Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Amyloid inhibitor octapeptide forms amyloid type fibrous aggregate and affect in microtubule motility

Atanu Biswas, Prashant Kurkute‡, Batakrishna Jana, Aparna Laskar and Surajit Ghosh*

Chemistry Division, CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Jadavpur, Kolkata-700032, West Bengal, India

Correspondence author: sghosh@iicb.res.in

Materials:

All the Fmoc-amino acids, HBTU and Fmoc-Rink Amide AM resin were purchased from Novabiochem. D-Biotin was purchased from Thermo Fisher. Pipyridine, Diisopropylethylamine (DIPEA) and 1-Hydroxybenzotriazole (HOBT) anhydrous were purchased from Spectrochem. Phenol, Dichloromethane (DCM), Ethanedithiol (EDT), Thioflavine-T and Trifluoroaceticacid, Hydrogen peroxide (30% solution), Acetone, Dichloromethane and N, N-Dimethylformamide were purchased from Merck. 3-glycidoxypropyltrimethoxysilane, *N,N′*-Diisopropylcarbodiimide (DIC) and Nickel(II) chloride hexahydrate and Amberlite IRA-400 were purchased from Sigma Aldrich. Diamino-polyethylene glycol with MW 2000 Da (PEG2000) and mono BOC-amino-polyethylene glycol with MW 3000 Da were purchased from Rapp Polymere. E,Z-Link Biotin-NHS was purchased from Pierce. Tris-NTA was received as gift from Dr. Thomas Surrey's lab at EMBL, Heidelberg (Currently at London Cancer Research Institute, UK). Pyridine and Ether were purchased from Fisher Scientific. For purification, we use Simadzu HPLC system with Symmetry C-18 (Waters) semi preparative reverse phase column. Vertis 4K freeze drier for Lyophilized the pure product after column purification was used. HPLC grade water and Acetonitrile were purchased from J. T. Baker.

Tubulin: Tubulin, labelled tubulin and Decahistidine-tagged kinesin612 were received as gift from Dr. Thomas Surrey's lab at EMBL, Heidelberg (Currently at London Cancer Research Institute, UK). Polymerisation of microtubules with GMP-CPP were performed as described in literature.[1]

Methods:

Peptide synthesis: Synthesis of Octapeptide ′X′ (NH_2-NAPVSIPQ-NH_2) and Biotin-Octapeptide ′HX′ (Biotin-NAPVSIPQ-NH_2) has been achieved by solid phase peptide synthesis method using Rink Amide AM resin.[2] Crude peptides were purified by HPLC and characterized by MALDI Mass Spectroscopy.

Transmission electron microscopy (TEM): A 10 μL aliquot of the 100nM peptide solution of both ′X′ and ′HX′ after 0, 7 and 30 days incubation was placed on a 300 mesh copper grid from ProSciTech. After 1 minute, excess solution was removed, washed with water and the grids were stained with 2% Uranyl acetate in water. Excess staining solution was removed from the grid after two minutes. Samples were viewed using a TECNAI G2 SPIRIT

BIOTWIN CZECH REPUBLIC 120 KV electron microscope operating at 80 kV. High resolution samples were viewed and analyzed with a JEOL JEM-2012 electron microscope operating at 200 kV.

Fourier Transform Infrared Spectroscopy: The lyophilized powder of samples were transferred into a mortar-pestle containing IR-grade KBr (ca. 30 mg) and mixed to prepare the pellet under strictly dry condition to prevent absorption of water vapour. This pellet was further dried by storing in a vacuum desiccator. Spectra of these pellets were recorded and accumulated of 5 times scan with speed 0.2 cm/s at a resolution of 1.6 cm^{-1} in a Perkin-Elmer Spectrum 100 series Spectrometer. The $LiTaO_3$ detector was used for data plotting. Each time background correction was performed to eliminate interference from air (or any other parameters)[3].

Thioflavin-T Assay: ThT fluorescence measurements were performed on a Varian Cary Eclipse luminescence spectrometer, using a 1 mm light path quartz cuvette. Fluorescence emission spectra were recorded from 465-600 nm with excitation at 446 nm. 0, 7 and 30 days incubated peptide solutions (10μL) as well as 7 days incubated solution of Aβ alone, Aβ with 'X' and Aβ with 'HX' were thoroughly mixed with 1 mL of 10 μM ThT in water followed by immediate acquisition of the fluorescence emission spectrum. The excitation and emission slit width were 10 nm[4].

