Interfacial Interaction of Amyloid Beta Peptide 1-40 with ThT

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BACKGROUND

One of the main causes of many neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease, is the deformation of a specific protein, called amyloidogenic protein. While extensive studies of these proteins have been conducted, an enormous amount of effort is needed to interpret the study outcome due to the complexity of the proteins involved. The amyloid beta peptide (Aβ) is highly regarded as a critical monomer that reversibly conforms an insoluble fibril that leads to Alzheimer's disease. The mechanism of fibril formation is considered to be a polymerization of a nucleus unit consisting of Aβ oligomers under a reversible process. While extensive studies have been conducted on fibril properties and character, only limited information is available on these initial oligomers. The proteins, when immobilized at an interface, can possess different properties than their counterparts dispersed in solution. The sheet structure was confirmed on fluorinated nanoparticles, implying that self assembly over nanoscale interfacial environment plays a key role in fibrillogenesis. A folding or unfolding of monomer peptide is highly associated with the critical stage of intermediate oligomer formation, leading to fibrillogenesis. While direct probe of plausible interfacial conformation may be challenging, monitoring a change in the dynamics of fluorophore attached to a folding peptide is possible.

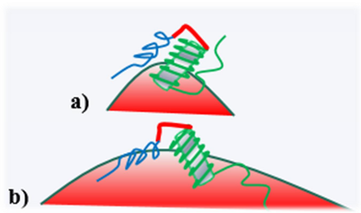


Figure 1. The stable conformation of Aβ may depend on the interfacial environment. Images **a** and **b** illustrate the folded and unfolded conformations that may be experienced by Aβ attached to different nanocolloidal interfacial environments.

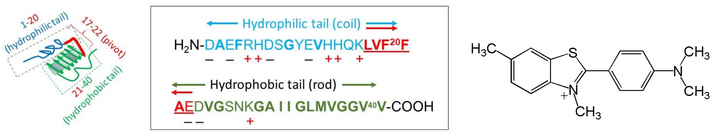
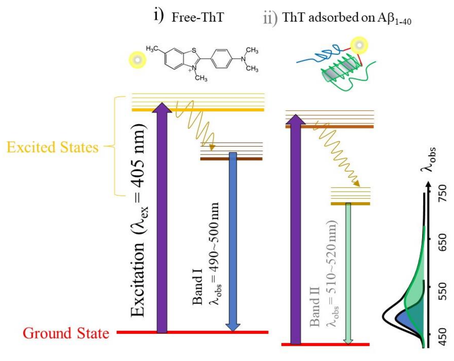


Figure 2. Sketch showing sequence of $A\beta_{1\text{-}40}$ monomer (left) and molecular structure of ThT (right)

METHODS

The Tht was attached to gold nanocolloids of various sizes ranging from 10 to 100 nm in diameter which provided the peptide with a nanoscale interfacial environment to conform on. Additionally, changes to the chemical environment were made by altering the pH from 2 to 12 and its influence on the solution was studied.

Two approaches were used to study the dynamics of the peptide fluorescence, spectroscopy, and subnanosecond fluorescence decay time measurements. Fluorescence spectroscopy was used to understand the possible oligomeric structures formed by the $A\beta_{1\text{-}40}$. Time correlated single photon counting (TCSPC) was used to measure the energy relaxation time of the fluorescein in order to indirectly probe the time scale of the conformational changes exhibited by the $A\beta_{1\text{-}40}$.



RESULTS

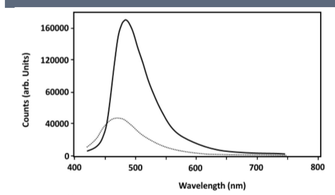


Figure 3: The fluorescence spectra of ThT alone (dotted line) and ThT with $A\beta_{1\text{-}40}$ (solid line).

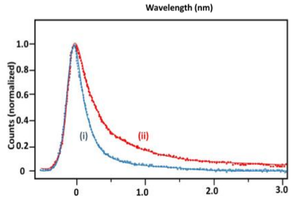


Figure 4: The fluorescence decay time of band II of ThT with $A\beta_{1\text{-}40}$ coated over 10 nm gold colloid (i) in blue and ThT with $A\beta_{1\text{-}40}$ coated over 100 nm gold colloid (ii) in red at pH 6.5. The decay time (i) profile was almost identical with that of ThT alone.

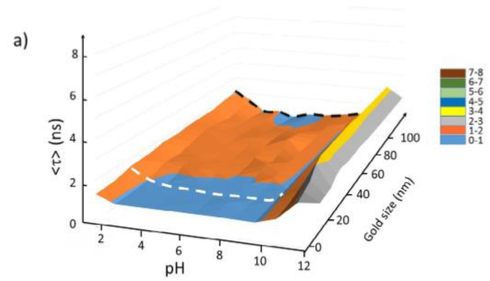


Figure 5a: A surface plot of pH dependent average fluorescence decay time, <t>, for gold colloidal size ranging between 10 nm and 100 nm size. The value of <t> was monitored at a band I.

