

Chapter 2

Application of Gold-Nanocluster-Based Fluorescent Sensors for Highly Sensitive and Selective Detection of Cyanide in Water

Abstract Cyanide is a highly toxic substance and can invade the human body through many routes. Nevertheless, cyanide is still used in many practical fields. Thus, there is an urgent need to develop highly sensitive and selective sensing systems for the detection of cyanide in the environment, especially in water and biological samples. To overcome the problems of currently studied cyanide sensors such as poor water solubility, poor selectivity, and complex preparation procedures, we develop an innovative gold nanocluster-based fluorescent sensor for cyanide in aqueous solutions. Owing to the unique Elsner reaction between cyanide and the gold atoms of gold nanoclusters, this sensor shows high sensitivity and strong tolerance to other interferences. More impressively, this sensor can be directly used for the detection of cyanide in aqueous solution with excellent recoveries. Thus, this gold-nanocluster-based sensor may provide an effective new tool for highly sensitive and selective detection of cyanide in biological samples.

2.1 Introduction

In USA, approximately 5000–10,000 people each year die of inhalation of the smoke emitted from burning of plastics, woolens, or other nitrogen-containing substances [1, 2]. Cyanide is the most important prime culprit. Cyanide is one of the most-concerning anions in the environment, the toxicity of which is known because of its binding to a3 cytochromes and inhibition of the electron transport chain in mitochondria [3]. Different from toxic metal ions, which induce some diseases mainly by accumulating in the human body, cyanide can directly lead to the death of human beings as well as aquatic life in several minutes, even at a low concentration, by depressing the central nervous system. In addition, cyanides can invade human body through various routes including inhalation, skin contact, or injection. Therefore, cyanides are widely used for murder by criminals. Despite its high toxicity, cyanide is still widely used in electroplating, gold mining and other fields, and approximately 14,00,000 t of toxic cyanide is produced per year [4]. Accidental

cyanide release can thus result in serious contamination of the groundwater and even drinking water, leading to human death.

Conventional detection of cyanide relies on several methods including electro-metric, chromatographic, titrimetric, and voltammetric techniques. However, these methods are generally time-consuming, have complicated procedures, and request significant special skills. To overcome these issues, colorimetric strategies, a cost-effective and naked-eye detection method have been used for cyanide sensing, based on the strong nucleophilicity or high binding affinity of cyanide with the sensors [5–7]. Nevertheless, these methods also showed limitations, such as complicated organic synthesis and a high detection limit. Fluorescence, in contrast, has been emerging as a more-powerful optical technique for the detection of low concentration of analytes, owing to its simple, inexpensive, and rapid implementation. As a result, varieties of fluorescent cyanide chemosensors have been developed. Although these optical chemosensors have made great contributions in cyanide sensing, these strategies also pose limitations, such as environmentally harmful systems, water-insolubility, poor photostability, a high detection limit or easy interference from other anions (especially by F^- , Ac^-) [8–15]. For the detection of cyanide in the biosamples, it requires the sensors to have good water solubility, high sensitivity, and selectivity. Moreover, facile synthesis is also needed for practical applications.

To achieve this goal, in this chapter, we describe a novel gold-nanocluster (Au NC)-based fluorescent sensor for the recognition and determination of cyanide in aqueous solutions, based on the fluorescence quenching effect of Au NCs by cyanide. Notably, several advantages of this method make it especially attractive for the detection of cyanide in the real samples: (i) the sensor was synthesized with a facile and environmentally friendly synthetic route, minimizing the cost and avoiding the use of toxic organic reagents; (ii) the fluorescence of Au NCs is highly size-dependent, and thus highly sensitive to cyanide etching; (iii), the fluorescent, nontoxic metal nanoclusters can be applied directly as a sensing moiety, which enables the facile detection of cyanide; and (iv) gold is chemically inert, and few anions can react with it, except cyanide, enabling a high selectivity toward cyanide. Importantly, this sensing system can work directly in aqueous solution, thus enabling monitoring of practical samples, such as biofluids.

2.2 Experimental Section

2.2.1 Materials

BSA was purchased from Beijing Dingguo Chemical and Biotechnology Co. Ltd. $HAuCl_4 \cdot 3H_2O$ was obtained from Alfa. All other reagents and solvents are of analytical grade and used without further purification.

2.2.2 Preparation of the BSA-Stabilized Au NCs

The BSA-stabilized Au NCs were synthesized according to a method reported previously [16]. Briefly, HAuCl₄ solution (5 mL, 10 mM) was mixed with BSA solution (5 mL, 50 mg/mL) under vigorous stirring at 37 °C. Two minutes later, NaOH solution (0.5 mL, 1 M) was added, and the reaction was allowed to proceed for 12 h under vigorous stirring at 37 °C.

