Electronic Supplementary Information (ESI)

Water-soluble and phosphorus-containing carbon dots with strong green fluorescence for cell labeling

Wei Wang,[a,b] Yongmao Li[a], Lu Cheng[a], Zhiqiang Cao[b], and Wenguang Liu[a]

Experimental

Materials

Phytic acid (70% solution) and fluorescein (99%, suitable as reference) were purchased from Aladdin Industrial Corporation. Ethylenediamine (EDA, 98%), acetonitrile (99%), sulfuric acid (98%) and sodium hydroxide (solid) were obtained from Guang Fu Technology Development Co., Ltd, China. Tetrahydrofuran (99%), methanol (99%), ethanol (99%) were supplied by Kaiyingte Reagent, Tianjin Jie'er Chemical Co., Ltd, China. Acetone (99%) was obtained from Tianjin Jiangtian chemical technology Co., Ltd. All other reagents were of analytical grades and used without further purification.

Synthesis of PCDs

The procedure for synthesizing PCDs was as follows. 2 ml 70% phytic acid (PA) and 1 ml ethylenediamine (EDA) were added to 25 ml ultrapure water under vigorously stirring. The clear solution became white and opaque due to the insolubility of the salt formed during acid-base reaction. Some opaque solution was lyophilized and the white-powder reactant was collected for characterization. The turbid mixture was then put at the center of the rotation plate of a domestic microwave oven (700W) for about 8 minutes while the solution changed from opaque to transparent and dark brown, indicating the carbonization of the reactant. After cooling to room temperature, 30 ml ultrapure water was poured into the beaker to dissolve the product and the obtained dark brown solution was centrifuged under 9000 r/min for 10 min. The clear brown solution was utilized for further purification.

Purification of PCDs

For further purification of synthesized PCDs, 0.1 g/ml crude PCDs water solution was poured into 5 times volume amount of organic solvent and stirred for 6 h. Then, the clear organic solution underwent centrifuge and rotary evaporation to remove all the organic solvent. The remaining PCDs were re-dissolved in ultrapure water, which was called purified PCDs water solution. The PCDs were only dissolved in organic solvent which was miscible with water. We tested the purification efficiency of PCDs by low-boiling-point organic solvents miscible with water(acetone, acetonitrile, methanol, ethanol, tetrahydrofuran and glacial acetic acid).

Characterization

Photoluminescence (PL) emission measurements were performed using FLS920 fluorometer (Edinburgh Instruments, Britain) equipped with both hydrogen and xenon lamp. PL performance in different pH environment of PCDs was characterized by dropping 50 μL concentrated PCDs solution into 5 ml different pH solution. The Raman spectrum was measured by the DXR Microscope Raman spectrometer (Thermo Electron Corporation, America) with 532 nm laser. UV-Vis absorption was measured on a TU-1810 UV–Vis Spectrophotometer (Pgeneral, China). The morphology and microstructure of the PCDs were examined by high-resolution transmission electron microscopy (HRTEM) on a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. The sample for HRTEM was made by dropping an aqueous solution onto a 300-mesh copper grid coated with a lacy carbon film. The XPS spectrum of the sample was measured on a Kratos AXIS Ultra DLD X-ray Photoelectron Spectroscopy (Shimadzu, Japan). X-Ray diffraction (XRD) pattern of the PCDs were recorded on a Rigaku-D/MAX 2500 diffractometer (Rigaku, Japan) equipped with graphite monochromatized CuKα (λ = 1.54 Å) radiation at a scanning speed of 4°/min in the range from 10° to 90°. The FT-IR spectra of the white-powder reactant and purified PCDs were measured on a Nicolet 380 spectrometer (Thermo, America).

MTT assay and confocal microscopy

We measured the cytotoxicity of PCDs through MTT assay. L929 cells(mouse fibroblasts) were seeded and incubated in a 96-well plate overnight, at a density of 2×104 cells/well. Then, the culture medium was removed and the PCDs were added

into each well with the increasing concentrations from 0.5 to 10 mg/ml and incubated for 24 h before replacing the medium with 200 μl fresh complete medium containing 20 μl MTT (5 mg/ml in PBS). The plate was incubated for another 4 h before all medium was removed and 150 μl/well DMSO was added, followed by shaking for 15 min. The absorbance of each well was measured at 570 nm using a Synergy HT Multi-Mode Microplate Reader (BioTek, USA) with pure DMSO as a blank. Non-treated cell was used as a control and the relative cell viability (mean% ± SD, n = 3) was expressed as $Abs_{sample}/Abs_{control}\times100\%$.

