Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Theranostic Saponin Nano-assembly based on FRET of an Aggregation Induced Emission Photosensitizer and Photon Up-conversion Nanoparticles

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1. Experimental section

1.1 Reagents and chemicals

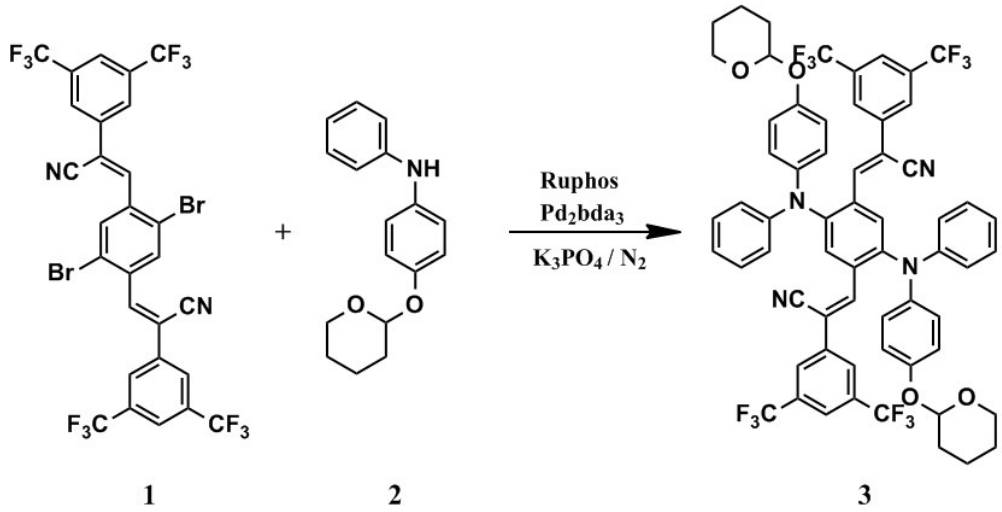
2',7'-Dichlorofluorescein diacetate (DCF-DA), 3,5-bis(trifluoromethyl)phenylacetonitrile, bis(dibenzylideneacetone)palladium(0)[$Pd_2(dba)_3$], 2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl (Ruphos), oleic acid(OA), 1-octadecene(ODE), ammonium fluoride(NH_4F), rare earth oxides yttrium (III) oxide (Y_2O_3), ytterbium (III) oxide (Yb_2O_3) and thulium (III) oxide (Tm_2O_3) were purchased from Energy Chemical Co., Ltd. (Shanghai, China). 1,4-dibromo-2,5-dimethylbenzene, ethyl bromoacetate, N-pheny-4-(tetrahydro-2H-pyran-2-yi)oxy)aniline, 4-hydroxydiphenylamine, pyridinium p-toluenesulfonate were purchased from Heowns (Tianjin, China). 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used in the cell cytotoxicity assay (Dojindo, Japan). 9,10-Anthracenediylbis(methylene)dimalonic acid (ABDA) was purchased from Sigma-Aldrich. All other reagents were obtained from Tianjin Chemical Reagent Co. (Tianjin, China).

^{1}H-NMR spectra of AIEgen derivatives and the polymers were recorded on a 400 MHz Bruker Avance-400 spectrometer (400 MHz, Bruker, Freemont, CA). The chemical shifts were referred to the solvent peaks, δ = 2.50 ppm for DMSO. UV-Vis spectra were recorded on a Nanophotometer NP80 Touch spectrophotometer. Fluorescence spectra were carried out with a Hitachi F4500 spectrofluorophotometer and Maya2000Pro optical fiber spectrophotometer. Upconversion luminescence spectra in near-infrared (NIR) range were collected on an Edinburgh FLS920 spectrometer (collected at range of 400-880 nm) with 980 nm laser as the excitation source.

1.2 Synthesis of (2Z,2'Z)-3,3'-(2,5-bis(phenyl(4-((tetrahydro-2H-pyran-2-yl)oxy)phenyl) amino)-1,4-phenylene)bis(2-(3,5-bis(trifluoromethyl)phenyl)acrylonitrile) (3)（AIEgen）

