

## Electronic Supplementary Information

### **Excitation-Dependent Ratiometric Dual-Emission Strategy for the Large-Scale Enhancement of Fluorescent Tint Control**

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**General.** Cetylpyridinium chloride monohydrate (CPC) and polymethylmethacrylate (PMMA) was obtained from Sigma-Aldrich (Shanghai, China). Calf thymus DNA was purchased from Macklin (Shanghai, China). Hydrochloric acid (HCl), sodium hydroxide (NaOH), dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) and sodium borohydride ( $\text{NaBH}_4$ ) were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). CCK-8 assays were purchased from Dojindo (Shanghai, China). All reagents were analytical or reagent grades and used without further purification. Deionized water (18.2 M $\Omega$  cm) was obtained from a F'DEER water purification system.

The ultraviolet-visible (UV-vis) absorption spectra was measured in the region of 220-620 nm on a Shimadzu 1800 spectrophotometer. The photoluminescence (PL) excitation and emission spectra were recorded on a Shimadzu RF-5301. The lifetime and quantum yield were measured on Edinburgh Instruments FLS 1000 and Photo Technology International QM40 spectrofluorophotometer. The Fourier transform infrared (FT-IR) spectra was recorded with a Thermofisher Nicolet 6700 spectrometer using KBr pellets as the sample matrix in the wavenumber range of 500–4000  $\text{cm}^{-1}$ . The X-ray photoelectron spectroscopy (XPS) was performed on a PHI5300 using a magnesium Ka source (250 W, 14 kV). The Raman spectra was carried out for CDs and nanohybrids in aqueous droplets on the slide. The spectra were recorded on a Horiba Jobin Yvon XploRA spectrometer equipped with a 10x objective and a laser with a wavelength of 532 nm. Data were collected between 500  $\text{cm}^{-1}$  and 3600  $\text{cm}^{-1}$  using a grating of 600 grooves per mm. The high contrast transmission electron microscopy images (TEM) were obtained on a Hitachi HT7800 field emission electron microscope with 100 kV field emission. 0.1 mg CDs were dissolved in 1 mL DI water. The solutions were then dropped on an ultra-thin carbon membrane coated copper grid and the excess of the solution was removed with filter paper. The Circular Dichroism spectroscopy was measured in Applied Photophysics Chirascan. The confocal microscopic images were captured by Nikon C2<sup>+</sup> confocal microscope. Dynamic light scattering (DLS) and zeta potential were carried out with Nano-Zeta Potential Analyzer ZS-90. The synthesis experiments were carried out in the ultrasonic machine Kunshan KQ5200DE with output power of 200 W and a

frequency of 40 kHz. Photographs of CDs/PMMA film sheets were taken using an iPhone 7 under different excitation wavelengths of Shimadzu fluorometer.

**Preparation of dual-emission CDs.** Dual-emission CDs (W-CDs) were prepared by one-pot hydrothermal reaction based on a similar report.<sup>S1</sup> Briefly, 0.358 g of CPC was dissolved in 30 mL of deionized water and pH of the solution was adjusted to 11.5 with 1 M NaOH. Then the solution was transferred into a stirred Teflon-lined stainless steel autoclave (100 mL) and put it in the oven. The autoclave was heated at 150 °C for 2 h and cooled to room temperature naturally. W-CDs were produced with the color change of the solution to brown. The same purification process was applied in the brownish solution. After dialysis for 24 h (Spectrumlabs, CA, USA, the cutoff of molecular weight was 3500) and freeze-dried, neutral brown CDs powders were obtained and stored at -4 °C for further use.

**Preparation of the multicolor luminescent CDs.** Based on the improvement of previous reports,<sup>S2</sup> the multicolor luminescent CDs were synthesized by adjusting the ratio of reactants and the reaction was speeded up progress with the help of ultrasonic. In detail, 50 mL of CPC aqueous solution (15 mM) was mixed well with different concentrations of NaOH and then ultrasonic treatment was carried out at room temperature. With the use of 0.1 M, 2 M, 6 M of NaOH, the molar ratio between base and CPC was 0.1, 10, 2000, respectively. The color of solution darkened gradually, which indicated the formation of blue CDs (B-CDs), green CDs (G-CDs) and yellow CDs (Y-CDs), respectively. After treating with ultrasonic wave for 30 min, all reactions were terminated by adjusting pH of the solution to neutral (pH = 7.0) with diluted HCl aqueous solution. The solutions were purified with ultrapure water through dialysis membranes for 24 h (Spectrumlabs, CA, USA, the cutoff of molecular weight was 3500). The dialysis-purified solutions were freeze-dried to obtain the CDs powders. These CDs powders were redispersed in ultrapure water and stored at -4 °C before further use.

