Supporting Information for

Intermolecular Structural Change for Thermo-Switchable Polymeric Photosensitizer

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Experimental section

Materials*:* Pheophorbide-a (PPb-a) was purchased from Frontier Scientific (Logan, UT, USA). hydroxypropyl cellulose (HPC, average M_w: ~80,000, average M_n: ~10,000), *N,N'*-Dicyclohexylcarbodiimide (DCC), 4-(Dimethylamino)pyridine (DMAP), *N,N*-Dimethylformamide (DMF), and Dimethyl Sulfoxide (DMSO) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). These chemical products were used without further purification process.

Synthesis of a temperature-responsive polymeric photosensitizer (T-PPS): T-PPS was synthesized via conventional carbodiimide reaction.[1] HPC (0.2 g, 2.50 μmol) was dissolved in 5 mL of DMF and stirred for 4 h. Then, mixture of PPb-a (2.5 mg, 4.22 μmol), DCC (12.5 mg, 60.60 μmol), and DMAP (12.5 mg, 102.30 μmol) in 5 mL of DMF was added to the as-prepared HPC solution and reacted for 24 h at room temperature. After reaction, the reactant was precipitated in cold diethyl ether (0.2 L) and repeated three times. The resultant was dialyzed against deionized water (DW) using a cellulose membrane (molecular cut-off, 1000 Da, Spectrum Laboratories, Rancho Dominguez, CA, USA) to remove unreacted impurities. The final products were lyophilized and stored in refrigerator at -20 °C. Chemical structure of the as-synthesized T-PPS was analyzed by ^{1}H-NMR spectroscopy (Bruker 500 MHz NMR Spectrometer, Bruker, Germany). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.43 (m, H1 of HPC), 3.04-4.00 (m, H2-8 of HPC), 2.27 (s, 17^1-H_2 of PPb-a), 2.04 (s, H17^2-H_2 of PPb-a), 1.05 (m, H9 of HPC). The degrees of substitution (DS) of PPb-a of the T-PPS was estimated by the relative peak intensities between the protons of HPC (δ 4.43, H1 of HPC) and the protons of PPb-a (δ 2.04, H17^2-H_2 of PPb-a) in the ^{1}H-NMR spectrum of T-PPS (Figure S2). To double-check the DS of PPb-a of the T-PPS, absorbance spectra of T-PPS was measured in DMSO at 413 nm using a UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan).

Optical transmittance change of the T-PPS at different temperatures: The T-PPS (10 mg/mL) was

dissolved in DW. The optical transmittance change of the solution was measured at 500 nm of wavelength using UV–Vis spectrometer (UV-1601, Shimadzu, Japan) with increasing temperature. The transmittance value was normalized to 100 % with the transmittance of the sample at 35 °C.

Transmission electron microscopy (TEM) measurement: TEM images were obtained from filtered (0.45 μm syringe filter) sample solutions (HPC or T-PPS, 1 mg/mL). Small droplets (~10 μL) of the solution were deposited onto 400 mesh formvar-coated copper TEM grids (Ted Pella Inc., Redding, CA, USA). The samples were dried in a vacuum oven at 45 °C for 2 hr. TEM images were recorded using an FEI Tecnai G2 Spirit transmission electron microscope (FEI, Hillsboro, OR, USA) at 60 kV.

Fluorescent intensity change of the T-PPS at different temperatures: The T-PPS (10 mg/mL) was dissolved in DW. The fluorescent intensity change of the solution was measure at excitation (670 nm) and emission (700 nm) wavelength using fluorescence spectroscopy (RF-5301; Shimadzu, Japan) with increasing temperature.

Singlet oxygen generation (SOG) of the T-PPS at different temperatures: The SOG from T-PPS was quantitatively measured using a fluorescence probe (*i.e.,* singlet oxygen sensor green (SOSG, Molecular Probes, Eugene, OR, USA)). One mL of the T-PPS solutions (10 mg/mL, in DW) at different temperature were mixed with SOSG (2.0 mM, 100 μL) and then irradiated with a 670 nm laser source (fiber optic-coupled laser system, Laser Lab®, Korea) at 5 mW/cm^2 intensity for 4 minutes. After laser-irradiation, the fluorescent intensity change of the solution was measure at excitation (494 nm) and emission (534 nm) wavelength using the fluorescence spectroscopy.

Dynamic light scattering measurement at different temperature: Hydrodynamic size of the T-PPS (10 mg/mL, in DW) as different temperature was measured using a Zetasizer Nano ZS (Nano-ZS, Malvern Instruments, Ltd., UK).