L929 cells were seeded on a coverslip in 6-well plate 12 h before confocal microscopy detection. Then, the culture medium was replaced by 2.5 ml fresh medium containing 2 mg/ml PCDs and the cells were incubated for another 24 h. Subsequently, the cells were washed with isotonic PBS (pH=7.4) three times, and fixed with 4 % paraformaldehyde solution in PBS at 4 ºC overnight. The PCDs labeled samples were examined by laser confocal microscope (Olympus FluoView FV1000) with a fixed excitation wavelength at 405 nm and the corresponding emission wavelength ranged from 500-530 nm.

Measurement of fluorescence quantum yields

The quantum yield (QY) of the PCDs was calculated by comparative method. Fluorescein in 0.1 M was utilized as the standard whose QY was reported to be 90%. The integrated fluorescence intensity is the area under the PL curve in the wavelength range from 410 to 750 nm. Absolute values were calculated according to the following equation:

$$QY_{PCDs} = QY_{ST} * \frac{Grad_{PCDs}}{Grad_{ST}} * \frac{\eta^2_{PCDs}}{\eta^2_{ST}}$$

Where ST denotes the standard, Grad is the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η is the refractive index of the solvent. To prevent the re-absorption effect, absorbance in the 10 mm fluorescence cuvette should never exceed 0.1 at the excitation wavelength.

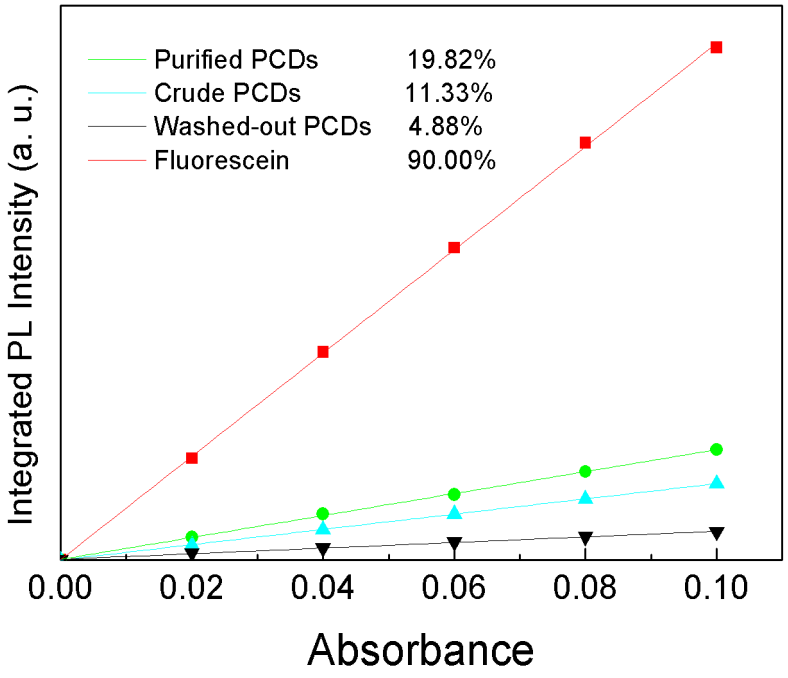

Fig. S1. Photoluminescence and absorbance of PCDs for QY calculation.
(Washed-out PCDs: black substance not soluble in acetone)

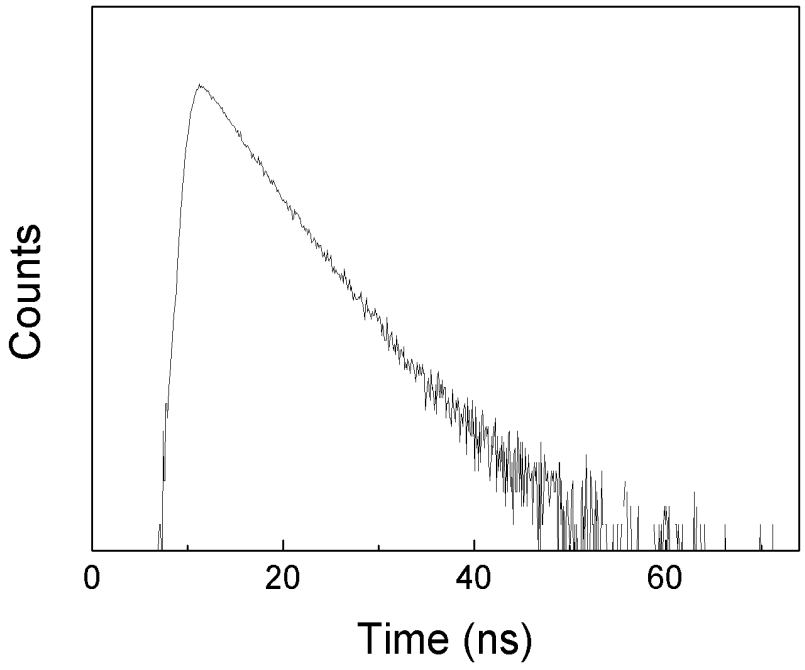

Fig. S2. Photoluminescence decay curve of PCDs at the excitation of 400 nm.

