Electronic Supplementary Information

Synthesis of Fluorescent Carbon Nanoparticles from Polyacrylamide for Fast Cellular Endocytosis

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Experimental Details

1. Chemicals:

Polyacrylamide solution (average Mw=10000, 50wt% in H_2O) was purchased from Aldrich. All aqueous solutions were prepared with deionized water (18 MΩ•cm^{-1}) obtained from the Millipore system.

2. Preparation and purification of the fluorescent carbon nanoparticles (CNPs):

In a typical experiment, 8g polyacrylamide solution was diluted with 40mL deionized water and stirred for 10 minutes to become homogeneous and clear. Then the mixture was transferred to a 100 mL Teflon equipped stainless steel autoclave and sealed. The autoclave was placed in a oven at 260 ^oC for given hours to complete the hydrothermal treatment with heating rate at 5 ^oC •min^{-1}. When the reaction was completed, the autoclave is cooled down at room temperature. The obtained brown solution without any deposit was neutralized and dialyzed for 3 days (MWCO =3.5 kD for 24h-CNPs and 96h-CNPs, MWCO =7 kD for 72h-CNPs) to precipitate out small molecules. In addition, the remaining 72h-CNPs solution was processed with ultrasonic disruptor to disintegrate particle aggregates. Finally, the yellow solution was freeze dried to obtain the pure fluorescent CNPs.

3. Characterization:

Transmission electron microscopy (TEM) images were taken by a JEM-1011 electron microscope (JEOL, Japan) at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) result was obtained by a Malvern MasterSizer 2000. The X-ray diffraction (XRD) patterns were measured by a D8 Advance X-ray diffractometer (Bruker) with Cu Ka radiation (40 kV, 40 mA, λ =1.54051 Å). Fourier transform infrared (FTIR) spectroscopy were performed on an Vector-22 spectrometer (Bruker) in the KBr pellet， ranging from 400 to 4000 cm^{-1}. Zeta potential measurements were obtained on a Malvern Nano-Z instrument. Elemental analysis was measured with a CHN-O-Rapid elemental analyzer (Heraeus, Germany). Ultraviolet-visible (UV-vis) absorption of the obtained CNPs solution was carried on a UV-1800(PC) UV-vis

spectrophotometer (Mapada, China). All fluorescence spectra of the CNPs were measured with a FluoroMax-4 spectrofluorometer (HORIBA Scientific, Japan) with slit width of 5 nm for both excitation and emission. The fluorescence images were acquired with a Leica TCS SP5 confocal scanning microscope (Leica Microsystems, Heidelberg GmbH, Mannheim, Germany). The specimens were excited at 488 nm, and the emission was detected between 500 and 530 nm.

4. Quantum yield (QY) measurements:

The quantum yield (QY) of fluorescent CNPs was obtained by the following steps. We chose quinine sulfate dissolved in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) as reference. Then UV-vis absorption and PL emission spectra (with 360nm excitation) of CNPs and reference were measured respectively. The accurate QY value was calculated according to the given equation:

$$Q_{sam} = Q_{ref} \frac{I_{sam} A_{ref} n_{sam}^2}{I_{ref} A_{sam} n_{ref}^2}$$

In here "*sem*" and "*ref* " refer to sample and reference respectively. Q means Quantum yield. I is the integrated emission intensity, which could be calculated from the emission spectra at 360nm excitation. A represents UV-vis absorbancies at 360 nm were control under 0.1 in the 10 mm quartz absorbance cell to avoid re-absorption effect. And n is the refractive index with 1.33 as the default for both quinine sulfate and CNPs solvent.

Table S1 Quantum yield (QY) calculation of the fluorescent CNPs at 360nm

Sample	Intergrated emission intensity (I)	UV Absorbance	Refractive index of solvent (n)	Quantum yield (Q)
Quinine sulfate	30950715	0.0067	1.33 (default)	0.54
24h-CNPs	53281110	0.0501	1.33 (default)	0.124
72h-CNPs	64858990	0.0597	1.33 (default)	0.127
96h-CNPs	46717285	0.0438	1.33 (default)	0.125

5. Cytotoxicity test:

LnCaP cells (10^5 cells/mL) in Dulbecco's modified Eagle's medium (DMEM) with high glucose were incubated in a 96-well microplate (100 μL/well) for 6 h to adhere in an incubator at 37 ^oC in 5% CO_2. Then 100 μL of the CNPs solution with different concentration was introduced into per well to obtain the final concentration of 10, 50, 200, 400, 600, 800, 1000 μg/mL and were cultured for 24 h. The medium was removed and the cells were washed with phosphate-buffered saline (PBS, pH 7.4). 100 μL DMEM and 20 μL of 5 mg/mL MTT solution were added to every cell well. The 96-well microplate was further incubated for 4 h, followed by removing the culture medium with MTT, and then 150 μL of DMSO was added. The resulting mixture was shaken for 10 min at room temperature. The optical density (OD) of the mixture at 490 nm was measured in the En-Spire multimode plate reader. The PBS was introduced into the cells in the control group. The cell viability was calculated according to the givien equation:

$$\text{Cell viability} = \frac{OD_{sam}}{OD_{con}} \times 100\%$$

In here ODcon refers to OD of sample, and ODsam refers to OD of control group.

