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Fabrication and biomedical applications of AIE active nanotheranostics through the combination of ring-opening reaction and formation of dynamic hydrazones

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1. Experimental procedure

1.1 The determination of cisplatin standard curve

Firstly, the experimental procedure for preparing 100 μg mL^{-1} cisplatin solution is described in follows: 10 mg cisplatin is dissolved in a little number of distilled water and removed into 100 mL volumetric flask and standardization (named as solution A). Secondary, 120 mg OPD is dissolved in a small quantity of DMF and removed into 100 mL volumetric flask and standardization (named as solution B). Finally, seven different concentration solution A (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mL) are added into volumetric flask (10 mL), and respectively added 5 mL B soliton, these mixed solutions are demarcated to 10 mL with HCl solution (pH=6.3). putting these volumetric flasks into water bath (90 ^{o}C) for 10 min, and detecting their absorbancy using solution B as reference under 710 nm.

1.2 Loading and release of cisplatin using HTPEG@ATPHE-*co*-BTDA

The loading efficiency of HTPEG@ATPHE-*co*-BTDA FONs for cisplatin is detected in this work. 10 mg cisplatin and 20 mg HTPEG@ATPHE-co-BTDA FONs are dissolved in PB buffer solution (Ph = 7.4), and stirring at dark environment for 24 h. Afterwards, the fluorescent materials loaded cisplatin are centrifuged. The solid materials and centrifugation (named as solution C) are preserved in dark. On the other hand, the solid materials with loading cisplatin are dissolved in 20 mL PB buffer solution (pH=5.5) and removed into dialysis bag. The dialysis bag is place in 150 mL beaker with 100 mL PB solution and stirring in dark environment. The solution in beaker is named as solution D.

1.3 The measurement of load and release efficiency of HTPEG@ATPHE-*co*-BTDA for cisplatin

The 0.3 mL solution C is diluted to 5 mL with HCl solution (pH = 6.3) and demarcated to 10 mL with solution B. This solution is put into water bath (90 °C) for 10 min, and used to determine its absorbance under 710 nm. This result is used to calculate the loading quantity of cisplatin. The solution D is used to detect release quantity of cisplatin and the experimental process is the same as previous.

1.4 Preparation of HTPEG@ATPHE-co-BTDA FONs

Thus-obtained HTPEG@ATPHE-co-BTDA polymers (230 mg) further dissolved in the mixture solution of THF (10 mL) and distilled water (10 mL) after ultrasonic treatment for 10 min. When solution was cooling, the THF solvent of mixed solution was removed by rotary evaporation, leading to form emulsion. The red solid could be obtained after centrifugation under 8000 rpm for 6 min, and red solid power could be obtained via freeze drying for further characterization and imaging application.

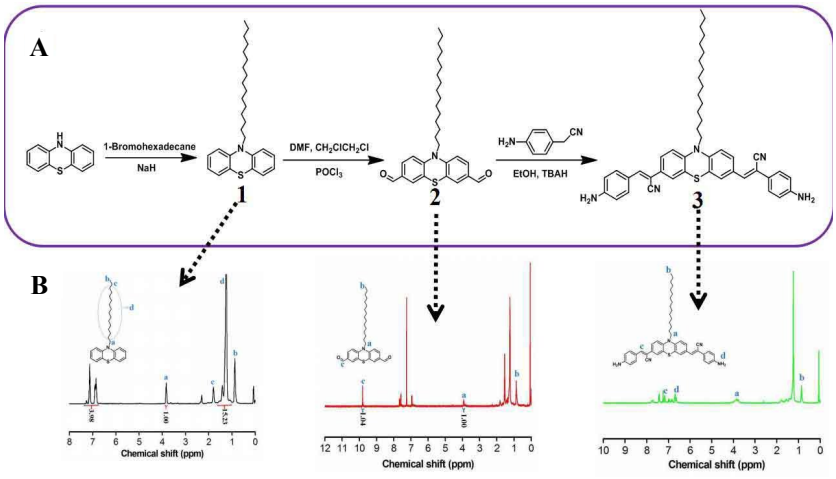

Fig. S1 (a) The synthetic route of ATPHE and its intermediates; (b) ^{1}H NMR spectra of ATPHE and its intermediates.

