Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information (ESI)

Amyloid β Derived Switch-Peptides as a Tool for Investigation of Early Events of Aggregation: A Combined Experimental and Theoretical Approach

Ashim Paul, Bhanita Sharma, Tanmay Mondal, Kishore Thalluri, Sandip Paul and Bhubaneswar Mandal*

Department of Chemistry, Indian Institute of Technology Guwahati, Assam - 781039, India.

Email: bmandal@iitg.ernet.in

Materials and Methods:

Reagents and Solvents:

Rink amide MBHA resin was purchased from Fluka (Loading 1.1 mmol/g). BOP [(Benzotriazole-1-yloxy) tris (dimethylamino) phosphoniumhexafluorophosphate], PyBOP [(Benzotriazole-1-yl-oxy-tris-pyrolidine-phosphonium hexafluorophosphate], Diisopropyl ethylamine (DIPEA) were purchased from Sigma. Dimethylformamide (DMF, extrapure grade), dichloromethane (extrapure grade) and acetonitrile of HPLC grade were obtained from Merck (India). Acetic anhydride (synthesis grade), N-methyl imidazole (extrapure), Trifluoroacetic acid (TFA) of extra pure grade were purchased from SRL (India). Milli-Q water at 18.2 Ω was used. All Fmoc amino acids and Boc-Ser-OH were purchased from GL Biochem (Shanghai) with following side chain protecting groups: tert-butyl for Asp, Glu, and Ser; trityl for His and Gln; and Boc for Lys.

Peptide Synthesis:

All the described peptides were synthesized by standard Fmoc/tBu orthogonal protection strategy based 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 were monitored by Kaiser's test and in cases of incomplete 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.

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 4 mL/ min. Binary solvent system was used, solvent A (0.1 % TFA in H_2O) and solvent B (0.1 % TFA in CH_3CN). A Waters 2489 UV detector was used with an option of dual detection at 214 and 254 nm. 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 C18 analytical column at a flow rate of 1 ml/min, linear gradient of 5-100% CH_3CN over 18 minutes with a total run time of 20 min was used. Dual wavelength was selected at 214 nm and 254 nm.

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 of the switch-peptides was monitored by Waters UPLC-MS (ESI +ve mode) Micromass Q-TOF equipped with Masslynx software.

Sample Preparation:

Potassium phosphate buffer solution (PBS) of pH 7.4 was prepared (50 mM) using potassium di-hydrogen phosphate and di-potassium hydrogen phosphate salts. Purified solid peptide samples (amyloidogenic peptide alone and in some cases mixed with breaker peptides) were dissolved in PBS (50 mM, pH 7.4) and incubated at 37 °C over a water bath. The amyloidogenic natures of the peptides were characterized by various bio-physical tools, e.g. TEM, Congo-Red Stained Birefringence, Thiflavin-T assay, UV, CD, etc., as described below.[1]

Transmission Electron Microscopy:

After incubation of 5 days, 10 μL of the peptide stock solution was added over the carbon coated copper grid and allowed to float for 1 min. Then 10 μL of 2% uranyl acetate solution was added over 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 performing TEM analyses on JEOL instrument at 200 kV.

Congo-Red Stained Birefringence:

Commercially available (purchased from Sigma) Congo red was dissolved in 80 % aqueous ethanol solution 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 incubation of the stock an aliquot of 20 μL was placed over a glass slide followed by 40 μL of the saturated Congo red solution. The excess solution was removed using blotting paper; the sample was dried at room temperature, and was kept in desiccator before birefringence analysis under a Leica ICC50 HD polarizable microscope.

Thioflavin T Assay:

Commercially available Thioflavin T (ThT) was purchased from Sigma Aldrich and a stock solution of 50 μM concentration in PBS (50 mM, pH 7.4) was prepared. It was stored at 4 °C with dark cover to prevent degradation from light. To perform the fluorescence study, 40 μL of peptide sample was taken out from the stock and was mixed with 200 μL of thioflavin T solution (50 μM), final volume was made up to 400 μL with PBS. For each experiment, three

different replicate solutions were prepared. For ThT fluorescence assay, emission was measured at 485 nm and excitation at 440 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 replicates were scanned separately and average was taken with observed standard deviation.

