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Supporting Information for

Rod-like BODIPY nanomaterials with Enhanced Photodynamic Activity

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1. Experimental Procedures

Materials. IBDP were prepared following the protocol has been reported. Pluronic F127 was purchased from Shanghai Yuanye Biological Technology Co., Ltd. 2',7'-Dichlorofluorescence diacetate (DCFH-DA) and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) were purchased from Shanghai Beyotime Biotechnology Co., Ltd.. Cell viability (live dead cell staining) assay kit was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. All of the other Chemicals and reagents were acquired from commercial sources without further purification, unless otherwise noted. All the solvents were purified according to the standard methods whenever needed. Milli-Q water was collected from a Milli-Q system (Millipore, USA).

The instruments for characterizations. Diameter and diameter distribution of the nanoparticles were determined by Malvern Zeta-sizer Nano for dynamic light scattering (DLS). The measurement was carried out at 25 ^{o}C and the scattering angle was fixed at 90^{o}. Transmission electron microscopy (TEM) images were taken by a JEOL JEM-1011 (Japan) at the accelerating voltage of 100 kV. To prepare specimens for TEM, a drop of NPs solution (0.1 mg mL^{-1}) was deposited onto a copper grid with a carbon coating. The specimens were air-dried and measured at room temperature. UV−vis absorption spectra were recorded via a Shimadzu UV-2450 UV−vis scanning spectrophotometer. Fluorescence emission spectra were conducted on a LS-55 fluorophotometer. The cell confocal images were obtained using Zeiss confocal laser microscope (ZEISS LSM 700). Flow cytometry was carried out on Guava easyCyte 6-2L Base System (Merck Millipore, USA).

Preparation of nanoparticles. IBSA NRs were prepared using a reprecipitation method. In a typical procedure, we prepared the IBDP solution (200μg/mL) with acetone as solvent. Then the IBDP solution (400 μL) was quickly dropwise dispersed into 4 mL of milli-Q water with vigorous stirring at room temperature for 30 min. Then the solution was dialyzed against Milli-Q water for 24 h, the cutoff molecular weight of the dialysis bags is 3500.

IBPF NPs were prepared using Pluronic F127 to assemble IBDP in water. In a typical procedure, firstly, the Pluronic F127 solution (200 μL) was mixed with the IBDP solution (400 μL), then the mixing solution was added dropwise to the 4 mL of milli-Q water with vigorous stirring at room temperature for 30 min. Then the solution was dialyzed against Milli-Q water for 24 h, the cutoff molecular weight of the dialysis bags is 3500.

Singlet oxygen $^1O_2$ generation ability detection. To evaluate the singlet oxygen generation ability of IBDP, indocyanine green (ICG) was employed as scavenger and monitored by time-dependent electronic absorption spectroscopy. IBDP dissolved in DMF (4 μg/mL) were blended with 21 μL ICG water solution (1 mg/mL) and then irradiated with 20 mW/cm^2 of a 450 nm light source. The absorption intensity of the ICG was detected every 30 seconds.

The singlet oxygen $^1O_2$ generation abilities of the corresponding nanoparticles IBPF NPs and IBSA NRs were also tested. The procedures were the same for that of the small organic molecules, unless the solution was changed to water.

Cell culture. HeLa cells and HepG2 cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cells were propagated to confluence in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma) and heat-inactivated fetal bovine serum (FBS, GIBCO), and maintained at 37^{o}C in a humidified atmosphere of 5% CO_2 for further cell experiments.

Biocompatibility of IBDP, IBPF NPs and IBSA NRs in vitro by MTT Assay. Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 8×10^3 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL of DMEM containing predetermined concentrations of IBDP, and then incubated for 24 h, followed by MTT assays to measure the live cells. Cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. The cells incubated with DMEM were used as the control. The cell viability (%) =A sample /A control ×100%. We also detected the cytotoxicity of IBDP for different culture time. The procedures were the same for that of IBPF NPs and IBSA

NRs.

Cellular uptake and tracking in vitro. The Cellular uptake of nanoparticles was examined by using a confocal laser scanning microscope (CLSM). Cells harvested in a logarithmic growth phase were seeded in 6-well plates (a sterile cover slip was put in each well) at a density of 2.5×10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by 2 mL of DMEM containing nanoparticles and incubated for different hours at 37°C, and further washed using PBS for 3 times.

For the CLSM detection, the cells were fixed with 4% of paraformaldehyde solution for 10 min. After that, DAPI (4,6-diamidino-2-phenylindole) was added for another 5 min incubation to locate the nucleus. Later, the cells were washed with PBS and observed using confocal laser scanning microscopy (CLSM, Zeiss LSM 700).

For the flow cytometry detection, the cells were washed with PBS and treated with trypsin. The harvested cells were suspended in PBS and centrifuged at 1000 rpm for 5 min. The supernatants were discarded and the cells were washed again with PBS to remove the medium. After washing, the cells were re-suspended in 500 μL PBS before analysis by Guava easyCyte 6-2L Base System (Merck Millipore, USA).

Intracellular ROS assays. To investigate the intracellular $^1O_2$ production ability of IBDP, IBPF NPs and IBSA NRs, the intracellular generation of ROS was determined by the fluorescence by CLSM. HeLa or HepG2 cells were treated with IBDP/ IBPF NPs/ IBSA NRs for 6 h and then irradiated with 450 nm LED lamp at a power density of 20 mW/ cm^2 for 30 min. Then, the medium was replaced. DCFH-DA with a final concentration of 10 μM was added and the cells were incubated for 20 min. Later, the DCFH-DA solution was removed and then repeatedly washed 3 times with PBS. Finally, the cells were observed as soon as possible *via* CLSM (ex= 488 nm).

