Electronic Supplementary Information

Red Emitting Conjugated Polymer based Nanophotosensitizers for Selectively Targeted Two-photon Excitation Imaging Guided Photodynamic Therapy

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	λ_{abs} / nm	λ_{em} / nm	ε / $M^{-1}cm^{-1}$	Φ / %[a)]
PPBF	415	480	16.6 × 10^4	84 (THF)
TPP	416	654	55.4 × 10^4	10 (THF)
TPD	506	665	2.64 × 10^4	44 (Hexane)

Table S1. Optical Properties of PPBF, TPP and TPD. [a)] Fluorescein in NaOH water solution (PH = 11) was used as a standard for quantum yield measurements.

2PA Cross Section Measurement

2PA cross sections of PPBF, TPD and TPP were measured by comparing with the reference, fluorescein in water (pH 11), which has been well characterized in the literature.[1-3] 2PA cross section was calculated according to $\delta = \frac{S_s}{S_r} \times \frac{\varphi_r}{\varphi_s} \times \frac{C_r}{C_s} \times \delta_r$, where S is the integrated 2PE emission intensity, φ is the fluorescence quantum yield, and C is the concentration of the sample (s) and reference (r). The experimental

uncertainty in the measurement of the cross sections was about 15 %. [1]

Calculation of the Number (n) of Conjugated Polymer Repeat Unit in NPs

Considering one NP contains many molecules composed of many repeat units, 2PA cross section value of CPNs is estimated to be 8.6×10^6 GM per NP based on the two-photon light harvesting ability of PPBF. Calculation of the number of conjugated polymer PPBF repeat unit in NPs have been well characterized in the literature.[4] The density (ρ) of the NP suspension could be roughly estimated to be ~1 g/cm^3. The weight (m) of a single NP can be calculated by $m = V\rho = \frac{4}{3}\pi r^3 \rho$ where the units of m, r and ρ are g, cm, and g/cm^3, respectively. The number (n) of conjugated polymer repeat unit in a single polymer NP was calculated by $n = \frac{mA}{M} N_A = \frac{4\pi r^3 \rho A N_A}{3M}$ where M, N_A, and A represent the repeat unit weight of conjugated polymer, Avogadro constant, and the weight ratio of the conjugated polymer in the NP, respectively.

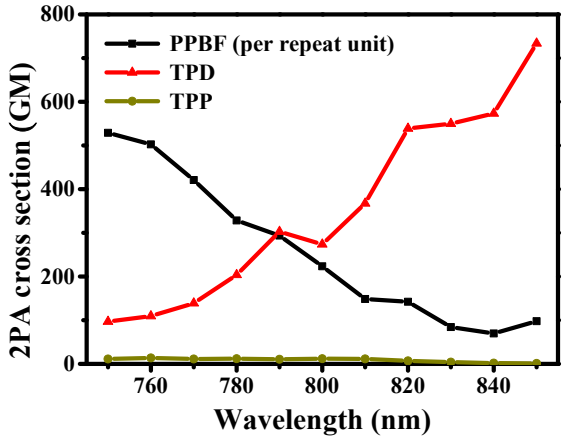

Figure S1. Two-photon absorption cross section values (per repeat unit) of PPBF, TPD and TPP (from 750 nm to 850 nm).

