A High Affinity Red Fluorescence and Colorimetric Probe for Amyloid β Aggregates

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A major challenge in the Alzheimer’s disease (AD) is its timely diagnosis. Amyloid β (Aβ) aggregates have been proposed as the most viable biomarker for the diagnosis of AD. Here, we demonstrate hemicyanine-based benzothiazole-coumarin (TC) as a potential probe for the detection of highly toxic Aβ₄₂ aggregates through switch-on, enhanced (~30 fold) red fluorescence (Eₘₐₓ = 654 nm) and characteristic colorimetric (light red to purple) optical outputs. Interestingly, TC exhibits selectivity towards Aβ₄₂ fibrils compared to other abnormal protein aggregates. TC probe show nanomolar binding affinity (Kₐ = 1.72 × 10⁷ M⁻¹) towards Aβ₄₂ aggregates and also displace ThT bound to Aβ₄₂ fibrils due to its high binding affinity. The Aβ₄₂ fibril-specific red-shift in the absorption spectra of TC responsible for the observed colorimetric optical output has been attributed to micro-environment change around the probe from hydrophilic-like to hydrophobic-like nature. The binding site, binding energy and changes in optical properties observed for TC upon interaction with Aβ₄₂ fibrils have been further validated by molecular docking and time dependent density functional theory studies.

The misfolding driven aggregation process of Aβ peptides in the brain is one of the main causes of Alzheimer’s disease (AD)¹⁻³. The neurodegeneration and subsequent progressive deterioration in cognitive ability are hallmark symptoms of this incurable syndrome. The Aβ₁₄₂ peptide with 42 amino acids has been shown to be highly susceptible to aggregation and toxic behavior among all the Aβ peptides (36–43)³⁻⁵. The aggregation process of Aβ peptides leads to formation of polymorphic oligomers, protofibrils, and fibrils which individually display a range of cellular toxicities⁶. Initially, Aβ fibrils were considered the neurotoxic form and causative agent of AD, whereas research in the last decade has revealed that oligomers are the most toxic form of Aβ causing oxidative stress, interacting with signaling receptors, disturbing metal homeostasis and disrupting neuronal cell membrane¹⁷. Membrane disruption is one of the major pathway of toxicity induced by Aβ oligomers⁸. Ramamoorthy et al. have recently shown that Aβ exhibit two-step mechanism for membrane disruption, i) Aβ interacts with gangliosides present on the cell membrane to form ion channel-like pores and ii) Aβ fibrillization itself induce membrane fragmentation of lipid bilayer⁹. Aβ₁₄₂ aggregates are an attractive biomarker to target for diagnosis and therapeutics of AD. One of the major problems in the diagnosis of AD is the lack of effective methods for the selective detection of Aβ₁₄₂ aggregates. While diagnosis of AD is traditionally based on behavioral tests or cognition in patients, several imaging technologies such as positron emission tomography (PET)¹⁰, magnetic resonance imaging (MRI)¹¹, and single-photon emission computed tomography (SPECT)¹² have been developed for the detection of Aβ₁₄₂ aggregates. However, these technologies are still limited by several obstacles, like long data acquisition time, radioactive exposure, poor resolution and need of expensive equipment. Optical imaging using fluorescence and colorimetric probes has emerged as a potential alternative technique as it offers real-time, non-radioactive, high-resolution imaging for inexpensive diagnostics and screening of drugs for AD¹³⁻¹⁵. Thioflavin T (ThT) is the most extensively used fluorescence probe for the in vitro detection and staining of Aβ₁₄₂ fibrillar aggregates however, it suffers from poor selectivity and often leads to false detection¹⁶⁻¹⁷. In the past few years, derivatives of oxazine¹⁸, BODIPY¹⁹, curcumin²⁰, styryl²¹ fluorescein²² and benzothiazole²³ have been developed and used as fluorescence probes for Aβ₁₄₂ fibrillar aggregates, these probe lack selectivity for Aβ₁₄₂ fibrils over other peptide/protein based aggregates.

