Supporting information

Materials and Measurements

Most reagents, including perylene and 5,10,15,20-tetra(4-pyridyl)-21H,23H-porphine (H2TPyP), polystyrene (MW, 250000 Da), THF, dimethyl sulfoxide (DMSO), and the disodium salt of 9,10-anthracenedipropionic acid (ADPA), were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was also obtained from Sigma-Aldrich and was used after drying in vacuo for 24 h. DMEM culture medium, fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS), trypsin-EDTA (0.5% trypsin, 5.3 mM EDTA tetra-sodium), and the antibiotic agents penicillin–streptomycin (100 U/ml) were purchased from Invitrogen (USA). High-purity water with resistivity greater than 18.4 MΩ. cm was obtained from an in-line Millipore RiOs/Origin water purification system. Unless otherwise noted, all chemicals were obtained from commercial suppliers and used without further purification.

Preparation of Doped NPs

Doped NPs were prepared using an anti-solvent precipitation technique[1]. In a typical procedure, 6 mM perylene and 6 mM H2TPyP were prepared in THF solution. These two solutions were then mixed in a 10 mL volumetric flask with different volume ratios. Next, 200 μL of the mixture solution was quickly dispersed into 5 mL high-purity water under vigorous stirring at room temperature.

Characterization of Doped NPs

The size and morphology of doped NPs were investigated using SEM (HITACHI S-4300) and TEM (JEM-2100). The SEM samples were prepared by placing a few drops of sample solutions onto a Si substrate and then drying in air. Before SEM examination, a 2 nm layer of Au was deposited on samples. The TEM samples were prepared by dripping the NPs solution onto a carbon-supported copper grid (FEI Tecnai, Inc.) and drying it at room temperature before observation. The hydrodynamic size of NPs was measured in an aqueous solution using a DLS instrument (Malvern Zetasizer Nano ZS).

UV-vis and Fluorescence Measurements

All UV-vis and fluorescence spectra in this work were respectively recorded in Hitachi U-3900 and Hitachi F-4600. The perylene NPs, H_2TPyP NPs, and doped NPs were excited at 438 nm with a xenon lamp. The autocorrected emission spectra were recorded from 460 nm to 700 nm. The free perylene and H_2TPyP in THF were respectively examined at an excitation light of 438 and 408 nm. The fluorescence lifetime data of perylene NPs, H2TPyP NPs, and doped NPs were obtained using Edinburgh Analytical Instruments F900.

$^1O_2$ Detection by ADPA

The generation of $^1O_2$ was detected chemically according to literature[2] using the disodium salt of ADPA (Sigma) as a $^1O_2$ sensor. ADPA was bleached by $^1O_2$ to its corresponding endoperoxide. The reaction was monitored spectrophotometrically by recording the decrease of OD at 378 nm. The ADPA (70 μL) in high-purity water (1 mg/mL) was mixed with 2 mL of the doped NPs diluted 50 times. Pure perylene NPs and pure H_2TPyP NPs dispersed in high-purity water were used as the control samples. The solutions were irradiated with a xenon lamp light source, and their optical densities at 378 nm were recorded every 10 min in a UV-Vis spectrophotometer.

$^1O_2$ Detection by ESR Spectrum

The ESR spectra were obtained using a Bruker ESP-300E spectrometer operating at room temperature. Using 2,2,6,6-tetramethylpiperidine as the spin-trapping agent[3], which is selective for $^1O_2$. The operating conditions were as follows: microwave bridge, X-band with 100 Hz field modulation; sweep width, 100 G; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; and microwave power, 5 mW. The samples were injected into the specially made quartz capillaries for ESR analyses. They were directly irradiated in the quartz capillaries by a xenon lamp laser light source. The ESR spectra were obtained after 10 min irradiation, which show the typical three-line ESR spectra.

In Vitro Studies with Tumor Cells

The HeLa cells were cultured with DMEM (Invitrogen, USA) supplemented with 10 % FBS (Hyclone Company, South Logan, UT), penicillin (100 μg/mL), and streptomycin (100 μg/mL; Gibco, Grand Island, NY, USA) in 5% CO_2 at 37 °C in a humidified incubator.

