Nano-scale Description through Dynamical Investigation of Adsorption Orientation

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Background

Alzheimer's disease (AD) is the sixth leading cause of the death in the USA. The "*fibrillogenesis*" of amyloidogenic peptides causes amyloid formation, which is commonly understood as the cause of many neurodegenerative diseases (*e.g.,* AD) Previous studies have looked at the folding pathways of different monomeric peptides; however, the studies lack the information of peptide networking. Our group uses a unique approach to study peptide networking by attaching one end of a peptide to the nano-gold surface allowing the networking end to be wide open for any peptide-peptide interaction. However, one big problem is that there is no information regarding peptide orientation yet.

It is hypothesized that a specific segment possesses a higher affinity to the nano-metal than the interaction between monomers.

To clearly identify which segment is responsible, fluorophore (fluorescein dye) was attached to amyloid beta 1-40 ($A\beta_{1-40}$) - FAβ in this study. Gold nanoparticles are expected to act as an effective quencher for fluorescein over nano surface area, by examining the fluorescence quenching, an identification of contacting sites can be conducted. The distance between the hydrophilic site (N-terminal) and hydrophobic site (C-terminal) changes as FAβ changes its conformation.

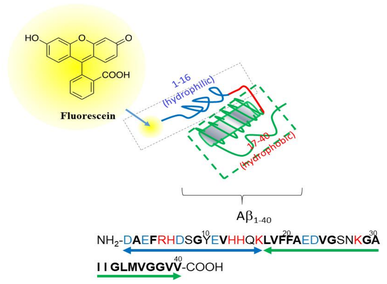

Figure 1. Molecular structure of fluorescein and the location of its attachment to $A\beta_{1-40}$ (FAβ). The section of fluorescein attached at the N-terminal and the sequences of $A\beta_{1-40}$ are shown. (Positively charged and negatively charged at pH 7.)

Methods

The energy relaxation process of an electronically excited fluorescein dye embedded to the $A\beta_{1-40}$ was examined as FAβ interacted with various sizes of gold nano-colloidal particles ranging in size between 10 nm and 100 nm in diameter at 25 ℃ under DMSO (Dimethyl Sulfoxide) environmemnt. There were two approaches used to study the dynamics of the peptide: [1] fluorescence spectroscopy, and [2] Time correlated single photon counting (TCSPC) for picosecond fluorescence decay time measurements. (λ_{ex}: excitation wavelength = 405 nm)

[1] Fluorescence spectroscopy was used to understand the energetics levels prepared by interacting with $A\beta_{1-40}$. The ratio between the fluorescence of FAβ and that of a free Fluorescein in DMSO (I/I_0) was examined in order to extract the effect of $A\beta_{1-40}$.
[2] TCSPC revealed energy relaxation time of the fluorescein. The average decay time, <τ>, was calculated by $\langle\tau\rangle = \sum_i^n a_i e^{-\tau i/t}$, where a_i and τ_i are the weighting factor and life-time of the *i*-th component after the deconvolution.

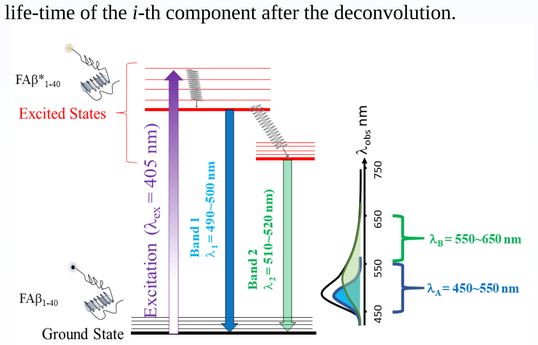

Figure 2. Sketch of the energy level diagram for fluorescein dyes in FAβ. Two fluorescence components are labeled as λ_1 and λ_2, and the fluorescence decay was monitored at two different wavelength regions given as λ_A and λ_B (550~650 nm).

Results

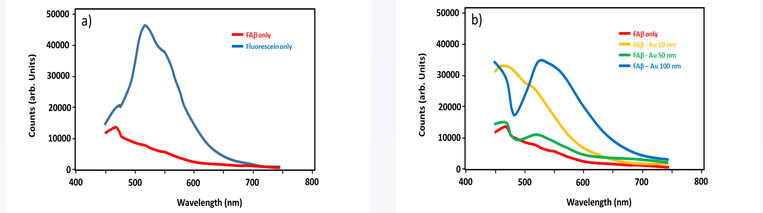

Figure 3. a) The fluorescence spectrum of FAβ a) in DMSO at pH ~7. b) The fluorescence spectrum of FAβ attached over gold nano-particles of the diameters of 10, 50, and 100 nm in DMSO at pH ~7.

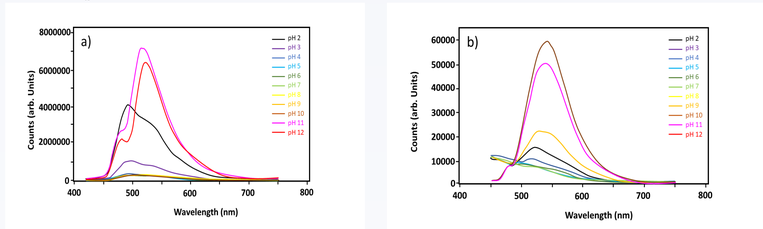

Figure 4. The fluorescence spectrum of fluorescein only (a) and FAβ only (b,) in DMSO monitored under pH 2~12 conditions.