2.2.3 Fluorescent Detection of Cyanide

The KCN stock solution was prepared by dissolving KCN (0.12 M) in a NaOH–NaHCO₃ buffer (0.01 M) at pH 12.0. CN[−] solutions with various concentrations were obtained by serial dilution of the stock solution with the buffer. For CN[−] detection, the CN[−] solutions with different concentrations were added into the Au NCs solution in the NaOH–NaHCO₃ buffer at pH 12.0; the mixture solution was equilibrated for 20 min before measurements.

2.2.4 Selectivity Measurement

The selectivity of this sensing system for cyanide was evaluated by monitoring the fluorescence response of Au NCs to other common anions using the following salts: Na₂S, KSCN, NaBr, Na₂CO₃, NaCl, NaNO₃, Na₂SO₄, NaN₃, KCN, EDTA disodium salt, CH₃COONa (NaAc), Na₃PO₄, NaNO₂, NaIO₃, sodium citrate, Na₂C₂O₄, NaF, and NaI. The above salt solutions were respectively mixed with the Au NCs solutions in a NaOH–NaHCO₃ buffer (0.01 M, pH of 12.0) and equilibrated for 20 min before measurements.

For the detection of selectivity of this sensing system over cations, Fe³⁺, Zn²⁺, Ni²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Cu²⁺, Co²⁺, Ag⁺, K⁺, Na⁺, Li⁺, Mg²⁺, Ca²⁺, Ba²⁺, Al³⁺ were used.

2.2.5 Detection of Cyanide in Real Samples

The tap-water and groundwater samples were used without any pretreatment, while the lake-water and pond-water samples were filtered twice using a syringe filter (pore diameter 0.02 mm) to remove oil and other organic impurities prior to analysis. Water samples spiked with various concentrations of cyanide were added to the Au NCs, and the fluorescence detections were performed within 20 min.

2.2.6 Characterization

The fluorescence spectra were obtained using a Perkin–Elmer LS 55 luminescence spectrometer. XPS was performed using a VG ESCALAB MKII spectrometer. The XPSPEAK software (Version 4.1) was used to deconvolute the narrow-scan XPS spectra of the Au 4f of the Au NCs, using adventitious carbon to calibrate the C1s binding energy (284.5 eV). The elemental analysis was performed using an ELAN 9000/DRC ICP-MS system. The molecular weights of the Au NCs in the absence and presence of cyanide were determined using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS).

2.3 Results and Discussion

2.3.1 Mechanism for Cyanide Detection with Au NCs

Au NCs are composed of several to tens of atoms with the size less than 1 nm, which is close to the Fermi wavelength of an electron. The spatial confinement of free electrons in Au NCs results in discrete and size-tunable electronic transitions, thus offering molecular like properties including fluorescence. Interestingly, the fluorescence of Au NCs is size-dependent, which we hypothesized that these fluorescent nanoclusters could be used for the detection of cyanide.

First, we prepared BSA-stabilized Au NCs. The aqueous solution of the Au NCs was deep brown in color and exhibited bright-red fluorescence under irradiation with the 365 nm UV light. To investigate whether these fluorescent nanoclusters could be used for the detection of cyanide, 5 mM cyanide was added in the aqueous solution of the Au NCs. We found that the original deep-brown color of the Au NCs became colorless, and very-weak blue fluorescence was observed under irradiation with a 365 nm UV light (Fig. 2.1). The corresponding characteristic fluorescence emission of the Au NCs at 640 nm was also disappeared, suggesting the reaction between Au NCs and cyanide (Fig. 2.2).

Given these phenomena, a series of experiments were carried out to reveal the mechanisms behind the reaction of Au NCs and cyanide. First, X-ray photoelectron spectroscopy (XPS) was used to investigate the oxidation states of the Au NCs with vs without addition of cyanide. For the Au NCs, the Au 4f7/2 spectrum could be deconvoluted into two peaks centered at 83.5 eV and 85.1 eV, which are corresponding to the binding energies of Au(0) and Au(I), respectively; whereas, upon the addition of 5 mM cyanide, only an enhanced Au(I) peak was observed at 85.1 eV (Fig. 2.3), indicating the oxidation of Au atoms in the Au NCs by the cyanide.

