Silver Nanoclusters Encapsulated into Metal−Organic Frameworks with Enhanced Fluorescence and Specific Ion Accumulation toward the Microdot Array-Based Fluorimetric Analysis of Copper in Blood

Chuan Fan, Xiaoxia Lv, Fengjuan Liu, Luping Feng, Min Liu, Yuanyuan Cai, Huan Liu, Jingyi Wang, Yanli Yang, and Hua Wang*  
Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, P. R. China

ABSTRACT: Silver nanoclusters (AgNCs) were first coated with bovine serum albumin (BSA) and then encapsulated into porous metal−organic frameworks of ZIF-8 by the protein-mediated biomineralization process. Unexpectedly, the fluorescence intensities of the yielded AgNCs-BSA@ZIF-8 nanocomposites were discovered to be continuously enhanced during each of the BSA coating and ZIF-8 encapsulation steps. Compared to common AgNCs, greatly improved photostability and storage stability of AgNCs could also be expected. More importantly, having benefited from the ZIF-8 shells, the prepared nanocomposites could possess the specific accumulation and sensitive response to Cu^{2+} ions, resulting in the rational quenching of their fluorescence intensities. Moreover, AgNCs-BSA@ZIF-8 nanocomposites were coated onto the hydrophobic arraying slides toward a microdots array-based fluorimetric method for the fast and sensitive evaluation of Cu^{2+} ions. It was discovered that the developed fluorimetric strategy could ensure the high-throughput analysis of Cu^{2+} ions in wide pH range, and especially some harsh and high-salt media. It can allow for the detection of Cu^{2+} ions in blood with the concentrations ranging from 4.0×10^{-4} to 160 μM, thus serving as a new copper detection candidate to be widely applied in clinical test, food safety, and environmental monitoring fields.

KEYWORDS: silver nanoclusters, metal−organic frameworks, biological mineralization, fluorimetric analysis, copper

Although copper (Cu^{2+}) as a microelement is useful for human health, excessive Cu^{2+} ions in the body may exert long-term adverse effects on liver, kidney, and neurological systems. At the present time, many classic detection methods have been applied to probe Cu^{2+} ions; most are known as the fluorimetric detection methods. It is established that the performance of fluorimetric methods can depend on the sensing properties of fluorescent probes like fluorescence (FL) intensities, environmental stability, and target-recognition selectivity. In recent years, noble metal nanoclusters like gold and silver nanoclusters (AgNCs) have been increasingly applied as the probes for the fluorimetric sensing of various environmental and medical targets including Cu^{2+} ions. In particular, AgNCs or their alloys with the intriguing structures, low cost, and unique luminescence properties have been preferentially used for probing some molecules and heavy metal ions. For example, Xiong et al. reported the AgNCs-based colorimetric detection for ascorbic acid. Xie’s group employed AgNCs for the sensitive analysis of cysteine based on the FL quenching of AgNCs. Also, AgNCs were utilized as the fluorescent probes in our group for the sensitive detection of Cu^{2+} ions. Nevertheless, silver nanomaterials, especially size-small AgNCs, are commonly noted for susceptible oxidation, low environmental stability, and poor quantum yields as the fluorescent probes, which may limit their applications in the biomedical, environmental, and catalysis fields. It is well established that the environmental stability, optical performance, and photophysical properties of noble metal clusters like AgNCs can largely depend on their cores, surface ligands, and dispersion media. As a result, many efforts have been devoted to the improvement of the aqueous stability and luminescence performances of these nanomaterials such as the modifications or surface passivation with some ligands containing S, P, and N elements but receiving still limited research advances.
Scheme 1. Schematic Illustration of the Main Mechanism and Procedure of the Fluorimetric Assay for Cu²⁺ Ions through the Step-by-Step Enhancement of the Fluorescence of AgNCs First by BSA Coating and Then ZIF-8 Encapsulation, of Which the Changing Morphological Structures and Fluorescent Intensities of Corresponding Products Were Manifested (Top Panel)⁴⁴

