Supplementary Information

Photosensitizer-doped Conjugated Polymer Nanoparticles with High Cross-sections for One- and Two-photon Excitation

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1. Fluorescence Lifetime Determination

Fluorescence lifetimes were measured using the time-correlated single-photon counting technique (TCSPC). The sample was excited by the second harmonic (400 nm, ~100 fs pulses) of a mode-locked femtosecond Ti:Sapphire laser (Coherent Mira 9000). The output of a fast PIN diode (Thorlabs, DET210) monitoring the laser pulse was used as the start pulse for a time-to-amplitude converter (TAC, Canberra Model 2145). Fluorescence signal from the aqueous nanoparticle dispersion was collected perpendicular to the excitation, passed through a 600 nm longpass filter, and detected by a single photon counting module (id Quantique, ID100-50). The output of the detector was used as the stop pulse for the TAC. The laser was attenuated to maintain the count rate below 20 kHz in order to minimize nonlinearities in the detector and electronics. The signal from the TAC was digitized using a multichannel analyzer (FastComTec, MCA-3A). The instrument response function was measured before and after each fluorescence lifetime measurement using the scattered laser light from a dilute suspension of polystyrene beads. The combination of the detector and electronics results in an instrument response function with a width of ~60 ps (FWHM). Custom software employing nonlinear least-squares minimization and convolution of a single exponential with the instrument response function yielded an estimated excited state lifetime of 6.1 ± 0.5 ns for TPP-doped PDHF nanoparticles. The time resolution of the instrument was insufficient to determine the rise time associated with the energy transfer rate.

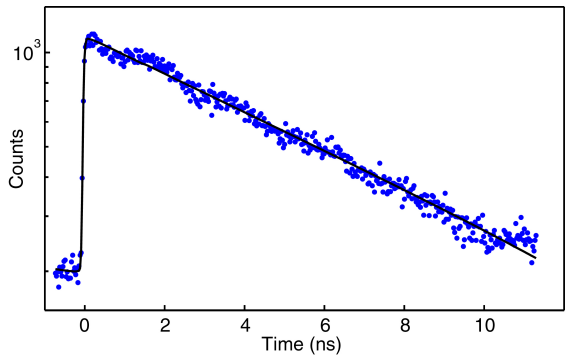

Figure S1. TCSPC fluorescence decay of TPP-doped PDHF nanoparticles. The scattered data are measured by a TCSPC technique and the solid curve represents the fit obtained by an iterative deconvolution method.

2. Particle Height Histogram of TPP doped PDHF nanoparticles

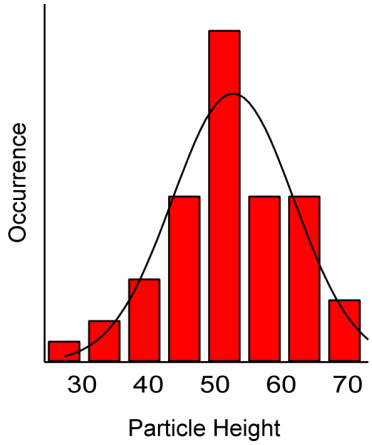

Figure S2. Particle height histogram of the TPP doped PDHF nanoparticles shown in Fig. 1(c). 50 particles were analyzed.

2. Gel Electrophoresis of Damaged DNA

Purification of plasmid DNA. Plasmid DNA (pBSSK) in Tris-EDTA (TE) buffer was purchased from Aldevron and dialyzed against 130 mM NaCl for 24 h at 4 ^{o}C to remove metal ions. The resulting DNA concentration was found using UV-vis measurements (Shimadzu UV-3101 PC spectrophotometer) at A_{260} (1 A_{260} = 50 ng/μL). Purity of plasmid DNA was determined via gel electrophoresis of a digested sample, and all absorbance ratios were within acceptable limits ($A_{250/260} < 0.95$, and $A_{260/280} > 1.8$).

Gel electrophoresis experiments with nanoparticles. These experiments were conducted to determine the amount of DNA backbone damage produced upon irradiation of DNA and nanoparticles as a function of irradiation time. As a positive control, hydroxyl-radical-damaged DNA was generated using Fe^{2+} and H_2O_2 as follows:, 0.1 pmol plasmid DNA, 130 mM NaCl, 2 μM $FeSO_4$•$7H_2O$ maintained at pH 6 with phosphate buffer were combined and allowed to stand for 5 min at room temperature. H_2O_2 (50 μM) was then added and the DNA-damaging reaction was allowed to continue for 30 min. EDTA (50 μM) was added at this time to halt the reaction, for a total sample volume of 10 μL. For the experimental lanes, plasmid DNA (0.1 pmol) was added to a suspension of nanoparticles to yield a final volume of 300 μL. Aliquots (10 μL) were taken for electrophoresis at various time intervals (0, 50, 100, and 200 min) during irradiation.