Uptake and Imaging

The cells were trypsinized and resuspended on 120 mm culture plates, and 2 mL of the medium was combined with 1.0 mL cell suspension. The cells were then seeded on 24-well plates, which were then placed in an incubator at 37 °C overnight with 5% CO_2 for 24 h. The cells were carefully washed with PBS, and 2 mL of fresh medium was added on the plates. Pure perylene NP, pure H2TPyP NP, and doped NP solutions were added to each plate and carefully mixed. The treated cells were returned to the incubator (37 °C, 5% CO_2) for 4 h. After incubation, the plates were washed thoroughly with sterile PBS. Finally, confocal imaging of cells was performed using a Nikon laser scanning confocal microscope. Imaging was carried out under 458 nm laser excitation, and the emission was collected in the range of 600 to 700 nm. All images were recorded separately in each fluorescence channel and merged afterwards.

In Vitro PDT Efficacy Assay

The cells were seeded on 96-well plates. After growing overnight, the cells were used for experiments. The predetermined concentrations of pure perylene NPs, pure H_2TPyP NPs, and doped NPs were added to the designated wells. The final concentration of these NPs on each plate ranged from 0.4688 to 60 μM. After 24 h incubation in the dark at 37 °C, the wells were rinsed three times with sterile PBS, and then 0.2 mL of fresh medium was added. The cells were photoirradiated for 10 min with broadband visible light using a xenon lamp (150 W) equipped with a filter passing light of 400 to 700 nm. The power at the cell level was 100 mW/cm2. The plates were incubated at 37 °C in the dark for 24 h. Cell viability was investigated by MTT assay. The doped NPs without light radiation were operated for comparative study.

MTT assay

The absorbance of formazan (produced by the cleavage of MTT by dehydrogenases in living cells) at 490 nm is directly proportional to the number of live cells. MTT was dissolved in sterile PBS at 5 mg/mL, and 20 μL was added to each well and incubated at 37 °C for 4 h with 5% CO_2. The medium was carefully removed and 150 μL of DMSO was added. The absorbance was measured at 490 nm using a microplate reader (Multidkan MK3, Thermo). The cells incubated with serum-supplemented medium represent 100% cell survival. Four replicate wells were run for each concentration and light dose. Each experiment was repeated three times.

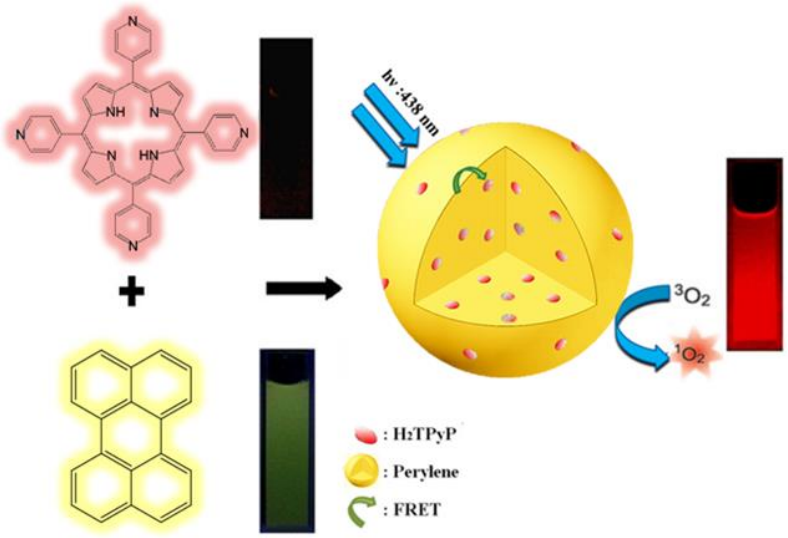

Fig. S1 The illustration of FRET process between H_2TPyP and perylene.