**Preparation of CDs/PMMA film sheets.** 100 mg of polymethylmethacrylate (PMMA) was completely dissolved in 5 mL of dichloromethane, then the CDs solutions (dispersed in 1 mL of dichloromethane or ethanol) were added and mixed

uniformly. The CDs/PMMA mixture formed a film after the solvent naturally evaporated. Based on our pre-made triangle mold, the film was trimmed into special-shaped sheets for matching the light source of the ultraviolet light detection.

**Reduction reaction of G-CDs.** An excess of NaBH<sub>4</sub> (~0.2 g) was added to G-CDs aqueous solution (~30 mL) and the mixture was stirred for 2 h at room temperature. Then, the solution was transferred into a dialysis membrane and dialyzed for 24 h. CDs were collected by freeze-dried and redispersed in ultrapure water.

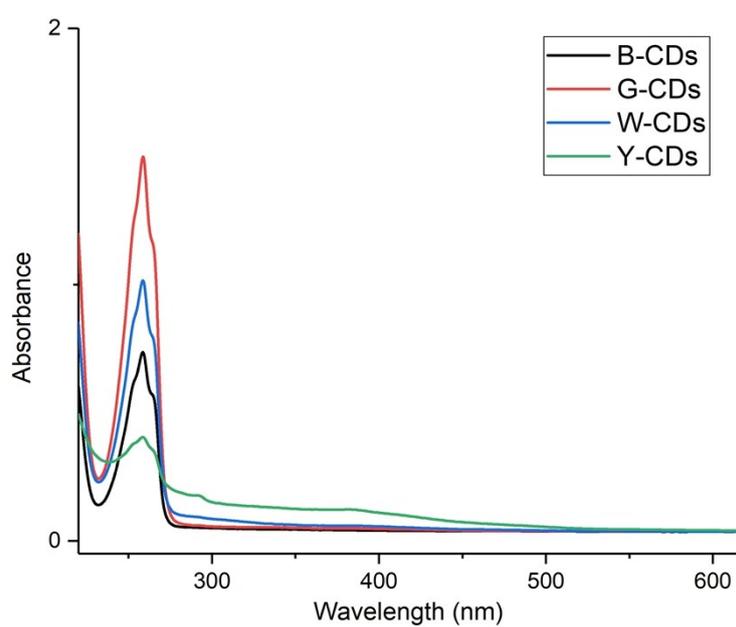
**Fluorescence tint variation:** In order to reflect the change of fluorescence tint variation in theory,<sup>s3</sup> the distance (d) between CIE coordinates at different excitation wavelengths ( $x_n, y_n$ ) and their center point ( $\bar{x}, \bar{y}$ ) can directly reflect the magnitude of the fluorescent tint variation (similar formula shown in literature<sup>s3</sup>). The distance (d) was calculated by using equation:

$$d = \sqrt{(\bar{x} - x_n)^2 + (\bar{y} - y_n)^2} \quad n = 1, 2, 3, 4$$

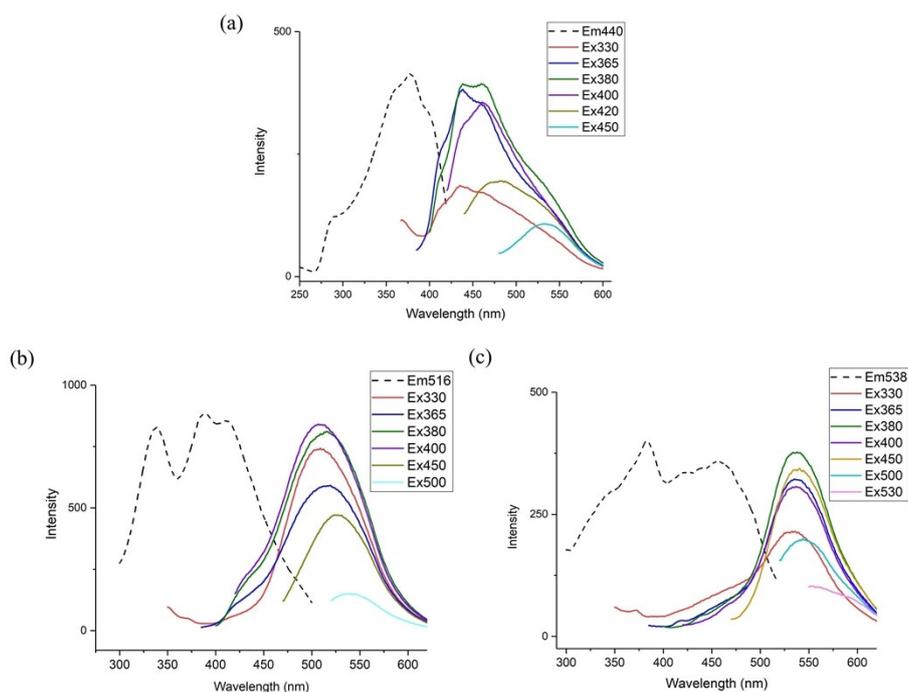
**In Vitro cytotoxicity assay.** The cell viabilities of G-CDs and W-CDs were quantitatively determined by cell counting kit-8 (CCK-8) assays. Hela cells were purchased from the cell bank of the Chinese academy of sciences (Shanghai, China), and were seeded into a 96-well plate at a density of  $1 \times 10^4$  cells per well in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco) under 5% CO<sub>2</sub> at 37°C. After the cells grew for 12 h, the medium was changed into a new medium (200 μL/well) containing G-CDs and W-CDs with 20, 10, 5 and 2 μg/mL, respectively. After the cells were incubated with the sample for 24 h, the medium was replaced with 100 μL of fresh medium. Subsequently, 10 μL of CCK-8 was added to each well and homogeneously mixed, followed by incubation at 37 °C for 4 h in a CO<sub>2</sub> incubator, and finally, 80 μL of the solutions were put into a new 96-well plate. After incubation for 2 h with G-CDs and W-CDs, the absorbance at 450 nm in each well was determined using a microplate reader (Multiskan Mk3). The relative cell viability was calculated to quantify the cytotoxicity.