6. Cellular imaging experiments:

LnCaP cells were cultured in three confocal dishs with 1 mL DMEM with high glucose at 37 ^oC in 5% CO_2 for 24 h. 0.25 mL CNPs aqueous solution (3 mg•mL^{-1}) was added into the medium and the final concentration of CNPs were 0.6 mg•mL^{-1}. The cells were cultured with CNPs at 37 ^oC for 1 or 2 h. The cells were washed with the physiological extracellular buffer (ECB: 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4) for three times before the fluorescent imaging. The fluorescent intensity of the cells was analyzed using the software Image J.

7. Explanation for using quality concentration to survey the nanoparticle's size dependent uptake:

The density of CNPs was difficult to measure, not like SiO_2 nanoparticles and gold nanoparticles. The CNPs were consisted of the core and polymer chains on the surface, but the ratio and density of these two parts were unknown and had not been calculated in related research yet. Additionally, as the CNPs grew, the influence of increased ratio of core on the density of CNPs was unclear. There was not an efficient and direct way to detect the amount of CNPs in cells, but to observe the fluorescence.

Some previous reports have used the same quality concentration to survey the size-dependent uptake of NPs, for example mesoporous silica nanoparticles[1], TiO_2 nanoparticles[2], and silica particles[3], Especially for silica particles, they used RITC to label SiO_2 and evaluated the uptake amount by the average fluorescence intensity of RITC- SiO_2 per cell, which was similar to our study. The fluorescence intensity of CNPs was not as high as RITC, so it's hard for us to measure the average fluorescence intensity per cell by using their method. In view of the differences of the CNPs solution at 0.6 mg/mL, we normalized the average green brightness of each pixel of cells with the fluorescent intensities of CNPs in the solution to compare the uptake amount of CNPs.

References:

1. F. Lu, S.H Wu, Y. Hung and C.Y Mou, *Small*, 2009, **5**: 1408–1413.
2. K.Y. Cai , Y.H Hou , Y. Hu , L. Zhao , Z. Luo , Y.S. Shi , M. Lai ,W.H. Yang , and P. Liu, *Small*, 2011, **7**: 3026–3031.
3. Y.Y. Zhang, L. Hu, D.H Yu and C.Y Gao, *Biomaterials*, 2010, **31**: 8465-8474.

Table S2 Elemental analysis results of the CNPs and polyacrylamide

Sample	C(%)	H(%)	N(%)	O(%,calculated)
Polyacrylamide	43.31	7.04	16.75	32.90
24h-CNPs	57.28	7.30	8.68	26.82
72h-CNPs	63.66	6.94	6.90	22.50
96h-CNPs	65.85	7.12	6.72	20.31