Fluorescence microscopy: 20 μL of 2 mM Thioflavin-T solution was taken into a 0.5 mL PCR tube and added 20 μL of fibrillated peptide solution into it and mixed well. Incubate the mixed solution at 37 °C water bath for 4-5 hr. Then the solution was placed on a microscopic glass slide, dried and washed with plenty of water to remove the excess dye. Fibrils were imaged under inverted fluorescence microscope (NIKON Ti-U) in 40× magnification using ANDOR iXON3 camera.

Preparation of biotin and Tris-NTA functionalized Surface: Glass cover slips (50X50 mm) were cleaned with 3M NaOH followed by sonication for 30 minutes. Next, cover slips were cleaned with plenty of water and further treated with piranha (2:3 mixture of hydrogen peroxide and sulphuric acid) followed by ultra-sonication for 45 min under fume hood. Piranha solution was discarded and glass slides were thoroughly cleaned with water and were dried under stream of nitrogen gas. Silanisation of glass surfaces has been achieved by treating with 3-Glycidoxypropyltrimethoxysilane (GOPTS) at 75 °C. Next, silanised glass surfaces were treated with 1:1 mixture of diamino-polyethylene glycol and Mono-BOC protected polyethylene glycol amine followed by heating at 75 °C for overnight. The polyethylene glycol functionalised surfaces were washed with DMF and plenty of water for complete removal of excess and unreacted polyethylene glycols from surfaces. Next, surface was treated with Biotin-NHS for 2 hour at 75 °C followed by washing with DMF and plenty of water. For removal of BOC protection from polyethylene glycol surface was treated with TFA for 3 hours and followed by washing with water and dried under N_2 gas stream. Dried glass surface was treated with 2:1 (v/v) mixture of 15 mg/mL Tris-NTA solution in dry chloroform and DIC at room temperature and incubated at 75 °C for 4 hours. Glass surface was washed with DMF and water and treated with TFA for removal of tertiary butyl group from Tris-NTA. Glass surfaces were washed with plenty of water dried under N_2 gas stream and stored at 4 °C.

Octapeptide-tubulin interaction study on biotin micropatterned surface: Biotin micropattern surfaces were prepared as previously described method.[5] A flow chamber of around 5 µL was built from one biotin-patterned glass surface and one poly-L-lysine (PLL)-PEG passivated counter glass, separated by two strips of double sticky tape (Tesa). Flow chamber was equilibrated with BRB80 and incubated with *β*-casein for 10 min followed by washing with 20 µL BRB80 for complete removal of unbound *β*-casein. 100 nM neutravidin was flowed into the flow chamber and incubated for 10 min followed by removal of excess neutravidin with 20 µL BRB80. 1 mM biotinylated-octapeptide (HX) in BRB80 was flowed into the flow chamber and incubated for 10 min and washed unbound peptide by 20 µL BRB80. Then, the flow chamber was filled with 18.5 µM tubulin mix (80:20 unlabelled tubulin and Alexa568 tubulin) in BRB80 supplemented with 3 mM GTP, 10 mM MgCl2, and an oxygen scavenger system (50 mM glucose, 1 mg mL^{-1} glucose oxidase, and 0.5 mg mL^{-1} catalase) on an ice cold metal block and placed to the TIRF microscope at 37 °C. Then flow chamber was imaged using an IX-81 total internal reflection fluorescence (TIRF) microscope (Olympus) with a 60x TIRFM objective (Olympus; Hamburg, Germany) and an Andor iXon3 897 Camera. Control experiment was performed, following previous described method without immobilized 'HX' on neutravidin loaded micropatterned surface.

Preparation of AlexaFluro 568 labeled GMP-CPP microtubules: Preparation of Alexa Fluor 568 labeled GMP-CPP microtubules: **1. (A)** Tubulin mix on ice: Alexa Fluor 568 labeled tubulin (0.75 µL, 15 mg/mL, 65% labeling ratio), tubulin (4.75 µL, 20 mg/mL) and BRB80 (44.5 µL; 80 mM PIPES, 1 mM MgCl2, pH adjusted to 6.8 by using KOH solution) were mixed on ice; **(B)** Final mix on ice: GMP-CPP (5 µL, 10 mM), $MgCl_2$ (1 µL, 100 mM), tubulin mix (10 µL) and BRB80 (34 µL) were mixed and incubated for 2 hour at 37 °C. **2.** The final mix was centrifuged for 7 min at 14,000 rpm in a tabletop centrifuge. **3**. The colored pellet was resuspended in 50 µL warm BRB80 at 37 °C. Microtubules were stored at room temperature.

