Supporting Information

Gold Nanorods Enhanced Conjugated Polymer/Photosensitizer Composite Nanoparticles for Simultaneous Two-photon Excitation Fluorescence Imaging and Photodynamic Therapy

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Materials

Poly[9,9'-bis(6"-bromohexyl)fluorine-2,7-ylene-vinylene-co-alt-1,4-phenylene] (PFV) was synthesized following the methods in the literature.[37] All other related chemicals and solvents were purchased from Sigma Aldrich. All solvents are analytical grade and used as-received without further purification.

Preparation of CPNs

TPP/PFV NPs and TPP NPs were prepared utilizing a re-precipitation method in an earlier report.[13] Briefly, PSMA, TPP and PFV were mixed together to fabricate nanoparticles decorated with carboxyl groups. Various amounts of TPP (0.4 mM) were blended with PFV (2.0 mL; 40 μM in RU) and PSMA (48 μL, 2 mM) in THF. The feed solution was quickly injected into the deionized water (8.0 mL) under sonication for 30s before vacuum evaporation to remove THF. The obtained nanoparticle dispersions

are yellow-green color. TPP NPs were prepared by replacing PFV with the same amounts of PSMA.

Preparation of Au NRs and Au NR@SiO_2

Au NRs were prepared according to a previously reported seed-mediated growth method.[38] 100 μL of 0.1 M NaOH solution was then added into 10.0 mL of Au NRs solution under stirring to adjust the pH value to c.a. 10. Different amounts of pure TEOS were subsequently injected three times under stirring at 90 min intervals. A silica layer was formed on the surface of AuNR through hydrolysis and condensation of TEOS after a few hours. The obtained AuNR@SiO_2 NPs were isolated by centrifugation, washed with ethanol several times and finally dispersed in 10.0 mL of ethanol. 100 μL APTES in chloroform solution (25% v/v) was added into 10.0 mL of AuNR@SiO_2 NPs in ethanol under stirring. The mixture reacted overnight at room temperature to afford the amino group functionalized AuNR@SiO_2. The as-prepared AuNR@SiO_2-NH_2 NPs were isolated by centrifugation and washed with ethanol several times, and then redispersed in ethanol.

Preparation of Au NR@SiO_2-CPNs

SiO_2 NPs were first prepared according to the Stöber method[39] by overnight reaction of the mixture of aqueous ammonia (25%, 3.0 mL) and pure TEOS (1.5 mL) in 50.0 mL of ethanol solution. The prepared SiO_2 are quite monodisperse with an averaged diameter of c. a. 100 nm. Surface modification of SiO_2-NH_2 NPs was conducted by the same method of AuNR@SiO_2-NH_2. After conjugation with CPNs or TPP NPs, SiO_2-NH_2 NPs were used to replace Au NR core to serve as a reference for a direct comparison.

AuNR@SiO_2-CPNs were prepared by crosslinking reaction between amino group on AuNR@SiO_2 NPs and carboxyl groups on CPNs. Briefly, 100 μL of 3 nM AuNR@SiO_2, 850 μL of as-prepared CPNs and 50 μL of 0.2 mM phosphate buffer (PB, pH = 7.2) were mixed together in ice bath. Excess amount of freshly prepared ice-cold sulfo-NHS and EDC were injected into the mixture to catalyze the crosslinking reaction at neutral

pH. The reaction mixtures were under rotation for 1 hrs followed by addition of 50 μL of 0.1% wt Tween 20 for stabilization. Excess CPNs were removed by centrifugation at 10,000 rpm, 4 min for three times. The final product was suspended in 0.01% wt Tween 20 solutions.

Characterizations

UV-vis and 1PE fluorescence spectra were measured by using a double-beam spectrophotometer (Hitachi, UH5300, Japan) and a Horiba JobinYvon FluoroMax-4 Spectrophotometer, respectively. Transmission electron microscopic (TEM) images were taken on a JEOL 2010 microscope. The fluorescence lifetimes were measured by using a time-correlated single-photon counting (TCSPC) technique. The frequency-doubled output (410 nm) of an Avesta TiF-100M femtosecond Ti:sapphire oscillator was used as the excitation source. Emission from the sample was collected at 90º angle with respect to the excitation beam by an optical fiber that was connected to a monochromator (Acton, Spectra Pro 2300i) coupled Photomultiplier tubes (PMT) (PicoQuant, Berlin, Germany). The signals were processed by a TCSPC module (PicoQuant, PicoHarp 300) with temporal resolution of 100 ps.