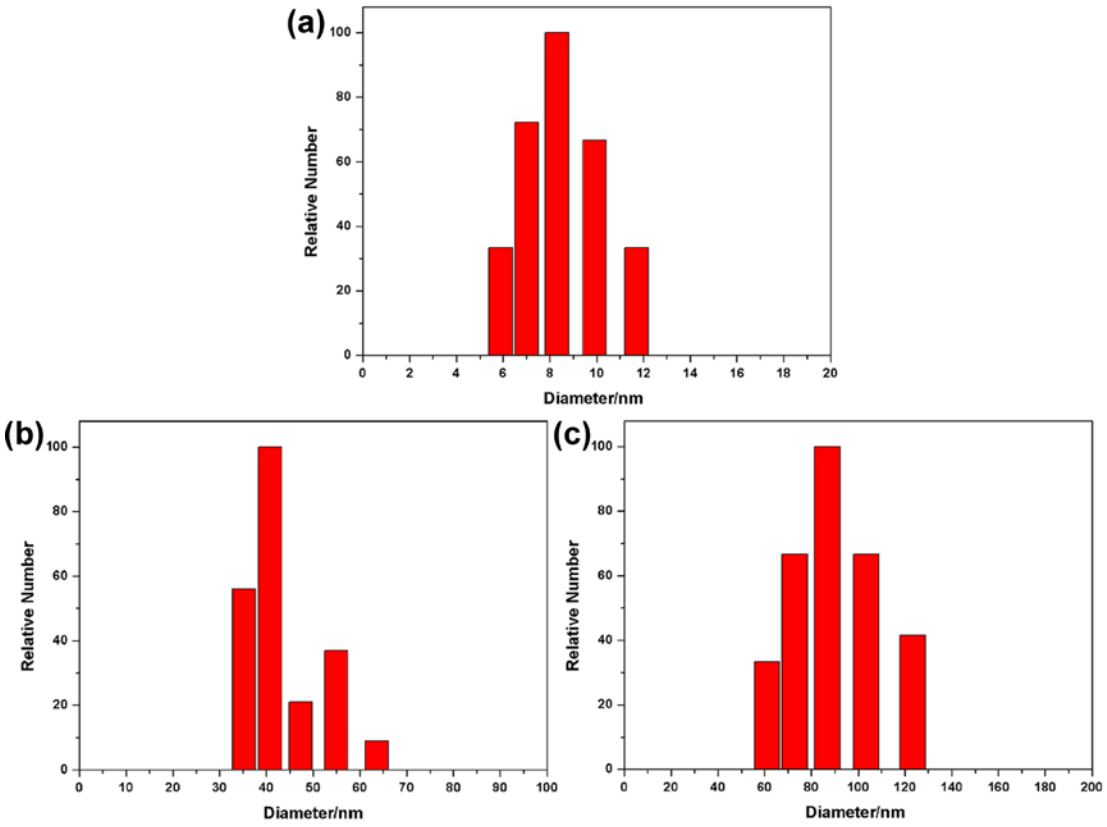


Fig. S1 Particle size distribution histograms of CNPs obtained by DLS. (a): 24h-CNPs, (b): 72h-CNPs, (c): 96h-CNPs.

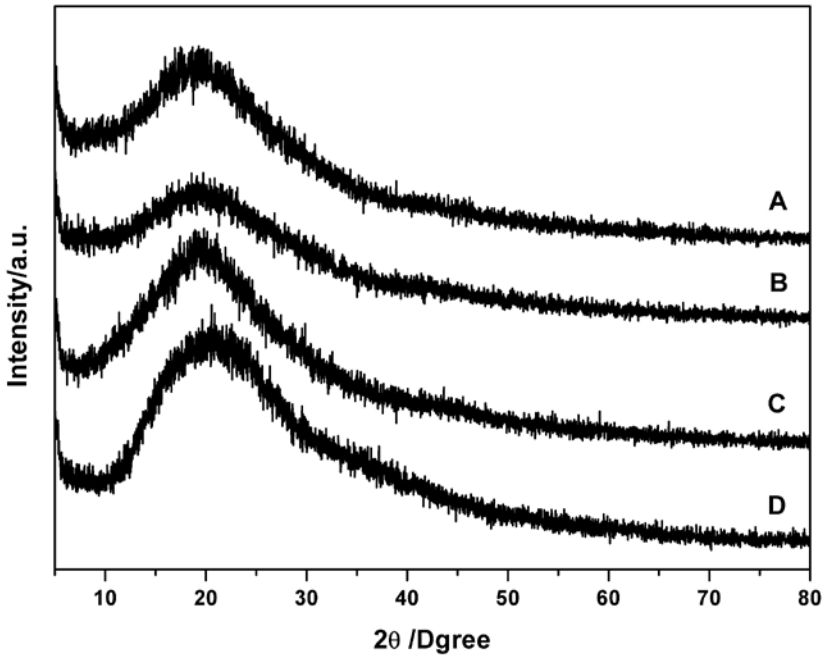


Fig. S2 XRD patterns of the CNPs and polyacrylamide. (A): 96h-CNPs, (B):

72h-CNPs, (C): 24h-CNPs, (D): polyacrylamide.