Motor-mediated microtubule transport on unpatterned kinesin and octapeptide immobilized dual functionalized glass surface: A flow chamber of around 5 µL was built from one dual functionalised Ni-loaded un-patterned Tris-NTA-PEG and neutravidin loaded biotin glass and one poly-L-lysine (PLL)-PEG passivated counter glass, separated by two strips of double sticky tape (Tesa). The flow chamber was equilibrated with BRB80 and incubated with β-casein for 5 min and excess β-casein was removed by washing with BRB80. Next, 1 mM Biotin-tagged octapeptide in BRB80 was flowed into the flow chamber and incubated for 10 min. After 10 min excess unbound peptides were washed out with 20 µL BRB80. Next, 50 nM kinesin612-His_{10} in 20 µL BRB80 was flowed into the chamber and incubated for 10 min. After 10 min, unbound motors were washed out with 20 µL BRB80. Now, the flow chamber was allowed to warm up to room temperature and five chamber volumes of preformed GMP-CPP stabilized Alexa568-microtubules in motility buffer (BRB80 containing 3 mM Mg-ATP and an oxygen scavenger system (50 mM glucose, 1 mg/mL glucose oxidase and 0.5 mg/mL catalase) were flowed into the chamber. Microtubules gliding were observed by time-lapse TIRF microscopy at 37 °C. Control experiment was performed maintaining similar experimental procedure as described before without addition of biotinylated octapeptide.

TIRF Microscopy: After the final solution was flowed into the flow chamber, it was placed on a TIRF microscope equipped with an environment box kept at 37 °C. Binding of Alexa568-tubulin and motility of Alexa-568 labeled GMP-CPP seeds were observed by recording time-lapse movies using an IX-81 total internal reflection fluorescence (TIRF) microscope (Olympus) with a 60x/1.45 NA TIRFM objective (Olympus) and an Andor iXon3 897 camera.

Data Analysis: Mean gliding speed of GMP-CPP stabilised microtubules on surfaces with immobilised kinesin in presence and absence of octapeptide ′HX′ were measured from kymographs

MD Simulation: The single peptide was kept at the center of cubic box solvated by 2970 Simple Point Charge (SPC) water model with one Chlorine atom in 4.5 nm cubic box to neutralize the system. Two random coil peptides, separated by 2.0 nm were solvated by 2929 Simple Point Charge (SPC) water model with two Chlorine atom to neutralize the system in 4.5 nm cubic box. GROMACS 4.5.5 package was used for simulation study.[6] Gromos 96 53a6 force field was applied for peptides.[7] Periodic boundary conditions were applied in all three directions. Cut-off radii were set at 0.9 nm for electrostatic interactions and 1.4 nm for Lennard-Jones interactions. Long-range electrostatics interactions were tested using Particle-Mesh Ewald (PME) method.[8] Simulation was performed at time step of 2 fs. The first phase involved the simulating for 500 ps under a constant volume (NVT) ensemble. Protein and non-protein atoms were coupled to separate coupling baths and temperature maintained to 310 K using V-rescale coupling method. Following NVT equilibration, 1 ns of constant-pressure (NPT) equilibration was performed using Parrinello-Rahman coupling method. Relaxation time of 0.1 ps and 1 ps were used for NVT and NPT respectively. Then production run was performed for 200 ns. LINCS algorithm was used to constrain bond lengths.[9]

Docking: Autodock-Vina version 1.1.2 was used for blind docking simulation.[10] 98x60x64 affinity grids were centred on the receptor (1JFF).[11] The 2D interaction plot between Protein-Peptide is plotted with the help of Ligplot.[12]

References:

