Supporting Information for

Modulation of Amyloid Aggregates into Nontoxic Coaggregates by Hydroxyquinoline Appended Polyfluorene

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1. EXPERIMENTAL SECTION

1.1. Materials

All the reagents and chemicals were purchased from Aldrich Chemicals, Merck or Ranbaxy (India) and were used as received. Milli-Q water and HPLC grade solvents were used in all the experiments. Solvents were degassed using three freeze thaw cycles or flushed with nitrogen for at least 1 h prior to use when necessary. β-Amyloid (1-40), human was purchased from G L Biochem Ltd., Shanghai, China.

1.2. Instrumentation

UV–Vis absorption spectra were recorded on a Perkin Elmer Lambda–25 spectrometer. Fluorescence spectra were carried out on a Varian Cary Eclipse Spectrometer. A 10 mm x 10 mm quartz cuvette was used for solution spectra and emission was collected at 90° relative to the excitation beam. FT–IR spectra were recorded on a Perkin Elmer spectrophotometer with samples prepared as KBr pellets. A fresh glass slide was used for every experiment. Deionized water was obtained from Milli–Q system (Millipore). Atomic force microscopy (AFM) was recorded on **Agilent, Model 5500 series** with non-contact mode. The PF-HQ NPs were examined using an ultrahigh resolution transmission electron microscope (TEM; JEM 2100; Jeol, Peabody, MA, USA). DLS and Zeta Potentials were measured by Malvern Zetasizer Nano series Nano-ZS90 instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend^{TM} 600 MHz spectrometer using chloroform-d as solvent.

1.3. Preparation of PF-HQ stock solution

PF-HQ stock solution was prepared at the concentration of 1.0 X 10^{-3} mL^{-1} in 10 mL THF. This stock solution was diluted to desire concentration for each titration in 1mL cuvette.

1.4. Preparation of HEPES buffer solutions

All the experiments like UV-Visible, FT-IR and fluorescence titrations were performed in 10 mM HEPES buffer and pH maintained at 7.4 by using 4M NaOH or 5M HCl solution.

1.5. TFA/HFIP treatment of A*β* (1–40)

A*β* (1–40) was disaggregated using trifluoroacetic acid/1,1,1,3,3,3-hexafluuor-2-propanol (TFA/HFIP) by an established method. 0.1 mg of Aβ (1–40) was added to a 2.5 mL eppendorf tube and dissolved in TFA to obtain a homogeneous solution free of aggregates. TFA was then evaporated using argon gas. Any left-over TFA was further removed by adding HFIP followed by evaporation using argon gas flow to obtain a film like material. This process was repeated

twice. To the eppendorf tube, 2.5 mL of HEPES (10 mM, pH 7.4) was added followed by sonication and vortexing to obtain a final concentration of 1.6 X 10^{-4} M. Fibril formation was monitored using a ThT binding assay.

1.6. Preparation of Aβ1–40 aggregates and ThT Binding Assay

For the preparation of amyloid peptide aggregates, after the TFA/HFIP treatment for amyloid peptide, the Aβ1–40 (5 μM) was initially incubated with ThT (20 μM) at 37 °C for 72 hours (pH 7.4 in HEPES) with steady agitation. Further, dose dependent aggregation of Aβ1–40 amyloid fibrils were monitored with different time incubations by monitoring ThT (20 μM) fluorescent enhancement peak at λ_{em} 484 nm (λ_{ex} 440 nm).

1.7. Preparation of PF-HQ

PF-HQ (10 μM) polymer was regularly injected into molecular biology grade water with vigorous stirring at room temperature, using a syringe. After the injection of PF-HQ, the solution was filtered by membrane filter with 0.2 μm pore size. Then the collected PF-HQ was used for other studies.

1.8. PF-HQ sample preparation for experiments.

5 μM, 10 μM and 20 μM of PF-HQ NPs were maintained in 1 mL of 10 mM HEPES buffer solution (pH 7.4) at room temperature. The mixtures of this solution were used for all the experiments like UV-Visible and fluorescence titrations.

1.9. Modulating experiment for Aβ1–40

The red shifted emission spectra at 520 nm from 509 nm for PF-HQ (5 μM, 10 μM and 20 μM) with Aβ1-40 (10 μM) were measured while exciting at 355 nm. Aβ1-40 (10 μM) were mixed with different molar ratio of PF-HQ (1:0.5, 1:1 and 1:2) in 1 mL of 10 mM HEPES buffer

solution (pH 7.4). Then, fluorescence spectra were monitored for all the samples in different interval of incubation time from 0-25 days at 37 °C in water bath.

1.10. Inhibition of Aβ1–40 fibrils formation monitored by ThT assay experiment

ThT emission changes at 484 nm were measured for inhibition of Aβ1–40 fibrils formation for the mixture of PF-HQ with Aβ1-40 monomer in presence of ThT while exciting at 440 nm. Aβ1-40 monomer (10 μM) was mixed with PF-HQ (5 μM) in 1 mL of 10 mM HEPES buffer solution (pH 7.4) in presence of Thioflavin T (20 μM). Then, fluorescence spectra were monitored in different interval of incubation time from 0-25 days at 37 °C in water bath.

