Electronic Supplementary Information

for

One-step prepared fluorescent copper nanoclusters for reversible pH-sensing

Wei Wang,[a] Fei Leng,[b] Lei Zhan,[b] Yong Chang,[b] Xiao Xi Yang,[a] Jing Lan,[a] Cheng Zhi Huang*[a,b]

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Experimental Section

Reagents and Chemicals. Trypsin was purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA). $CuCl_2$ and all other chemicals were analytical grade without further purification before used. Milli-Q purified water (18.2 MΩ) was used throughout the experiments.

Characterization. The absorption and fluorescence spectra of CuNCs were measured with U-3010 spectrophotometer and F-2500 spectrofluorometer (Hitachi, Japan), respectively. The absolute quantum yield (Φ) of CuNCs was measured by using an absolute photoluminescence quantum yield measurement system (Quantaurus-QY, Hamamatsu Photonics, Japan) with an excitation wavelength of 363 nm. A high resolution transmission electron microscopy (Tecnai G2 F20 S-TWIN, FEI, USA) was used to characterize the morphology of CuNCs, which was operated at an accelerating voltage of 200 kV. The circular dichroism (CD) spectrum was collected with a JASCO J-810 spectropolarimeter (Jasco, Japan). The pictures of CuNCs were taken with a digital camera (Olympus, Japan). The pH values of various buffers were measured with a pH 510 precision pH meter (Eutech, USA). Elemental analysis of CuNCs was made on ESCALAB 250 X-ray photoelectron spectrometer (Thermo, Japan). The fluorescence life time of CuNCs was measured with FL-TCSPC fluorescence spectrophotometer (Horiba Jobin Yvon Inc., France). A QL-901 vortex mixer (Haimen, China) was employed for solution blending adequately, and a high-speed H1650-W centrifuge (Xiangyi, China) was used for centrifugation.

Preparation of CuNCs. The CuNCs were synthesized by a hydrothermal method. In a typical synthesis, an aqueous solution of trypsin (8 mL, 100 mg) was mixed with an aqueous solution of $CuCl_2$ (2 mL, 100 mM) at room temperature. The solution was stirred at room temperature for 2-3 min. Finally, the mixture was allowed to reflux for 12 h at 100 ^{o}C, and the color changed from light blue to brownish yellow gradually. After reaction, the CuNCs were purified by centrifugation (13000 rpm, 5 min) in order to remove the solid from the supernatant and stored at 4 ^{o}C before use.

pH Response Experiments. Briefly, 50 μL of buffer solutions, 430 μL of water, and 20 μL of CuNCs were mixed thoroughly. After 1 h reaction, the solutions were centrifuged for 5 min at 10000 rpm. The fluorescence intensity of supernatants was recorded by fluorescence spectrophotometer with the excitation wavelength of 363 nm.

pH Cycling Experiments. Firstly, 3.2 mL of water and 800 μL of CuNCs were mixed together, followed by adding 10 μL NaOH (2 M). At this time, the pH of the solution was 11.41. After incubating for 20 min at room temperature, the solution was centrifuged for 5 min at 10000 rpm. The fluorescence intensity of supernatant was recorded by fluorescence spectrophotometer with the excitation wavelength of 363 nm. Subsequently, 20 μL of HCl (2 M) was added to the above solution and incubated for 10 min. The pH of the solution was changed to 2.40 and the fluorescence intensity of CuNCs was measured again. In this way, the cycles were repeated 7 times by using acid and base as modulators.

