

*Electronic supplementary information*

**One-step synthesis of novel phosphorus nitride dots for two-photon imaging  
in living cells**

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## **1. Experimental**

### **1.1 Materials**

Phosphonitrilic chloride trimer (PCT) and melamine (MA) were obtained from Adamas Beta. Dulbecco's Modified Eagle's medium (DMEM) and Trypsin-EDTA (0.25%) were purchased from GE Healthcare Life Sciences. Fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco. All other chemical reagents were of analytical grade and used as received without further purification.

### **1.2 The Preparation of PNDs**

The PNDs were synthesized by modified solvothermal method.<sup>1</sup> 20 mg PCT were mixed with 20 mL ethanol and transferred into a Teflonlined autoclave and reacted under 180 °C for 12 h. When cooled to the room temperature, the mixture solution was purified by dialysis using 500 Da dialysate bag and then obtained PNDs were kept at 4 °C for further use.

As a control, g-C<sub>3</sub>N<sub>4</sub> QDs was prepared with a method reported previously.<sup>2</sup> 3.0 g MA and was grinded uniform in an agate mortar for 20 min, transferred to ceramic crucible and calcined at 520 °C for 2 h in muffle furnace to prepare bulk g-C<sub>3</sub>N<sub>4</sub>. Before temperature decreased from 520 °C, the resulted g-C<sub>3</sub>N<sub>4</sub> was rapidly added to 30 mL pure water and disrupted by ultrasonic for 30 min. Through centrifuged at about 7000 rpm, and dialyzed in a dialysis bag (500 Da), the obtained g-C<sub>3</sub>N<sub>4</sub> QDs were kept at 4 °C for further use.

### **1.3 Characterizations**

The crystal structures of samples was characterized by X-ray diffraction (XRD) using Bruker D2 (Bruker D8, using Cu K $\alpha$  radiation operating at 30 kV and 10 mA, scanning from  $2\theta = 10^\circ$  to  $80^\circ$ ). Transmission electron microscope (TEM) images of the nanoparticles were obtained by a Tecnai G20 (FEI, USA) instrument, which was operated at 200 kV. The particle size and zeta potential of the

nanoparticles were measured at room temperature by dynamic light scattering (DLS) using a zeta particle size analyzer (Nano-ZS, Malvern, UK) with a detection angle of scattered light at 173°. X-ray photoelectron spectroscopy (XPS) was measured on a VG Multilab 2000 system. Raman spectra were taken with a confocal microprobe Raman system (LabRAM HR Evolution, Japan), and the laser of 633 nm was used as the excitation source. Scattering spectra were recorded in the range of 40-4000  $\text{cm}^{-1}$  and the data acquisition time was 3.0 s. Fourier transform infrared spectra (FT-IR) of these samples were measured on Bruker Equinox 55 instrument. Photoluminescence (PL) spectra were recorded on Hitachi F7000. Fluorescence images were recorded by total internal reflection fluorescence microscopy (TIRFM, Nikon Eclipse Ti, Japan). Two-photon spectra and images were taken by two-photon imaging system constructed by our group.

TPA cross section was determined by Super-resolution multiphoton confocal microscope (TCS SP8 STED 3X, Leica, Germany) using Rhodamine B as the reference.<sup>3</sup> An IR laser (Chameleon Ultra II, 680 nm-1080 nm) that produced 140 fs (HW1/e) pulses at a repetition of 80 MHz was used to excite the two-photon fluorescence in TCS SP8 STED 3X. And the TPA cross section ( $\delta$ ) of PNDs can be calculated utilizing the equation of  $\delta_2 = [\delta_1 F_2 \phi_1 c_1] / [F_1 \phi_2 c_2]$ , where  $\delta$  represented the TPA cross section,  $F$  was the fluorescence intensity,  $\phi$  was the fluorescence quantum yield, and  $c$  was the molar concentration. 1 and 2 represented the Rhodamine B and PNDs, respectively.<sup>2</sup>

## 2.4 In vitro cytotoxicity of PNDs

Human breast cancer cell line (MCF-7 cells) were cultured in DMEM supplemented with 10 wt% FBS, 100 units/mL of penicillin and 100 mg/mL of streptomycin. The cells were incubated at 37 °C in a humidified atmosphere containing 5% of  $\text{CO}_2$ .