Small-angle X-ray scattering (SAXS) measurement[2]: The SAXS measurements were performed with X-ray energy of 14 keV at the 12-ID-B station in the Advanced Photon Source of the Argonne National Laboratory (Lemont, IL, USA). A Pilatus 2M detector (Dectris Ltd., Baden, Switzerland) was used to acquire scattering data with exposure times typically in a range of 0.1–1.0 s. SAXS experiments are performed both for solution and solid samples with varying temperature. Each of T-PPS solution sample (10 mg/mL, in DW) was prepared in an aluminum pan and sealed. Solid samples (approximately 20 mg) were prepared between a pair of Kapton tape. They were placed on the THMS 600 heating stage (Linkam Scientific Instruments, Tadworth, Surrey, UK) modified for X-ray experiment. The temperature was varied from 30 to 60 °C with a rate of 1 °C/min and dwelling time of 5 min in each run end.

In vitro* cytotoxicity test*: PANC-2 (human pancreatic cancer) cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA). For photo-cytotoxicity study at different temperature, PANC-2 cells (2×10^5 cells/well) were seeded in 35 mm^2 cell culture dishes and incubated (37 °C, 5% CO_2) for 12 h. After rinsing twice with Dulbecco's phosphate buffer saline (DPBS; Invitrogen Corp., Carlsbad, CA, USA), the cells were treated with the T-PPS dissolved in the DMEM cell media for 4 h and then irradiated with the 670 nm laser. Finally, to access an additional cytotoxicity effect of hyperthermia, the cells were further incubated at either 37 °C or 45 °C for 1 h. After 24 h incubation at normal condition, cell viability was evaluated with both a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD, USA) and a LIVE/DEAD two-color fluorescence assay (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instruction.

In vivo fluorescent imaging: All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Catholic University of Korea (Republic of Korea). Five-week-old male BALB/c nude mice (Orient Bio, Seongnam, Korea) were used for the imaging study. Either free PPb-a

(Control) or T-PPS (injection dose; equivalent to 0.25 μg of PPb-a) was subcutaneously injected into the left and right flank regions of BALB/c nude mice, respectively. After the injection, left flank region was treated heat treatment with 45 °C for 5 min by using a pre-heated gel pack (Moore Medical Corp, New Britain, CT, USA). The fluorescent images were taken by *in vivo* image station (IVIS, Kodak, New Haven, CT, USA).

Statistical analysis*:* Data are expressed as the mean ± standard deviation (SD). Student's t-test was performed with SigmaPlot software (Version 10, Systat, Chicago, IL, USA) for statistical analyses.

Table S1. Characterization of T-PPS

Sample	Conjugates	Yield (wt%)[a)]	DS of PPb-a[b)]	DS of PPb-a[c)]	LCST[d)] [°C]
HPC	HPC	-	-	-	42.8 ± 0.7
T-PPS	PPb-a conjugated HPC	80 ± 5.0	1.0 ± 0.1	1.1 ± 0.1	43.4 ± 0.6

[a)]Yield (wt%) = weight of obtained compounds (PPb-a conjugated HPC, g) / weight of added initial materials (HPC + PPb-a, g) × 100 (n=3).
[b)]Degree of substitution (DS) of PPb-a molecules per one HPC molecule, calculated by ^{1}H-NMR analysis (Figure S2, n=3).
[c)]Degree of substitution (DS) of PPb-a molecules per one HPC molecule, calculated by comparing the absorption value to the standard curve (Figure S14, n=3).
[d)]Lower critical solution temperature (LCST) values of T-PPS were determined at the temperatures showing an optical transmittance (at 500 nm) of 50% by UV–vis spectrophotometer (Figure 1(a)).

