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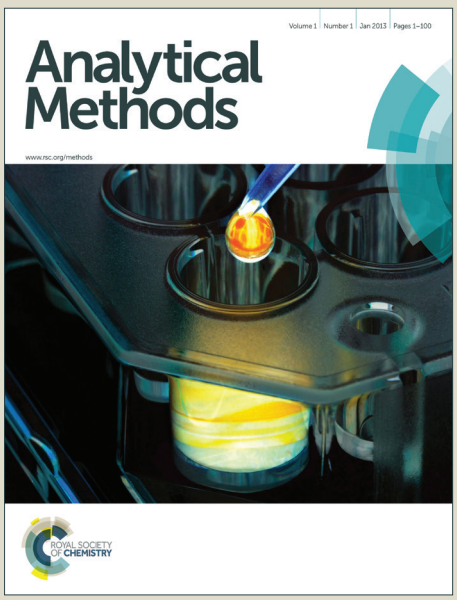

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Ratiometric Fluorescence Silver Nanoclusters for Determination of Mercury and Copper Ions

Xiangjun Liu[a,*] , Linlin Wang[a,b], Nan Zhang[a,b], and Dihua Shangguan [a,*]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

Corresponding author. Fax/ tel: +86 10 62528509.

E-mail address: xjliu@iccas.ac.cn (X. Liu); sgdh@iccas.ac.cn (D. Shangguan)

ABSTRACT

A new ratiometric fluorescence nanoprobe based on DNA-stabilized silver nanoclusters (AgNCs) was one-pot synthesized by a simple reduction method. The obtained nanoprobe exhibited two typical green and red emissions of FAM and AgNCs, using as reference and response signal respectively. Upon addition of Hg^{2+} or Cu^{2+}, the red emission of AgNCs was quenched greatly, whereas the green emission of FAM remained constant, resulting in the ratiometric fluorescence determination of Hg^{2+} and Cu^{2+}. This ratiometric nanoprobe showed good selectivity to Hg^{2+} and Cu^{2+} over many other metal ions, and exhibited high sensitivity with a detection limit as low as 1.03 nM and 2.77 nM for Hg^{2+} and Cu^{2+} respectively. Moreover, by the aid of chelating agent EDTA, this ratiometric fluorescence nanoprobe can discriminate and realize exclusive determination of Hg^{2+} and Cu^{2+}. This ratiometric fluorescence nanoprobe was successfully applied for the determination of Hg^{2+} and Cu^{2+} in real tap water samples.

Introduction

Fluorescence noble metal nanoclusters, which consists of a few to several hundreds of metal atoms, have attracted much attention in recent years because of their unique optical properties, large stoke's shift, good biocompatibility and low toxicity.[1-3] Silver

nanoclusters (AgNCs), especially oligonucleotide-stabilized AgNCs, become more popular recently owing to the facile synthesis, and regulated emissions from visible to near-IR regions simply through varying the sequence and length of the oligonucleotides.[4,5] Now, DNA-stabilized AgNCs have been widely applied in optical sensing and biological imaging, such as metal ions (Hg^{2+}, Cu^{2+}),[6,7] small molecular compounds (thiolated molecules, ATP),[5,8] proteins (thrombin, glutathione reductase),[9,10] targeted DNA,[5,9,11] and cell imaging.[12-14]

Heavy metal pollution has become more and more seriously along with the industry development, and received increasing attention throughout the world because of their high toxicity and potential threat to the ecosystem and human health. Mercury is one of the most toxic heavy metals without biological role, and extensively exists in environment and food. Additionally, mercury is a cumulative poison, and its accumulation in human body can cause adverse effects on human, resulting in a variety of serious diseases such as fatigue, deafness, hyperirritability, and memory or motor disorders.[15] Copper is an essential metal of human and plays important role in many biological processes. However, unregulated copper intake can result in many diseases such as kidney damage, Wilson disease, and Alzheimer's disease.[16]