Ultra Violet- Visible Spectroscopy (UV-Vis):

Purified solid peptide samples were dissolved in PBS (50 mM, pH 7.4) to obtain a stock solution of 50 μM and incubated at 37 °C on a water bath. To perform the UV-VIS study, stock solution was diluted to 5-20 μM (as required) and kinetics were performed at 220 nm and 257 nm respectively. 400 μL of the sample was taken in a cuvette with path length of 1 cm. For UV-Vis absorption study Spectra were recorded from 200 nm to 450 nm on Perkin Elmer (lamda 750). The text files were taken from the instrument and were plotted using OriginPro 8 software.

Circular Dichroism (CD):

To perform the CD studies, stock solution (50 μM) was directly used without further dilution. 200 μL of the sample was taken in a cuvette with path length of 1 mm. Three measurements were accumulated. Spectra were recorded from 190 nm to 300 nm on a JASCO J-1500 instrument. For the kinetics study peptide solutions were diluted further to 5-20 μM and kinetics were performed at 220 nm and 257 nm respectively. Observed ellipticity (mDeg) was converted to mean residue molar ellipticity using the following equation:

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

N = Number of peptide bond present in the peptide. The text files were taken from the instrument and were plotted using OriginPro 8 software.

Raman Spectroscopy:

To perform the Raman spectroscopy stock solution was diluted to 20 μM and 600 μL of the sample was taken in a fluorescence cuvette with path length of 1 cm. The samples were excited at 488 nm with an Ar^+ ion laser and spectra were recorded from 500 to 2000 cm^{-1}on HORIBA Jobin Yvon, Model LabRAM HR Raman spectroscope. The spectra were recorded after 20 accumulations and plotted using OriginPro 8 software.

Molecular Dynamics (MD) Simulations:

All MD simulations were carried out using sander from Amber 12 molecular dynamics package.[2] Amber ff99SB force-field parameters were used for switch-peptide as well as for β-breakers.[3] SPC/E[4]model was employed for water and sodium ions were added to neutralize the total charge of the systems. MD simulations were carried out in NPT ensemble at 310 K temperature and 1 atm pressure with a time-step of 2 fs. The starting configurations were energy minimized for 5000 steps with 2500 steps of steepest decent minimization, followed by 2500 steps of conjugate gradient minimization. The systems were then heated slowly from 0 K to 310 K in NVT ensemble. Then the systems were equilibrated in NPT ensemble for 100 ns. Periodic boundary conditions were applied for all the simulations. Temperature was controlled using Langevin dynamics method with a collision frequency of 1 ps^{-1} and the Berendsen barostat[5] was used to maintain the pressure with a pressure relaxation time of 2 ps. We used SHAKE algorithm[6] to constrain the bonds involving hydrogen. A cut off distance of 12.0 Å was applied for all nonbonding interactions and the long-range electrostatic interactions were treated using particle mesh Ewald method.[7]

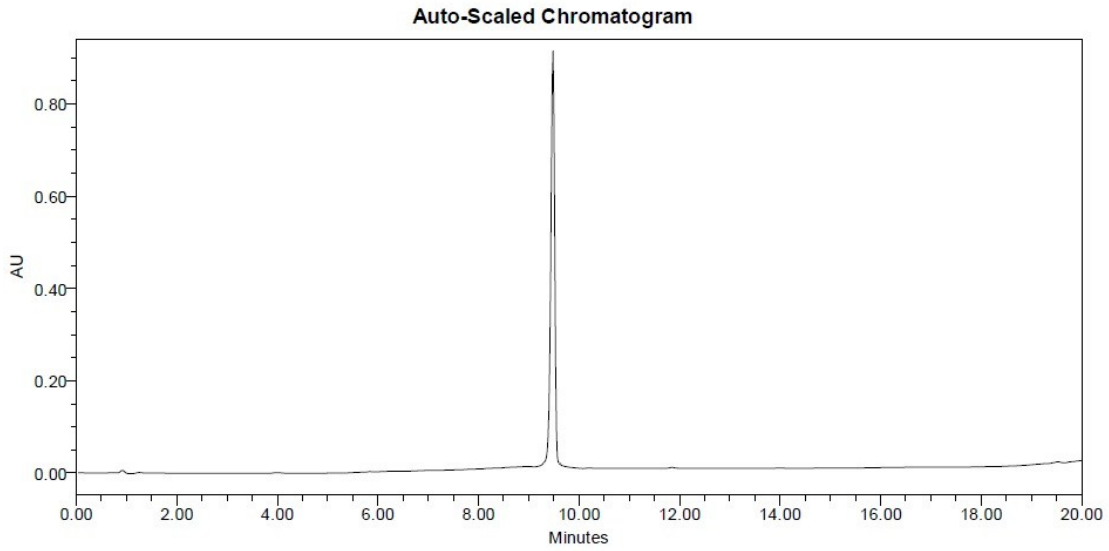

Fig. S1. HPLC profile of the purified peptide **1**.