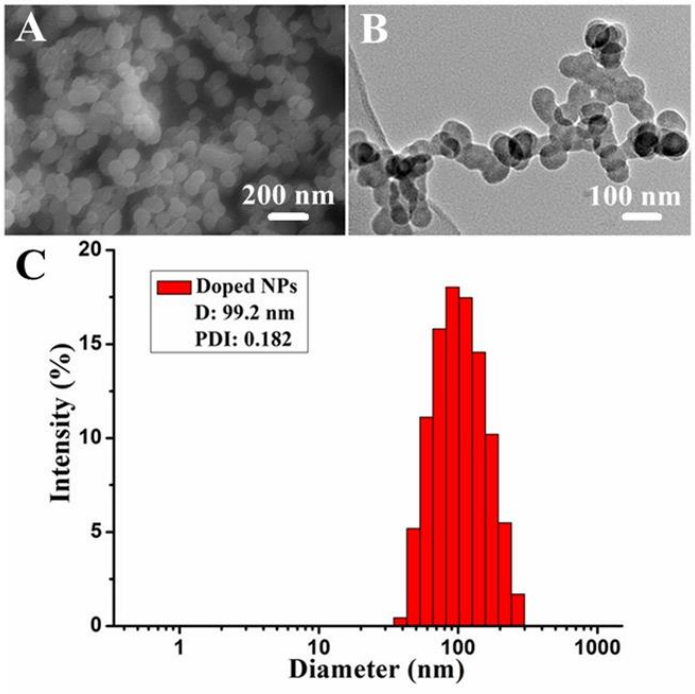

Fig. S2 Characterization of doped NPs. A) SEM micrograph of doped NPs. B) TEM micrograph of doped NPs. C) DLS measurement of doped NPs in water.

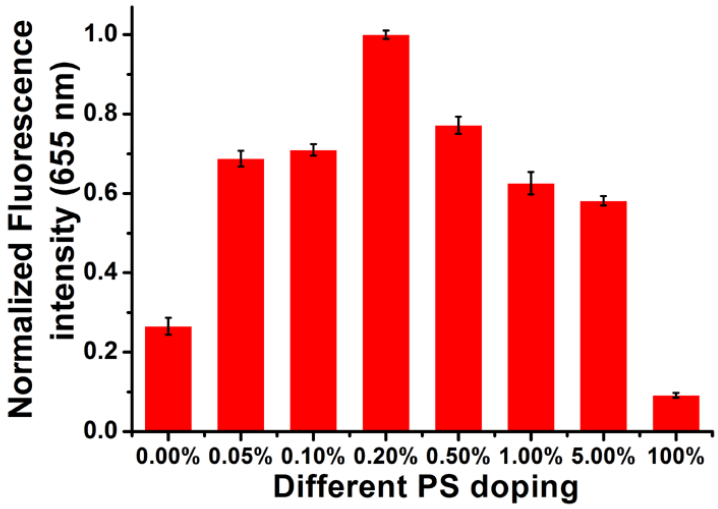

Fig. S3 Fluorescence intensity of doped NPs (655 nm) as a function of dopant concentration.

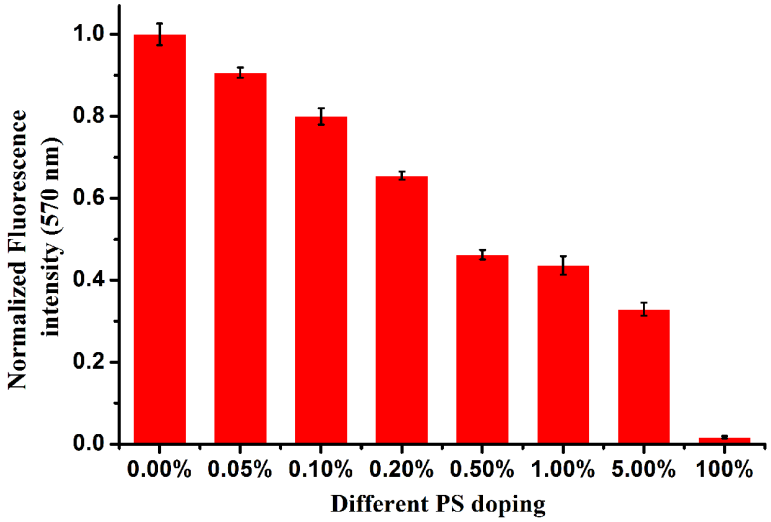

Fig. S4 Fluorescence intensity of doped NPs (570 nm) as a function of dopant concentration.

