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Electronic Supplementary Information

A facile and versatile approach to biocompatible "fluorescent polymers" from polymerizable carbon nanodots

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Experimental section

Materials

Acrylic acid (99%), N,N,N',N'-Tetramethylethylenediamine (TEMED, 99%), glycidyl methacrylate (GMA, 97%) and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) were purchased from Alfa Aesar. 1,2-ethylenediamine (EDA, 98%), 2, 2'-azodiisobutyronitrile (AIBN, 99%), methyl methacrylate (MMA, 99%) and ammonium persulfate (APS, 98%) were obtained from GuangFu Technology Development Co., Ltd. N-isopropylacrylamide, (NIPAM, 97%), polyethylene glycol diacrylate (PEGDA, Mn = 575), Oligo(ethylene glycol) methacrylate (OEGMA, 99%) were purchased from Sigma Aldrich. Quinine sulfate (98%, suitable for fluorescence), Fluorescein (Standard Fluka) were supplied by Fluka. All other reagents were of analytical grades and used without further purification.

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Characterization

UV-Vis absorption was measured on a TU-1810 UV–Vis Spectrophotometer (Pgeneral, China). Photoluminescence (PL) emission measurements were performed using FLS920 fluorometer (Edinburgh Instruments, Britain). The normalized spectrum was obtained by divided each intensity of the PL spectrum by the maximum value of its own. The morphology and microstructure of the CNDs 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) profile of the CNDs 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 5° to 80°. The composition of CNDs was further confirmed by elemental analysis with Vanio-EL (Elementar Analysensysteme GmbH, Germany). Thermogravimetry (TG) curves were recorded on a Perkin–Elmer Pyris TG/DTA Thermal Analyzer (Perkin–Elmer, America) in N_2 atmosphere. The FTIR spectra of the samples were measured on a Perkin–Elmer spectrum 100 spectrometer (Perkin–Elmer, America). ^{1}H NMR spectra of the samples were measured with a UNITY plus-500 NMR spectrometer (Varian, USA).

Synthesis of the PCNDs

Experimentally, 5.49 ml acrylic acid (80 mmol) and 5.35 ml 1,2-ethanediamine (80 mmol) were dissolved in 40 ml water in a common 100 ml beaker under vigorous stirring. Then the transparent solution was put into a 700W domestic microwave oven, heated for 7 minutes until a light smoke appeared. After cooled down to room temperature, 20 ml water was added to dissolve the formed CNDs. To synthesize polymerizable carbon nanodots (PCNDs), 10 ml glycidyl methacrylate (GMA) was added to the above CNDs solution, and stirred for 24 hours at 30 °C). Then, the oil phase of the solution was removed, and the remaining water phase was further washed by n-hexane to remove the unreacted GMA molecules. Finally, the dry PCNDs were obtained by lyophilization of the remaining water solution.

Preparation of the fluorescent P(CND-NIPAM) polymers

The P(CND-NIPAM) was synthesized by radical polymerization. First, 600 mg N-isopropylacrylamide and 60 mg PCNDs were dissolved in 30 ml anhydrous ethanol under N_2 atmosphere by magnetic stirring. When the solution was heated to 80 ºC, 5 mg AIBN was added to the reaction system to initiate the polymerization. After continuously refluxed for 6 hours, the

obtained solution was cooled down to room temperature and dialyzed (MWCO 3500) against water for 5 days. Finally, the aqueous solution was lyophilized to collect dry polymers.

Fabrication of CND-containing hydrogels

The nanoparticle-containing hydrogels were prepared by radical copolymerization of PCNDs, oligo(ethylene glycol) methacrylate (OEGMA) and polyethylene glycol diacrylate (PEGDA575) in the presence of tetramethylethylenediamine (TEMED) as catalyst and ammonium persulfate (APS) as initiator in water. Initial monomer concentration in feed was kept constant at 15 wt%. A series of hydrogels with different CND contents were obtained by varying monomer feed ratio (Table S1). The reaction mixture was injected into glass molds, and then kept in 37 ºC for 24 hours. The resultant hydrogels were purified by immersing in distilled water, which was refreshed daily, for one week to remove reagent residues completely.

Table S1 Preparation of fluorescent hydrogels with different CND contents

CND contents (%)	PCND (mg)	PEGDA (mg)	OEGMA (mg)	TEMED (µl)	APS (mg)	H_2O (µl)
0	0	50	100	5	8	850
0.1	0.15	50	100	5	8	850
0.5	0.75	50	100	5	8	850
1	1.5	50	100	5	8	850
5	7.5	42.5	100	5	8	850
10	15	35	100	5	8	850
50	75	25	50	5	8	850
100	150	0	0	5	8	850

Synthesis of bulk P(CND-MMA) nanocomposites

The synthesis of bulk CND-PMMA nanocomposites was carried out by a two-step procedure. First, PCND-MMA prepolymers with different CND content (0, 2% and 15%) were synthesized by solution polymerization. 10g MMA monomers and a certain amount of PCNDs were dissolved in 50 ml N,N-dimethylformamide (DMF) under magnetic stirring, with N_2 bubbling for 15 minutes. Then the reaction system was heated to 80 ºC, followed by the addition of 100 mg AIBN to initiate polymerization. The reaction was kept for 6 hours, and then the polymers were precipitated by 1000 ml water, and washed by water for five times. Finally, PCND-MMA prepolymers were dried under vacuum over night (yield > 90%).