In vitro **PDT studies of IBPF NPs and IBSA NRs.** The dark cytotoxicity as well as photocytotoxicity of IBDP/IBPF NPs/IBSA NRs were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa or HepG2 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 8×10^3 cells per well and incubated in DMEM for 24 h. The media were then replaced by different concentrations of IBDP/IBPF NPs/IBSA NRs. After 6 h

incubation, the medium was replaced by fresh DMEM and the cells were irradiated by a LED lamp with a wavelength of 450 nm at 20 mW/cm^2 for 30 min. The dark cytotoxicity (without irradiation) was monitored without irradiation at the same time as control. After that, the cells were incubated for another 24 hours. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plates were incubated for another 4 h at 37 °C, followed by removal of the culture medium containing MTT and addition of 150 μL of dimethyl sulfoxide (DMSO) to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 5 min, and the absorbance of formazan product was measured at 490 nm by a Bio-Rad 680 microplate reader. Cell viability (%) was calculated based on the following equation: (Asample/Acontrol) × 100 %, where Asample and Acontrol were denoted as absorbancies of the sample well and control well, respectively.

Live/ dead cell staining assays. To further confirm the phototoxicity and PDT efficacy of IBDP/IBPF NPs/IBSA NRs, HeLa or HepG2 cells were stained with the calcein-AM/propidium iodide (PI) to determine their viabilities. Briefly, the cells were incubated with IBDP/IBPF NPs/IBSA NRs for 6 h and then irradiated with 450 nm LED lamp at a power density of 20 mW/cm^2 for 30 min. The cells treated with the same concentration of IBDP/IBPF NPs/IBSA NRs were set as the controls at the same time. After that, the cells were further incubated at 37^{o}C for additional 24 h. The cells were stained with calcein AM (green for living cells) and PI (red for dead cells) for 30 min at room temperature, and imaged with a confocal fluorescence microscope.

2. Figures and Tables

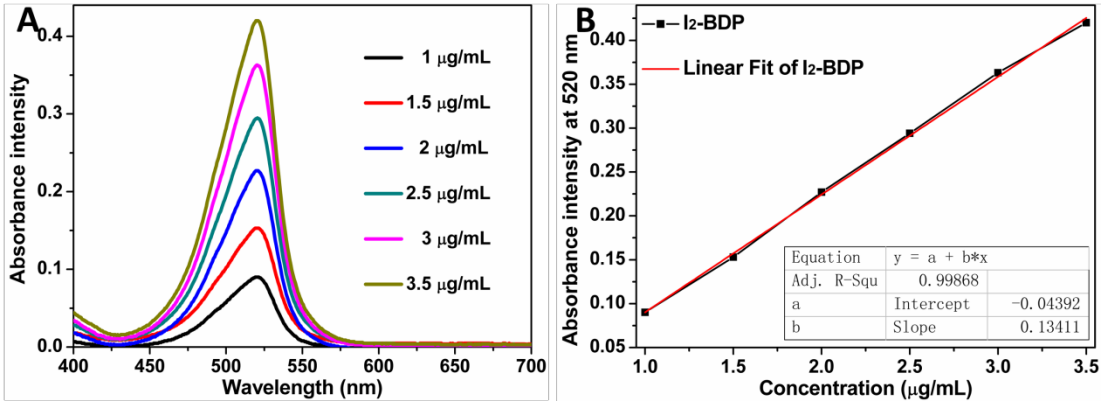

Figure S1. A) and B) Standard absorbance curve of IBDP. (The absorbance of IBDP molecules at 520 nm (from a mixture of acetone and water (v/v = 4:1)) as a function of IBDP concentration.)

