Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Intracellular thermometry nanoprobe based on biosynthesized fluorescent copper nanoclusters

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The Supplementary Information includes:

1. Experiment of the repetitive freeze-thaw method (S1)
2. Supplementary Figures
3. Equations

1. Experiment of the repetitive freeze-thaw method (S1)

(1). Incubated MDA-MB-231 cells with 20 μg/mL copper precursor solution for 24 h, removed the medium, trypsinize the cells.

(2). Harvest the cells with PBS in Eppendorf tubes, centrifuge the mixture at 1200 rpm for 5 min.

(3). Removed the supernatant and collected the cells.

(4). Disperse the collected cells in 2 mL Milli-Q water.

(5). Put the cells into liquid nitrogen for 30s, then put it into in 37℃ water for thawing.

(6). By a frequently repetitive freeze-thaw method for 1 h.

(7). Centrifuge the mixture at 2500 rpm for 5 min. Discard the precipitated cell debris and collect the supernatant with CuNCs.

2. Supplementary Figures

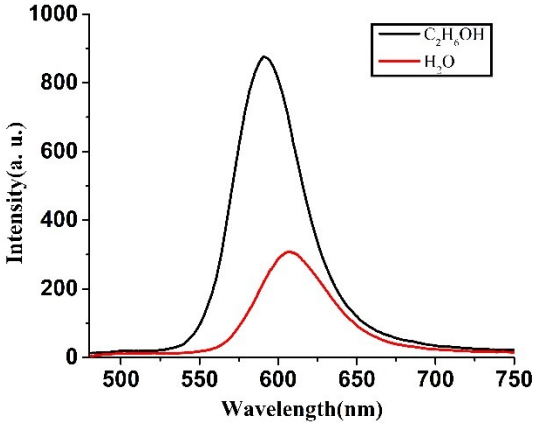

Figure S1 Emission spectrum of the biosynthesis CuNCs extracted from cancer cells in different solvent. Red: aqueous solution; black: ethanol.

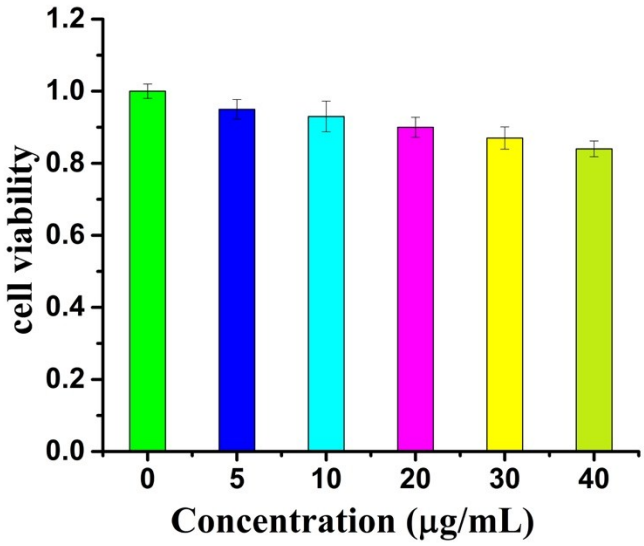

Figure S2 MTT assay for the viability of MDA-MB-231 cells after incubation with copper precursor solution for 24 h.

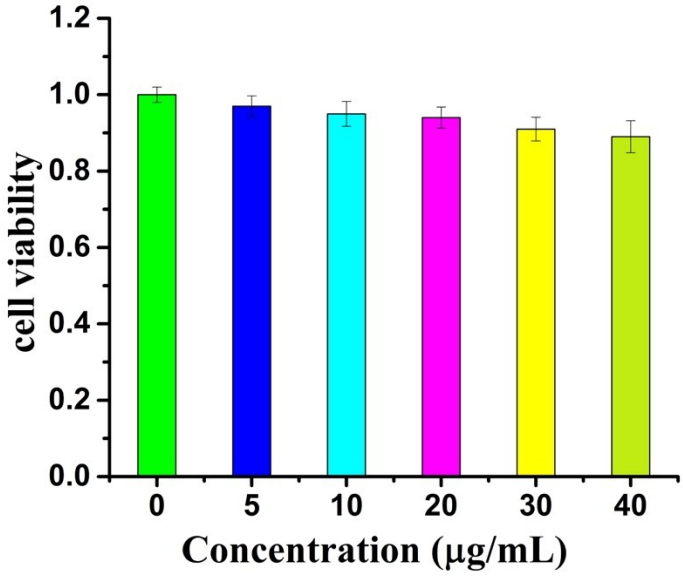

Figure S3 MTT assay of dose-dependent cytotoxicity towards L02 cells 24 h after incubation with copper precursor solution. The data represent three individual experiments done in triplicates.