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The fabrication of bulk P(CND-MMA) nanocomposites was realized by bulk polymerization. PCND-MMA prepolymers and initiator AIBN were dissolved in MMA monomers to form a viscid solution, and then kept under 50 ºC for 3 days, followed by keeping under 80 ºC for another 3 hours, obtaining the bulk P(CND-MMA) material. By varying the feed ratio of differentPCND-MMA prepolymers to MMA ratios, P(CND-MMA) with different CND contents could be obtained (Table S2).

Table S2 Preparation of bulk P(CND-MMA) with different CND contents

CND contents	PCND-MMA prepolymer with different CND contents (mg)			MMA	AIBN
(%)	0	2%	15%	(ml)	(mg)
0	500	0	0	1.6	6
0.05	450	50	0	1.6	6
0.1	400	100	0	1.6	6
0.2	300	200	0	1.6	6
0.5	0	500	0	1.6	6
0.8	47	400	53	1.6	6
1	20	400	80	1.6	6

Cell culture, confocal microscopy and cytotoxicity assay

L929 cell line was obtained from Peking Union Medical College (Beijing, China). The cells were cultured in RPMIi-1640 Medium (1640, HyClone), containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 ºC in 5% CO_2 humidified atmosphere.

For confocal microscopy, L929 cells were seeded on a coverslip in 6-well plate 12 h before use. Then, the culture medium was replaced by 2.5 ml fresh medium containing 2 mg/ml PCNDs and the cells were incubated for another 24 h. The cells were then washed with isotonic PBS (pH 7.4) three times, and fixed with 4 % paraformaldehyde solution in PBS at 4 ºC overnight. The samples were examined under a Leica confocal laser scanning microscope (Mannheim, Germany) equipped with a UV laser (351/364 nm), an Ar laser (457/488/514nm), and a HeNe laser (543/633 nm).

The cytotoxicity of PCNDs was assessed through MTT assay. L929 cells were seeded in a 96-well plate, at a density of 2×10^4 cells/well and incubated overnight. Then, the culture medium was removed and the PCNDs at the increasing concentrations from 0.5 to 10 mg/ml were added

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into each well and incubated for 24 h before refreshing the medium with 200 μl fresh complete medium containing 20 μl MTT (5 mg/ml in PBS). The plate was further incubated for 4 h. Finally, 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 490 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 $Abs_{sample}/Abs_{control}\times100\%$.

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Supplementary data and discussion

To understand the formation process and optimize the synthesis conditions of the CNDs, we have investigated the product obtained under different microwave time periods. Interestingly, it was found that before a particular time point (*e.g.*, 7 minutes) when the water in the reaction system has not been totally evaporated, the obtained solution exhibited totally different PL behavior (Fig. S4). Under 360-nm excitation, unlike the normal CNDs, the solutions obtained before 7 minute microwave-treatment all emitted green color fluorescence at the wavelength of 510 nm, with quantum yield around 18%, and did not exhibit wavelength-dependent PL under different excitations. However, this strange PL emission was not stable as it disappeared when the solution was dried either by heating or lyophilization. We speculate that, it is some organic molecules formed by acrylic acid and 1,2-ethanediamine via Michael addition reaction under heating conditions, that should be responsible for the green color fluorescence, as no nanoparticle could be found when these samples were examined under HRTEM. When the microwave heating lasted for 7 minutes, most of the water has been evaporated; and the appearance of a light smoke indicated the formation of CNDs. We have also studied the CNDs obtained under microwave for longer than 7 minutes (Fig. S5-S7 and Table S3). The characterization results, including TG, FTIR and elemental analysis, all demonstrate that with longer microwave heating time, more organic structure of the CND surface would be destroyed. This result is also reflected by the hydrophilicity of the obtained CNDs: the CND-7min sample has so excellent solubility that it can easily form 50 wt% solution in water, while the CND-13min sample has a poor solubility of less than 0.1 wt%. Fig. S7 displays the PL spectra of CNDs obtained under different microwave pyrolysis periods. It is obvious that the wavelength-dependent phenomenon of the CND-11min and CND-13min samples has been diminished, which could be explained by the decreased surface state diversity induced by the destructive effect on the surface structure. In summary, the CND-7min sample remains more surface functional groups and best PL performance, thus is favorable for the fabrication of PCNDs.

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Table S3 The results of elemental analysis of the CNDs obtained under different microwave treatment time.