Digestion with Fpg enzyme. These gel electrophoresis experiments were conducted to determine the amount of DNA purine base damage produced upon irradiation of DNA and nanoparticles over time. The Fpg (formamidopyrimidine [fapy]-DNA glycosylase) enzyme was used in these experiments since it is specific for cleaving oxidized purine (G

and A) bases in DNA.[1,2] Plasmid DNA (0.1 pmol) in the absence and presence of nanoparticles irradiated for varying amounts of time (0, 50, 100, and 200 min) were incubated with a bovine serum albumin (BSA) and NEB buffer 1 solution (0.5 μL) and Fpg enzyme (0.5 μL) for 1 h at 37°C. After this time, 10% SDS (0.5 μL) was added to each sample and allowed to stand for 5 min before performing gel electrophoresis separations.

Separation and analysis of DNA. After preparation of the samples, the DNA was separated on 1% agarose gels via electrophoresis, stained with ethidium bromide for 30 min, and imaged on an UVIproDBT-8000 gel imager (UVITec, Cambridge, UK). Quantification of undamaged and damaged DNA bands was performed using the UviPro software (Jencons Scientific Inc., Bridgeville, PA, 2003). Since ethidium bromide stains undamaged DNA less efficiently than damaged DNA, band intensities for undamaged DNA were multiplied by 1.24 prior to comparison.[3,4] The intensities of the DNA bands for each lane were then normalized so that addition of damaged and undamaged DNA equals 100 %.

Table S1. DNA backbone damage upon irradiation with (lanes 2-8) and without (lanes A-C) nanoparticles. Data are from gel image in Figure 3.

Lane	Contents	% Circular DNA	% Nicked DNA	p-value
2	plasmid DNA	96.7 ± 0.2	3.3	-
3	DNA + H_2O_2	94 ± 3	6	-
4	DNA + H_2O_2 + Fe^{2+}	3 ± 2	97	0.008
5	DNA + nanoparticles (irr for 0 min)	97.5 ± 0.1	2.5	0.04
6	DNA + nanoparticles (irr for 50 min)	99.9 ± 0.1	0.1	0.06
7	DNA + nanoparticles (irr for 100 min)	50.0 ± 0.5	50.0	0.002
8	DNA + nanoparticles (irr for 200 min)	31.0 ± 0.5	69.0	0.0001
A	DNA (irr for 50 min)	96 ± 2	5	0.67
B	DNA (irr for 100 min)	96 ± 3	4	0.77
C	DNA (irr for 200 min)	95.1 ± 0.8	5.0	0.11

Combining nanoparticles and plasmid DNA without irradiation caused no DNA base damage (Table S2, lane 3). Irradiation of nanoparticles for 50 min also produced a negligible amount of base damage compared to irradiated DNA base damage alone (lanes 4 and A, respectively), indicating little production of reactive oxygen species. As the irradiation time of plasmid DNA with CP dot nanoparticles increased to 100 and 200 min, DNA base damage significantly increased to 36% and 47%, respectively.

Table S2. DNA backbone damage upon irradiation with (lanes 2-8) and without (lanes A-C) nanoparticles.

Lane	Content	% Circular DNA	% Nicked DNA

2	plasmid DNA + Fpg	96.0	4.0
3	DNA + Fpg + nanoparticles (irr for 0 min)	99.3	0.7
4	DNA + Fpg + nanoparticles (irr for 50 min)	98.0	2.0
5	DNA + Fpg + nanoparticles (irr for 100 min)	63.6[a]	36.4[a]
6	DNA + Fpg + nanoparticles (irr for 200 min)	52.8[a]	47.2[a]
A	DNA + Fpg (irr for 50 min)	91 ± 7	9.5
B	DNA + Fpg (irr for 50 min)	83 ± 2	17
C	DNA + Fpg (irr for 50 min)	75 ± 11	25

[a]Corrected for DNA damage resulting from irradiation alone (lanes B and C, respectively).

References

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