Near-Infrared Light Triggered ThT acid-based Upconversion Nanocomposites for early stage Alzheimer's disease detection

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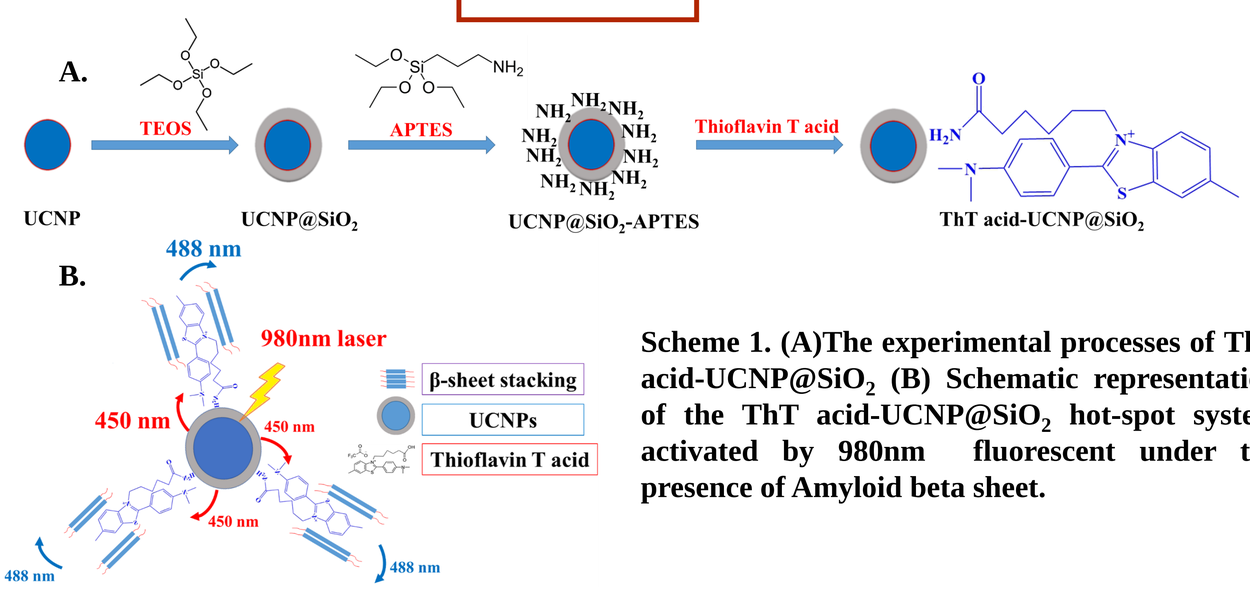
Abstract

Fast-growing numbers in people aged above 65 inevitably results in needs to tackle problems in long-term care and aging-related comorbidity diseases such as high blood pressure, diabetes and especially Alzheimer's disease (AD). Therefore, we propose a novelty platform combining nanotechnologies and optical technology to develop early detection and diagnosis for patients with AD. The AD-related Amyloid-β (Aβ) is known to polymerize into oligomers that serve as the nuclei for subsequent elongation into fibrils via a nucleation dependent polymerization mechanism. Thus, Thioflavin T (ThT) is a fluorescent probe frequently used to monitor the fibrillization process, but not oligomer formation. Above the reasons, we come up with a concept to fabricate ThT acid carrying upconvesion nanocomposites (ThT acid-UCNP@SiO_2) to make them as optical "hot-spots" to achieve early detection of ThT acid signals by enhancing its fluorescence during Förster resonance energy transfer (FRET) procedure. In this report, we successfully synthesized ThT acid-UCNP@SiO_2 nanocomposites and further incubated with $A\beta_{1-42}$ peptides for ThT assay. Base on our results, the fluorescence intensity (λex= 488 nm) of ThT acid dramatically increases after 8 days incubation within 21.6μM $A\beta_{1-42}$ peptides which provided by ThT acid molecule insert into long-term β sheet structure but not presents in low concentration (10 μM). Remarkably, the ratio of fluctuation emission intensity (λex= 488 nm / 450 nm) of ThT acid-UCNP@SiO_2 gradually increased during incubation of $A\beta_{1-42}$ peptides (21.6μM) within 2 to 4 days and 10 μM with 25 to 30 days under 980 nm laser irradiation, separately. In short, ThT acid-UCNP@SiO_2 nanocomposites can be ThT acid fluorescence enhancer to detect short-term β sheet structure of Aβ and as a potentially optical sensing platform for Alzheimer disease detection in the early stage.