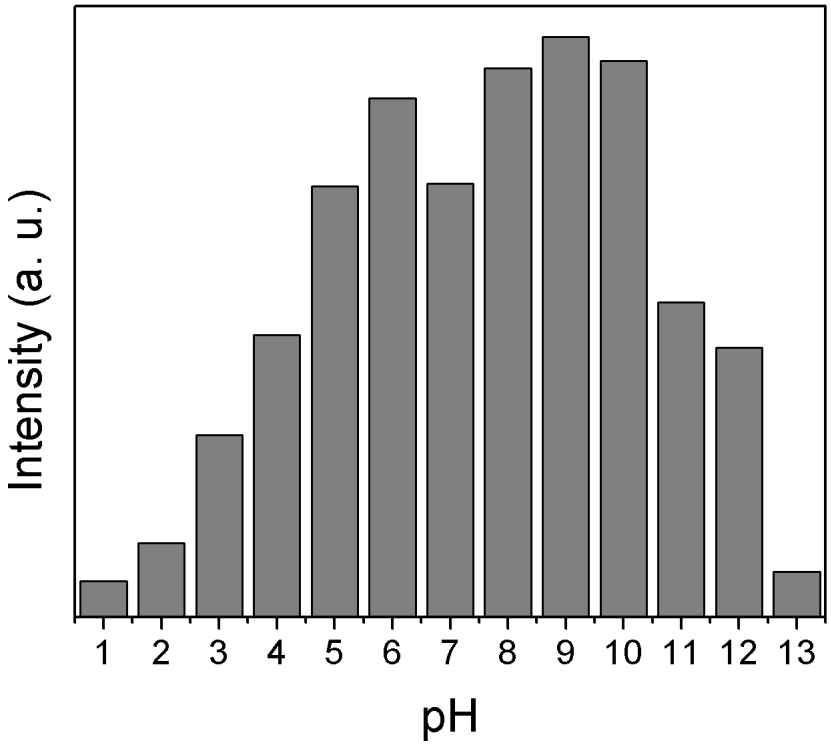

Fig. S3. Integrated photoluminescence intensity of PCDs in different pH solutions.

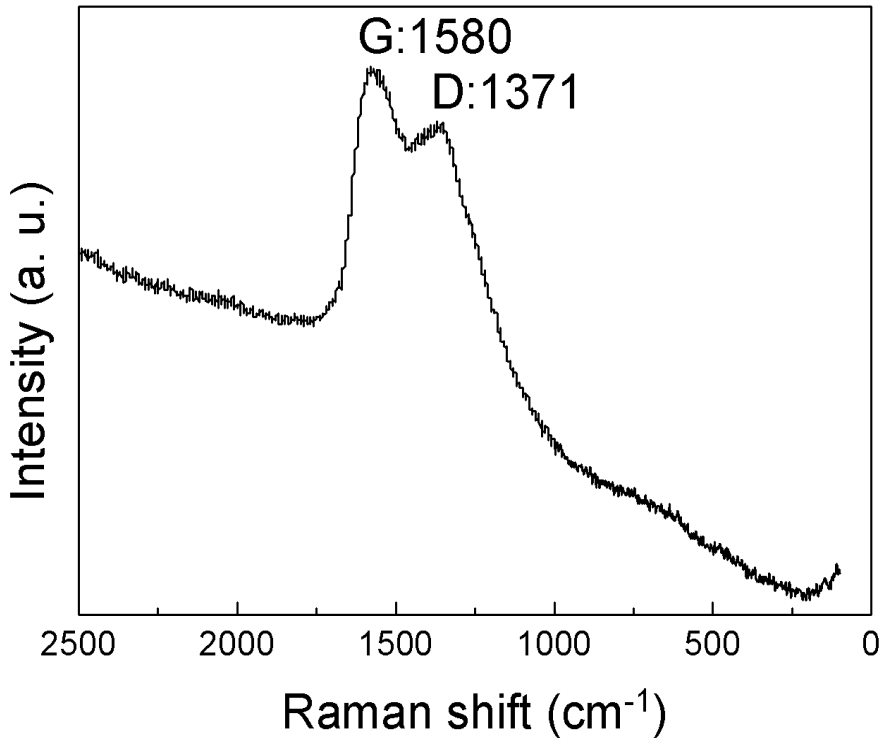

Fig. S4. Raman spectrum of the PCDs.