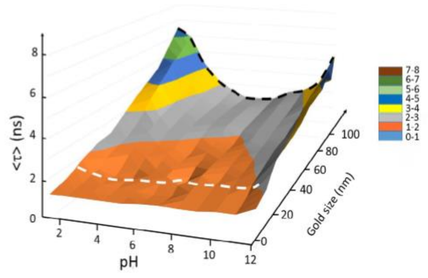


Figure 5b: . A surface plot of pH dependent average fluorescence decay time, <t>, for gold colloidal size ranging between 10 nm and 100 nm size. The value of <t> was monitored at a band II.

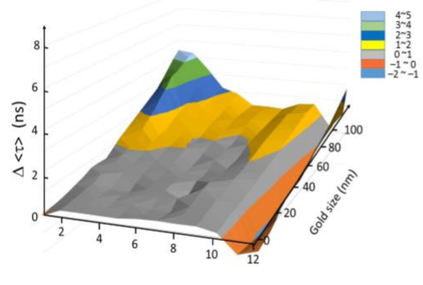


Figure 5c: A surface plot of pH dependent average fluorescence decay time, Δ<τ>; differences between band I and band II.

DISCUSSION

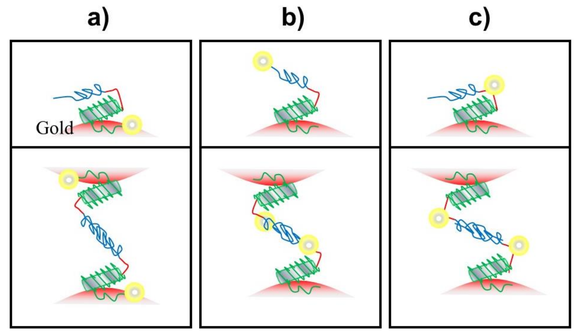


Figure 6. Representative sketch indicating the position of ThT dye attached to an unfolded (at acidic condition) $A\beta_{1\text{-}40}$ adsorbed over gold colloidal surface. a) ThT is adsorbed at the hydrophobic section of $A\beta_{1\text{-}40}$. b) ThT adsorbed at the hydrophilic segment exposed to the outward for aggregate formation. c) ThT adsorbed at the pivotal section of $A\beta_{1\text{-}40}$. In the lower box, the situation of $A\beta_{1\text{-}40}$ adsorbed gold colloid are illustrated for each case.

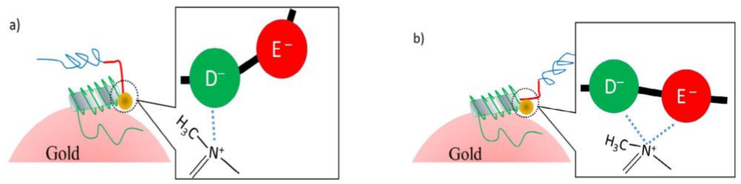


Figure 7. A schematic sketch indicating a particular segment(s) used for ThT attachment in the case of folded (a) and unfolded (b) $A\beta_{1\text{-}40}$. Here, yellow circle indicates ThT dye and D– and E– are ^{22}Glu– and ^{23}Asp–, respectively. Each box shows a blow up of the ThT attachment site.

An identification of the ThT attachment site confirms that both 1) $A\beta_{1\text{-}40}$ adsorbs on the gold colloidal surface through any parts of hydrophobic segments (23-40) and that 2) hydrophilic segments (1-16) are used for networking with the other $A\beta_{1\text{-}40}$ monomer adsorbed on gold colloid to form colloidal aggregates. The formation of networking with the other $A\beta_{1\text{-}40}$ is a very significant implication of the formation of oligomers at an interface between adjacent gold colloids. This allows these oligomers to act like a networking chain for the colloidal aggregates. However, an extraction of a particular oligomeric form was not conclusive through this study. Currently, a morphology of amyloidogenic peptide coated gold colloidal aggregates are being studied by TEM (Transmission Electron Microscopy). This networking conformation can provide a great hint of how networking segment is spatially (geometrically) oriented.

CONCLUSIONS

The Aβ1-40 adsorbed gold colloid provided the ThT fluorescence assay with monitoring free ThT as well as the Aβ1-40 interacted with ThT. Through exploration of fluorescence probing over various sizes of gold colloidal surfaces in the pH range of 1 to 12, the plausible section of ThT-Aβ1-40 binding is concluded to be a sequence of 22Glu and 23Asp through electro-static interaction (–N+⋯ [-22Glu– - 23Asp – -]), avoiding the direct interaction between ThT and Ab1-40,as it took a conformational change by folding. The folded conformation was estimated to shorten an interaction distance between attachment sites and ThT, resulting in a stabilization of excited states of ThT.