1.11. Sample preparation for AFM images

To monitor the morphology, pristine solutions of Aβ1-40 and PF-HQ and PF-HQ coincubated Aβ1-40 were dissolved in 10mM HEPES (pH 7.4) and diluted 10 times and 5 μL of the sample was mounted onto the freshly cleaned glass slide and dried at room temperature for overnight. The data were recorded on an **Agilent, Model 5500 series** AFM with non-contact tapping mode and analyzed with WSxM 5.0 Develop 8.0 software.

1.12. Sample preparation for FT-IR spectra

FT-IR spectra of Aβ1-40 solutions incubated with or without modulators were recorded on a Perkin Elmer 100 series FT-IR Spectrophotometer. 10 μL of each sample was mounted onto the freshly cleaned glass slide and dried at room temperature for overnight and samples prepared as KBr pellets before recording.

1.13. Sample preparation for DLS and Zeta Potential

Zeta potential and DLS of pristine Aβ1-40 and PF-HQ samples and PF-HQ coincubated Aβ1-40 were determined with a Malvern Zetasizer Nano-ZS. A suspension of above incubated solutions of different concentrations were dissolved in 10mM HEPES (pH 7.4) and transferred into 3 mL

zeta potential cuvette (DTS1060, Malvern) and DLS cuvette (DTS1060, Malvern). The data were collected and analyzed with Zetasizer software (version 7.11, Malvern).

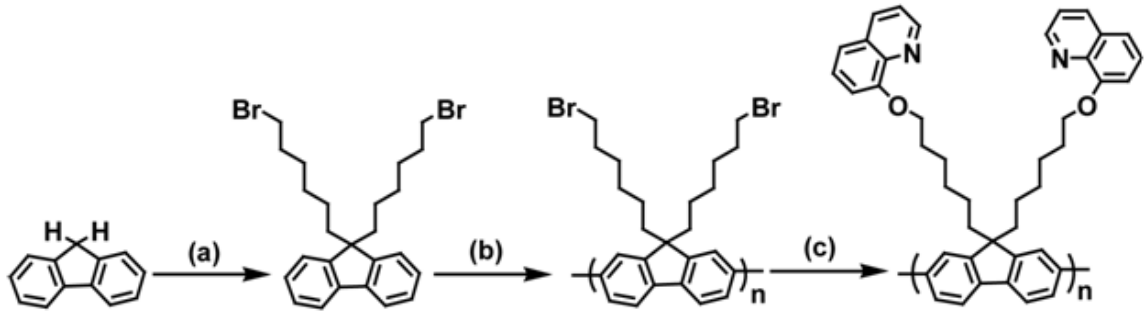
1.14. Sample preparation for Circular Dichroism (CD) Spectroscopy

CD Spectra of pristine Aβ1-40 and PF-HQ samples and PF-HQ coincubated Aβ1-40 were determined in a JASCO J-815 Spectrometer (JASCO Co., Tokyo, Japan), using a quartz cuvette (1 mm path length). The concentration of Aβ1-40 solution for CD analysis was 10 μM and the Aβ40 solutions incubated without or with modulators were prepared at 0.5, 1 and 2 equiv (we define equiv as the molar ratio of modulators to Aβ1-40 (10 μM)). All the samples were incubated at 37 °C in 10 mM HEPES buffer solution with a continuous agitation speed of 100 rpm for different time intervals before analyses. Spectra were calibrated by subtracting the buffer or sample solution baseline.

2. Synthesis of PF-HQ

2.1. 9, 9-Bis-(6-bromohexyl)-9*H*-fluorene : Fluorene (2 g, 12.032mmol), 50% aq. NaOH and a catalytic amount of tetra-butyl ammonium Iodide (TBAI) (0.888 g, 2.406 mmol) were added to a 100 mL round bottom flask and then degassed 3 times by applying freeze-thaw cycles. 1,6-dibromohexane (20.632 g, 84.227mmol) was added through a syringe (degassed) and the mixture was stirred continuously for 6 hours at 70 °C. The reaction mixture was cooled to room temperature and extracted with chloroform. The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and excess 1, 6-dibromohexane was removed through shortpath distillation and the crude was purified using Column Chromatography over a pad of silica gel using hexane as an eluent to get the desired doubly alkylated product as yellow oil (4.8 g, 81%).^{1}H NMR (600MHz, $CDCl_3$), δ (ppm): 7.7(m, 2H), 7.32(m, 6H), 3.26(t, 4H), 1.96(m, 4H), 1.64(m, 4H), 1.18(m, 4H), 1.07(m, 4H), 0.62(m,

4H);^{13}C NMR (150MHz, $CDCl_3$), δ (ppm): 150.5, 141.3, 127.3, 127.1, 123, 119.9, 55.1, 40.4, 34.1, 32.8, 29.3, 28, 23.7.



(a) 1,6-Dibromohexane, 50% aq. NaOH, TBAI, (b) $FeCl_3$, Nitrobenzene, (c) 8-Hydroxyquinoline, K_2CO_3, DMF.