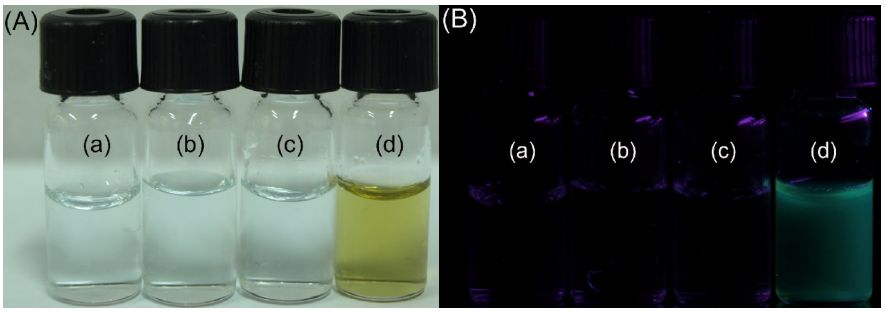

Figure S1. Photographs of both trypsin (a) and $CuCl_2$ alone (b) refluxed for 12 h at 100 ^oC, the mixture of trypsin and $CuCl_2$ before reaction (c) and the as-prepared CuNCs (d) under the daylight (A) and 365 nm UV light irradiation (B).

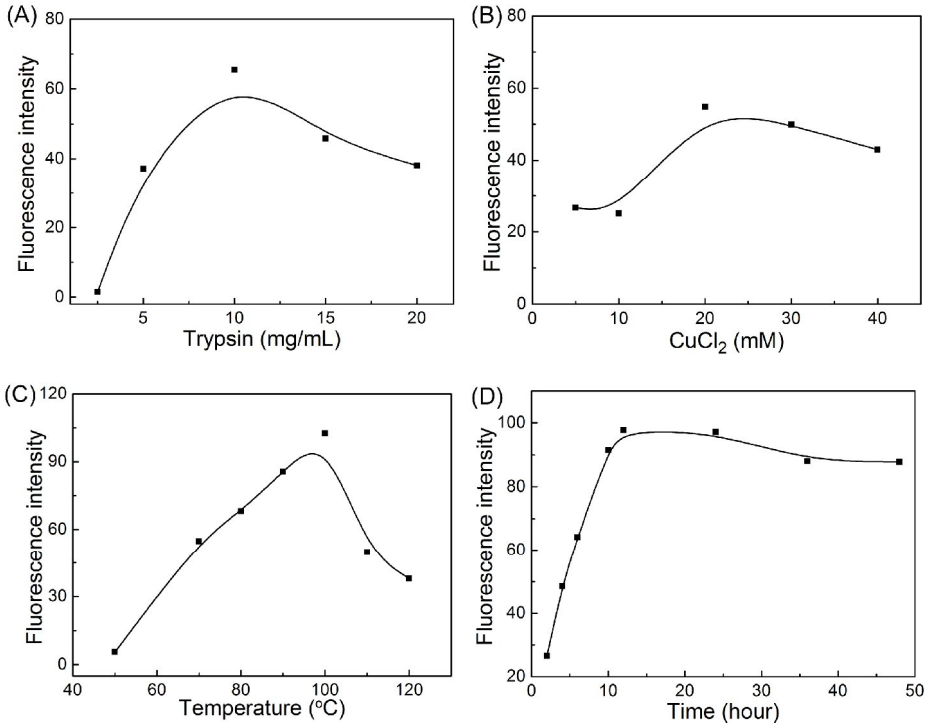

Figure S2. Optimization of reaction conditions of the fluorescent CuNCs. (A) The fluorescence intensity of the CuNCs under the different concentrations of trypsin (2.5, 5, 10, 15 and 20 mg/mL). The concentration of $CuCl_2$ was fixed at 10 mM, and the reaction temperature and time were 70 ^oC and 12 h, respectively. (B) The fluorescence intensity of the CuNCs under the different concentrations of $CuCl_2$

(5, 10, 20, 30, 40 mM). The concentration of trypsin was fixed at 10 mg/mL, and the reaction temperature and time were 70 ^{o}C and 12 h, respectively. (C) The fluorescence intensity of the CuNCs under different reaction temperatures (50, 70, 80, 90, 100 ^{o}C). The concentrations of $CuCl_2$ and trypsin were fixed at 20 mM and 10 mg/mL, respectively. The reaction time was 12 h. (D) The fluorescence intensity of the CuNCs under different reaction times (2, 4, 6, 10, 12, 24, 36 and 48 h). The concentrations of $CuCl_2$ and trypsin were fixed at 20 mM and 10 mg/mL, respectively. The reaction temperature was 100 ^{o}C.

