Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

DNA tetrahedron-loaded natural photosensitizer with aggregation-induced emission characteristics for boosting fluorescence imaging-guided photodynamic therapy

Wei Zhu, Ke Ma, Zhi-Chao Yan*, Qian Wu, Dong Wang*, and Ben Zhong Tang*

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Experimental section

Job plot

Job plot was established Job plot was established to determine binding ratio between DNA-T and PaH in the complex.[1] While keeping the total concentration of the PaH and DNA-T at 1 uM in TM buffer (10 mM Tris-HCl, 5 mM $MgCl_2$, pH=8.0), the difference in absorbance of the PaH@DNA-T complexs and the free PaH was recorded after incubation for 0.5 h at room temperature. Linear regression analysis was carried out with curve fitting software.

ROS generation measurement in solution

A commercial ROS indicator DCFH-DA was employed to detect the ROS generation of PaH and PaH@DNA-T in aqueous solution under white light irradiation (50 mW/cm^2).[2] Firstly, the indicator was activated: 2 mL NaOH (1×10^{-2} M) and 0.5 mL DCFH-DA in ethanol (1×10^{-3} M) were mixed and stirred under dark at room temperature for 30 min. The solution was added to 10 mL of 1×PBS and stored in dark at 4 ^{o}C until use. By the time, DCFH-DA was hydrolyzed to DCFH. Then DCFH (4 uM) in PBS was further diluted to 5×10^{-6} M in the sample solution of PaH (1×10^{-6} M) or PaH@DNA-T (PaH, 1×10^{-6} M and DNA-T, 1.2×10^{-6} M) for measurement by PL instrument in a dark room. The fluorescence of DCFH triggered by ROS under white light irradiation was measured at different time intervals. The PL spectra were obtained with 488 nm excitation and emission was collected from 500 nm to 620 nm. The fluorescence intensity at 525 nm was recorded to indicate the ROS generation rate.

Cell culture and staining

Human hepatoma cells (HepG2), human normal hepatocyte cells (LO2), human hepatoma cells (SMMC-7721), and mouse breast cancer cells (4T1) were purchased from ATCC company, and cultured in 1640 culture medium containing 10% FBS and 1% antibiotics (penicillin-streptomycin). The culture condition was 37 °C in a cell incubator containing 5% CO_2. For the cell stain study, different cells were seeded and cultured in a glass-bottom dish for 24 h. PaH and PaH@DNA-T were respectively added into medium and incubated with cancer cells at 37 °C for 0.5 h-2 h. Then, the cells were gently washed with PBS for five times. Finally, the cells were measured by CLSM. Conditions: excitation wavelength was 405 nm, emission range was 500 nm - 700 nm for PaH and PaH@DNA-T.

Intracellular ROS detection

HepG2 cells were seeded and cultured in glass bottom dish for 24 h. The cells were first stained with PaH or PaH@DNA-T at 37 °C for 2 h, then washed with PBS for five times. Afterward, the cells were stained by 10 μM DCFH-DA at 37 °C for 30 min to transfer DCFH-DA to DCFH. After washing for three times, the cells were exposed to white light (50 mW/cm^2) for 5 min, followed by CLSM measurement. Conditions: excitation wavelength was 405 nm, emission range was 500 nm - 700 nm for PaH and PaH@DNA-T; excitation wavelength was 488 nm, emission range was 500 nm - 550 nm for DCFH.

Data Analysis

The investigators were not blinded to the group allocation. Quantitative data was expressed as mean ± standard deviation. Statistical comparisons were made by unpaired Student's t-test between two groups. P value < 0.05 was considered statistically significant. The Origin 9.0 software was used for graph plotting. Each experiment included at least three replicates.

Table S1. DNA-T and ds-DNA oligonucleotides details.