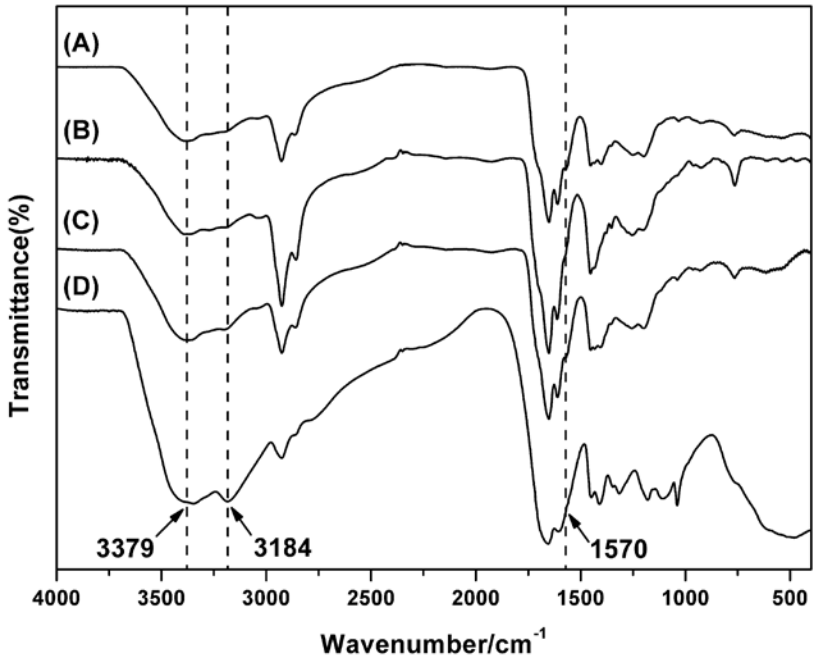


Fig. S3 FTIR spectra and of the CNPs and polyacrylamide. (A): 96h-CNPs, (B): 72h-CNPs, (C): 24h-CNPs, (D): polyacrylamide.

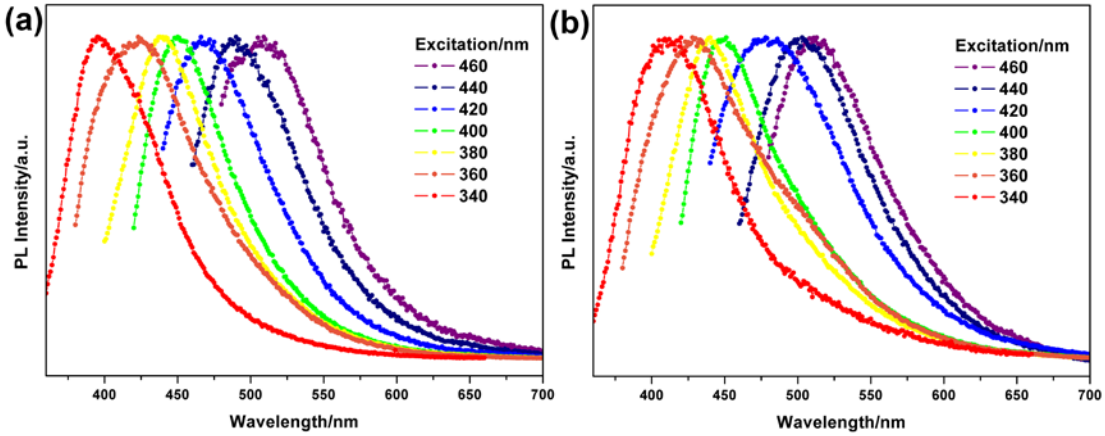


Fig. S4 Normalized photoluminescence emission spectra of 24h-CNPs (a) and 96h-CNPs (b). Excitation wavelengths start from 340 nm and increase with 20 nm increments.

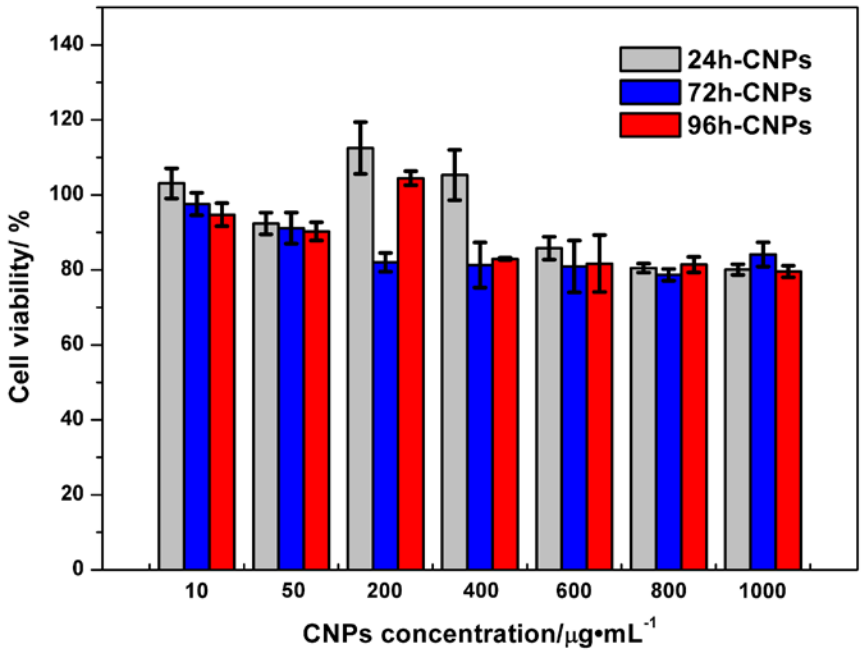


Fig. S5 Cytotoxicity test of LnCaP cells with different concentration of 24h-CNPs, 72h-CNPs and 96h-CNPs after 24 h incubation. The error bars corresponded to standard errors (n≥3).