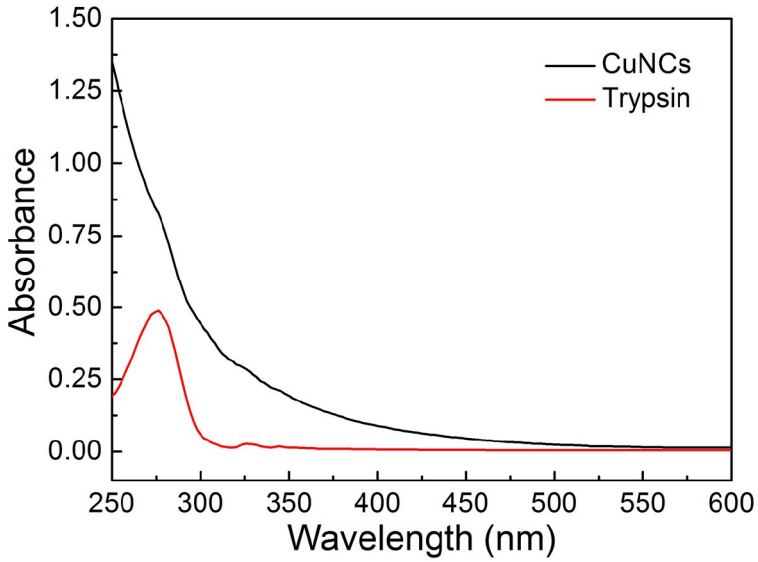

Figure S3. The UV-vis absorption spectrum of CuNCs (black line) and trypsin (red line) in aqueous solution. All the parameters are the same in both cases.

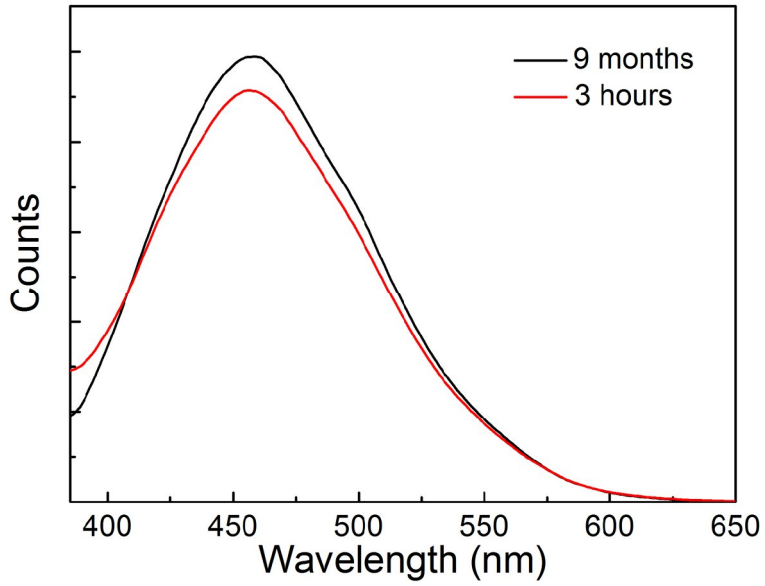

Figure S4. Fluorescence spectrum of CuNCs at different time indicating the high stability of CuNCs. The excitation wavelength was 363 nm.

