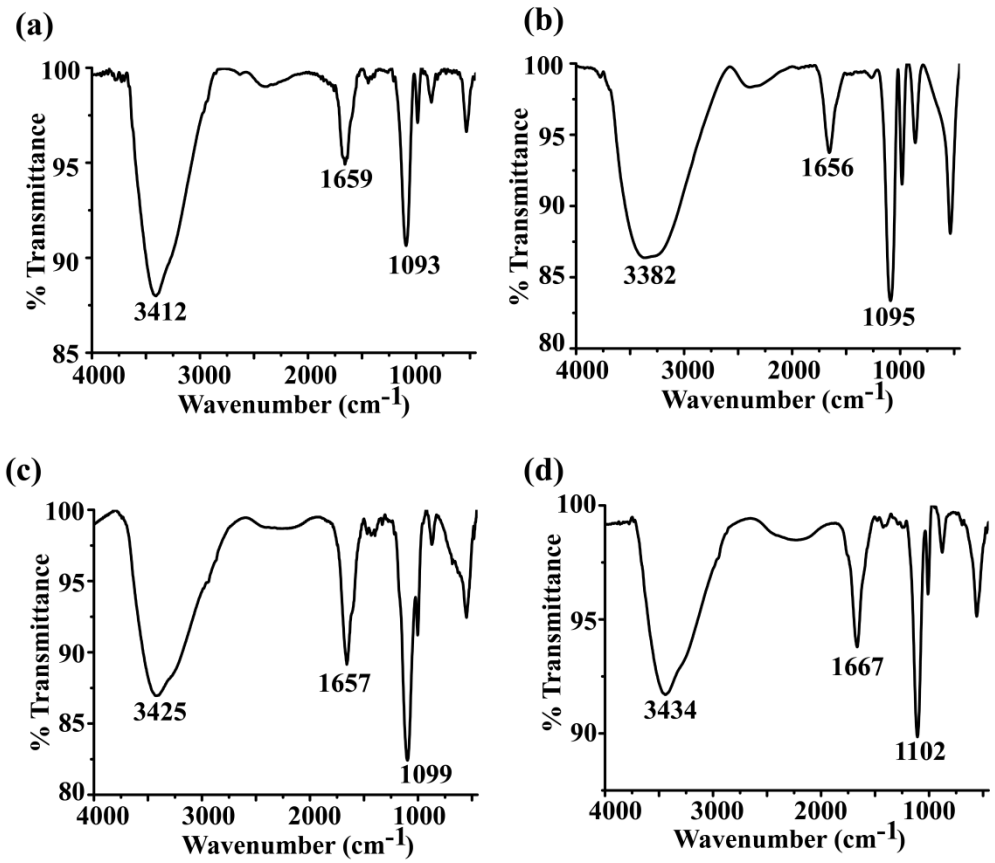


**Fig. S4.** FT-IR spectra of (a) peptide **1**, (b) peptide **2**, (c) peptide **3** and (d) peptide **4** in PBS pH 7.4 at 37 °C.

## Biophysical studies for the inhibition of amyloid formation of model aggregating peptide

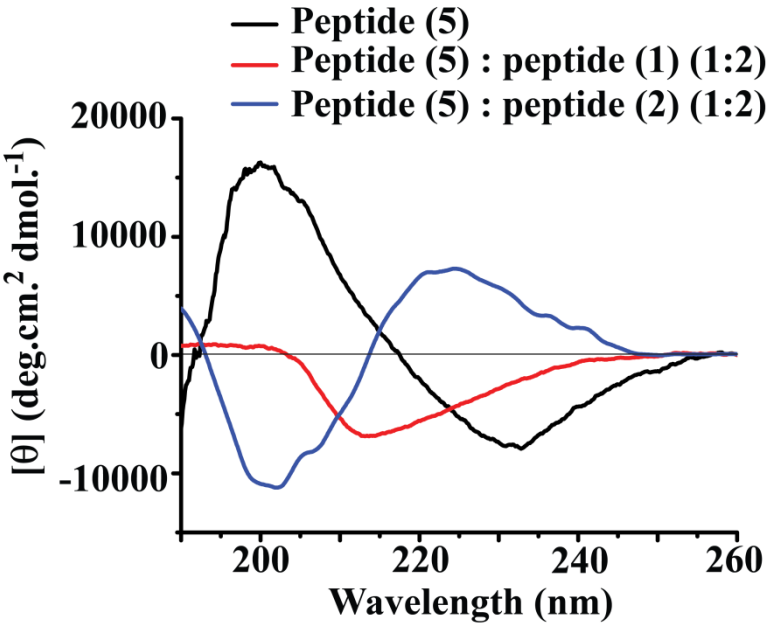


**Fig. S5.** CD spectra of peptide **5** in absence (black) and presence of 2 fold molar excess of peptide **1** (red) and peptide **2** (blue) The stock concentration of the peptide **5** solutions were 500 μM.

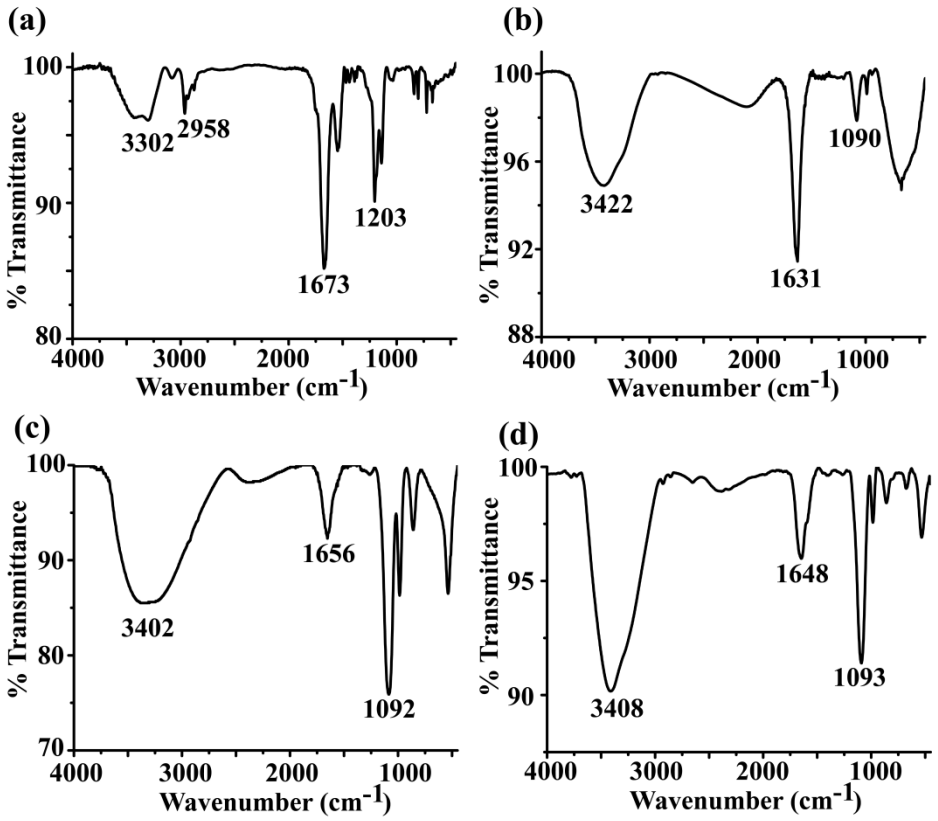


**Fig. S6.** FT-IR spectra of peptide **5** (500 μM) in absence (a) at 0 time, (b) after 5 days of incubation and in presence of 2 fold molar excess of (c) peptide **1** and (d) peptide **2** after 5 days. The peptides were incubated in PBS of pH 7.4 at 37 °C

## Biophysical studies for the inhibition of amyloid formation of $A\beta_{1-40}$ peptide

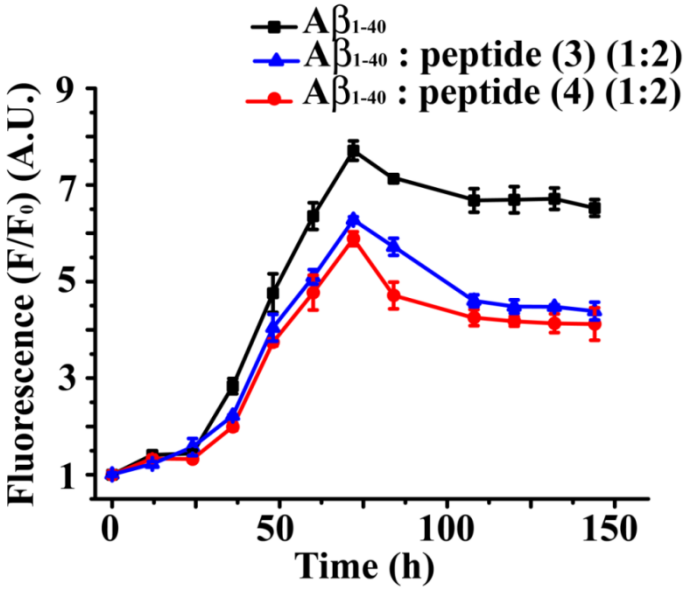


**Fig. S7.** Time dependent Thioflavin T-fluorescence assay of $A\beta_{1-40}$ in absence (black) and presence of 2 fold molar excess of peptide **3** (blue) or peptide **4** (red). Peptide solutions were incubated in PBS pH 7.4 (50 mM) at 37 °C.