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An ideal fluorescence probe must exhibit certain characteristic properties to be used as a diagnostic probe for Aβ42 fibrillar aggregates in AD viz., i) high specificity and strong binding affinity, ii) emission in the optical window of 500–750 nm with a large Stokes shift, iii) switch-on fluorescence change upon binding with Aβ42 fibrillar aggregates, and iv) ability to rapidly cross the blood brain barrier (BBB). Further, mixed dementia is another state in which abnormal characteristics of more than one type of dementia occur simultaneously and in such cases, determining the specific type of neurodegenerative disorder in the patient is very crucial. Therefore, there is an urgent need for developing probes which could selectively differentiate toxic aggregates responsible for specific neurodegenerative disease. Unfortunately, there is lack of studies on probes that selectively differentiate plaques responsible for any specific disorder. We lack fluorescence probes which selectivity binds to specific aggregates, as most of them fluoresce upon binding to forced or artificially formed protein aggregates generally observed in all kinds of dementia. Recently, J. Yang et al. reported an amino naphthalene 2-cyanoacrylate based fluorescence probe, which discriminates between Aβ and Prion plaques by means of differential mode of binding attributed to microenvironments in the binding pockets. However, still there is a need for many more probes which can selectively differentiate other important neurodegenerative disorders. Oligomers being the most toxic form of Aβ causing neuronal death in AD, much efforts are devoted towards studying its structure and designing probes for detection of oligomers. Recently, Knowles et al. revealed that the formation of Aβ oligomers depends on the amount of both Aβ monomers and Aβ fibrils. Initially, Aβ aggregates formed through primary nucleation where Aβ monomers self-assemble to fibrils through oligomers as intermediate state. Once a certain concentration of Aβ fibrils is reached they act as secondary nucleation site and initiate the formation of Aβ oligomers on their surface this phenomenon is called as secondary nucleation. Therefore designing inhibitors and probes for both Aβ fibrils and oligomers are essential for treating AD and studying its progression. Colorimetric detection of Aβ fibrillar aggregates using antibodies has been demonstrated, but this technique is complicated and expensive. With this background, the need for developing selective fluorometric and colorimetric probes based on simple organic molecules which are easy to handle and offer quick detection is strongly indicated. In this context, we report a hemicyanine derivative as a high affinity, selective, switch-on red fluorescence and colorimetric probe TC for Aβ42 fibrillar aggregates. TC exhibits better detection properties over previously reported fluorescent probes (Table S1).

Results and Discussion
ThT has been extensively used to stain Aβ42 fibrillar aggregates for the past few decades. This probe mainly consists of electron donating (N,N-dimethylaniline) and electron withdrawing (benzothiazole) moieties. The benzothiazole group is known to play a crucial role in the interaction of ThT on the surface of Aβ42 fibrillar aggregates. The major drawbacks of ThT and many other probes involves lack of selectivity and low affinity, which encouraged us to search for a new, more effective fluorescence probe for Aβ42 fibrillar aggregates based on the benzothiazole platform, with high selectivity and affinity. We chose to investigate the hemicyanine-based (benzothiazole-coumarin (TC) conjugate as a ‘fluorescence-ready’ probe for Aβ42 fibrillar aggregates (Fig. 1). A benzothiazole conjugate with hydrophobic pyrene chromophore (TP) was also included in our studies (Fig. S1). These compounds are recently reported by our team and discovered TC as an effective switch-on red fluorescence probe for DNA containing AT sequences. To our surprise, TC with benzothiazole and coumarin moieties was found to exhibit highly enhanced fluorescence with superior selectivity and sensitivity for Aβ42 aggregates with higher affinity compared to DNA. Furthermore, the TC and TP probes with molecular weights in the optimum range of ~350–550 Da, and possesses appropriate log P values and number of hydrogen bond donors and acceptors (Fig. S1).

Initially we studied the molecular interactions of TC and TP in the absence and presence of Aβ42 aggregates through the absorption and emission measurements in PBS buffer (10 mM, pH = 7.4). Mature Aβ42 fibrillar aggregates were prepared following the procedure reported in the literature (Supplementary Information). TC and TP showed absorption bands at 537 nm and 460 nm, respectively, and very weak emissions at 638 nm and 623 nm, respectively, in the absence of Aβ42 fibrillar aggregates (Figs 1b and S1). In the presence of Aβ42 fibrillar aggregates (10 μM), TC (2 μM) showed a remarkable increase in the absorption maxima (hyperchromicity) with an enormous bathochromic shift (Δλ_max ≈ 59 nm) relating to solution color change from pale pink to purple (Fig. 2). To elucidate the observed spectral changes of TC, we carried out concentration-dependent studies of Aβ42 fibrillar aggregates against a fixed concentration of TC (2 μM). Initially, TC exhibited a decrease in absorption intensity in the concentration range 0–1 μM of Aβ42 fibrillar aggregates. In addition, a shoulder band was observed for 0.8 μM of Aβ42 at 595 nm. Further, with increasing concentration of Aβ42 fibrillar aggregates (1–10 μM) the shoulder band at 595 nm became more prominent with strong absorption (Fig. 2a). The bathochromic shift in the absorption band of TC, in the presence of Aβ42 fibrillar aggregates indicated their favorable interactions. The observed colorimetric change (pale pink to purple) as a consequence of binding of TC to Aβ42 fibrillar aggregates may be attributed to aggregate-induced changes in the intramolecular alignment and electronic structure of TC (Fig. 1). In similar absorption studies with Aβ42 fibrillar aggregates, TP failed to exhibit any detectable change in absorption and in the color of the solution.

