Protein-Localized Bright-Red Fluorescent Gold Nanoclusters as Cyanide-Selective Colorimetric and Fluorometric Nanoprobes

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ABSTRACT: Herein, we describe a bright-red-emitting ovalbumin-protected gold nanoclusters (OVA–AuNCs) that were prepared and applied as a luminescent probe for a simple, rapid, and highly sensitive determination of cyanide ions (CN⁻ ions) based on an emission quenching and colorimetric method. Initially, an intense red-emissive fluorescence of the OVA–AuNCs successfully disappeared upon the addition of CN⁻ ions. The resultant emission-quenching process involved CN⁻ ions etching the OVA–AuNC surface, which produced AuCN₂⁺ complexes in the presence of ambient oxygen. Under optimized experimental conditions, the relative emission intensity is inversely relative to CN⁻ ion concentrations ranging from 5.00 × 10⁻⁹ to 75.00 × 10⁻⁷ mol/L with a linear correlation coefficient of 0.9932. Furthermore, OVA–AuNC-based optical detection systems on both colorimetric and fluorometric assays were tested, which expose highly sensitive and specific determination of CN⁻ ions, and it is easily visualized by the naked eye (day light and UV light). Because of the distinct Elsner reaction between Au atoms of OVA–AuNCs and CN⁻ ions, the recent nanoprobe offered ultrasensitivity and good selectivity with the lowest limit of detection value of 68.00 × 10⁻⁹ mol/L. In addition, this fluorescence “turn-off” CN⁻ ion detection method was executed in real water samples. The demonstrated route of OVA–AuNC preparation is extremely easy and quick, making the proposed selective and sensitive CN⁻ ion sensing assay based on the fluorescence response of the OVA–AuNCs for numerous practical applications.

1. INTRODUCTION

In recent decades, frequent attempts were allotted for the progress of specific chemosensors for the determination of various anions. Among them, chemosensors for cyanide ion (CN⁻ ions) quantifications are mainly of considerable interest owing to environment, security, and public health concerns. Nowadays, in the industry, a bulky amount of cyanide composites is manufactured and used. For example, CN⁻ ions are utilized in a lot of chemical methods, namely, plastics manufacturing, electroplating, tanning, metallurgy, and gold and silver extraction. Hence, CN⁻ is a usually encountered pollutant in soil and water. Generally, CN⁻ is a very poisonous agent ensuing in metabolic asphyxiation; extremely low levels of CN⁻ groups are a deadly poison to people, which is caused by its tendency to connect with the ferric atom of the hemoglobin component consequentially the inhibition of cytotoxic hypoxia. Furthermore, anaerobic metabolisms provoked by CN⁻ ions are directed to accretion of lactates in the human blood. Mutual consequences of lactate acidosis and hypoxia trouble the vital nervous arrangement, ensuing in respiratory arrest and fatality.

On account of the eminent lethality in a specific type of CN⁻ ions, the tolerable amounts of CN⁻ ions in soil and water are normally extremely low. As stated by the United States Environmental Protection Agency (USEPA) and World Health Organization (WHO), the highest tolerable amounts of CN⁻ ions in drinkable water are 200 μg/L and 1.90 × 10⁻⁶ mol/L, respectively. Because of the intense lethal poisonous nature of CN⁻ ions, from the ecological point of view, attracting attention using simplistic and highly sensitive analytical tactics for CN⁻ ion sensing is very necessary.

The broad occurrence and acute lethal poisonous nature of CN⁻ ions, several conservative recognition approaches, including electrochemical, titrimetric, chromatography, chemiluminescence, surface-enhanced Raman spectroscopy, and other techniques, were demonstrated for CN⁻ ions’ quantitative testing. On the other hand, as an outcome of these analytical methods are dependent on instrument facility, low sensitivity, time-consuming characteristics with high limit of detection (LOD) values, high cost, and normally multifaceted. Advanced development of the tactics for rapid and trace-level determination of CN⁻ ions is still anticipated. CN⁻ ions are familiar for the ability to disperse in metals, such as gold and silver, in the existence of oxygen, leading to the...
development of water-soluble metal–CN compounds via the etching process.16