In previous reports, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) was often used to determine the number of Au atoms in Au NCs [17]. Thus, the number of Au atoms in our Au NCs before versus after reaction with

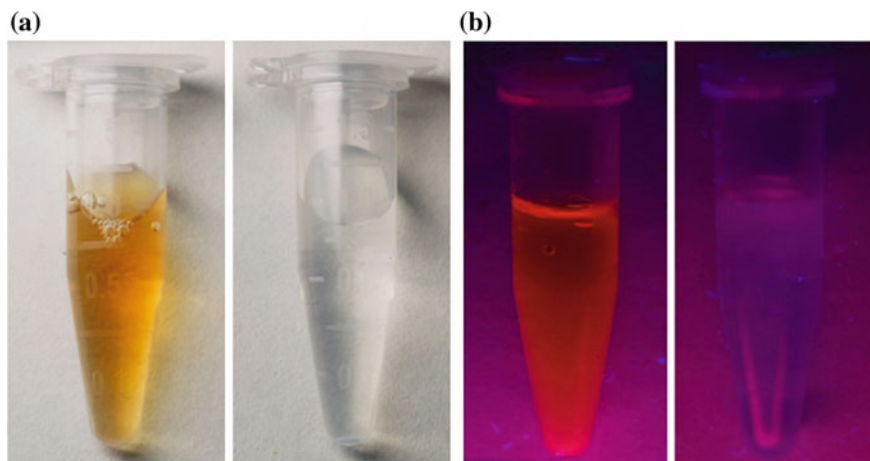
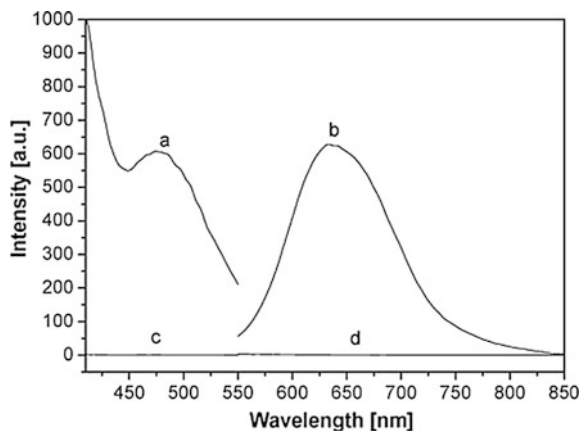


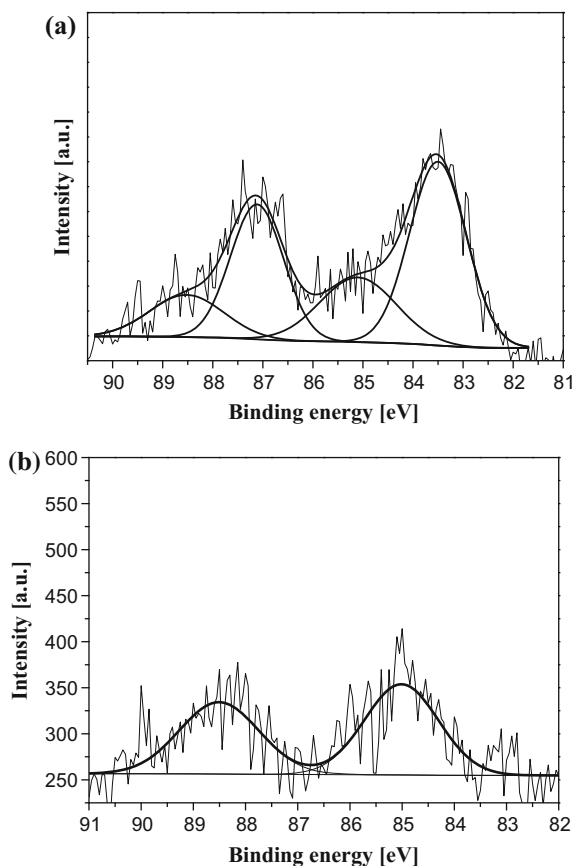
Fig. 2.1 **a** The digital pictures of BSA-stabilized Au NCs in the absence versus presence of 5 mM cyanide. **b** The corresponding fluorescent pictures of BSA-stabilized Au NCs in the absence versus presence of 5 mM cyanide under irradiation with the 365 UV light. (Copyright 2010 Wiley-VCH)

Fig. 2.2 Excitation and emission spectra of BSA-stabilized Au NCs in the absence versus presence of 5 mM cyanide. (Copyright 2010 Wiley-VCH)