Samples	C%	H%	N%	O (Calculated, %)
CND-7min	48.82	8.05	16.85	26.28
CND-9min	53.78	7.91	18.77	19.55
CND-11min	59.37	7.23	18.26	15.15
CND-13min	70.34	5.98	18.66	5.02

The elemental analysis results displayed above reflect the elemental composition changes of the CNDs with the prolonged microwave time in the synthesis of the nanoparticles. Obviously, with longer heating time, the combined dehydration and carbonization effect results in a significant increase of C content, as well as the decrease of O content. This is in accordance with the TGA and FTIR results that with longer microwave heating time, less organic structure remained.

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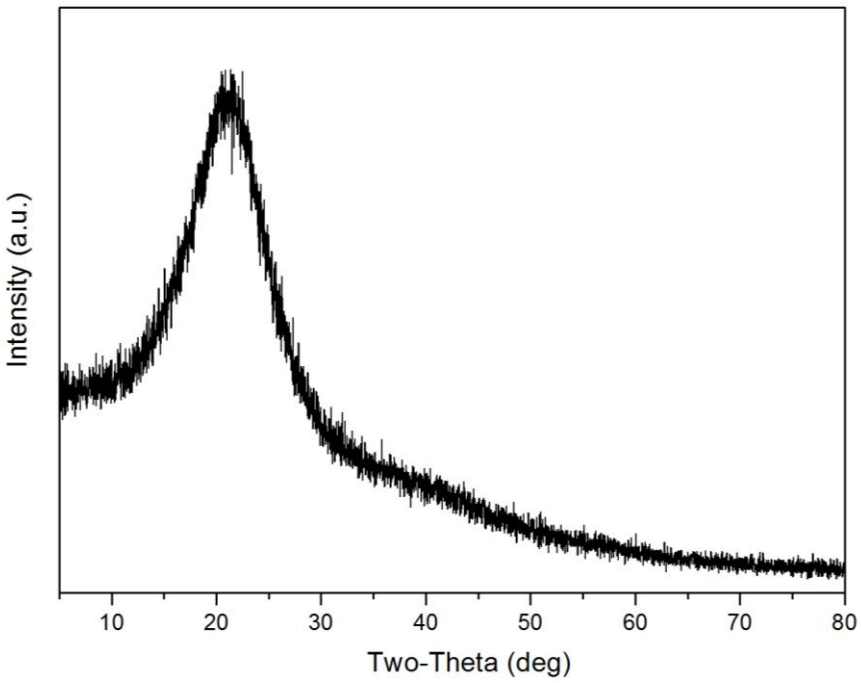

Figure S1. A typical XRD profile for CNDs.

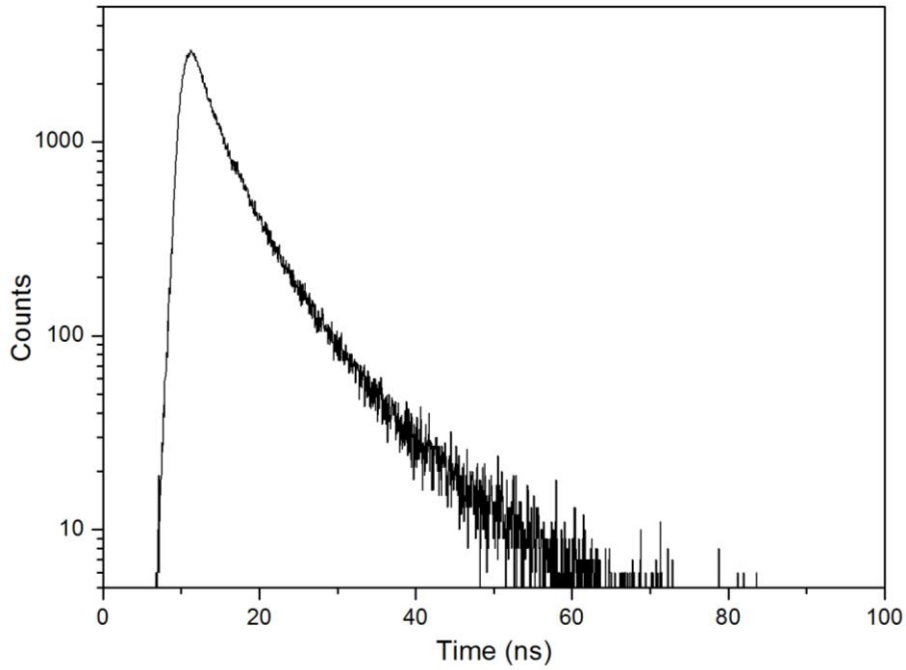

Figure S2. Photoluminescence decay curve for CNDs. The three decay processes consist of a short-lived component 2.70 ns, a moderate-lived component 5.92 ns and a long-lived component of 13.39 ns, with the average lifetime of 5.93 ns. ($Fit = A + B_1 e^{(-t/\tau_1)} + B_2 e^{(-t/\tau_2)} + B_3 e^{(-t/\tau_3)}$, while A=0.368, B_1=1975.8, B_2=1049.4, B_3=173.0, χ^2=1.019).