P1	5'–ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA–3'
P2	5'–TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC–3'
P3	5'–TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC–3'
P4	5'–TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT–3'
S1	5'–GTTTCTGTTCTCCCTGC–3'

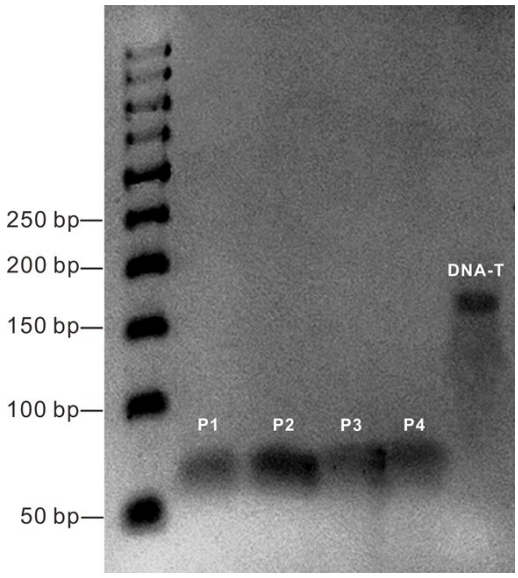

Figure S1. Native PAGE (2%) to verify assembly of DNA-T.

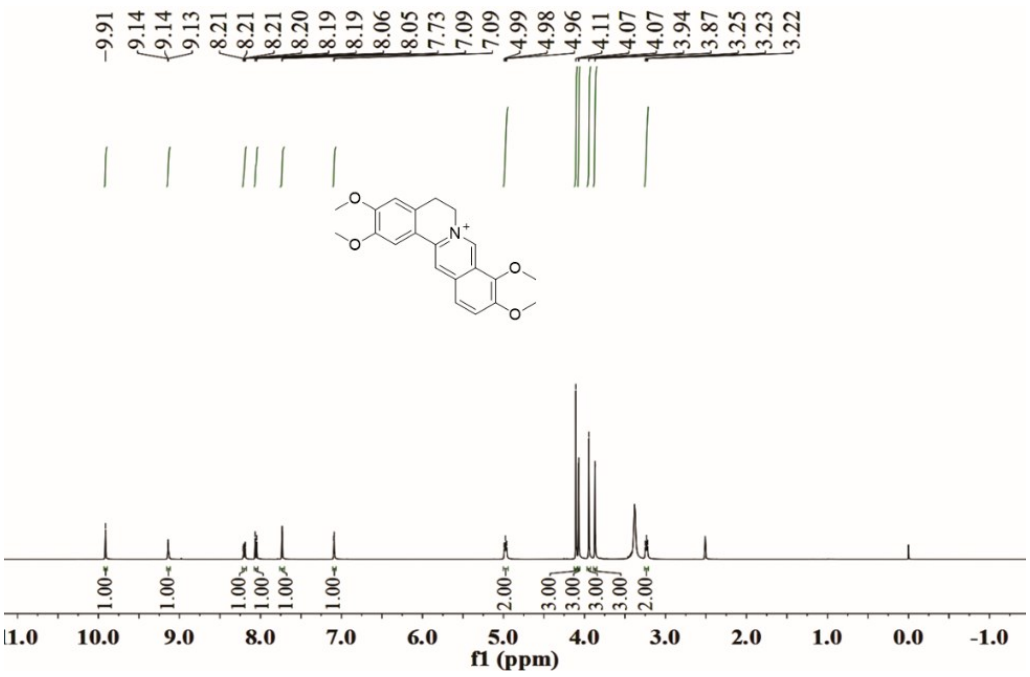

Figure S2. ^{1}H NMR spectrum of PaH.

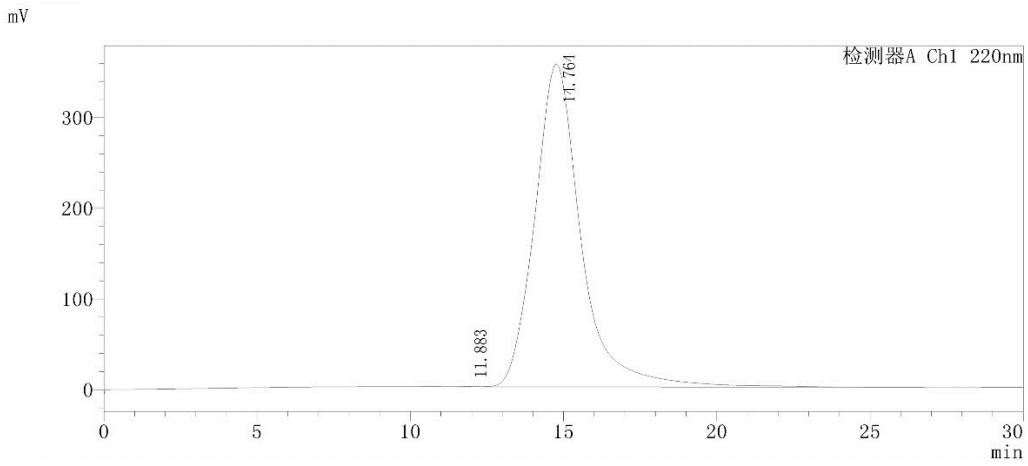

Figure S3. HPLC spectrum of PaH.