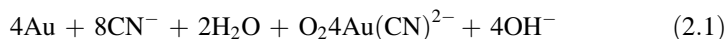
cyanide was determined by MALDI-MS to further confirm the oxidation reaction between Au NCs and cyanide. Prior to detection, a dialysis procedure was performed to purify the Au NCs, followed by freeze-drying. As shown in Fig. 2.4, BSA-stabilized Au NCs exhibited a characteristic peak at 70 kDa. Given the molecular weight of BSA (67 kDa), the number of gold atoms in Au NCs is calculated to be 25. We noticed that the peak at 70 kDa, characteristic of the BSA-stabilized Au NCs, disappeared upon addition of 5 mM cyanide. We reasoned that when the BSA-stabilized Au NCs were dialyzed against water using a 10 kDa cut-off dialysis bag, they could not leach into the filtrate due to their large size. In contrast, cyanide reacted with the BSA-stabilized Au NCs, resulting in the release of Au atoms from the BSA-stabilized Au NCs, thus allowing the resulting Au atoms

Fig. 2.3 Au 4f XPS spectrum of BSA-stabilized Au NCs in the absence (a) versus presence (b) of 5 mM cyanide. (Copyright 2010 Wiley-VCH)



to be filtrated through the cut-off dialysis bag. To further confirm our hypothesis, ICP analysis was carried out to detect the concentration of gold atoms in the filtrate of BSA-stabilized Au NCs in the absence vs presence of 5 mM cyanide. Results showed that the concentration of Au ions in the filtrate of BSA-stabilized Au NCs in the presence of 5 mM cyanide was 648.7 mg/mL, strongly indicating the etching reaction between BSA-stabilized Au NCs and cyanide.

Previous studies have proved that cyanide reacts with Au to generate a very stable $\text{Au}(\text{CN})_2^-$ complex through strong covalent bonding, which is referred to as the Elsner reaction [18, 19]:



According to Eq. 2.1, cyanide can etch the Au NCs, thus leading to the fluorescence quenching of the Au NCs at 640 nm. The reaction mechanism is demonstrated in Fig. 2.5. Notably, the very-weak blue fluorescence, as aforementioned, is characteristic emission of the aromatic side groups in the amino acid residues of the BSA (tryptophan, tyrosine and phenylalanine).

