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COMMUNICATION

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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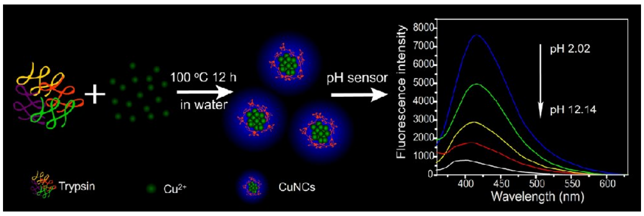
A one-step synthesis of water soluble and pH-responsive trypsin-stabilized fluorescent copper nanoclusters (CuNCs) was reported without using additional protective or reducing agents, and the as-prepared CuNCs exhibited highly stable properties including oxidation resistance, thermal stability and photostability.

In recent years, fluorescent metal nanoclusters with the ultra-small size near to the Fermi wavelength of electrons, have attracted great attention owing to their superior optical, electrical and catalytic properties,[1-3] and their potential applications in optoelectronic devices,[4] biosensors,[5-8] bioimaging[9] and novel catalysts.[10] So far, extensive studies have concentrated on the synthesis of luminescent gold and silver nanoclusters[11-13] using various kinds of templates such as peptides,[14] proteins,[15, 16] DNA,[17] polymers,[18] dendrimers[19] and thiols[20]. As far as we know, copper is highly conductive and extremely cheap, and widely used in daily life. CuNCs, as potential materials, can be used in various industries such as catalysis and sensing. However, only a few researches[21-24] were devoted to CuNCs at present, mainly due to their susceptibility to oxidation and the difficulty in preparing ultra-small CuNCs. For example, Chen group developed a one-pot synthesis of CuNCs. However, an argon stream was necessary in the synthesis process and the obtained CuNCs were insoluble in water.[23] Lopez-Quintela group described a electrochemical synthesis of CuNCs with a relatively high quantum yield of 13 % under a nitrogen atmosphere,[24] but the as-prepared CuNCs were not dissolved in water as well which limited their application. Therefore, to establish a simple method for the synthesis of water soluble CuNCs with excellent property is necessary.

Herein, we report a novel one-step synthesis of trypsin-stabilized fluorescent CuNCs in aqueous solution without using additional protective or reducing agents. Trypsin is a serine protease found in the digestive system of many vertebrates, which was widely used in industrial and biomedical areas.[25] Moreover, trypsin is also a strong candidate for the synthesis of gold and silver nanoclusters, since trypsin includes rich amino acid residues with 7 cysteine (Cys) and 10 tyrosine (Tyr).[16, 26] In this study, the resulting CuNCs of approximate 2 nm in size were highly resistant to oxidation and greatly stable in aqueous solution. Interestingly, trypsin-stabilized CuNCs were employed as an effective and reversible fluorescent pH indicator. Scheme 1 illustrated the synthesis and application of CuNCs.

As a typical synthesis, 8 mL of trypsin solution (100 mg) was mixed with an aqueous solution of $CuCl_2$ (2 mL, 100 mM) by magnetically stirring for 3 min at room temperature. Then, the mixture was allowed to reflux for 12 h at 100 °C, and the color of the solution changed from light blue to brownish yellow gradually, indicating the formation of CuNCs. However, both trypsin and $CuCl_2$ alone were refluxed for 12 h at 100 °C and no fluorescent substances were generated (Figure S1). Besides, the optimization of synthesis conditions including the amount of trypsin and $CuCl_2$, the reaction time and temperature was shown in Figure S2.



Scheme 1 Illustration of the synthesis and application of CuNCs.

Transmission electron microscopy (TEM) showed that the average size of the as-prepared CuNCs was 2.03±0.46 nm (Figure 1A). The high-resolution TEM (HRTEM) image indicated that the crystal lattice fringes were 2.02 Å apart which agreed with the *d* value of the (111) planes of the metallic Cu. Figure 1B and Figure S3 showed the UV-vis absorption spectrum of the as-prepared CuNCs as well as pure trypsin. A strong absorption of the CuNCs was exhibted around 350 nm which was totally different from the 276 nm peak of pure trypsin. Figure 1B showed that the CuNCs in aqueous solution had a blue emission at 455 nm under 363 nm excitation, which further confirmed the formation of CuNCs. Moreover, the absolute quantum yield of CuNCs in aqueous solution was 1.1 %. It is worth mentioning that the as-prepared CuNCs exhibited highly stable property. To be specific, the fluorescence intensity of CuNCs remained unchanged after 9 months (Figure S4) and it showed the property of antiphotobleaching as well (Figure S5). Furthermore, the photostability of CuNCs in human serum was examined by contacting CuNCs with different dilutions of serum. It is gratifying to see that the fluorescence of CuNCs could remain constant even in the two-fold diluted serum (Figure S6). What's more, the fluorescence intensity of CuNCs was hardly changed even if H_2O_2 solutions with different concentrations have been introduced into the CuNCs solution (Figure S7). In order to study the thermal stability of the CuNCs, temperature-dependent luminescence has been monitored (Figure S8). Surprisingly, the fluorescence intensity of CuNCs also remained unchanged in the temperature range from 4 to 100 °C. All the results revealed that they have amazing performances of photostability, antiphotobleaching, antioxidation and thermal stability.