Compound 1 and 2 were synthesized according to the literature's protocol.[1,2] A flask was charged with compound 1 (0.227 g, 0.298 mmol), 2 (0.802 g, 2.747 mmol), $Pd_2(dba)_3$ (0.027 g, 0.028 mmol), Ruphos (0.038 g, 0.081 mmol), K_3PO_4(0.632 g, 2.974 mmol) and toluene (10.0 mL). The mixture was then degassed, purged with N_2

and heated at 110 °C for 24 h. The reaction mixture was cooled to room temperature, followed by addition of water (60.0 mL) and chloroform (100 mL). An organic layer was separated and washed with brine, dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was purified bysilicagel chromatography (petroleum:ethyl acetate = 5:1), and then recrystallized from CH_2Cl_2/ethanol to give 3 (AIEgen)[1,2]. Yield: 68.4%. ^{1}H NMR (400 MHz, DMSO) δ 8.17 (d, J = 14.4 Hz, 1H), 7.88 (s, 1H), 7.58 (s, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.03 (d d, J = 25.8, 8.5 Hz, 2H), 6.90 (d, J = 9.0 Hz, 1H).



Scheme S1 Synthetic route of the compound 3 (AIEgen).

1.3 Synthesis of NaYF4:Yb/Tm upconversion nanoparticles (UCNPs)

Y_2O_3 (90.1 mg, 0.798 mmol), Yb_2O_3 (39.4 mg, 0.200 mmol) and Tm_2O_3 (0.400 mg, 0.002 mmol) were dissolved in hydrochloric acid. The mixture is heated to dissolve completely, then cooled to room temperature, and dried to obtain chloride. YCl_3, $YbCl_3$, $TmCl_3$ obtained above were dispersed in OA and ODE, and heated at 160 °C for 30 minutes and then cooled to room temperature. Then, 10 mL of a methanol solution of NaOH (100 mg, 2.50 mmol) and NH_4F (148 mg, 4.00 mmol) was added, and the mixture was rapidly stirred for 30 minutes. The mixture was heated to 100 °C to remove methanol and water, then the system was raised to 295 °C for 90 minutes under nitrogen atmosphere. After cooled to room temperature, excessive ethanol was added. The insoluble matter was obtained by remove the liquid from the emulsion by

centrifugation. UCNPs could obtained by washed above insoluble matter with cyclohexane and ethanol and finally dispersed in THF and stored at 4 °C.[2]

1.4 Preparation of UCNPs@AIE constructs

To prepare UCNPs@AIE constucts, 100 μL of THF was used to dissolve monomer (AIEgen, 10.0 μg). UCNPs dispersed in THF (900 μL, 100 μg/ml) was added into the formed solution and then mixed under sonication for 5 min. Then the solution was added dropwise to Millipore DI water (5.00 mL) under ultrasonicating for 5 min with the help of anultrasonicator (60 W).[2] Treatment under ultrasound conditions (80 W) was continued for 2 minutes to form UCNPs@AIE constructs and stored at 4 °C.

1.5 Preparation of UCNPs@AIE@Sap nanoparticles

The above prepared UCNPs@AIE (200 μL) was added to DI water (1.00 mL) and stirred for 20 minutes. N_2 was continuously passed during the reaction to remove THF. After THF was removed, the saponin (Sap) solution (40.0 μL, 1.00 mg/mL) was added to the system, and stirred for another 10 minutes to obtain UCNPs@AIE@Sap nanoparticles, which was sealed and stored at 4 °C.[3]

1.6 DLS measurements of UCNPs@AIE@Sap NPs

The size of UCNPs@AIE@Sap NPs was determined by Zetasizer Nano ZS90 instrument (Malvern Instruments, Southborough, MA) at 25 °C. UCNPs@AIE@Sap NPs were prepared in DI water according to the aforementioned procedures.

1.7 Fluorescence experiment

The AIEgen, UCNPs, UCNPs@AIE solution were pipetted into 1 mL of quartz fluorescence cuvette, and then placed in a fluorescence spectrophotometer connected with an external 980 nm laser with a power of 2.5 W and the emission was collected from 500 to 650 nm.

1.8 Detection of ROS production

2',7'-Dichlorofluorescein diacetate (DCF-DA) was used to detect the production of ROS under light irradiation. Concisely, 0.500 mL ethanol solution of 1 mM DCF-DA was added to 2 mL of NaOH (10 mM) aqueous solution and the mixture was stirred at room temperature for 30 min. The hydrolysate (dichlorodihydrofluorescein, DCFH)

was then neutralized with 10 mL of PBS at pH 7.4 and stored on ice for use. The above-mentioned solution (100 μL) was added to 0.9 mL of UCNPs@AIE@Sap NPs (20 μg/mL) and exposed to NIR laser irradiation for different time intervals. The fluorescence intensity change of the solution was measured with excitation at 488 nm and the emission was collected from 500 to 650 nm.[2,4]