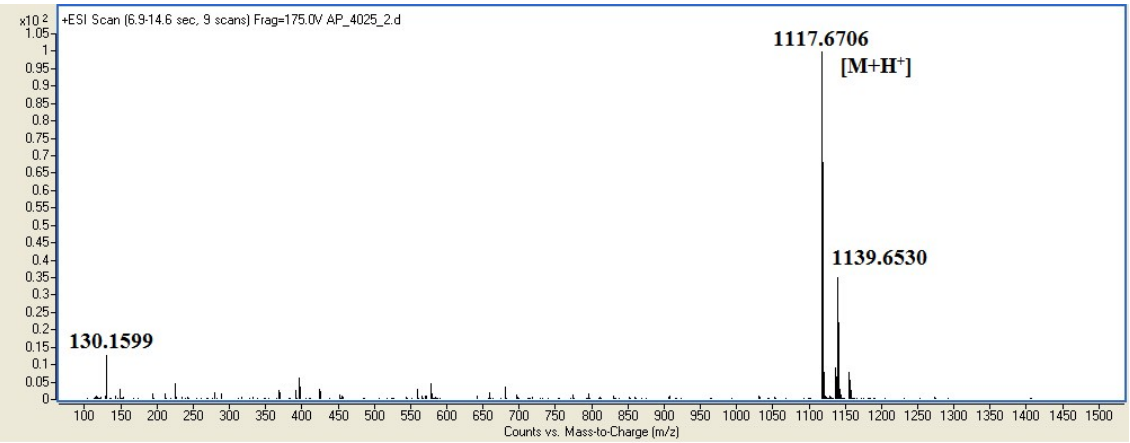

Fig. S2. Mass spectrum of peptide **1**, Calculated mass for $C_{49}H_{89}N_{12}O_{17}$ is 1117.64 $[M+H]^+$, observed 1117.67 $[M+H]^+$ and calculated mass for $C_{49}H_{88}N_{12}O_{17}Na$ is 1139.63 $[M+Na]^+$, observed 1139.63 $[M+Na]^+$.

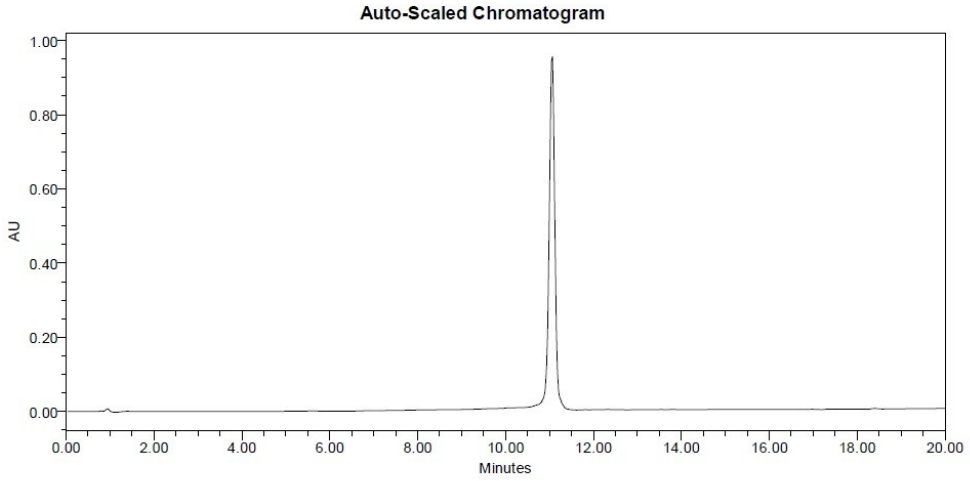

Fig. S3. HPLC profile of the purified peptide **2**.