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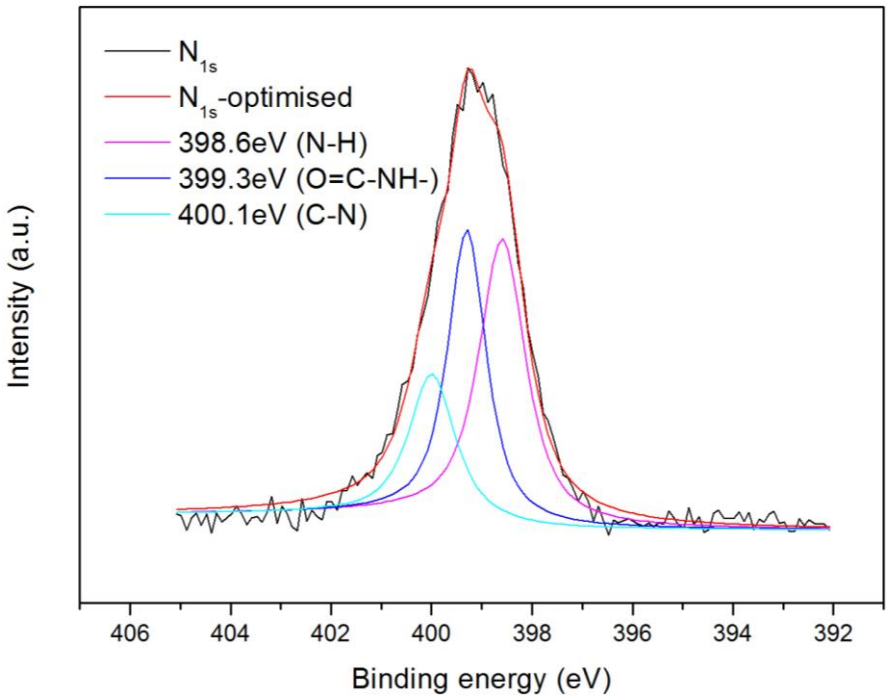

Figure S3. XPS N1s spectra of CNDs.

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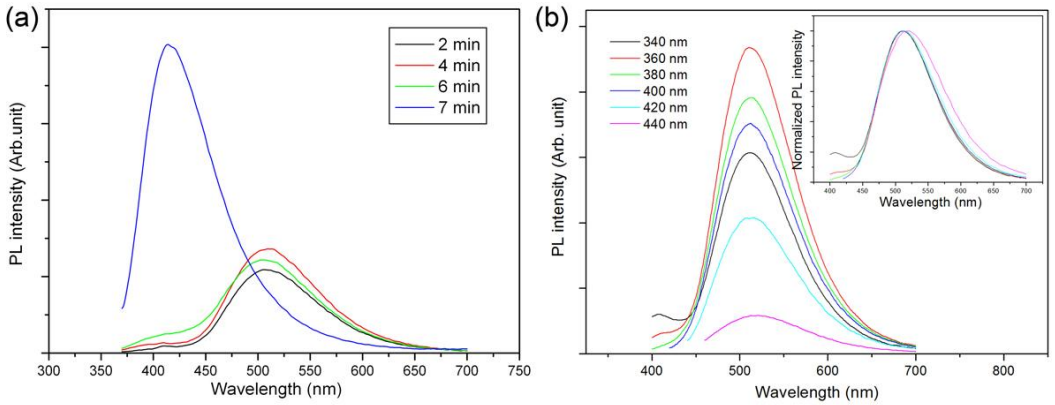

Figure S4. (a) PL spectra of the samples obtained when microwave time is less than 7 minutes; (b) detailed PL emission spectra (with progressively longer excitation wavelengths from 340 nm to 440 nm in 20 nm increments) of the 4 min sample (the inset is the normalized PL emission spectra).

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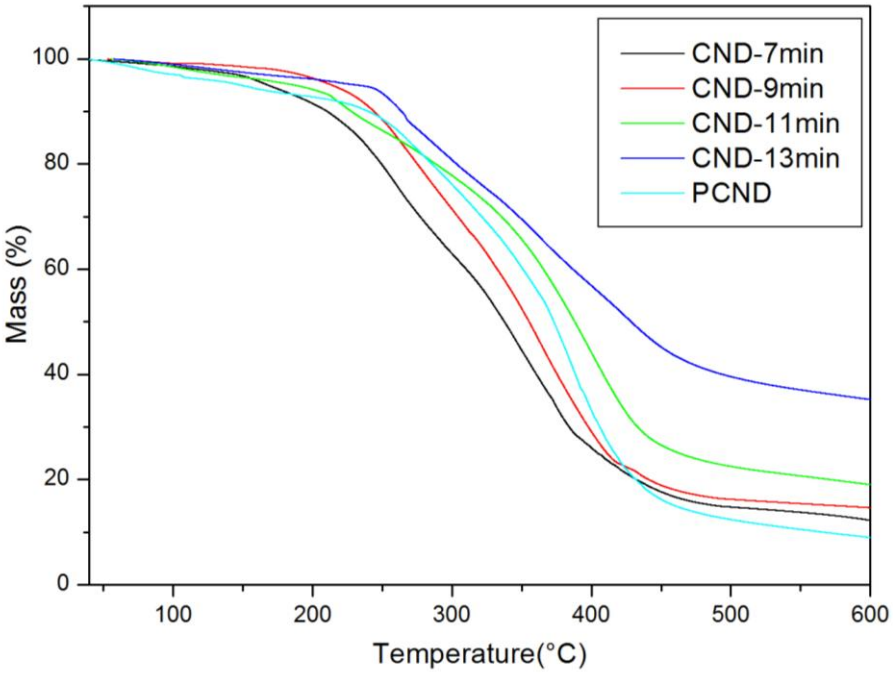