Two-photon excitation fluorescence measurements were performed by using a femtosecond Ti: sapphire oscillator (Avesta TiF-100M) with output laser pulses centered at 820 nm and average power of 100 mW as the excitation source. The laser pulses have pulse duration of 80 fs and repetition rate of 84.5 MHz. The laser beam was focused onto the sample that was contained in a cuvette with a path length of 1.0 cm. The emission from the sample was collected at a 90° angle by a pair of lenses and an optical fiber that was connected to a monochromator (Acton, Spectra Pro 2300i) coupled CCD (Princeton Instruments, Pixis 100B) system. A shortpass filter with cut-off wavelength of 750 nm was placed before the monochromator to minimize the scattering from the pump beam.

Detection of $^1O_2$ Generation

$^1O_2$ generation under 2PE was characterized by photo-oxidation of ABDA in aqueous

environment due to an irreversible oxidation of ABDA by $^1O_2$, resulting in decrease in the absorbance of ABDA at 378 nm. A solution with total volume of 400 μL and concentration of 60 pM (with respect to Au NR) was used in this experiment. The concentration of ABDA at the beginning of the photo-oxidation was set as 8.0 μM. The two-photon excitation (2PE) light source for $^1O_2$ generation is fs laser pulsed at 820 nm with laser power of 100 mW and repetition rate of 84.5 MHz. The absorbance at 378 nm was monitored at 15 min intervals after continuous laser irradiation. The same irradiation conditions were applied to AuNR@SiO_2-TPP NPs, SiO_2-TPP NPs and SiO_2-TPP/PFV NPs for comparison.

Cell Culture and Cell Viability

HeLa cell line was chosen as the model system. The HeLa cells were cultured in DMEM containing 1% penicillin-streptomycin and 10% FBS at 37 °C in a humid atmosphere with 5% CO_2. HeLa cancer cells were seeded on a 96 well plate at a density of 5×10^4 cells per mL. After 24 h of culture, different concentrations of AuNR@SiO_2(9nm)-8%CPNs were added and further incubated for 24 h. The sample and control wells were washed twice with PBS buffer and added with freshly prepared MTT medium solution (0.5 mg mL^{-1}, 100 μL). After 3 h of incubation at 37 °C, the MTT medium solution was carefully removed and washed twice with PBS buffer. DMSO (150μL) was then added into each well and the plate was gently stirred for 10 min at room temperature to dissolve all the precipitates that were formed. The absorbance of sample and control wells at 570 nm was then measured by a microplate reader. Cell viability was then calculated by the ratio of the absorbance of the sample wells to control cells.

2P-PDT Activity on Cancer Cells

HeLa cancer cells were seeded on a 96 well plate and grown until 70-80% confluence, then treated with AuNR@SiO_2(9nm)-8%CPNs and SiO_2-8%CPNs suspension for 3 h in cell culture media, followed by changing with fresh cell culture media. 2P-PDT activities on cancer cells were performed by using an unfocused femtosecond laser beam (0.33 cm^2) and pulse energy of 300 mW at 800 nm as the illumination source.

Cells were exposed to the femtosecond laser beam from 0 to 8 min irradiation period. The irradiated plates were sent back to the incubator overnight and cell viability was measured with the MTT assay.

2P Excitation Cell Imaging

HeLa cancer cells were grown in glass-bottom dishes at 37 °C. After incubation with AuNR@SiO_2(9nm)-8%CPNs (40 pM) for 3 h, the cells were washed with DMEM medium for three times before imaging measurement. The 2PE fluorescent images of cells were conducted by using a home-made microscope system consisting of an Olympus IX73 microscope and a PMA Hybrid 40 as detector. The imaging was collected under an excitation wavelength of 820 nm and a power of 5 mW over a detection emission range of 510-750 nm and processed by a SymPho Time 64 software.