Moreover, metal–organic frameworks (MOFs), a prominent class of porous crystalline materials, have recently concentrated numerous interests for the separation, storage, catalysis, and biological or chemical sensing.⁴⁻²⁰ As the most interesting representative, zeolitic imidazolate frameworks (ZIFs) like ZIF-8 can feature some outstanding advantages such as easy preparation, large surface area, ultrahigh porosity, and aqueous dispersability.,²⁷,²⁸ thus serving as the carriers or delivery vehicles separately for drugs, fluorescent probes, and enzymes in the biomedical fields.²⁹⁻³¹ For example, carbon dots were embedded into ZIF-8 to act as the fluorescent probes for sensing Cu²⁺ ions.³² Enzymes were encapsulated into porous ZIF-8 to obtain unprecedented protection against harsh environmental damages.³³ Especially, metal nanomaterials shelled with MOFs can expect improved thermodynamic stability with minimized agglomeration.³³ More importantly, the prepared nanocomposites could possess specific accumulation and sensitive response to Cu²⁺ ions with rational quenching fluorescence. Subsequently, a microdot array-based fluorimetric method was developed using AgNCs-BSA@ZIF-8 nanocomposites for the evaluation of Cu²⁺ ions in some high-salt media like blood.

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**Apparatus.** The fluorescence (FL) measurements were conducted using FL spectrophotometer (Horiba, Fluoromax-4, Japan) operated at an excitation wavelength at 425 nm, with both excitation and emission slit widths of 5.0 nm. UV-3600 spectrophotometer (Shimazu, Japan) was used to measure the UV–vis spectra of different materials such as AgNCs, BSA-coated AgNCs, and AgNCs-BSA@ZIF-8 with and without copper (Cu²⁺) ions.

**Fluorimetric Cu²⁺ Measurements with AgNCs-BSA@ZIF-8 Nanocomposites.** The selective detections of Cu²⁺ ions were conducted by the following procedure. AgNCs-BSA@ZIF-8 nanocomposites (containing 0.035 mM AgNCs) were dispersed in dihydroxylic acid (DHLA) at room temperature.¹¹ The synthesis of AgNCs-BSA@ZIF-8 nanocomposites was conducted as follows. First, under vigorous stirring, an aliquot of BSA (240 μL, 20 mg mL⁻¹) was added separately into 200 μL AgNCs of different concentrations. Then, ZIF-8 precursor solution of 2-methylimidazole (400 μL, 160 mM) and zinc acetate (400 μL, 40 mM) were introduced and mixed for 2 h. After the aging treatment was performed overnight, the yielded products of AgNCs-BSA@ZIF-8 nanocomposites were centrifuged (5000 rpm, 15 min) and washed several times with deionized water, and then diluted to 2.0 mL to be stored at 4 °C for future usage. Optical fluorescent microscopy was employed to characterize the AgNCs-BSA@ZIF-8 nanocomposites in the presence and absence of Cu²⁺ ions. An aliquot of 5.0 μL AgNCs-BSA@ZIF-8 suspensions with and without Cu²⁺ ions (5.0 μM) was separately dropped on a glass slide. After being dried at room temperature, the fluorescent images were taken separately by using optical filters of UV light (λ = 340–380 nm) and blue light (λ = 450–490 nm) for the excitation of photoluminescence.

The obtained AgNCs-BSA@ZIF-8 nanocomposites were further coated onto the glass slides with hydrophobic pattern to yield the high-throughput microdots array for the fluorimetric analysis of copper ions.