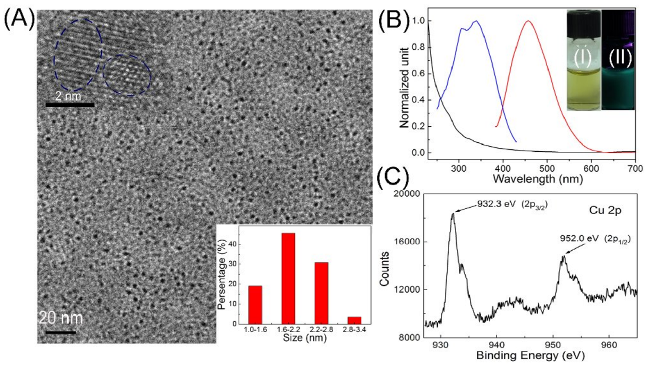


Figure.1 Caption characterization of trypsin-stabilized CuNCs. (A) TEM image of the CuNCs. Upper left inset: HRTEM image of CuNCs (scale bar: 2 nm), lower right inset: the size distributions of CuNCs based on statistics of TEM image. (B) Uv-vis absorption (black line) and fluorescence excitation (blue line) and emission spectrum (red line) of the CuNCs. Inset: the photographs of CuNCs under visible light (I) and 365 nm UV light irradiation (II), respectively. (C) XPS spectrum in the Cu 2p region of CuNCs.

The oxidation state of Cu was also investigated through X-ray photoelectron spectroscopy (XPS). Figure 1C depicted two intense peaks at 932.3 and 952.0 eV, which were assigned to the binding energies of the $2p_{3/2}$ and $2p_{1/2}$ electrons of Cu(0). Meanwhlie, a shake-up can be observed at 942 eV, indicating very minimal Cu(II) was presented in this system which was derived from the unreacted $CuCl_2$. Furthermore, it's of great importance to mention that the $2p_{3/2}$ binding energy of Cu(0) is only about 0.1 eV away from that of Cu(I). Therefore, the valence state of the obtained CuNCs most likely lied between 0 and +1. Previous study also reported that atoms can have different oxidation states in clusters depending on their position inside the clusters.[27] The XPS spectrum of other elements containing O, N, C and Cl were showed in the Figure S9. Additionally, the conformational behavior of trypsin before and after the reaction were observed by circular dichroism (CD) spectroscopy (Figure S10). After the formation of CuNCs, the peak at 209 nm was shifted to 206 nm, revealling that α-helix were transformed into β-sheets. The decay profile of the as-prepared CuNCs was monitored at an excitation wavelength of 370 nm and the fluorescence intensity was collected at 455 nm. The numerical fitting of the luminescence reveals time constants of 0.79 ns (28.26 %), 3.5 ns (56.17 %), and 15.8 ns (15.58 %) which were attributed to emission from singlet excited states (Figure S11).

The thermo gravimetric analysis (TGA) was used to further demonstrate the successful synthesis of trypsin-stabilized CuNCs. The mass loss of water was below 150 °C and the protein mass loss was above 200 °C. Moreover, the slope of CuNCs in the range of 300 to 800 °C was gentler than that of pure trypsin. At 780 °C, the mass of the protein became zero, but there was still certain quality of CuNCs which also provided supporting evidence for the formation of the CuNCs (Figure S12).[28]

These trypsin-stabilized CuNCs described in this paper were intrinsically pH sensitive and could be employed as an effective fluorescent pH indicator. We observed the fluorescence intensity of CuNCs under a series of buffer solutions with different pH values. The fluorescence emission spectra of CuNCs in Britton-Robinson (BR) buffers of different pH values were showed in Figure 2A. Moreover, the fluorescence intensity of CuNCs exhibited a linear fashion over the pH range of 2.02-12.14 (Figure 2B) which was extremely superior to the reported nanoscaled pH sensors.[29-32] To be specific, the fluorescence intensity of CuNCs reached the maximum value at a low pH value (2.02) and it decreased approximately 7-fold at the pH of 12.14. Interestingly, in alkaline medium, blue precipitation was formed in CuNCs solutions and the color of the solution became colorless and turbid. However, the precipitation can be dissolved in acid medium and the color of the solution became brownish yellow and transparent again. Accordingly, the reason for the fluorescence quenching of CuNCs may probably be the formation of reversible aggregates at high pH values.