In recent times, gold nanoclusters (AuNCs) saturated discrete physiochemical qualities that made them outstanding colorimetric assays for the invention of new chemosensors.17−20 Liu et al. proposed a bovine serum albumin-functionalized AuNCs, and Dong et al. reported lysozyme-stabilized AuNCs for highly selective sensing of CN− ions based on an etching-tempted emission quenching method.19,20 Encouraged by the realization that specific and highly sensitive determination of CN− ions is obtained via the CN− ion-etching process affected emission quenching of AuNCs.21 In this sense, we have chosen highly fluorescent AuNCs which are a perfect aspirant for the quantification of CN− ions. Furthermore, AuNCs are produced through a simple wet chemical method, high fluorescence quantum yield, large Stokes shift, good photostability, and excellent biocompatibility, which demonstrate a distinctive chance to optimize the sensing of CN− ions in ways impracticable in other usual detection methods.22

For the synthesis of AuNCs, amino acids, biomolecules, and polymers that operate as functionalizing and reducing reagents are essential for the synthesis of stable and highly luminescent AuNCs.18 Biologically important moieties, including proteins and peptides, are employed as structure-clear scaffolds to facilitate the growth and nucleation of AuNCs.18−20 On this background, we have chosen easily available and very cheap ovalbumin (OVA) protein as a surface-stabilizing agent for the preparation of AuNCs. OVA is an N-linked glycoprotein originated from chicken egg white, and it contains 385 amino acid residues. It is a sturdy nominee for the preparation of protein-functionalized AuNCs because OVA holds 6 cysteine acid residues. It is a sturdy nominee for the preparation of AuNCs. OVA is an N-linked glycoprotein originating from chicken egg white, and it contains 385 amino acid residues. However, the luminescence mechanism and the atomic structure of these AuNCs are still unidentified after some years of growth. Very recently, Zhang et al. reported the results for luminescent features, atomic structure, and biomolecular self-assembly of AuNCs functionalized by the bulky globular protein and bovine serum albumin.31 Significantly, such AuNCs are in a rigidified state inside the protein scaffold, presenting a description for their highly fluorescent nature.31 During the synthesis of AuNCs, OVA act both as a stabilizing agent and as a reducing agent, which is similar to bovine serum albumin, and maintaining the physical temperature and alkaline conditions is compulsory.25 In addition, OVA was successfully utilized as a surface-functionalizing agent for the preparation of silver and gold nanoparticles.28 OVA-stabilized Au-based nanomaterials are applied in various fields such as sensing4,29 and bioimaging30 studies.

In this paper, water-soluble, highly red-emissive OVA-conjugated AuNCs were effectively prepared and established for the fluorometric and colorimetric sensing of CN− ions in water medium for the first time. Morphological properties and photophysical behaviors of OVA-stabilized AuNCs were characterized by appropriate techniques. Upon the addition of CN− ions to OVA−AuNCs, the fluorescence intensity of OVA−AuNCs is quickly and linearly reduced, whereas other common interfering ions did not affect the fluorescence intensity. OVA−AuNCs demonstrate more specificity for CN− ions against the other common interfering anions. The achieved superb selectivity of CN− ions is due to the fine etching of the OVA-stabilized AuNC surface and after decay of the AuNCs through the growth of Au−CN complex, leading to the emission quenching of OVA−AuNCs. An additional exciting attribute of the proposed approach is the double visual signal alteration, OVA−AuNCs in the presence of CN− ions, as well the emission quenching; the color of the AuNC colloidal solution under day light and ultraviolet (UV) lamp also faded. The twin visual color change in one sensing methodology applied for the identification of CN− ions both colorimetrically and fluorometrically offers a further rectification of possible falsified signals in a single modal system. In addition, this fluorescence “turn-off” CN− ions detection method was executed for real-water samples.