To data, many methods have been developed for the detection of mercury and copper ions, such as atomic absorption/emission spectrometry, inductively coupled plasma mass spectroscopy, colorimetry, and fluorescent chemosensors.[17-22] Due to the simplicity, high sensitivity, good selectivity and low-cost, fluorescent chemosensors have attracted increasing attention recently. Up to now, some DNA-stabilized AgNCs nanoprobes have been developed for the detection of mercury and copper ions. For example, Guo et al. developed an Hg^{2+} determination method with a low detection limit and high selectivity using oligonucleotide-stabilized AgNCs;[6] Zhang et al. developed a label-free detection method for Cu^{2+} using DNA-templated highly luminescent AgNCs;[7] Su et al. developed a Cu^{2+} detection method through recovery of the fluorescence of DNA-templated Cu/AgNCs.[23] Recently, Liu et al. developed a ratiometric and visual Hg^{2+} detection method using dual emissive DNA-AgNCs.[24]

However, most of the reported DNA-stabilized AgNCs sensors for Hg^{2+} or Cu^{2+} used the change of fluorescence intensity as a single responsive signal. These single-intensity-based sensors were usually interfered by some factors, such as probe concentration, instrument efficiency, and determination conditions. Ratiometric fluorescence technique can avoid these interferes effectively, and has attracted significant attentions in the determination of complex samples in recent years. Additionally, ratiometric fluorescence probe also have the advantages of improved sensitivity and built-in correction for environment effects of the complex sample.[22,25]

Herein, we developed a ratiometric fluorescence nanoprobe based on DNA-stabilized AgNCs for the analysis of Hg^{2+} and Cu^{2+} with high sensitivity and selectivity. The ratiometric fluorescence nanoprobe was easily prepared by using a facile, one-pot synthesis method through reducing $AgNO_3$ with sodium borohydride ($NaBH_4$) in the presence of a designed FAM-labeled DNA sequence.[7] The sensing mechanism of the ratiometric nanoprobe is illustrated in Scheme 1, the fluorescence of FAM remains constant before and after preparation of AgNCs, which is used as reference signal for providing built-in correction to overcome the environment effects; while the red emission AgNCs is selectively quenched by Hg^{2+} or Cu^{2+}, which is used as responsive signal. Using the ratio of fluorescence intensity, a simple ratiometric fluorescence determination method for Hg^{2+} and Cu^{2+} was established. The selectivity and sensitivity of this ratiometric fluorescence nanoprobe to Hg^{2+} and Cu^{2+} were studied. The application in real water samples was also investigated.

Experimental

Chemicals

FAM-labeled oligonucleotide was purchased from Sangon Inc. (Shanghai, China) with the sequences of 5'-FAM-AAAAAAAAAAATCCTCCCACCGGGCCTCCCACCATAAAAACCCTTAATCCCC-3'. The stock solutions of oligonucleotides were prepared in phosphate buffer (PB, 10 mM, pH 7.4, 5.0 mM $MgCl_2$) and the concentration was accurately quantified based on their absorbance at 260 nm. Prior to use, the oligonucleotides

were treated by heating at 95 °C for 10 min, followed with rapidly cooling to 4 °C. Sodium borohydride ($NaBH_4$) was purchased from J&K (Beijing, China). EDTA, silver nitrate ($AgNO_3$) and other reagents were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Beijing, China).

Instrumentations

Fluorescence was measured on a Hitachi F-4600 fluorescence spectrophotometer. Ultraviolet-visible (UV-Vis) absorption spectra were collected on a Hitachi UH-5300 spectrophotometer. Transmission electron microscopy (TEM) measurements were performed on a JEM-2011F electron microscope.

Synthesis of DNA-stabilized AgNCs

Briefly, $AgNO_3$ (20 μM) were added to FAM-labeled DNA (3 μM) solution in PB (10 mM, pH 7.4, 5.0 mM $MgCl_2$). After 20 min of mixing at room temperature in the dark, 20 μM $NaBH_4$ was added and the reaction mixture was incubated at room temperature for another three hours to form DNA-stabilized fluorescent AgNCs.