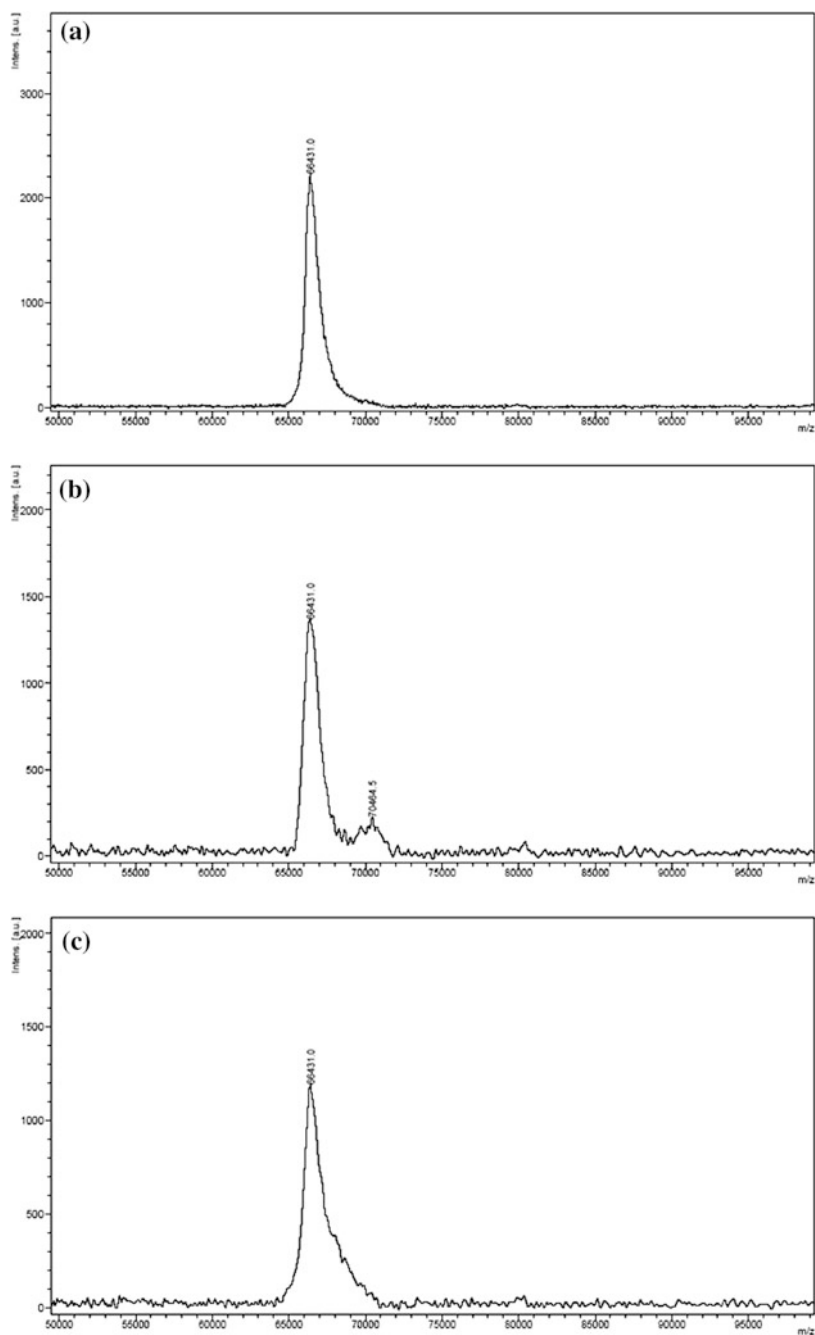


Fig. 2.4 MALDI-MS spectrum of BSA (a), BSA-stabilized Au NCs in the absence (b) versus presence (c) of 5 mM cyanide. (Copyright 2010 Wiley-VCH)

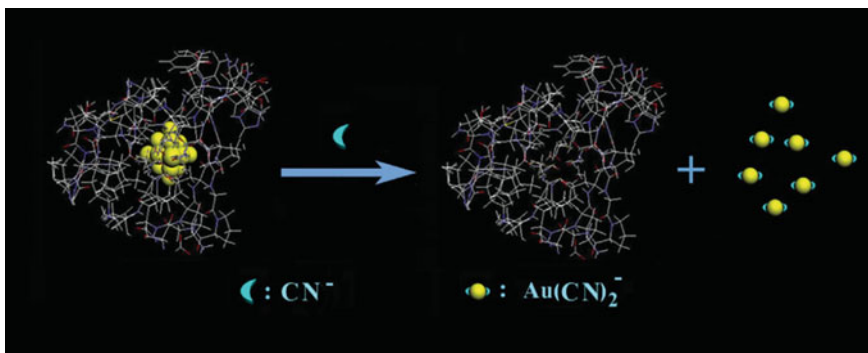


Fig. 2.5 Schematic representation of the Au NC-based sensor for CN^- . (Copyright 2010 Wiley-VCH)

2.3.2 Optimization of the Detection

Before using our fluorescent sensor for the detection of cyanide, we first optimized the detection time, given the slow etching process between Au NCs and cyanide. The time-dependent fluorescence change upon addition of 50 μM cyanide was first monitored. As shown in Fig. 2.6, the fluorescence intensity at 640 nm was quenched by approximately 47% within 1 min and, remained constant after 20 min etching, indicating that the etching reaction was complete within 20 min. Thus, all the following tests were performed at 20 min after addition of cyanide unless otherwise specified.

Another crucial factor is the pH value of the detection system. Cyanide is known to be a weak acid, and a thus may exist in forms in aqueous solution, including hydrocyanic acid (HCN) and cyanide ions (CN^-):

Fig. 2.6 Time-dependent fluorescence response of BSA-stabilized Au NCs upon addition of 5 mM cyanide. (Copyright 2010 Wiley-VCH)

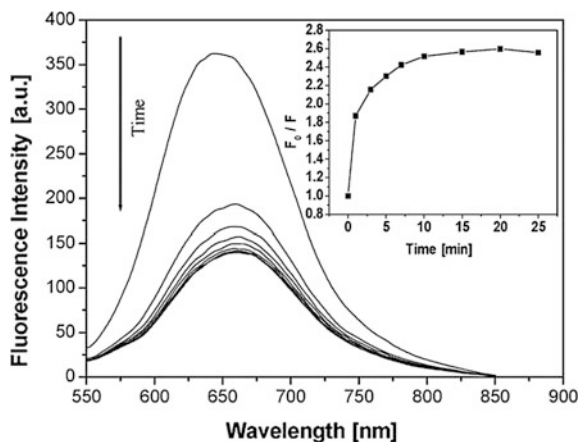
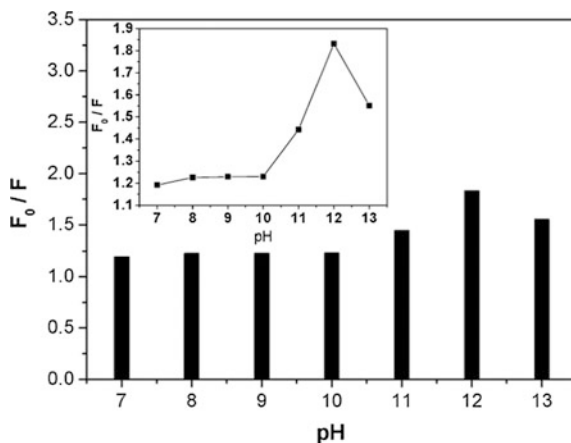