**Confocal Microscopic Images.** Hela cells were seeded in 35 mm plastic-bottomed m-dishes and grown in DMEM medium for 24 h. Then the cells were treated with G-

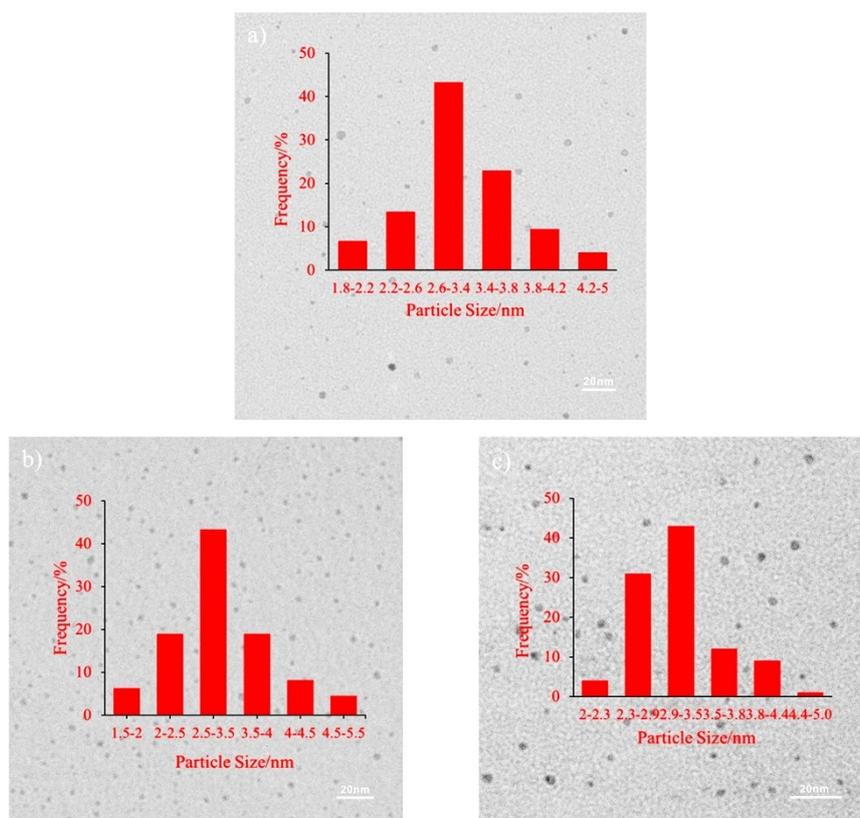
CDs and W-CDs for another 2 h. The cells were washed with a phosphate buffer saline (PBS) solution (pH 7.4) three times and fixed with polyformaldehyde at 4 °C for 15 min. The luminescence images of the cells were captured using a Nikon laser scanning confocal microscope C2<sup>+</sup> with DAPI and FITC channel.



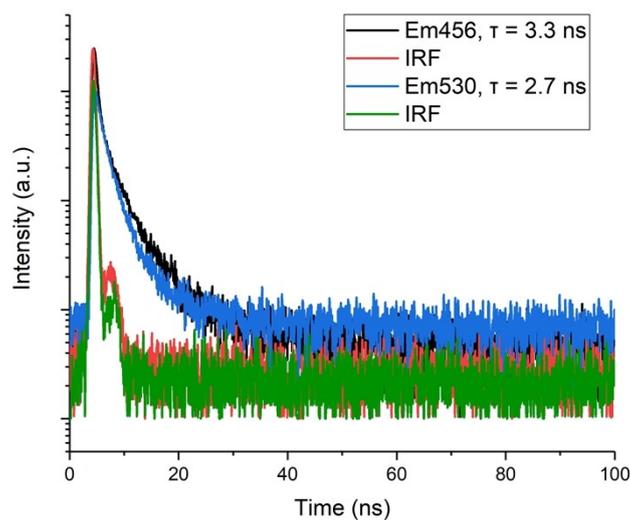
**Figure S1.** UV-vis spectra of B-CDs, G-CDs, W-CDs and Y-CDs.