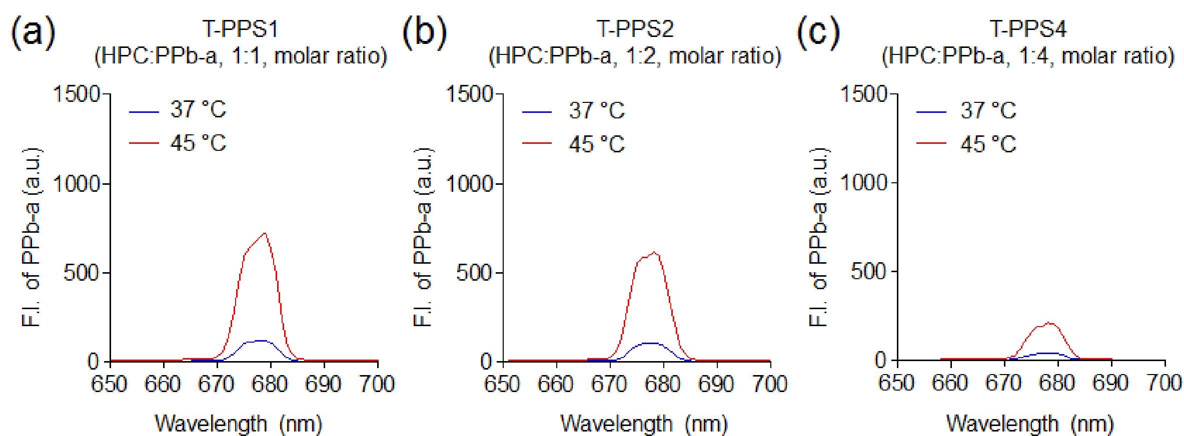

Figure S1. Fluorescent emission spectra of T-PPS with different PS conjugation ratio at different temperature conditions. The fluorescent emission spectra of (a) T-PPS1 (with 1:1 molar ratio of HPC to PPb-a), (b) T-PPS2 (with 1:2 molar ratio of HPC to PPb-a), and (c) T-PPS4 (with 1:4 molar ratio of HPC to PPb-a) were measured at 37 and 45 °C. As increasing PS conjugation ratio, maximum fluorescent emission both at 37 and 45 °C was decrease, which could be attributed to self-quenching of the PSs conjugated in the HPC polymer chain.[3]

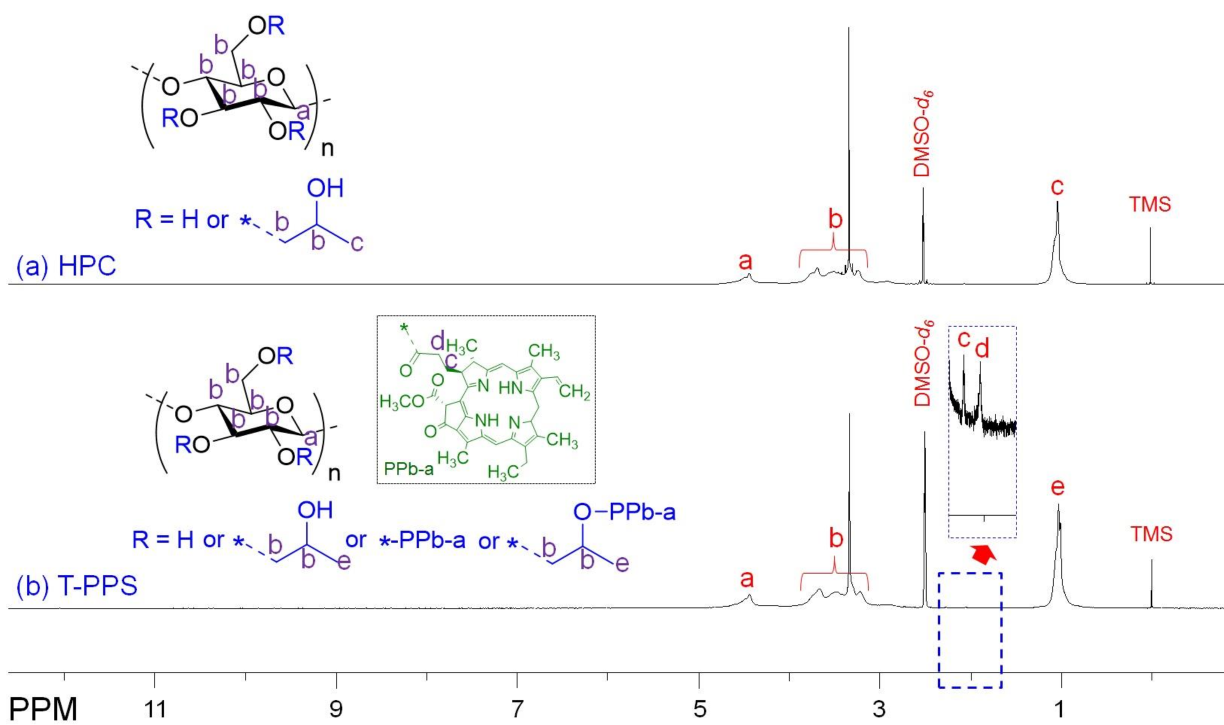

Figure S2. ^{1}H-NMR analysis of (a) native HPC and (b) T-PPS in DMSO-d_6.

Figure S3. Photograph of T-PPS aqueous solution (10 mg/mL in water) after cooling down for 30 minutes at room temperature: T-PPS solution without (1) or with (2) heating.

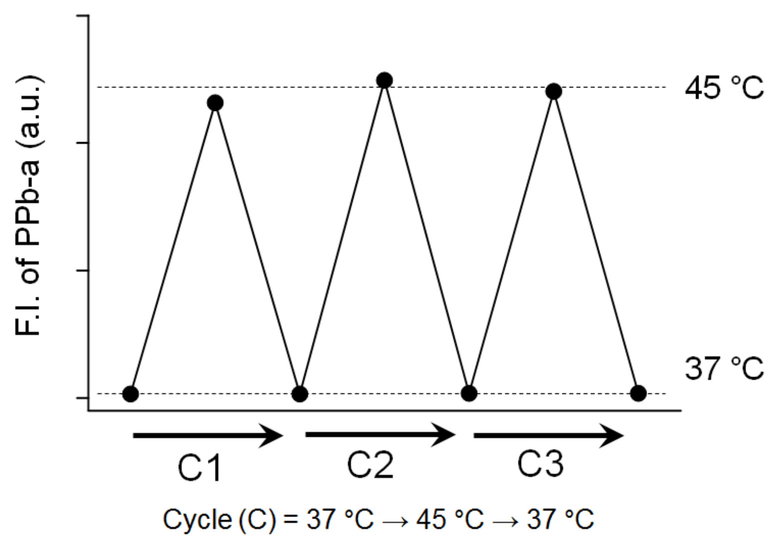

Figure S4. Reversible change of fluorescent intensity of T-PPS aqueous solution (10 mg/mL) between at 37 °C and at 45 °C.