2. RESULTS AND DISCUSSION

2.1. Synthesis, Characterization, and Stability of OVA−AuNCs. To achieve the highly fluorescent and stable OVA−AuNCs, three significant reaction factors, such as the weight of the OVA, effect of pH on the reaction mixture, and reaction time, were investigated, and the corresponding results are displayed in Figure S1. As depicted in Figure S1a, with the increasing OVA content from 2.5 to 10 mg mL−1, the emission intensity of OVA−AuNCs is increased. Once exceeding the addition of 10 mg mL−1, the emission intensity of OVA−AuNCs is decreased because free OVA molecules are adsorbed on the surface of AuNCs, which affects the fluorescence nature of OVA−AuNCs. Therefore, we chose 10 mg mL−1 as a characteristic amount of our AuNCs synthetic procedure. The effect of the reaction time of AuNCs synthesis, the value emission intensity enhanced with the rise in time from 1 to 12 h, and the maximum emission intensity were obtained at 12 h reaction time (Figure S1b). It was designated that Au3+ was reduced to Au0 by tyrosine residues of OVA protein, and AuNCs were grown in situ. Moreover, these emission spectral observations demonstrated that the emission intensity of OVA−AuNCs diminished after 12 h. Hence, we set the reaction time to the synthesis of highly fluorescent AuNCs for 12 h. Furthermore, it is essential to manage the end point by examining the pH value of the reaction mixture. It can be noted from Figure S1c that OVA−AuNCs show that the emission intensity appeared only in a basic condition at a pH value above 9, and the highest emission intensity was observed at a pH value of 12. Thus, the value of pH = 12 was preferred as the most favorable synthetic condition of AuNCs. The resultant OVA−AuNCs are highly red emissive in nature. However, the luminescence mechanism and the atomic structure of these AuNCs are still unidentified after some years of growth. Very recently, Zhang et al. reported the results for luminescent features, atomic structure, and biomolecular self-assembly of AuNCs functionalized by the bulky globular protein and bovine serum albumin.31 Significantly, such AuNCs are in a rigidified state inside the protein scaffold, presenting a description for their highly fluorescent nature.31 During the synthesis of AuNCs, OVA act both as a stabilizing agent and as a reducing agent; this is analogous to the bovine serum albumin.32 Therefore, in the present study, it is believed that AuNCs are formed within the OVA protein because the collected OVA−AuNCs are highly emissive in nature.

An extremely bright-red-fluorescent OVA−AuNCs were achieved through a one-pot, “green” synthetic approach. Absorption and emission spectra of OVA−AuNCs are given in Figure S2. As visualized in Figure S2, OVA−AuNCs show no noticeable surface plasmon resonance (SPR) peak in the range of 500 to 535 nm, representing the growth of AuNCs instead of large-sized Au nanoparticles. Xie et al. previously reported that the absence of the SPR band in the protein-stabilized AuNCs is attributed to the creation of NCs within the protein template.33 The inset photographic images of Figure 1 demonstrate that the OVA−AuNCs colloidal dispersion is a clear yellowish brown under ambient light and show a highly intense red fluorescence under a UV lamp.
Figure 1. Excitation and emission spectra of OVA–AuNCs, and the inset shows photographs of the color of the OVA–AuNCs under day light (i) and UV light (ii).

Emission features of OVA–AuNCs were recorded periodically during 25 days, and the obtained observations are given in Figure S4b. As viewed in Figure S4b, it is important to state that the colloidal nature and optical properties are the same after 20 days storage of the nanoprobe in the refrigerator at 4 °C. After 20 days storage, the colloidal nature and emission characteristics of OVA–AuNCs were changed because of aggregation or flocculation.

2.2. Fluorescence Detection of CN⁻ Ions Using OVA–AuNCs. The interaction time of OVA–AuNCs was also evaluated, and the result is depicted in Figure S5a. As shown in Figure S5a, when CN⁻ ions toward CN⁻ ions was studied in the range of pH from 9 to 12 and is displayed in Figure S5b. The maximum emission quenching of OVA–AuNCs with CN⁻ ions was reached at the value of pH about 11 (Figure S5b). Moreover, free CN⁻ ions form a feeble acid and hence can subsist in dual forms in a solution medium, such as hydrocyanic acid (HCN) and CN⁻ ions. When pH > pKₐ + 1, for example, pH > 10.36, the CN⁻ ion form is major, and when pH < pKₐ − 1, for example, pH < 8.36, the HCN form becomes important because the value of pKₐ is 9.36. Hence, pH 11 was selected as the most appropriate pH, and this pH was employed in other investigations.

The utility of the resultant OVA–AuNCs was appraised for quantification of trace amounts of CN⁻ ions in a water medium. Under the most favorable experimental settings, the effects of the incremental amounts of CN⁻ ions on the emission intensity changes of the OVA–AuNCs were tested. The emission spectral response of OVA–AuNCs with the incremental amounts of CN⁻ ions is depicted in Figure 3. It can be noted from Figure 3 that when aliquots of CN⁻ ion

Figure 2. HR-TEM image (a) and the corresponding statistical diagram (b) of OVA–AuNCs.