Fluorescence assay of metal ions

Typically, 20 μL of as-synthesized ratiometric fluorescence nanoprobe mixed with different concentration of Hg^{2+} or Cu^{2+} in the PB (10 mM, pH 7.4) with a total volume of 200 μL. After incubation in the dark for 1 hour, the fluorescence spectra were recorded at the excitation wavelength of 495 nm and 562 nm respectively. For selectivity study, other metal ions, such as Ba^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, and Al^{3+}, were selected and the determination was conducted accordingly. Moreover, for exclusive determination of Hg^{2+} and Cu^{2+}, EDTA was added to the mixture together with metal ions.

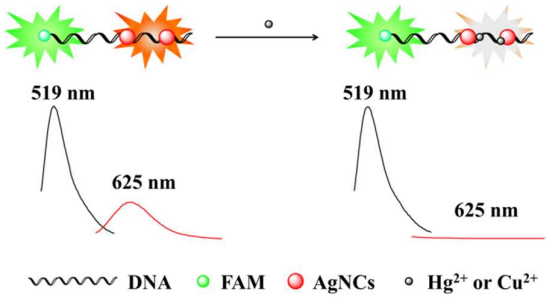
Real tap water analysis

Tap water samples were collected from our lab and filtered through a 0.22 μm membrane prior to use. Different concentrations of Hg^{2+} or Cu^{2+} were spiked in the water samples and analyzed by the ratiometric nanoprobe according to the procedure described above.

Results and discussion

Determination principle and Characterization of the ratiometric fluorescence nanoprobe

The determination principle of the ratiometric fluorescence nanoprobe is shown in scheme 1. DNA-stabilized AgNCs was synthesized facilely in the presence of a designed FAM-labeled DNA by reducing $AgNO_3$ with $NaBH_4$. Initially, the obtained fluorescence nanoprobe showed green and red emissions, which were assigned to FAM and AgNCs respectively. After the addition of Hg^{2+} or Cu^{2+}, the red fluorescence of AgNCs was quenched due to the interaction of AgNCs with Hg^{2+} or Cu^{2+}, whereas the fluorescence of FAM almost did not change, providing reliable reference signal for the ratiometric detection.



Scheme 1 Scheme illustration of the DNA-stabilized AgNCs for the ratiometric fluorescence detection of Hg^{2+} and Cu^{2+}.

The UV-Vis absorption spectra of before and after the formation of AgNCs are shown in Fig. 1a. The absorbance at 260 and 495 nm remained constant, indicating that the synthesis process did not affect the absorption spectrum of FAM-labeled DNA. A new peak appeared around 565 nm after the reduction reaction (Fig. 1a, inset), which confirmed the formation of AgNCs successfully. HR-TEM image showed that the average size of the DNA-stabilized AgNCs was about 2.1 nm (Fig. 1b). Moreover, the fluorescence spectra of FAM-labeled DNA and FAM-labeled DNA-stabilized AgNCs are shown in Fig. 1c. The FAM fluorescence with excitation at 495 nm almost did not change before (Fig. 1c, black line) and after reaction (Fig. 1c, red line), illustrating its good reliability as reference of the ratiometric nanoprobe.

Otherwise, compared with FAM-labeled DNA (Fig. 1c, blue line), a strong fluorescence emission at about 625 nm (Fig. 1c, cyan line) with excitation at 562 nm (Fig. 1d) appeared after reaction, indicating the successful formation of AgNCs.