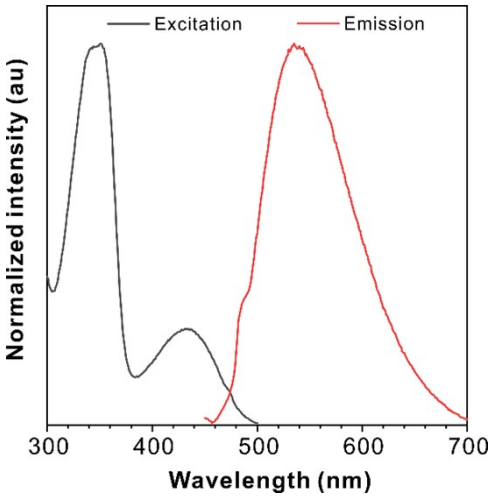

Figure S4. The excitation and emision spectra of PaH in EtOH.

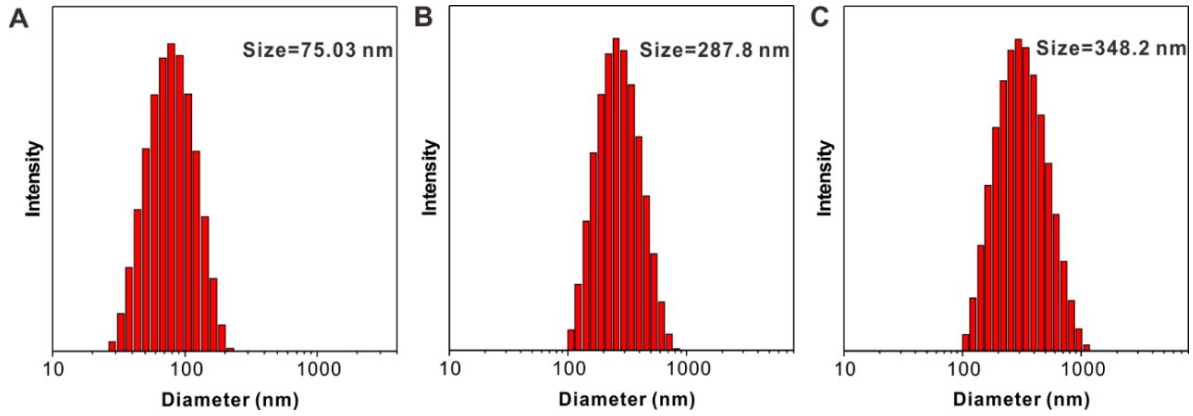

Figure S5. DLS results of PaH in different THF/water mixtures: f_{THF} = 10% (A); f_{THF} = 50%(B); f_{THF} = 80% (C). Concentration: 10 uM.

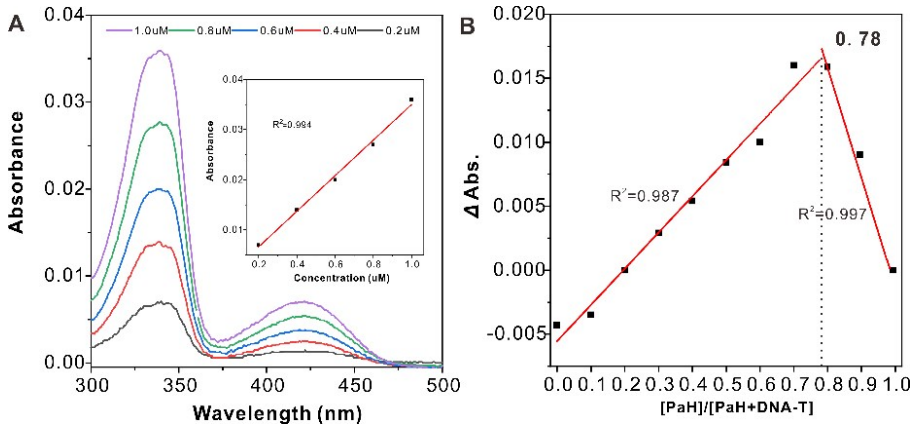

Figure S6. (A) UV–vis absorption spectrum of different concentrations of PaH in water. (B) Job's plot for the PaH@DNA-T complex.