Figure 3. Emission spectral response of OVA–AuNCs with incremental amounts of CN⁻ ions. [CN⁻ ions]: (a) 0.00, (b) 5.00 × 10⁻⁷, (c) 10.00 × 10⁻⁷, (d) 15.00 × 10⁻⁷, (e) 20.00 × 10⁻⁷, (f) 25.00 × 10⁻⁷, (g) 30.00 × 10⁻⁷, (h) 35.00 × 10⁻⁷, (i) 40.00 × 10⁻⁷, (j) 45.00 × 10⁻⁷, (k) 50.00 × 10⁻⁷, (l) 55.00 × 10⁻⁷, (m) 60.00 × 10⁻⁷, (n) 65.00 × 10⁻⁷, (o) 70.00 × 10⁻⁷, and (p) 75.00 × 10⁻⁷ mol/L, and the inset demonstrates the photographic images of the color for OVA–AuNCs in the absence (a) and presence (b) of 75.00 × 10⁻⁷ mol/L of CN⁻ ions.
aqueous solution were successively mixed into the OVA–AuNCs, the emission intensity of OVA–AuNCs is reduced steadily with a distinctive alteration (Figure 3) in the emission spectral profile (hypsochromic shift, peak position, and shape). OVA–AuNCs show a bright-red-fluorescence nature which is highly sensitive to CN\(^-\) ions concentration (inset of Figure 3). The sensitivity of this nanoprobe toward CN\(^-\) ions is caused by the strong interaction between the CN\(^-\) ions and Au atoms on the NC surface, which demonstrates the high effectiveness in the emission quenching of OVA–AuNCs. Furthermore, it is reasonable that CN\(^-\) ions entirely reduce the emission features of OVA–AuNCs via the etching of Au atoms from the NC surface and decayed the highly red-emissive AuNCs into small fragments and leads to the formation of nonemissive AuCN\(_2\)\(^-\) complexes, which is shown in Scheme 1. Similar kinds of

**Scheme 1. Schematic Illustration of the Mechanism for the Interaction of CN\(^-\) Ions and OVA–AuNCs**

![Scheme 1](image)

observations have been previously reported in the case of various molecule-functionalized Au nanomaterials.\(^{19,20,35,36}\) Emission quenching results were quantitatively subjected to the traditional Stern–Volmer equation (eq 1)\(^{37–40}\)

\[
\frac{F_0}{F} = 1 + K_v [Q] = 1 + K_v r [Q]
\]

(1)

where \(F\) and \(F_0\) denote the emission intensities of the OVA–AuNCs with and without the addition of CN\(^-\) ions, \([Q]\) is the concentration of CN\(^-\) ions, and \(K_v\) is the Stern–Volmer quenching constant. The Stern–Volmer plot of emission quenching of OVA–AuNCs versus the concentration of CN\(^-\) ions is given in Figure S6. Unambiguously, emission quenching is directly connected to the quantity of CN\(^-\) ions mixed into the OVA–AuNC colloidal solution. From the slope of the linear Stern–Volmer plot (Figure S6), the \(K_v\) value is estimated to be 9.74 \(\times\) 10\(^5\) mol\(^-1\) dm\(^3\), which reveals the strong emission quenching of OVA–AuNCs with CN\(^-\) ions (Figure S6).

**2.3. Mechanistic Studies for Fluorescence-Based Sensing Approach.** AuNCs consisting of few to tens of atoms are smaller than 2 nm in the average size, which is close to the Fermi wavelength of an electron. Thespacial intertainment for free of charge electrons in AuNCs results in size-tunable and distinct electronic conversions; hence, it is similar to the molecular features, for instance fluorescence.\(^{41}\) Fascinatingly, the emission activities of AuNCs are extremely size-dependent, which stimulated us to believe whether the present bright-red-emitting OVA–AuNCs are prospective appliance in the quantification of CN\(^-\) ions. Upon the addition of CN\(^-\) ions to OVA–AuNCs, the CN\(^-\) ions etched the AuNCs surface, and it is responsible for the emission quenching (97%). To get further insight into the emission quenching mechanism, the fluorescence lifetime decay studies of OVA–AuNCs before and after the addition of CN\(^-\) ions were examined, and the resultant lifetime profiles are presented in Figure 4. The typical