Fig. 2.7 Fluorescence response of BSA-stabilized Au NCs upon addition of 5 mM cyanide under different pH values. The insert is the relative fluorescence changes of BSA-stabilized Au NCs upon addition of 5 mM cyanide under different pH values. (Copyright 2010 Wiley-VCH)



Nevertheless, only CN^- can etch Au NCs. According to the Eq. 2.2, when pH is decreased, the equilibrium will move to right, leading to decreased CN^- . In contrast, when pH is increased, this equilibrium will move to left, thus resulting in increased CN^- . To this end, the pH effect on the sensing system was examined. Figure 2.7 is the response of Au NCs to cyanide under different pH values, and the Au NCs have demonstrated highest sensitivity against cyanide at pH 12.

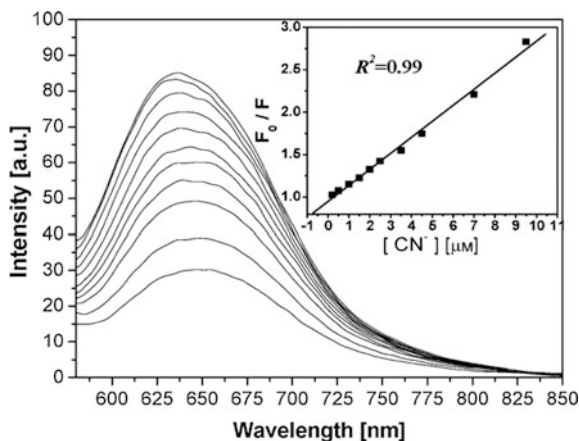
2.3.3 Sensitivity Analysis

Given the optimized reaction time and pH, we proceeded to test the sensitivity of the Au NCs against cyanide. Different concentrations of cyanide were respectively added into the aqueous solution of Au NCs and the fluorescence intensity of Au NCs was recorded. As seen in Fig. 2.8, a dose-dependent decrease in the fluorescence intensity of Au NCs was observed with increasing concentration of cyanide. The quenching efficiency was fitted to the Stern–Volmer equation [20]:

$$F_0/F = 1 + K_{\text{SV}}[Q] \quad (2.3)$$

where F_0 and F are the fluorescence intensities at 640 nm in the absence and presence of cyanide, respectively, K_{SV} is the Stern–Volmer quenching constant, and $[Q]$ is the concentration of analyte quencher. According to Eq. 2.3, the K_{SV} for cyanide was determined to be $1.0 \mu\text{M}$. In this case, the lowest detection concentration of cyanide is 200 nM , which is about $14 \times$ lower than the maximum level ($2.7 \mu\text{M}$) of cyanide in drinking water permitted by the World Health Organization (WHO), strongly suggesting the great potential of Au NCs as a highly sensitive cyanide probe.

Fig. 2.8 Fluorescence response of BSA-stabilized Au NCs upon addition of different concentrations of cyanide. The insert is the F_0/F value as a function of the concentration of cyanide. (Copyright 2010 Wiley-VCH)