In order to characterize the aggregate-specific shift in the absorption spectrum of TC and to propose its absorption maximum as a “colorimetric signature” for amyloidosis, its one photon absorption properties were computed by employing time-dependent density functional theory (at the B3LYP/TZVP level) in polar, non-polar and fibril-like environments. In particular static and dynamic results were presented where the former one involves a single optimized geometry of TC in the specific solvent environments while the latter results are obtained as average over numerous configurations from Car-Parrinello QM/MM molecular dynamics. These models are respectively referred to as TD-DFT/PCM and TD-DFT/MM. For further details, we refer to the computational details section of supplementary Information. The calculation only for the most stable binding mode of TC in fibril as shown in Fig. 1c has been carried out. Representative snapshots used in TD-DFT/MM calculations
for TC/fibril and TC/water systems are shown in Fig. S2. The spectra computed only for dynamic models (by convoluting the absorption bands of six lowest energy excitations) are shown in Fig. 1d. The absorption spectrum is characterized by a single dominant band in the visible region which is due to the lowest frequency excitation.
of $\pi \cdot \pi^*$ character. The molecular orbitals involved in this excitation are shown in the supplementary information (Fig. S3). The absorption maximum ($\lambda_{\text{max}}$) for TC from the aforementioned models is listed in Table 1 along with the experimental results which show a red shift by 58 nm for the TC probe going into the fibril-like environment.

The simplistic polarizable continuum model reproduces the trend of a red-shift in the absorption spectra of TC when going from water-like to non-polar, chloroform environment even though the size of the shift is small (14 nm) when compared to experiment (24 nm). Based on this result, it can be suggested that the hydrophilic-like to hydrophobic-like change in the micro-environment may be a feasible mechanism for the fibril-induced red-shift in the absorption spectra of TC. The more sophisticated TD-DFT/MM approach which accounts for electrostatic and polarization interactions between TC and its fibril-like and aqueous environment also confirmed this and reproduce the red shift (56 nm) in excellent agreement with experiment. Usually, the change in the micro-environment alters the molecular structure and conformation of the probe which also significantly contributes to the shift in the spectra\(^3\). For this reason, we investigated the fibril-induced changes in conformation and molecular structure (along the conjugation pathway) of TC and fibril-like and aqueous environment also confirmed this and reproduce the red shift (56 nm) in excellent agreement with experiment. Usually, the change in the micro-environment alters the molecular structure and conformation of the probe which also significantly contributes to the shift in the spectra\(^3\). For this reason, we investigated the fibril-induced changes in conformation and molecular structure (along the conjugation pathway) of TC and fibril-like and aqueous environment also confirmed this and reproduce the red shift (56 nm) in excellent agreement with experiment. Usually, the change in the micro-environment alters the molecular structure and conformation of the probe which also significantly contributes to the shift in the spectra\(^3\). 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fibrillar aggregates leading to enhanced (~30-fold) red fluorescence. Aβ42 fibrillar aggregates (30 μM) were incubated with ThT (5 μM) and TC (5 μM) for 5 min and imaged under fluorescence microscope. Characteristic Aβ42 fibrillar aggregates can be distinctly seen in TEM images, whereas fluorescence images show large clumps of Aβ42 aggregates owing to low resolution of the technique (Fig. S7). Probe TC did not show appreciable changes in the absorption and emission properties under different buffer conditions which indicate that solvent (buffer solution) has no significant effect on the conformational or aggregation tendency of the probe (Fig. S4). Furthermore, a pH-dependent study showed that photophysical properties of probe TC is not affected in the pH range of 3–8, which reaffirm the utility of the probe in most physiological conditions (Fig. S5).