1. P. Bieling, I. A. Telley, C. Hentrich, J. Piehler and T. Surrey., *Methods Cell Biol.*, 2010, **95**, 555.
2. H. Zheng, D. Blat and M. Fridkin, *J. Neural. Transm.*, 2006, **71**, 163.
3. (a) T. M. Doran, E. A. Anderson, S. E. Latchney, L. A. Opanashuk and B. L. Nilsson, *J. Mol. Biol*., 2012**, 421**, 315. (b) R. V. Ulijn and A. M. Smith, *Chem. Soc. Rev*., 2008, **37**, 664. (c) R. J. Mart, R. D. Osborne, M. M. Stevens and R. V. Ulijn, *Soft Matter*, 2006, **2**, 822. (d) F. Fulop, T. A. Martinek and G. K. Toth, *Chem. Soc. Rev*., 2006, **35**, 323. (e) J. M. Holub and K. Kirshenbaum, *Chem. Soc. Rev*., 2010, **39**, 1325. (f) K. Channon and C. E. MacPhee, *Soft Matter*, 2008, **4**, 647. (g) S. Maiti, K. Das, P. K. Das, *Chem. Commun*., 2013, **49**, 8851.
4. (a) G. Thakur, M. Micic, Y. Yang, W. Li, D. Movia, S. Giordani, H. Zhang and R. M. Leblanc, *Int. J. Alzheimer's Disease*, 2011, doi:10.4061/2011/502386. (b) S. A. Hudson, H. Ecroyd, T. W. Kee, J. A. Carver, *FEBS J*., 2009, **276**, 5960.
5. A. Biswas, A. Saha, B. Jana, P. Kurkute, G. Mondal and S. Ghosh, *ChemBioChem.*,2013, **14**, 689.
6. B. Hess, C. Kutzner, D. van der Spoel and E. Lindahl, *J. Chem. Theor. Comput.*, 2008, **4**, 435.
7. C. Oostenbrink, A. Villa, A. E. Mark and W.F. van Gunsteren, *J. Comput. Chem.*, 2004, **25**, 1656.
8. U. Essmann, L. Perera, M.L. Berkowitz, T. Darden, H. Lee and L.G. Pedersen, *J. Chem. Phys.*, 1995, **103**, 8577.
9. B. Hess, H. Bekker, H. J. C. Berendsen and J. G. E. M. Fraaije, *J. Comput. Chem.*, 1997, **18**, 1463.
10. O. Trott and A. J. Olson, *J. Comput. Chem.*,2010, **31**, 455.
11. J. Löwe, H. Li, K. H. Downing and E. Nogales, *J. Mol. Bio*., 2001, **313**, 1045.
12. R. A. Laskowski and M. B. Swindells., *J. Chem. Inf. Model*, 2011, **51**, 2778.

Supplementary Figures

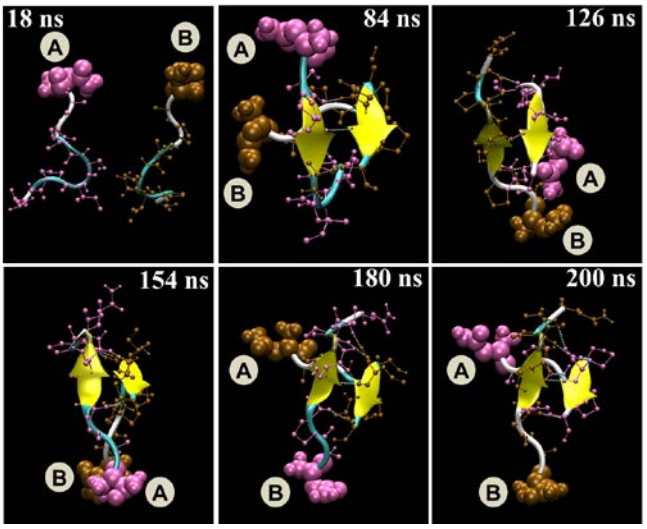

Figure S1. Time lapse images from MD simulation movie of ′X′ reveal that two octapeptides slowly transform from *β*-turn to *β*-sheet rich structure.