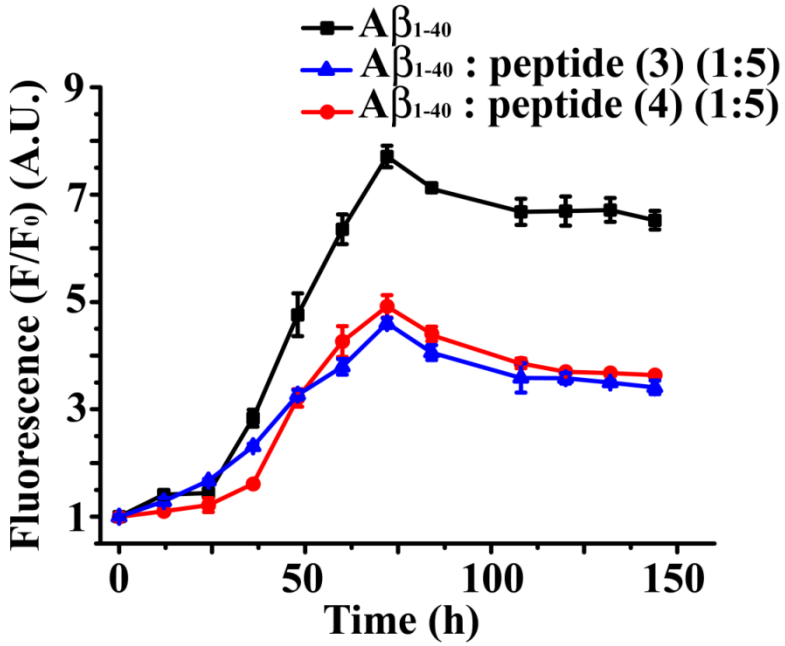


**Fig. S8.** Time dependent Thioflavin T-fluorescence assay of $A\beta_{1-40}$ in absence (black) and presence of 5 fold molar excess of peptide **3** (blue) or peptide **4** (red). Peptide solutions were incubated in PBS pH 7.4 (50 mM) at 37 °C.

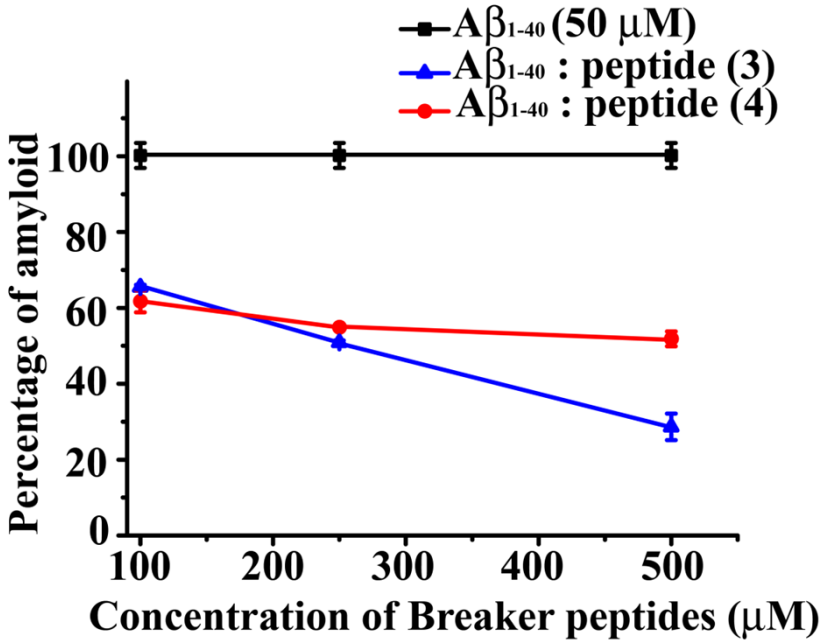


**Fig. S9.** Concentration dependent inhibition of amyloid fibril formation of $A\beta_{1-40}$ in absence (black) and presence of peptide **3** (blue) or peptide **4** (red). Peptide solutions were incubated in PBS pH 7.4 at 37 °C.

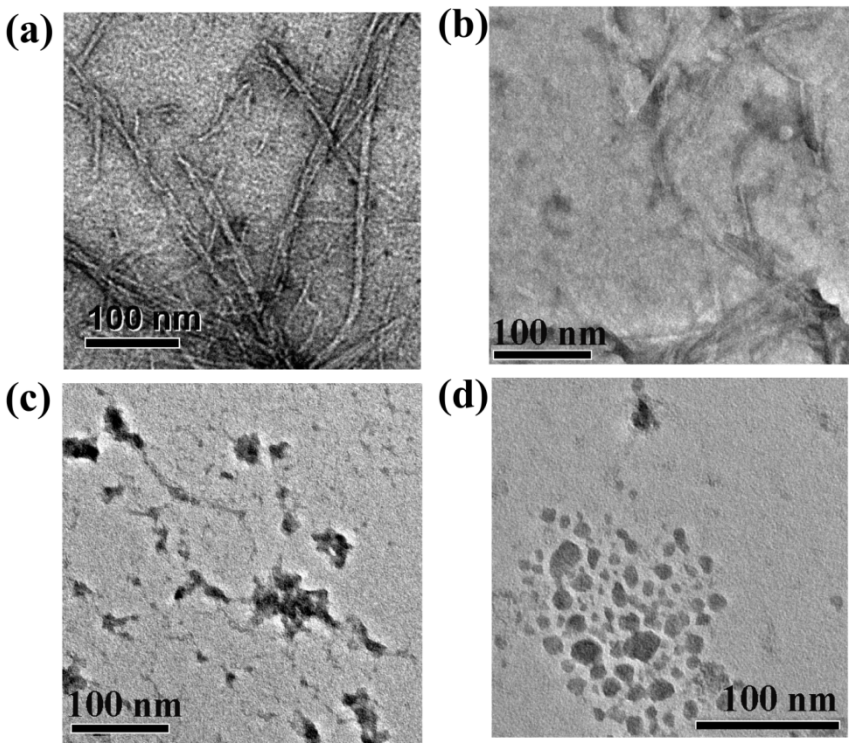


**Fig. S10.** TEM images of $A\beta_{1-40}$ (a) in absence and presence of (b) 2 fold, (c), 5 fold or (d) 10 fold molar excess of breaker peptide **4**. Images were taken after 7 days of incubation in PBS pH 7.4 (50 mM) at 37 °C. Scale bar is indicated as 100 nm

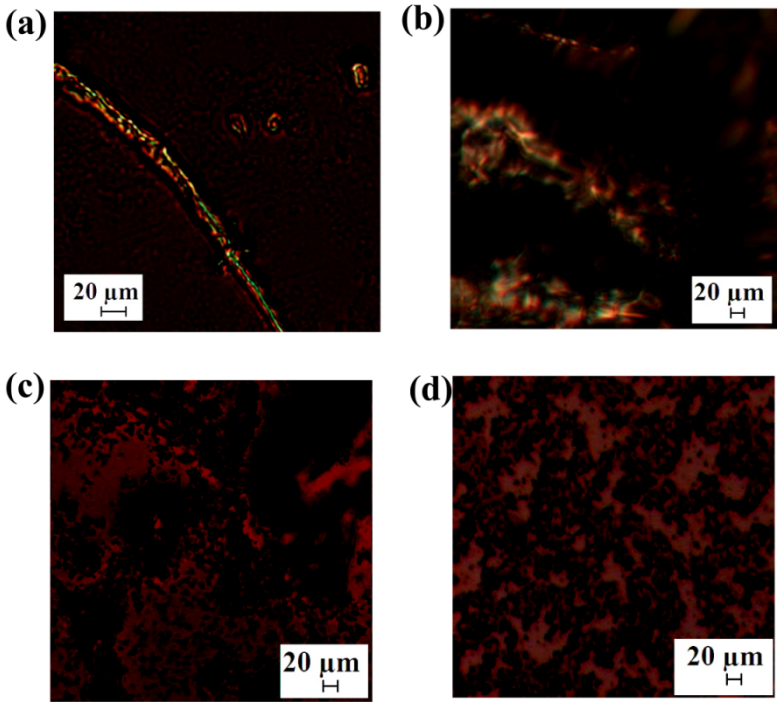


**Fig. S11.** Congo red stained birefringence images of (a) $A\beta_{1-40}$ (a) in absence and presence of (b) 2 fold, (c), 5 fold or (d) 10 fold molar excess of breaker peptide **4**. Images were taken after 7 days of incubation in PBS pH 7.4 (50 mM) at 37 °C. Scale bar is indicated as 20 µm.

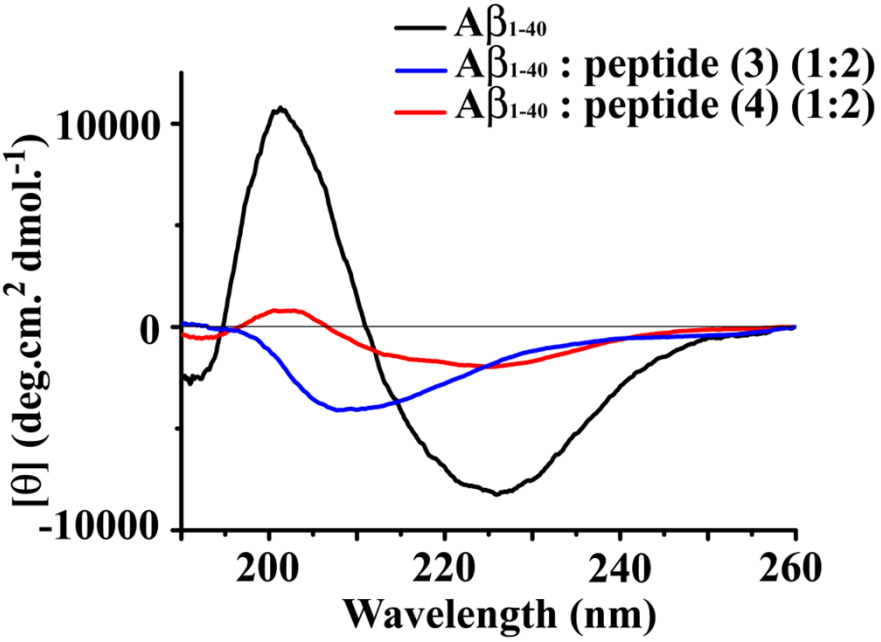


**Fig. S12.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 2 fold molar excess of peptide **3** (blue) or peptide **4** (red). The spectra were recorded after 7 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 µM in PBS pH 7.4 (50 mM).