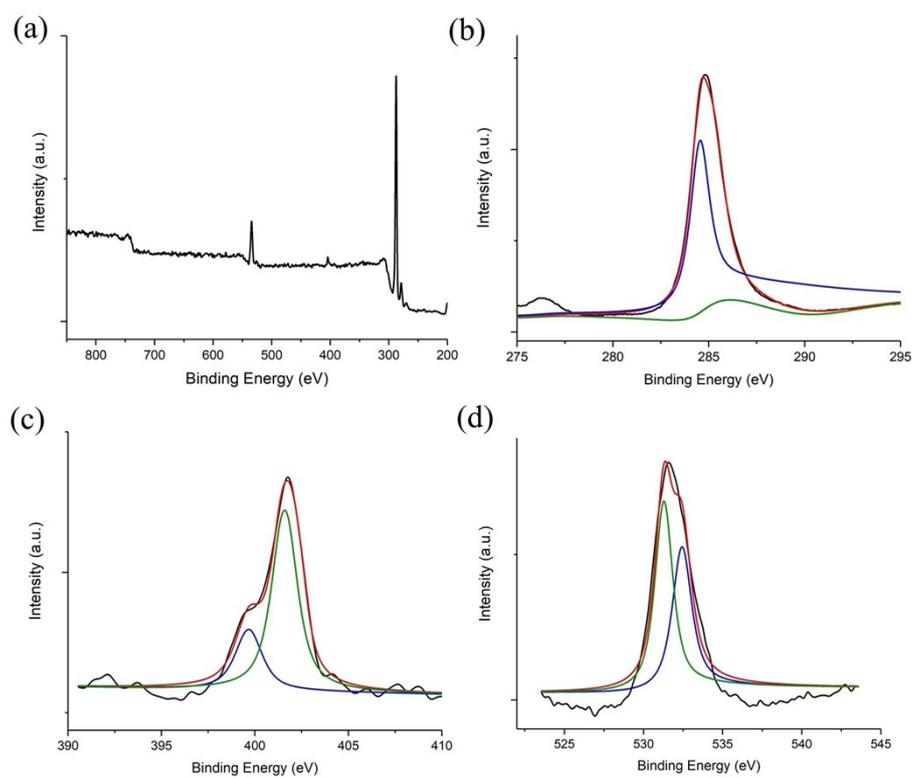
**Figure S2.** Photoluminescent excitation (dotted curve) and emission (full curve) spectra of (a) B-CDs; (b) G-CDs; (c) Y-CDs.



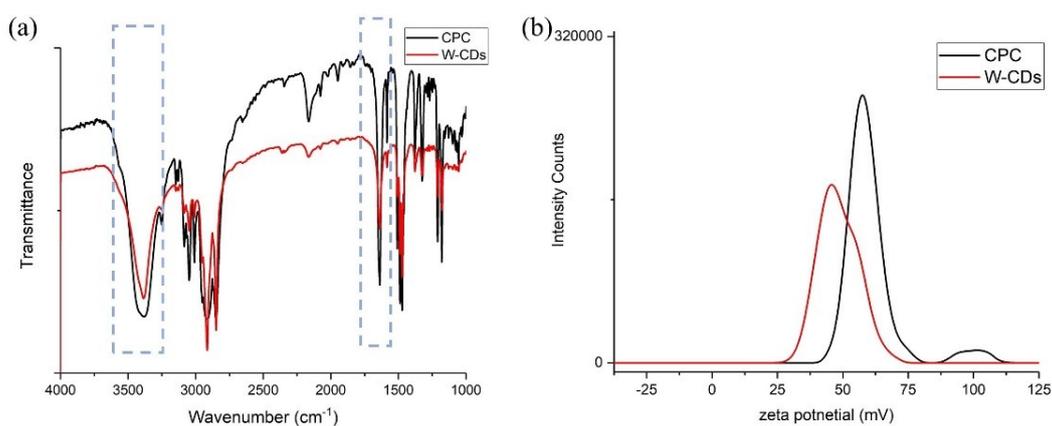
**Figure S3.** TEM images of (a) B-CDs; (b) G-CDs; (c) Y-CDs. Scale bar: 20 nm. Insets are the corresponding size distribution calculations based on 100 dots.