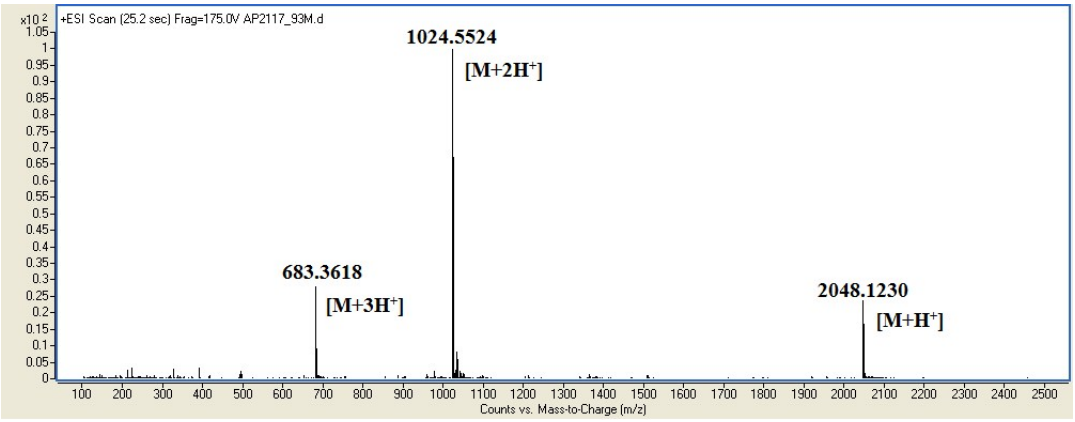

Fig. S4. Mass spectrum of peptide 2, Calculated mass for $C_{94}H_{148}N_{23}O_{28}$ is 2048.09 $[M+H]^+$, observed 2048.12 $[M+H]^+$, 1024.55 $[M+2H]^{2+}$ and 683.36 $[M+3H]^{3+}$.

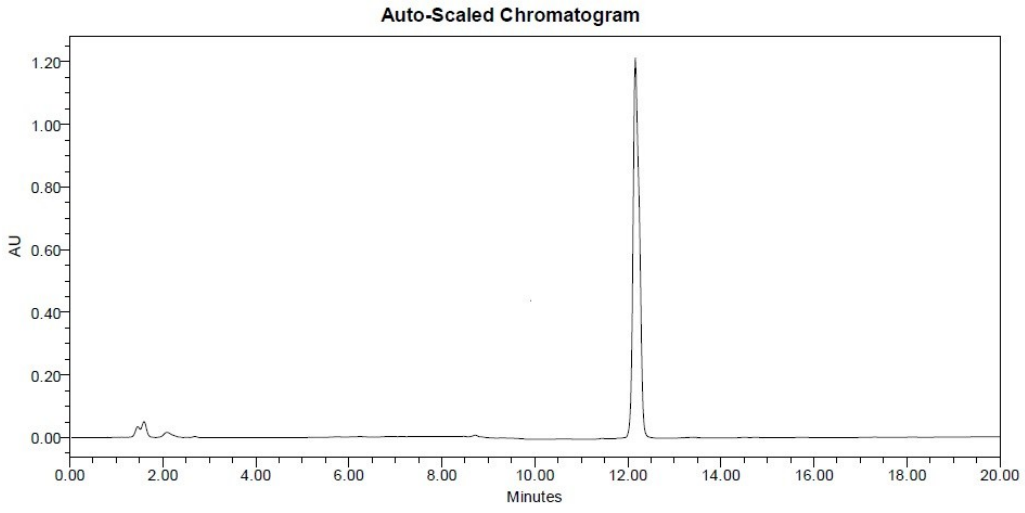

Fig. S5. HPLC profile of the purified peptide 3.