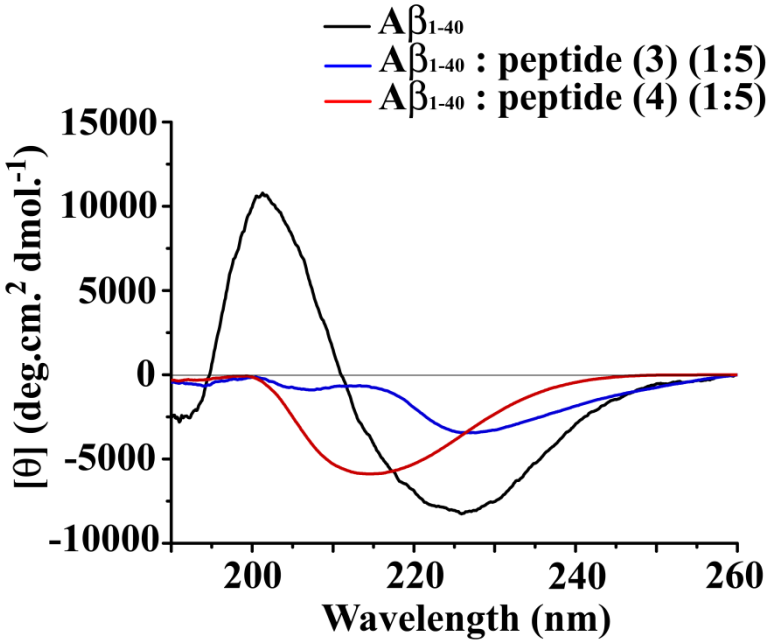


**Fig. S13.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 5 fold molar excess of peptide **3** (blue) and in presence of 5 fold molar excess of peptide **4** (red). The spectra were recorded after 7 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 µM in PBS pH 7.4 (50 mM).

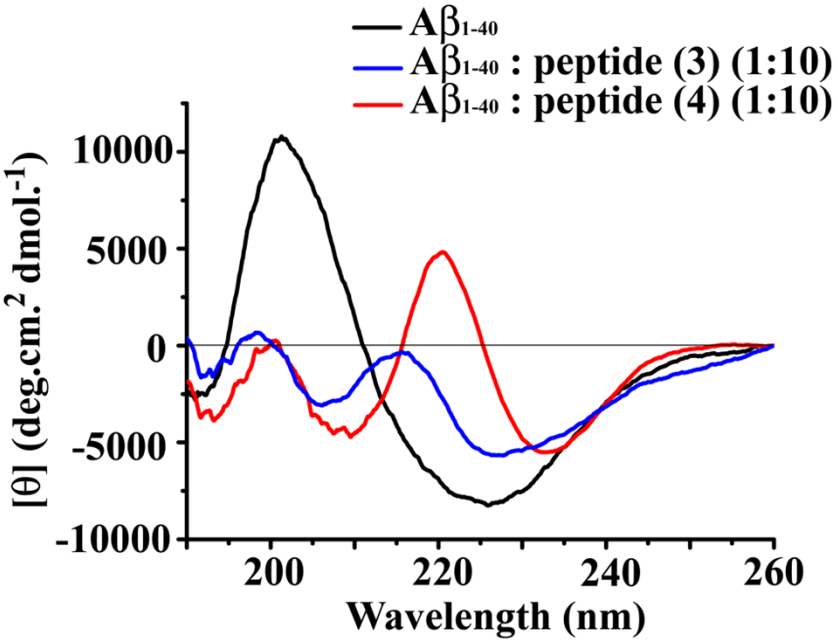


**Fig. S14.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 10 fold molar excess of peptide **3** (blue) and in presence of 10 fold molar excess of peptide **4** (red). The spectra were recorded after 7 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 µM in PBS pH 7.4 (50 mM).

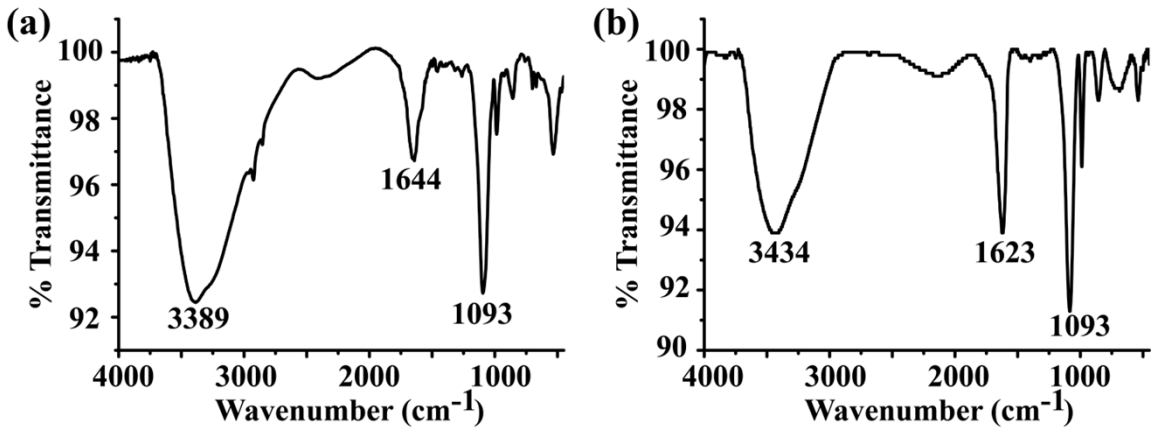


**Fig. S15.** FT-IR spectra of $A\beta_{1-40}$ peptide (50 µM) (a) at 0 time and (b) after 7 days of incubation in PBS pH 7.4 at 37 °C.

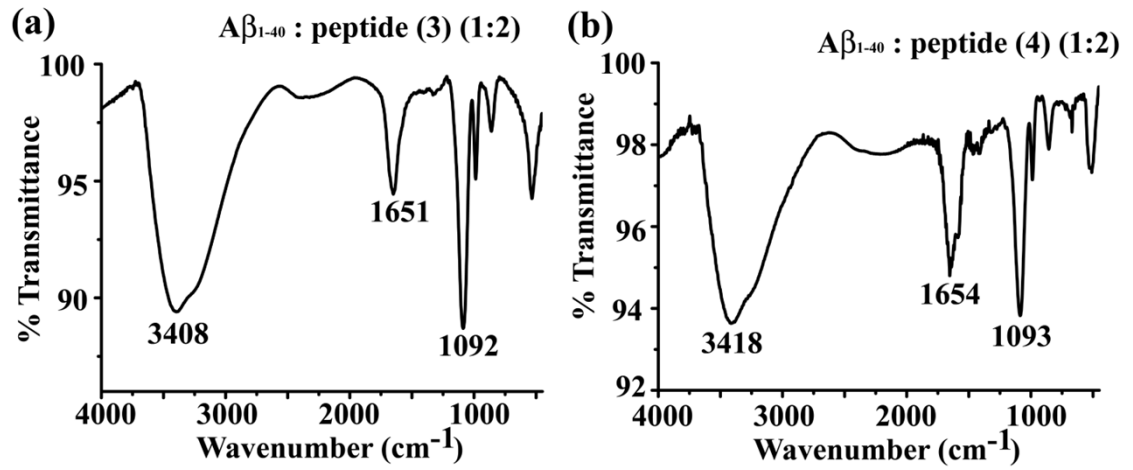


**Fig. S16.** FT-IR spectra of $A\beta_{1-40}$ peptide after 7 days incubation in PBS pH 7.4 at 37 °C, (a) in presence of 2 fold molar excess of peptide **3** and (b) in presence of 2 fold molar excess of peptide **4**.

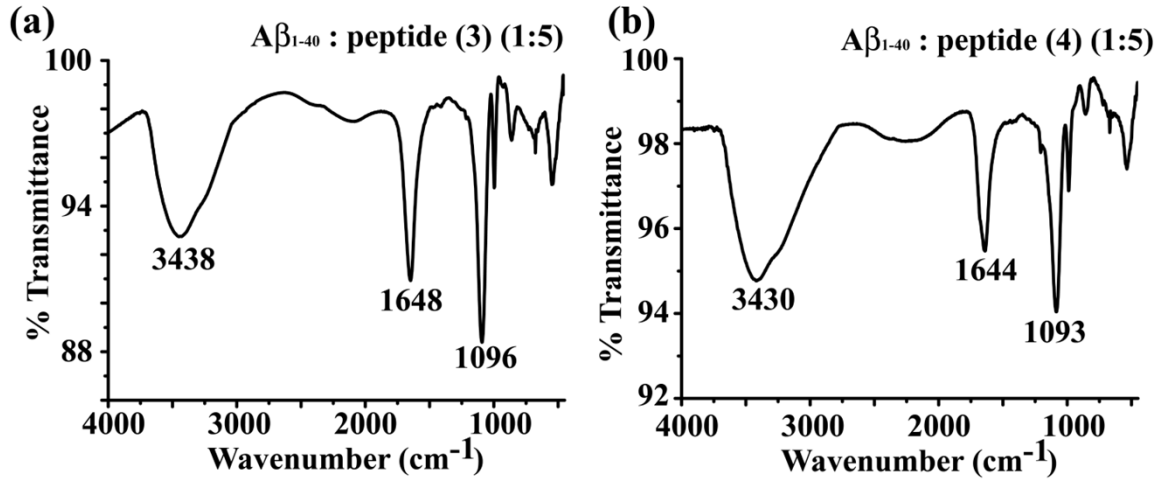


**Fig. S17.** FT-IR spectra of $A\beta_{1-40}$ peptide after 7 days incubation in PBS pH 7.4 at 37 °C, (a) in presence of 5 fold molar excess of peptide **3** and (b) in presence of 5 fold molar excess of peptide **4**.