Figure S5. TG curves of the CND and PCND samples in N_2 atmosphere.

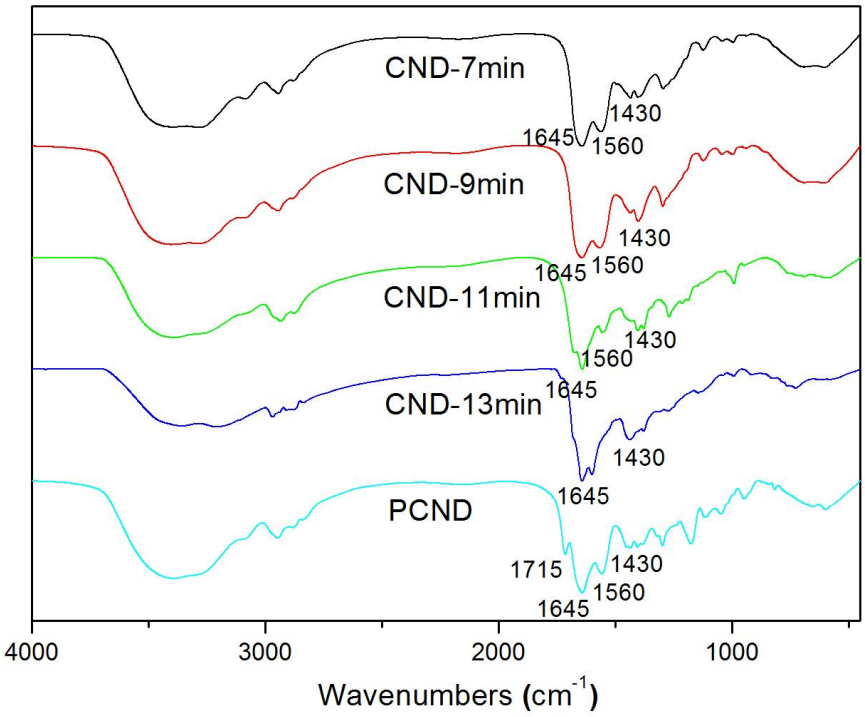

Figure S6. FTIR spectra of the CND and PCND samples.

As shown in the above Figure S6, the CND-7min, CND-9min and CND-11min samples all showed absorption peaks at 1645 cm^{-1} (amide I, C=O), 1560 cm^{-1} (amide II, N-H) and 1430 cm^{-1} (amide III, C-N), indicating the amide bond on the CND surface. This characteristic absorption decreased with the longer heating time, which confirms the destruction of the surface organic structure of the CNDs. The new absorption appeared at 1715 cm^{-1} for the PCND sample demonstrates the successful surface functionalization of the CND nanoparticles.