Figure S1. Synthetic outline of PF-HQ.

2.2. Poly 9, 9-Bis-(6-bromohexyl)-9*H*-fluorene (PF-Br) : Anhydrous ferric chloride ($FeCl_3$) (1.30g, 8.12 mmol) and 9, 9-Bis-(6-bromohexyl)-9H-fluorene (2.0 g, 4.06 mmol) were dissolved in 15 mL nitrobenzene in a 100 mL three-necked round-bottom flask equipped with a nitrogen inlet. The reaction mixture was stirred at room temperature for 36 hours, followed by precipitation from methanol. The resulting polymer, poly 9, 9-Bis-(6-bromohexyl)-9H-fluorene was dried under reduced vacuum to obtain 1.3 g (65%) as dark brown powder.

^{1}H NMR (600MHz, $CDCl_3$), δ (ppm): 7.74(bp), 7.31(bp), 3.29(bp), 2.1(bp), 1.64(bp), 1.25(bp), 0.88(bp); bp: broad peak.

2.3. Poly8,8'-(((9*H*-fluorene-9,9-diyl)bis(hexane-6,1-diyl))bis(oxy))diquinoline (PF-HQ): To synthesize PF-HQ, PF-Br (0.1 g, 0.203 mmol), 8-hydroxyquinoline (0.14 g, 1.01 mmol) and K_2CO_3 (0.28 g, 2.03mmol) were dissolved in 20 mL dry DMF and refluxed for 48 hours in a 50 mL round bottom flask. Then the solvent was removed through a rotavapor under reduced

vacuum and dried under a high vacuum, followed by precipitated from methanol and acetone to obtain the final polymer (57 mg, ~60%). ^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm): 0.8–1.0(–CH2), 1.2–1.3(–CH2–), 1.4–1.6(–CH2–), 1.6–1.8 (–CH2–), 2–2.1(–CH2–), 4.0 (–CH2-O–), 6.9–7.1(ArH), 7.2–7.3(ArH), 7.7–8.1(ArH), 8.9(ArH). ^{13}C NMR (150 MHz, CDCl3), δ (ppm): 155.0, 149.4,143.0, 140.6, 136.0, 135.4, 130.0, 129.6, 126.8, 124.2, 123.7, 121.6, 119.5, 116.1, 114.3, 108.8, 40.6, 34.5, 31.8, 29.4, 25.9, 24.2, 24.1, 22.9, 14.3, 11.6. ArH : aromatic hydrogen.

FT-IR (KBr pelettes), wavenumber (cm^{-1}): 3432.09, 2930.65, 2853.24, 1724.16, 1630.77.

The weight average molecular weight (M_w) of PF-HQ was found to be 40.2 kDa with polydispersity 1.8.