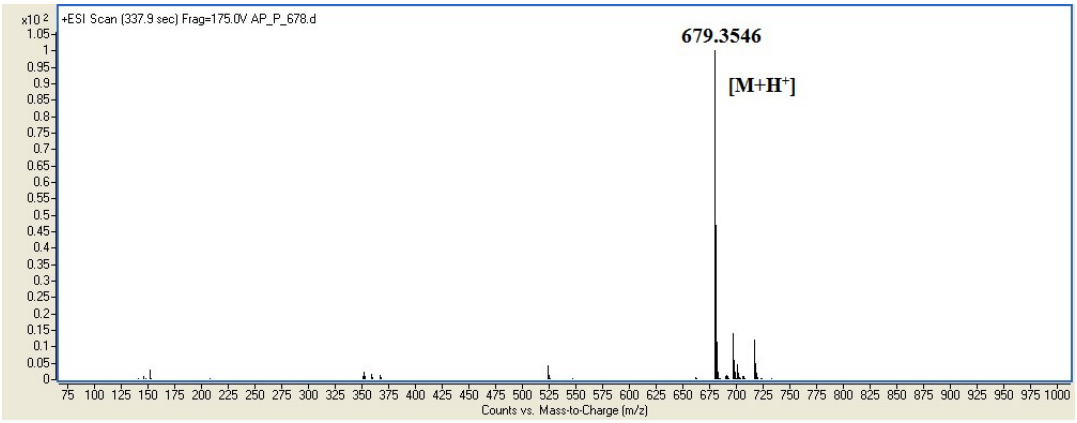

Fig. S6. Mass spectrum of peptide 3, Calculated mass for $C_{35}H_{47}N_6O_8$ is 679.34 $[M+H]^+$, observed 679.35 $[M+H]^+$.

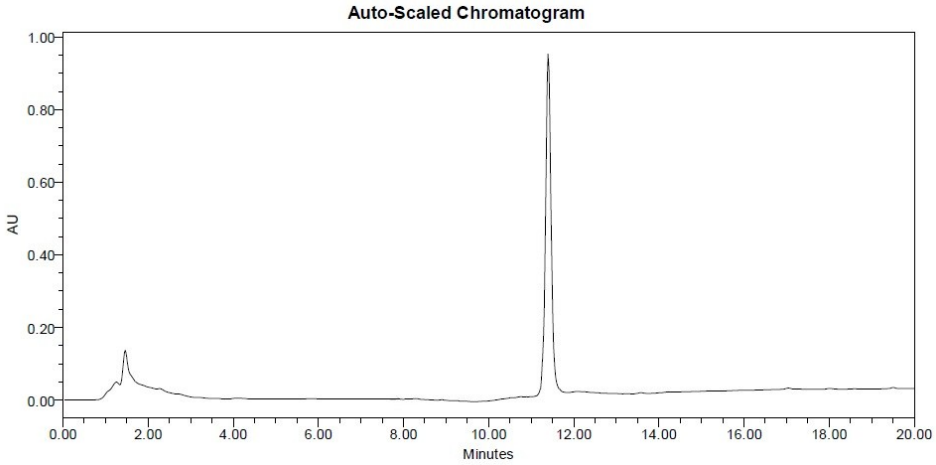

Fig. S7. HPLC profile of the purified peptide 4.

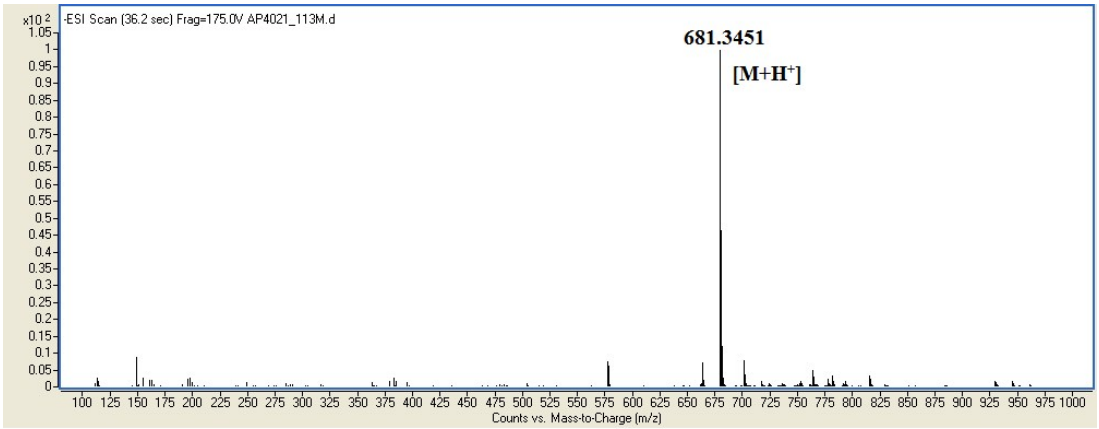

Fig. S8. Mass spectrum of peptide 4, Calculated mass for $C_{35}H_{49}N_6O_8$ is 681.36 $[M+H]^+$, observed 681.34 $[M+H]^+$.