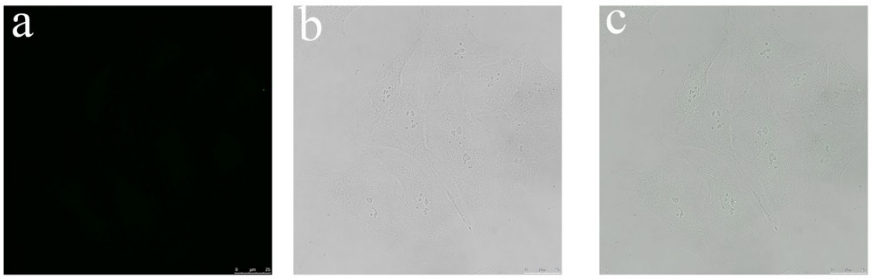

Figure S4 Laser confocal fluorescence imaging of L02 cells incubated with 20 μg/ml copper precursor solution for 24 h at 37°C. (a) dark-field (b) bright-field. (c) image-overlay.

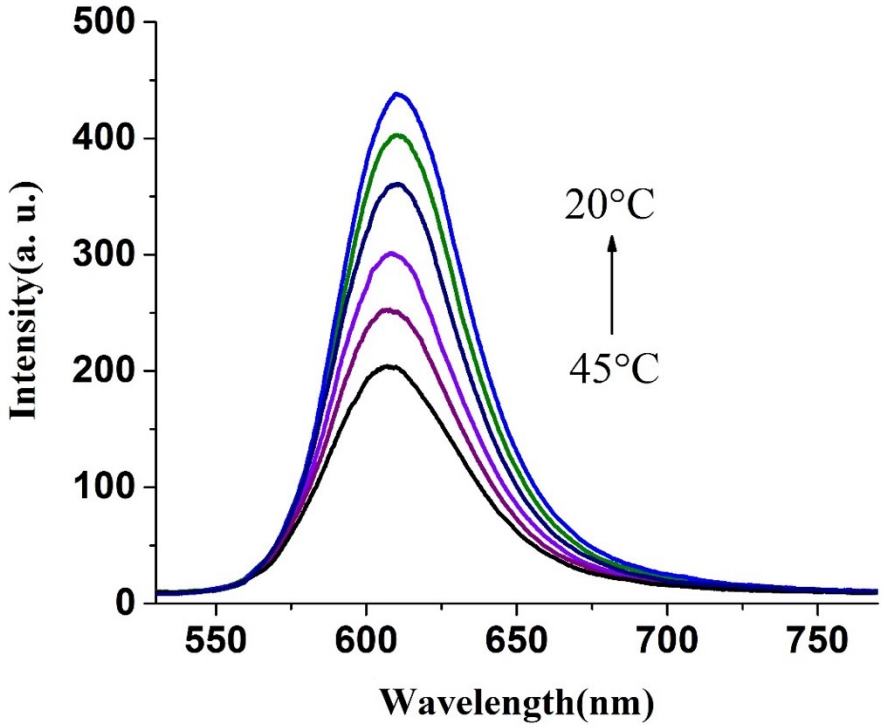

Figure S5 Fluorenscence emission spectra measured under the excitation at 400nm with the decrease of the temperature from 45 to 20 ˚C(from bottom to top)

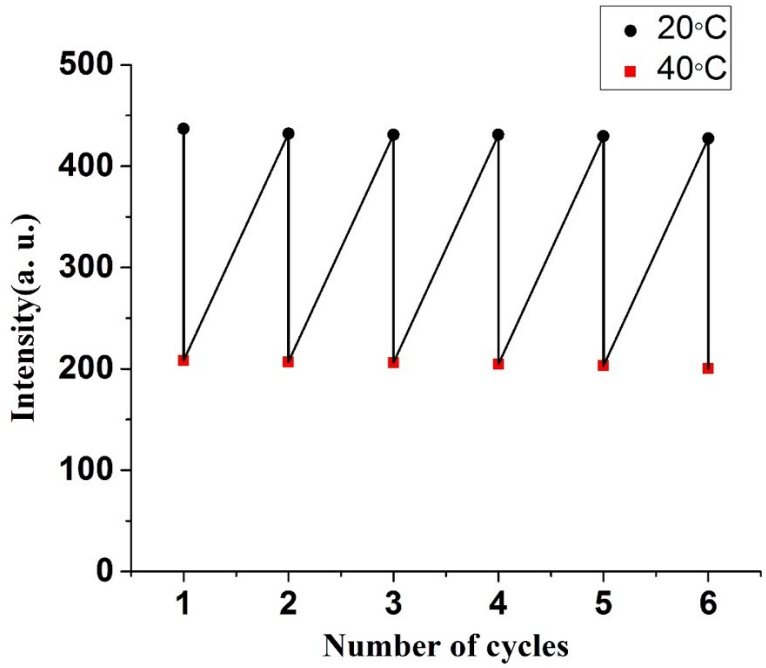

Figure S6 Fluorescence intensity of Cu nanoclusters extracted from MDA-MB-231cells upon cycling the temperature six times between 20 and 40 ˚C.