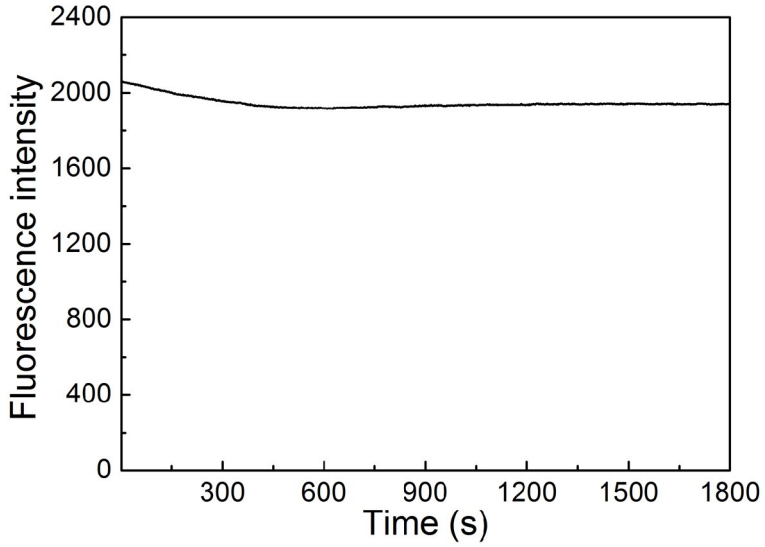

Figure S5. Kinetic study for the fluorescence change of CuNCs. The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

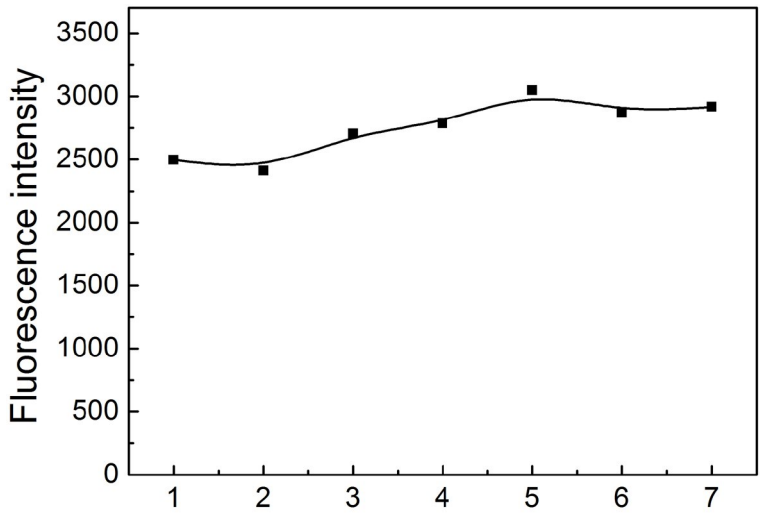

Figure S6. The photostability of CuNCs in human serum with different dilutions. The abscissa of 1 represented CuNCs in serum-free aqueous solution, and 2 to 7 represented CuNCs in serum with different dilutions (2: 500-fold diluted serum, 3: 100-fold diluted serum, 4: 50-fold diluted serum, 5: 10-fold diluted serum, 6: 5-fold diluted serum, 7: 2-fold diluted serum). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

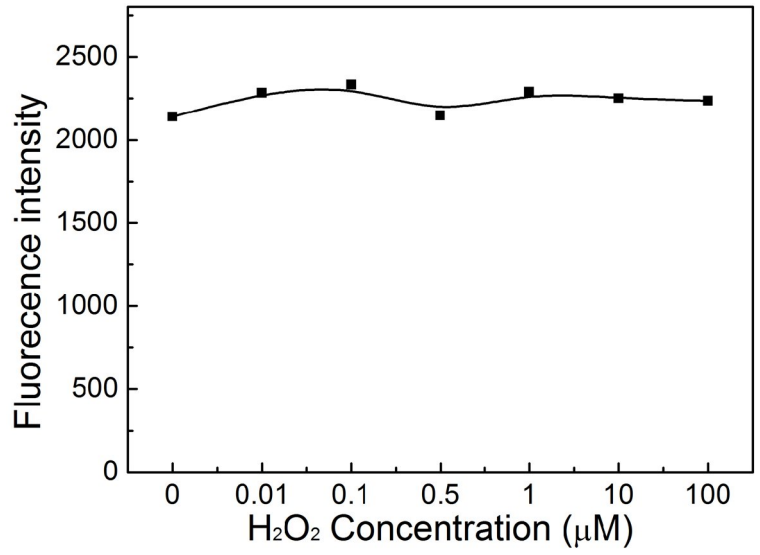

Figure S7. The fluorescence intensity changes of CuNCs with the addition of H_2O_2 (0, 0.01, 0.1, 0.5, 1, 10, 100 μM). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