“AgNCs-BSA@ZIF-8 nanocomposites were synthesized in water according to our previous work using dihydroxylic acid (DHLA) at room temperature.¹¹ The synthesis of AgNCs-BSA@ZIF-8 nanocomposites was conducted as follows. First, under vigorous stirring, an aliquot of BSA (240 μL, 20 mg mL⁻¹) was added separately into 200 μL AgNCs of different concentrations. Then, ZIF-8 precursor solution of 2-methylimidazole (400 μL, 160 mM) and zinc acetate (400 μL, 40 mM) were introduced and mixed for 2 h. After the aging treatment was performed overnight, the yielded products of AgNCs-BSA@ZIF-8 nanocomposites were centrifuged (5000 rpm, 15 min) and washed several times with deionized water, and then diluted to 2.0 mL to be stored at 4 °C for future usage. Optical fluorescent microscopy was employed to characterize the AgNCs-BSA@ZIF-8 nanocomposites in the presence and absence of Cu²⁺ ions. An aliquot of 5.0 μL AgNCs-BSA@ZIF-8 suspensions with and without Cu²⁺ ions (5.0 μM) was separately dropped on a glass slide. After being dried at room temperature, the fluorescent images were taken separately by using optical filters of UV light (λ = 340–380 nm) and blue light (λ = 450–490 nm) for the excitation of photoluminescence.”
In addition, the optimization of the Cu\(^{2+}\) detection conditions were optimized for the developed fluorimetric assays, including the dosages of AgNCs probes (0.010, 0.015, 0.020, 0.035, 0.040, 0.080, and 0.120 mM), pH values (1.0, 3.0, 5.0, 7.0, 9.0, 11.0, and 13.0), ionic strengths (0, 100, 250, 500, 750, and 1000 mM NaCl), and response time (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 min).

Fluorimetric Analysis of Cu\(^{2+}\) Samples with the AgNCs-BSA@ZIF-8 Microdots Array. The AgNCs-BSA@ZIF-8 microdots arrays were fabricated according the experimental procedure reportedly previously.\(^{13}\) Typically, glass slides (72 × 24 mm\(^2\)) were cleaned by fresh piranha solution of H\(_2\)SO\(_4\):H\(_2\)O\(_2\) = 7:3 \((\text{Caution: piranha solution as a strong oxidant must be handled with extreme care})\) to activate the substrate surface, and then thoroughly washed with deionized water to be further dried in nitrogen. After that, those cleaned glass slides were dipped into the HDS solutions of 5.0% in ethanol to be reacted for 6 h at room temperature. Then, the resulting glass slides with the hydrophobic patterns were washed twice in ethanol and further dried to be kept in the sealing drier for future usage. Furthermore, AgNCs-BSA@ZIF-8 nanocomposites (containing 0.035 mM AgNCs) were dispersed and then mixed with an aliquot of Nafton (5.0%) for 1 min. Following that, an aliquot of 1.0 µL AgNCs-BSA@ZIF-8 mixture was spotted onto the surface of hydrophobic-patterned glass slides to be air-dried in the dark overnight. The as-prepared AgNCs-BSA@ZIF-8 microdot arrays were kept in the dark at 4 °C for future usage.

The samples of different concentrations of Cu\(^{2+}\) ions spiked in blood were separately dropped onto the testing area of nanocomposite-spotted microdots of the arrays. Then, the microdot array was inserted into the testing hold, where the solid-phase reflection fluorescence intensities for each of the testing microdots were recorded separately.

**RESULTS AND DISCUSSION**

Main Procedure for Synthesis and Characterization of AgNCs-BSA@ZIF-8. AgNCs were encapsulated into a ZIF-8 matrix through the protein-mediated biological mineralization process (Scheme 1). Herein, AgNCs were first coated with bovine serum albumin (BSA) and then encapsulated into ZIF-8 to yield the AgNCs-BSA@ZIF-8 nanocomposites with changing morphologies, as witnessed from the corresponding images. Importantly, the FL intensities of AgNCs so encapsulated could be continuously enhanced during the BSA coating and ZIF-8 MOFs shelling steps, together with the

![Figure 1. FL intensities of AgNCs-BSA@ZIF-8 depending on (A) BSA dosages and (B) the Ag-to-Zn ratios used.](image1)