2.3.4 Specificity Analysis

To investigate whether our system is specific for cyanide, we measured the fluorescence response of this sensing system with eighteen common anions under the same conditions including S^{2-} , SCN^- , Br^- , CO_3^{2-} , Cl^- , NO_3^- , SO_4^{2-} , N_3^- , CN^- , $EDTA^{2-}$, Ac^- , PO_4^{3-} , NO_2^- , IO_3^- , citrate, $C_2O_4^{2-}$, F^- , and I^- . As shown in Fig. 2.9, only cyanide could induce a drastic decrease in the fluorescence intensity, whereas, no obvious fluorescence changes were observed in the presence of any other anion. The tolerance concentrations of these anions were at least $20 \times$ the CN^- concentration for detecting CN^- using Au NCs. The above results indicate that our sensing system is highly selective towards cyanide over other anions. This excellent selectivity can be attributed to the aforementioned Elsner reaction between the cyanide and Au atoms. In contrast, for currently studied fluorescence-based cyanide-sensing systems that are generally based upon the strong nucleophilicity of cyanide towards the sensor, the strong competition of sensors with cyanide and fluoride may result in poor selectivity, as fluoride is also a strong nucleophile. Moreover, the response of Au NCs to various environmentally relevant metal ions was also investigated (Fig. 2.10). The results showed excellent selectivity for CN^- over K^+ , Na^+ , Mg^{2+} , Ca^{2+} , and Al^{3+} . Also, in the presence of a chelating ligand, 2,6-pyridinedicarboxylic acid (PDCA), BSA and glutathione, Au NCs showed high selectivity against Zn^{2+} , Ni^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Cu^{2+} , Co^{2+} , and Ag^+ . All these results revealed the great potential of Au NCs for highly specific detection of cyanide in the real samples, such as drinking water or biological samples.

Fig. 2.9 **a** Fluorescence response of BSA-stabilized Au NCs to different anions at the concentration of 5 μM at pH 12. **b** F_0/F plotted against 5 μM CN^- with the coexistence of other anions at a concentration of 100 μM and a pH of 12. (Copyright 2010 Wiley-VCH)

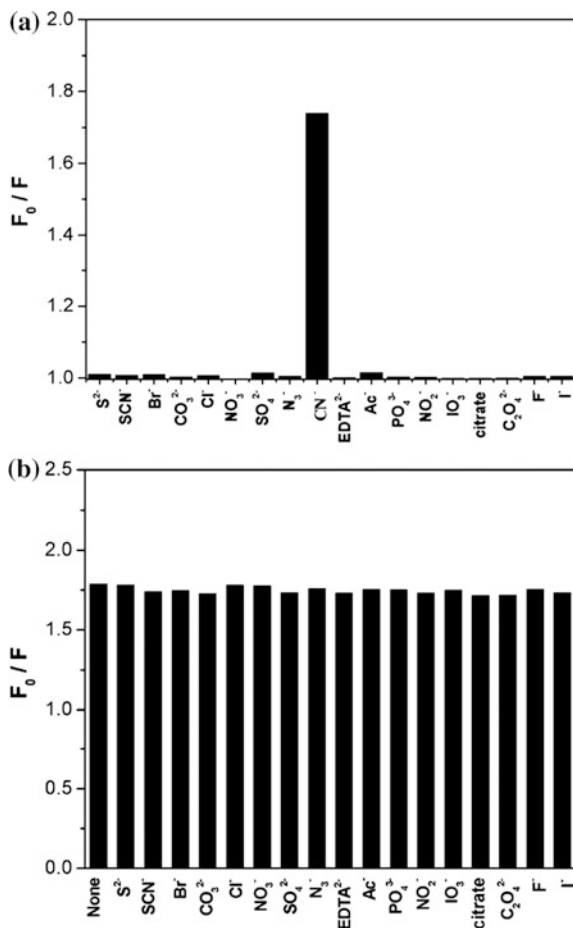
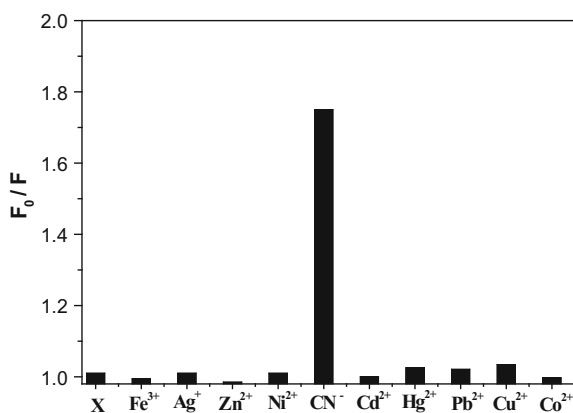


Fig. 2.10 Fluorescence response of BSA-stabilized Au NCs to different cations in the presence of chelating ligand, PDCA (2 mM), BSA (0.4 mg/mL), and GSH (0.4 mM) (CN^- : 5 μM , Fe^{3+} : 2 μM , Zn^{2+} , Ni^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , Cu^{2+} , Co^{2+} ; Ag^+ : 0.1 μM ; X: 0.01 mM; X represents the mixture of K^+ , Na^+ , Li^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} and Al^{3+}). (Copyright 2010 Wiley-VCH)