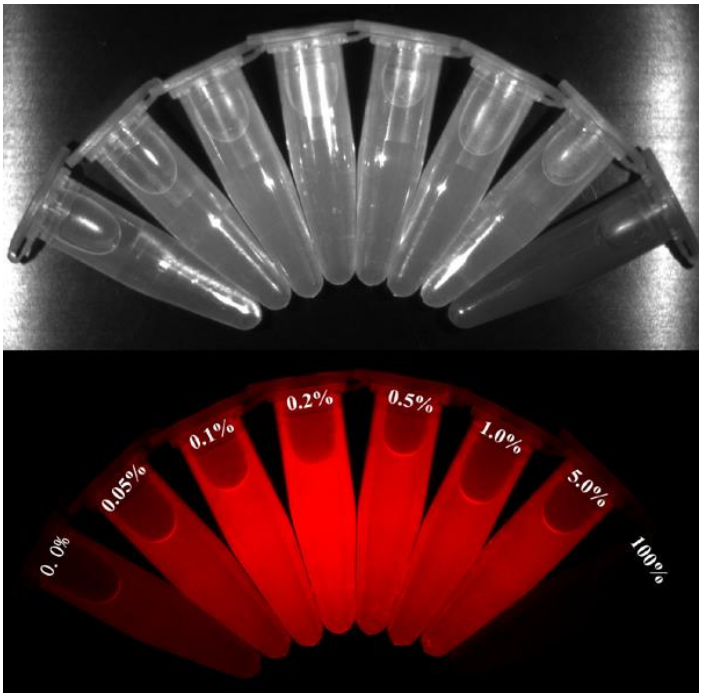

Fig. S5 The optical photograph of doped NPs with different doping ratios.

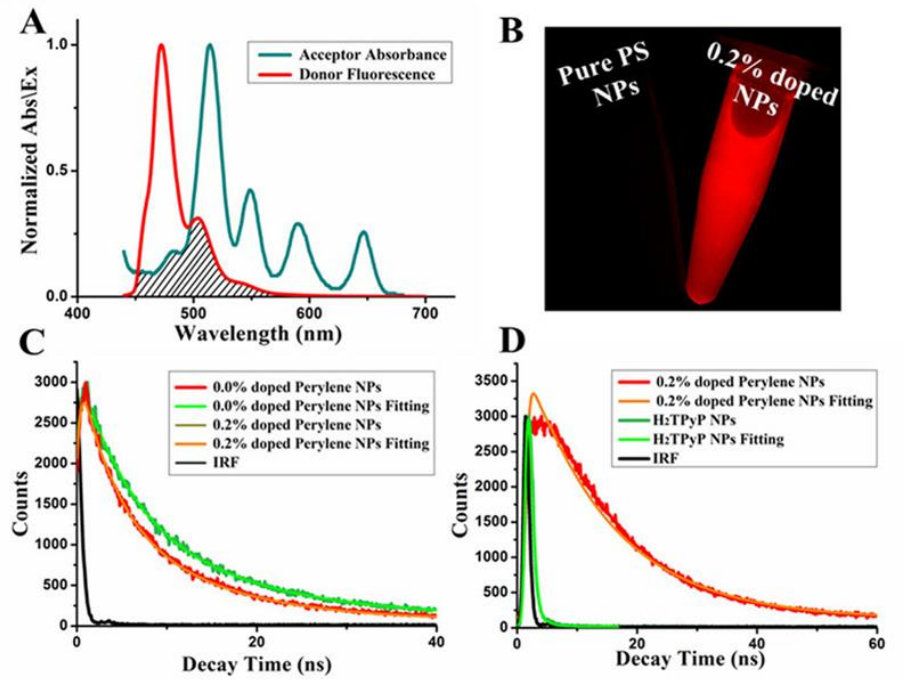

Fig. S6 FRET occurrence in 0.2% doped NPs. A) Spectral overlap between the normalized emission spectrum of perylene matrix (donor, red line) and the normalized absorption spectrum of porphyrin (acceptor, dark green line). B) Fluorescence photo of 0.2% doped NPs in comparison to pure H_2TPyP NPs. C) Fluorescence lifetime measurements of 0.2% PS-doped perylene NPs before the doping of PS H_2TPyP, D) and after the doping of PS H_2TPyP.