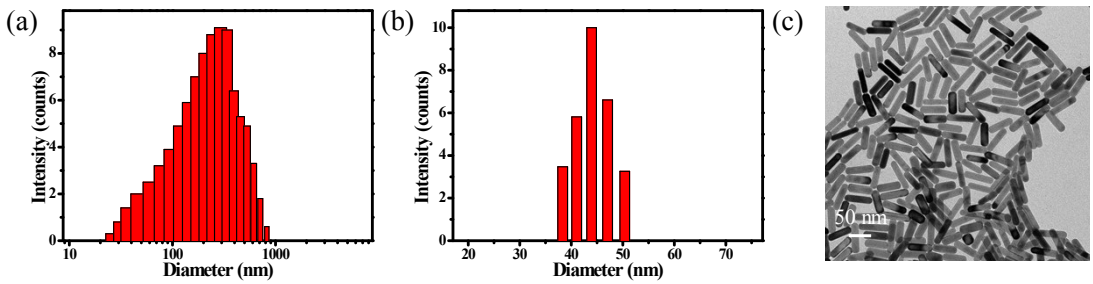

Figure S1. Size distribution of AuNR@SiO_2(9nm)-TPP(8%)/PFV NPs (a), and TPP(8%)/PFV NPs (b) measured by DLS; (c) TEM images of Au NRs.

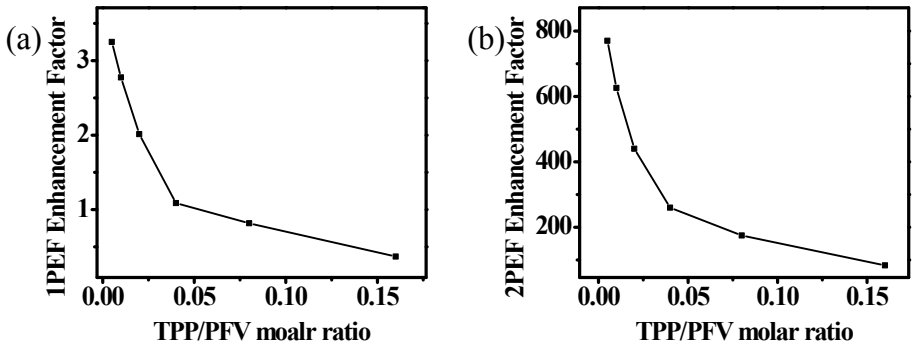

Figure S2. 1PEF and 2PEF enhancement factors of TPP in various TPP/PFV NPs.