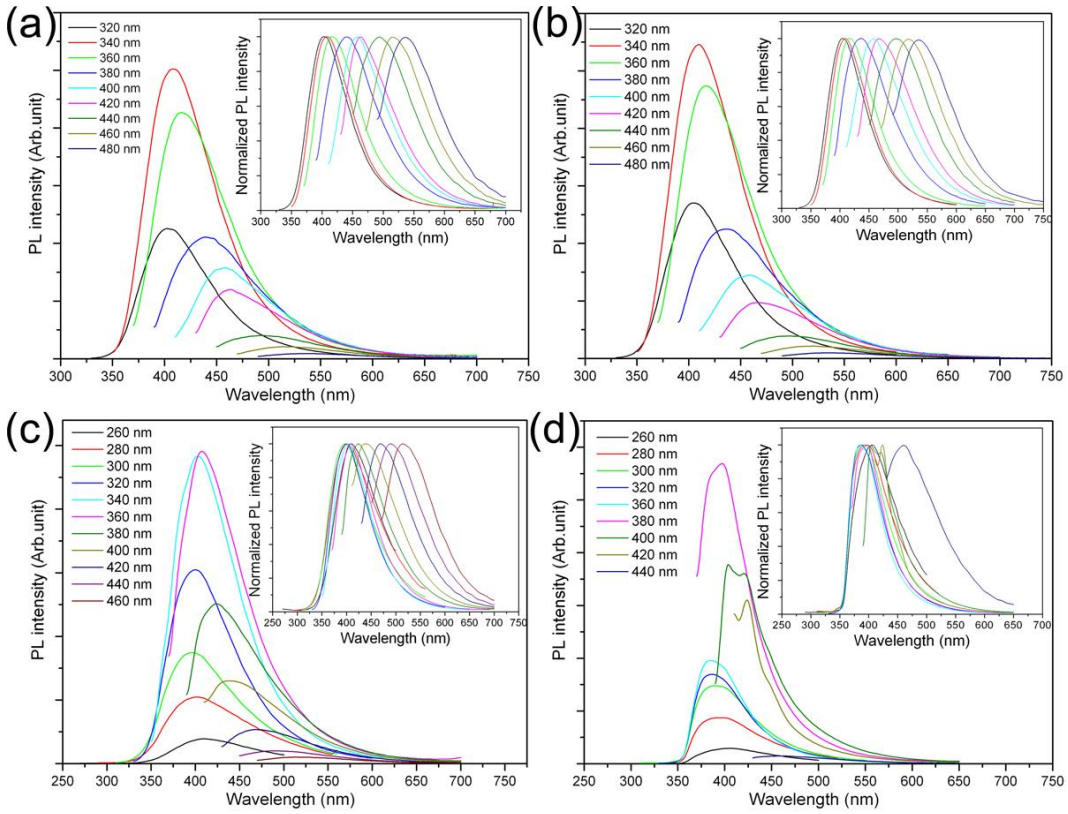

Figure S7. PL emission spectra of the (a) CND-7min, (b) CND-9min, (c) CND-11min, (d) CND-13 samples with different excitations (the inset is the normalized PL emission spectra).

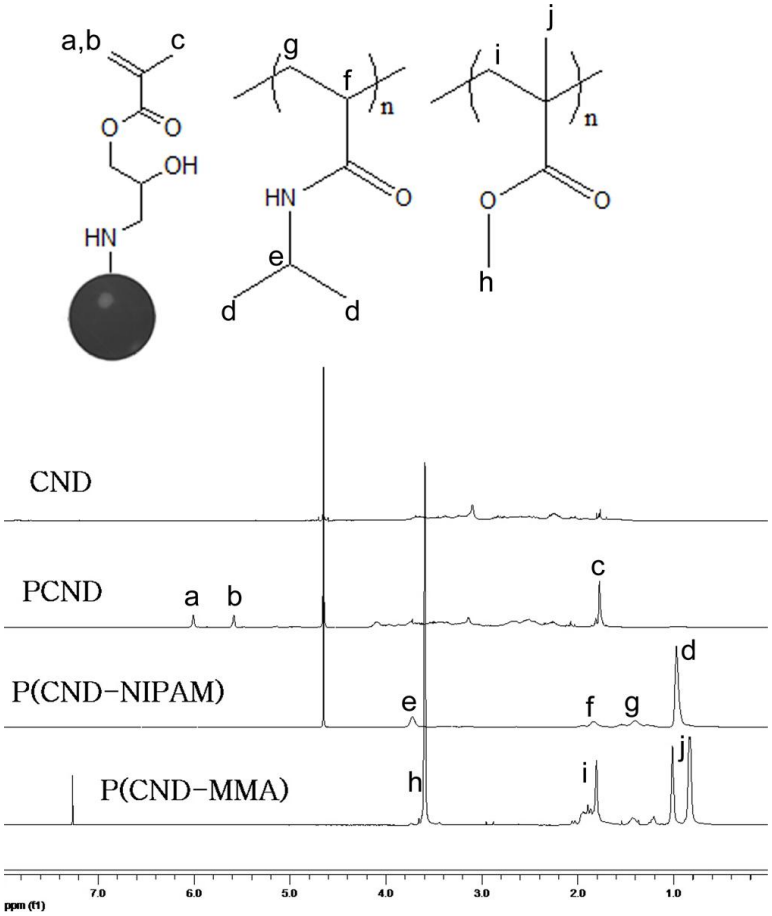

Figure S8. ^{1}H NMR spectra of the samples. CND, PCND, P(CND-NIPAM) samples were dissolved in D_2O; while PCND-MMA was measured in $CDCl_3$.