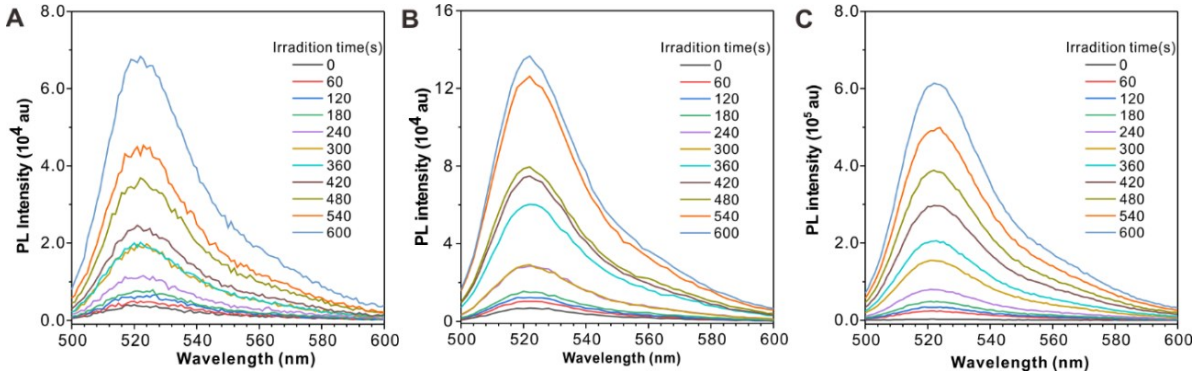

Figure S7. PL spectra of DCFH (A), mixtures of DCFH and PaH (B), and mixtures of DCFH and PaH@DNA-T (C) under white light irradiation for different times.

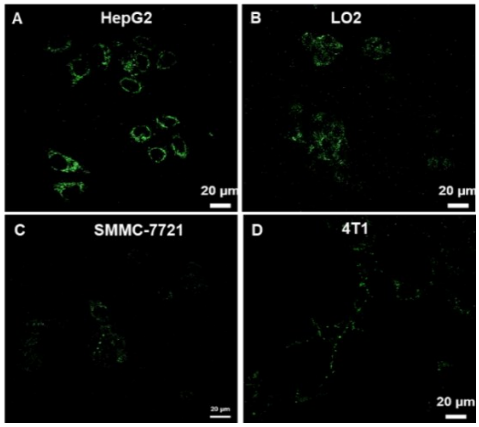

Figure S8. CLSM images of HepG2 (A), LO2 (B), SMMC-7721 (C), and 4T1 (D) stained with PaH (40 μM) for 30 min. Scale bar: 20 μm.

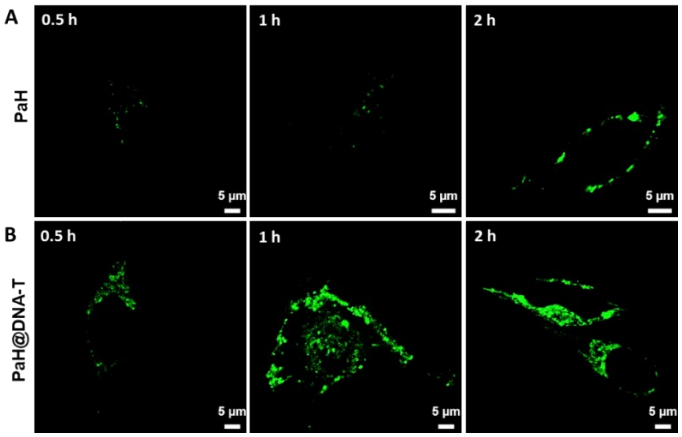

Figure S9. CLSM images of HepG2 cells after incubated with PaH (5 μM) (A)and PaH@DNA-T (PaH/DNA-T=5 mM/100 nM) (B) for 0.5 h, 1 h and 2 h respectively. Scale bar: 5 μm.