Method



Scheme 1. (A)The experimental processes of ThT acid-UCNP@SiO_2 (B) Schematic representation of the ThT acid-UCNP@SiO_2 hot-spot system activated by 980nm fluorescent under the presence of Amyloid beta sheet.

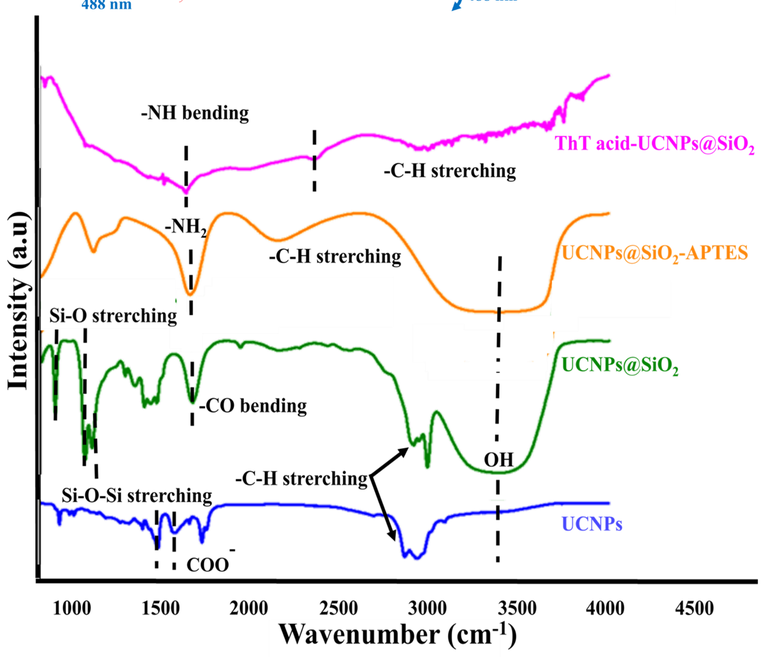


Figure 2. FTIR spectra of UCNPs (blue line), UCNP@SiO_2 (green line), UCNP@SiO_2-APTES (orange line), ThT acid- UCNP@SiO_2 (pink line).

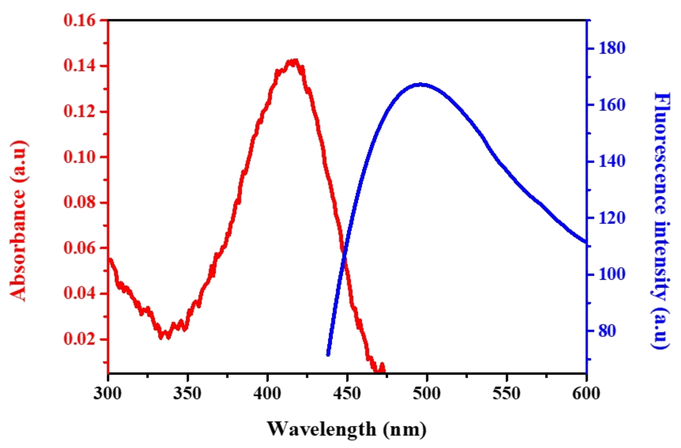
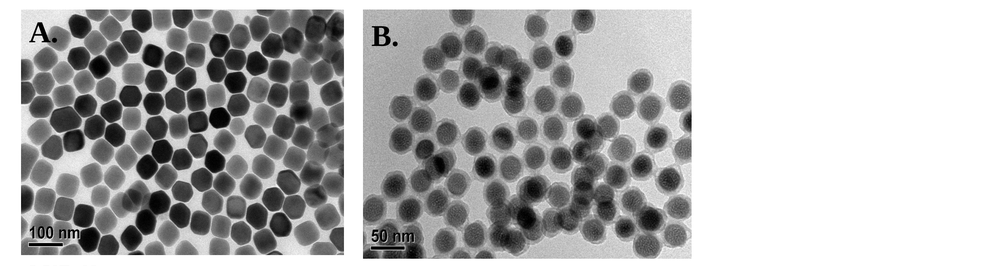


Figure 3. UV-VIS absorbance (red line) and fluorescence intensity (blue line) of ThT acid – UCNP@SiO_2