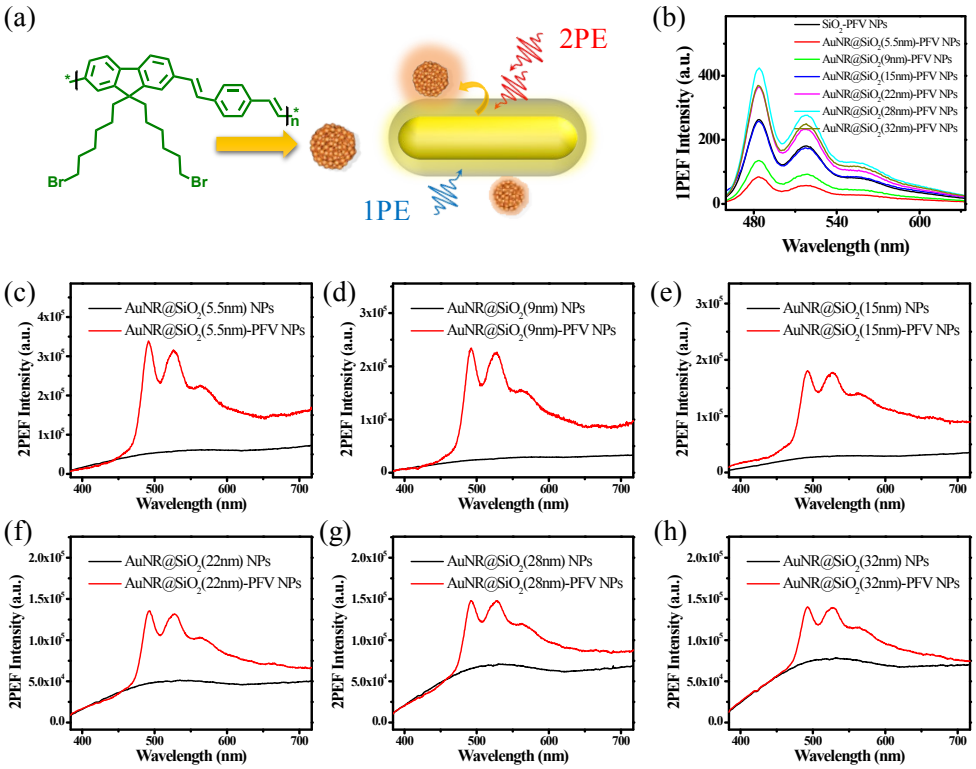

Figure S3. (a) Illustration of Au NR mediated 1PEF and 2PEF properties of PFV NPs, (b) 1PEF of PFV NPs conjugated to various AuNR@SiO_2, (c-h) 2PEF of AuNR@SiO_2-PFV NPs and 2PPL of AuNR@SiO_2.

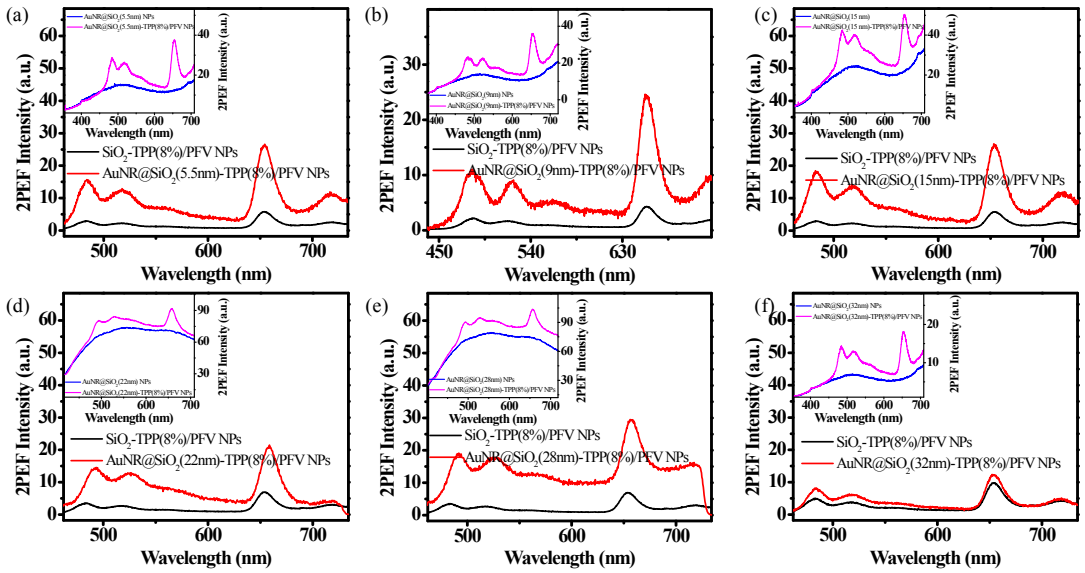

Figure S4. 2PE fluorescence spectra of SiO_2-TPP(8%)/PFV NPs and AuNR@SiO_2-TPP(8%)/PFV NPs with various SiO_2 shell thicknesses after subtraction of two-photon photoluminescence of AuNR@SiO_2 NPs. The insets are 2PE fluorescence spectra before subtraction and two-photon photoluminescence spectra of corresponding AuNR@SiO_2 NPs.

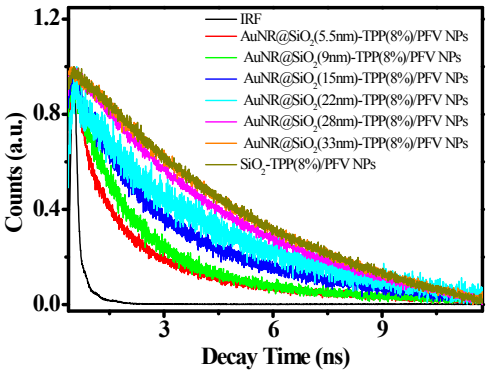

Figure S5. Fluorescence lifetime decays of TPP molecules in various nanoparticles.