To evaluate the in vitro cytotoxicity, MTT assays were performed on the MCF-7 cells. 100  $\mu\text{L}$  of MCF-7 cells were seeded in a 96-well plate at a density of  $1 \times 10^5$  cells per mL and incubated for 24 h. 100  $\mu\text{L}$  of PNDs (0–200  $\mu\text{g}/\text{mL}$ ) dispersed in the culture medium were added in 96-well plate and

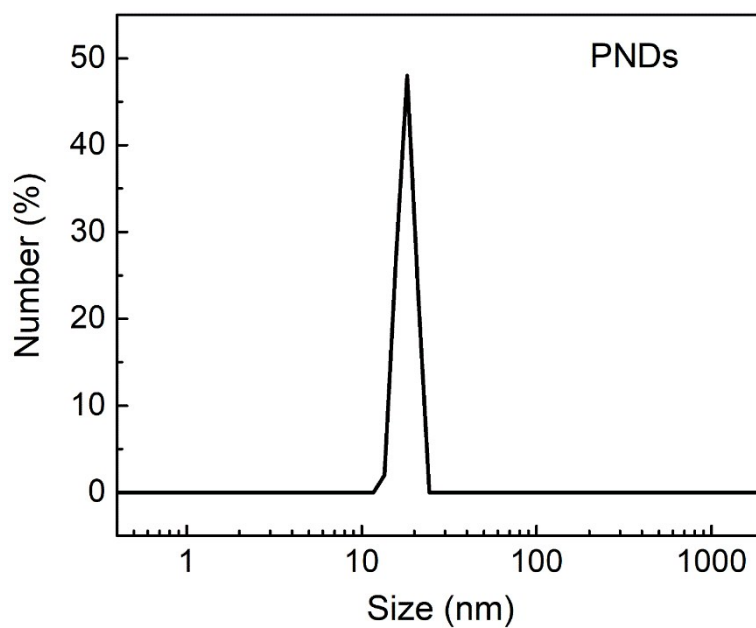
incubated for another 24 h. 10  $\mu$ L of MTT (5 mg/mL) was added to each well. After an additional 4 h incubation, the medium and MTT were removed, and the MTT-formazan crystals in each well were dissolved in 100  $\mu$ L of DMSO. The absorbance of the suspension was recorded using a microplate reader (Thermo MultiskanFC, USA) at a wavelength of 570 nm and 620 nm.

## **2.5 Two-photon and one-photon fluorescence imaging**

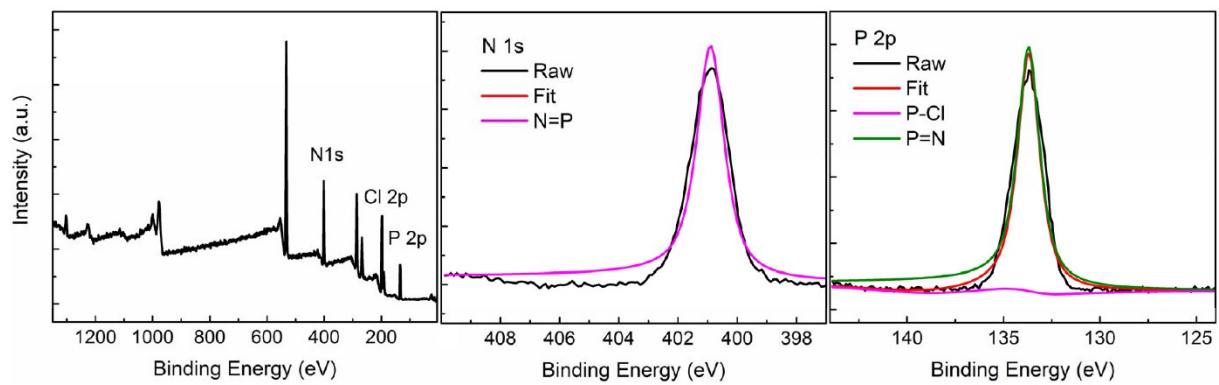
For the two-photon fluorescence imaging, 2 mL of MCF-7 cells were seeded in a glass bottom dish with a density of  $1 \times 10^5$  cells per mL, and incubated at 37 °C in 5% CO<sub>2</sub> for 24 h. Then, the culture medium was replaced with 2 mL of fresh culture contained PNDs (100  $\mu$ g/mL). After being incubated for another 4 h, the cells were washed three times with PBS to remove the free nanoparticles for the next living cells imaging. The PNDs were excited by 800 nm NIR femtosecond laser at the wavelength of 800 nm, and the two-photon fluorescence images at emission wavelengths of 400–500 nm were collected using a TPI system constructed by our group.