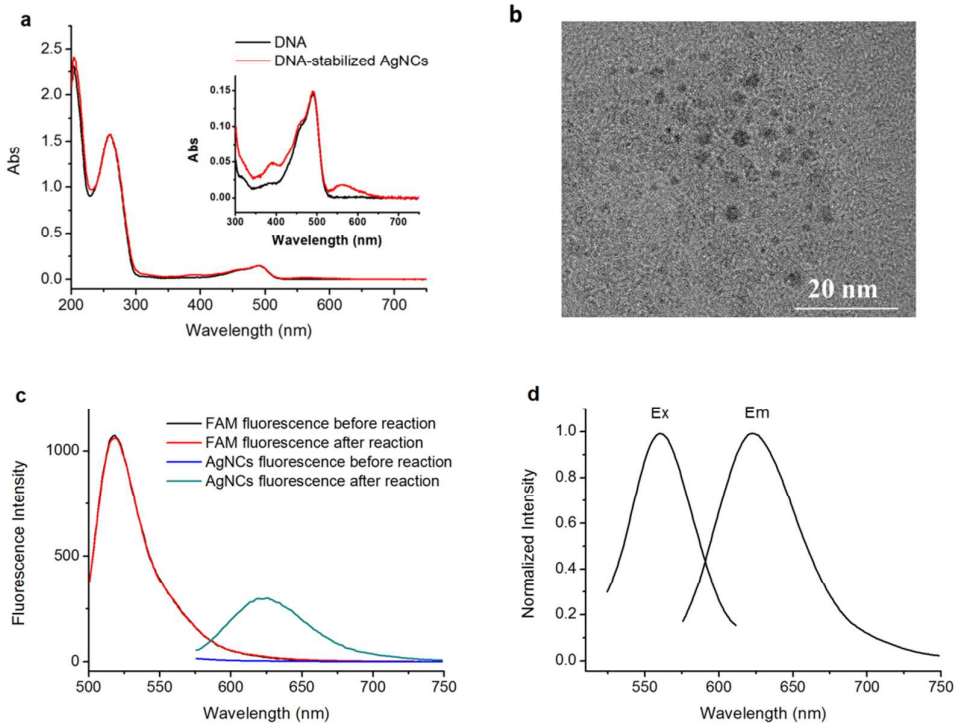


Fig. 1 (a) UV-Vis absorption spectra of the DNA and DNA-stabilized AgNCs. (b) TEM image of DNA-stabilized AgNCs. (c) Fluorescence spectra of FAM (Ex: 495 nm, Em: 519 nm) and AgNCs (Ex: 562 nm, Em: 625 nm) before and after reaction. (d) Excitation and emission spectra of DNA-stabilized AgNCs.

Ratiometric fluorescence determation of Hg^{2+} and Cu^{2+} using the DNA-stabilized AgNCs

Many studies have reported that Hg^{2+} and Cu^{2+} can quench the fluorescence of AgNCs through the metal-metal interaction with AgNCs.[6,7,23,24,26] From Fig. 2, the fluorescence of AgNCs was quenched upon the addition of Hg^{2+} or Cu^{2+}, while the fluorescence of FAM almost did not change. Compared with the single turn-off mode, the change of fluorescence ratio was more obviously, which may improve the sensitivity of this nanoprobe. Moreover, the labeled FAM on DNA as internal

reference signal can avoid the effects of probe concentration and instrument efficiency. And the fluorescence of FAM almost did not change when the Cu^{2+} concentration high to 200 μM. These results verified that our designed FAM-labeled DNA-stabilized AgNCs can be employed as a ratiometric nanoprobe to detect Hg^{2+} or Cu^{2+}. Additionally, all the detections were performed in buffer without any organic solvent, suggesting the potential application of the ratiometric nanoprobe in real environmental or biological samples.

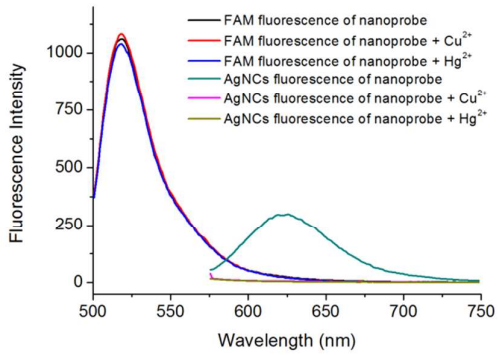


Fig. 2 Fluorescence spectra of DNA-stabilized AgNCs before and after addition of 2.0 μM Hg^{2+} or Cu^{2+}.