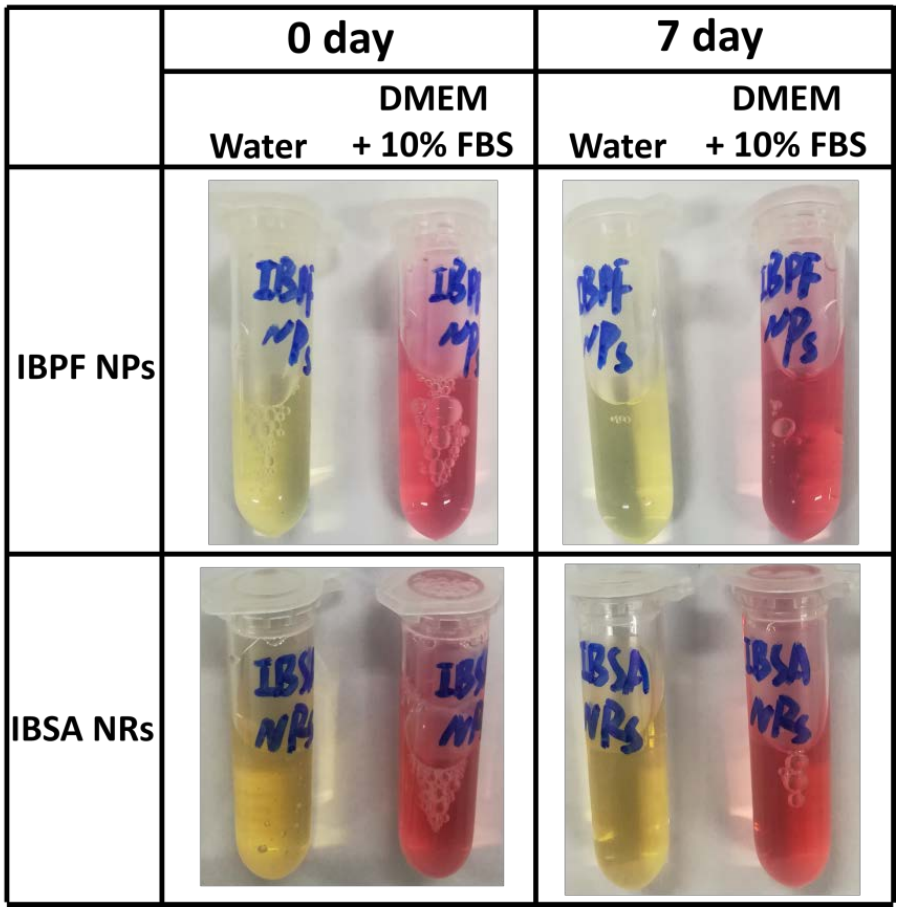

Figure S2. Photographs of IBPF NPs and IBSA NRs under different conditions at 0 day and 7 day, respectively.

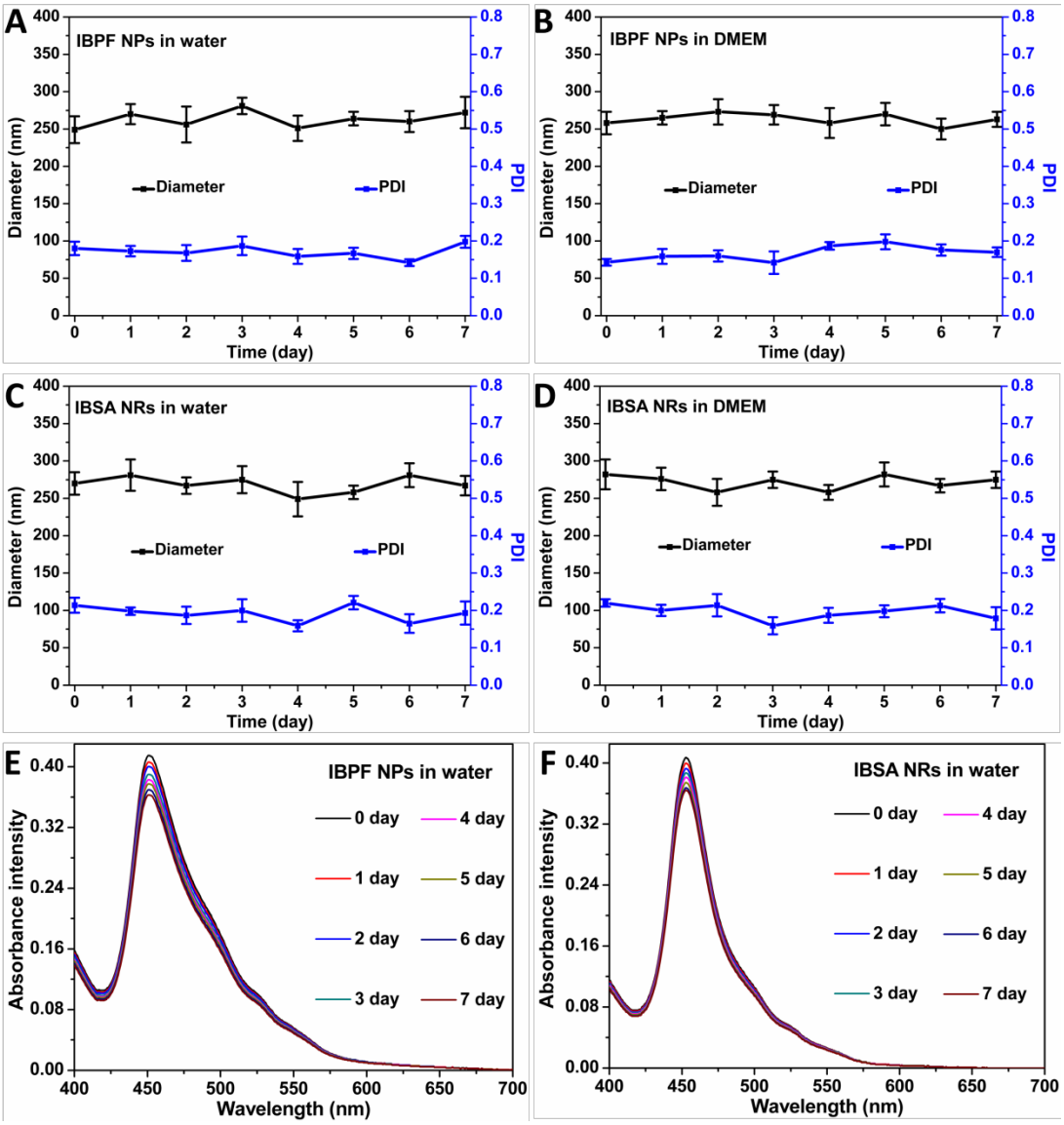

Figure S3. Average size changes of IBPF NPs (A and B), IBSA NRs (C and D) during 7 days in different solutions. The data are shown as the mean values ± standard deviation (SD) (n=3). The absorbance intensity of E) IBPF NPs and F) IBSA NRs during 7 days.