![Figure 2. TEM images of (A) AgNCs, (B) BSA-coated AgNCs, and AgNCs-BSA@ZIF-8 in the (C) absence and (D) presence of copper ions (inset: hydrodynamic diameters).](image2)
greatly improved environmental stability. The BSA dosages and Ag−Zn ratios used in the synthesis reactions were optimized to be 3.8 mg mL\(^{-1}\) and 1/2, respectively (Figure 1). Moreover, the porous ZIF-8 shells of the nanocomposites could allow for the adsorption-based accumulation of Cu\(^{2+}\) ions, which would then act as the specific energy quenchers adaptable to the fluorescent probes of AgNCs encapsulated in the MOFs. The obtained nanocomposites were further spotted onto the hydrophobic-pattern glass slides resulting in a microdots array for the high-throughput and sensitive fluorescent analysis of Cu\(^{2+}\) ions in blood afterward. To the best of our knowledge, this is the first attempt to encapsulate AgNCs into ZIF-8 by the protein-mediated biomineralization process with enhanced fluorescence, storage stability, and specific Cu\(^{2+}\) accumulation. The changing topological structures of AgNCs with the BSA coating and ZIF-8 encapsulation were monitored using transmission electron microscopy (TEM) (Figure 2). It was found that both AgNCs (Figure 2A) and BSA-coated AgNCs (Figure 2B) could present uniform monodispersion in water. They could display basically the same average hydrodynamic diameters of about 4.4 nm by DLS (Figure 2, insert). Furthermore, once BSA-coated AgNCs were encapsulated into the ZIF-8 matrix, the yielded AgNCs-BSA@ZIF-8 exhibited the defined structure of the ZIF-8 profile (Figure 2C). However, after Cu\(^{2+}\) ions were introduced, most of the AgNCs-BSA@ZIF-8 structure would collapse toward the aggregation or precipitation (Figure 2D). Such a phenomenon was also confirmed using fluorescent inverted microscopy (Figure 3), where the red FL properties (dark field) and topological structures (bright field) of AgNCs-BSA@ZIF-8 would be apparently changed in the absence and presence of Cu\(^{2+}\) ions. Besides, energy dispersive spectroscopy (EDS) was employed to explore the morphological structure and chemical composition of AgNCs-BSA@ZIF-8 (Figure 4A) in comparison with AgNCs and BSA-coated AgNCs (Figure 4B), showing the changing chemical composition at each of the formation steps. The well-defined particle shape could also be witnessed for the nanocomposites by SEM imaging (Figure 4A, inset). Especially, the C, N, O, Zn, and Ag elements could be uniformly dispersed throughout the nanocomposites by a discretely mixed way (Figure 4C), thus confirming the elemental profile of AgNCs-BSA@ZIF-8 nanocomposites.

A comparison of UV−vis absorption spectra was carried out among AgNCs, BSA-coated AgNCs, and AgNCs-BSA@ZIF-8 (Figure 5). One can note that BSA-coated AgNCs (curve b) could display the similar absorption peaks of AgNCs (curve a) characteristically at about 330, 430, and 490 nm, except for 280 nm of BSA protein. The results indicate that the BSA coating might not change the morphological properties of AgNCs such as the particle sizes and structures. Surprisingly, AgNCs-BSA@ZIF-8 might not display any obvious UV−vis absorption peaks in the absence (curve c) and presence of Cu\(^{2+}\) ions (curve d), presumably due to the fact they were embedded deeply into the dense ZIF-8 matrix. In addition, no significant color change was observed for all of the testing solutions (Figure 5, inset). Yet, the AgNCs-BSA@ZIF-8 with and without Cu\(^{2+}\) ions could feature obvious suspension properties. Based on the evidence, the fluorescence quenching of AgNCs-BSA@ZIF-8 was thought to result from the Cu\(^{2+}\)-triggered aggregation of nanocomposites as validated by the TEM images above.