Similar method was used to study the one-photo fluorescence imaging. Additionally, PNDs were excited at 405, 488 and 535 nm, and the fluorescence images at emission wavelengths of 430–490, 512–558 and 550–660 nm were obtained using a TIRFM.

## 2. Figures



**Fig. S1.** Size distribution of the PNDs in MilliQ water measured by DLS at a scattering angle of  $173^\circ$  (backscatter detection) at room temperature.



**Fig. S2.** XPS spectra of PNDs: survey spectra, N 1s spectra and P 2p spectra.

**Table S1.** The element content of PN QDs by XPS analysis.

<b>Element</b>	<b>P</b>	<b>N</b>	<b>O</b>	<b>C</b>	<b>Cl</b>
<b>Atomic (%)</b>	7.56	17.03	31.96	32.06	11.38

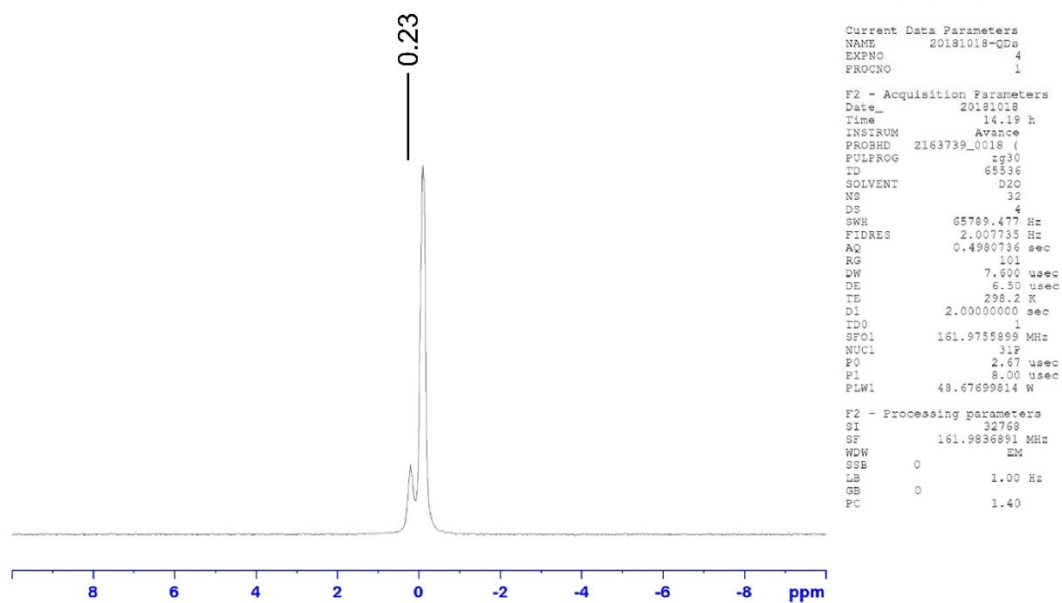
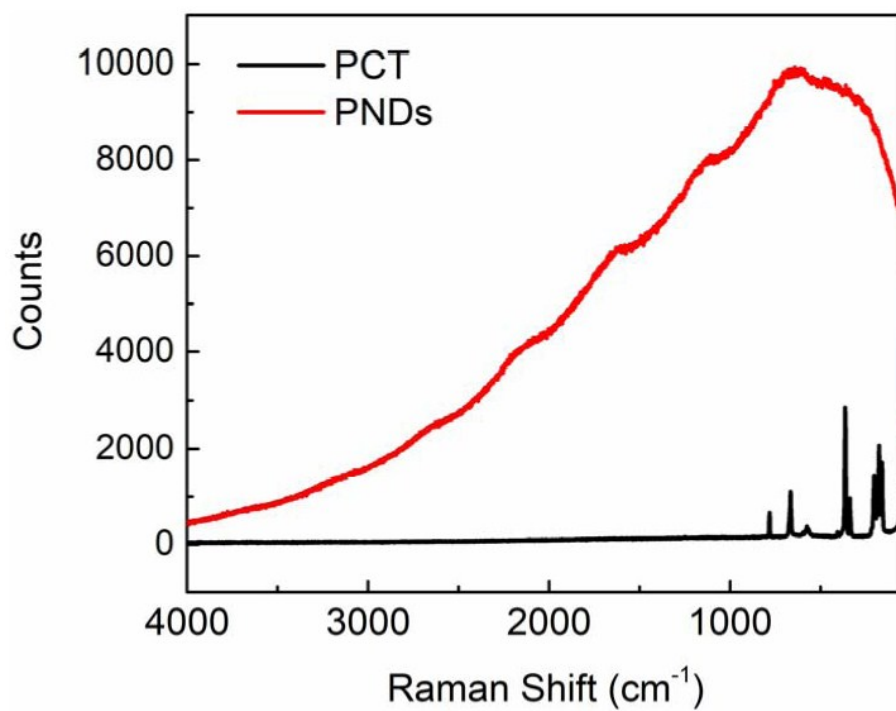
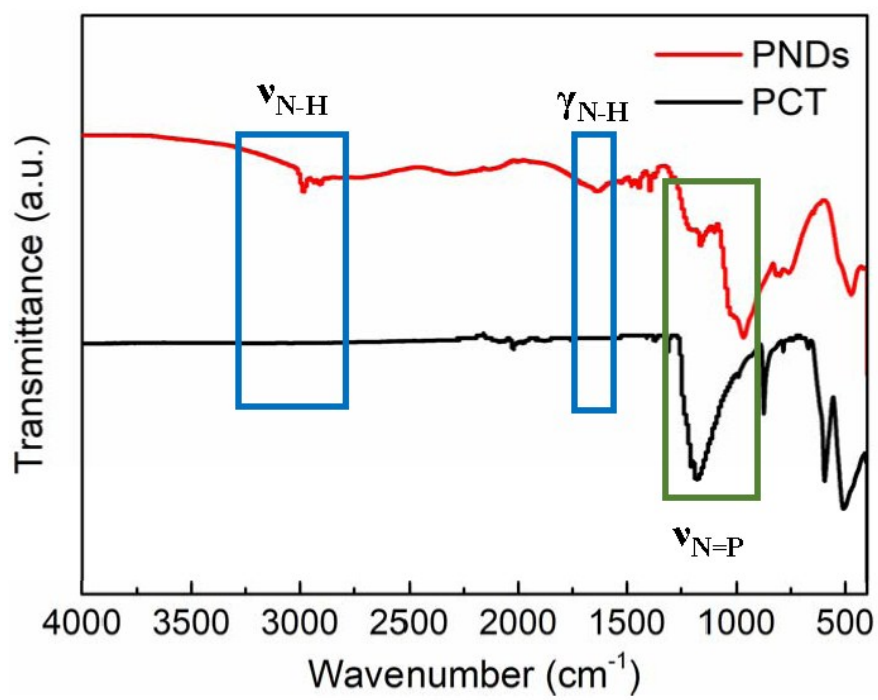


Fig. S3. 162 MHz  $^{31}\text{P}$  NMR spectra of PNDs.

