Supporting Information

For

Fluorescent Carbon Dots an Effective Nano-Thermometer in Vitro Applications

Waheed Ullah Khan[1a], Liying Qin[1b], Abid Alam[a], Ping Zhou[b]*, Yong Peng[c], and Yuhua Wang[a]*

[a] National and Local Joint Engineering Laboratory of Optical-Conversion Materials and Technology, Lanzhou University, Lanzhou 730000, P.R. China.
[b] School of Stomotology, Lanzhou University, Lanzhou 730000, P.R. China.
[c] Key Laboratory of Magnetism and Magnetic Materials of the Ministry of Education, School of Physical Science and Technology and Electron Microscope Center of Lanzhou University, Lanzhou 730000, P.R. China.

Corresponding Authors

Tel: +86931-8912772; Fax: +86931-8913554

Email: zhoup@lzu.edu.cn (P. Zhou); wyh@lzu.edu.cn (Y. Wang)

Characterization

High-resolution transmission electron microscopy (HRTEM) images were observed by a FEI Tecnai F30 transmission electron microscope (TEM) operating at 300 kV. Further the morphology of the sample was observed by atomic force microscopy (AFM) multimode nanoscope, DI, USA. The ultraviolet-visible (UV-Vis) absorption spectra were recorded using a Perkin-Elmer (USA) Lambda 950 UV-Vis-NIR spectrophotometer. Fourier transform infrared spectroscopy (FT-IR) of CDs was measured by Nicolet 670 spectrophotometer, and KBr crystals were used as matrix for sample preparation. The phase purity of fluorescent CDs was analyzed by X-ray diffraction (XRD) using a Bruker D2 PHASER X-ray Diffractometer with graphite monochromator using Cu Kα radiation (λ = 1.54056 Å), operating at 30 kV and 15 mA. The emission and excitation spectra were recorded at room temperature by using a Fluorolog-3 Spectrofluorometer equipped with a 450W Xenon light source. X-ray photoelectron spectroscopy (XPS, PHI- 5702, Physical Electronics) was recorded using a monochromatic Al Kα irradiation. Raman spectroscopy (JY-HR800 micro-Raman, using a 532 nm wavelength YAG laser with a laser spot diameter of about 600 nm). The decay curves were measured by a FLS-920T fluorescence spectrophotometer with an nF900 nanosecond flash lamp as the light

source. Fluorescent images of cells are obtained on a Nikon Confocal laser scanning microscope A1R+Ti2E.

Biological Experiments

The biological experiments are performed with the permission of School of Stomotology, Lanzhou University, Lanzhou 730000, P.R. China under the project name/grant no# (National Science Foundation of China (U1905213)).

Quantum Yield (QY) measurement

Quantum yield (QY) of the obtained fluorescent CDs was determined by a relative slope method.[1] Specially, quinine sulfate (QY=54% in 0.1 M H_2SO_4) was selected as a standard for the prepared CDs. The aqueous solution of CDs and quinine sulfate ware diluted to keep the absorption intensity below 0.1 at the best excitation wavelength of 360. The QY of the prepared CDs was calculated according to the following equation (1).

$$\varphi_x = \varphi_{st} (K_x/K_{st}) (\eta_x/\eta_{st})^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where φ is the quantum yield, K is the slope of the fitted line and η is the refractive index of the solvent. The subscript "x" refers to the testing sample and "st" refers to the standards (QS). The value of refractive index is 1.33 for water.

Cytotoxicity Assay

Tongue squamous Cell (T-ca) cells were incubated with different concentration of CDs (25-400μg/ml) at 37°C in 5% CO_2 for 24 hours. Then the cell viability was determined by cell counting kit-8 (CCK-8). In detail, cells after treatment were incubated with CCK-8 diluents for 1 hour, and then measured the optical densities (OD) at 450 nm. The experiments were performed 3 times.

Cell Imaging

CDs (300 μg/mL) were mixed with living T-ca cells in 24-well plates. After incubation for 4 h, the culture medium with free CDs was then discarded and the T-ca cells are washed with phosphate buffered saline (PBS) for several times. Fluorescent images of cells were obtained on a Nikon Confocal laser scanning microscope A1R+Ti2E.

In Vitro Intracellular Thermal Sensing with CDs.