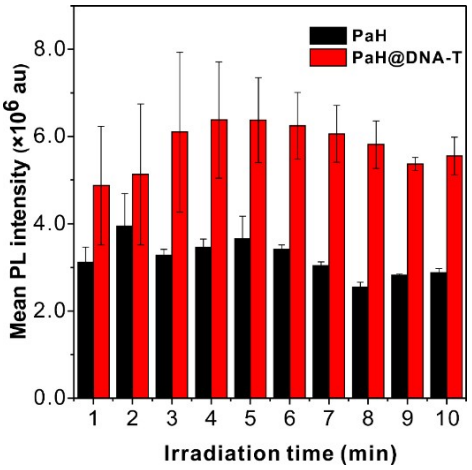

Figure S10. Mean PL intensity of HepG2 cells staining with PaH and PaH@DNA-T upon 405 nm laser irradiation with different time (1-10 min).

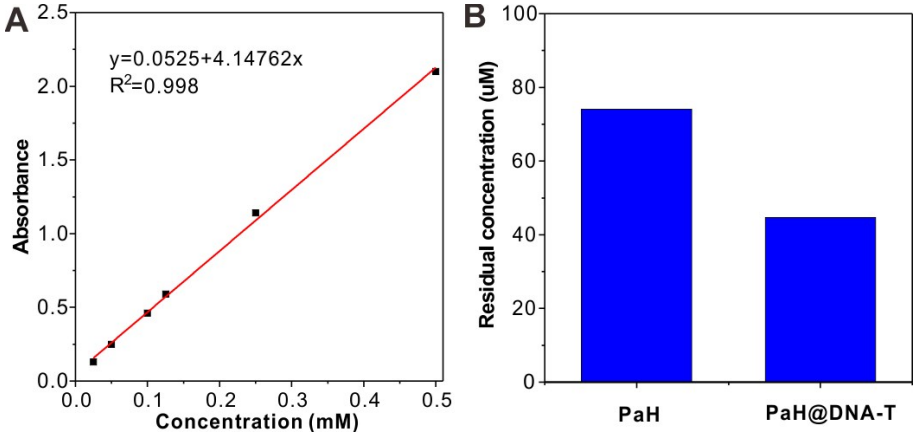

Figure S11. (A) UV–vis absorption spectrum of different concentrations (0.0125 to 0.5 mM) of PaH in water. (B) The residual concentration of PaH and PaH@DNA-T stained with HepG2 cells for 2 h.

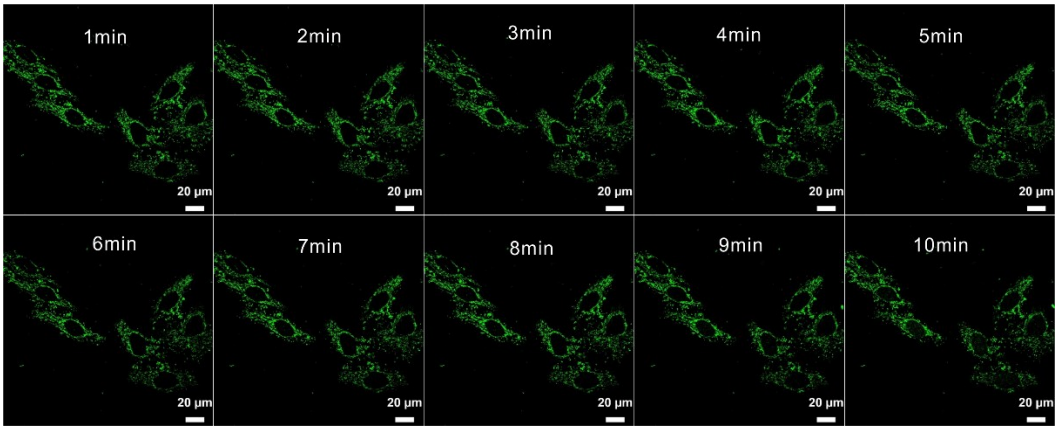

Figure S12. Confocal images of HepG2 cells stained with PaH@dsDNA under different irradiation time (1-10 min) with 405 nm laser irridination (0.5 W) . Scale bar = 20 μm.