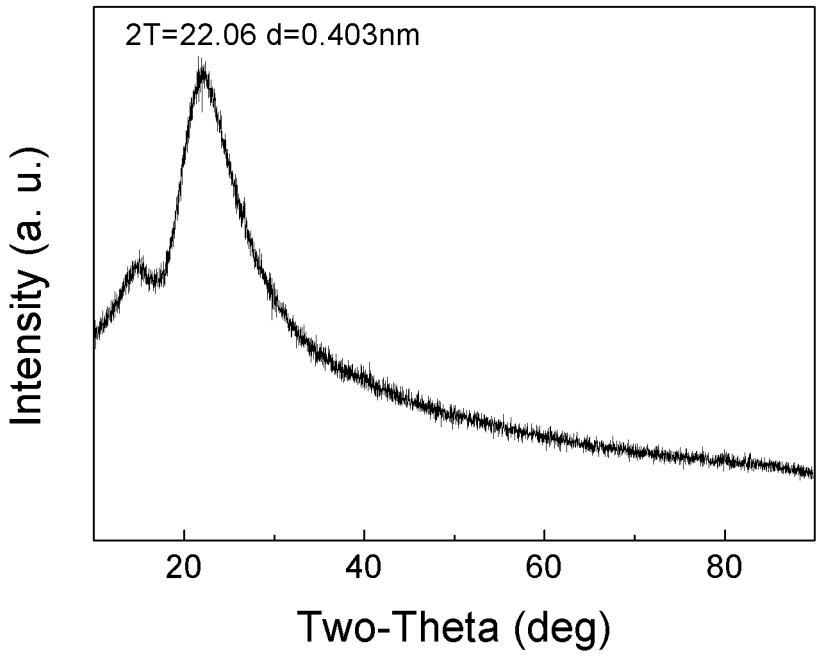

Fig. S5. Typical XRD pattern of PCDs.

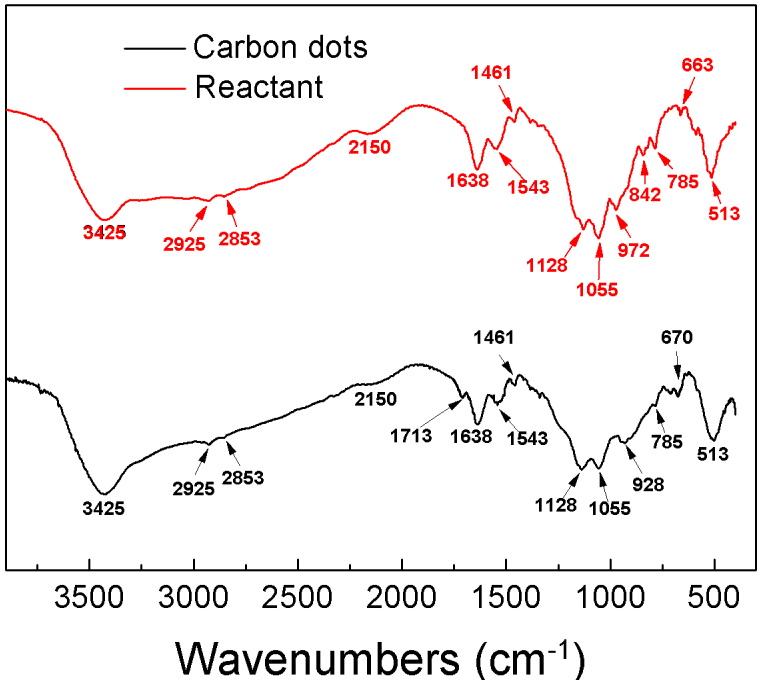

Fig. S6. FT-IR spectra of the reactant (mentioned in the Experimental part) and the PCDs.