T-ca cells were plated into 24-well plates (1 × 105 cells/well) and cultivated for 24 h. Then, medium containing 300 μg/mL of CDs was added to each well. After 24 h of incubation, the supernatant was removed and the cells were washed five times with PBS. The cells were then detached with trypsin (0.25% in EDTA) and centrifuged (1600 rpm for 5 min). The pellet of sedimented cells from each well was resuspended in 200 μL of fresh medium. PL emission and PL decay measurements of T-ca cells incubated with CDs at different temperature were performed in a quartz.

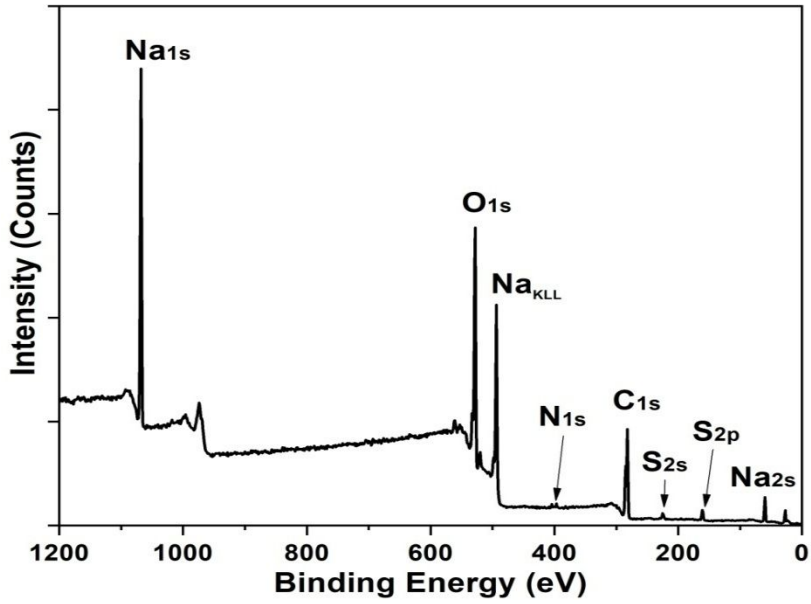

Figure S1. The wide XPS spectrum of CDs

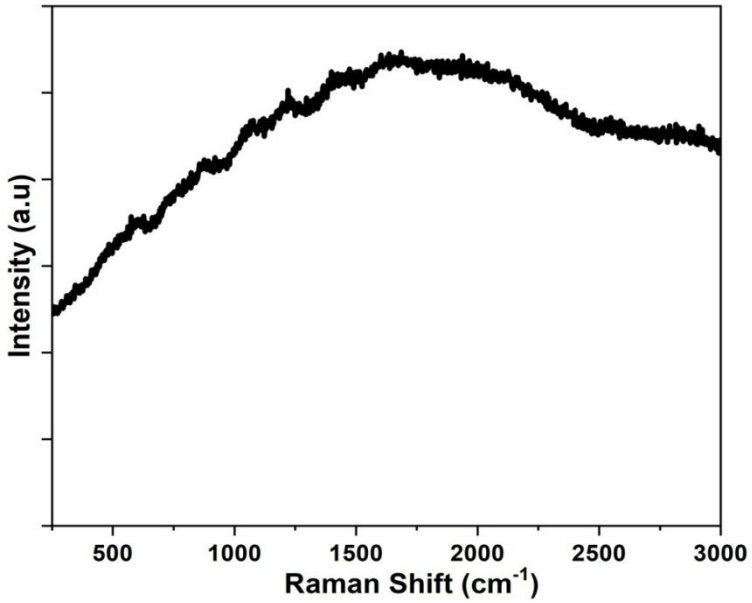

Figure S2. The Raman Shift of the prepared CDs.