2.3.5 Cyanide Detection in the Real Samples

To evaluate whether the Au-NC-based fluorescent sensor developed here is applicable to natural systems, real water samples, including local groundwater, tap water, pond water, and lake water collected from South Lake at Changchun City, were analyzed using our cyanide-sensing system. The experimental results showed that this sensing system gives no obvious fluorescence response to the above water samples, suggesting that, similar to deionized water, these real water samples had little interference in the performance of this sensing system (Fig. 2.11). Nevertheless, the addition of the water samples spiked with 50 μM cyanide led to a significant decrease in the fluorescence intensity of Au NCs. Interestingly, similar fluorescence response was observed for different cyanide-spiked water samples (Fig. 2.12), further indicating the high selectivity of our sensing system over other compositions in real water samples.

Fig. 2.11 Fluorescence response of the Au NCs to different water samples and water samples spiked with 50 μM cyanide at a pH of 12. (Copyright 2010 Wiley-VCH)

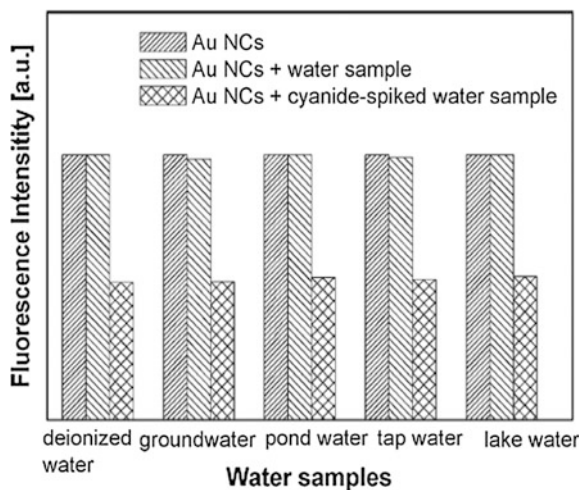


Fig. 2.12 Fluorescence response of different batches of Au NCs to the water samples spiked with 50 μM cyanide at a pH of 12. (Copyright 2010 Wiley-VCH)

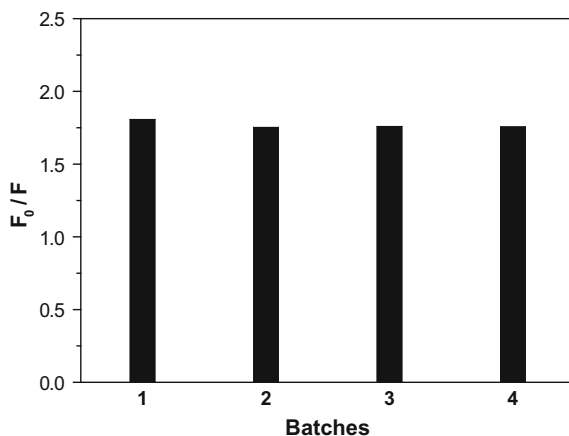


Table 2.1 Recovery of Au NCs in the detection of cyanide in the real samples. (Copyright 2010 Wiley-VCH)

CN [−] added to the water sample [μ M]	CN [−] detected [μ M]	Recovery (%)
2	1.97	98.5
4	3.72	93.0
6	5.90	98.3
8	7.82	97.7

Based on the above results, a standard addition method was performed to determine the concentration of cyanide in the cyanide spiked water samples. Taking the cyanide-spiked lake water as an example, a linear correlation was obtained between F_0/F and the concentration of cyanide over the range from 1 μ M to 9 μ M ($R^2 = 0.98$). The recoveries were 93–98.5% (Table 2.1), demonstrating that this novel sensing system has great potential for quantitative analysis of cyanide levels in the environmental samples.

2.4 Conclusions

In summary, in this chapter, we have described an Au-NC-based fluorescent sensor for highly sensitive and selective detection of cyanide in aqueous solution, based on the cyanide etching-triggered fluorescence quenching of the Au NCs. Different from traditional detection methods, this method is relatively simple, involving no complex organic synthesis and complicated instruments, while enabling a high sensitivity and excellent selectivity toward cyanide over other common anions. Also, this method can work directly in aqueous solution and does not require toxic organic reagents as an assistant solvent. These unique features make this simple, cost-effective sensing system very attractive for reliable detection of cyanide in real samples such as food, soil, water and biological samples.

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From Design Validation to Biomedical Applications

Liu, Y.

2018, XVI, 151 p. 107 illus., 88 illus. in color., Hardcover

ISBN: 978-981-10-6167-7