**Main Principle of MOF-Induced Enhancement and Cu\(^{2+}\)-Triggered Quenching of Nanocomposite Fluorescence.** The changing FL intensities of AgNCs-BSA@ZIF-8 were recorded during the step-by-step BSA coating and ZIF-8 encapsulation (Figure 6A), with the corresponding photographs of the products (inset). Accordingly, the FL intensities of the resulted AgNCs-BSA@ZIF-8 (curve c) were about 6- and 10-fold larger than those of BSA-coated AgNCs (curve b)
and AgNCs (curve a), respectively. Herein, the red FL intensities of AgNCs enhanced by the protein coating presumably resulted from the AgNC–protein interaction that might induce the energy transfer from the tryptophan residues (i.e., Trp 214) of BSA to AgNCs, as also confirmed elsewhere. A further, ZIF-8 with the N-containing organic ligands like imidazole might conduct the well-known “electron donor effects” so as to increase the FL intensities of AgNCs, as observed previously for the amine-containing ligands. A particularly, the ZIF-8 matrix of the nanocomposites might spatially separate AgNCs in certain orientations through the modulation of AgNCs within the rigid framework of MOFs, thus endowing them with further improved luminescence and aqueous stability of AgNCs. Moreover, comparable studies were conducted on the fluorescent responses to Cu²⁺ ions among AgNCs, BSA-coated AgNCs, and AgNCs-BSA@ZIF-8 (Figure 6B–D). It was observed that the Cu²⁺-induced FL responses of AgNCs-BSA@ZIF-8 (Figure 6B) were over 3- and 7-fold larger than those of BSA-coated AgNCs (Figure 6C) and AgNCs (Figure 6D), respectively. That is, the prepared nanocomposites could possess the largest FL responses to Cu²⁺ ions, as visually disclosed in the corresponding photographs (Figure 6, inset). Herein, the imidazole groups on ZIF-8 might functionalize as the specific recognition elements to selectively capture Cu²⁺ ions from the sample media. Meanwhile, the porous ZIF-8 shells of AgNCs-BSA@ZIF-8 might help to strongly adsorb and accumulate Cu²⁺ ions as already mentioned. In addition, BSA coatings of AgNCs-BSA@ZIF-8, with the abundant amino acid residues (i.e., lysine, cysteine, and glycine), might also interact with Cu²⁺ ions, as disclosed elsewhere for the glutathione-coated AgNCs or BSA-conjugated ZnO. Hence, once Cu²⁺ ions

Figure 4. EDS spectra of (A) AgNCs-BSA@ZIF-8 (inset, the SEM image) and (B) AgNCs (a) and BSA-coated AgNCs (b); (C) element mapping images of AgNCs-BSA@ZIF-8 composed of C, N, O, Zn, and Ag elements and their superimposed image.
were introduced, the FL intensities of AgNCs-BSA@ZIF-8 would be acutely quenched to facilitate the sensitive and selective fluorimetric analysis of Cu$^{2+}$ ions.

### Photostability and Storage Stability of AgNCs-BSA@ZIF-8

The environmental stability of AgNCs-BSA@ZIF-8 was investigated under the harsh testing conditions of either strong light exposure or stored in the dark, using AgNCs and BSA-coated AgNCs for the comparison (Figure 7). As shown in Figure 7A, AgNCs-BSA@ZIF-8 could maintain the FL intensities after the 1 min strong exposure of xenon lamp, in contrast to the others showing greatly decreased FL intensities. Furthermore, the FL intensities of AgNCs-BSA@ZIF-8 could survive even stored up to six months, whereas those of AgNCs and BSA-coated AgNCs might be mostly lost (Figure 7B). The results validate that the ZIF-8 shells could endow AgNCs with the greatly improved photostability and storage stability in addition to the enhanced FL emissions already mentioned.

### Optimization of Fluorescence Analysis Conditions of the AgNCs-BSA@ZIF-8

It is widely recognized that silver nanomaterials are very sensitive to sulfides and thiol-containing or reductive molecules because of the strong Ag$\sim$S binding or the oxidization property of Ag$^{+}$ ions. A comparable investigation was thus made for AgNCs-BSA@ZIF-8 and AgNCs that were mixed separately with cysteine, S$^{2-}$ ions, and ascorbic acid (Figure 8). One can find from Figure 8B that no significant change in the FL intensities was observed for AgNCs-BSA@ZIF-8 in the presence of any of these tested substances especially ascorbic acid, in contrast to AgNCs for which FL intensities could largely decrease (Figure 8A). Therefore, benefiting from the ZIF-8 shells, the AgNCs-BSA@ZIF-8 might achieve the enhanced specific responses to Cu$^{2+}$ ions with minimized interference from the formidable sulfides and thiol-containing or reductive substances. Moreover, Figure 9 illustrates that the fluorimetric responses of AgNCs-BSA@ZIF-8 to Cu$^{2+}$ ions by comparing some possibly coexisting common ions and molecules. As expected, only Cu$^{2+}$ ions could trigger the immediate quenching of the FL emissions of AgNCs-BSA@ZIF-8, as witnessed in corresponding photographs (Figure 9, inset). Also, the fluorescent responses to Cu$^{2+}$ ions separately coexisting from other foreign ions were investigated showing no significant interference with the Cu$^{2+}$ detection. The data indicate that the AgNCs-BSA@ZIF-8 could serve as robust fluorescent probe for the selective detection of Cu$^{2+}$ ions. Besides, the main conditions for the fluorimetric Cu$^{2+}$ analysis were explored (Figure 10), showing the optimal conditions of 0.035 mM AgNCs-BSA@ZIF-8 probes (Figure 10A) and pH 5.0–9.0 (Figure 10B). More interestingly, the fluorimetric method developed could enable the detection of Cu$^{2+}$ ions in the buffer containing NaCl.

