Electronic Supplementary Material (ESI) for Nanoscale.


# Gadolinium-porphyrin based polymer nanotheranostics for fluorescence/magnetic resonance imaging guided photodynamic therapy

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## EXPERIMENTAL

**Biocompatibility.** The cytotoxicity of Gd-PNPs was evaluated against HeLa cells and CT 26 cells using a standard MTT assay. In brief, the cells were seeded in 96-well plates at a density of $3.0\times10^3$ cells per well and allowed to adhere overnight. Then, the cells were treated with Gd-PNPs at various concentrations (0, 50, 100, 150, 200, 250, 300, 350 and 400 μg $mL^{-1}$) for 24 h, the cell viabilities were measured by MTT assay.

The hemocompatibility of Gd-PNPs was evaluated using human blood cells. 0.5 mL of blood cells suspension ($1.0 \times 10^8$ cells $mL^{-1}$) was incubated with 0.5 mL of Gd-PNPs with different concentrations (0, 50, 100, 150, 200, 250, 300, 350 and 400 μg $mL^{-1}$) for 2 h at room temperature under mild shake. Then, the samples were centrifuged with 1000 rpm for 10 min, and the upper supernatants are collected for measuring the absorbance at 541 nm to calculate the hemolysis percentage using the following equation: $Hemolysis(\%) = \frac{A - A_{PBS}}{A_{TritonX-100} - A_{PBS}} \times 100\%$. In this hemolysis assay, 0.5 mL of PBS and ultrapure water are used as negative and positive control, respectively. In addition, the sediments were re-dissolved in PBS for erythrocyte morphology observation (**Figure S4B**).

The biotoxicity of Gd-PNPs is evaluated using zebra fishes and Balb/c mice. All experiments are approved by the Animal Experimentation Ethics Committee of Sun Yat-Sen University and conducted in accordance with guidelines for human care. 6 days old zebra fishes are added in 24 wells with 10-12 larvae per well, followed by incubation with 2 mL Gd-PNPs solution (dissolved in E3 medium, 0, 100, 200, 400 μg $mL^{-1}$) for 8 h. At different stages of embryonic development (from 24 to 96 hpf), the

number of live embryos were counted to evaluate the *in vivo* cytotoxicity. Bright field and FL images of the zebrafish are then recorded using a fluorescent microscope (Olympus IX71, Japan) under excitation at 535 nm. The survival rate and hatching rate were calculated following the given equations:

$$Survival\ rate = \frac{the\ number\ of\ viable\ embryos}{the\ number\ of\ embryos} \times 100\%$$

$$Hatching\ rate = \frac{the\ number\ of\ hatched\ embryos}{the\ number\ of\ embryos} \times 100\%$$

*In vivo* toxicity of Gd-PNPs is further evaluated using Balb/c mice (18-22g, n=3). After intravenous injection of 0.8 mg $mL^{-1}$ Gd-PNPs, the mice anesthetized with 4 % chloral hydrate (6 mL $kg^{-1}$) are dissected at 3 days, and the main organs are collected for H&E staining. The mice injected with 100 μL of normal saline are used as control.

**Tumor Models**. To establish CT 26 tumor-bearing mice models, 100 μL of CT 26 cell suspension ($1 \times 10^7$ cells·$mL^{-1}$) were injected subcutaneously in the back of Balb/c mice (4-5 weeks, 16-20 g). The mice were used when the tumor reached about 150-200 $mm^3$ in volume. All experiments are approved by the Animal Experimentation Ethics Committee of Sun Yat-Sen University and conducted in accordance with guidelines for human care.

**Mechanism for singlet oxygen detection.** A well-established protocol was used to evaluate the capability of Gd-PNPs to generate singlet oxygen under illumination. The mechanism for singlet oxygen detection can be explained using the following chemical equations:

$$^1_0O_2 + imidazole(A) \rightarrow [AO_2] \rightarrow AO_2$$

$$AO_2 + RNO \rightarrow -RNO + Products$$

In detail, imidazole (A) ring could capture $^1O_2$, resulting in the formation of a trans-annular peroxide intermediate ($AO_2$). $AO_2$ could induce the bleaching of N,N-Dimethyl-4-nitrosoaniline (RNO) to -RNO, resulting in the absorbance change at 440 nm which can be recorded with a UV-VIS spectrometer.