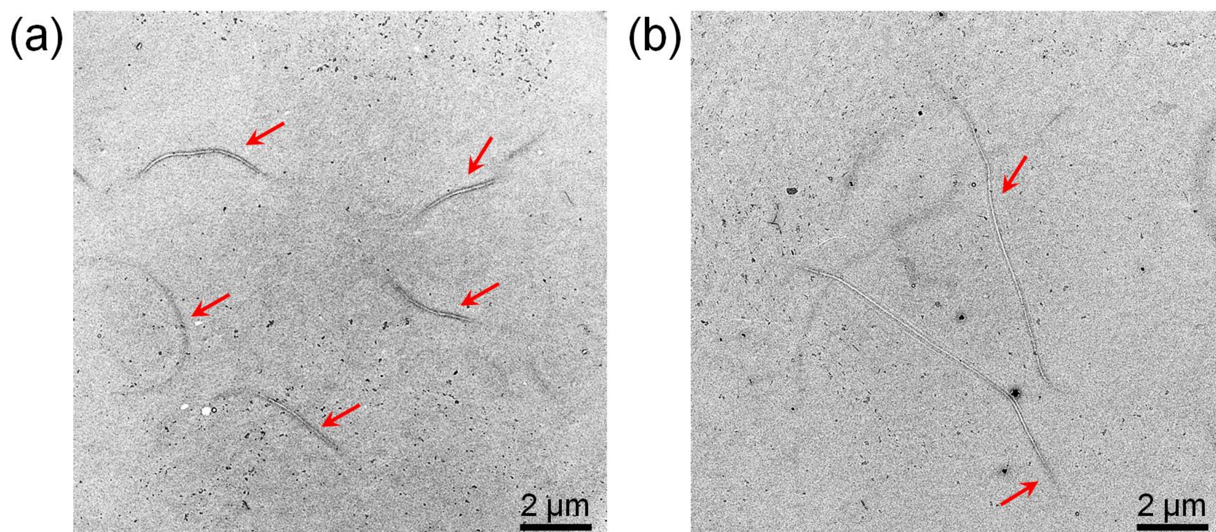

Figure S5. TEM image of (a) native HPC and (b) T-PPS sample dried at 45 °C on a Formvar-coated copper grid. The scale bar presents 2 μm. Fibril structure (red arrow) was obviously observed both in native HPC and T-PPS sample at the dehydrated condition.

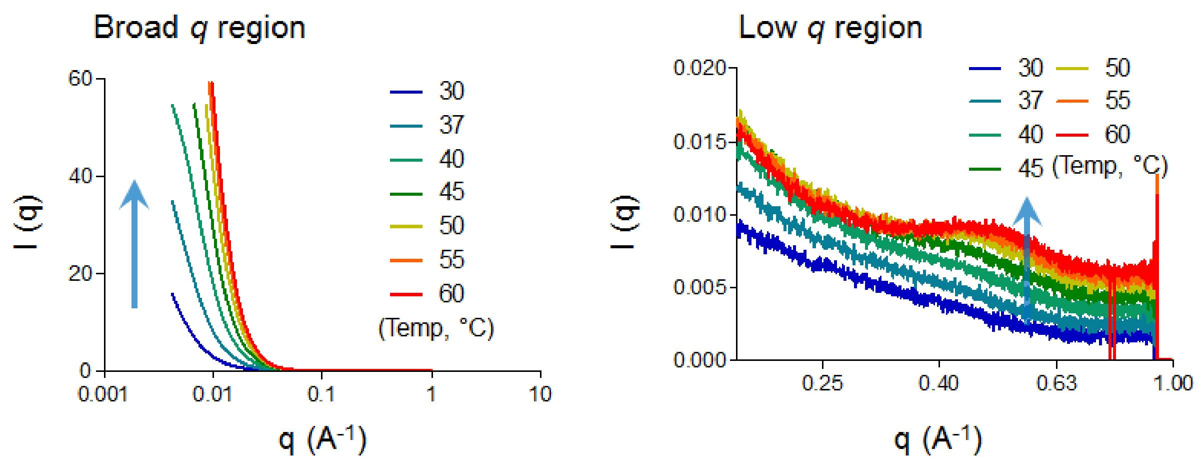

Figure S6. SAXS curve for native HPC in aqueous solution (10 mg/mL) at different temperature (from 30 to 60 °C).