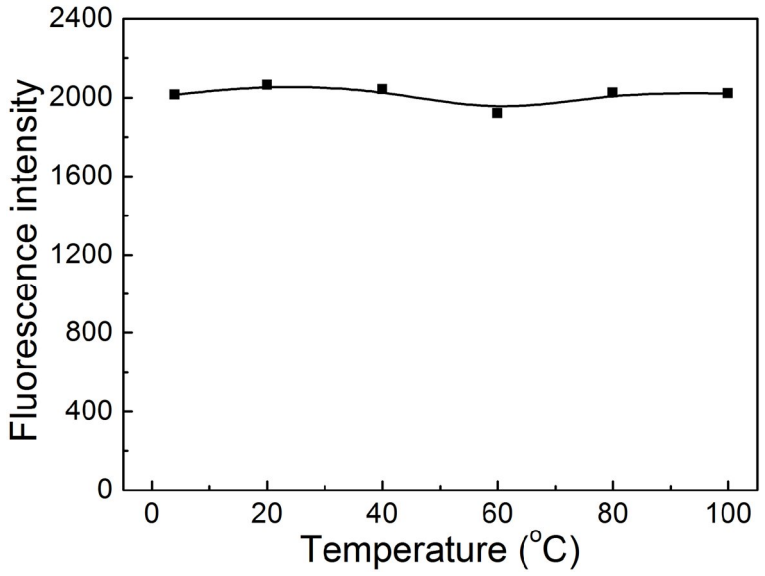

Figure S8. Effect of temperature on the fluorescence intensity of CuNCs in aqueous solution (4, 20, 40, 60, 80, 100 ^{o}C). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

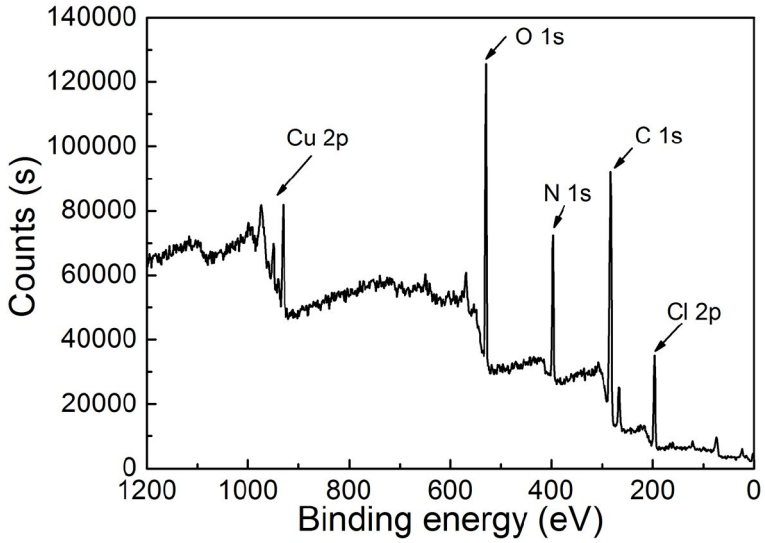

Figure S9. XPS survey spectrum of Cu 2p, O 1s, N 1s, C 1s, and Cl 2p involved in CuNCs.