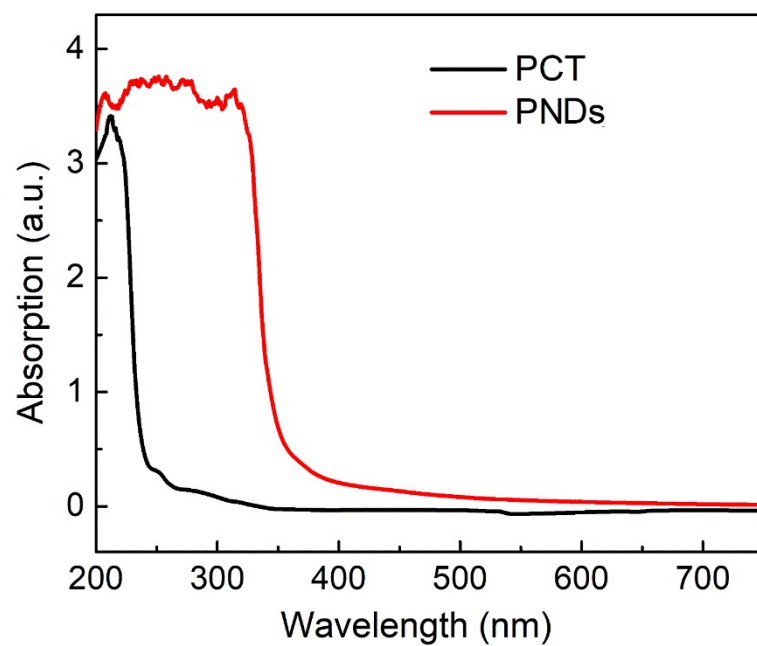




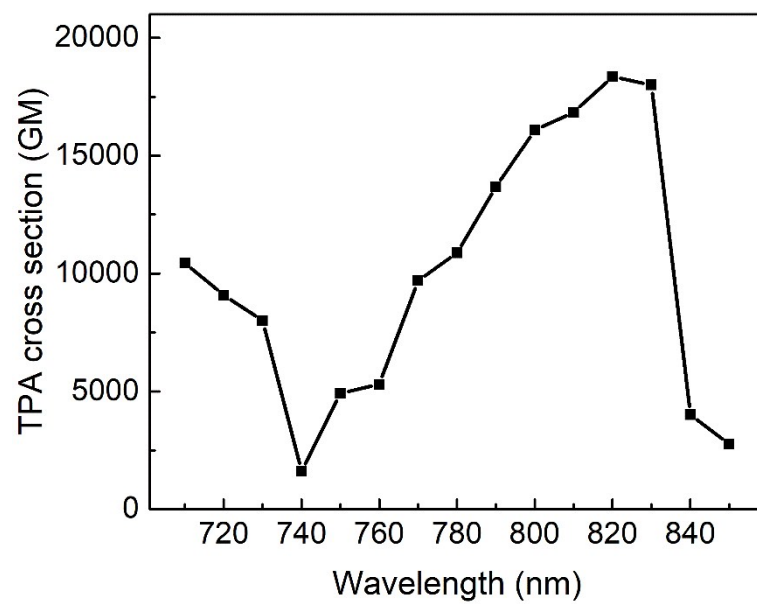
**Fig. S4.** The characterization of Raman scattering for PCT and PNDs under the laser of 633 nm.



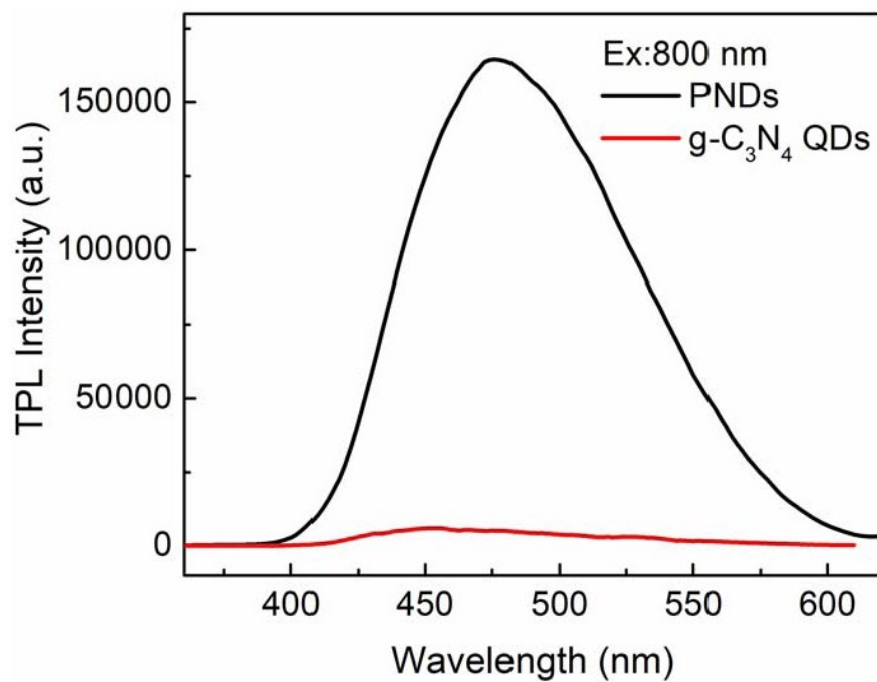
**Fig. S5.** The FT-IR spectra of PCT and PNDs.