1.9 ROS quantum yield

The ROS quantum yield of UCNPs@AIE@Sap nanoparticles in water (Φ) upon 980 nm irradiation (2.5 W) was determined using ABDA as an indicator and using Rose Bengal (RB) as the standard reference. ABDA solid (200 μM) was dissolved in DI water. The UCNPs@AIE@Sap nanoparticles (100 μg mL^{-1}) or RB (25 μg mL^{-1}) was then added in aqueous solution. The absorbance decrease of ABDA at 400 nm was recorded for different durations of light irradiation to obtain the decay rate of the photosensitizing process. The ROS yield is calculated using the following equation:

$$\Phi_{\text{nanoparticles}} = \Phi_{\text{RB}} (K_{\text{nanoparticles}} \bullet A_{\text{RB}}) / K_{\text{RB}} \bullet A_{\text{nanoparticles}}$$

Where $K_{\text{nanoparticles}}$ and K_{RB} are the decomposition rate constants of the photosensitizing process determined by the plot ln (A_0/A) versus irradiation time. A_0 is the initial absorbance of ABDA while A is the ABDA absorbance after different irradiation times. $A_{\text{nanoparticles}}$ and A_{RB} represent the light absorbed by UCNPs@AIE@Sap nanoparticles and RB, which are determined by integration of the absorption bands in the wavelength range of 400-800 nm. Φ_{RB} is the ROS quantum yield of RB, which is 75% in water. The decomposition rate constants of UCNPs@AIE@Sap nanoparticles ($K_{\text{nanoparticles}}$) and RB (K_{RB}) were calculated as 0.0003 and 0.0011, respectively. The integrations of the optical absorption in the wavelength range of 400-800 nm for RB (A_{RB}) and UCNPs@AIE@Sap ($A_{\text{nanoparticles}}$) were 78.513 and 23.636, respectively.

1.10 Cell culture and imaging

HeLa cell lines were incubated in RPIM 1640 media (Thermo Fisher Scientific from Shanghai, China) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in humidified environment containing 5% CO_2 at 37 °C. Prior to experiments, the medium was removed and the adherent cells were washed twice with PBS buffer to remove the remnant growth medium. The UCNPs@AIE@Sap nanoparticles were then added to the plates and final concentration of nanoparticles was 20 μg/mL. After 6 h post incubation,

the cells were washed three times with PBS buffer. The stained cells were then washed three times with PBS buffer, cells were fixed with 1 ml of 75% alcohol for 20 min, then the stained cells were then washed three times with PBS buffer to remove alcohol. 200μL of DAPI was added, stained for 30 minutes, the stained cells were then washed three times with PBS buffer and finally the cells were retained in 1 mL of PBS for bioimaging.

1.11 Intracellular ROS detection

The intracellular ROS generation under NIR irradiation was detected by using DCF-DA as an indicator and studied by confocal laser microscope system (CLMS). HeLa cells were cultured in the 35 mm glass-bottom dishes at 37 °C. After 80% confluence, the culture medium was removed and washed twice with PBS buffer. Following incubation with UCNPs@AIE@Sap nanoparticles (20 μg/mL) for 6 h in the dark, the cells were rinsed with PBS for three times and stained with 10 μM of DCF-DA. At 20 min post incubation, cells were washed three times with PBS and then exposed to 980 nm laser irradiation (1.5 W/cm^2) for different time. After irradiation, the cells were observed by CLMS. Pertaining to UCNPs@AIE@Sap nanoparticles detection, the excitation wavelength was 488 nm while the fluorescence emission range was collected at 598-662 nm; for DCF-DA detection, the excitation wavelength was 488 nm while fluorescence emission range was collected at 500-530 nm.

1.12 Cytotoxicity assays

To determine cytotoxicity, MTT-based cell viability assays were performed in 96-well plates. HeLa cells were seeded at a density of 5000 cells per well. After overnight incubation, cells were treated with UCNPs@AIE@Sap nanoparticles (20 μg/ml). After 4 hours of incubation, cells were exposed to 980 nm laser irradiation (1.5W/cm2) for 0-10 min. Then the cell medium was replaced with the fresh one and the cells were further cultured for 44 h. Then, the medium in the wells were removed and the cells were washed with PBS buffer and then incubated MTT solution (5 mg mL-1) in fresh medium (100 μL) for 3 h. After removing MTT solution, 100 μL of filtered DMSO was added into each well to dissolve all the formed crystals. The cell viability was accessed by means of MTT absorbance at 570 nm recorded using a microplate reader (Epoch, BioTek, Gene company Limited). The cell viability in each well was calculated from the obtained values as a percentage of control wells. The results were presented as a mean and standard deviation obtained from samples. Cell

viability of HeLa cells incubated with different concentrations of UCNPs@AIE@Sap nanoparticles in a dark environment was tested according to a similar protocol.