Results



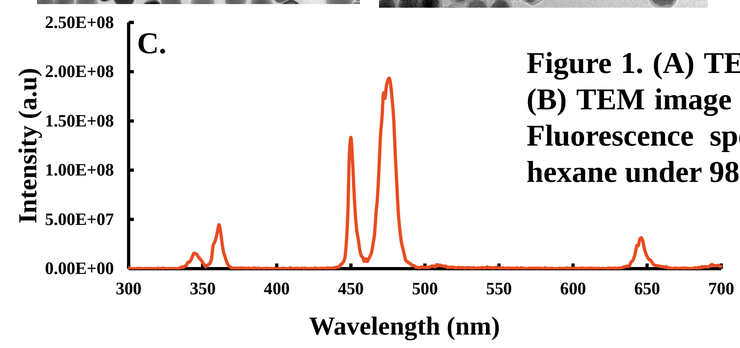


Figure 1. (A) TEM image of UCNPs; (B) TEM image of UCNP@SiO_2 ;(C) Fluorescence spectra of UCNPs in hexane under 980 nm excitation.

21.6μM

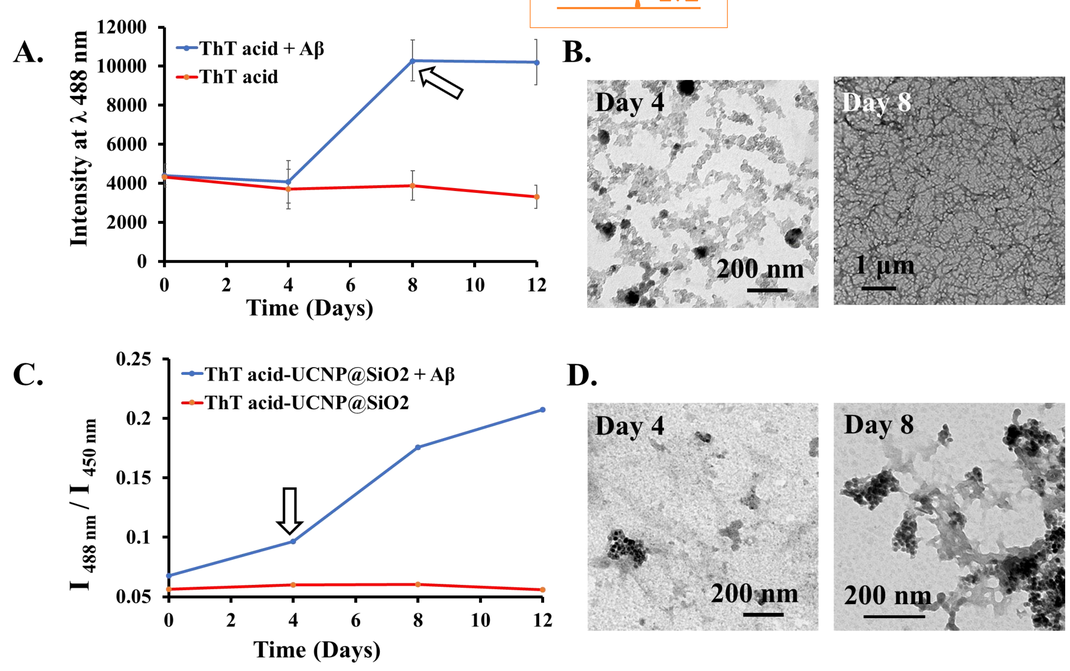


Figure 4. (A) Fibrillization kinetics of 21.6μM $A\beta_{1-42}$ peptides monitored by detecting fluorescence intensity of ThT acid at 488 nm emission under 450 nm excitation. ; (B)TEM images of ThT acid + $A\beta_{1-42}$ in 4 and 8 days incubation, separately; (C)Fibrillization kinetics of 21.6μM $A\beta_{1-42}$ peptides monitored the fluctuation emission intensity of ThT acid-UCNP@SiO_2 from 450 nm to 488 nm by 980 nm laser irradiation.(D)TEM images of ThT acid-UCNP@SiO_2 + $A\beta_{1-42}$ in 4 and 8 days incubation, separately. (The mixture all dispersed in Tris buffer (pH = 7) and incubation 12 days at room temperature)