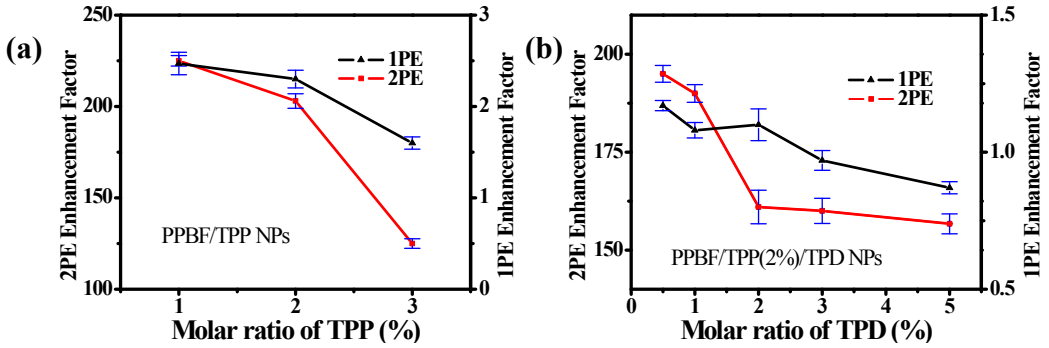

Figure S2. Enhancement factors of 1PE and 2PE emission of (a) TPP in PPBF/TPP NPs with different molar ratios of TPP and (b) PPBF/TPP(2%)/TPD NPs with different molar ratios of TPD.

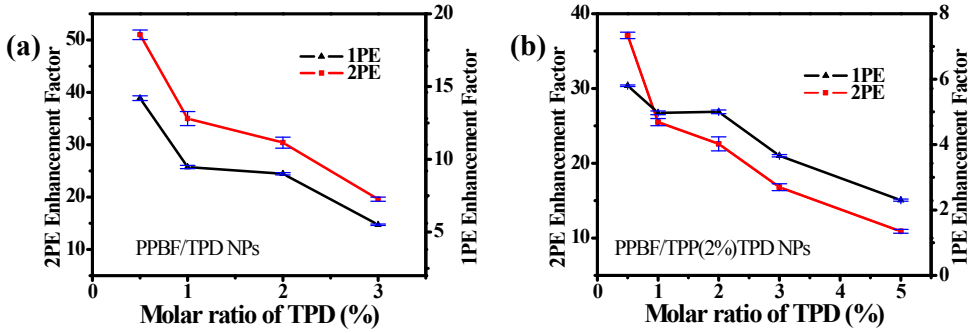

Figure S3. Enhancement factors of 1PE and 2PE emission of TPD in (a) PPBF/TPD NPs and (b) PPBF/TPP(2%)/TPD NPs with different molar ratios of TPD.

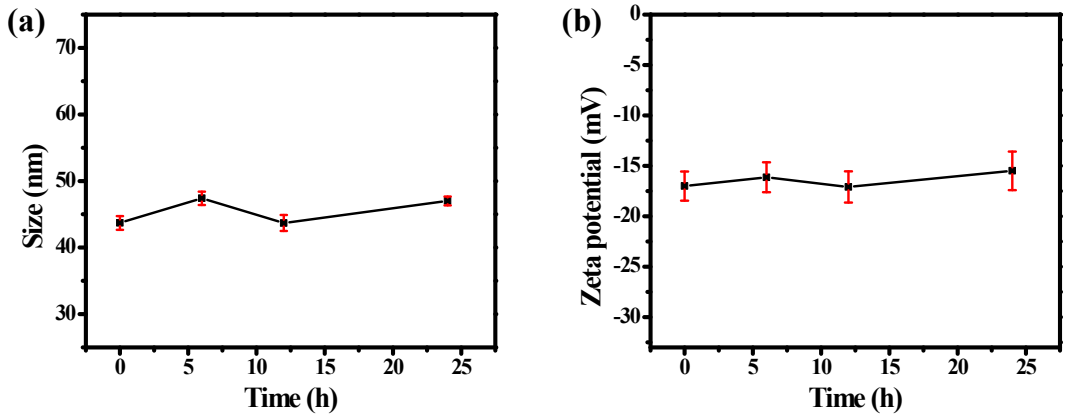

Figure S4. Hydrodynamic diameter (a) and zeta potential (b) of FA-PPBF/TPP/TPD NPs in cell culture media for 24 hours after preparation.