**Figure 4. Fluorescence lifetime decay profile of OVA–AuNCs with and without the addition of 75.00 \(\times\) 10\(^{-7}\) mol/L of CN\(^-\) ions.**
lessen the surface offered for the adsorption of OVA. Thus, OVA protein molecules may slowly desorb from the surface of OVA–AuNCs which then lead to recover the CD spectral signal at 208 and 222 nm of OVA (Figure S8). In addition, CN− ions etching the OVA–AuNC surface were further identified by HR-TEM analysis and are shown in Figure 5. As noted in Figure 5, OVA–AuNCs after the addition of CN− ions show that the average sizes of OVA–AuNCs varied from 2.1 ± 0.3 to 1.3 ± 0.4 nm, which clearly indicates that CN− ions etch the Au atom of OVA–AuNCs. A similar type of the HR-TEM result has been reported in the case of AuNPs in the presence of CN− ions. Optical properties of OVA–AuNCs were significantly dependent on the concentration of CN− ions. The CN− ion-etching process induced emission quenching of OVA–AuNCs recognized from the fluorescence lifetime, absorption, and CD spectral outcomes.

### 2.4. Selectivity of the Present Approach

Along with the prerequisite for sensitivity, superb specificity is also a crucial characteristic for nanoprobes applied in analytical purposes. To inspect the specificity of the present approach for the OVA–AuNC-based fluorescence sensing of CN− ions, the emission spectral changes of OVA–AuNCs after the addition of different appropriate anions were appraised under optimum conditions, and typical outcomes are given in Figure 6. As illustrated in Figure 6, environmentally relevant probable interfering anions showed a weak interference to CN− ion quantification and did not lead to any obvious fluorescence spectral changes at other anions, which makes a good selective determination of CN− ions. Selective and well-built CN−Au interactions offered superb selectivity for the recent OVA–AuNCs toward CN− ions over other relevant anions. Furthermore, the present method exhibits that the selectivity was further visualized by the naked eye. The colorimetric nature of OVA–AuNCs under day light and UV light was investigated, and the corresponding photographs are displayed in the inset of Figure 6. The inset of Figure 6a demonstrates that the brownish yellow color of the OVA–AuNC colloidal solution was only obliterated by CN− ions, whereas no remarkable colorimetric alterations were monitored in the existence of the various probable interfering anions. Similarly, the bright-red-emissive feature of OVA–AuNCs was not altered by various interfering anions, and CN− ions only led to changes from red to a slight blue color emission of OVA–AuNCs (inset of Figure 6b). In addition, the recent high-intense red-emitting OVA–AuNCs were extremely specific in the occurrence of different anions even at 10-fold high concentrations that of CN− ions, which assists the recognition of the proposed nanoprobe for illustrations in different analytical applications. Several anions were treated to OVA–AuNCs; only distinctive color change of CN− ions was noted and easily identified by naked eyes.

### 2.5. Sensing Performance

According to the emission-quenching experiments, CN− ions concentrations were calculated. The calibration curve plotted between the emission intensities and different concentrations of CN− ions and the resultant calibration curve are displayed in Figure 7. As depicted in Figure 7, the calibration curve exhibits a well-linear regression of emission intensity on CN− ion concentrations ranging from 5.00 × 10−7 to 75.00 × 10−7 mol/L with a linear correlation coefficient value (R) of 0.9932. The lowest LOD of OVA–AuNCs at a signal-to-noise ratio of 3 for CN− ions was calculated as 68.00 × 10−9 mol/L, which was much less than the highest tolerable amount of CN− ions (1.90 × 10−6 mol/L) in drinkable water, which is allowed by the WHO. This means that in this, the OVA–AuNCs-based fluorescent approach is highly sensitive and sufficient to examine CN− ion concentrations in drinkable water. The relative standard deviation (RSD) was 2.05% for five repeated experiments, signifying the fine repeatability of the present fluorescent approach. The analytical performance of OVA–AuNC-based CN− ion-sensing fluorescence approach compared with that of previously reported other fluorescence methods for CN− ions is listed in Table S1. It can be observed from Table S1 that the current methodology had an acceptable sensitivity with simple experimental procedures and no need for
complicated apparatus. Furthermore, compared to the lysozyme and bovine-serum-stabilized AuNC-based CN− ion detection methods,20,21 the proposed method has several outstanding advantages, including wide linear range, lowest LOD value, and comparatively low-cost protein OVA that is used as a surface-functionalizing agent for the synthesis of AuNCs. In addition, this recent approach is highly sensitive and specific for CN− ions detection with eco-friendly and easy-handling procedures.