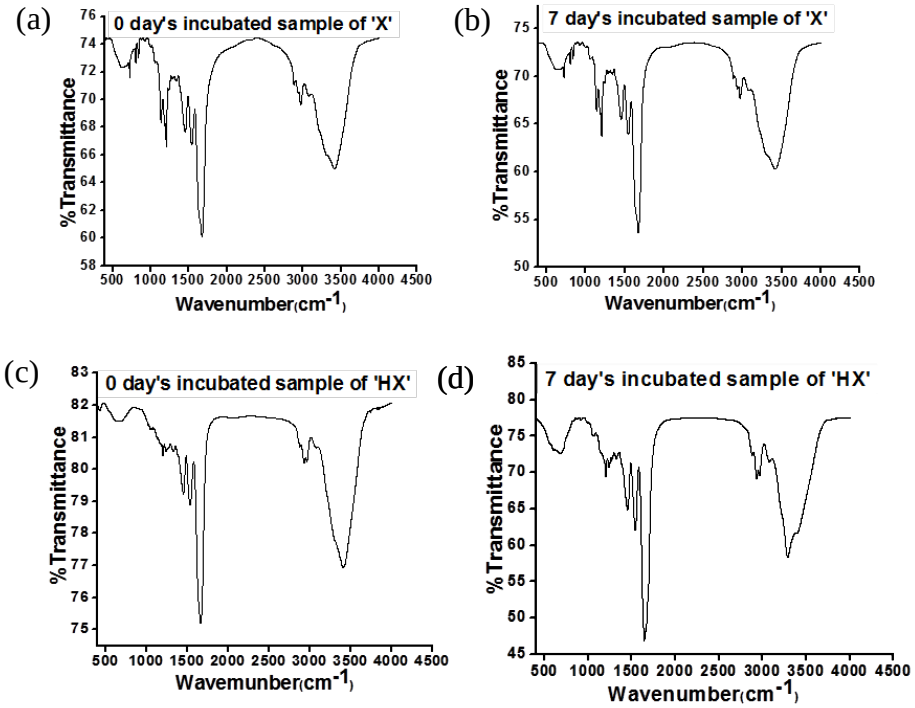

Figure S2. FT-IR Spectra of ′X′ and ′HX′ after 0 (a,c) and 7 days (b,d) incubation.

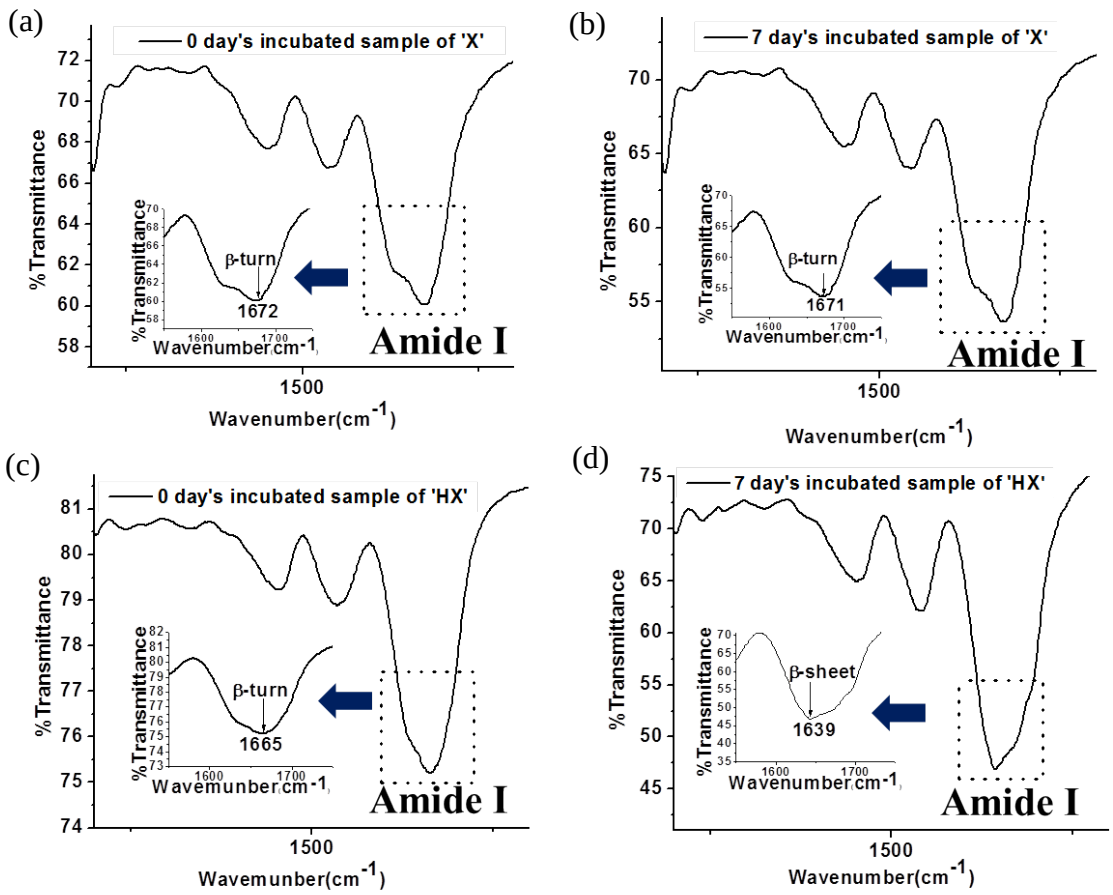

Figure S3. Enlarged view of FT-IR Spectra of ′X′ and ′HX′ after 0 (a,c) and 7 days (b,d) incubation.