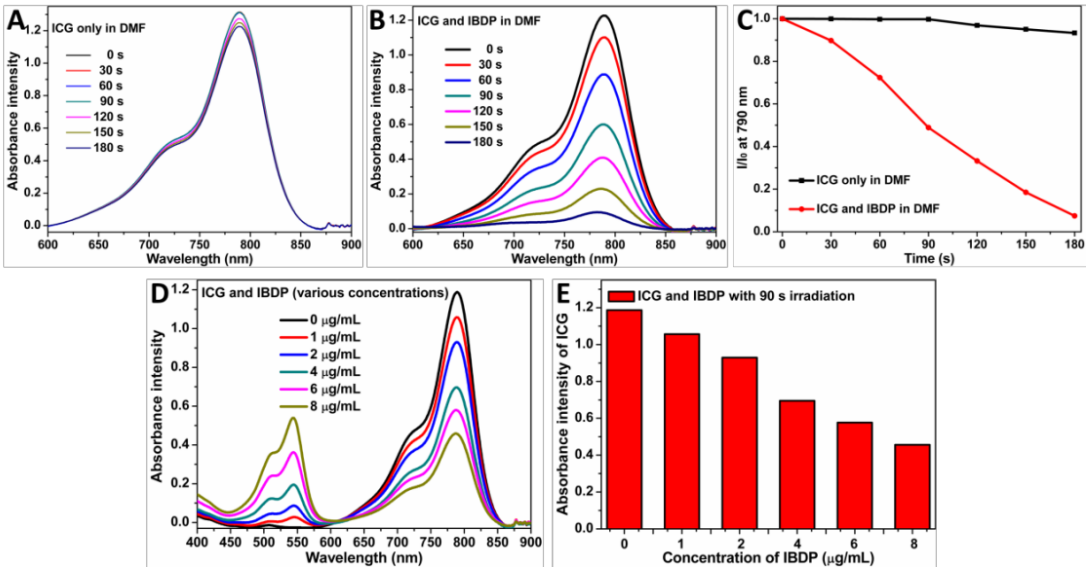

Figure S4. A-B) Singlet oxygen generation by IBDP in DMF monitored by disappearance of UV absorbance of ICG with an LED light power density of 20 mW/cm^2 over 180 seconds. C) The quantification of the $^1O_2$ generation ability of ICG blank (black), IBDP (red). D) Singlet oxygen generation by IBDP with different concentrations in DMF monitored by disappearance of UV absorbance of ICG with an LED light power density of 20 mW/cm^2 for 90 seconds.

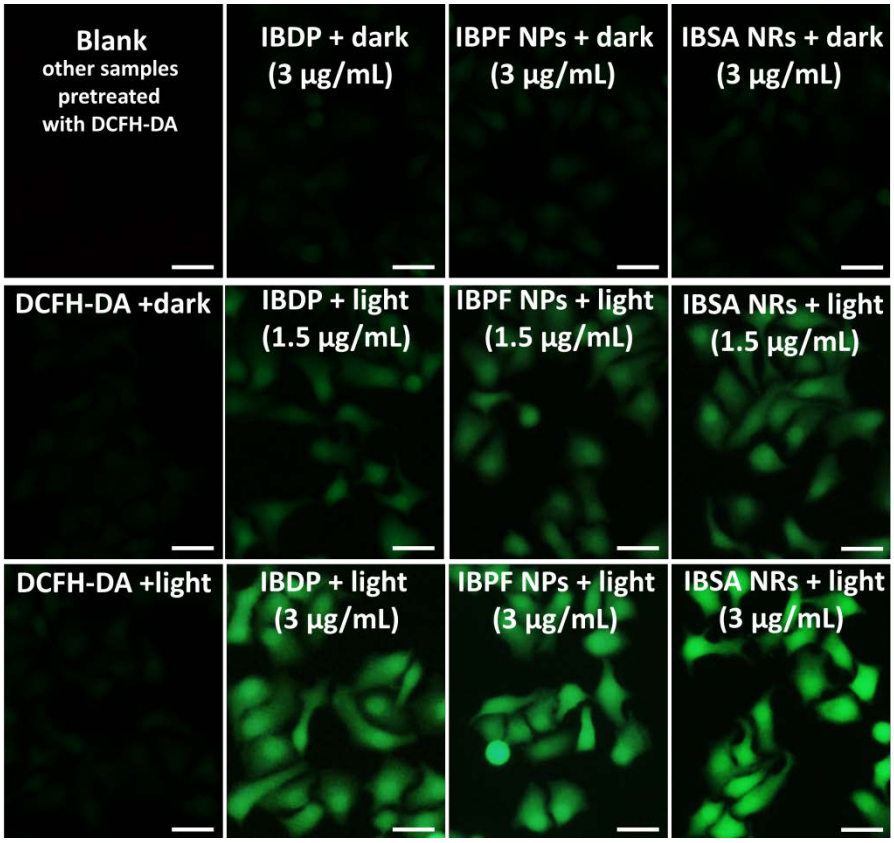

Figure S5. Intracellular DCF fluorescence detection by CLSM of HepG2 cells (pretreated with DCFH-DA) after incubation with IBDP, IBPF NPs and IBSA NRs (IBDP: 1.5 or 3 μg/mL) for 6 h at 37 °C, and then under light irradiation (20 mW/cm^2) for 25 min. The cells without any treatment were set as the controls. Scale bars represent 20 μm in all images.