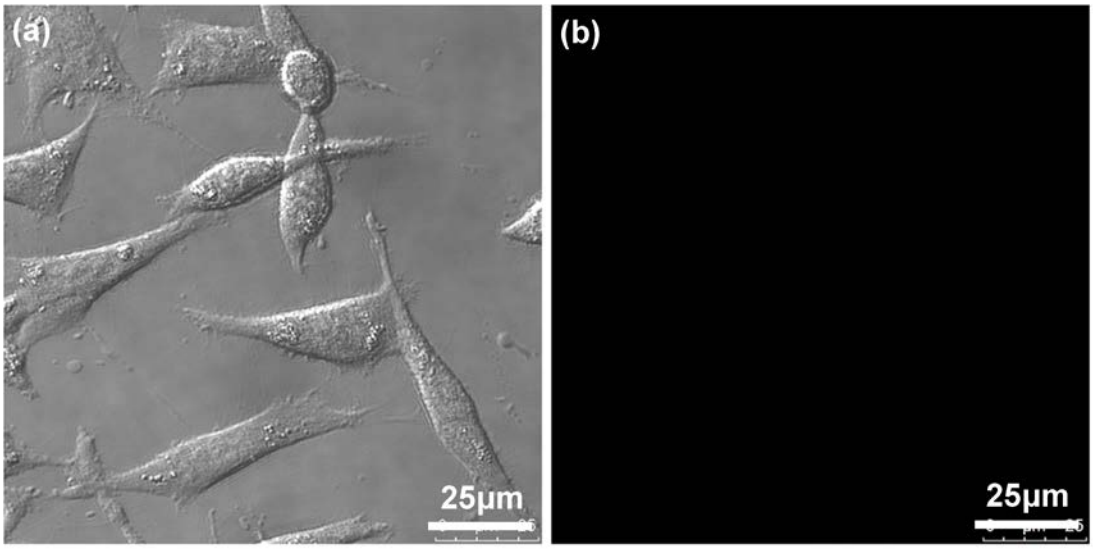


Fig. S6 Confocal scanning microscope microphotographs of LnCaP cells labeled with the CNPs drived from orange juice (QY=26%) for 1 h (λex= 488 nm). (a): brightfield, (b): fluorescence.