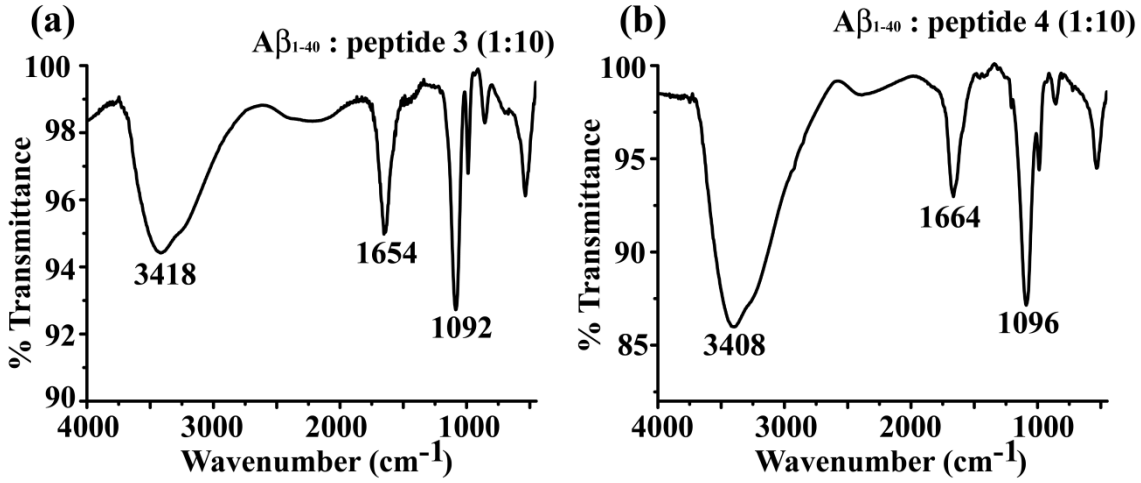


**Fig. S18.** FT-IR spectra of $A\beta_{1-40}$ peptide after 7 days incubation in PBS pH 7.4 at 37 °C, (a) in presence of 10 fold molar excess of peptide **3** and (b) in presence of 10 fold molar excess of peptide **4**.

## Biophysical studies for the disruption of preformed amyloid fibrillar aggregates of $A\beta_{1-40}$ peptide

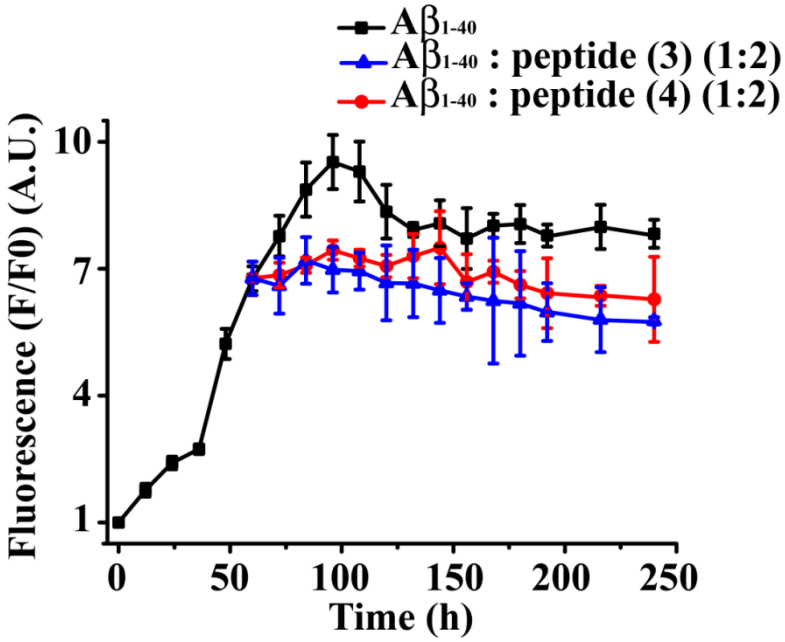


**Fig. S19.** Time dependent Thioflavin T assay of $A\beta_{1-40}$ in absence (black) and presence of 2 fold molar excess of peptide **3** (blue) or peptide **4** (red). Peptide solutions were incubated in PBS pH 7.4 (50 mM) at 37 °C.

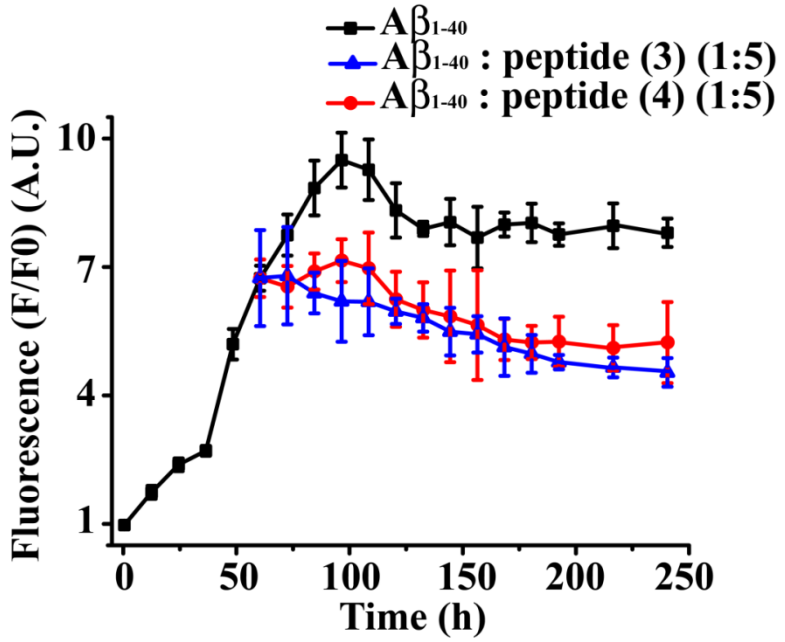


**Fig. S20.** Time dependent Thioflavin T assay of $A\beta_{1-40}$ in absence (black) and presence of 2 fold molar excess of peptide **3** (blue) or peptide **4** (red). Peptide solutions were incubated in PBS pH 7.4 (50 mM) at 37 °C.

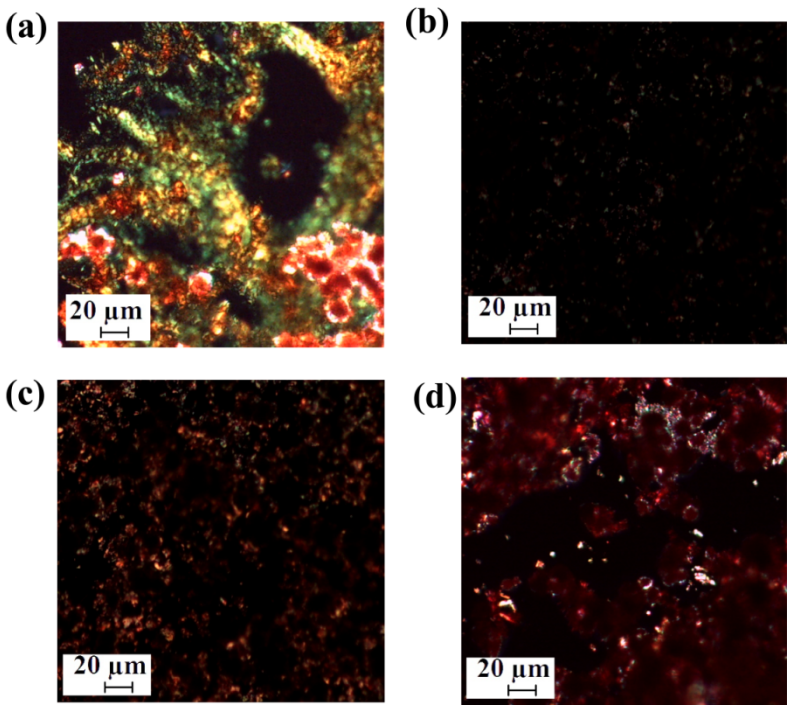


**Fig. S21.** Congo red stained birefringence images of (a) $A\beta_{1\text{-}40}$ (a) in absence and presence of (b) 2 fold, (c), 5 fold or (d) 10 fold molar excess of breaker peptide **4**. Images were taken after 10 days of incubation in PBS pH 7.4 (50 mM) at 37 °C. Scale bar is indicated as 20 µm.

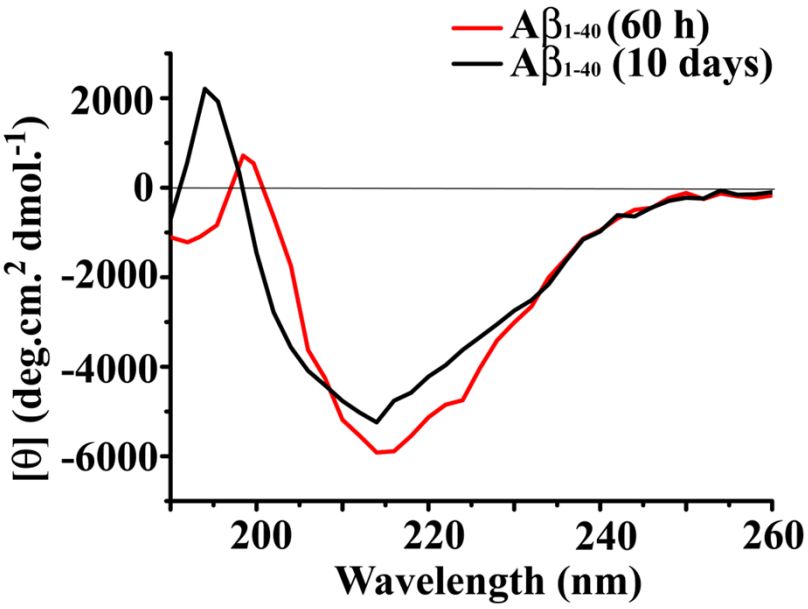


**Fig. S22.** CD spectra of $A\beta_{1\text{-}40}$ peptide at 60 h (red) and after 10 days (black) of incubation at 37 °C. The concentration of $A\beta_{1\text{-}40}$ was 50 µM in PBS pH 7.4 (50 mM).