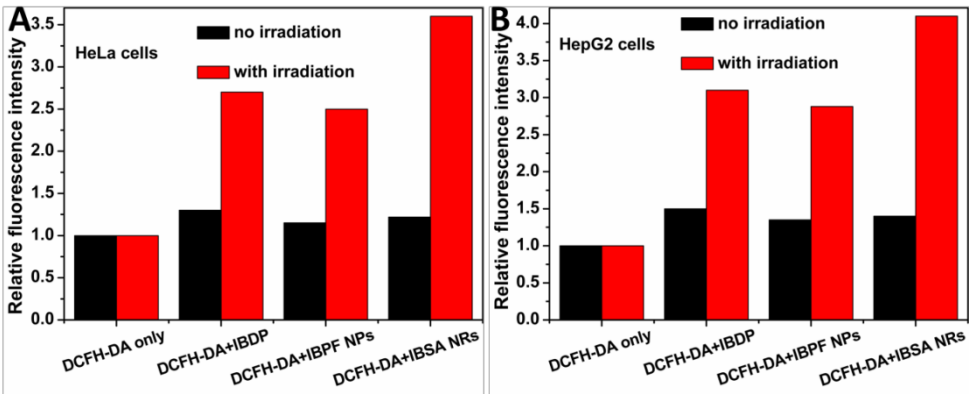

Figure S6. Semi-quantitative results of intracellular fluorescence intensity of DCF after the treatment of IBDP, IBPF NPs and IBSA NRs in dark or under irradiation via the image analysis software ImageJ.

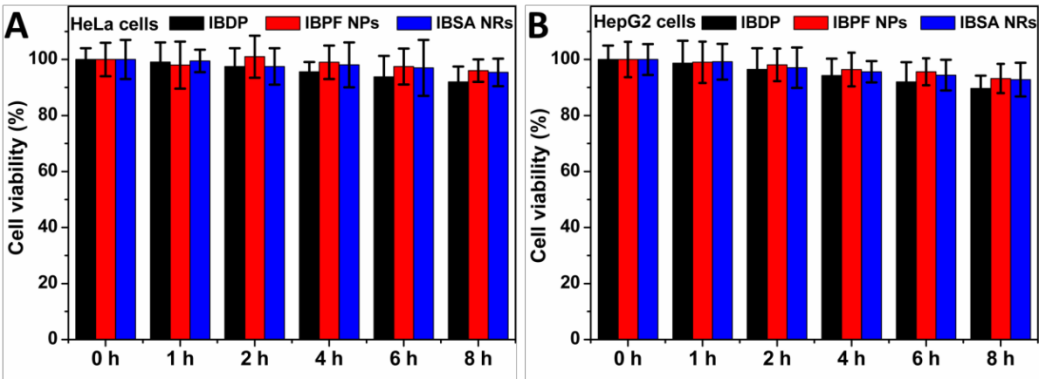

Figure S7. A) Cell viability of HeLa cells after incubation with IBDP, IBPF NPs and IBSA NRs for different hours, respectively. B) Cell viability of HepG2 cells after incubation with IBDP, IBPF NPs and IBSA NRs for different hours, respectively. Data represent mean values ± standard deviation, n=3.