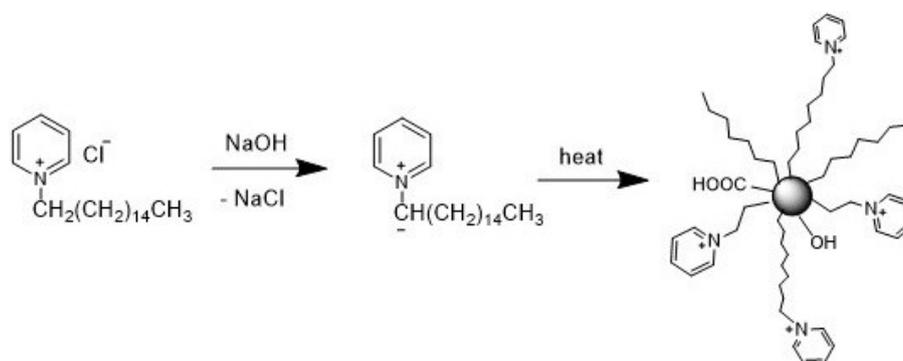
**Figure S4.** Fluorescence decay (black and blue lines) and IRF (red and green lines) curves of W-CDs recorded at emission wavelengths of 460 nm and 530 nm with excitation at 365 nm, respectively, solved in deionized water.



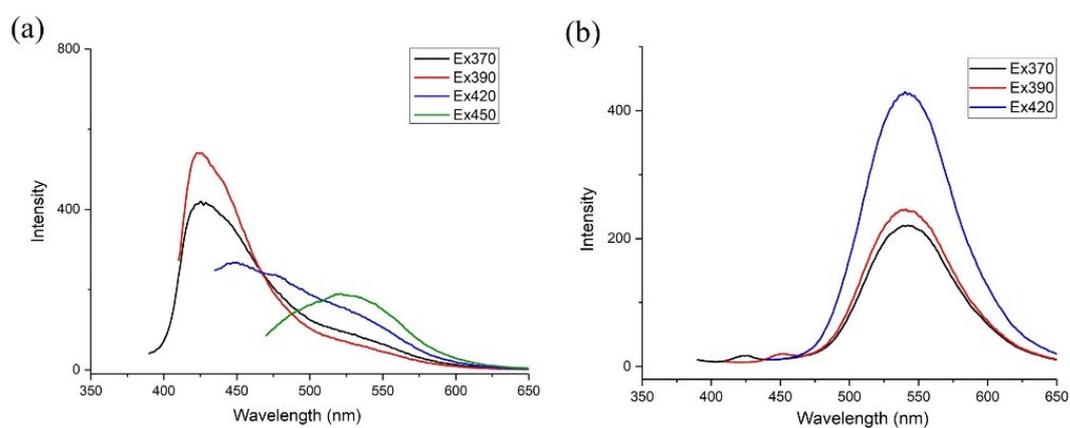
**Figure S5.** (a) XPS survey scan and (b) C1s, (c) N1s and (d) O1s of W-CDs, respectively.



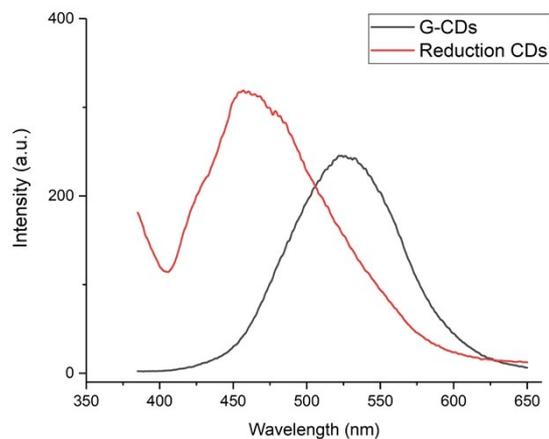
**Figure S6.** (a) FT-IR spectra of CPC and W-CDs. (b) Zeta potential of CPC and W-CDs.



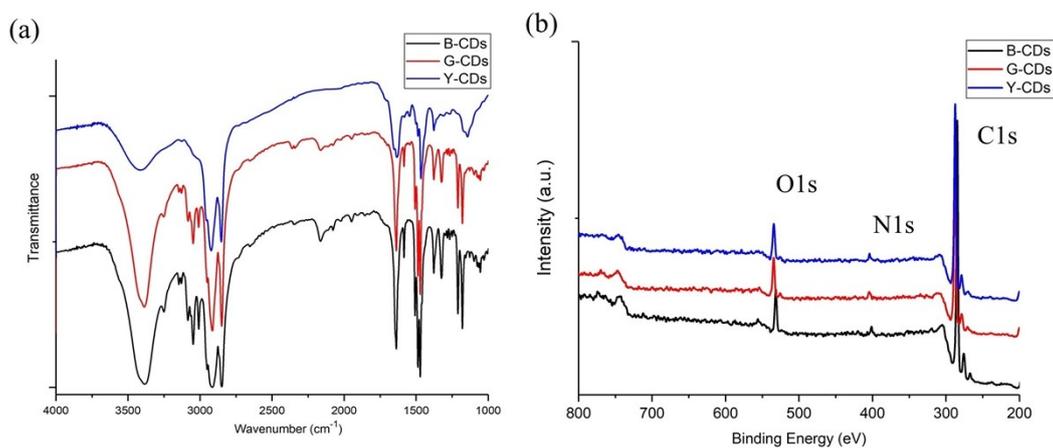
**Figure S7.** The proposed mechanism of CDs formation with CPC and NaOH.



