

Supramolecular Self Assembly of AIE-active Nanoprobes: Fabrication and Bioimaging Applications

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1. Experiment

1.1 Materials and characterization

All chemicals were of analytical grade and were used as received without any further purification. 1-Adamantanecarbonyl chloride (MW = 198.69, 97%), β -Cyclodextrin (MW = 1134.98, 98%) and 4-hydroxybenzophenone (MW = 198.22, 98%) were purchased from Aladdin company (Shanghai China). Zinc powders, titanium tetrachloride and anhydrous THF were provided from Heowns (Tianjin, China). Ammonium chloride and ethylacetate solution were suffered from Sinopharm Chemical reagents Co., Ltd. (Shanghai, China). ^1H NMR spectra was recorded on Bruker Avance-400 spectrometer with CDCl_3 as the solvent. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV and the TEM samples were made by placing a drop of nanoparticle ethanol suspension on a carbon-coated copper grid. The Fourier transform infrared (FT-IR) spectra were obtained by using a Nicolet 380 Fourier transform spectrometer with a resolution of 2 cm^{-1} which samples were pressed with KBr into a pellet before measuring the infrared absorption spectra.

1.2 Preparation of TPE-OH

The organic luminescence dye with AIE-active was prepared according to McMurry Coupling reaction. Zinc powder (3.9 g, 0.06 mol) was add to dry THF (60 mL) and stirring under nitrogen for 10 min in an ice water bath. After that, TiCl_4 (3.28 mL, 0.03 mol) was slowly added over a period of 30 min, then the ice water bath was removed and the reaction mixture was refluxed under N_2 for about 2 h. Afterwards, the solution which was 4-hydroxybenzophenone (2.97 g, 0.015 mol) dissolved in dry THF (15 mL) was slowly added by using a syringe and keeping reflux for about 24 h. The system was quenched with 5 % ammonium chloride aqueous solution (w/v) and the extracts that extracted with ethylacetate (3 x 200 mL) was dried with anhydrous magnesium sulfate (MgSO_4). After removing the solvent under reduced pressure distillation, the crude product was purified by silica gel column chromatography eluted to gain a white solid of 2.26 g. In the end, the product was refined in the vacuum for two days.

1.3 Preparation of TPE-Ad/ β -CD

The purified TPE-OH (0.182 g, 0.5 mM) and 1-Adamantoyl chloride (0.199 g, 1.0 mM) were dissolved in dry THF (40 mL). Subsequently, added TEA (0.101 g, 1.0 mmol) as an acid binding agent to accelerate the thermodynamic balance towards the forward reaction. Then TPE-Ad was obtained via filtration and further purified washing with water. After that, TPE-Ad (68.8 mg, 0.1 mM) was mixed with β -CD (227 mg, 0.2 mM) in water. After shaking at room temperature for 30 S, the suspensions were separated by centrifugation, and the

product (TPE-Ad/ β -CD) was purified after washing with deionized water three times to remove the free β -CD.

1.4 Confocal microscopic imaging of cells using TPE-Ad/ β -CD LPNs

The cell uptake behavior of TPE-Ad/ β -CD LPNs was conducted with a confocal laser scanning microscope (CLSM) Zeiss 710 3-channel (Zeiss, Germany) at 405 nm excitation wavelength. After A431 cells were cultured in culture dish overnight with a density of 1×10^5 cells, cells were treated with TPE-Ad/ β -CD LPNs ($20 \mu\text{g mL}^{-1}$) for 3 h. Then, cells were washed three times with phosphate buffered saline (PBS) to completely remove fluorescence nanoparticles that did not enter cells. The cell uptake of TPE-Ad/ β -CD LPNs was analyzed using CLSM and digital monochromatic images were acquired using Leica Confocal Software.

1.5 Cytotoxicity of TPE-Ad/ β -CD LPNs

The cytotoxicity effects of TPE-Ad/ β -CD LNP on A431 cells was determined through cell counting kits 8 (CCK-8) assay.¹ Briefly, A431 cells were seeded in 96-well tissue culture plates at a density of 5×10^4 cells/well in a final volume of 200 μL . After culturing for 24 h, cells were treated with 10, 20, 40, 80, 120 $\mu\text{g mL}^{-1}$ for 8 and 24 h respectively. Cells were seeded in 96-well microplates at a density of 5×10^4 cells per mL in 160 μL of the respective media containing 10% FBS. After 24 h of cell attachment, A431 cells were incubated with 10, 20, 40, 80, 120 $\mu\text{g mL}^{-1}$ of TPE-Ad/ β -CD LNPs for 10 and 24 h. Then the cells were washed with PBS for three times to remove the uninternalized nanoparticles. After that, 10 μL of CCK-8 dye and 100 μL of Dulbecco's Modified Eagle's Medium (DMEM) cell culture media was added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of WST was compared to the control (cells not exposed to nanoparticles), which represented 100% WST reduction. Three replicate wells were used for each control and test concentrations per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean \pm standard deviation (SD).