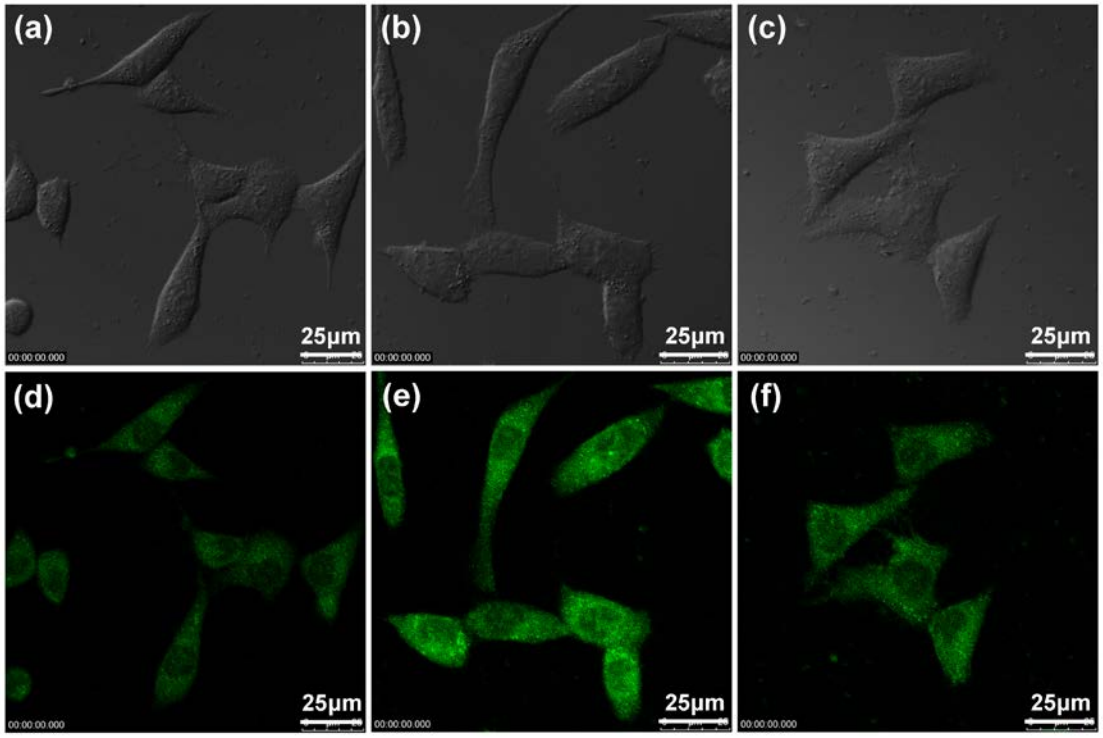


Fig. S7 Brightfield (a, b, c) and fluorescence (d, e, f,) microphotographs of LnCaP cells labeled with the CNPs for 2 hours loading (λex= 488 nm). (a)(d): 24h-CNPs, (b)(e): 72h-CNPs, (c)(f): 96h-CNPs. The concentration of CNPs was 0.6 mg•mL^{-1}.

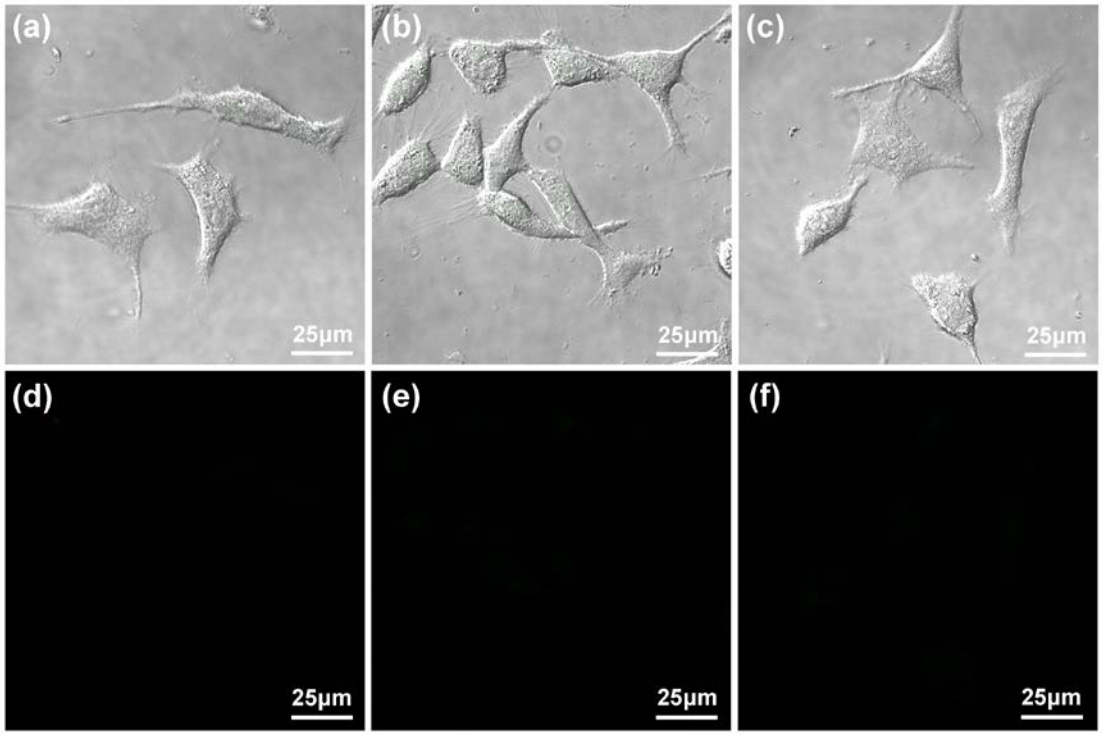


Fig. S8 Brightfield (a, b, c) and fluorescence (d, e, f,) microphotographs of LnCaP cells cultured with the CNPs of 0.6 mg•mL^{-1} for 1 h at 4^{o}C (λex= 488 nm). (a)(d):

24h-CNPs, (b)(e): 72h-CNPs, (c)(f): 96h-CNPs.