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**Figure 5.** UV–vis spectra of (a) AgNCs, (b) BSA-coated AgNCs, (c) AgNCs-BSA@ZIF-8, (d) AgNCs-BSA@ZIF-8 with Cu$^{2+}$, each of which contains AgNCs (8.0 μM) (inset: the photographs of the testing solutions).

**Figure 6.** (A) Comparison of fluorescence intensities among (a) AgNCs, (b) BSA-coated AgNCs, and (c) AgNCs-BSA@ZIF-8, each of which contains AgNCs (8.0 μM), corresponding to (B, C, and D) the changes of their fluorescence intensities in the (a) absence and (b) presence of Cu$^{2+}$ ions (10 μM).
concentrations up to 1.0 M (Figure 10C), indicating that the AgNCs-BSA@ZIF-8 probes could sense Cu²⁺ ions in some harsh media of high-salt samples like wastewater and blood. Besides, Figure 10D displays the comparison of Cu²⁺-response time between AgNCs-BSA@ZIF-8 and AgNCs. Unexpectedly, AgNCs-BSA@ZIF-8 could present much faster responses to Cu²⁺ ions (about 30 s) than AgNCs (about 60 s). Again, the porous shells of ZIF-8 MOFs might facilitate the large absorption and accumulation of Cu²⁺ ions from the samples, so that the Cu²⁺-AgNC interaction might be accelerated for the faster and enhanced responses to Cu²⁺ ions.

Fluorescence Analysis of Cu²⁺ Ions in Samples. The developed fluorimetric method with AgNCs-BSA@ZIF-8 probes was applied for the detection of Cu²⁺ ions with different concentrations in buffer (Figure 11). Figure 11A shows the relationship between the logarithms of Cu²⁺ concentrations and the quenching efficiencies of AgNCs-BSA@ZIF-8, with the Cu²⁺ concentrations linearly ranging from 2.0 × 10⁻⁴ to 80.0 μM, with the limit of detection of 0.05 nM, estimated by the 3σ rule. Subsequently, the AgNCs-BSA@ZIF-8 nanocomposites were spotted onto the hydrophobically patterned glass slides. The resulted fluorimetric microdot array was employed to probe Cu²⁺ ions with different levels spiked in blood (Figure 11B). Accordingly, Cu²⁺ ions could be multiply quantified over the concentrations from 4.0 × 10⁻⁴ to 160 μM, with the limit of detection (LOD) of about 0.10 nM. Moreover, the analyzed performances of the developed fluorimetric methods were compared with those of other detection methods previously reported for Cu²⁺ ions, with the data shown in Table 1. It was noted that the developed AgNCs-BSA@ZIF-8-based fluorimetric methods could facilitate better or comparable capacities for the analysis of Cu²⁺ ions in terms of linear concentration range and LOD. Therefore, the feasibility of the practical application of the developed fluorimetric array could be expected for the high-throughput analysis of Cu²⁺ ions in blood. In addition, the bright red FL of AgNCs-BSA@ZIF-8 might additionally circumvent any interference of other co-existing fluorescent substances in some complex samples like blood.
In summary, AgNCs were successfully encapsulated into MOFs matrix of ZIF-8 by the BSA-mediated biomineralization process, yielding the AgNCs-BSA@ZIF-8 nanocomposites for the microdots array-based fluorimetric analysis of Cu^{2+} ions in blood. Compared to the current fluorescent probes especially those with silver nanomaterials, the developed fluorimetric method with the multifunctional AgNCs-BSA@ZIF-8 could feature several outstanding advantages in sensing Cu^{2+} ions. First, the FL intensity of AgNCs probes could be step-by-step enhanced by the BSA coating and ZIF-8 encapsulation (about 10-fold larger than that of AgNCs), serving as the fluorescence probes for the sensitive fluorimetric analysis. Second, the introduction of ZIF-8 shells could endow the AgNCs-BSA@ZIF-8 with the increased specific responses to Cu^{2+} ions that