2. Results

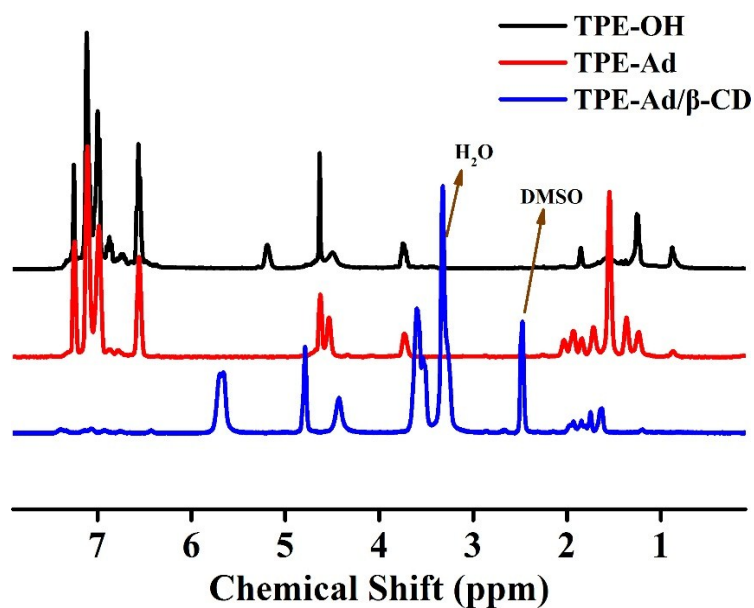


Fig. S1 ¹H NMR spectra of TPE-OH, TPE-Ad and TPE-Ad/β-CD samples. Of which TPE-OH and TPE-Ad were dissolved in CDCl₃. And TPE-Ad/β-CD was dissolved in deuterated DMSO. All of these supplied an evidence of the successful assemble into supramolecular LPNs.

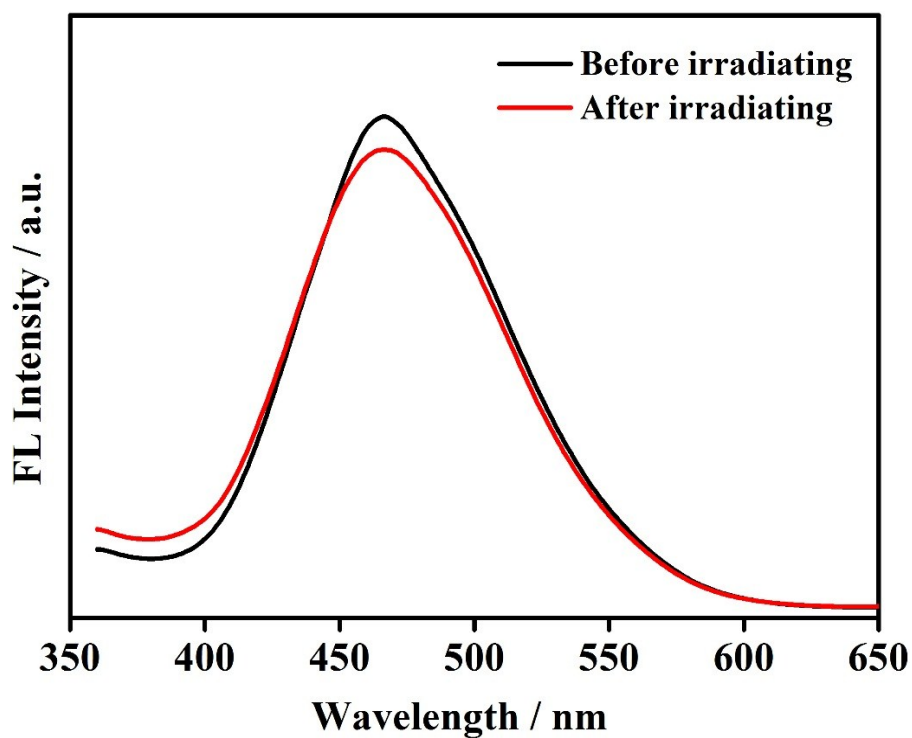


Fig. S2 PL spectra of TPE-Ad/β-CD LPNs before and after irradiation using UV lamp ($\lambda = 365$ nm) for 30 min.