2.6. Analytical Applications. As we recognize, unintentional CN− ions liberated from industrialized progressions and inappropriate waste discharge are a reason for brutal pollution of different water sources. Therefore, it is incredibly significant to construct a proper systematic approach to detect the CN− ions in ecological water samples. To assess whether the OVA–AuNCs-based fluorescent-sensing approach demonstrated here is suitable to ecosystems, natural water samples, such as tap water, drinking water, and dam water samples, were taken from our department, Bharathiar University, and Siruvani dam and monitored by the recent CN− ion detection assay. Under optimum reaction conditions, 0.1 mL of real-water samples was mixed into the reaction mixture, and the emission spectral response was monitored. No substantial alterations in the emission intensity were noted, which illustrates that the level of CN− ions in the natural water samples was lower than the LOD of the recommended analytical approach. However, subsequent to mixing the real water with CN− ions, a noteworthy reduction in the emission intensity was examined. Three levels of CN− ions concentrations of 25, 50, and 75.00 × 10−7 mol/L were executed for all samples to estimate the recovery (%), and the calculated recoveries (n = 3) are tabulated in Table 1. A standard addition technique was applied to compute the amount of CN− ions in the CN− ion-mixed water samples. From Table 1, the calculated concentration in CN− ions-added water samples by the current work was in fine concordance with the CN− ions spiked, together with reasonable recoveries of 97.75−99.44%. These outcomes illustrate that the recent simple fluorescent-based sensor has an immense prospective for quantitative methods of CN− ions levels in natural samples. It is worth noting that numerous merits of the proposed fluorescent approach make it particularly effective for the quantification of CN− ions, including the fact that this nanoprobe was constructed with an effortless, eco-friendly “green” synthetic method without any harmful organic solvents; emission features of AuNCs are extremely concentration-dependent to CN− ions etching, and Au is a chemically stable metal in the periodic table; also, only some anions react with it, except CN− ions, which permits that high specificity and sensitivity toward CN− ions were attained. Significantly, the present nanoprobe was working directly in water medium, hence facilitating the examination of natural water samples.

3. CONCLUSIONS

To summarize all, synthesis of OVA-protected AuNCs was performed by a simple eco-friendly “green” synthetic route and comprises the ability to sensitively and selectively determine CN− ions in 100% water medium. With the consecutive addition of CN− ions to OVA–AuNCs, emission intensities of OVA–AuNCs were reduced via a static quenching mechanism which reveals the very sensitive determination of CN− ions. The sensing method is found on the capability of CN− ions etching the OVA–AuNCs surface, triggering the reducing emission features of AuNCs. Moreover, both colorimetric and fluorometric detection of CN− ions by OVA–AuNCs are easily monitored by the naked eye (day light and UV light). Because of the distinctive (Elsner reaction) strong CN−–Au attraction, the recent luminescent OVA–AuNCs illustrated greater selectivity for CN− ions over different potential interfering anions. Particularly, the present nanoprobe allowed the highly specific and ultrasensitive detection of CN− ions with an estimated LOD value of 68 × 10−9 mol/L, which is much lesser than the highest permissible limit of CN− ions (1.90 × 10−6 mol/L) in drinkable water mandated by the WHO. Furthermore, these analytical experiments extended for some real-water samples spiked with CN− ions established the prospective sensible applications of the current nanoprobe in real-water samples. The developed OVA–AuNCs-based CN− ion fluorescence detection system is trouble-free, fast, sensitive, low-priced, and without any harmful organic solvent.

4. EXPERIMENTAL SECTION

4.1. Chemicals. Hydrogen tetrachloroaurate(III) trihydrate (HAuCl4·3H2O) was obtained from Sigma-Aldrich, USA. Sodium hydroxide (NaOH), OVA, and sodium cyanide (NaCN) were collected from Loba Chemie Pvt. Ltd., India. All reagent chemicals were of as-minimum analytical reagent grade and used as received. Double distilled water was utilized throughout investigation.