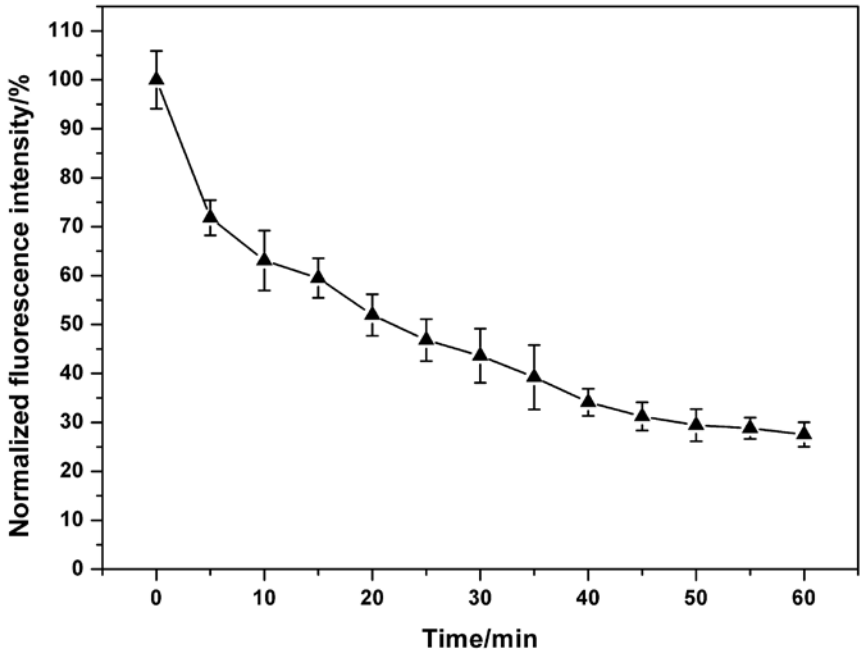
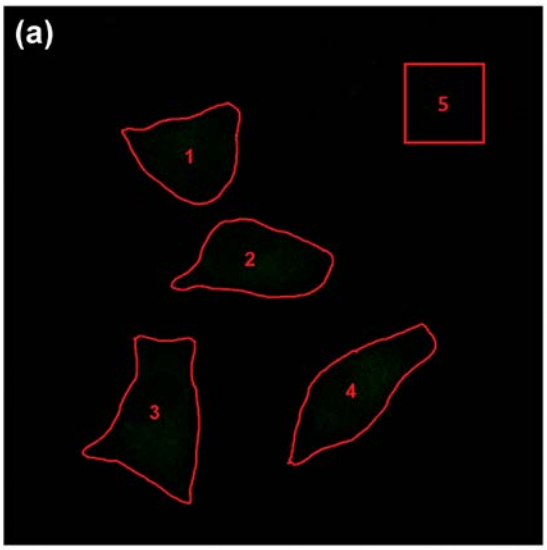
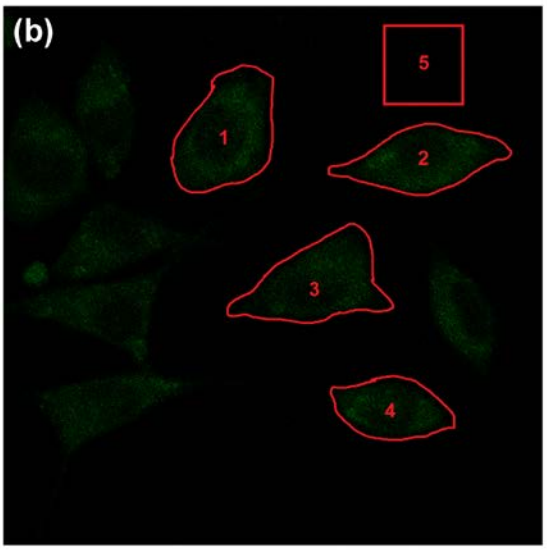
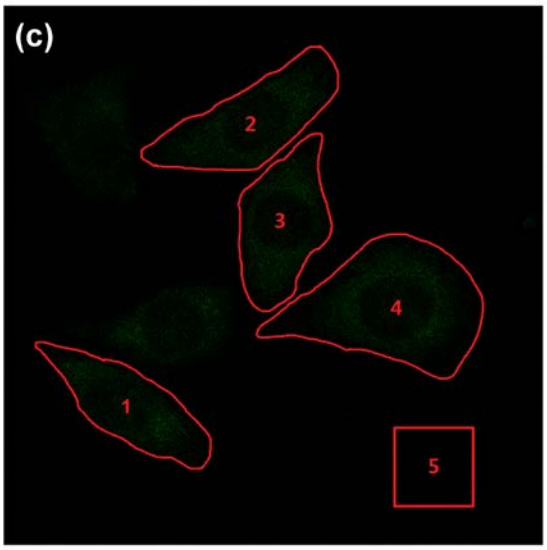


Fig. S9 Normalized mean fluorescent intensity of five cells loaded with 72h-CNPs after continuous exposure of the laser light at 488nm. The fluorescence images of the cells were recorded every 5 min.