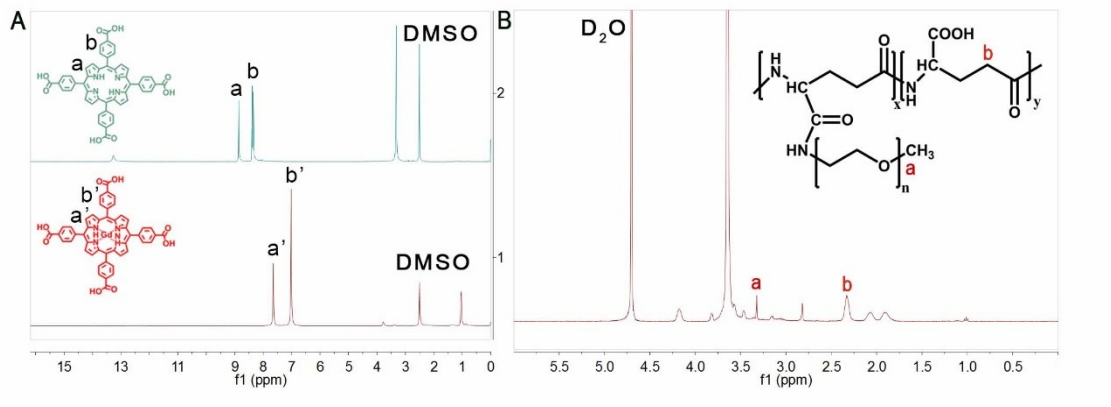


**Figure S1.** (A) $^{1}$H NMR spectra of TCPP and Gd-TCPP. (B) $^{1}$H NMR spectra of mPEG-PGA.

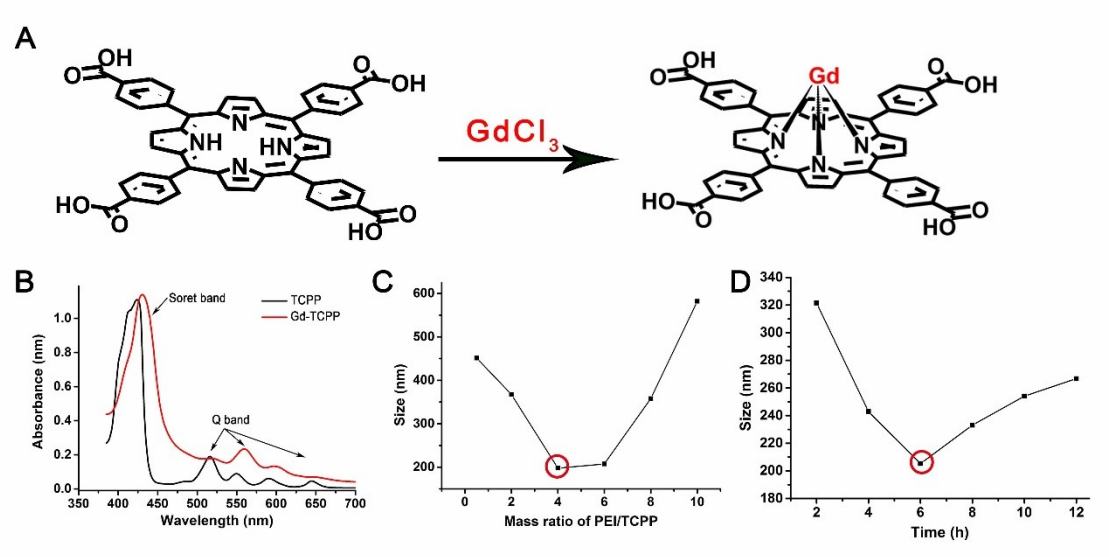


**Figure S2.** (A) The chelation reaction between TCPP and $Gd^{3+}$ to prepare Gd-TCPP. (B) UV-visible spectra of TCPP and Gd-TCPP. (C-D) Optimization for the synthesis of the supramolecular nanotheranostics using the diameter of the as-prepared nanoparticle as the key parameter: (C) the mass ratio of PEI/TCPP; (D) reaction time.