4.2. Synthesis of OVA-Stabilized AuNCs (OVA–AuNCs). OVA–AuNCs were synthesized by the following procedure. Initially, 2 mL of OVA solution (10 mg mL−1) was mixed with 2 mL of HAuCl4 (4.00 × 10−3 mol/L) solution under magnetic stirring at room temperature. After 5 min, 0.2 mL of NaOH (1.00 mol/L) was added into the reaction mixture to adjust the alkaline pH ≈ 11–12. Then, the reaction mixture was permitted to progress under stirring for 12 h at 37 °C, whereas, the color of the solution changes from whitish yellow to yellowish brown, proposing that the growth of AuNCs and the obtained OVA–AuNCs were treated to membrane dialysis for the purification process. The resultant OVA–AuNCs were stored in a refrigerator at 4 °C for further use.

4.3. Instruments. Fluorescence spectral data were monitored by a JASCO FP-8500 spectrophotometer attached with 150-W xenon light as an excitation resource. A double-beam JASCO V-630 UV–visible spectrophotometer was applied for the recording of the absorption spectral analysis.

Table 1. Quantification of Spiked CN− Ions in Tap and Drinking Water Samplesa

| real samples | CN− ions added × 10−7 mol/L | CN− ions estimated × 10−7 mol/L | recovery (%) | (n = 3) |
|--------------|-----------------------------|---------------------------------|--------------|--------|
| tap water    | 25.00                       | 24.46                           | 97.84        | 1.08   |
|              | 50.00                       | 48.88                           | 97.75        | 1.83   |
|              | 75.00                       | 73.58                           | 98.11        | 1.18   |
| drinking     | 25.00                       | 24.69                           | 97.60        | 0.98   |
| water        | 50.00                       | 49.16                           | 98.32        | 1.23   |
|              | 75.00                       | 74.13                           | 98.84        | 1.01   |
| dam water    | 25.00                       | 24.86                           | 99.44        | 1.87   |
|              | 50.00                       | 49.46                           | 98.92        | 2.12   |
|              | 75.00                       | 74.49                           | 99.32        | 1.56   |

“RSD: relative standard deviation.

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HR-TEM images were achieved on JEOL JEM 2100 at an accelerating voltage of 200 kV. For HR-TEM analysis, the samples were equipped by dipping OVA–AuNCs on the carbon-coated copper grid. The sample was investigated by HR-TEM images after drying. Time-resolved fluorescence decay investigation was achieved on a HORIBA Scientific time-correlated single-photon counting unit equipped with a 390 nm light source as an excitation. CD studies were tracked in the range from 200 to 260 nm with a scan speed of 50 nm min⁻¹ using a JASCO-810 spectrophotometer equipped with a quartz cuvette of 0.1 cm path length.

4.4. Detection Procedure for CN⁻ Ions. NaCN solution was freshly prepared prior to use. For the fluorescence sensing of CN⁻ ions, 0.5 mL of OVA–AuNC colloidal solution and different concentrations of CN⁻ ions were placed in a series of 5 mL standard measuring flask (SMF). Then, the reaction mixture was made up to the mark of SMF using doubly distilled water, thoroughly shaken well, and allowed for a 10 min equilibration time. Finally, emission spectra were examined by an excitation source at 390 nm, and values of emission intensities at the emission signal at 650 nm were plotted against the CN⁻ ion concentration. Selectivity experiments for CN⁻ ions were performed by the addition of other common ions rather than CN⁻ ions using the same procedure. In addition, photographs of OVA–AuNCs in the absence and presence of various potential interfering ions were achieved under day light and UV light.

4.5. Detection of CN⁻ Ions in Real-Water Samples. Real-water samples (tap water, drinking water, and dam water) were taken from our department, Bharathiar University, and Siruvani dam and were focused to the quantification of the spiked amount of CN⁻ ions using OVA–AuNCs as a fluorescence nanoprobe. Real-sampling-analysis-correlated procedures were as similar as those for standard sample solutions. All the measurements were carried out minimum three times.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02044.

UV–vis absorbance spectrum, optimized synthetic conditions, zeta potential result, fluorescence stability, sensing optimization, Stern–Volmer plot, absorption and CD spectral changes of OVA–AuNCs with CN⁻ ions, comparison of the present method with previously reported fluorescence detection methods, and general procedure for the calculation of LOD and quantum yield (PDF)

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Notes
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