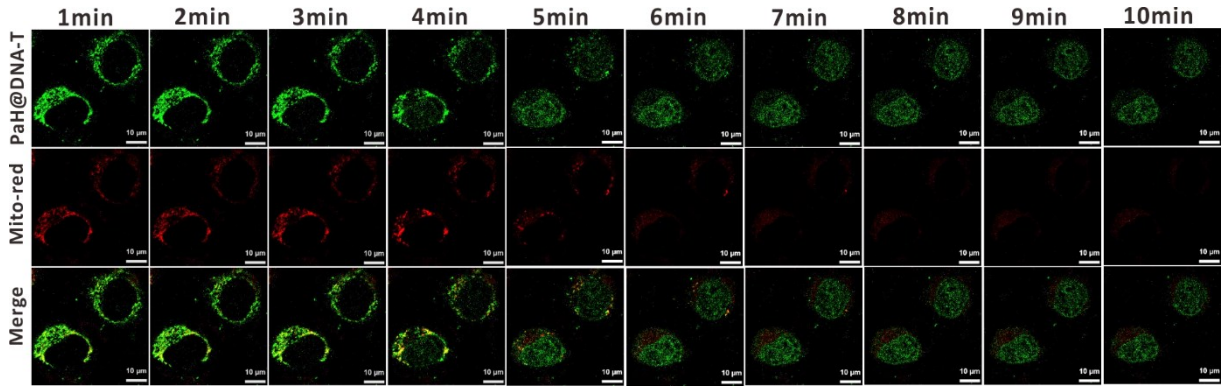

Figure S13. Representative mitochondria colocalization images of PaH@DNA-T (PaH/DNA-T=40 mM/100 nM) and MitoTracker Red by CLSM in HepG2 human liver cancer cells under different irradiation time (1-10 min) with 405 nm laser (0.5 W).

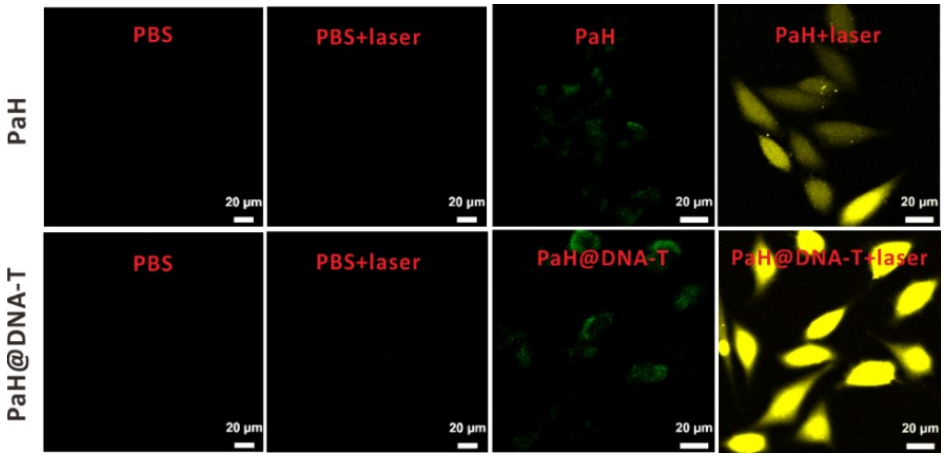

Figure S14. CLSM imaging of intracellular ROS in HepG2 cells using DCFH-DA (10 uM) with PaH (40 μM) and PaH@DNA-T (PaH/DNA-T=40 mM/100 nM) upon white light irradiation for 5 min. Scale bar: 20 μm.