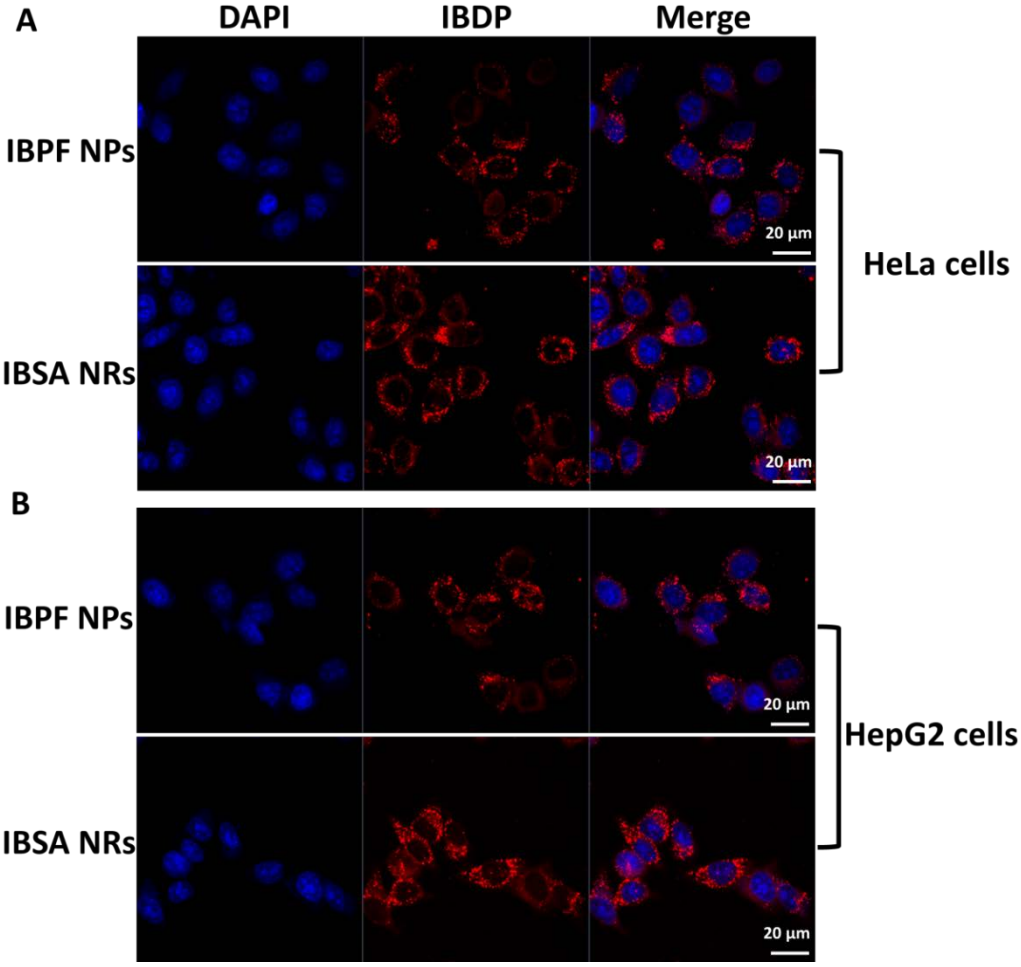

Figure S8. CLSM images of the cellular uptake of IBPF NPs and IBSA NRs in HeLa cells (upper pictures) and HepG2 cells (lower pictures) with 3 μg/mL of BDP at 2 h, respectively.

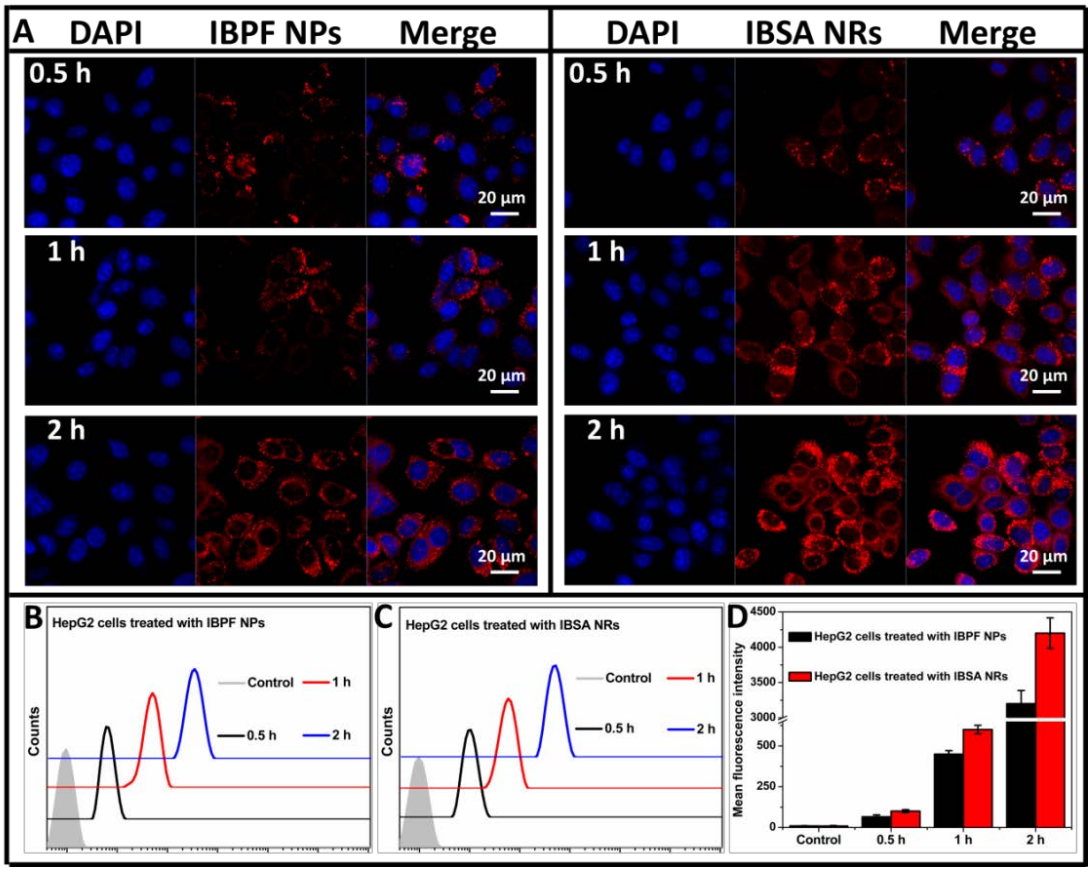

Figure S9. A) CLSM images of HepG2 cells incubated with IBPF NPs or IBSA NRs for 0.5 h, 1 h and 2 h at 37^{o}C, respectively. Cells are viewed in the blue channel for DAPI, the red channel for IBDP. Scale bars represent 20 μm in all images. B) Flow cytometry histograms of HepG2 cells treated with IBPF NPs and without treatment (control) for different hours, respectively. C) Flow cytometry histograms of HepG2 cells treated with IBSA NRs and without treatment (control) for different hours, respectively. D) Quantitative analysis of B) and C). The data are presented as the mean values ± standard deviation, n=3.