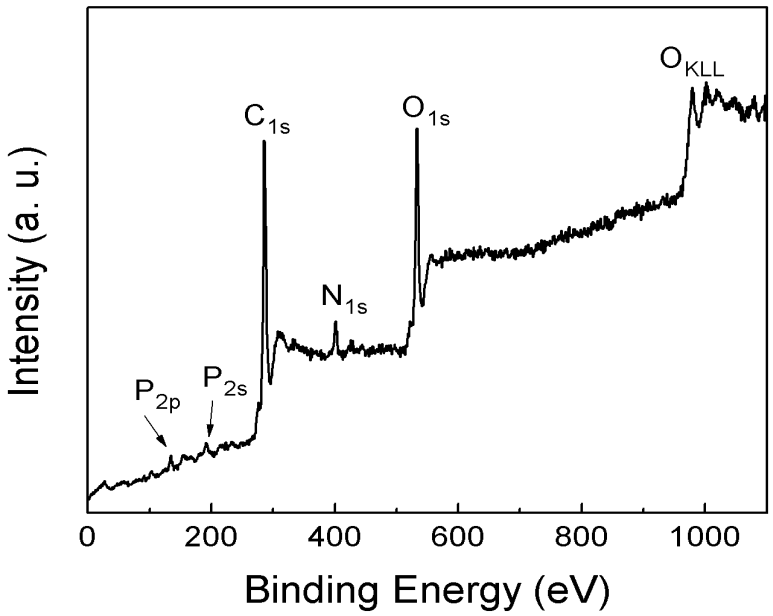

Fig. S7. XPS full spectrum of PCDs.

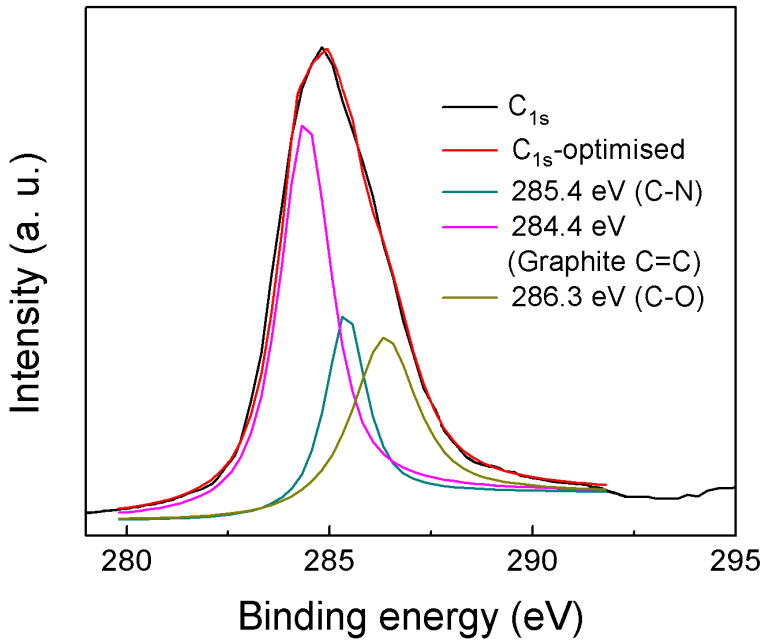

Fig. S8. XPS C 1s spectra of the PCDs.

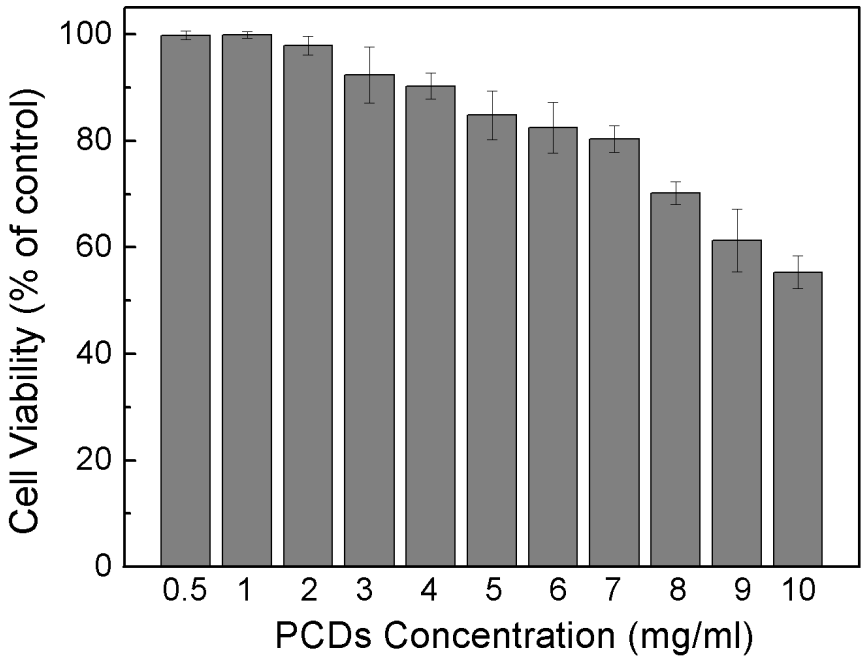

Fig. S9. MTT result of the PCDs. The values represent percentage of cell viability (means% ± SD, n = 3).