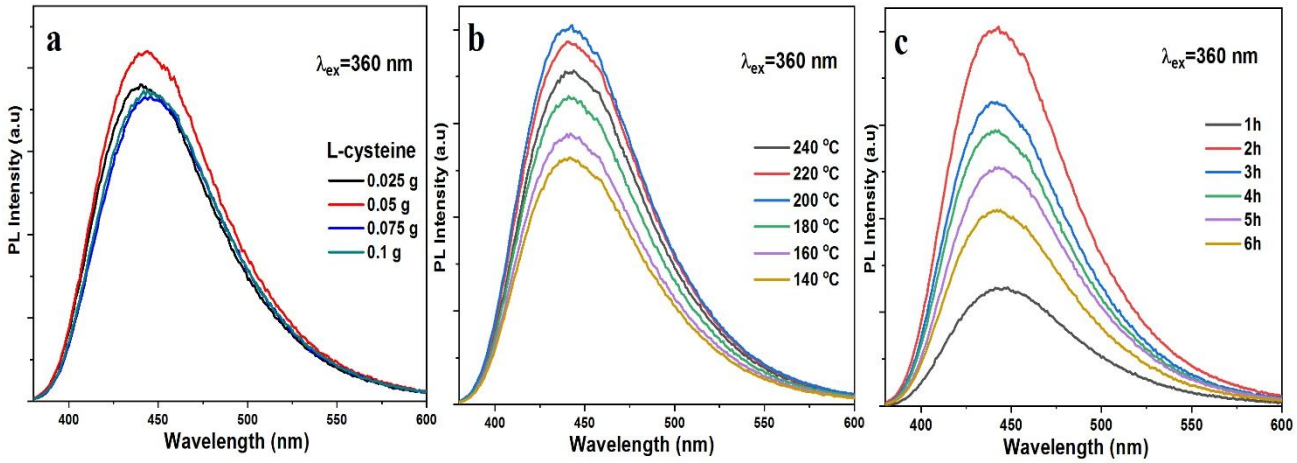

Figure S3. Optimal condition for CDs (a) ratio of trisoduim citrate and l-cysteine. (b) Different annealing temperatures. (c) Reaction time.

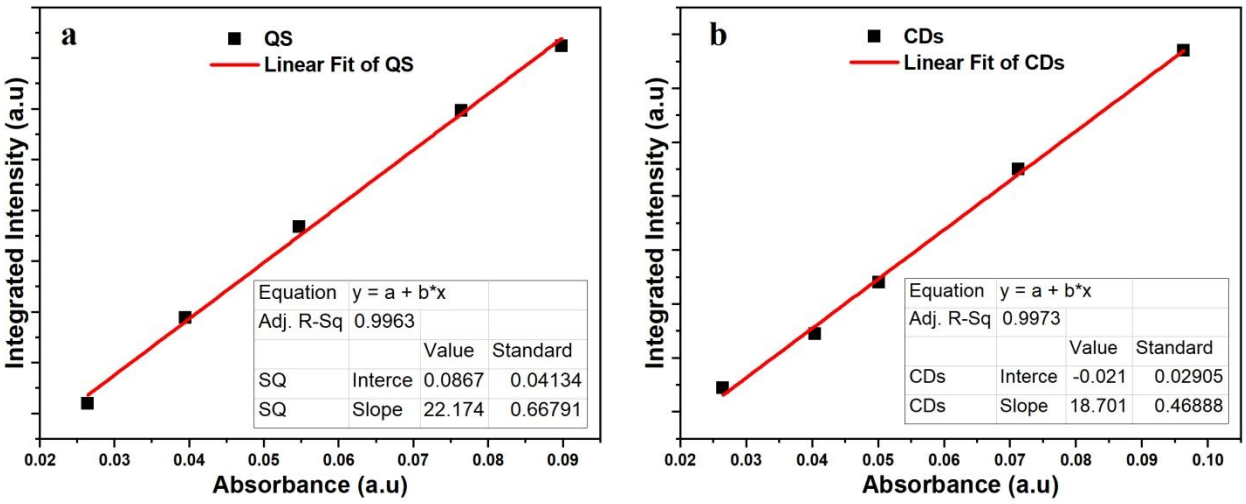

Figure S4. (a) Plots of integrated PL intensity of quinine sulfate and (b) fluorescence CDs excited at 360 nm.