Next, we calculated the binding constant by studying the fluorescence response with varying concentration of TC against a fixed concentration of Aβ42 fibrillar aggregates (dose-dependent saturation assay, Fig. S8). The obtained standard saturation curve was fitted to a single-binding site, which gave a dissociation constant $K_d = 58.43$ nM (the association constant was calculated to be $K_a = 1.72 \times 10^7$ M$^{-1}$ for 2 μM of Aβ42 fibrillar aggregates) (Fig. 4a). Notably, our recent study showed that AT-selective binding of TC to a DNA duplex generates a ~16-fold fluorescence enhancement and $K_d$ in the micromolar range (10 μM). Remarkably, the current study reveals a ~30-fold fluorescence enhancement with $K_d$ in the nanomolar range indicating a much higher binding affinity of TC towards Aβ42 fibrillar aggregates compared to DNA. To further evaluate high affinity of TC towards Aβ42 fibrillar aggregates compared to DNA, we have performed a competitive binding experiment. The competitive binding experiment is based on the fact that, when TC binds to either Aβ42 fibrillar aggregates or DNA show characteristic changes in both absorption and emission spectra corresponding to probe TC and changes observed in each case are substantially different. First, probe TC was saturated with excess DNA (calf thymus) which showed characteristic changes in both absorption and emission spectra of TC, which corresponds to DNA binding, but when the same sample was added with Aβ42 fibrillar aggregates (10 μM, incubate for 15 min), it exhibited changes in both absorption and emission spectra which were similar to absorption and emission features corresponding to TC bound to Aβ42 fibrillar aggregates alone. This observation highlights the fact that in the presence of both Aβ42 fibrillar aggregates and DNA it preferably binds to Aβ42 fibrillar aggregates over DNA (Fig. S9).

In addition, the $K_d$ of Aβ42 fibrillar aggregates bound TC is very low compared to that of the control probe ThT (~0.8 μM) and Congo red (~1.1 μM) confirming the superiority of the TC probe in terms of binding affinity towards Aβ42 fibrillar aggregates. Oligomers and fibrils are prominent polymorphic forms of Aβ42 aggregates. Probe TC showed selective fluorescence enhancement towards Aβ42 fibrils over oligomers which exhibited a slight red shift (8 nm) in the basal fluorescence and negligible fluorescence enhancement (Fig. S10). Further we have performed fluorescence studies in the presence of intracellular protein content bovine serum albumin (BSA), fibrillar aggregates of α-synuclein and islet amyloid polypeptide (IAPP, amylin) implicated in Parkinsons disease and type II diabetes, respectively. Incubation of TC with BSA, α-Syn aggregates and IAPP aggregates (20 μM) did not lead to significant fluorescence enhancements confirming the preferential selectivity of the probe towards Aβ42 fibrillar aggregates over other proteins and peptide aggregates (Fig. 4b).

Recently, Suzuki et al. have studied the competitive binding of Aβ inhibitor, EGCG and ThT towards Aβ aggregates using 19F NMR to understand its binding interactions. Similarly, to gain further insight into the binding interaction of TC probe, displacement assay was performed against ThT-bound Aβ fibrillar aggregates. The well-separated emission spectra of ThT (green region) and TC (red region) made it possible to observe fluorescence changes corresponding to individual probes during the displacement experiments (Fig. S12). Remarkably, a gradual addition of TC to the ThT/ Aβ fibrillar aggregate complex (ThT = 5 μM and Aβ = 10 μM) resulted in a steady decay in fluorescence at 483 nm ($\lambda_{ex} = 450$ nm) and a corresponding enhancement in the emission intensity at 654 nm ($\lambda_{em} = 537$ nm). This clearly suggested an effective displacement of ThT by TC owing to the formation of a much stronger TC/Aβ fibrillar aggregate complex (Fig. 5a). An interesting observation was made during the titration studies where spectral features corresponding to emission of TC (at 654 nm) were observed...
upon excitation of the sample (TC/ThT/Aβ42 fibrillar aggregates) at 450 nm (ThT excitation wavelength). Addition of TC (33 nM to 10.233 μM) to the ThT/Aβ42 fibrillar aggregate complex showed a gradual decrease in the fluorescence emission at 483 nm as expected. However, upon 450 nm (ThT) excitation, fluorescence was also observed at 654 nm (TC) with a slight red shift. The fluorescence intensity of this unprecedented emission band decreased slowly with further increase in the concentration of added TC and finally reached a constant value (Fig. 5b). These changes in the emission characteristics, particularly the fluorescence emission of TC upon excitation corresponding to ThT is attributed to fluorescence resonance energy transfer (FRET) between the Aβ42 fibrillar aggregates bound to ThT and TC. Evidently, the emission spectrum of ThT significantly overlaps with the absorption spectrum of TC making them a suitable donor-acceptor pair on the aggregate surface (Fig. S12)46,47. At the beginning of the titration, TC binds to the ThT/Aβ42 fibrillar aggregates complex by partially displacing ThT, leading to FRET between bound ThT (donor) and TC (acceptor) (Fig. 5b). For concentrations of TC > 150 nM, displacement of ThT by TC resulted in a decreased FRET-fluorescence of TC (Fig. S6). However, the FRET-based fluorescence at 654 nm was not quenched completely due to persistent residual ThT-TC pairs on an Aβ42 fibrillar aggregates. The quenching of fluorescence intensity of ThT (at 483 nm) to its basal level indicates that TC binds to similar primary binding pockets of Aβ42 fibrillar aggregates occupied by ThT. On the other hand, excitation at 537 nm (TC) showed a gradual increase in fluorescence independent of ThT displacement, confirming the presence of multiple binding sites for TC on Aβ42 fibrillar aggregates (Fig. 6). The displacement of ThT was almost instantaneous and did not require any incubation time. Addition of TC (1 μM) to the ThT (10 μM)/Aβ42 (50 μM) complex led to a complete change in emission color of the sample, from green to bright pinkish red, as seen under