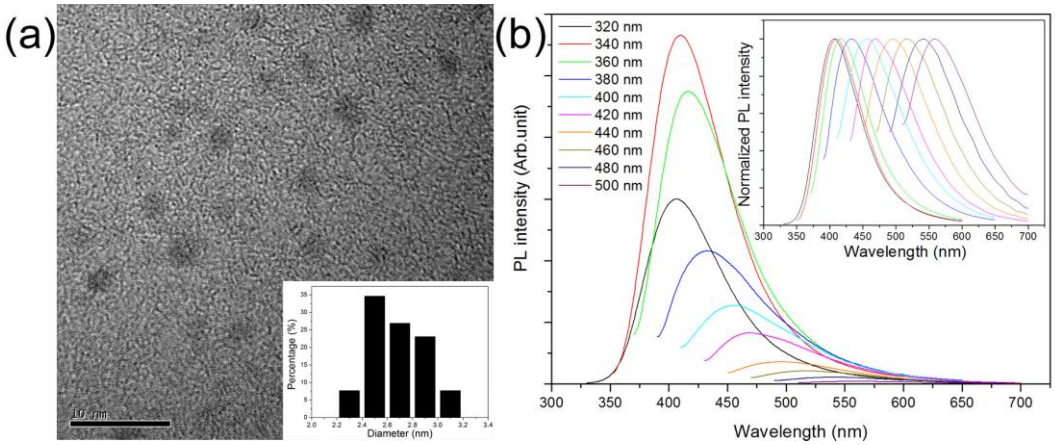

Figure S9. (a) HRTEM image of PCNDs (scale bar: 10 nm). (b) PL emission spectra of the PCNDs with different excitation wavelengths (the inset is the normalized PL emission spectra).

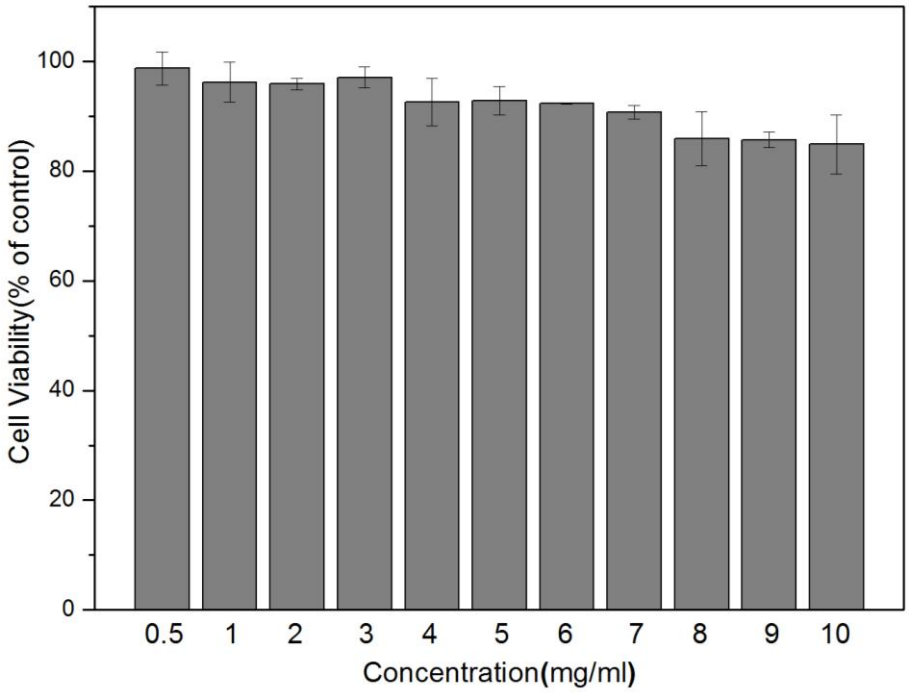

Figure S10. Cytotoxicity testing results via a MTT assay. The values represent percentage cell viability (means% ± SD, n = 3).