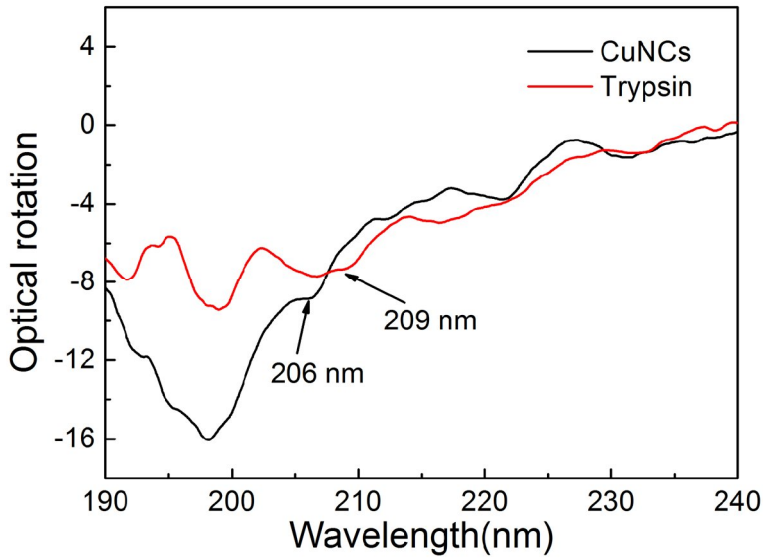

Figure S10. Circular dichroism(CD) spectrum of trypsin (red line) and CuNCs (black line) in aqueous solution.

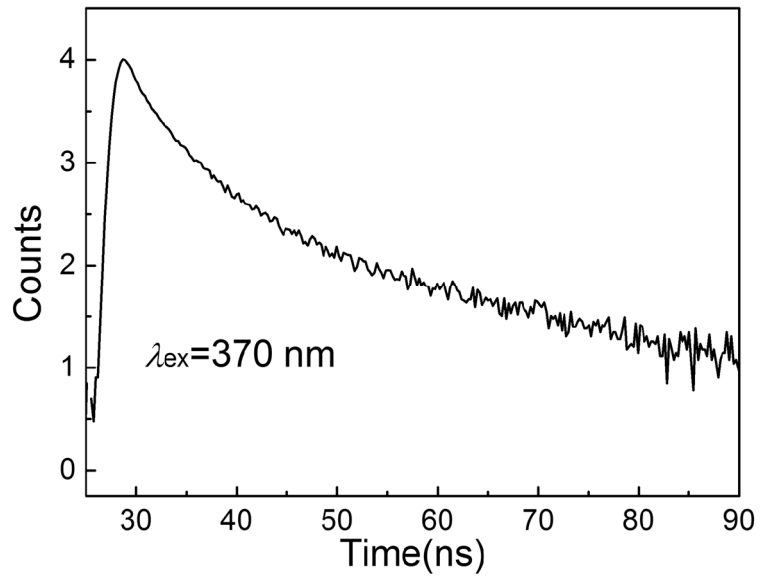

Figure S11. Fluorescence decay curves of the CuNCs with an excitation wavelength of 370 nm and the fluorescence intensity was collected at 455 nm.

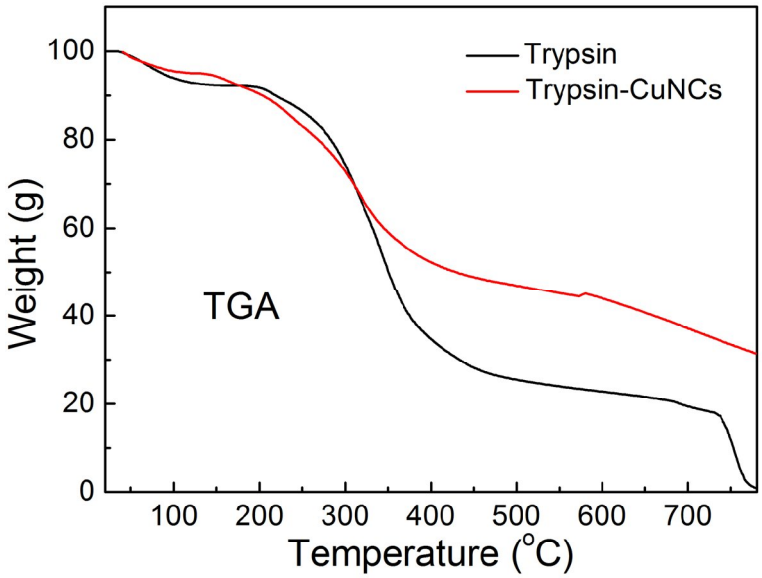

Figure S12. Thermo gravimetric analysis (TGA) of pure trypsin (black) and the as-prepared CuNCs (red).