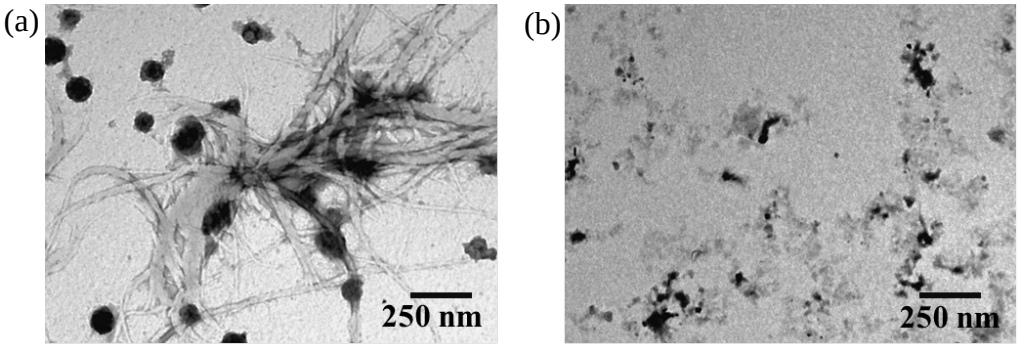

Figure S4. TEM image reveals the inhibition of Aβ42 fibrillation. (a) 100 nM Aβ42 peptide forms fiber after 7 days incubation at 37 ^{o}C in PBS buffer containing 1% ammonium hydroxide. (b) 7 days co-incubated sample of 100 nM Aβ42 peptide and 100 nM octapeptide in PBS buffer at 37 ^{o}C shows no fiber formation.