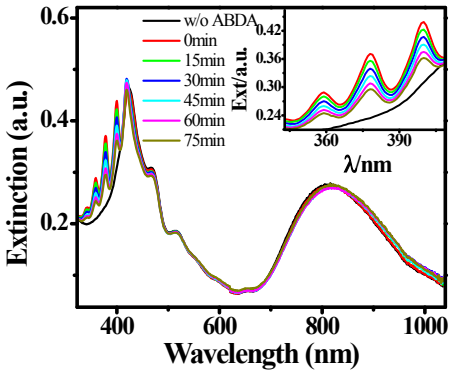

Figure S6. Extinction spectra of ABDA and AuNR@SiO_2(9nm)-TPP(8%)/PFV NPs under continuous laser irradiation with a fs laser with a power of 100 mW, a repetition rate of 84.5 MHz at 820 nm.

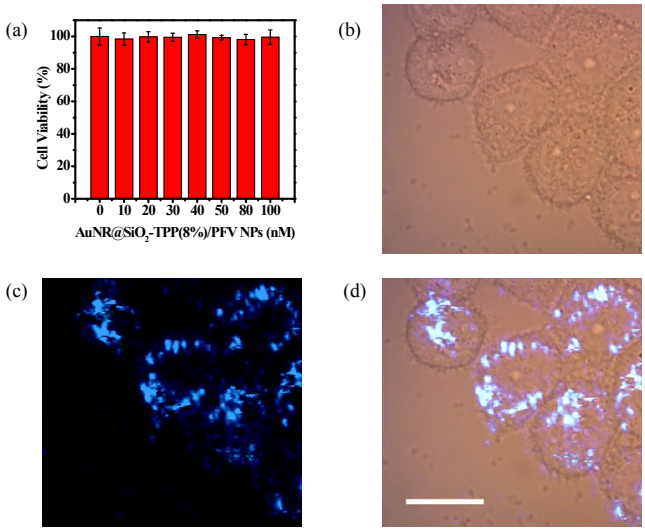

Figure S7. (a) Cell viability of HeLa cancer cells incubated with various concentrations of AuNR@SiO_2(9nm)-TPP(8%)/PFV NPs for 24 hrs, bright field (b), 2PEF (b) and merged images (c) of HeLa cancer cells treated with 40 pM AuNR@SiO_2(9nm)-TPP(8%)/PFV NPs . Scale bar is 20 μm.

Table S1. Optical properties of PFV NPs, and Au NR. [a]extinction maximum, [b]extinction coefficient, [c]two-photon absorption cross section, [d]singlet oxygen generation quantum yield, [e] fluorescence quantum yield.

	λ_{abs} (nm)[a]	ε ($M^{-1}cm^{-1}$)[b]	δ (GM)[c]	Φ_{Δ}[d]	Φ_{F}[e]
PFV NPs	437	7.5×10^4	256 (per RU)	5.6%	10%
Au NRs	820	5×10^9	$2\sim3\times10^8$	~0.1%	~0.01%

Table S2. Enhancement factors in 1PEF and 2PEF of PFV NPs by AuNR@SiO_2 with different SiO_2 shell thickness.

SiO_2 thickness	2PEF Enhancement	1PEF Enhancement
5.5	7.26	0.32
9	5.43	0.51
15	4.50	0.98
22	3.11	1.39
28	2.92	1.61
32	2.38	1.40

Table S3. Enhancement factors in 1PEF, 2PEF and 2P-$^1O_2$ of TPP by AuNR@SiO_2 with different SiO_2 shell thickness in terms of TPP(8%)/PFV NPs and TPP(8%) NPs.

SiO_2(nm)	Compared to TPP(8%)/PFV NPs			Compared to TPP(8%) NPs		
	1PEF	2PEF	2P-$^1O_2$	1PEF	2PEF	2P-$^1O_2$
5.5	0.36	4.47	2.92	0.30	752	485
9	0.54	5.61	4.77	0.44	980	792
15	0.93	4.53	3.27	0.76	805	543
22	1.31	2.99	1.35	1.07	508	224
28	1.41	2.69	1.09	1.16	455	181

33	1.25	1.29	0.96	1.02	226	159