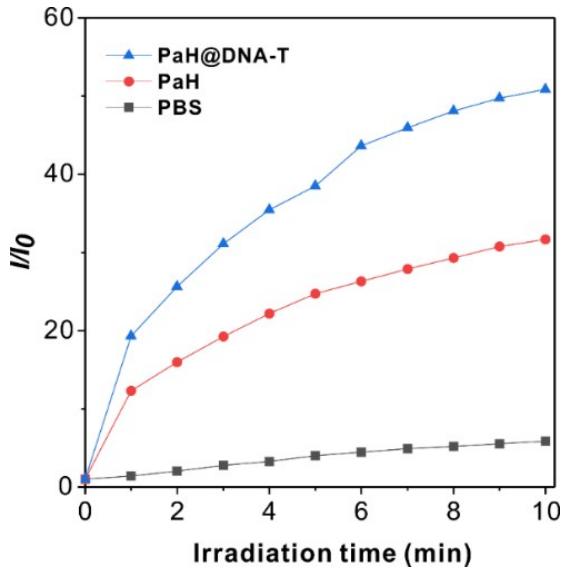

Figure S15. ROS generation of PBS, PaH, and PaH@DNA-T in HepG2 cells upon white light irradiation for 5 min.

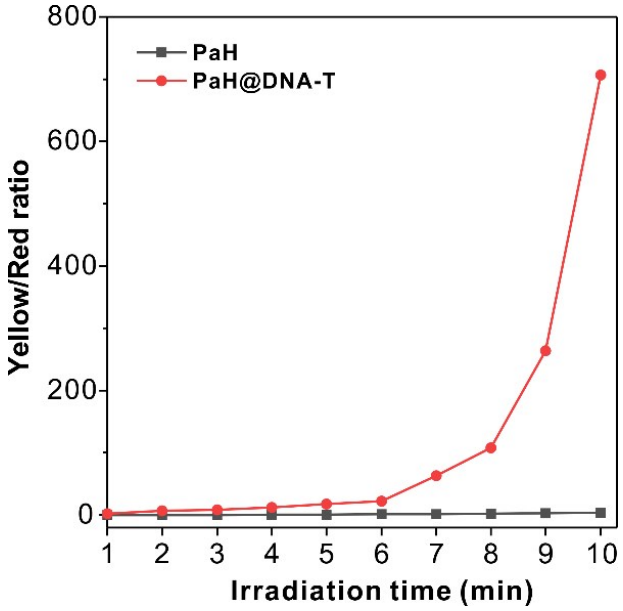

Figure S16. The plot of relative PL intensity (yellow/red) of HepG2 cells staining with PaH and PaH@DNA-T under different irradiation time (1-10 min) with 405 nm laser (0.5 W). Yellow represents monomers and red represents J-aggregates.

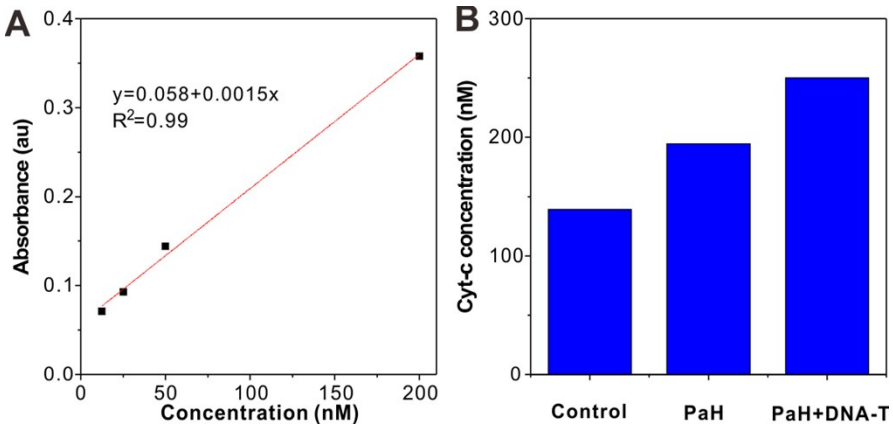

Figure S17. (A) Correlation relationship between absorbance intensity and the cyt-c concentration. (B) Cyt-c concentration of the control, PaH, and PaH+DNA-T.

Reference

[1] K. R. Kim, D. Bang, D. R. Ahn, Nano-formulation of a photosensitizer using a DNA tetrahedron and its potential for in vivo photodynamic therapy, *Biomater. Sci.*, 2016, **4**, 605-609.

[2] D. Wang, M. M. S. Lee, G. Shan, R. T. K. Kwok, J. W. Y. Lam, H. Su, Y. Cai, B. Z. Tang. Highly efficient photosensitizers with far-red/near-infrared aggregation-induced emission for in vitro and in vivo cancer theranostics. *Adv. Mater.* 2018, **30**, 1802105