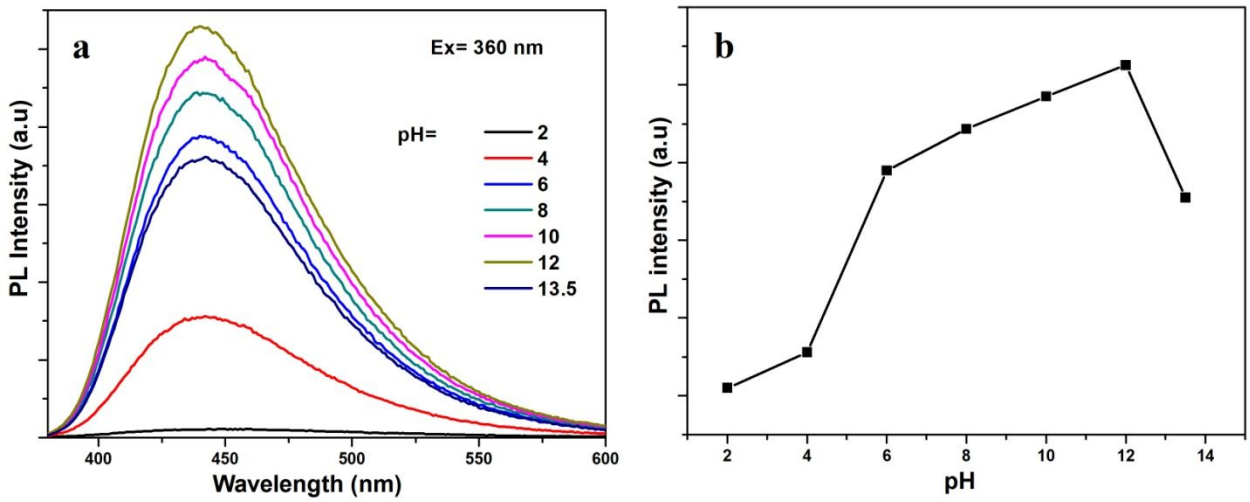

Figure S5. The pH study of CDs under the excitation of 360 nm (a) PL emission spectra, (b) Corresponding PL intensity under different pH values.

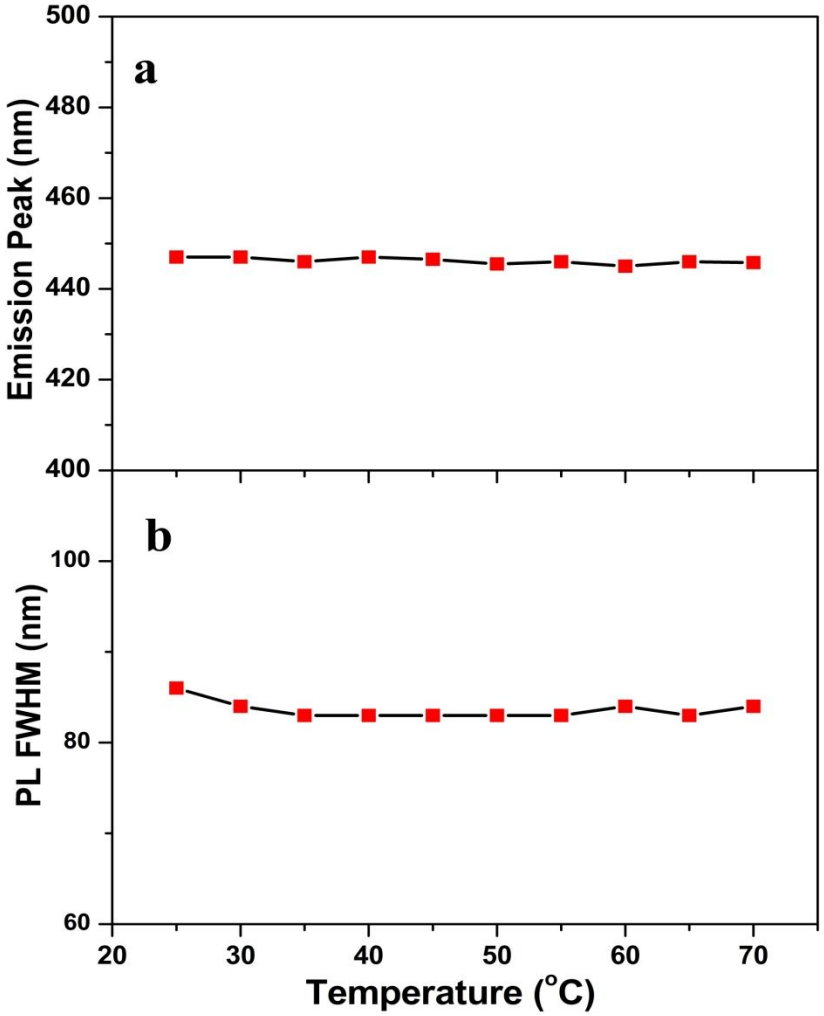

Figure S6. The temperature dependent changes in (a) PL emission peak and (b) PL fwhm.

Table. S1. Decay time of CDs at various temperatures.