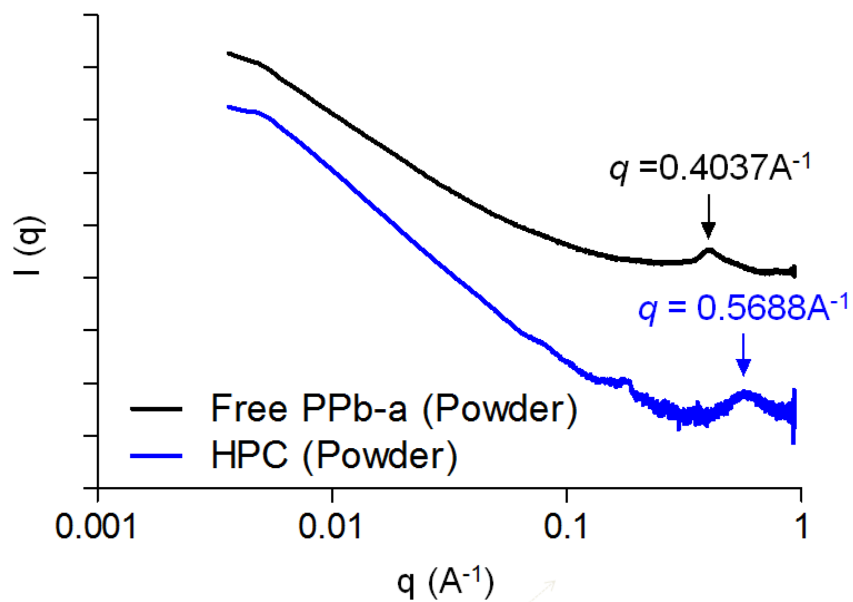

Figure S7. SAXS curve for free PPb-a powder and HPC powder (embedded in kapton film) at room temperature (25 °C). Peaks for PPb-a and HPC are at q = 0.4037 A^{-1} and 0.5688 A^{-1}, respectively, corresponding to *d*-spacings of 1.56 and 1.1 nm respectively.

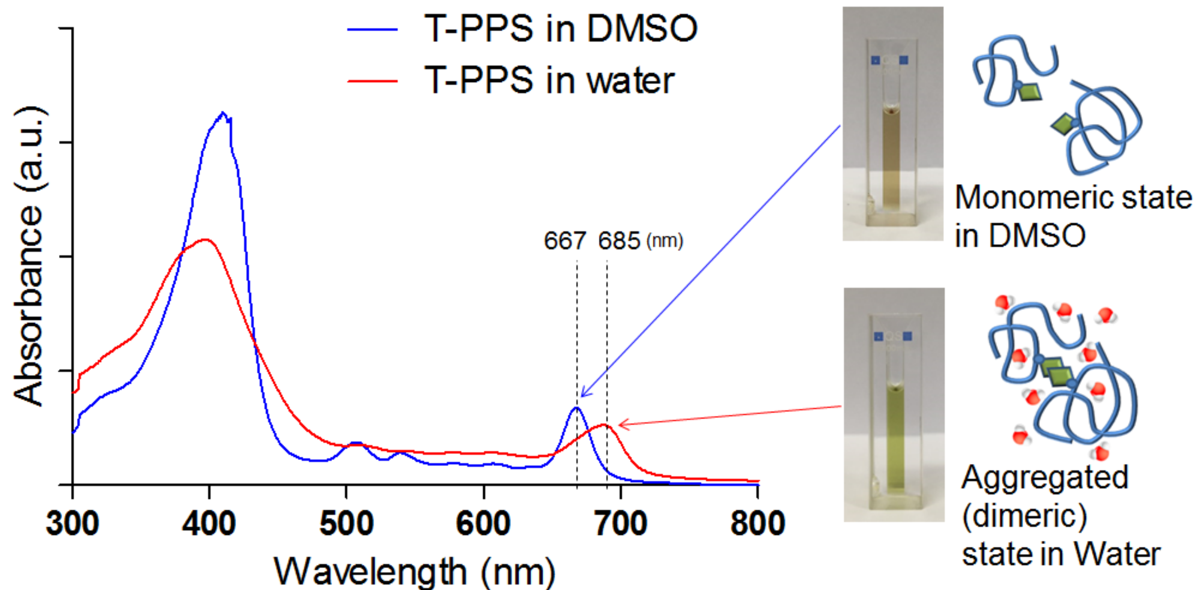

Figure S8. Light absorption spectra of T-PPS (10 mg/mL) at room temperature in water and DMSO, respectively (Inset: schematic illustration of inter-molecular structural change of T-PPS in different solvent media). The absorption band at 667 nm is characteristic for the corresponding the PPb-a monomer, while the band at 685 nm is assigned to absorption of the PPb-a dimer.[4]