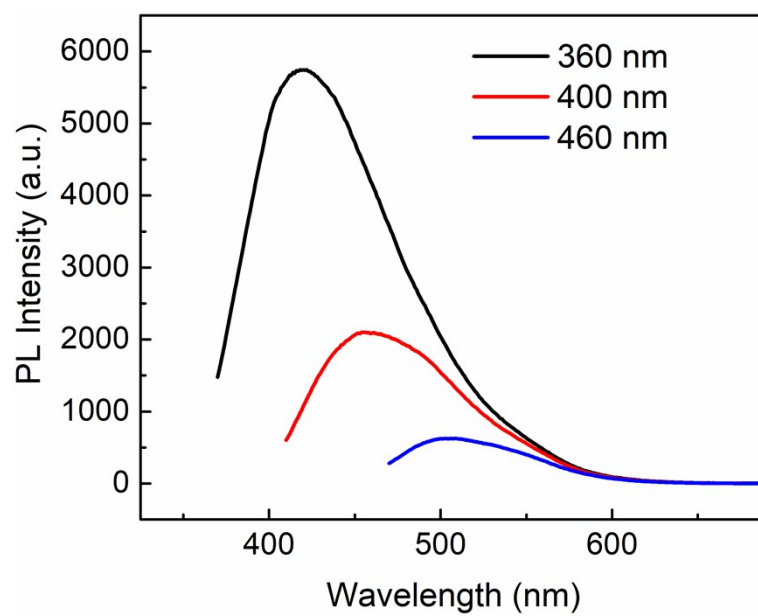
**Fig. S6.** UV-Vis spectra of the PCT and PNDs dispersed in ethanol.



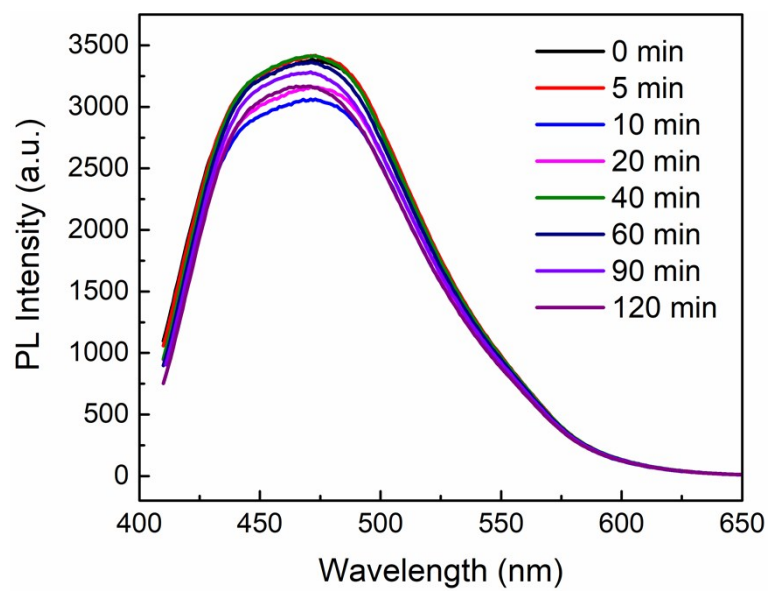
**Fig. S7.** The TPA cross section of PNDs at different wavelength using Rhodamine B as the reference.



**Fig. S8.** The two-photon emission spectra of PNDs and g-C<sub>3</sub>N<sub>4</sub> QDs with the excitation wavelength of 800 nm.



**Fig. S9.** The emission spectra of PNDs which the excitation wavelength were at  $360\pm 5$ ,  $400\pm 5$  and  $460\pm 5$  nm, respectively.



**Fig. S10.** The stability of PNDs under UV lights for different time. The emission spectra of PNDs which the excitation wavelength was at  $400\pm 5$  nm.

## References

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