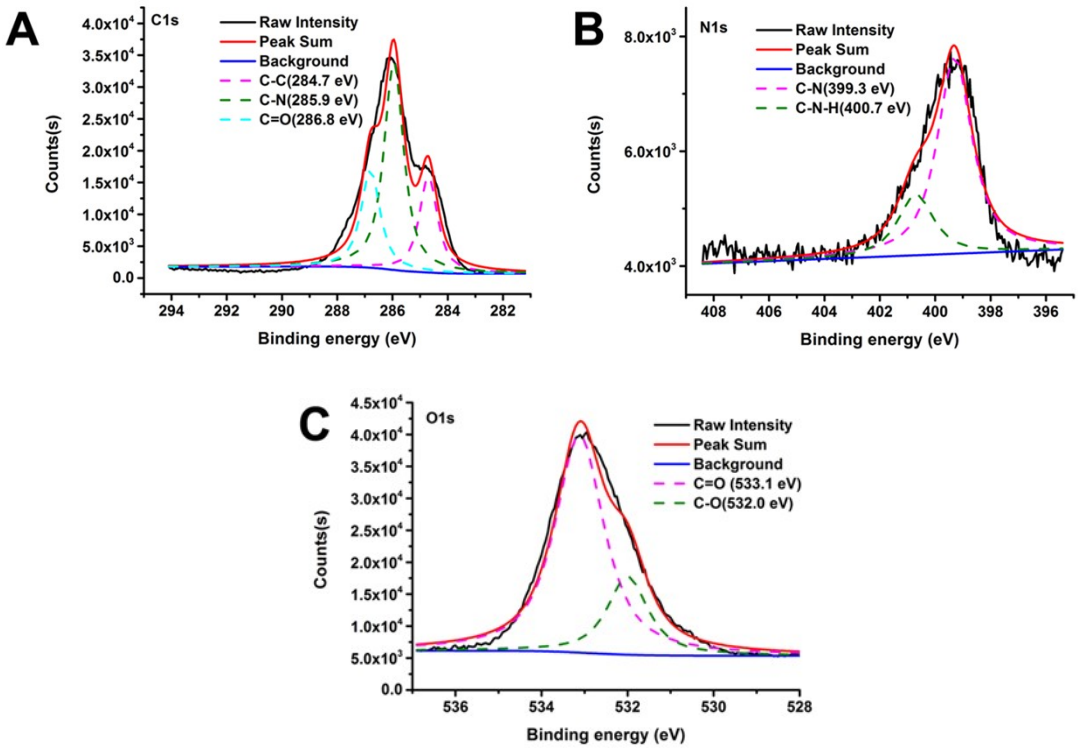


**Figure S3.** The deconvoluted XPS spectra of Gd-FPNPs: (A) C 1s, (B) N 1s, and (C) O 1s.

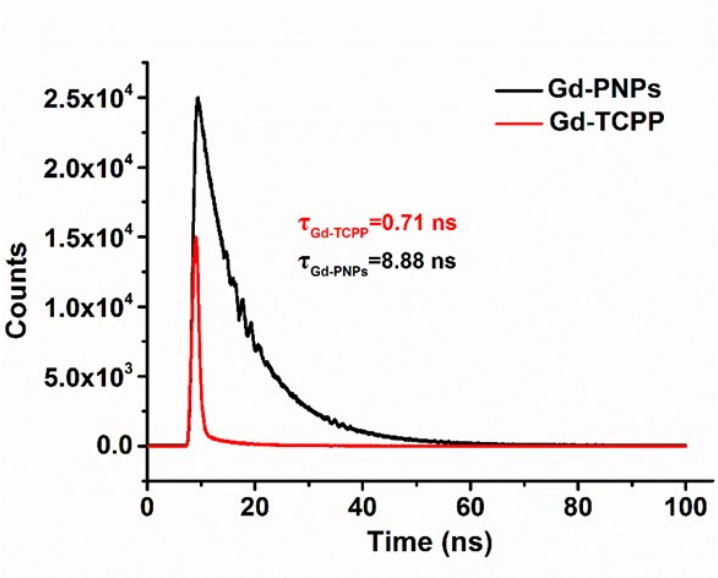


**Figure S4**. Fluorescence decay profiles of Gd-TCPP and Gd-PNPs.