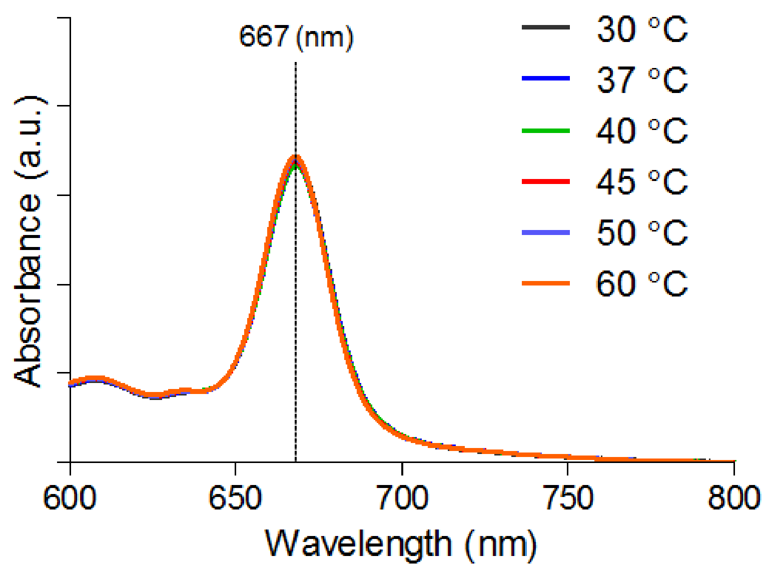

Figure S9. Light absorption spectra of T-PPS (10 mg/mL) as a function of temperature in DMSO.

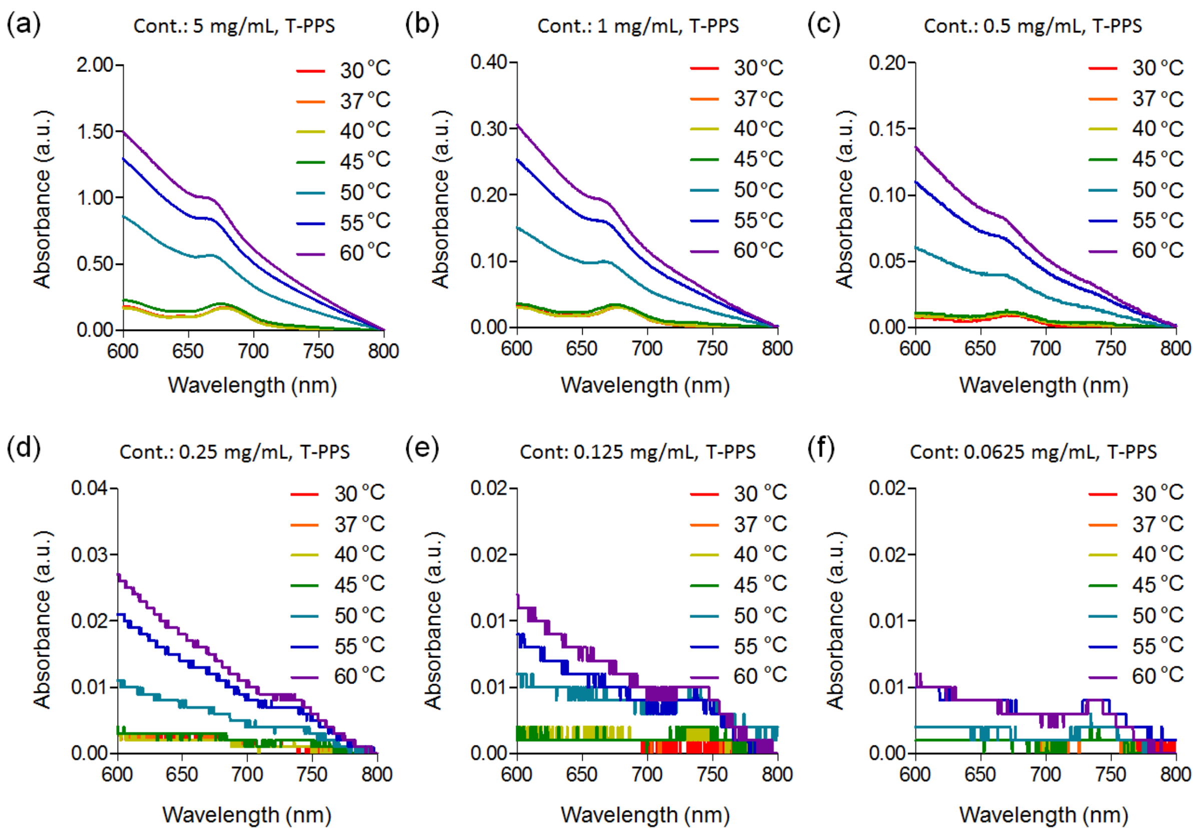

Figure S10. Temperature-dependent light absorption spectra change of different concentration of T-PPS in water.