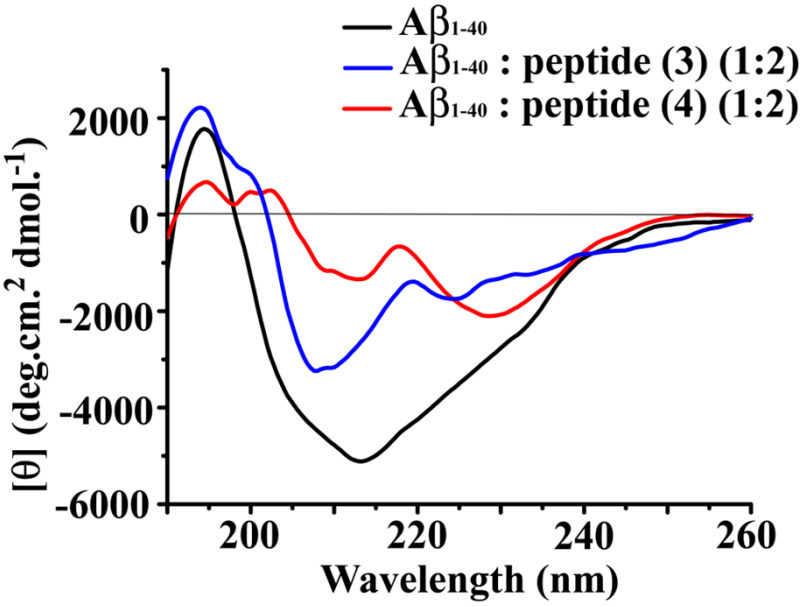


**Fig. S23.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 2 fold molar excess of peptide **3** (blue) or peptide **4** (red). The spectra were after 10 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 µM in PBS pH 7.4 (50 mM).

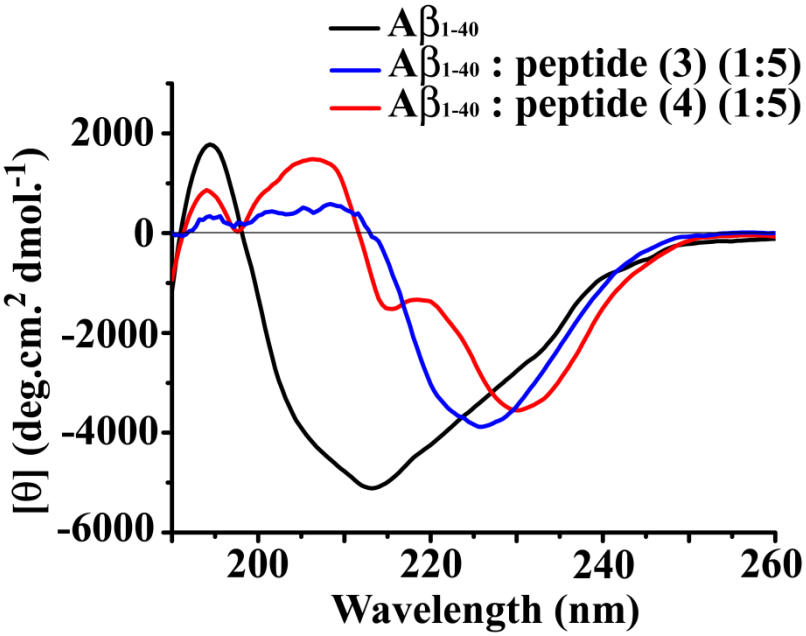


**Fig. S24.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 5 fold molar excess of peptide **3** (blue) or peptide **4** (red). The spectra were recorded after 10 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 µM in PBS pH 7.4 (50 mM).

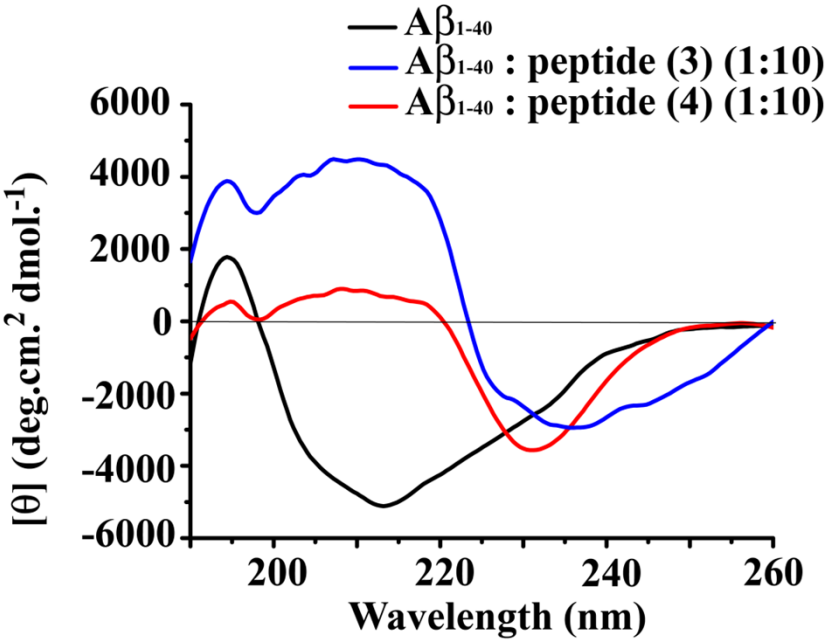


**Fig. S25.** CD spectra of $A\beta_{1-40}$ alone (black), in presence of 10 fold molar excess of peptide **3** (blue) or peptide **4** (red). The spectra were recorded after 10 days of incubation at 37 °C. The concentration of $A\beta_{1-40}$ was 50 μM in PBS pH 7.4 (50 mM).

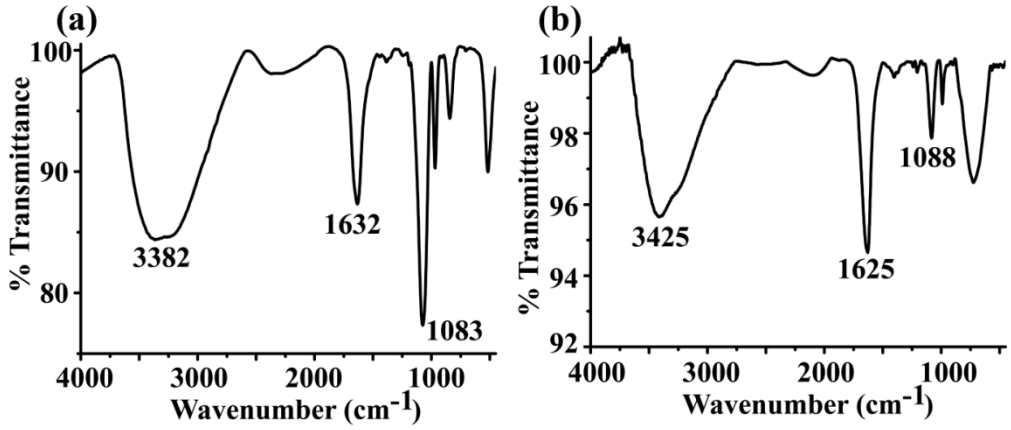


**Fig. S26.** FT-IR spectra of $A\beta_{1-40}$ peptide (a) at 60 h and (b) after 10 days of incubation. The peptide was incubated in PBS pH 7.4 at 37 °C.

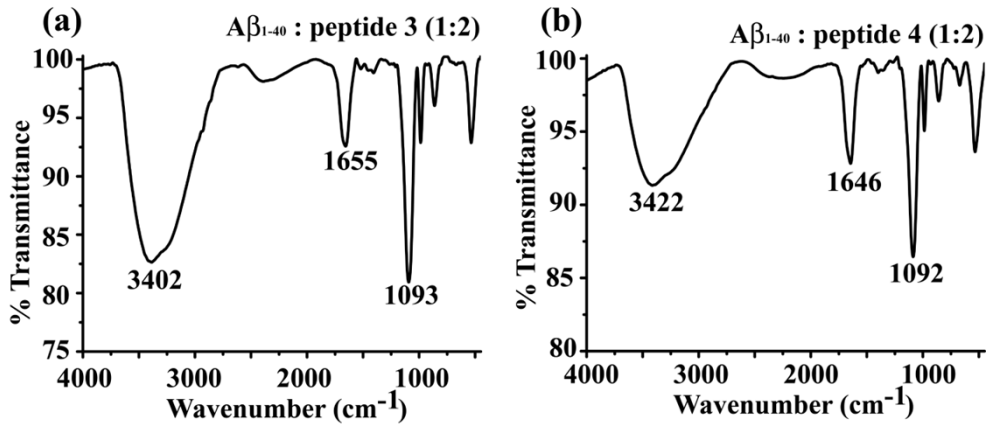


**Fig. S27.** FT-IR spectra of $A\beta_{1-40}$ peptide after 10 days incubation in PBS pH 7.4 at 37 °C, in presence of 2 fold molar excess of (a) peptide **3** or (b) peptide **4**.