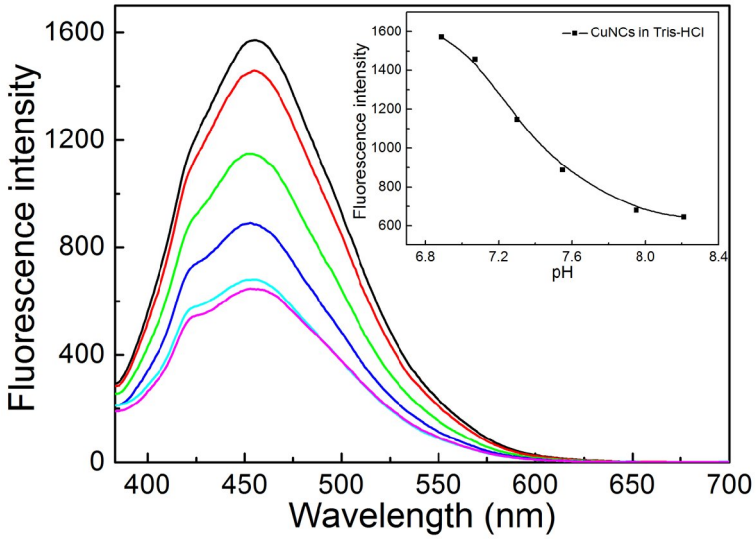

Figure S13. Fluorescence spectrum of CuNCs in Tris-HCl buffers of different pH values (6.89, 7.07, 7.30, 7.55, 7.95, 8.21).The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

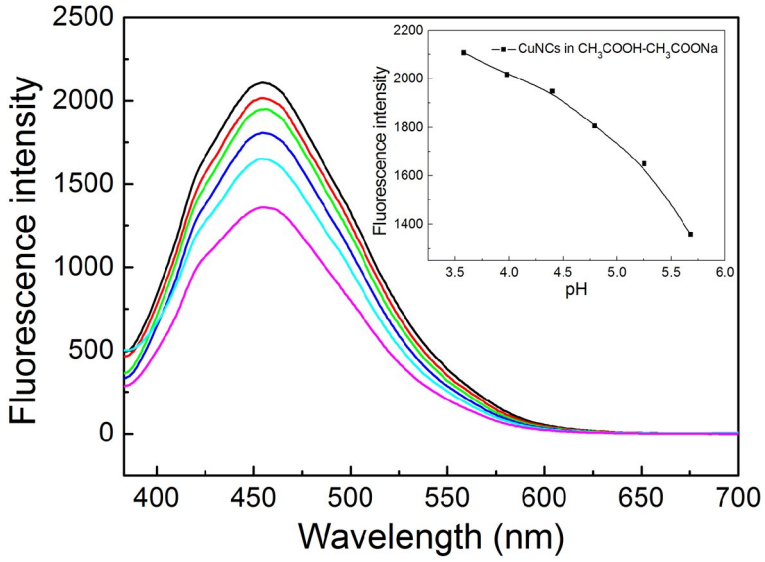

Figure S14. Fluorescence spectrum of CuNCs in CH_3COOH-CH_3COONa buffers of different pH values (3.58, 3.98, 4.40, 4.79, 5.25, 5.68). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