10μM

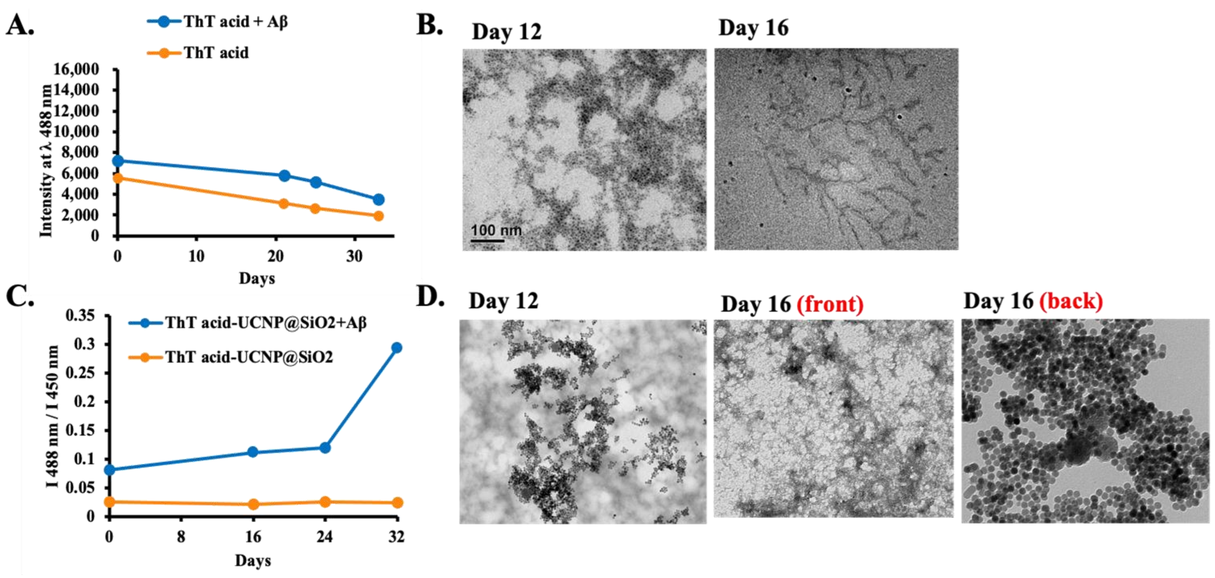


Figure 5. (A) Fibrillization kinetics of 10μM $A\beta_{1-42}$ peptides monitored by detecting fluorescence intensity of ThT acid at 488 nm emission under 450 nm excitation.; (B)TEM images of ThT acid + $A\beta_{1-42}$ in 12 and 16 days incubation, separately; (C)Fibrillization kinetics of 10μM $A\beta_{1-42}$ peptides was monitored the fluctuation emission intensity of ThT acid-UCNP@SiO_2 from 450 nm to 488 nm by 980 nm laser irradiation. (D)TEM images of ThT acid-UCNP@SiO_2 + $A\beta_{1-42}$ in 12 and 16 days incubation, separately. (The mixture all dispersed in Tris buffer (pH = 7) and incubation 35 days at room temperature)

Conclusion

In this report, we successfully synthesized UCNPs with thermal decomposition method and functionalized the ThT acid with surface chemistry process on the UCNPs surface. After that, the structure characterization and optical properties analysis of UCNPs and ThT acid-UCNP@SiO2 were done by a series of equipment. In order to simulate the actual amount of Aβ1-42 in human tissue, as well as present the stability of the sensor in low concentration of Aβ1-42 peptides, the experiment was conducted in 21.6μM and 10μM. Base on our results, the fluorescence intensity (λex= 488 nm) of ThT acid dramatically increase after 4 to 8 days within 21.6μM but not rising within 10μM of incubation with Aβ1-42 peptides. Significantly, the ratio of fluctuation emission intensity (λex= 488 nm / 450 nm) of ThT acid-UCNP@SiO2 gradually increased during 2 to 4 days within 21.6μM, and 25 to 30 days within 10μM, of Aβ1-42 peptides incubation by 980 nm laser irradiation. In summary, we provide a potentially optical sensing platform to detect short-term β sheet structure of Aβ by using ThT acid-UCNP@SiO2 nanocomposites for AD detection in the early stage. Further, we will plan to confirm the configuration of Aβ by FTIR spectroscopy and AFM images.

Reference

1. G. V. D. Ferrari, et. al., *Biochem.*, 2001, **40, 10447-10457.**
2. X. B. Mao, et., al., *ACS Chem. Neurosci.*, **2011, 2, 281–287.**
3. Jacky F. C. Loo, Y. H. Chien, F. Yin, S. K. Kong, H. P. Ho, K. -T. Yong*, *Coord. Chem. Rev.*, **2019, 400, 213042.**

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