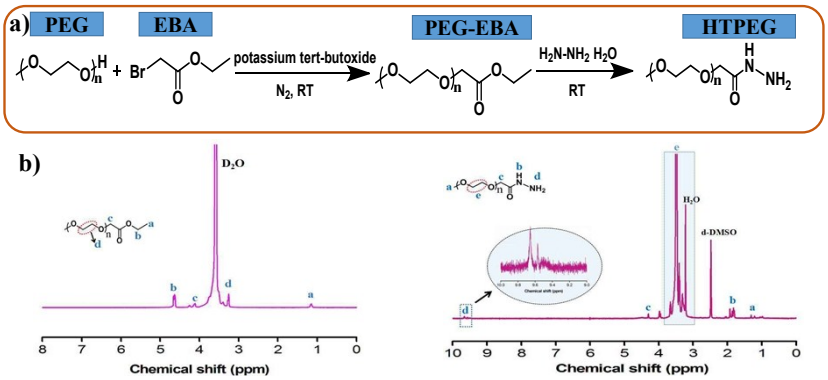

Fig. S2 The synthetic procedure of hydrazide-terminated PEG (HTPEG) and its intermediates was shown in **Fig. S2a**. **Fig. S2b** showed their ^{1}H NMR spectra. The obvious peak at 9.6 ppm was attributed to hydrazides, indicating the successful synthesis of HTPEG.

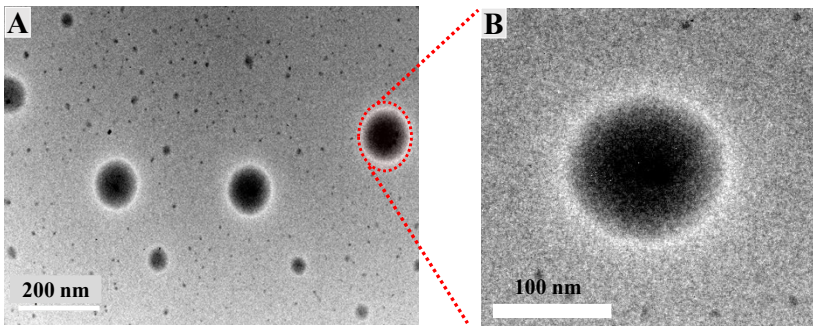

Fig. S3 (A) TEM images of HTPEG@ATPHE-*co*-BTDA FONs dispersed in water, scale bar = 200 nm; (B) amplifying TEM image of single FONs.

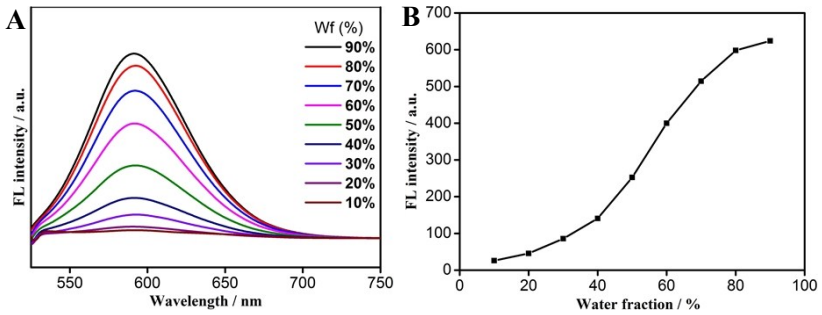

Fig. S4 the FL spectra of HTPEG@ATPHE-co-BTDA FONs dispersed in the mixed solvent of THF/H_2O with different ratios. (A) fluorescence spectra of fluorescent polymer in THF/H_2O with different valume ratios; (B) the trend curve of fluorescent intensity with addition of water.