As expected, the pH sensor was also sensitive to other commonly used buffer solutions, including tris(hydroxymethyl) aminomethane (Tris)-HCl buffers (Figure S13), CH_3COOH-CH_3COONa buffers (Figure S14), phosphate (PB) buffers (Figure S15), borax-HCl buffers (Figure S16). Good corresponding linear relationships were obtained between the pH values measured by pH meter and the fluorescence intensity of CuNCs (Figure 2C), revealing that the fluorescence intensity changes were primarily due to the variation of pH values rather than the reaction between CuNCs and ions contained in the buffer solutions. Importantly, CuNCs would react with some anions contained in the buffer solutions, resulting in deviations in the pH determination. In spite of this, the pH-responsive action would not be affected by the ionic strength. The fluorescence intensity of CuNCs remained almost constant when exposed to 0.2-1.0 M NaCl solutions (Figure S17), indicating the ionic strength had no significant effect on the pH sensor.

To further investigate the reversibility of CuNCs, the pH value was changed from 11.41 to 2.40 and again to 11.41 by using acid and base as modulators, and the fluorescence intensity was measured at each pH value. As shown in Figure 2D, the cycles can be repeated 7 times without fatigue. A little decrease of fluorescence intensity was observed for the reason that the volume of solution became larger after each cycle. These results indicated the excellent reversibility of the as-prepared CuNCs. All of these afforded significant evidences and advantages to the CuNCs for the applications in various environments.

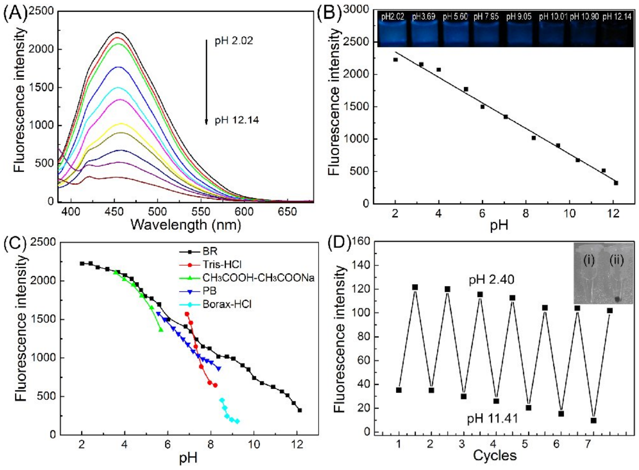


Figure.2 (A) Fluorescence emission spectra of CuNCs in BR buffers of different pH values: 2.02, 3.20, 4.01, 5.25, 6.02, 7.08, 8.36, 9.48, 10.38, 11.57, 12.14. (B) The calibration curve of the pH values in the range of 2.02-12.14 versus the fluorescence intensity of the CuNCs in BR buffers. The linear equation is F = -197.0 pH+2741.1 and the corresponding correlation coefficient is 0.994. Upper inset: the photographs of CuNCs in BR buffers of different pH values under the UV light irradiation. (C) The pH responses of CuNCs in various buffer solutions. (D) Fluorescence intensity of the CuNCs reversibly went upward and downward by alternating the pH value. One cycle meant that the fluorescent intensity was measured as the value changed from pH 11.41 to 2.40 and then from pH 2.40 to 11.41. Inset: photographs of CuNCs in acidic (i, pH 2.40) and alkaline medium (ii, pH 11.41), respectively. The excitation wavelength was 363 nm and the fluorescence intensity was monitored at 455 nm.

In conclusion, we successfully established a one-step approach for synthesizing water-soluble and antioxidant fluorescent CuNCs with unexceptionably stable properties by using a common protein, trypsin. Moreover, the obtained CuNCs exhibited rapid response, particularly wide range of pH response and superior reversibility as a pH sensor. Therefore, the trypsin-stabilized fluorescent CuNCs would be a promising candidate for various applications in biological, industrial, medical fields and so on.

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Notes and references

[a] *Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, P. R. China. Fax: (+86) 23 6886 6796; Tel: (+86) 23 6825 4659; E-mail: chengzhi@swu.edu.cn.*

[b] *College of Chemistry and Chemical Engineering Southwest University, Chongqing 400715, P. R. China.*

† Electronic Supplementary Information (ESI) available: experimental details, characterizations of CuNCs. See DOI: 10.1039/b000000x/

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Graphic abstract

for

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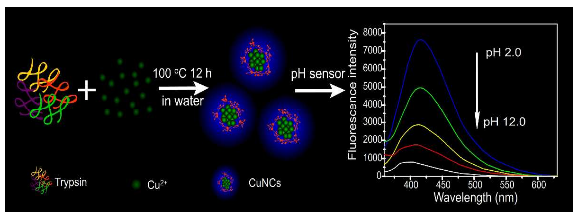

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