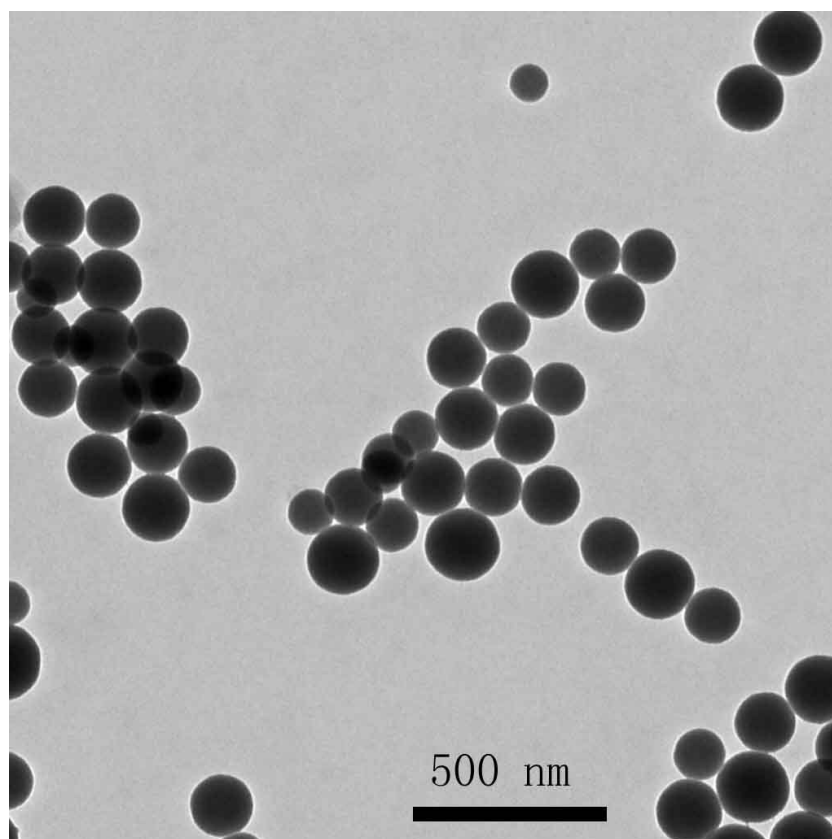


Fig. S3 Representative TEM image of TPE-Ad/ β -CD LPNs. The spherical nanoparticles with diameter of 100-200 nm can be observed (Scar bar = 500 nm).

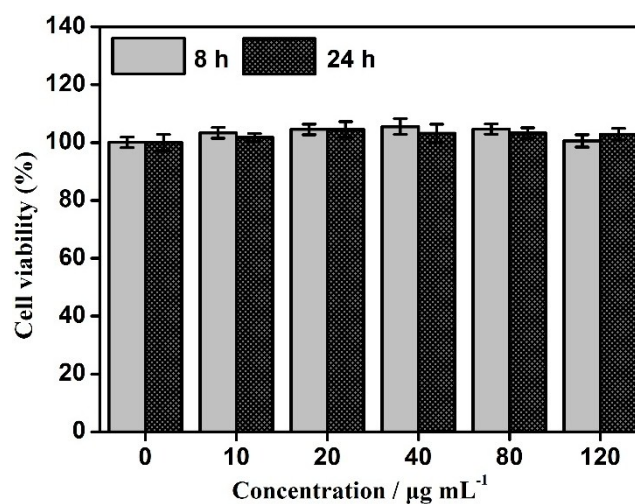


Fig. S4 Cell viability of A431 cells after incubation with TPE-Ad/ β -CD LPNs at various concentrations for 8 and 24 h, respectively.

3. References

1. H. Qi, M. Liu, L. Xu, L. Feng, I. Tao, Y. Ji, X. Zhang and Y. Wei, *Toxicol. Res.*, 2013, **2**, 427-

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