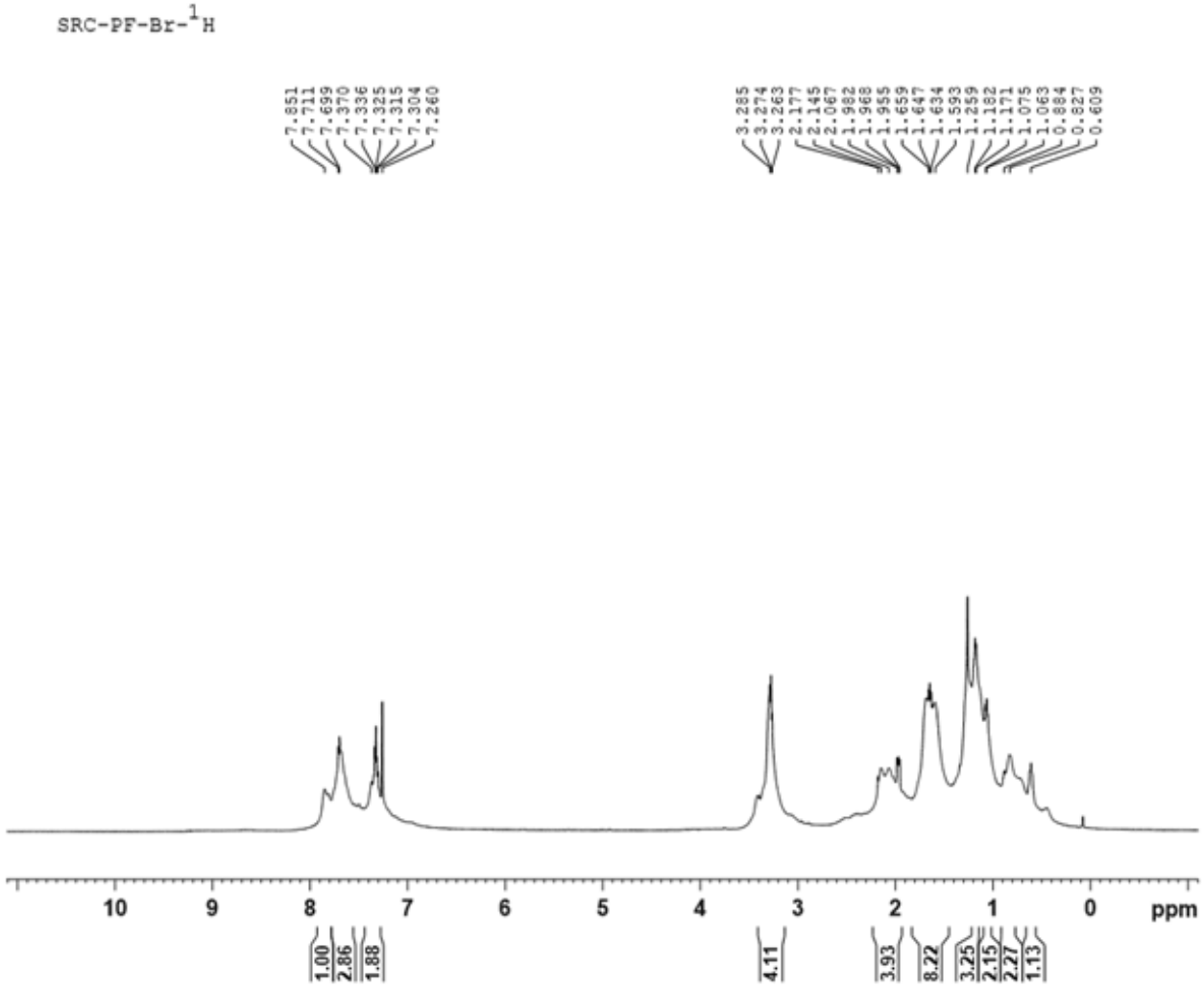


Figure S2. ^{1}H NMR of PF-Br

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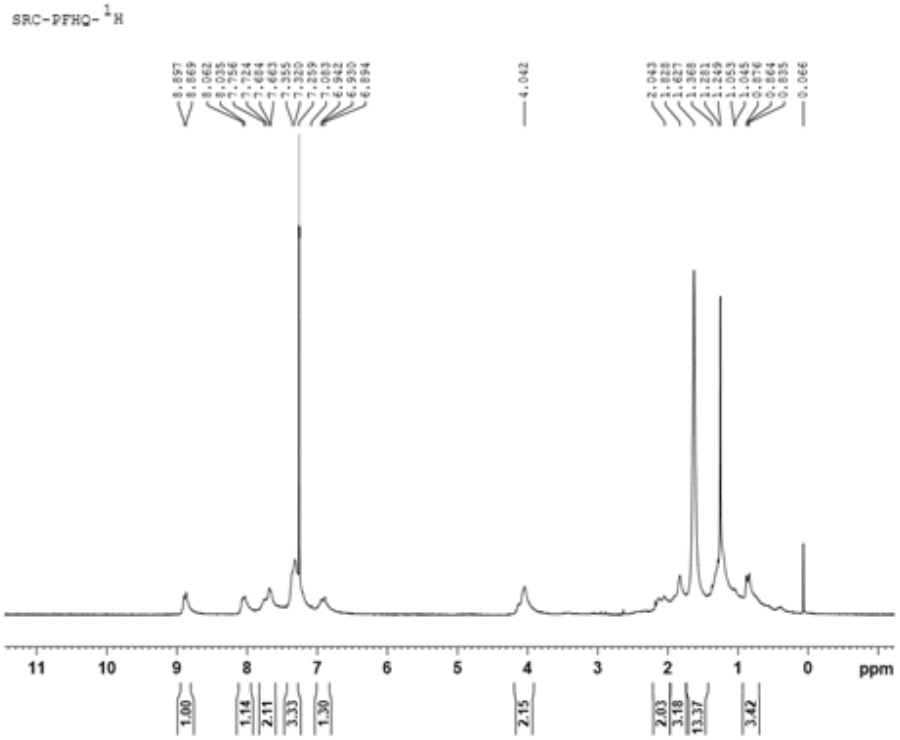


Figure S3. ^{1}H NMR of PF-HQ.

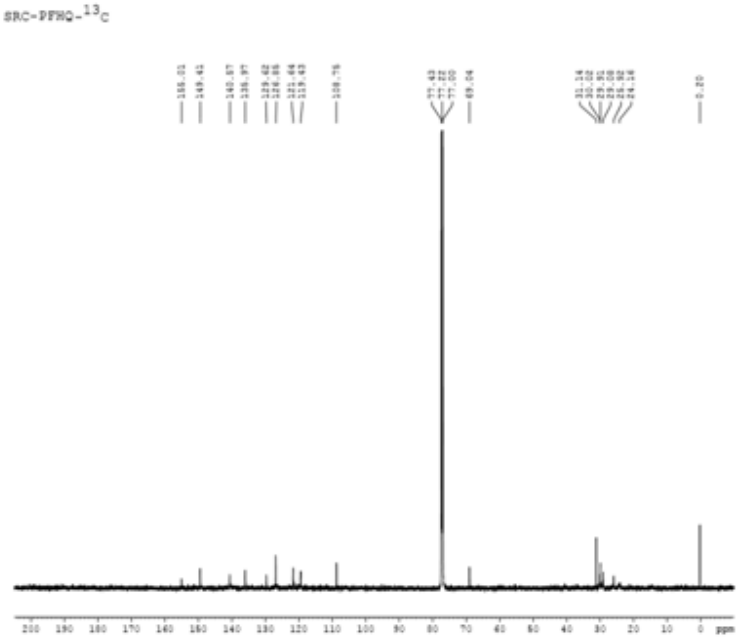


Figure S4. ^{13}C NMR of PF-HQ.