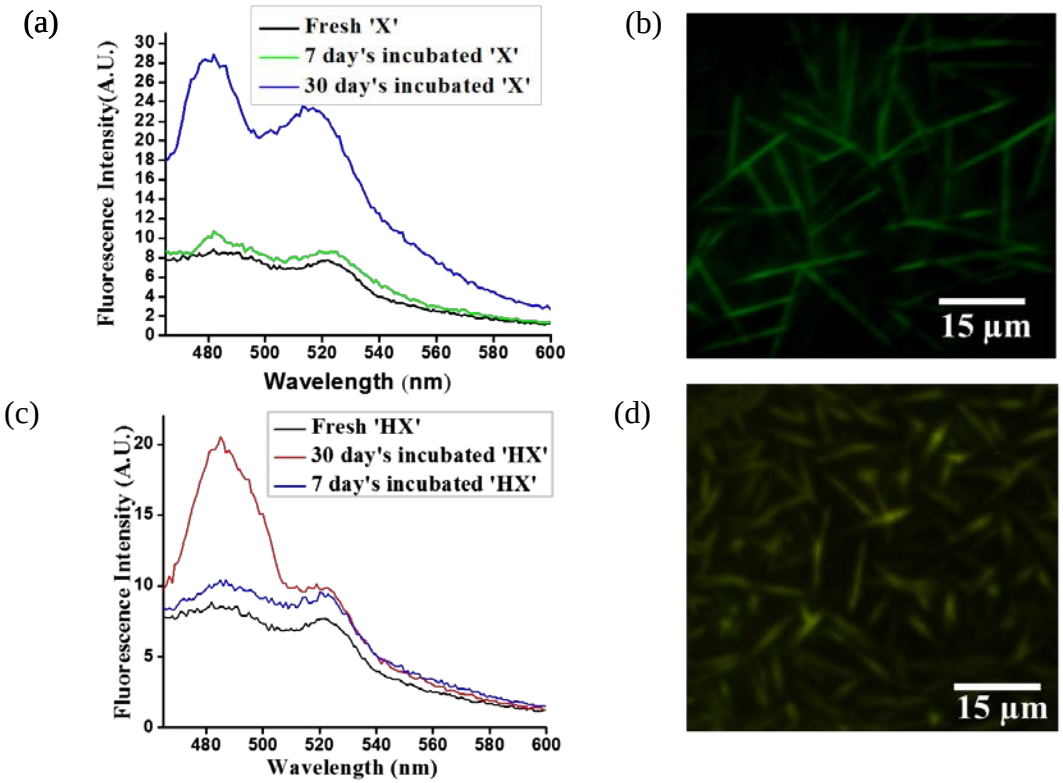

Figure S5. ThT test of ′X′ and ′HX′ self-assembled structure. (a,c) Fluorescence spectra reveal strong fluorescence enhancement upon addition of 30 days incubated ′X′ and ′HX′ solution into ThT solution, which indicate amyloid fibrill like structure. (b,d) Fluorescence microscopic images reveal strong green fluorescence fiber upon addition of ThT solution into 30 days incubated ′X′ and ′HX′ solution, which indicate amyloid fibrill like structure.

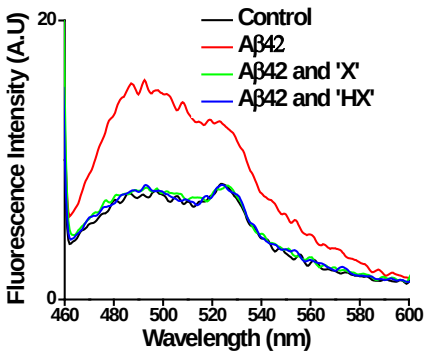

Figure S6: ThT assay of Aβ42 peptide in presence of ′X′ and ′HX′ as well as alone after 7 days incubation.

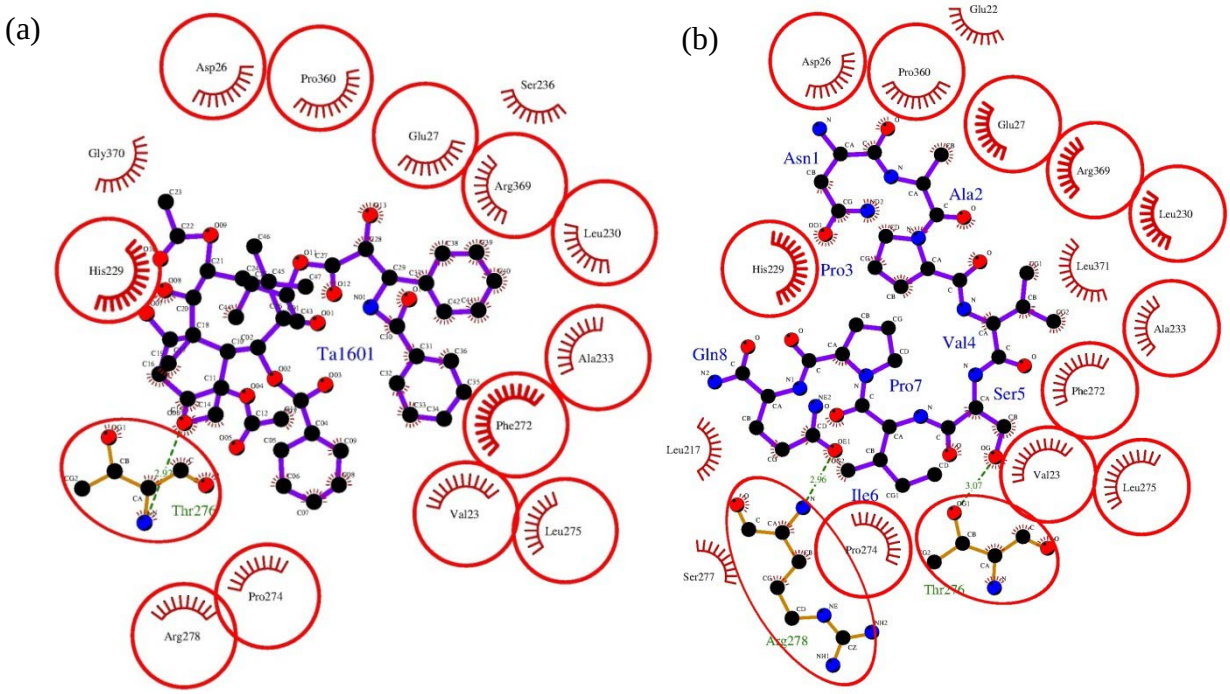

Figure S7. (a) 2D view of binding site structure of taxol with tubulin dimer. (b) 2D view of octapeptide binding site with tubulin dimer with the help of LIGPLOT.