Temperature (°C)	20	30	40	50	60	70
Decay time (ns)	7.94	6.87	5.89	4.97	4.12	3.3

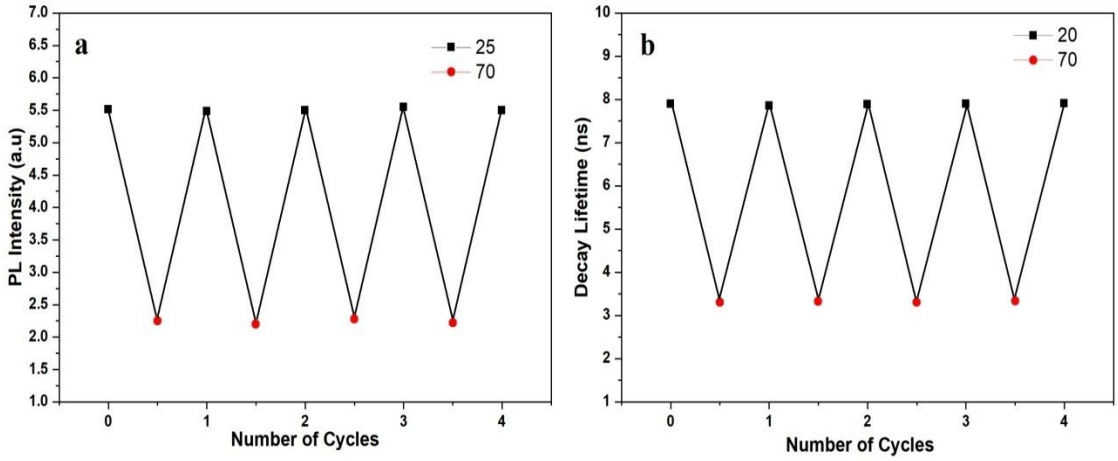

Figure S7. (a) PL intensity and (b) Decay lifetime upon the cycling swiching of temperture 20 °C and 70 °C.

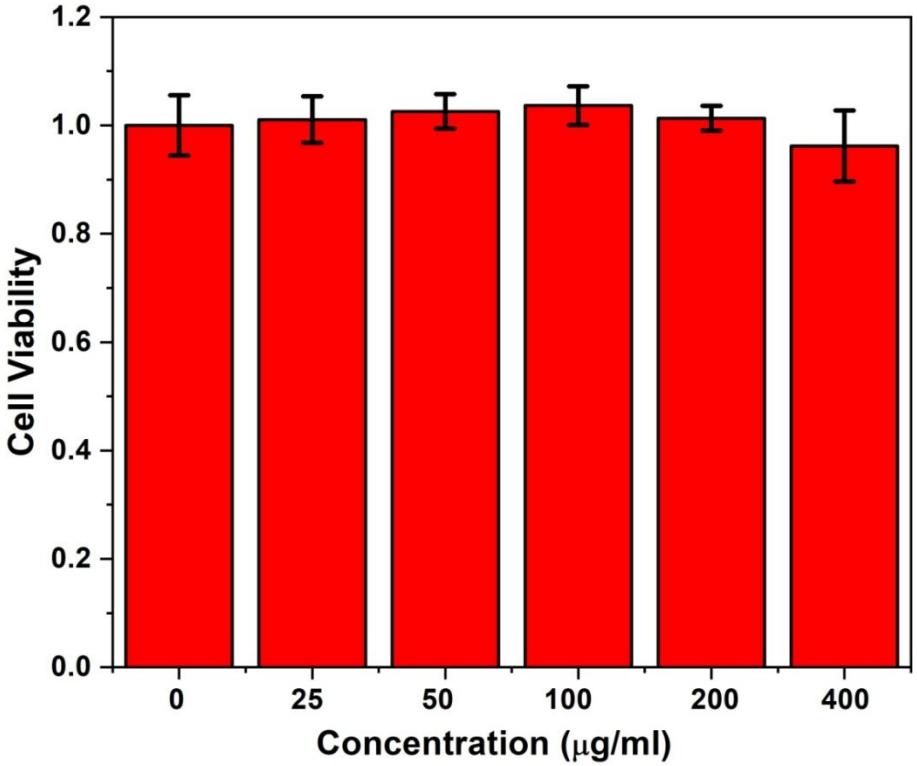

Figure S8. Toxicity test using different concentration of CDs (0-400μg/ml).