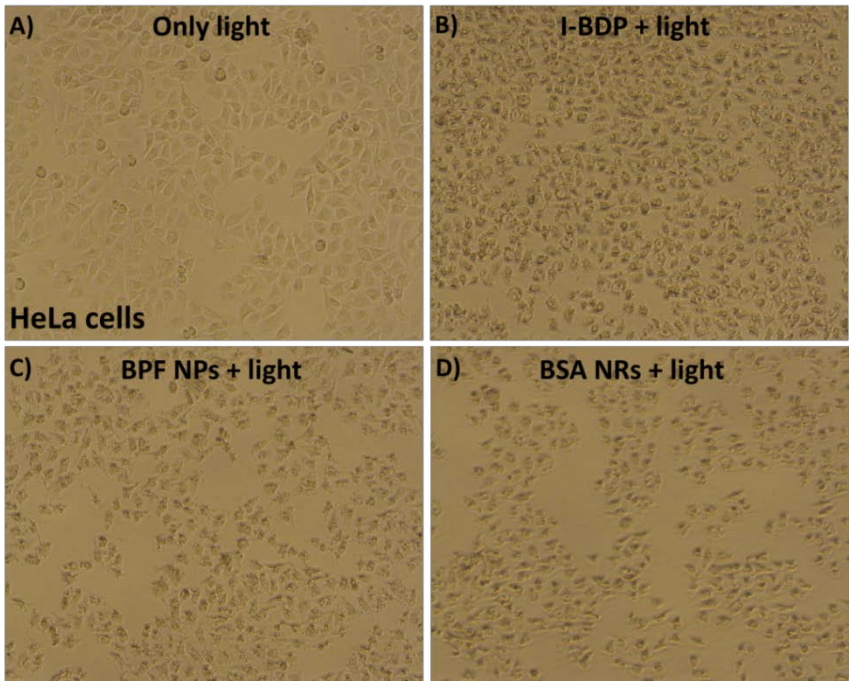

Figure S10. The morphology change of HeLa cells after incubation with IBDP, IBPF NPs and IBSA NRs (2 μg/mL) for 6 h at 37 °C, and then under light irradiation (20 mW/cm^2) for 25 min, and then continue incubation for 24 h, respectively.

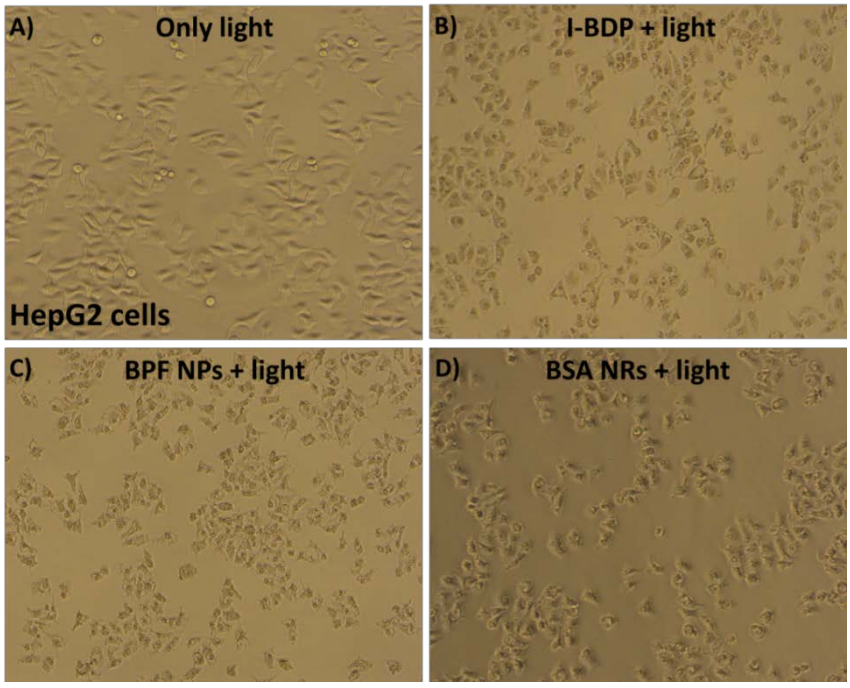

Figure S11. The morphology change of HepG2 cells after incubation with IBDP, IBPF NPs and IBSA NRs (2 μg/mL) for 6 h at 37 °C, and then under light irradiation (20 mW/cm^2) for 25 min, and then continue incubation for 24 h, respectively.