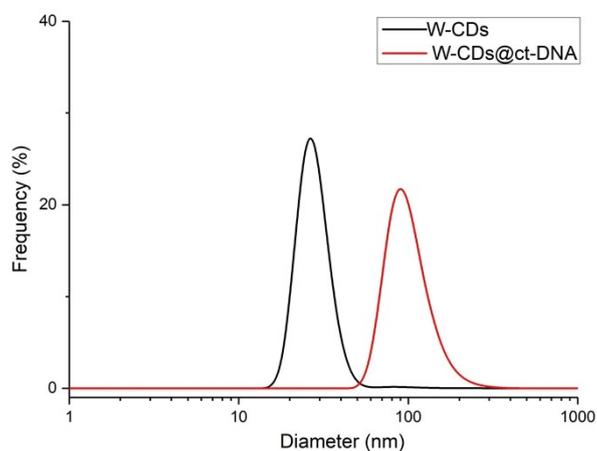
**Figure S8.** (a) Emission fluorescence spectra of W-CDs, measured in chloroform at different excitation wavelengths. (b) Emission spectrum of CPC aqueous solution treated with NaOH, measured at different excitation wavelengths.



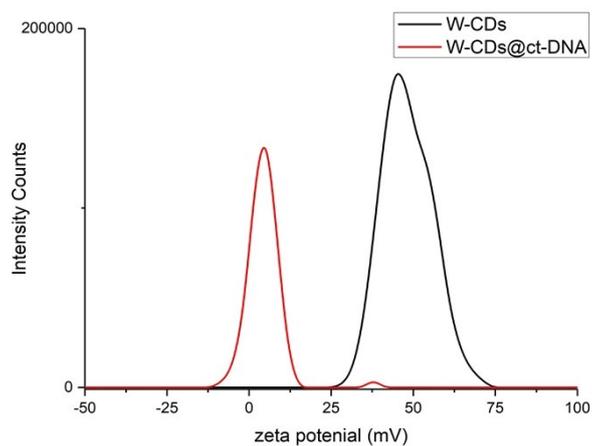
**Figure S9.** Emission spectra of G-CDs (black curve) and G-CDs after reduction (red curve) ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ).



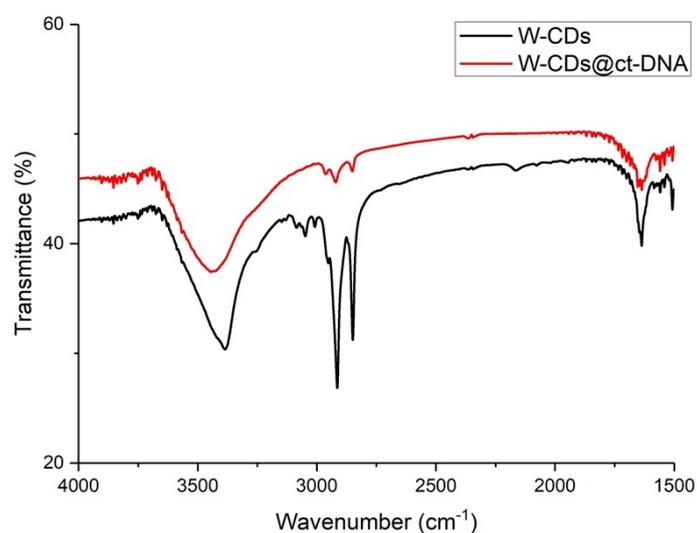
**Figure S10.** (a) FT-IR spectra of B-CDs, G-CDs and Y-CDs. (b) XPS survey scan of B-CDs, G-CDs and Y-CDs. The ratio of N/C is 0.025, 0.030, 0.032, respectively.



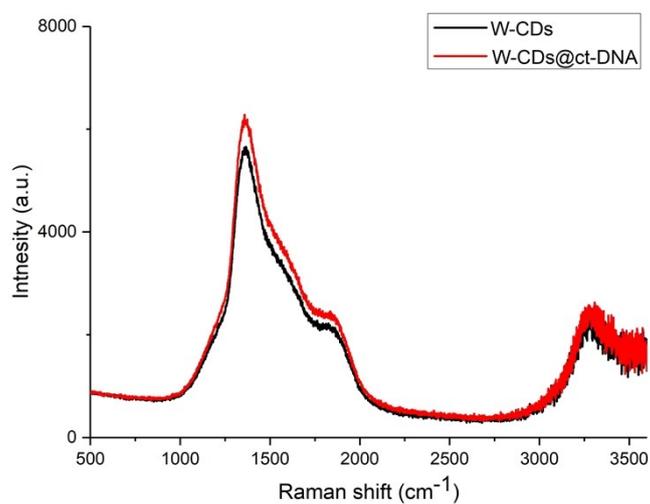
**Figure S11.** Average hydrodynamic radius of W-CDs (black curve) and W-CDs@ct-DNA nanohybrid (red curve).