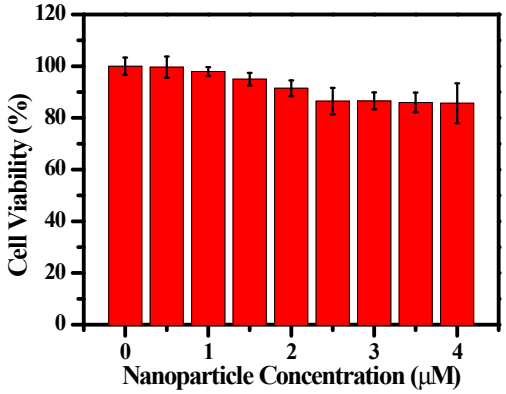

Figure S5. Cell viability assay of KB cancer cells treated with FA-PPBF/TPP(2%)/TPD(2%) NPs with different concentrations (in repeat unit of PPBF) for 24 h in the dark.

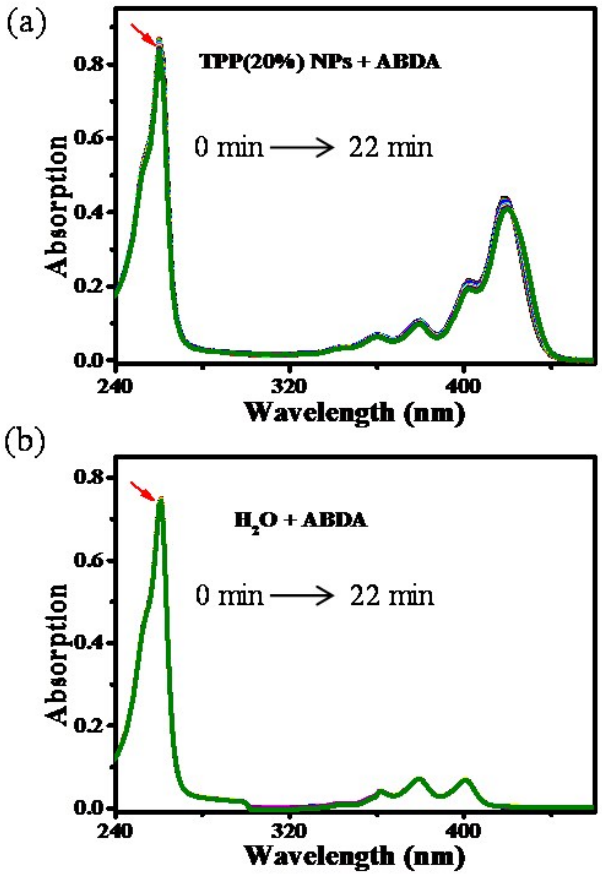

Figure S6. Change in ABDA absorption spectra in the presence of TPP (20%) NPs) or without any nanoparticles under femtosecond laser irradiation.