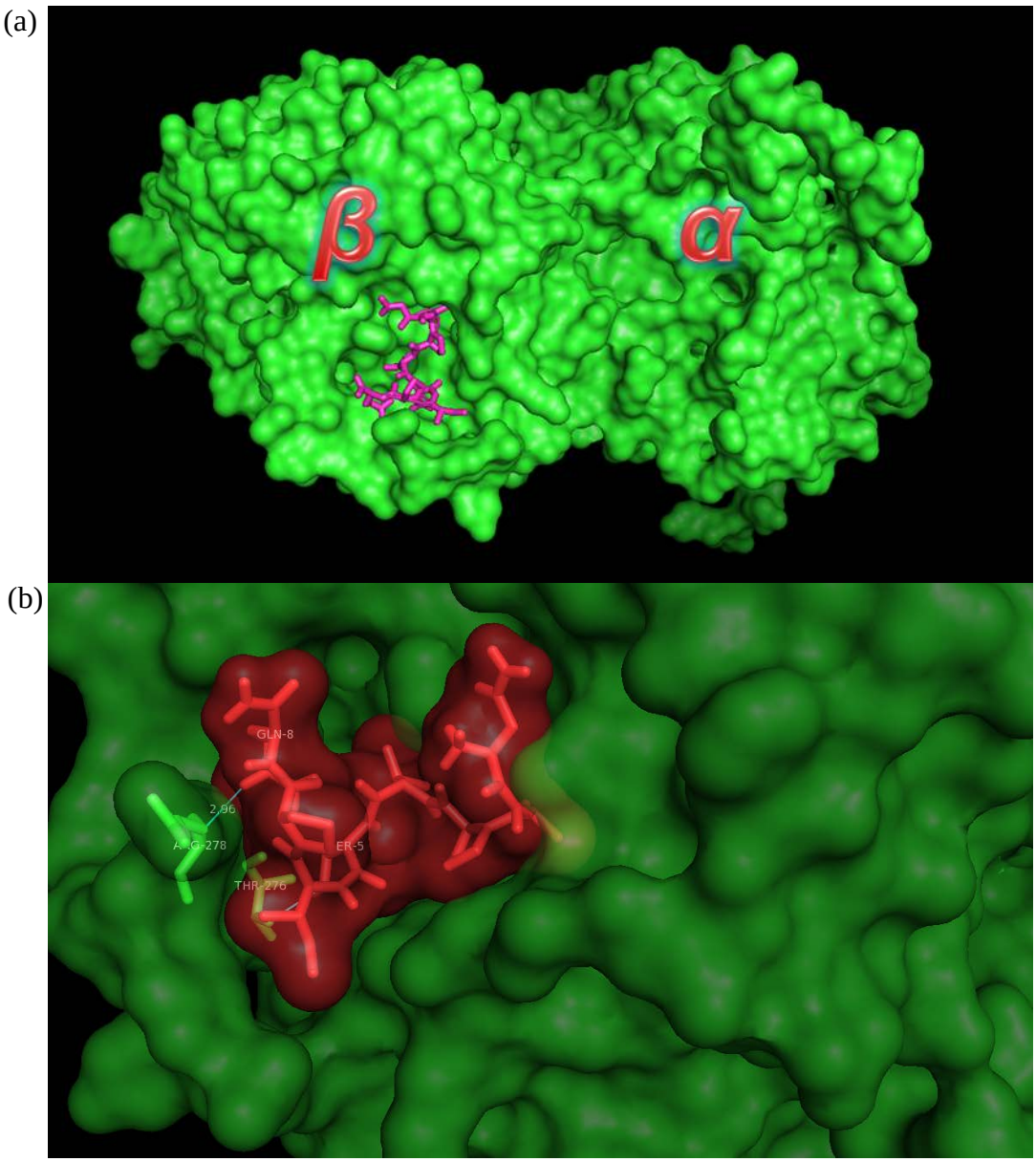

Figure S8. Docking results indicate the binding of the octapeptide with tubulin dimer (a,b).

Supplementary Movie

Movie S1. 200 ns MD Simulation movie of ′X′ reveals that it assembly behaviour switches β-turn to β-sheet rich conformation.

Movie S2. Gliding assay of GMP-CPP Alexa568 microtubules on Tris-NTA and biotin functionalised glass surface in presence of octapeptide ′HX′.

Movie S3. Gliding assay of GMP-CPP Alexa568 microtubules on Tris-NTA and biotin functionalised glass surface in absence of octapeptide ′HX′.

.