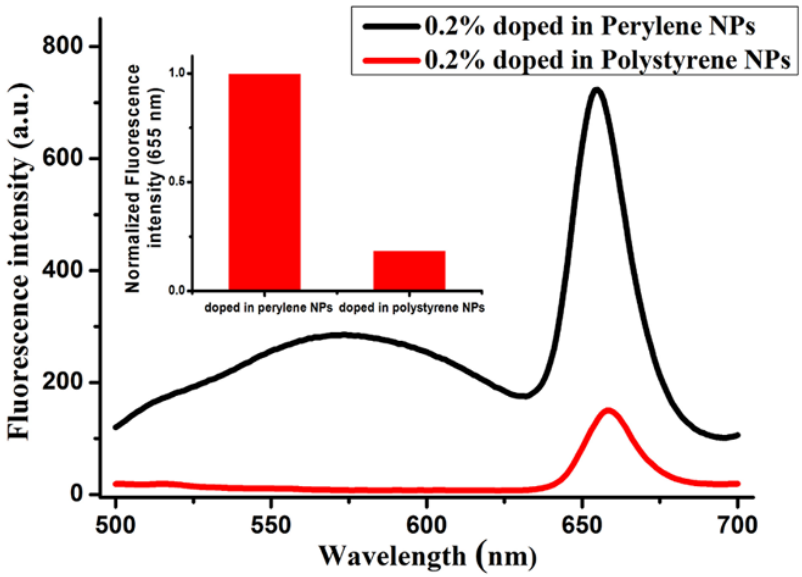

Fig. S7 Fluorescence emission intensity of 0.2% H_2TPyP-doped perylene NPs and 0.2% H_2TPyP-doped polystyrene NPs. The inner picture shows the intensity at 655 nm.

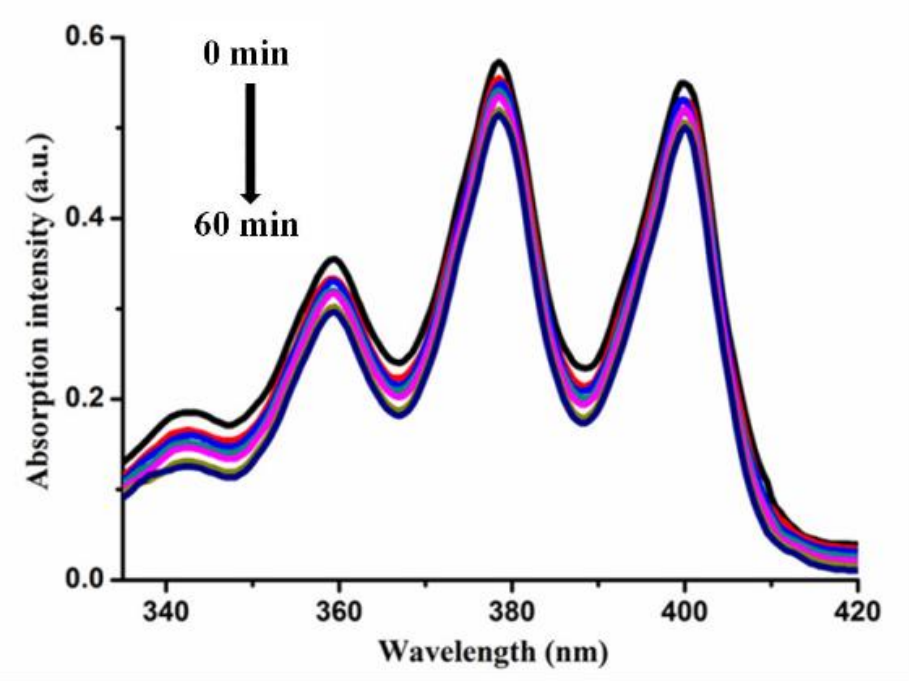

Fig. S8 Time-dependent bleaching of ADPA caused by $^1O_2$ generated by pure perylene NPs.