3. Aβ1-40 control studies

Thioflavin T (ThT) is generally utilized for the recognizable proof and measurement of amyloid fibrils and in addition for the investigation of fibrillization kinetics of Aβ utilizing fluorescence spectroscopy (Figure S5).[1,2] The observed emission at ~484 nm is thought to be specifically corresponding to the amount of amyloid fibrils present, and in this manner, the kinetics of fibril formation can be trailed by measuring the time-dependent increment in fluorescence. Then again, a decrease in the ThT fluorescence is frequently taken as a sign of restraint of the macromolecular amyloid self-assembly processes.[3]

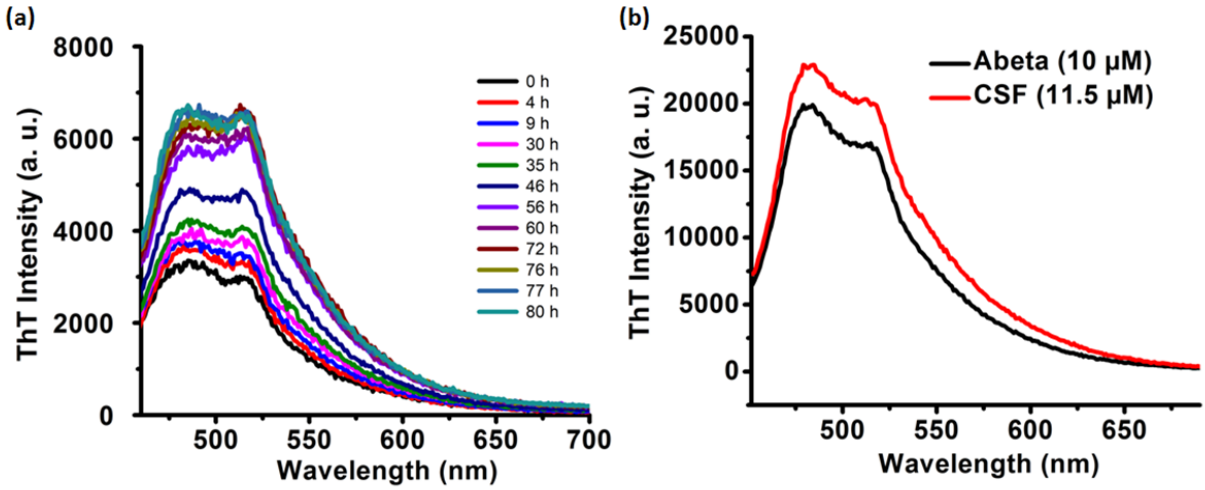


Figure S5. (a) ThT fluorescence profile to monitor Aβ1-40 (10 μM) fibrillation kinetics in absence of modulator (PF-HQ). (b) Similar enhancements were observed in presence of fibrillar aggregates in case of CSF (red line) and Aβ1-40 (black line). Samples were incubated in 10 mM HEPES buffer (pH 7.4) at 37^{o}C.

The AFM images show long unbranched mature fibrillar Aβ1-40 aggregates (Figure S7). For control, Aβ1-40 was incubated to aggregate and the final fibril formation was confirmed using CD and FT-IR studies. CD spectrum of preformed Aβ1-40 fibrils show a positive peak around 196 nm and a negative peak around 202 nm (Figure S8) confirming the β-sheet conformation of Aβ1-40. However, in presence of the polymer (PF-HQ), CD spectrum shows a negative peak around 193 nm and a positive peak around 199 nm indicating the conformational conversion of Aβ1-40. Analogously, the FT-IR spectrum of preformed Aβ1-40 fibrils shows a major band at 1631 cm^{-1} which indicates the parallel β-sheet conformation of Aβ1-40 aggregates (Figure. S9). The parallel β-sheet conformation of Aβ1-40 changed in presence of the inhibitory modulator (PF-HQ) and a new peak emerges at around 1651 cm^{-1} due to the formation of polymer-protein coaggregates.