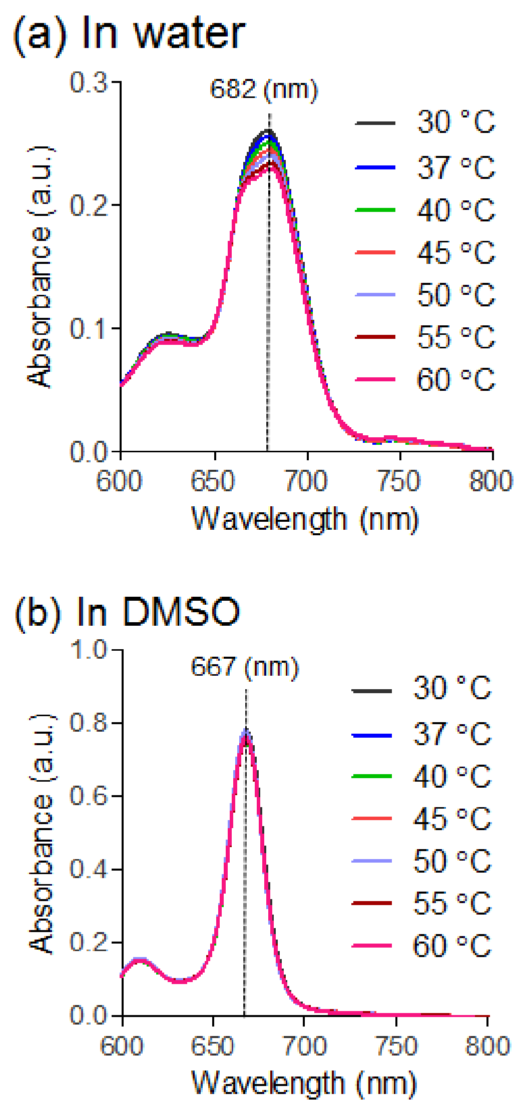

Figure S11. Light absorption spectra of free PPb-a (2 μg/mL) as a function of temperature in (a) water and (b) DMSO, respectively. Although the characteristic Q_y-band (at 682 nm) for aggregation was also observed in water due to their strong intermolecular hydrophobic interaction,[5] temperature-induced Q_y band shift was not observed in both water and DMSO.