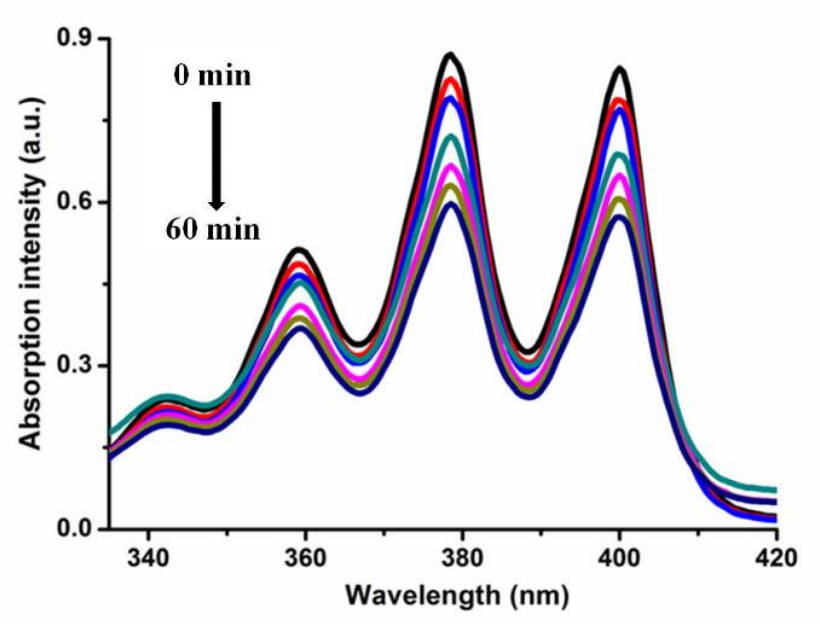

Fig. S9 Time-dependent bleaching of ADPA caused by $^1O_2$ generated by pure H_2TPyP NPs.

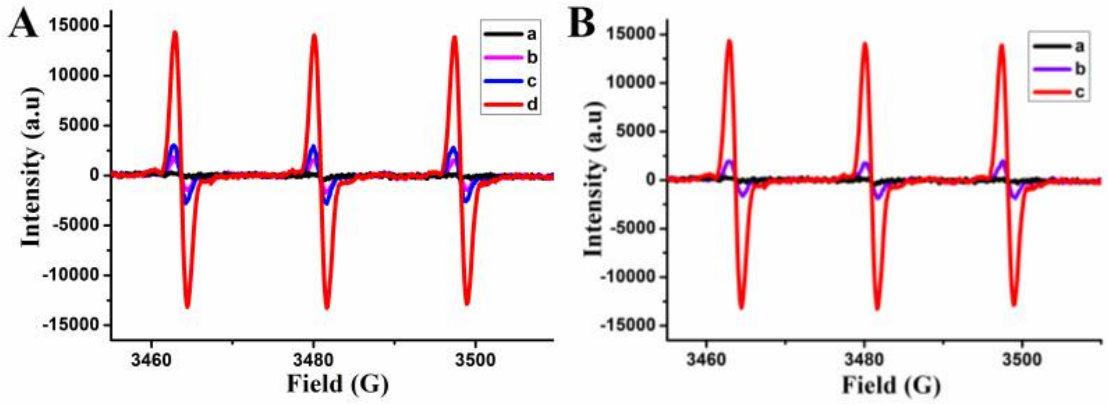

Fig. S10 A) ESR spectrum signal intensity of b) pure perylene NPs, c) pure H_2TPyP NPs, and d) 0.2% doped NPs. B) ESR spectrum signal intensity of b) 0.2% doped in polystyrene NPs, c) 0.2% doped in perylene NPs, and a) ESR spectrum signal intensity without light exposure.

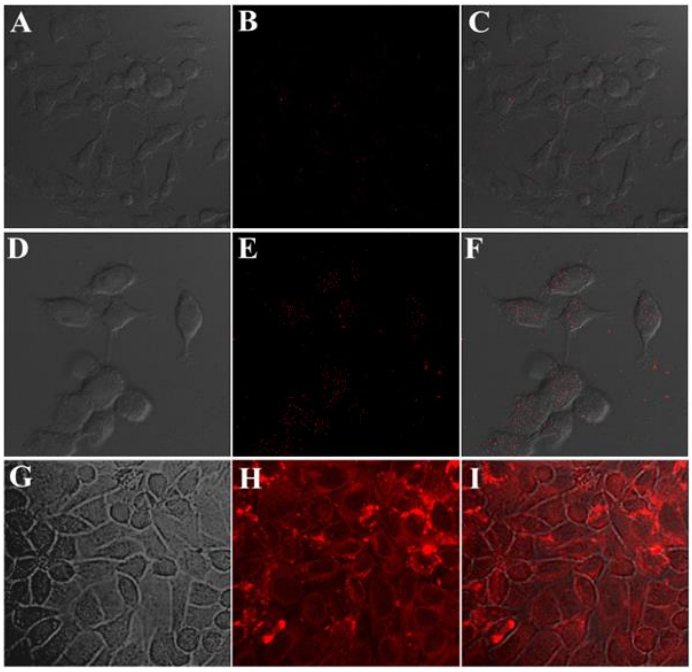

Fig. S11 Subcellular localization of pure H_2TPyP NPs (upper panel), pure perylene NPs (middle panel) and doped NPs (lower panel) observed by fluorescent CLSM. A), D), G) Bright-field channel. B), E), H) NPs channel. C), F), I) Overlap images.