(d)

Area	Average green brightness		
	24h-CNPs	72h-CNPs	96h-CNPs
1	5.774	17.491	11.559
2	6.12	20.255	11.334
3	5.912	15.51	11.38
4	6.868	17.343	9.804
5(background)	0.416	0.527	0.394
Actual mean	5.753	17.123	10.625
Standard deviation	0.488	1.957	0.816

Actual mean = average value of area 1 to 4 – value of area 5

Fig. S10 Comparison of actual average green brightness of each pixel in LnCaP cells in Fig. 3. Area mark of 24h-CNPs (a), 72h-CNPs (b) and 96h-CNPs (c). (d): Calculation of actual average green brightness of each pixel in LnCaP cells in Fig. 3.

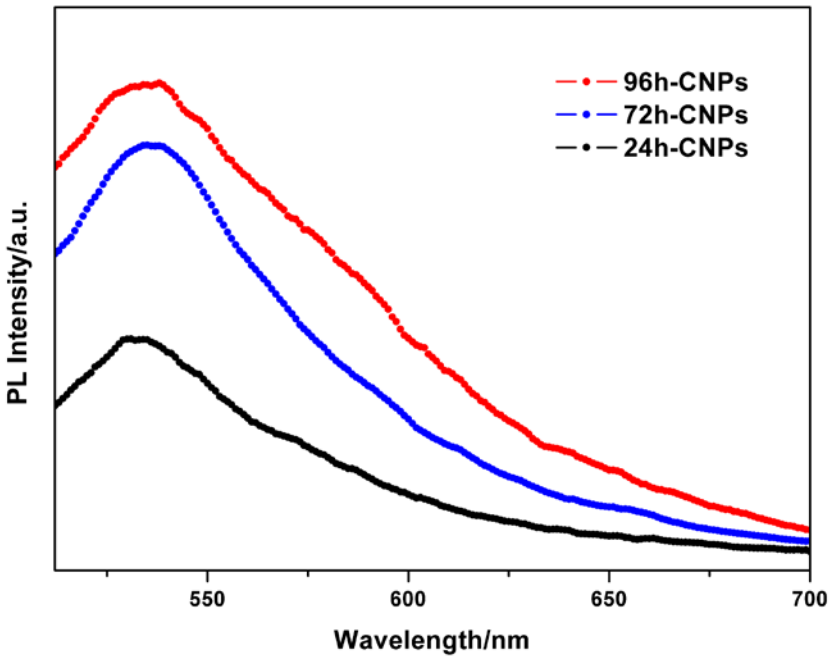


Fig. S11 Emission spectra of 24h-CNPs, 72h-CNPs and 96h-CNPs at excitation wavelengths of 488 nm.(the concentration of CNPs was 0.6 mg•mL^{-1}).

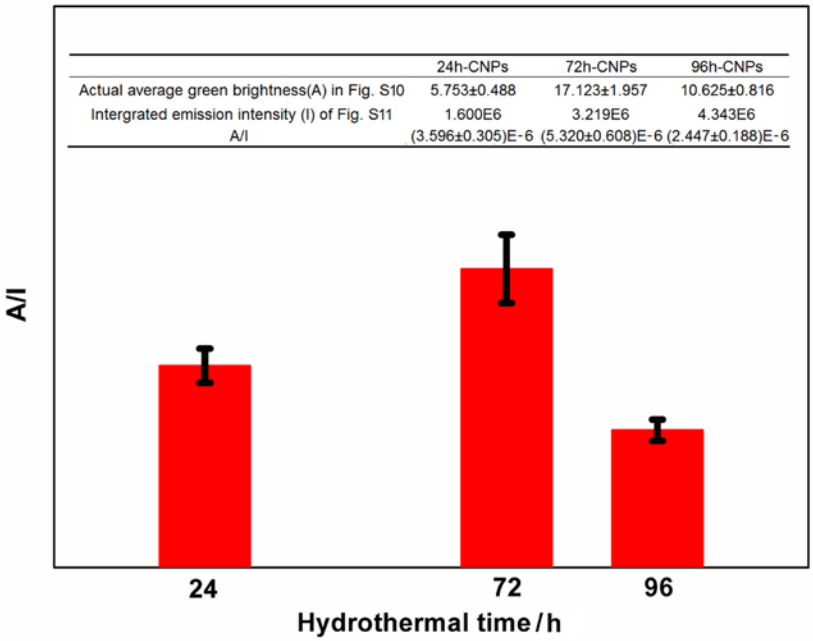


Fig. S12 The change of A/I with increase of hydrothermal time. A: Actual average green brightness in Fig. S10, I: Integrated emission intensity of Fig. S11. The inset

table was calculation of A/I value.