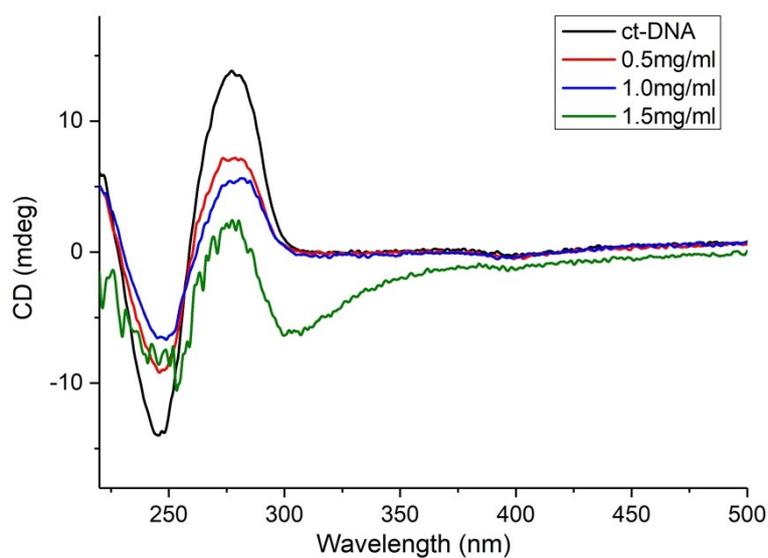
**Figure S12.** Zeta potential of W-CDs and W-CDs@ct-DNA.



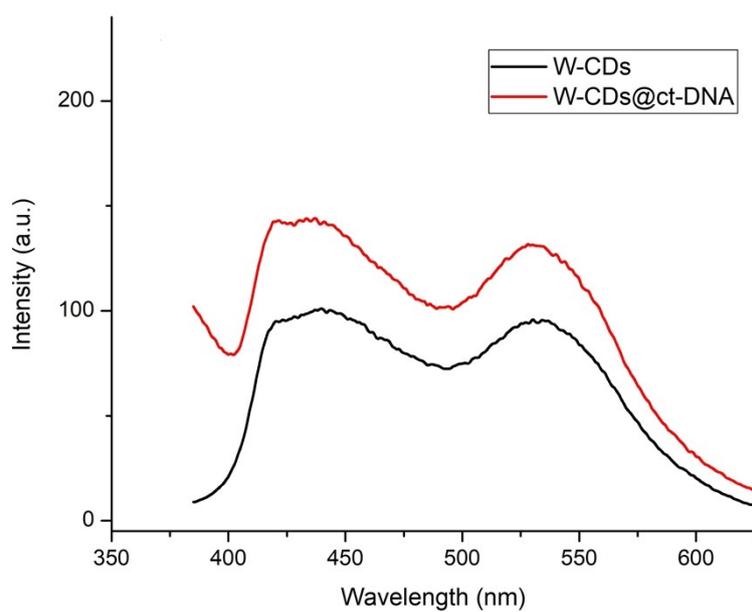
**Figure S13.** FT-IR spectra of WCDs and W-CDs@ct-DNA.