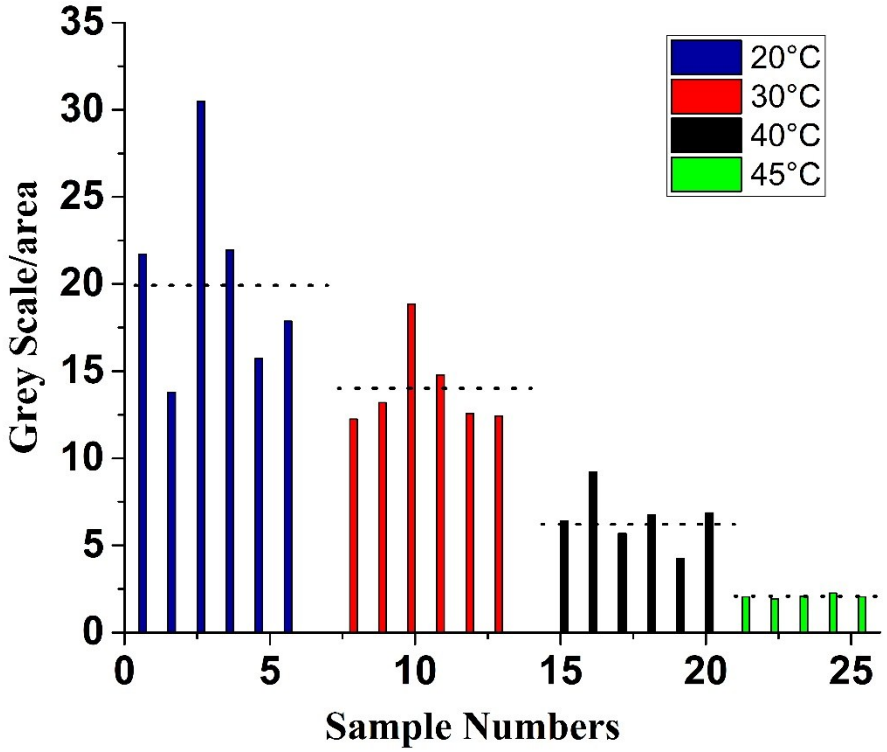

Figure S7 Comparison of gray scale values per unit area obtained from individual cells with different temperature in 20 ˚C (blue), 30 ˚C (red), 40 ˚C (black) and 45 ˚C (green) group. Scale bar: 50 μm.

3. Equations

The QY of the biosynthesis CuNCs was obtained using a 502 nm Xe laser calibrated with Rhodamine 6G dissolved in ethanol (QY = 95%). According to the emission peak area and absorbance of the biosynthesis CuNCs and Rhodamin 6G, the QY of the biosynthesis CuNCs could be calculated from Eq. (S1) below[1]

$$\phi_{sample} = \frac{A_{std}}{A_{sample}} \times \frac{F_{sample}}{F_{std}} \times \frac{\eta^2_{sample}}{\eta^2_{std}} \times \phi_{std} \quad \text{(S1)}$$

where Φ_{std} is the quantum yields (QY) of the standard compound, F_{sample} and F_{std} are the integrated areas of the fluorescence of the sample and the standard in the emission region of 450-750 nm respectively, A_{std} and A_{sample} are the absorbance of the standard and the sample at the excitation wavelength (400 nm) respectively, η is the refractive index of the solvent (equal to 1.33 for water and 1.36 for ethanol). All the samples were diluted to ensure that the optical densities were lower than 0.07 as measured by UV 3600 spectrophotometer to reduce the error.

The relative sensitivity(S) is defined as Eq. (S2): [2]

$$S = \frac{\left(\frac{\partial I}{\partial T}\right)}{I} \quad \text{(S2)}$$

where I is an experimental parameter corresponding with temperature (T). The S is usually expressed as percent change per degree Celsius ([%/∂ C]).

References

1. C. Zhang, Z. Zhou, Q. Qian, G. Gao, C. Li, L. Feng, Q. Wang and D. Cui, Journal of Materials Chemistry B, 2013, 1, 5045-5053.

2 C. D. S. Brites , P. P. Lima , N. J. O. Silva , A. Millan , V. S. Amaral , F. Palacio and L. D. Carlos , *Nanoscale,* 2012, **4**, 4799-4829.