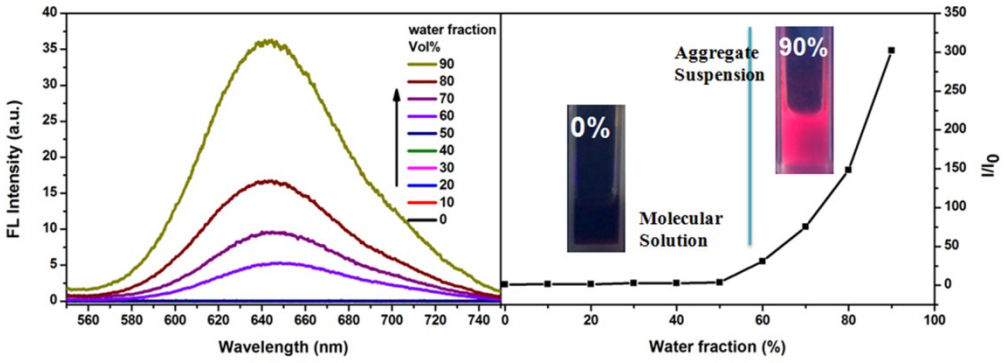


Fig. S1 Aggregation-induced emission properties of AIEgen.

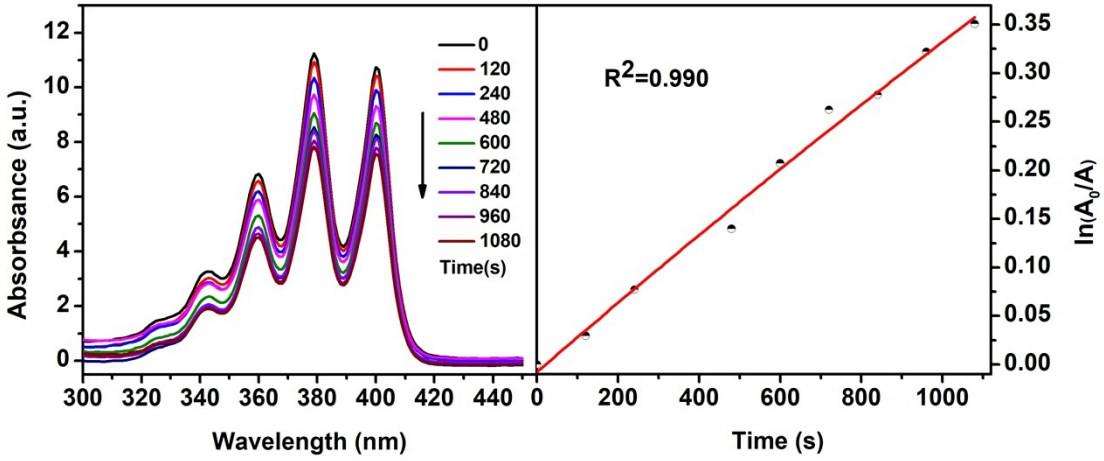


Fig. S2 UV-vis absorbance changes of the ROS indicator ABDA (200 mM) mixed with the UCNPs@AIE@Sap nanoparticles (100 μg mL^{-1}) upon varied irradiation periods at 980 nm and the decomposition rates of ABDA.

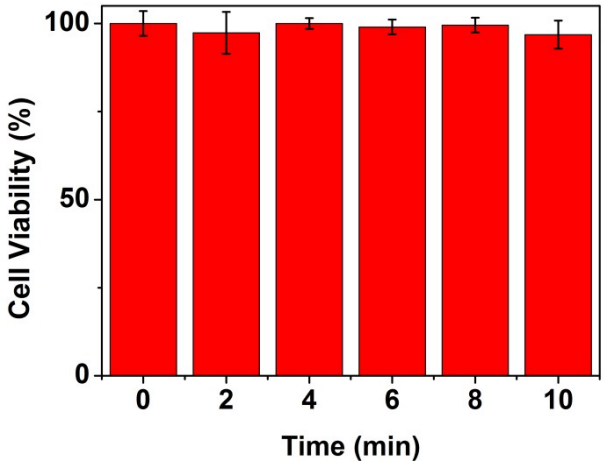


Fig. S3 Cell viabilities of HeLa cell under varied illumination duration periods in absence of UCNPs@AIE@Sap nanoparticles.

Reference

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