Figure 5. Displacement assay. (a) Titration of TC to ThT/Aβ42 fibrillar aggregate complex (ThT, 5 μM/Aβ42 fibrils, 10 μM) in 10 mM PBS buffer solution. High affinity TC effectively displaces ThT from the ThT/Aβ42 fibrillar aggregate complex, as monitored by the decrease in fluorescence emission at 483 nm (◊ green trace, λex = 450 nm) and corresponding increase in fluorescence emission at 654 nm (♦ red trace, λex = 537 nm). (b) In displacement assay (a) emission of TC monitored at 654 nm (Emax) upon excitation at 450 nm (λex of ThT). Region A: ThT/Aβ42 fibrillar aggregate complex. Region B: TC/ThT/Aβ42 fibrillar aggregate complex, at low concentration TC coexists with ThT leading FRET between them. Region C: TC displaces ThT, with residual ThT (possibly in the inner cleft of the Aβ42 fibril) which leads to residual FRET. (c) Proposed model for the TC displacement of ThT and FRET between them on the Aβ42 fibrils. NFI: Normalized fluorescence intensity.
to calculated by AutoDock is the highest in this site (about identified in the entry site formed by Leu17, Val18, Phe19, Gly38, Val39 and Val40 (Fig. 1c). The binding affinity cleft and surface) in the fibril accessible for binding of In agreement with experimental indications, our study shows that there are multiple binding sites (such as entry cleft and surface binding sites which have to be attributed to the larger van der Waals surface and ThT in the fibril. The TC probe also showed good specificity as it did not interact with other abnormal protein aggregates of α-synuclein and IAPP. Owing to high binding affinity, the TC probe displaced the ThT probe bound to Aβ42 fibril for Aβ42 aggregates along with a characteristic colorimetric response which can be attributed to a change in the dielectric nature of the micro-environment around TC from hydrophilic-like to hydrophobic-like. The TC probe also showed good specificity as it did not interact with other abnormal protein aggregates of α-Syn and IAPP. Owing to high binding affinity, the TC probe displaced the ThT probe bound to Aβ42 fibril for Aβ42 aggregates, conversely very high concentrations of ThT could not displace Aβ42 aggregates. The docking site in the Aβ42 fibril for TC has been revealed from molecular docking studies. We propose that optimization of TC as a lead probe for Aβ42 aggregates may afford novel, useful optical-based diagnostic probe for Alzheimer’s disease.

Conclusion
We demonstrated that the hemicyanine-based benzothiazole-coumarin (TC) probe binds to Aβ42 aggregates with nanomolar affinity (Kₐ = 1.72 × 10⁷ M⁻¹). The probe showed switch-on red fluorescence with a large Stokes shift (~117 nm) upon binding to Aβ42 aggregates along with a characteristic colorimetric response which can be attributed to a change in the dielectric nature of the micro-environment around TC from hydrophilic-like to hydrophobic-like. The TC probe also showed good specificity as it did not interact with other abnormal protein aggregates of α-Syn and IAPP. Owing to high binding affinity, the TC probe displaced the ThT probe bound to Aβ42 fibril for Aβ42 aggregates, conversely very high concentrations of ThT could not displace TC bound to Aβ42 aggregates. The binding site in the Aβ42 fibril for TC has been revealed from molecular docking studies. We propose that optimization of TC as a lead probe for Aβ42 aggregates may afford novel, useful optical-based diagnostic probe for Alzheimer’s disease.