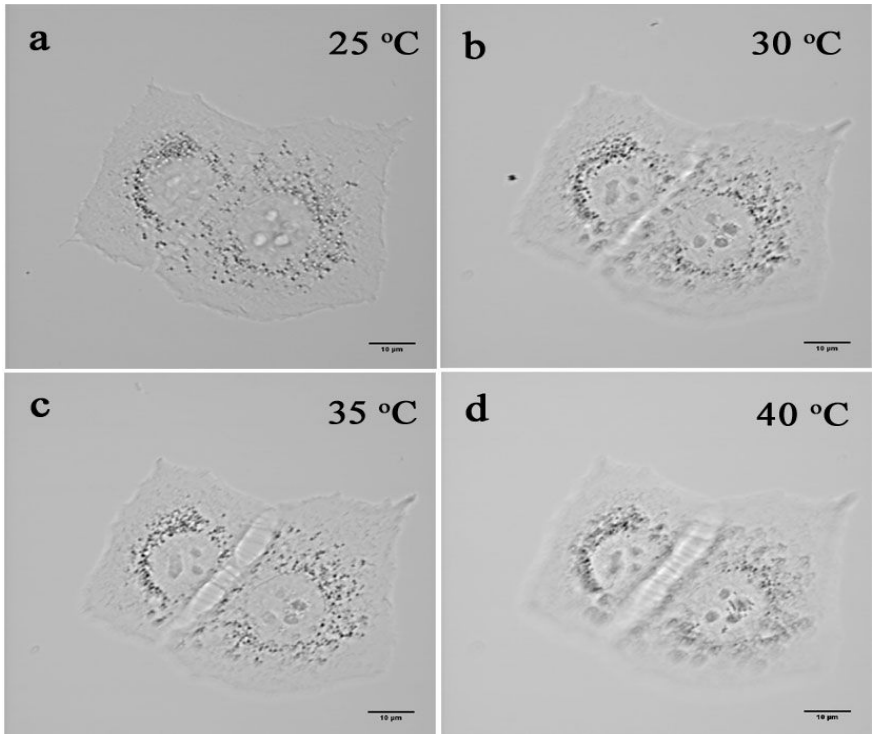

Figure S9. (a-d) Bright field images of T-ca cell under 25°C, 30°C, 35°C and 40°C, respectively. (All scale bars are 10 μm).

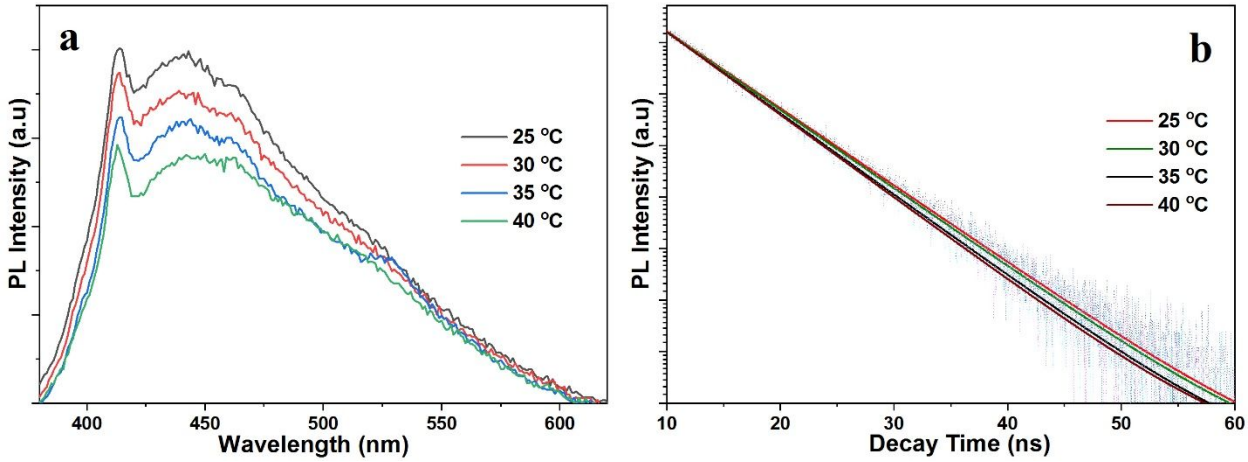

Figure S10. T-ca cells treated with CDs and measured their (a) Fluorescent spectra. (b) PL decay lifetime under different temperature ranging from 25 ºC-40 ºC.

Reference

1. L. Shi, J. H. Yang, H. B. Zeng, Y. M. Chen, S. C. Yang, C. Wu, H. Zeng, O. Yoshihito and Q. Zhang, Nanoscale, 2016, **8**, 14374-14378.