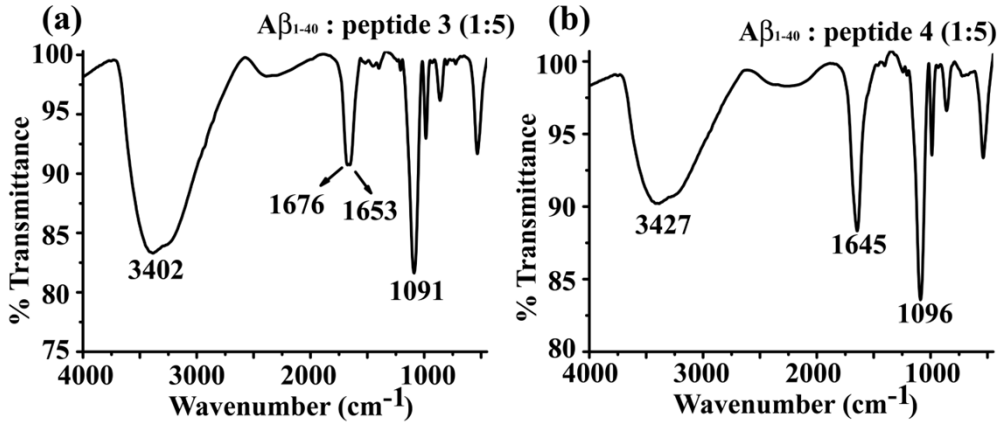


**Fig. S28.** FT-IR spectra of $A\beta_{1-40}$ peptide after 10 days incubation in PBS pH 7.4 at 37 °C, in presence of 5 fold molar excess of (a) peptide **3** and (b) peptide **4.**

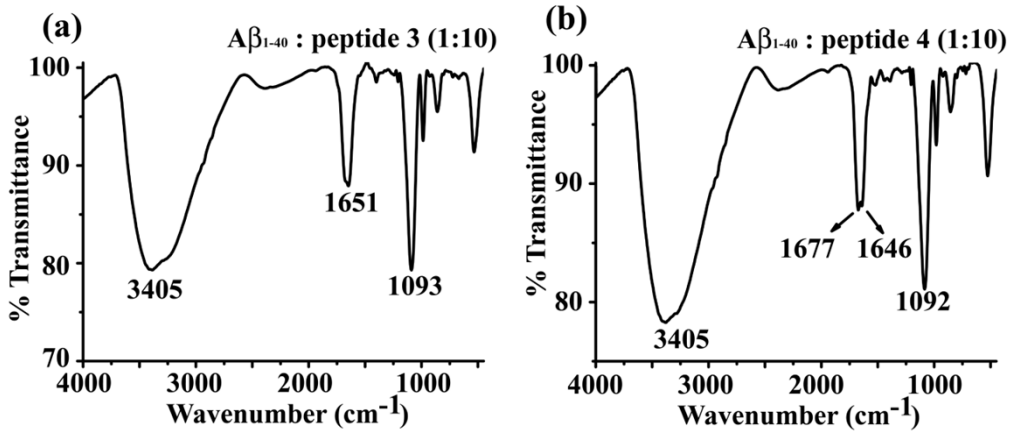


**Fig. S29.** FT-IR spectra of $A\beta_{1-40}$ peptide after 10 days incubation in PBS pH 7.4 at 37 °C, in presence of 10 fold molar excess of (a) peptide **3** or (b) peptide **4**.

## Characterization of prepared large unilamellar vesicles (LUVs) by transmission election microscope

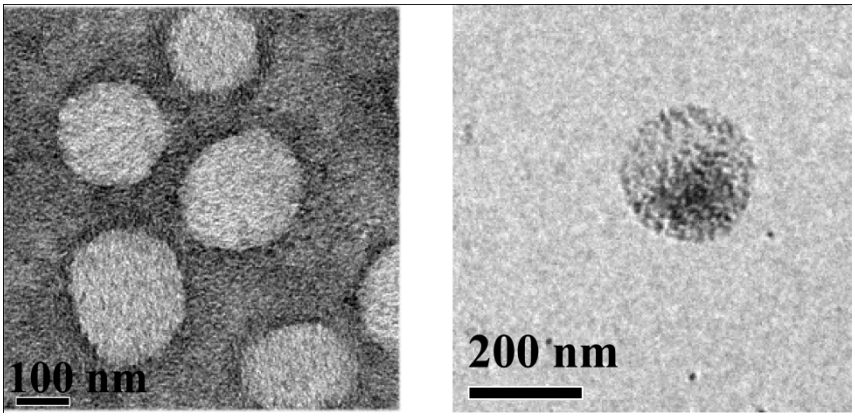


**Fig. S30.** TEM images of negatively stained LUVs at concentration of 100 μM in 50 mM HEPES buffer. Scale bars are indicated as 100 nm and 200 nm.

## Vesicle leakage study:[7, 9]

Soluble oligomers or protofibrils generated from Aβ peptide are believe to be more toxic as they are able to form pores in the cell membrane and may cause membrane disruption. To monitor such toxicity, leakage studies from dye entrapped large unilamellar vesicles (LUVs) are usually performed.

To perform the vesicle leakage study, LUVs were prepared first according to the method mentioned above (LUVs preparation section). Before performing the study, all peptide solutions were prepared in HEPES (50 mM) pH 7.4 and incubated for desired time period. The peptide solutions were prepared in a similar manner as described for CD analysis. There were four sets of peptide solutions used for the vesicle leakage studies:

Solution 1- $A\beta_{1\text{-}40}$ (incubated for 24 h),

Solution 2- $A\beta_{1\text{-}40}$ (incubated for 10 days),

Solution 3- $A\beta_{1\text{-}40}$ : peptide **3** (1:10) (peptide **3** was added to the preformed fibrillar aggregates after 60 h (3 days) and incubated for total 10 days).

Solution 4- $A\beta_{1\text{-}40}$ : peptide **4** (1:10) (peptide **4** was added to the preformed fibrillar aggregates after 60 h and incubated for total 10 days).

12.5 µL of dye loaded LUVs and 25 µL of peptide solution were taken and diluted to 500 µL to obtain final concentration of lipid solution 50 µM and $A\beta_{1\text{-}40}$ 2.5 µM. In the final solution, peptide and lipid were present in 1:20 molar ratios. To monitor the dye (carboxyfluorescein) released, we used Fluoromax-4, Horiba instrument and emission was recorded for each 5 min interval up to 20 min, then 10 min interval up to 100 min, further 12 h interval up to 72 h. The excitation was at 485 nm and emission was monitored at 516 nm with 3 nm of bandwidth. At the end of the experiment 10 µL of Triton X-100 was added to obtain complete dye release from the vesicle and the final fluorescence was measured. In addition to those, untreated LUVs (natural dye leakage) were studied and used as control. The % leakage (% dye release) was calculated as,[10]

$$\%\ Leakage = \frac{(observed\ fluorescence - initial\ fluorescence)}{(total\ fluorescence - initial\ fluorescence)} \times 100\ \%$$

From the instrument, text file was taken and graph was plotted using Origin Pro 8 software. For each data point 3 different sets of replicate solutions were scanned separately and average was taken with observed standard deviation.

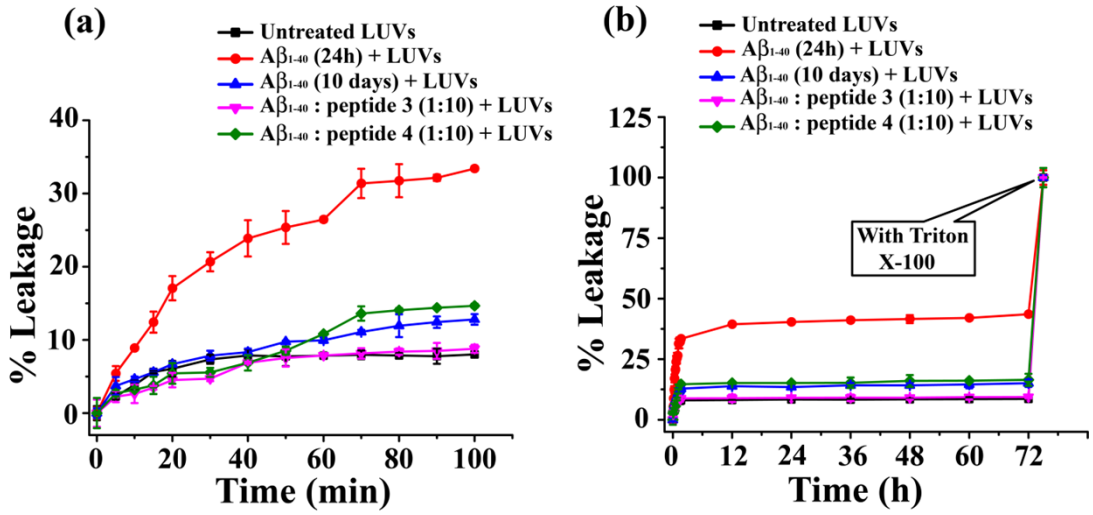


**Fig. S31.** Carboxyfluorescein dye emission showing the effect of $A\beta_{1\text{-}40}$ on LUVs with time and % of dye leakage. Panel (**a**) is the blown up vision of the first part of panel (**b**). We observed rapid increment of dye leakage of LUVs treated with $A\beta_{1\text{-}40}$ (solution 1) from 0 to 100 min but such increment was not observed after 36 h while the natural leakage of LUVs was saturated after 50 min. The spectra showing % of dye leakage by untreated LUVs (black), LUVs treated with solution 1 (red), LUVs treated with solution 2 (blue), LUVs treated with solution 3 (magenta) and LUVs treated with solution 4 (green). The 100 % dye release was obtained by treating the LUVs with triton x-100. The composition of solution 1, 2, 3 and 4 was mentioned above in the vesicle leakage study section.

From the vesicle leakage study it can be said that:

(a) Dye leakage by pore formation in the vesicle membrane was observed by 24 h old Aβ solution where possibility of the presence of the toxic soluble oligomers exists.

(b) The same was not observed from the solution where BSBHp **3** was added to the preformed fibrillar Aβ aggregates after 60 h and incubated for 10 days. That indicates peptide **3** disrupts the preformed fibrillar aggregates of $A\beta_{1\text{-}40}$ and changes it to possibly non-toxic monomers.