Methods
All reagents and solvents were obtained from Sigma-Aldrich and used without further purification. All air and moisture sensitive reactions were carried out under an argon atmosphere. Absorption spectra were recorded with Perkin Elmer Model Lambda 900 spectrophotometer. Fluorescence spectral measurements were carried out by using Perkin Elmer Model LS 55 fluorescence spectrophotometer. Incubation for fibril formation was performed in the Eppendorf Inova42 incubator.

Figure 6. Docking results of (a) TC and (b) ThT with Aβ42 fibril (all binding sites are shown). The fibril is shown in cartoon mode, the binding site residues in stick mode and TC or ThT in stick and ball mode.
Synthesis of probes. Probes TC and TP were synthesized following the literature procedure recently reported from our group.35

Preparation of Aβ42 fibrillar aggregates. Aβ42 peptide (0.25 mg) (Merck, calbiochem) was dissolved in hexafluoro-2-propanol (HFIP, 0.2 mL) and incubated at room temperature for 1 h. HFIP was then removed by a flow of nitrogen and further dried by vacuum. HFIP-treated Aβ42 was then dissolved in DMSO to a final concentration of 1 mM and diluted to 200 μM with 10 mM PBS buffer (pH 7.4). The solution was incubated at 37 °C for 48 h with gentle and constant shaking. The formation of Aβ42 fibrillar aggregates was confirmed by ThT assay, CD measurements and TEM (Fig. S11).

Preparation of amylin (IAPP) fibrillar aggregates and α-Synuclein fibrils. Amylin peptide (0.1 mg) (Merck, calbiochem) sample was dissolved in 100 μL of acetonitrile to disrupt any pre-existing aggregates, and taken up in 200 μL of 10 mM PBS buffer (pH 7.4). The final concentration of acetonitrile in the fibrillization buffer was 10% (v/v). The solution was sonicated continuously for 1 min to break up any potential aggregates. To form fibrils, the sample was incubated at 37 °C without agitation in an eppendorf tube for 120 h (5 days). α-Synuclein peptide (0.5 mg) (Sigma-Aldrich) was dissolved in hexafluoro-2-propanol (HFIP, 0.2 mL) and incubated at room temperature for 1 h. HFIP was then removed by a flow of nitrogen and further dried by vacuum. Then α-Synuclein peptide is dissolved TBS buffer to a concentration of 200 μM. Then the solution is incubated at 37 °C for 3–5 days with constant shaking of 150 rpm.

Determination of the binding constant of TC for Aβ42 aggregates. Increasing concentration of probe TC (0–1.15 μM) was titrated against a fixed concentration of Aβ42 aggregates (2 μM) and fluorescence intensity at 639 nm was recorded (λex = 537 nm). The Kd binding curve was generated by GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) by using below equation, where X is concentration of probe TC and Y is change in fluorescence intensity

\[ Y = \frac{B_{max} \cdot X}{K_d + X} \]

Bmax is the maximum specific binding has the same units as Y. Kd is the equilibrium binding constant.

Molecular Docking. Molecular docking was performed using AutoDock 4.2, and the AutoDock-Tools software was used to set up the necessary inputs for the docking program36. The structure of fibril consisting of 5 Aβ42 peptides (PDB code 2BEG)34 was taken from the Protein Data Bank and was used as the protein model for docking in this study. The geometry of fibril in gas phase was optimized at the level of B3LYP/6–31 × g* using the Gaussian09 software. A grid box centered on the protein was defined with a dimension of 90 × 70 × 60 Å using a 0.375 Å grid step, which is large enough to encompass the whole protein and leave enough space for docking ligand on the surface. The Lamarckian Genetic Algorithm was used for legend con-}

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**Author Contributions**
K.R. and T.G. designed the project. N.N synthesised the probe, K.R. undertook the photophysical studies and *in vitro* studies of the probe, N.A.M., G.K. and H.M. Performed computational studies. All authors contributed to writing the manuscript.
Additional Information

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