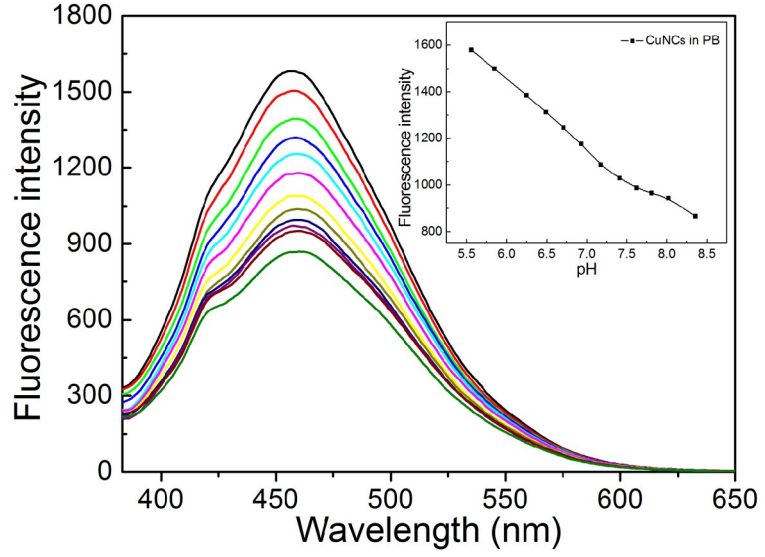

Figure S15. Fluorescence spectrum of CuNCs in PB buffers of different pH values (5.56, 5.85, 6.25, 6.49, 6.71, 6.93, 7.17, 7.41, 7.62, 7.81, 8.02, 8.36). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

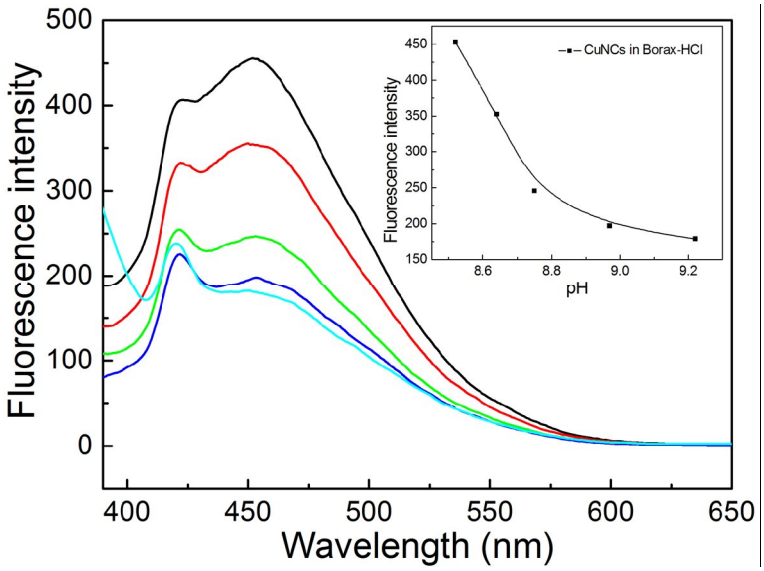

Figure S16. Fluorescence spectrum of CuNCs in Borax-HCl buffers of different pH values (8.52, 8.64, 8.75, 8.97, 9.22). The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

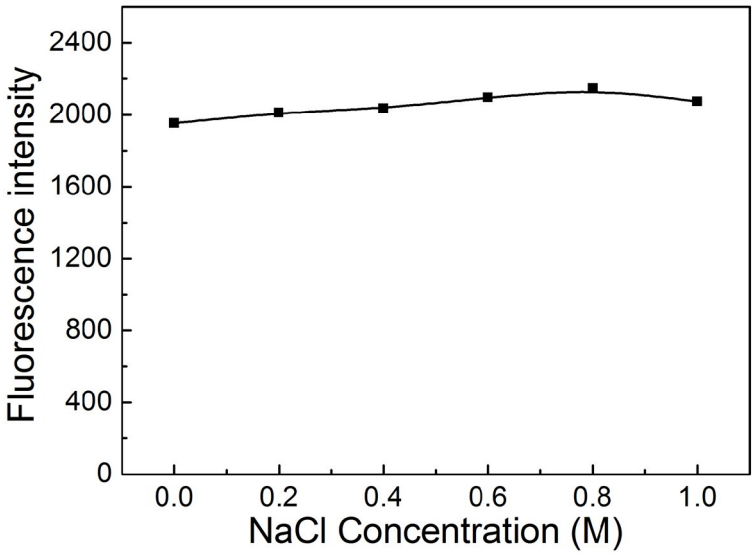

Figure S17. Effect of ionic strength on the fluorescence intensity of CuNCs by exposing the CuNCs to 0.2 -1.0 M NaCl in BR buffer with the pH of 3.20. The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.