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**CONCLUSIONS**

In summary, AgNCs were successfully encapsulated into MOFs matrix of ZIF-8 by the BSA-mediated biomineralization process, yielding the AgNCs-BSA@ZIF-8 nanocomposites for the microdots array-based fluorimetric analysis of Cu^{2+} ions in blood. Compared to the current fluorescent probes especially those with silver nanomaterials, the developed fluorimetric method with the multifunctional AgNCs-BSA@ZIF-8 could feature several outstanding advantages in sensing Cu^{2+} ions. First, the FL intensity of AgNCs probes could be step-by-step enhanced by the BSA coating and ZIF-8 encapsulation (about 10-fold larger than that of AgNCs), serving as the fluorescence probes for the sensitive fluorimetric analysis. Second, the introduction of ZIF-8 shells could endow the AgNCs-BSA@ZIF-8 with the increased specific responses to Cu^{2+} ions that

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**Figure 10.** Fluorimetric responses of AgNCs-BSA@ZIF-8 to copper ions depending on (A) AgNCs amounts (inset: the corresponding FL intensities in the (a) absence and (b) presence of copper ions), and (B) pH values and (C) ionic strength values in NaCl concentrations; (D) the comparison of reaction time between (a) AgNCs-BSA@ZIF-8 and (b) AgNCs (0.035 mM) separately with Cu^{2+} ions (10 μM).

**Figure 11.** (A) Calibration detection curve of quenching efficiencies versus different concentrations of Cu^{2+} ions spiked in water (2.0 × 10^{-4} to 80.0 μM) (inset: photographs of the testing solutions under UV light). (B) Microdot array-based fluorimetric analysis for Cu^{2+} ions spiked in blood (4.0 × 10^{-4} to 160 μM) (inset: photographs of the testing products under UV light).

**Table 1.** Comparison of Detection Performances among Different Methods for Probing Cu^{2+} Ions in Terms of Linear Ranges and Limit of Detections (LODs)

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would rationally quenched the FL intensities by triggering the aggregation or precipitation of nanocomposites. Particularly, the selective discrimination of Cu\(^{2+}\) ions could be expected with the minimized inference from other possibly coexisting substances including the formidable sulfides and thiol-containing or reductive molecules (i.e., cysteine, S\(^{2-}\) ions, and ascorbic acid). Third, compared to the common notorious AgNCs, high photostability and storage stability could be obtained for the AgNCs-BSA@ZIF-8 to ensure the improved analysis reproducibility for Cu\(^{2+}\) ions. Fourth, the porous ZIF-8 shells could help to largely absorb and accumulate Cu\(^{2+}\) ions from the samples so as to accelerate the Cu\(^{2+}\)-AgNC interaction to guarantee the faster and amplified responses to Cu\(^{2+}\) ions. Fifth, benefiting from the ZIF-8 shells, the AgNCs-BSA@ZIF-8 could allow for fluorometric analysis of Cu\(^{2+}\) ions in a wide pH range and especially some harsh and high-salt samples (i.e., wastewater and blood). Subsequently, the developed microdot array-based fluorometric method with AgNCs-BSA@ZIF-8 probes could facilitate the detection of Cu\(^{2+}\) ions in blood with the level down to 0.10 nM. Importantly, a protein-mediated MOF encapsulation route such as this may pave the way to the fabrications of various noble metal (i.e., Ag, Au, Cu) clusters with improved optical performance, environmental stability, and photophysical properties toward the extensive applications in the fields of fluorometric analysis, biological imaging, metal catalysis, and optoelectronic designs.

### AUTHOR INFORMATION

**Corresponding Author**

*E-mail: huawangqfnu@126.com. Tel: +86 537 4456306. Fax: +86 537 4456306.*

**ORCID**

Hua Wang: 0000-0003-0728-8986

**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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### REFERENCES


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