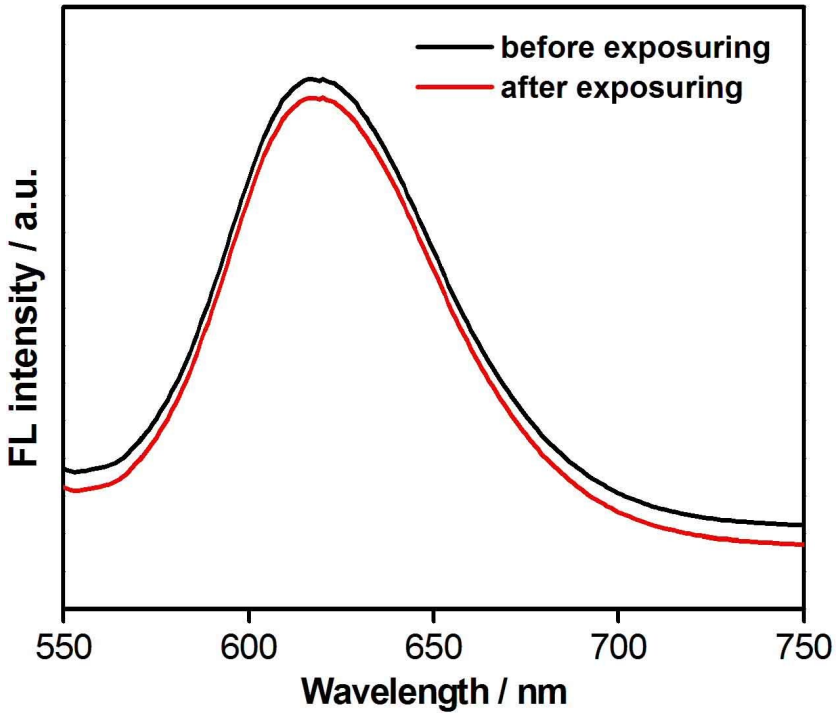

Fig. S5 Fluorescence spectra of HTPEG@ATPHE-*co*-BTDA FONs before and after continuously irradiated by UV lamp at 365 nm for 1 h. No obvious fluorescence intensity was found after UV irradiation, indicating the good photostability of HTPEG@ATPHE-*co*-BTDA FONs.

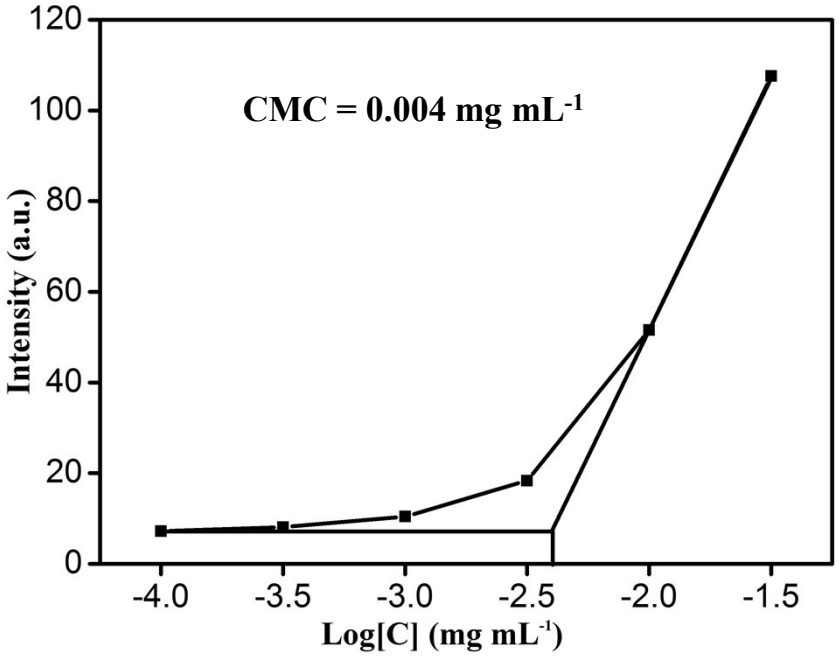

Fig. S6 The fluorescent intensity vs. the logarithm of the concentration of HTPEG@ATPHE-co-BTDA FONs dispersed in aqueous solution. (λ_{Ex} = 490 nm, λ_{Ex} = 591 nm). The final CMC is calculated about 0.004 mg mL^{-1}