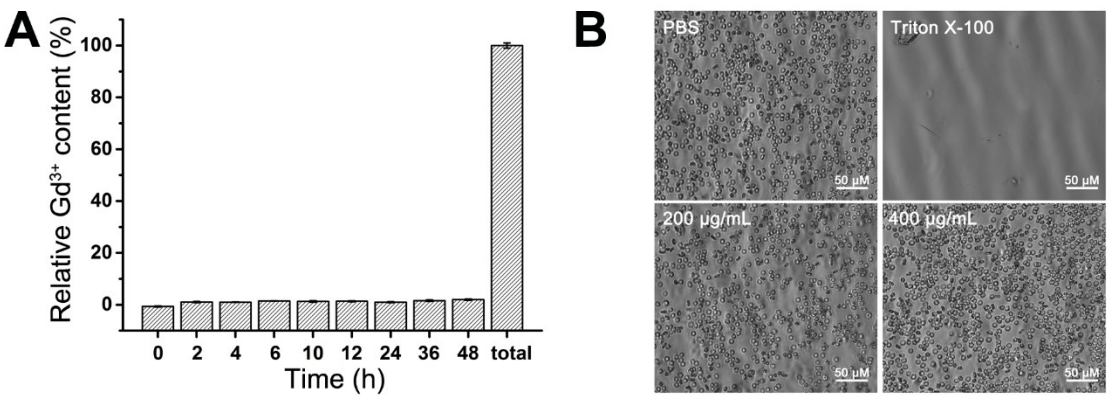


**Figure S5.** (A) $Gd^{3+}$ leakage from Gd-PNPs at different time points. (B) Morphology of human red blood cells upon treatment with PBS, Triton X-100, and Gd-PNPs (200, 400 μg $mL^{-1}$) for 2 h.