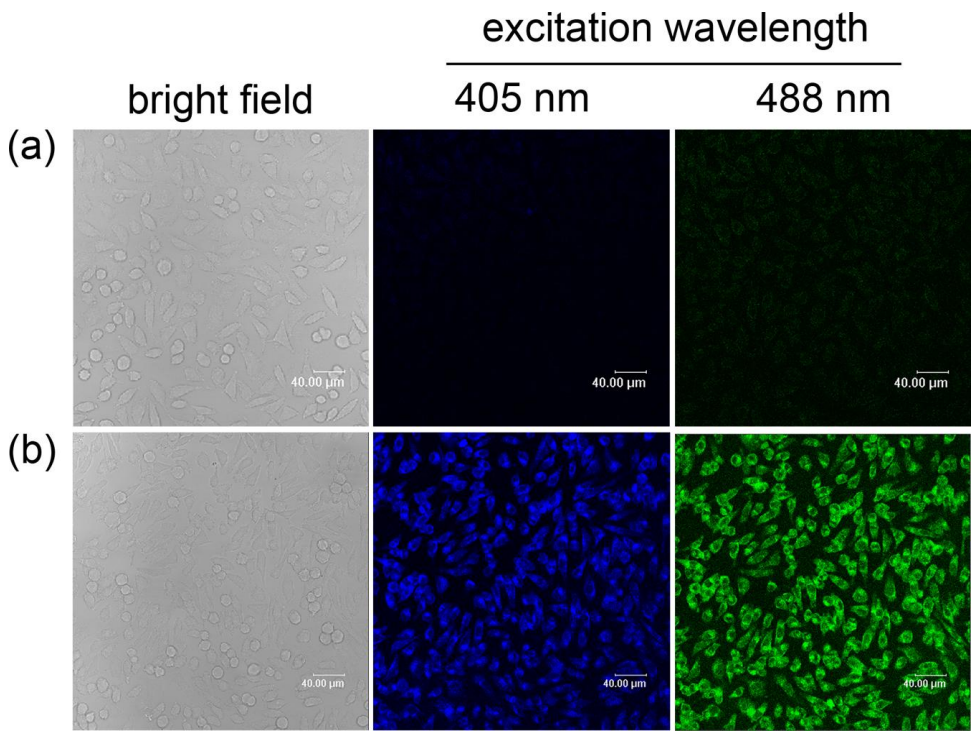

Figure S11. Laser scanning confocal microscopy images of L929 cells without labeling as a negative control (a) and PCNDs labeled L929 cells (b).

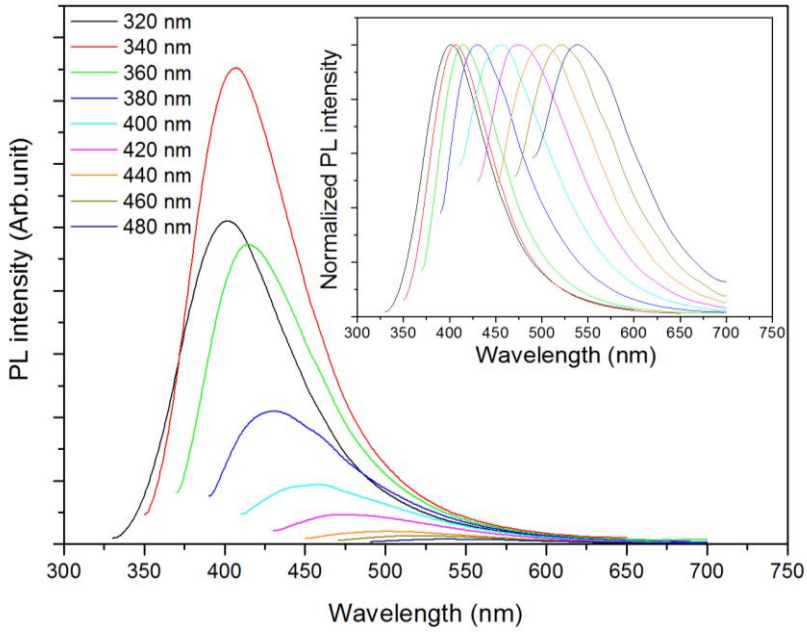

Figure S12. PL emission spectra of the P(CND-NIPAM) aqueous solution (Abs_{360nm} = 0.1, 25 ºC) with different excitation wavelengths (the inset is the normalized PL emission spectra).

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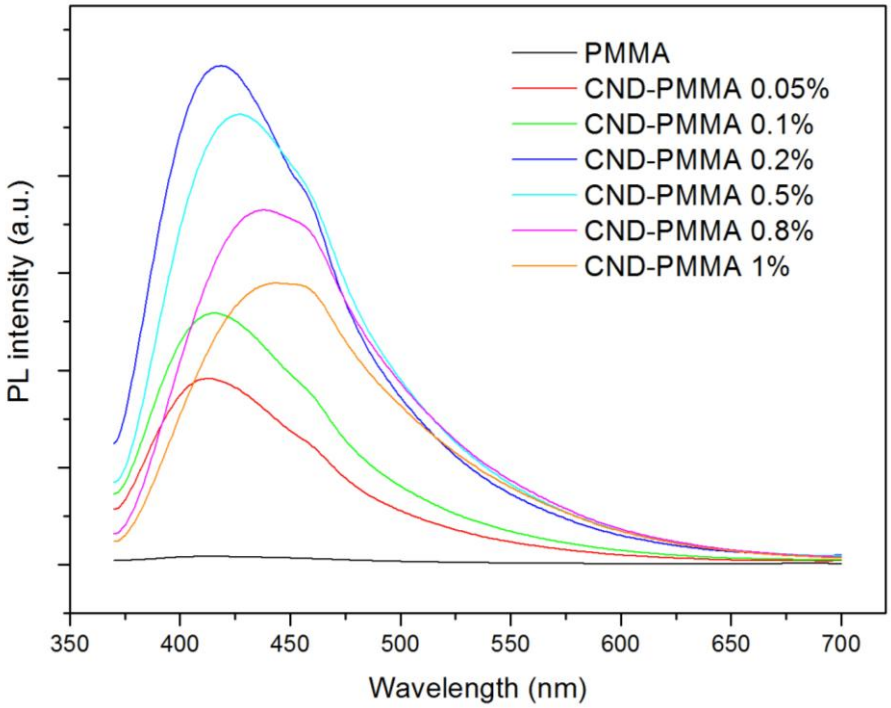

Figure S13. PL emission spectra of different P(CND-MMA) samples.