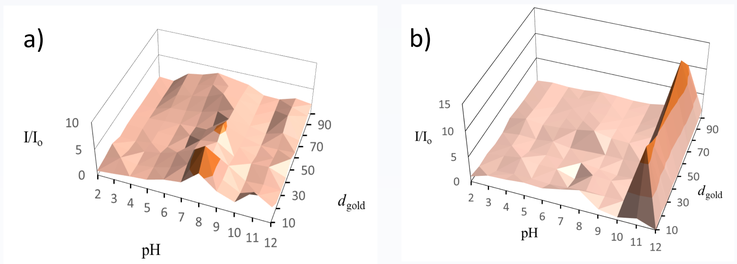

Figure 5. The normalized intensity plot of fluorescein attached $A\beta_{1-40}$ (FAβ) in DMSO as a function of pH and diameter of gold nanoparticle (d_{gold}): a) intensity map for the component λ_1, and b) intensity map for the component λ_2.

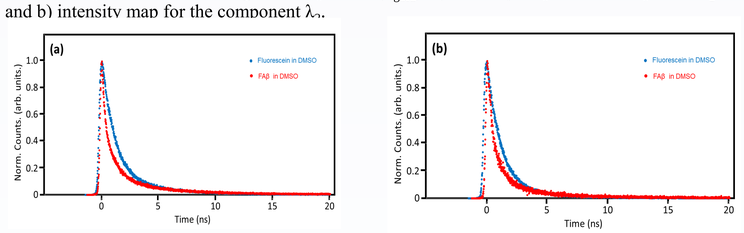

Figure 6. The fluorescein decay spectrum of fluorescein and FAβ in DMSO monitored at a) band λ_A and b) band λ_B under pH ~7.

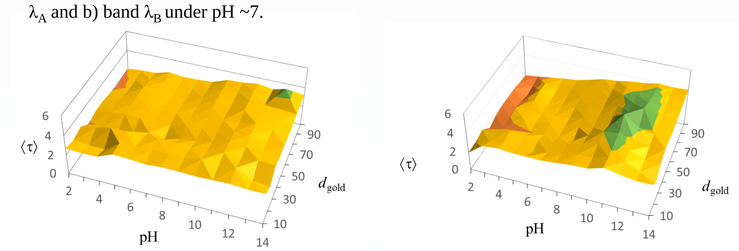

Figure 7. The weighted average fluorescence lifetime, 〈τ〉, of FAβ in DMSO as a function of pH and d_{gold}: a) 〈τ〉 map for the component λ_A, and b) 〈τ〉 map for the component λ_B.

Discussion

Absorption orientation of $A\beta_{1-40}$ to gold surface

A drastic reduction of fluorescence band of FAβ under DSMO strongly indicates that a significant restriction of fluorescein segment geometrically forced to go through quenching process. An enhancement of fluorescence was observed as FAβ mixed with nano-gold particles. The Fluorescein in FAβ in DMSO must receive direct steric hindrance from the rest of Aβ segment. This can be likely a hydrophobic segment highly interacted with fluorescein and prohibited a free motion of fluorescein or opened up relaxation channel of the excitation energy. Since we observed less quenching under basic condition, we conclude that the absorption of the $A\beta_{1-40}$ monomer is established by the hydrophilic segment (N-terminal: the side of Fluorescein attachment) of the monomer.

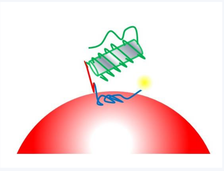

Figure 8. A schematic sketch for an orientation of an attachment of FAβ monomer over nano-gold particle surface under DMSO environment. The hydrophilic segments are color-coded by blue. The fluorescein dye is shown as a yellow sphere.

The DMSO environment created completely different relaxation environment between two energy levels. For DMSO environment, for the upper sate the profile was dominated by nano-size dependent feature and it was extremely conformational change dependent. Thus, it is plausible that the upper energy level could be originating from the fluorescein by highly impacted with an interaction of adjacent monomer and its conformational change. It is speculated that pH 7~8 area prepares folded conformation keeping the relatively further distance between adjacent monomers. On the other hand, lower energy state is concluded to be originating from the levels directly impacted by the nano-gold surface. As indicated in a sketch of Fig. 9, the distance between N-terminal fluorescein and nano-gold surface must be further located at pH 11.

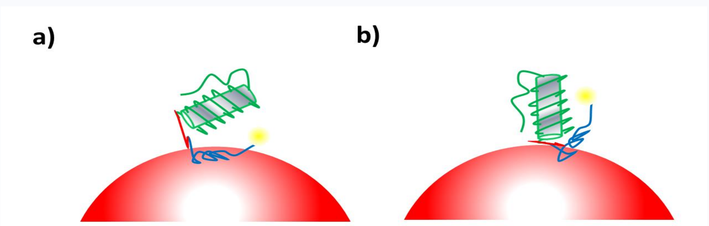

Figure 9. A schematic sketch of the attachment configuration of FAβ monomers over the nano-gold surface in DMSO environment for a) pH less than pH 10 and for b) pH 11. The hydrophilic segment and hydrophobic segments are color-coded by blue and green, respectively. The fluorescein dye attached to the N-terminal of $A\beta_{1-40}$ is shown as a yellow sphere.

Conclusions

1. The energy relaxation process of an electronically excited fluorescein dye embedded to the amyloid beta peptide 1-40 ($A\beta_{1-40}$) (Fluorescein attached Amyloid beta 1-40: FAβ) was examined as FAβ interacted with various sizes of gold nano-colloidal particles ranging in size between 10 nm and 100 nm in diameter.

2. The fluorescence profile accurately reflected which segment of $A\beta_{1-40}$ was used for the adsorption over the nano-gold surface. It was concluded that the FAβ adsorbed over the nano-colloidal surface through the hydrophilic (N-terminal side) segments under DMSO.

3. The entire dynamical profile of fluorescein was successfully explained by a relative degree of interaction between a fluorescein and the gold nano-particle surface.