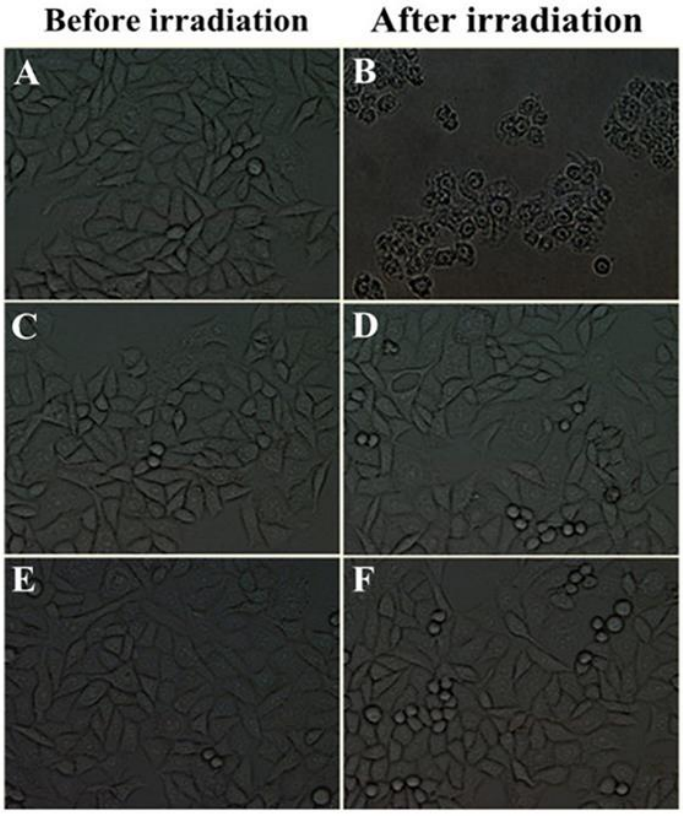

Fig. S12 Bright field images of HeLa cells treated with doped NPs (upper panel), pure perylene NPs (middle panel) and pure H_2TPyP NPs (lower panel), A), C), E) before irradiation; B), D), F) after irradiation with light of 400 nm to 700 nm for 20 min followed by 24 h of incubation at 37℃in the dark.

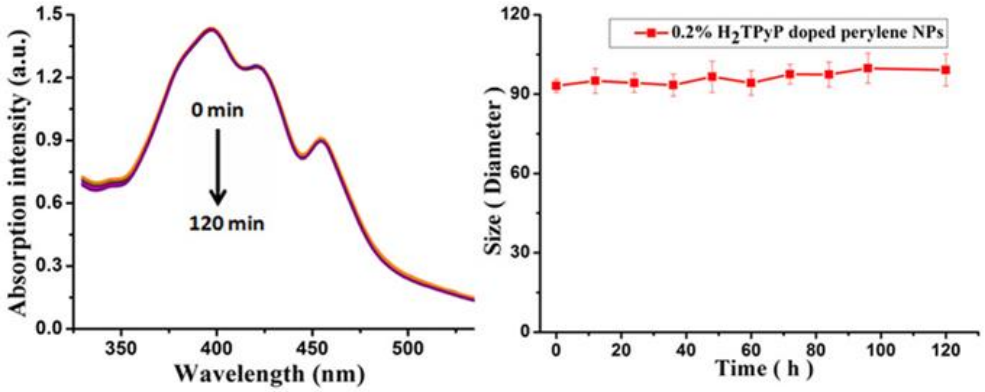

Fig. S13 Stability of doped NPs. A) Absorption intensity of 0.2% PS-doped perylene NPs after photoirradiation. B) DLS measurement of 0.2% PS-doped perylene NPs in the aqueous environment.

References:

1.W. Li, Y. Yang, C. Wang, Z. Liu, X. Zhang, F. An, X. Diao, X. Hao, X. Zhang, *Chem. Commun.*, 2012, **48**, 8120.
2. B. A. Lindig, M. A. J. Rodgers, A. P. Schaap, *J. Am. Chem. Soc.* ,1980, **102**, 5590.
3. J. Moan, E. Wold, *Nature*, 1979, **279**, 450.