**Figure S14.** Raman spectra of W-CDs (black curve) and W-CDs@ct-DNA nanohybrid (red curve).

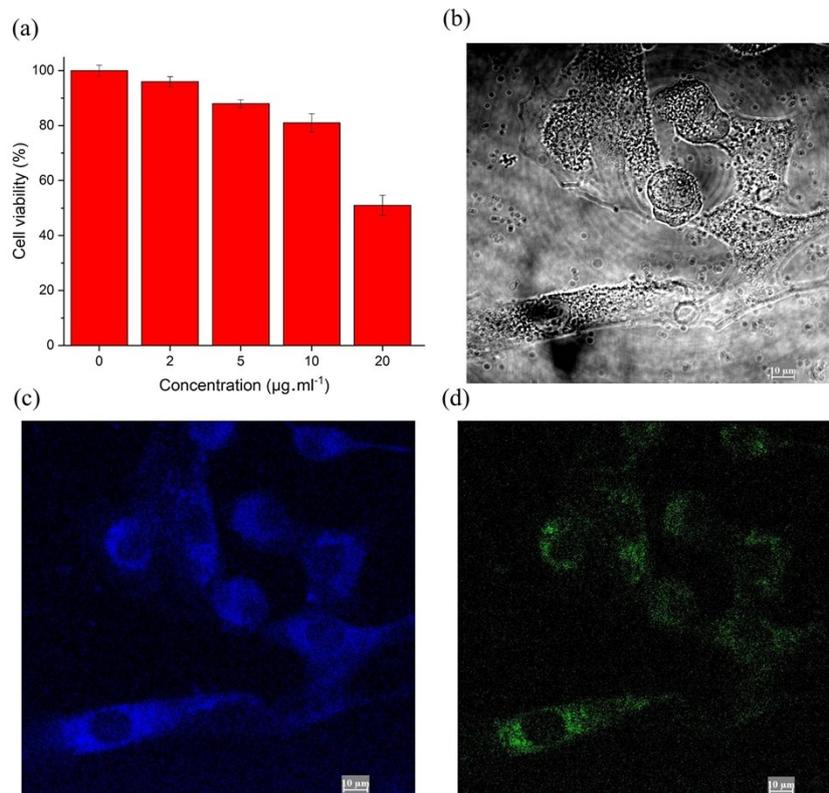


**Figure S15.** CD spectra of 0.5 mg/mL ct-DNA titrated with different concentrations G-CDs: 0, 0.5, 1.0, 1.5 mg/mL, respectively.

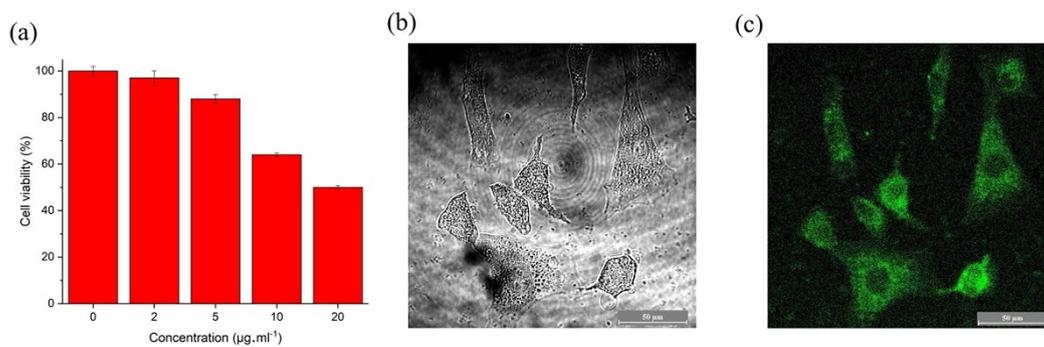


Category	PL QY (%)
W-CDs	~9.6%
W-CDs@ct-DNA nanohybrid	~15.3%

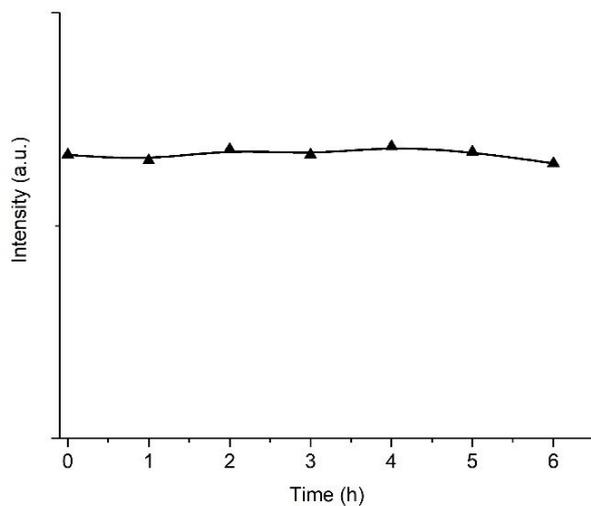
**Figure S16.** Fluorescence intensity of W-CDs (black curve) and W-CDs@ct-DNA nanohybrid (red curve) in water and corresponding photoluminescent (PL) quantum yield (QY).



**Figure S17.** (a) Cell viability of HeLa cells incubated with different concentration W-CDs via CCK-8 assay and confocal fluorescence microphotographs of HeLa cells incubated with W-CDs: (b) bright field; (c)  $\lambda_{ex} = 408$  nm and (d)  $\lambda_{ex} = 488$  nm.



**Figure S18.** (a) Cell viability of HeLa cells incubated with different concentration G-CDs via CCK-8 assay and confocal fluorescence microphotographs of HeLa cells incubated with G-CDs: (b) bright field and (c)  $\lambda_{ex} = 488$  nm.



**Figure S19.** The photostability of W-CDs solution under continuous UV irradiation ( $\lambda_{\text{ex}} = 365$  nm).

## Reference

- S1. O. Kozák, K. K. R. Datta, M. Greplová, V. Ranc, J. Kašlík and R. Zbořil, *J. Phys. Chem. C.*, 2013, **117**, 24991-24996.
- S2. W. Yang, J. Ni, F. Luo, W. Weng, Q. Wei, Z. Lin and Chen, G. *Anal. Chem.*, 2017, **89**, 8384-8390.
- S3. S. Abasi, M. A. Tehran and M. D. Fairchild. *Color Research and Application*, 2020, **45**: 208-223.