(c) Fully grown Aβ fibrils do not have pore formation ability, thus largely non-toxic (blue curve).

(d) The known breaker peptide **4** also breaks fibrils and makes it largely non-toxic.

## Characterization data of peptides 1 to 5

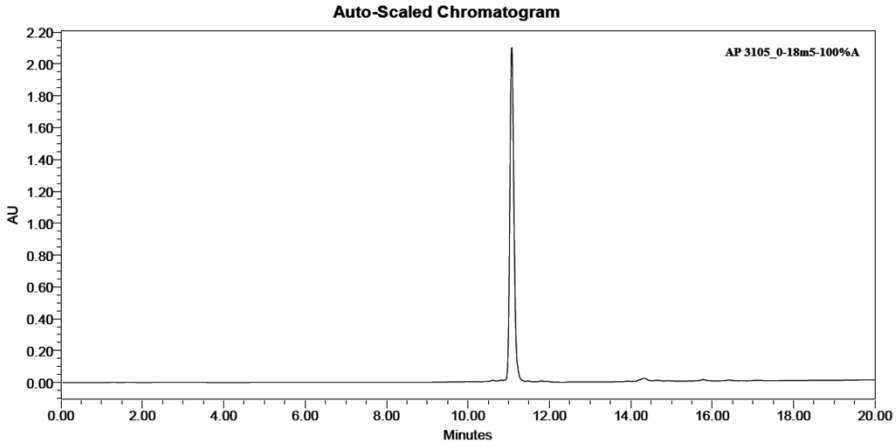


**Fig. S32.** HPLC profile of the purified peptide **1**.

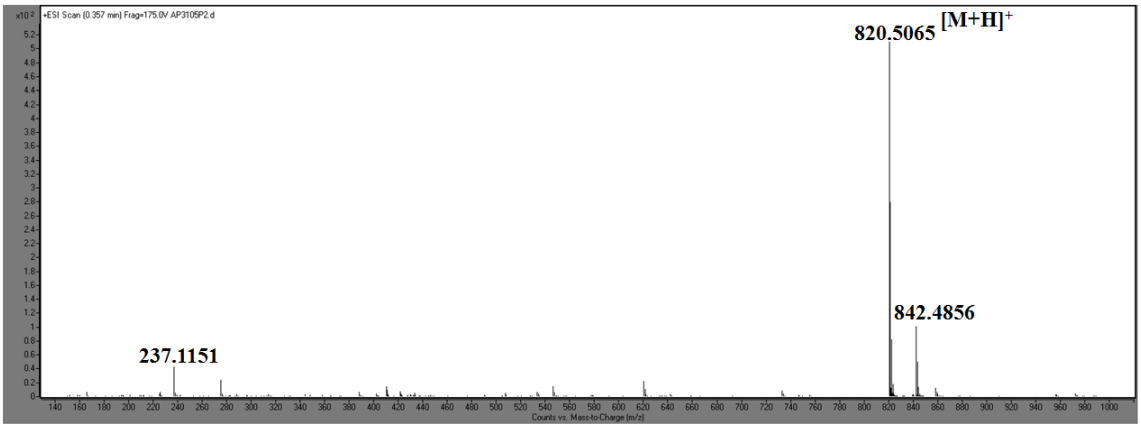


**Figure S33.** Mass spectrum of peptide **1**. Calculated mass for $C_{39}H_{66}N_9O_{10}$ is 820. 49 $[M+H]^+$, observed 820.50 $[M+H]^+$ and calculated mass for $C_{39}H_{65}N_9O_{10}Na$ is 842. 47 $[M+Na]^+$, observed 842.48 $[M+Na]^+$.

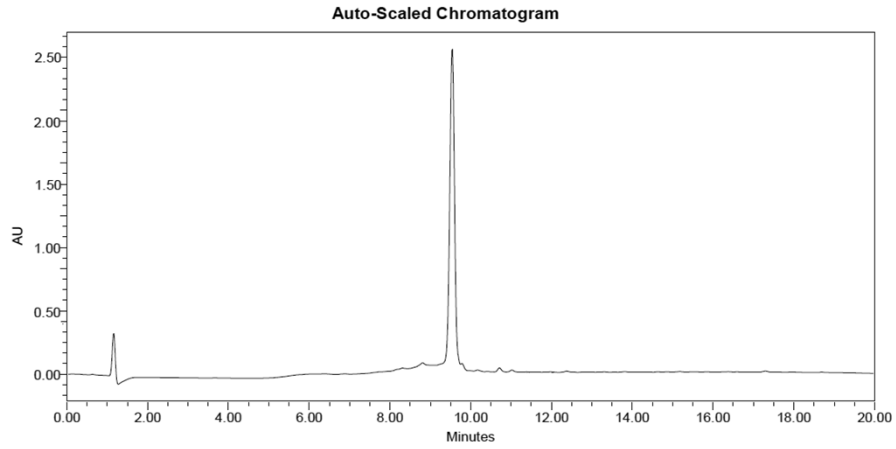


**Figure S34.** HPLC profile of the purified peptide **2**.

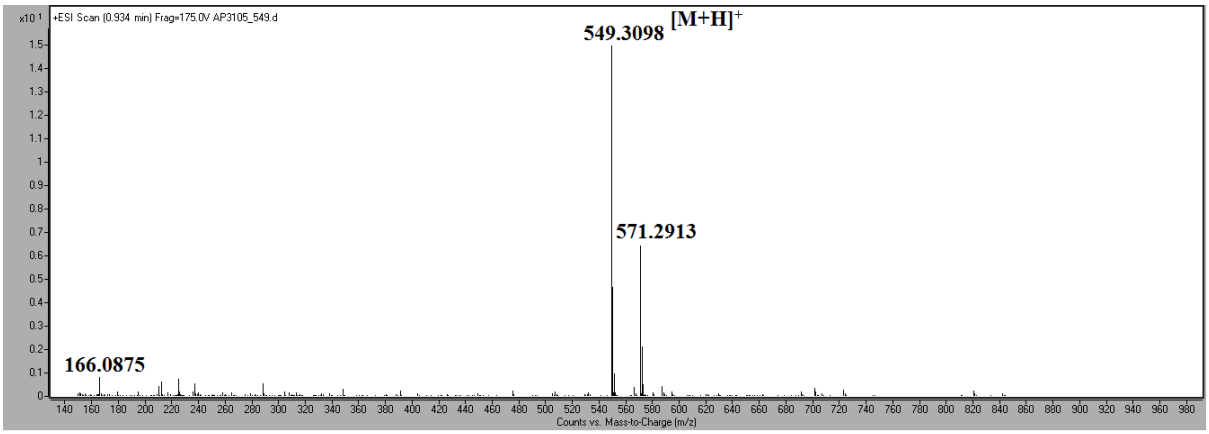


**Figure S35.** Mass spectrum of peptide **2**. Calculated mass for $C_{26}H_{41}N_6O_7$ is 549.30 $[M+H]^+$, observed 549.31$[M+H]^+$ and calculated mass for $C_{26}H_{41}N_6O_7Na$ is 571.28 $[M+Na]^+$, observed 571.29 $[M+Na]^+$.

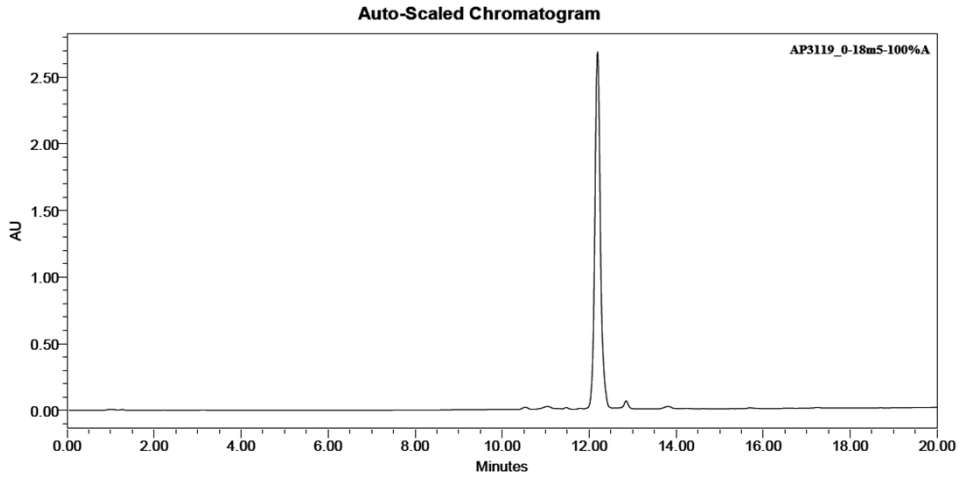


**Figure S36.** HPLC profile of the purified peptide **3**.

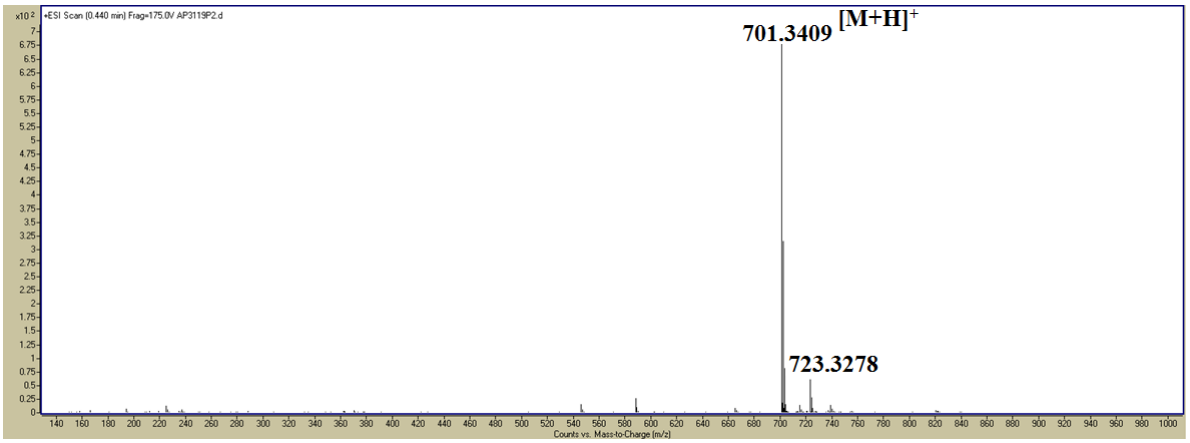


**Figure S37.** Mass spectrum of peptide **3**. Calculated mass for $C_{37}H_{45}N_6O_8$ is 701.32 $[M+H]^+$, observed 701.34$[M+H]^+$ and calculated mass for $C_{37}H_{44}N_6O_8Na$ is 723.31 $[M+Na]^+$, observed 723.32 $[M+Na]^+$.