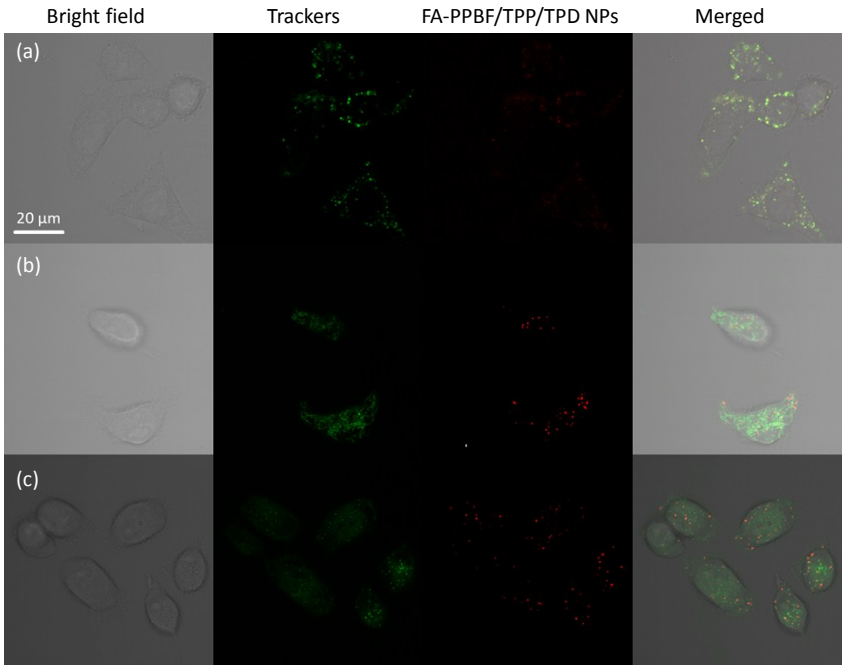

Figure S7. Cell images of KB cancer cells stained with FA-PPBF/TPP/TPD NPs (red), lysosome tracker (green, a), mitochondria tracker (green, b), lipid tracker (green, c).

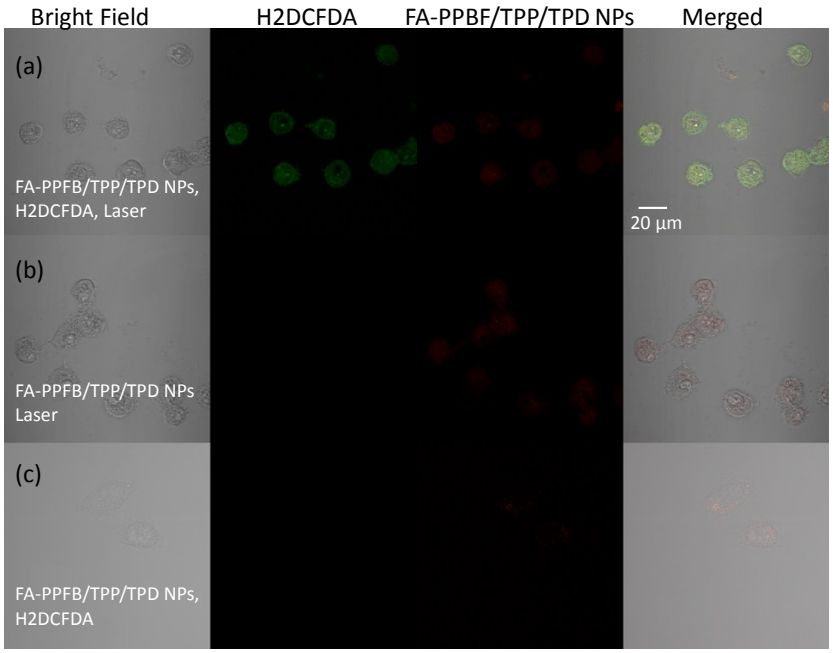

Figure S8. Cell images of KB cancer cells stained with FA-PPBF/TPP(2%)/TPD(2%) NPs (red), with H_2DCFDA (green) under laser irradiation (a), with laser without H_2DCFDA (b), and with H_2DCFDA without laser irradiation (c). Images containing green emission of H_2DCFDA were excited with a 488 laser and collected from 500 to 550 nm. Images containing red emission of FA-PPBF/TPP(2%)/TPD(2%) NPs were excited with a 405 laser and collected from 610 to 700 nm.

MTT assay for cell viability

Briefly, 100 μL of cells with a density of 5×10^4 cells/mL were seeded in 96-well plates and cultured for 24 h before the culture medium was removed. Cells were then incubated with media (100 μL) containing various concentrations of NPs. After 3 h of incubation in the dark, wells were washed twice by PBS buffer and then incubated with freshly prepared MTT medium (0.5 mg/mL, 100 μL) for 3 h at 37 ℃. MTT medium was then carefully removed and washed twice with PBS buffer. 100 μL of DMSO was first added into each well, followed by 10-min gentle stirring to dissolve all the precipitates. The absorbance of sample and control wells at 570 nm was then recorded by a microplate reader. Cell viability was then calculated by the ratio of the absorbance of the sample to control wells.

1. N. S. Makarov, M. Drobizhev and A. Rebane, *Opt. Express*, 2008, **16**, 4029-4047.
2. C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481-491.
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