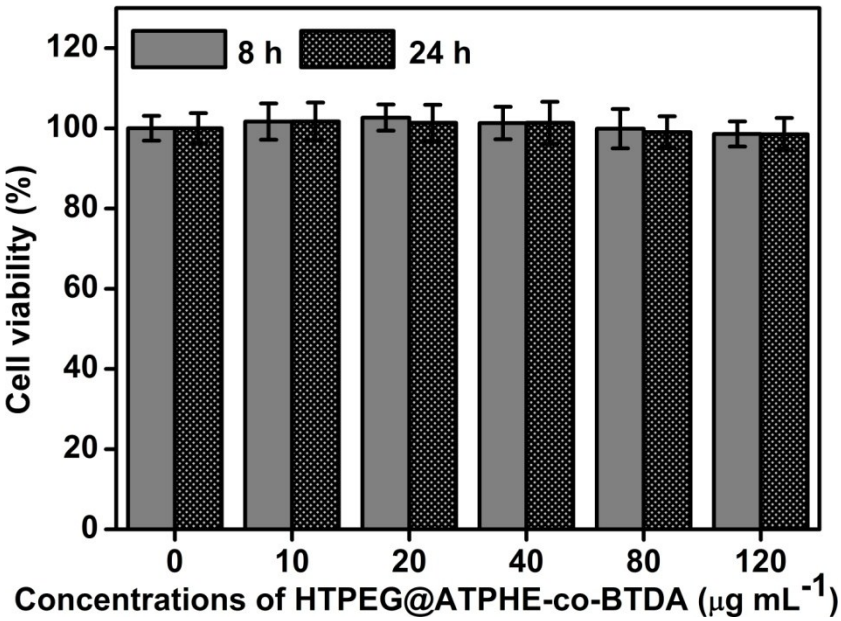

Fig. S7 Cell viability of HeLa cells after incubating with different concentration of HTPEG@ATPHE-*co*-BTDA FONs for 8 and 24 h.

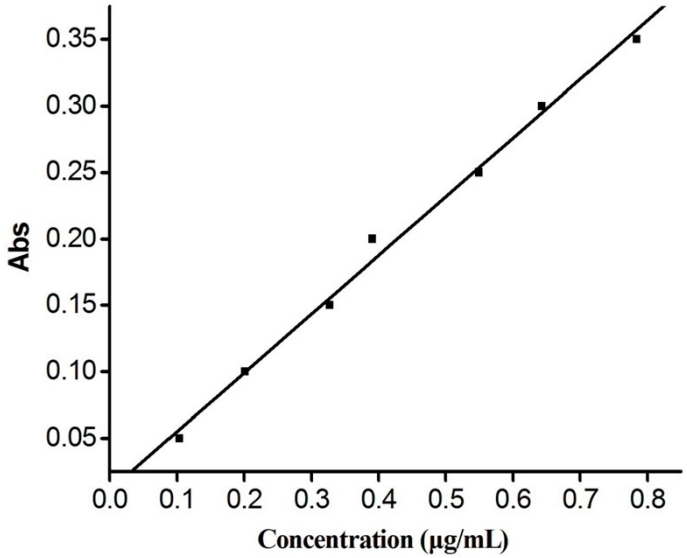

Fig. S8 The relationship of concentration/Abs in the sample of solution A.

Table S1 The absorbance of two solutions

	1	2	3
Solution A	0.005	0.005	0.006
Solution B	0.002	0.002	0.002

As shown in **Fig. S8**, we can see the direct relationship of sample concentration and absorbance, and the coefficient K in this picture is calculated as 0.4418. Based on this relationship, we can calculate the cisplatin quantity loaded on the surface of HTPEG@ATPHE-co-BTDA FONs is about 6 mg using 20 mg fluorescent materials. The loading efficiency of cisplatin is approximately 60% for HTPEG@ATPHE-co-BTDA FONs. The calculated formula is described by follows: $C_0/C_d=A_0/A_d$ (C_0 is original concentration of cisplatin, A_0 is absorbance of original cisplatin, A_d is absorbance of solution C).