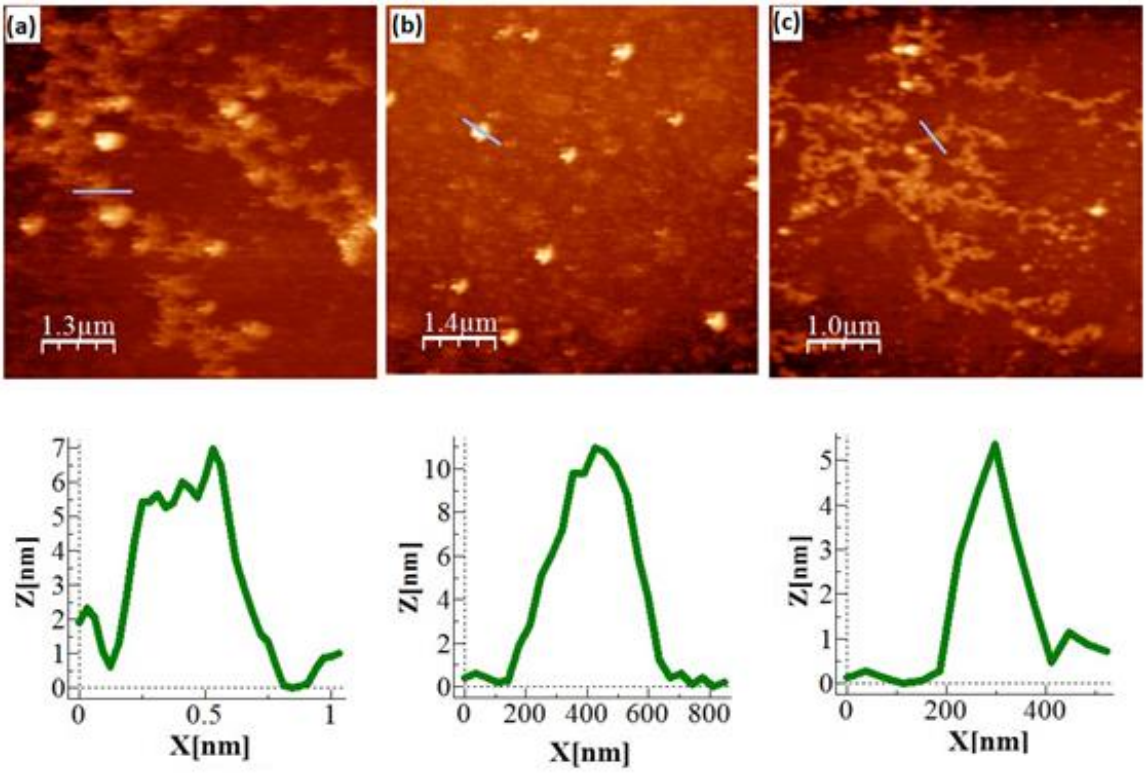


Figure S6. AFM images of Aβ1-40 fibrils. (a) 2D image of pre-mature Aβ1-40 fibril, (b) 2D image of Aβ1-40 oligomers. (c) 2D image of Aβ1-40 fibrils in CSF. Image size is 5 x 5 μM^2. Samples were incubated in 10 mM HEPES buffer (pH 7.4) at 37 ^oC.

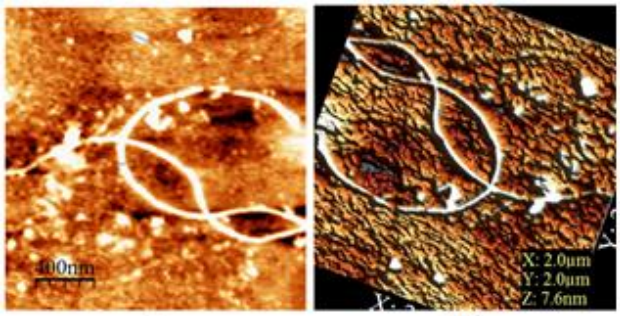
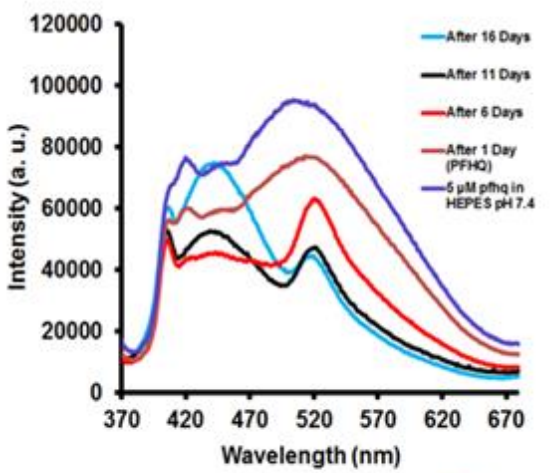


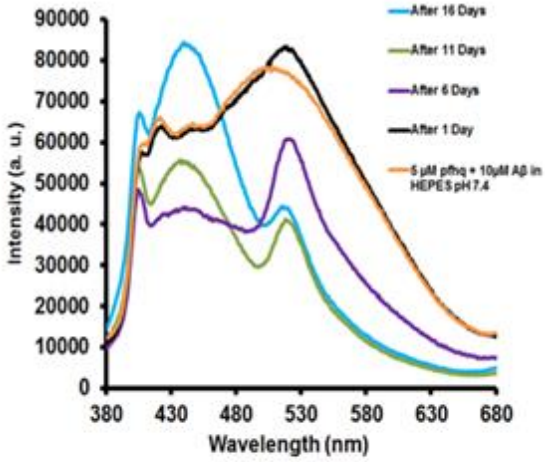
Figure S7. AFM images of Aβ1-40 fibrils. (a) 2D image of a long mature Aβ1-40 fibril, (b) 3D image of Aβ1-40 fibril. Image size is 2 x 2 μM^2. Samples were incubated in 10 mM HEPES buffer (pH 7.4) at 37 ^oC.