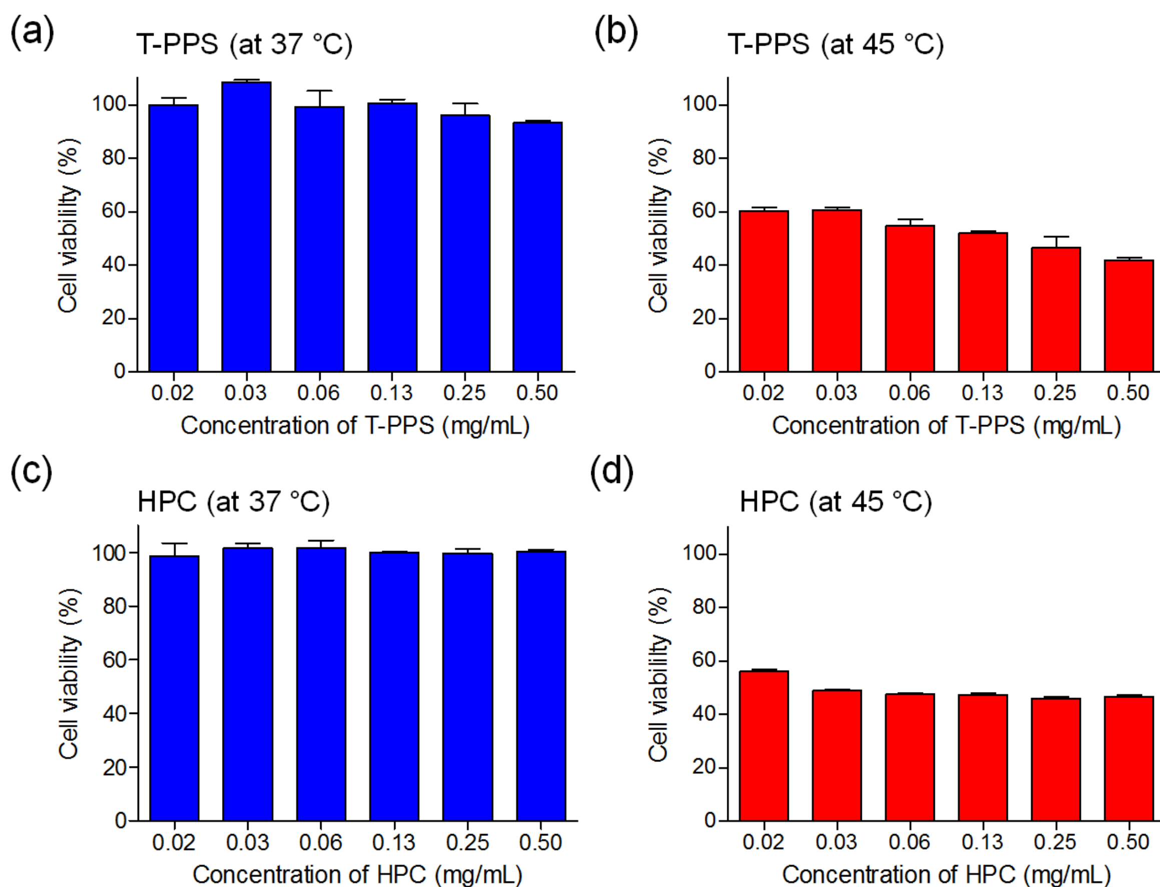

Figure S12. *In vitro* cytotoxicity of T-PPS and HPC against PANC2 cells. PANC2 cells were treated with either T-PPS or native HPC in various concentrations at physiological (37 °C) or hyperthermia (45 °C) temperature.

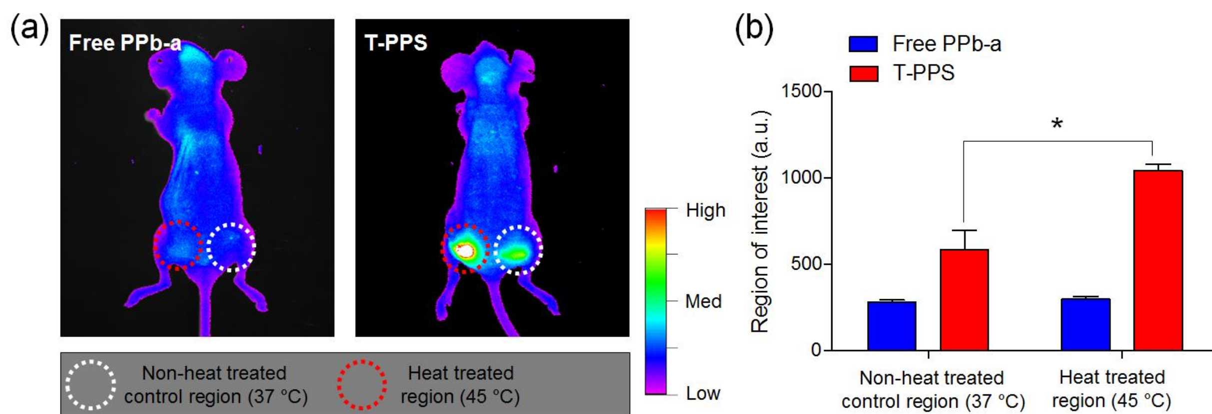

Figure S13. *In vivo* fluorescent imaging of heat-induced activation of T-PPS. (a) *In vivo* fluorescent image of free PPb-a or T-PPS treated BALB/c nude mice after heat treatment. (b) Quantitative analysis of the fluorescent signals obtained from *in vivo* fluorescent image. The fluorescent signal was quantified by measurement of region of interest (ROI) value for the injected sites (*$p < 0.05$, n=3).

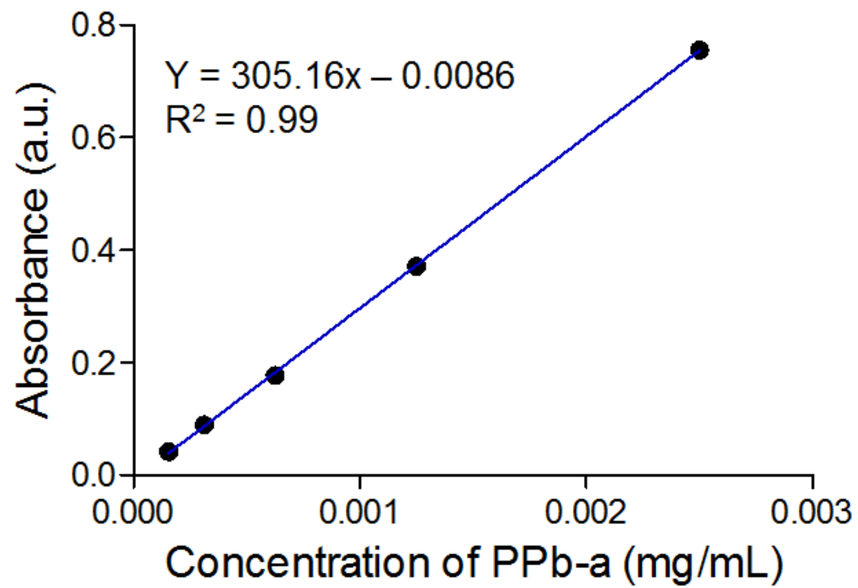

Figure S14. A linear regression curve obtained by plotting the absorbance (at 413nm) of free PPb-a at different concentration.

References for SI

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