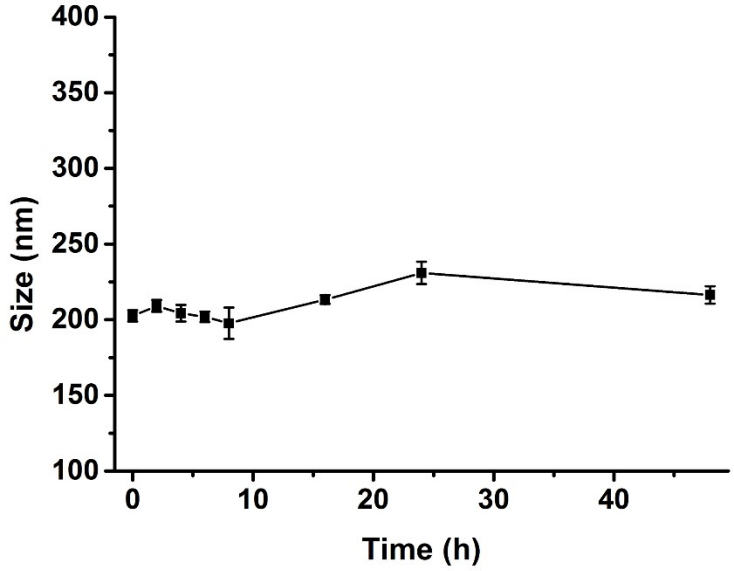


**Figure S6.** The size change of Gd-PNPs after incubated in FBS at different periods.

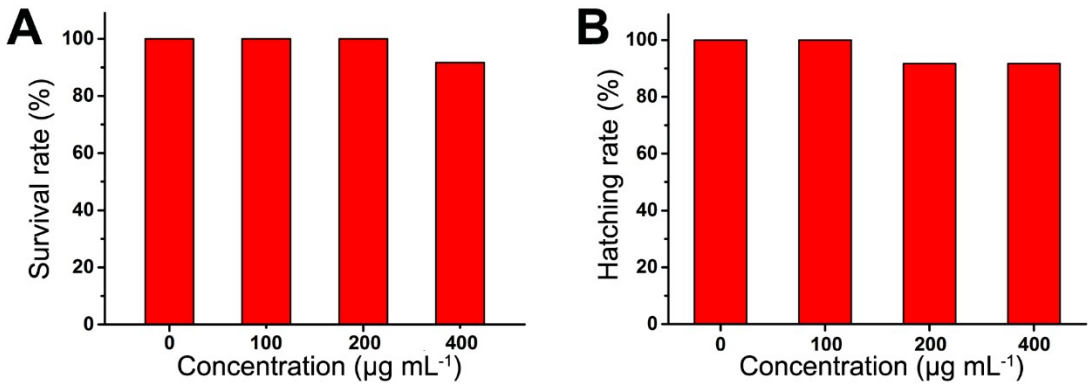


**Figure S7.** Survival rate (A) and hatching rate (B) of zebrafishes upon treatment without (0 μg $mL^{-1}$) and with Gd-PNPs (100, 200, 400 μg $mL^{-1}$).

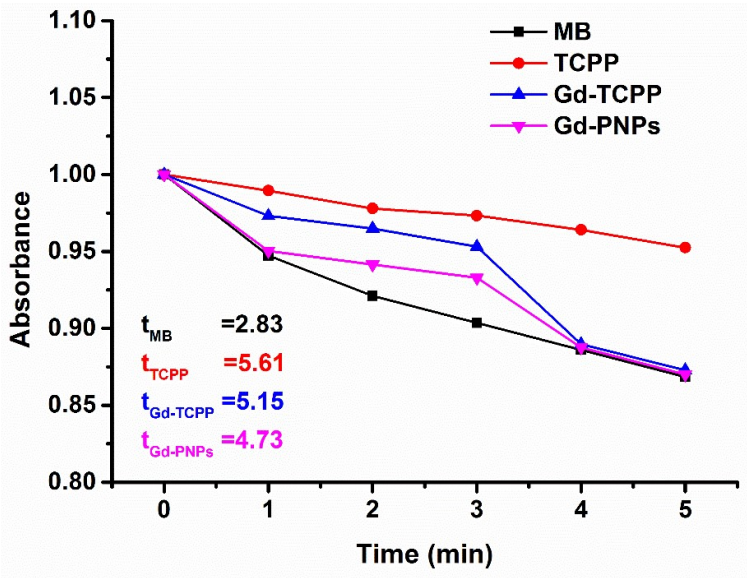


**Figure S8.** The $^1O_2$ quantum yield of TCPP, Gd-TCPP and Gd-PNPs.

**Table S1**. The exact values of MRI signals of tumor at each time point.

| Sample | 0 h | 1 h | 3 h | 6 h | 9 h | 12 h |
|---|---|---|---|---|---|---|
| Gd-PNPs (n=3) | 376.5 | 310.8 | 600.5 | 1242.9 | 985.1 | 767.4 |
| | 391.1 | 358.1 | 668.3 | 1367.9 | 1012.2 | 820.9 |
| | 289.3 | 250.4 | 552.1 | 1172.8 | 905.2 | 710.1 |
| Gadobutrol (n=3) | 303.6 | 812.2 | 433.8 | 358.2 | 322.7 | 418.2 |
| | 387.6 | 924.6 | 549.1 | 465.7 | 440.1 | 513.8 |
| | 303 | 938.1 | 551.9 | 457.8 | 454.4 | 535.1 |