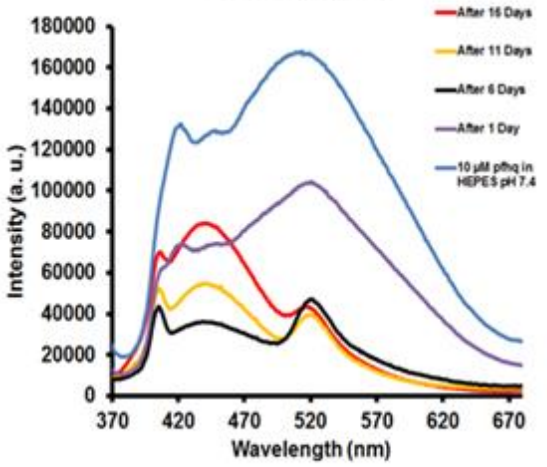
4. Fluorescence Measurements

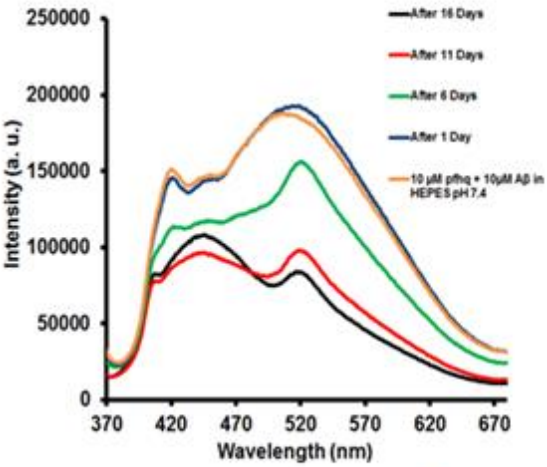
5 μM PF-HQ emits at 504 nm in HEPES buffer (pH 7.4) on exciting at 355 nm while 10 μM PF-HQ and 20 μM PF-HQ emit at 510 nm in HEPES buffer (pH 7.4) on exciting at 355 nm. After 0-6 days of incubation at 37 ^oC, we observed a marked decrease in fluorescence intensity along with a red shift of 6-13 nm in all the control spectrum of PF-HQ. On further incubation from 7-16 days, a new peak appears at 438 nm with continuous increment in its intensity. When we added 10 μM Aβ1-40 to 5 μM PF-HQ, we observed emission exactly at 504 nm along with a slight decrease in intensity compared to the spectrum seen for 5 μM PF-HQ only. This decrease in intensity may attribute to the formation of disordered coaggregate of Aβ1-40 and PF-HQ. After 24 hours of incubation, a slight increase in fluorescence intensity compared to the initial

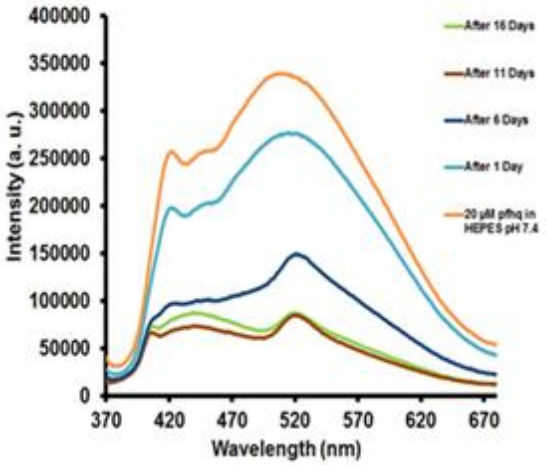
intensity along with a red shift of 12 nm (new peak at 516 nm) was observed. After 11 days of incubation, a new peak at 438 nm predominates and a small hump at 518 nm was seen.











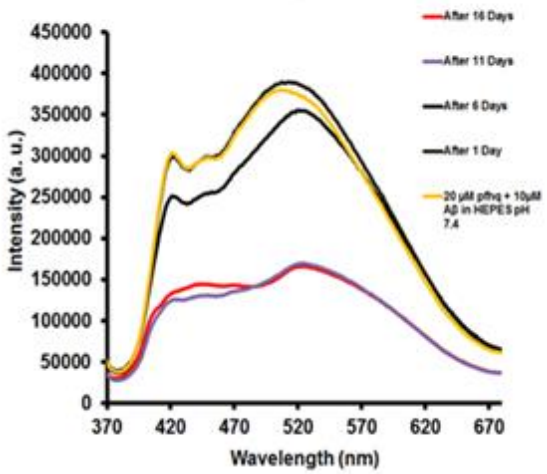


Figure S8. Fluorescence profile (Intensity vs Wavelength) of PF-HQ alone and PF-HQ coincubated Aβ1-40 over a period of 16 days. Samples were incubated in 10 mM HEPES buffer (pH 7.4) at 37 ^{o}C. Excitation wavelength: 355 nm.