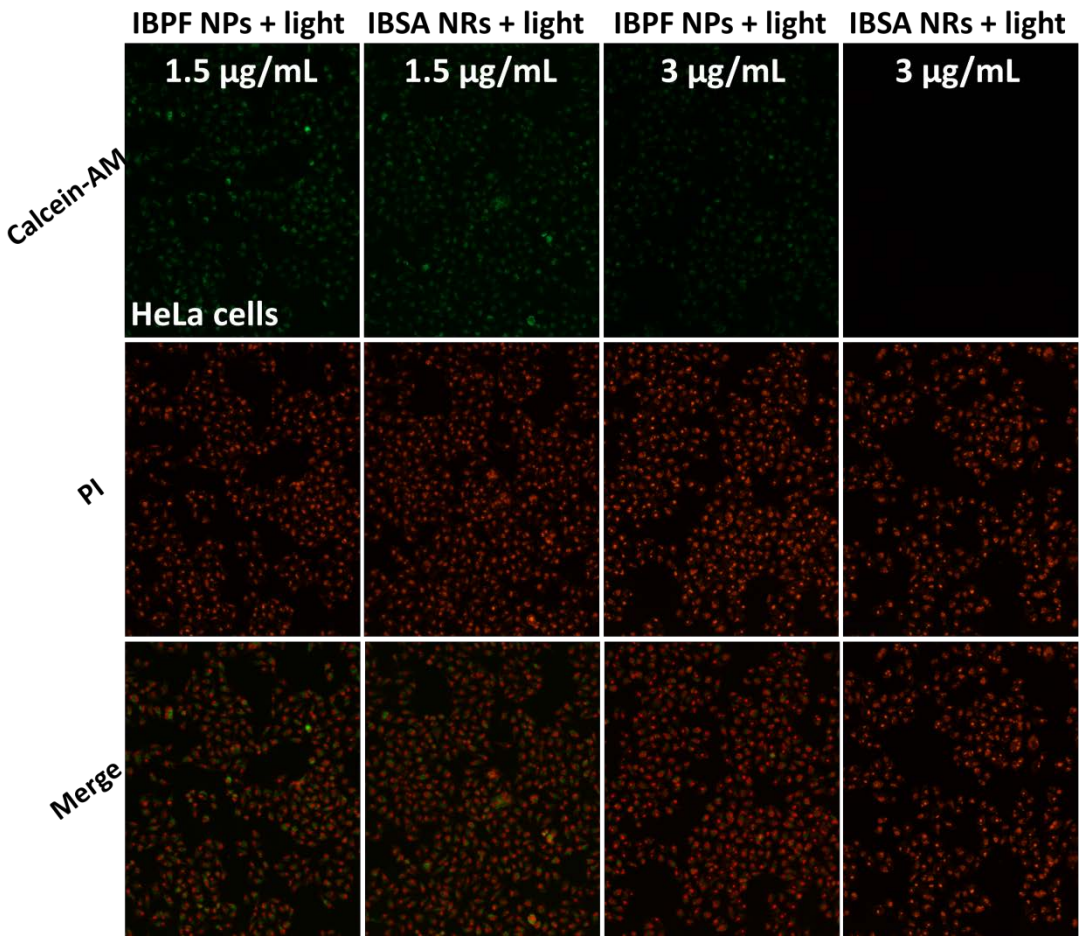

Figure S12. Fluorescence images of HeLa cells after incubation with different concentration of IBPF NPs and IBSA NRs for 6 h at 37 °C, and then under light irradiation (20 mW/cm^2) for 25 min, and then continue incubation for 24 h, respectively. Green calcein fluorescence indicated live cells. Red PI fluorescence indicated dead cells. The cells without any treatment were set as the controls.

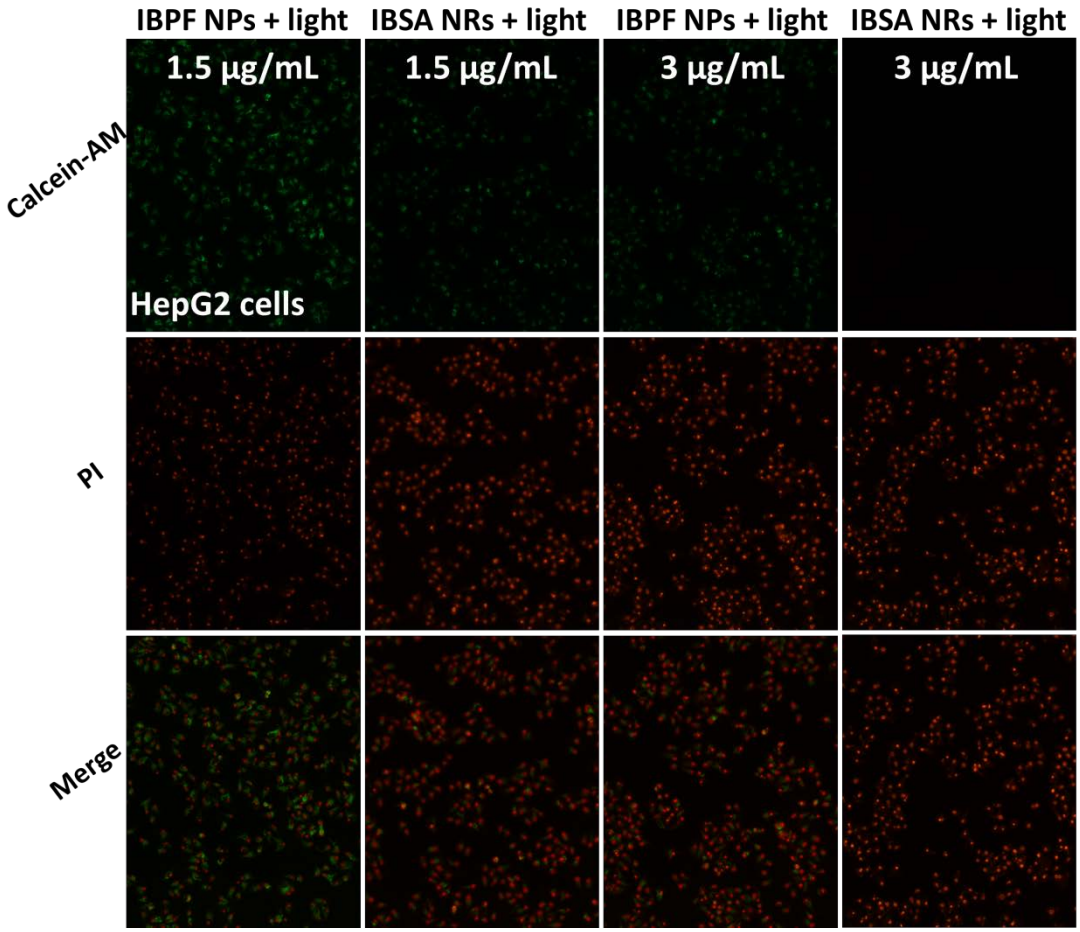

Figure S13. Fluorescence images of HepG2 cells after incubation with different concentration of IBPF NPs and IBSA NRs for 6 h at 37 °C, and then under light irradiation (20 mW/cm^2) for 25 min, and then continue incubation for 24 h, respectively. Green calcein fluorescence indicated live cells. Red PI fluorescence indicated dead cells. The cells without any treatment were set as the controls.