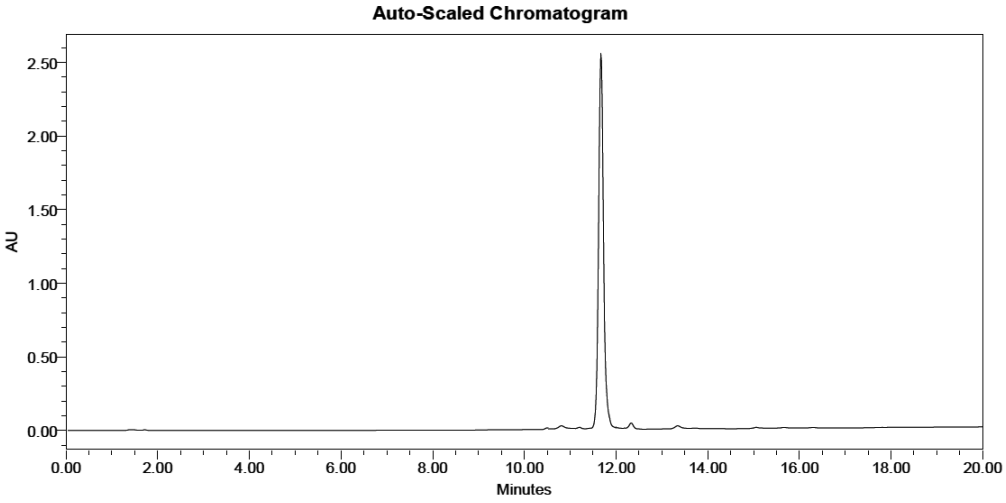


**Figure S38.** HPLC profile of the purified peptide **4**.

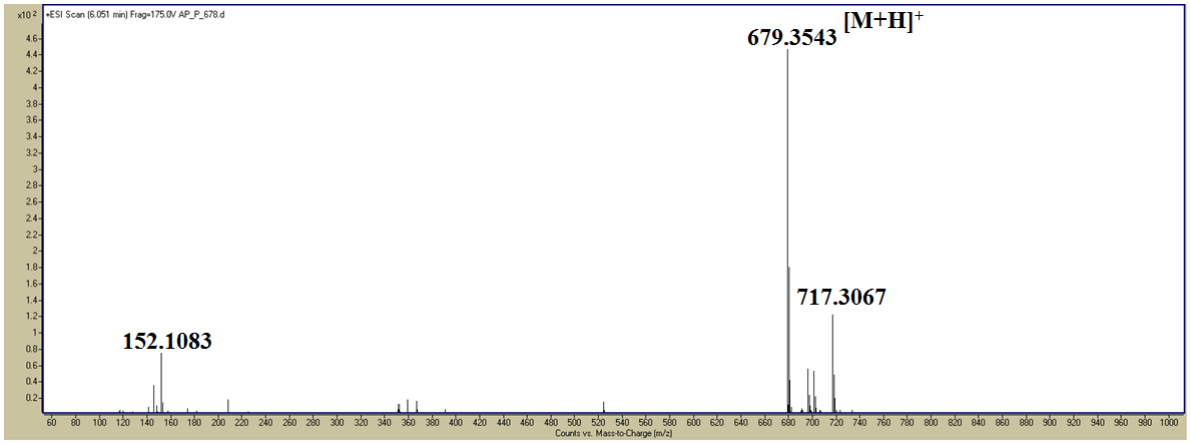


**Figure S39.** Mass spectrum of peptide **4**. Calculated mass for $C_{35}H_{47}N_6O_8$ is 679.34 $[M+H]^+$, observed 679.35$[M+H]^+$ and calculated mass for $C_{35}H_{46}N_6O_8K$ is 717.30 $[M+K]^+$, observed 717.30 $[M+K]^+$.

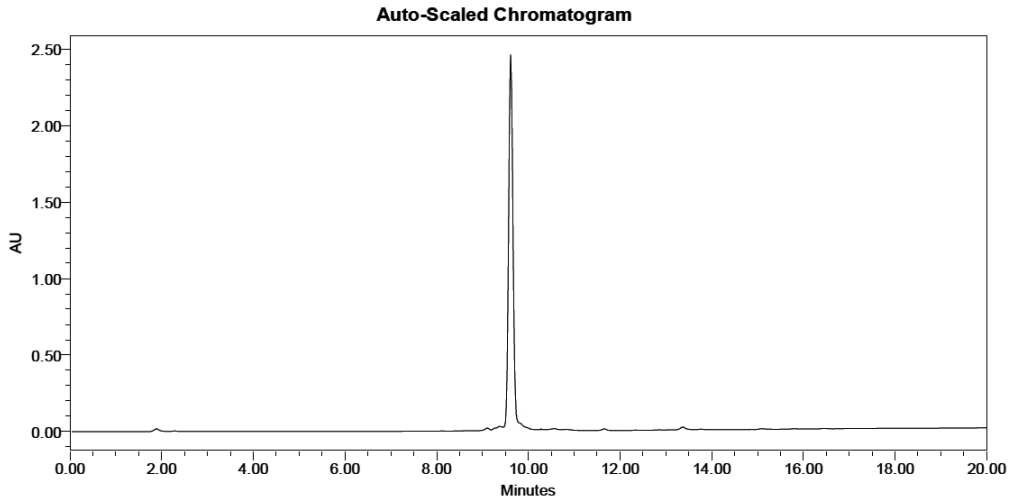


**Figure S40.** HPLC profile of the purified peptide **5**.

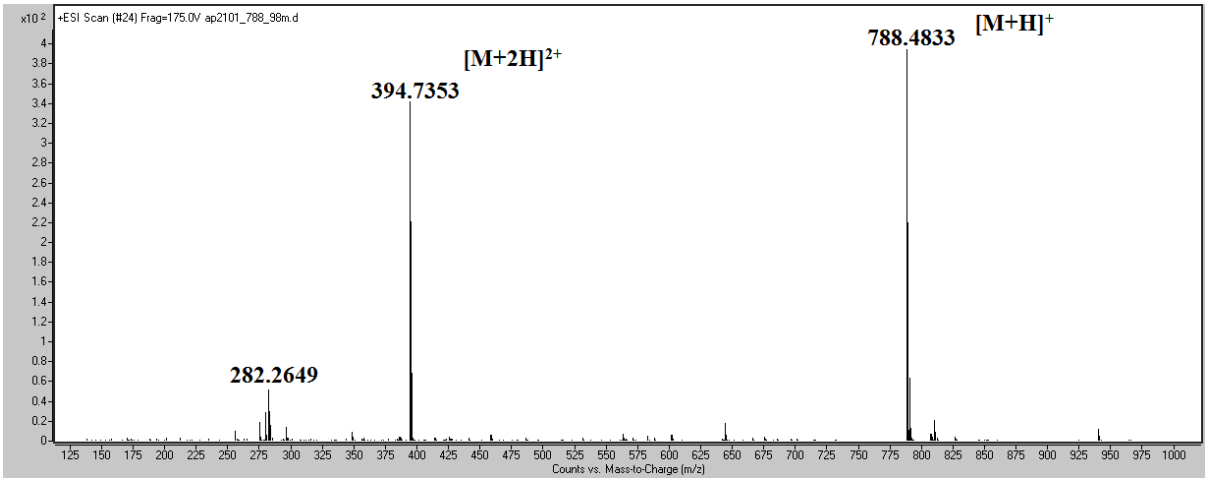


**Figure S41.** Mass spectrum of peptide **5**. Calculated mass for $C_{35}H_{66}N_9O_{11}$ is 788.49 $[M+H]^+$, observed 788.48 $[M+H]^+$ and 394.73 $[M+2H]^{2+}$.

## References:

1. S. Chen and R. Wetzel, *Protein Sci*., 2001, **10**, 887–891.

2. H. Levine, *Protein Sci*., 1993, **2**, 404–410.

3. P. Walsh and J. Yau, *Biochemistry,* 2009, **48**, 5779–5781.

4. M. R. Nilsson, *Methods,* 2004, **34**, 151–160.

5. B. A. Wallace and R. W. Janes, *Biochemical Society Transactions.* 2003, **31**, 631–633.

6. B. Shivu, S. Seshadri, J. Li, K. A. Oberg, V. N. Uversky and A. L. Fink, *Biochemistry,* 2013, **52**, 5176−5183.

7. T. L. Williams, I. J. Day and L. C. Serpell, *Langmuir,* 2010, **26**, 17260–17268.

8. M. Traikia, D. E. Warschawski, M. Recouvreur, J. Cartaud and P. F. Devaux, *Eur Biophys J.,* 2000, **29**, 184−195

9. T. Sheynis, A. Friediger, W. F. Xue, A. L. Hellewell, K. W. Tipping, E. W. Hewitt, S. E. Radford and R. Jeilinek, *Biophys. J.*, 2013, **105**, 745−755.

10. J. McLaurin and A. Chakrabartty, *J. Biol. Chem.,* 1996, **271**, 26482–26489.