Upon separate addition of 10 μM of Aβ1-40 to 10 μM PF-HQ and 20 μM PF-HQ solutions, a blue shift of 6 nm was observed in both cases. Again initial peak arises at 504 nm like in case of 1:0.5 molar ratio of Aβ1-40 to PF-HQ. After 24 hours of incubation, peak shifts at 514 nm with a slight increase in fluorescence intensity compared to the initial intensity. After 1-11 days of incubation, fluorescence spectrum of 1:1 and 1:2 molar ratio of Aβ1-40 to PF-HQ resemble the spectrum seen for 10 μM PF-HQ and 20 μM PF-HQ except an increase in intensity was observed in case of coincubated solutions. In case of 1 equiv of PF-HQ incubated Aβ1-40, a new peak arises at 440 nm along with a hump at 518 nm after 11 days of incubation while in case of 2 equiv of PF-HQ incubated Aβ1-40, peak appears at 438 nm predominates after 20 days of incubation. Higher concentration of polymer may lead to dominant attractive hydrophobic interaction among itself rather than the polymer-protein interaction.

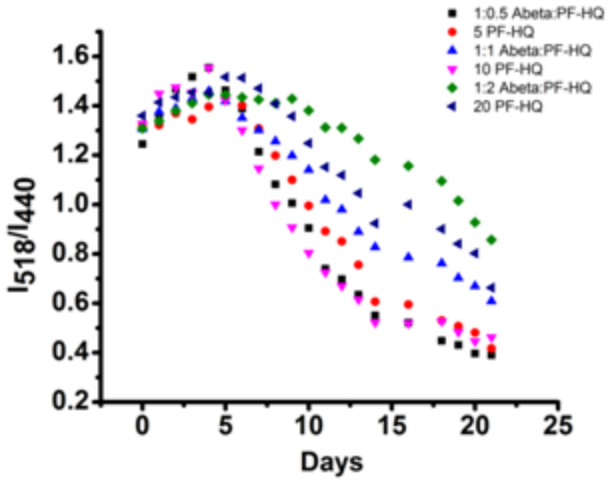


Figure S9. Fluorescence profile (I_{518}/I_{440} vs Days) of PF-HQ alone and PF-HQ coincubated Aβ1-40. Samples were incubated in 10 mM HEPES buffer (pH 7.4) at 37 ^{o}C. Excitation wavelength: 355 nm.

5. MTT Assay

5.1. Methods

Cell culture: U-87 MG cells is a glioblastoma, astrocytoma cell line derived from human malignant gliomas. U-87 MG cells were cultured in complete growth media, Dulbecco's Modified Eagle Medium (DMEM, HiMedia) with 10% fetal bovine serum (Gibco) and antibiotics (Anti-Anti, Gibco) at 37°C in 5% CO2 incubator. Cell line used in this study were gifted by National centre for cell science (NCCS), Pune.

5.2. Toxicity of PF-HQ alone

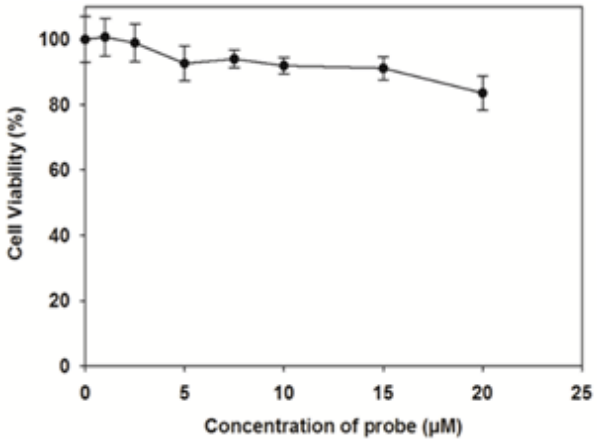


Figure S10. Toxicity of PF-HQ alone toward U-87 MG cells. The cell viability of U-87 MG cells in THF is taken to be 100%. Error bar corresponds to standard deviations of seven sets of experiments.

U-87 MG cells were seeded into 96-well plates at an initial seeding density of 10,000 cells/well in 100 μl medium. The cells were cultured for 24 h at 37°C in 5% CO_2. Then, cells of each well were treated with PF-HQ in a concentration range 0-20 μM in 100 μl serum free media. After incubation for 48 h, the 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (10 μl, 5 mg/mL in PBS) was added to each well. Plate is further incubated for 3 h at 37°C in 5% CO_2, wrapped in aluminum foil. After incubation, MTT-containing medium was replaced by 100 μl Dimethyl Sulfoxide (DMSO) to solubilize MTT-formazan crystals. After incubation for 5 min at 37°C, absorbance was measured at 570 nm and reference reading at 690 nm was recorded in ELISA microplate reader (Infinite 200 PRO, TECAN). Each of the samples was repeated with 7 replicates. The results were normalized by setting the cell viability of U-87 MG cells in tetrahydrofluran (THF) control to be 100%.

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