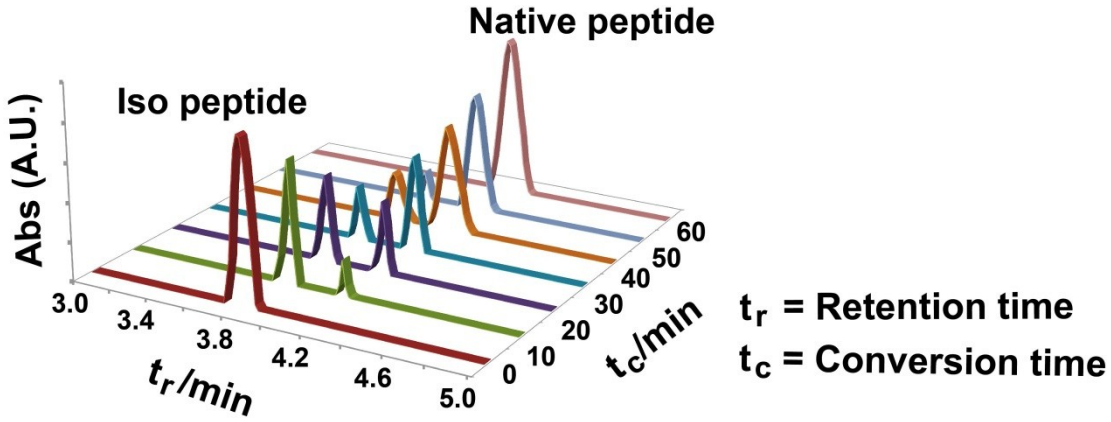

Fig. S9. O to N acyl migration of peptide **2** in PBS of pH 7.4 at 37 °C, (a) conversion profile and (b) decay of S_{off} state and formation of S_{on} state. Half life of conversion is 23.2 ± 0.5 min.

The peptide, **2** formed clear fibrillar structure when viewed under TEM and also exhibited characteristic green gold birefringence under cross polarized light when stained with Congo red.

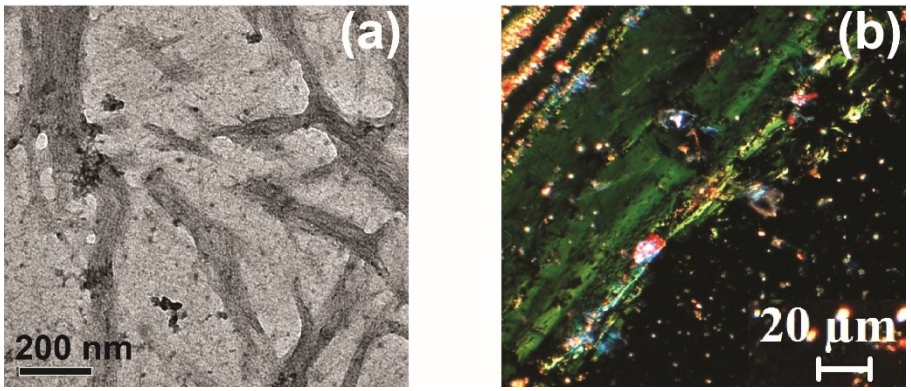

Fig. S10. (a) TEM and (b) Congo red stained birefringence images of peptide **2**. The peptide solution was incubated in PBS of pH 7.4 at 37 °C and images were taken after 5 days of incubation. Scale bar is indicated as 200 nm and 20 µm.

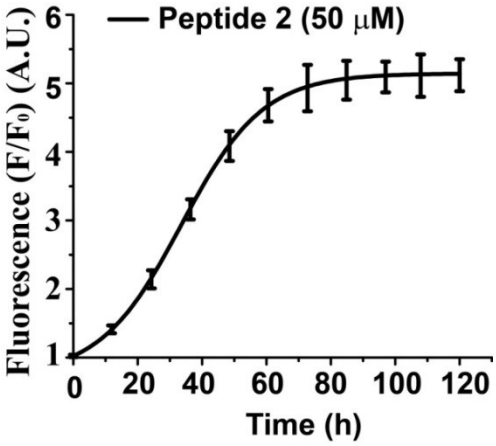

Fig. S11. Time dependent Thioflavin T assay of peptide **2** (50 μM) in PBS pH 7.4 (50 mM) at 37 °C.

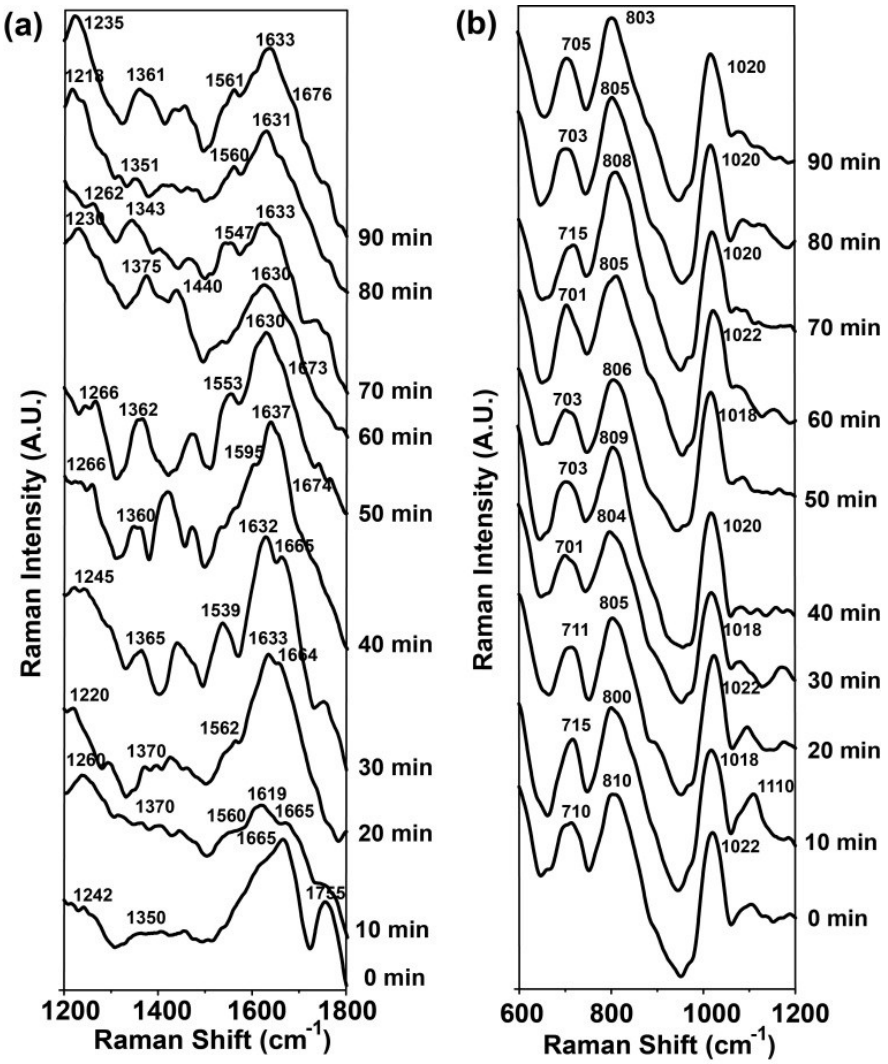

Fig. S12. Change in Raman bands at various time interval of **2** at (a) 1200-1800 cm^{-1} and (b) 600 -1200 cm^{-1} (c = 20 μM, PBS pH 7.4).

References.

1. A. Paul, K. C. Nadimpally, T. Mondal, K. Thalluri and B. Mandal, Chem. Commun., 2015, 51, 2245–2248.
2. D. A. Case, T. A. Darden, T. E. Cheatham, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, K. M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, Gtz, A. W., I. Kolossvary, K. F. Wong, F. Paesani, J. Vanicek, R. M. Wolf, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J.Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, R. Salomon-Ferrer, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, P. A. Kollman, *AMBER-12, University of California, San Francisco* 2012.
3. Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. Wang and P. Kollman, *J. Comput. Chem.*, 2003, **24**, 1999–2012.
4. H. J. C. Berendsen, J. R. Grigera and T. P Straatsma, *J. Phys. Chem.* 1987, **91**, 6269–6271.
5. H. J. C. Berendsen, J. P. M. Postma and van Gunsteren, *J. Phys. Chem.*, 1984, **81**, 3684–3690.
6. J. P. Ryckaert, G. Ciccotti and H. J. C. Berendsen, *J. Comput. Phys.*, 1977, **23**, 327–341.
7. T. Darden, D. M. York and L. Pedersen, *J. Chem. Phys.*, 1993, **98**, 10089–10092.