To evaluate the selectivity of the FAM-labeled DNA-stabilized AgNCs for Hg^{2+} and Cu^{2+}, the fluorescence response to many other metal ions was measured, including Ba^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, and Al^{3+}. As shown in Fig. 3, only Hg^{2+} and Cu^{2+} caused significant increase of the fluorescence intensity ratio of F_{519}/F_{625}, while other metal ions did not cause significant change of the ratio. These results indicate the good selectivity of the designed ratiometric fluorescence nanoprobe.

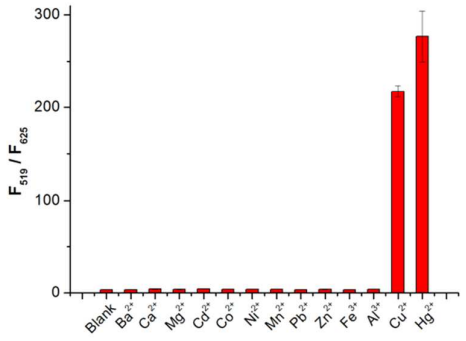


Fig. 3 Effect on the fluorescence intensity ratio of F_{519}/F_{625} upon addition of 2.0 μM different metal ions.

The dramatically increased ratios of F_{519}/F_{625} upon respective introduction of Hg^{2+} or Cu^{2+} indicate the feasibility of individual determination of Hg^{2+} and Cu^{2+}. Furthermore, it is more significant if the ratiometric fluorescence nanoprobe discriminate Hg^{2+} and Cu^{2+} to realize exclusive determination. It has been reported that Au-AgNCs can interact with Hg^{2+} to form more stable complex than Cu^{2+},[26] which inspiring us that the DNA-stabilized AgNCs may also possess similar performance. EDTA, a common used chelating agent, was selected to investigate the competitive chelation of Hg^{2+} and Cu^{2+} with AgNCs. From Fig. 4a, the green fluorescence of FAM was almost not changed before and after addition of Hg^{2+}, Cu^{2+}, Hg^{2+} + EDTA, and Cu^{2+} + EDTA. Otherwise, the red fluorescence of AgNCs was significantly quenched upon addition of Hg^{2+}, Cu^{2+}, Hg^{2+} + EDTA, but almost not quenched when simultaneous addition of Cu^{2+} and EDTA.[26-28] Additionally, the fluorescence intensity ratio of F_{519}/F_{625} was almost not increase upon the simultaneous addition of EDTA and Cu^{2+} owing to the stable complex of Cu^{2+}-EDTA (Fig. 4b). However, the ratio of F_{519}/F_{625} was dramatic increased upon the simultaneous introduction of EDTA and Hg^{2+} as well as the only addition of Hg^{2+}. Surprisingly, the stability constant of EDTA with Hg^{2+} is 21.5, and higher than that with Cu^{2+} (18.8). These results suggest that Hg^{2+}-AgNCs complex is more stable than Cu^{2+}-AgNCs complex. Moreover, the Cu^{2+}-EDTA complex is more stable than Cu^{2+}-AgNCs complex, which prevents the interaction of Cu^{2+} with AgNCs, resulting in that the fluorescence of AgNCs remains

unchanged in the presence of EDTA. Otherwise, the complex of Hg^{2+}-AgNCs is much more stable than Hg^{2+}-EDTA, thus the introduction of EDTA cannot effect the interaction of Hg^{2+} with AgNCs. These results suggest that the designed nanoprobe can discriminate Hg^{2+} and Cu^{2+} through the simple addition of EDTA, and realize the exclusive determination in complicated samples.

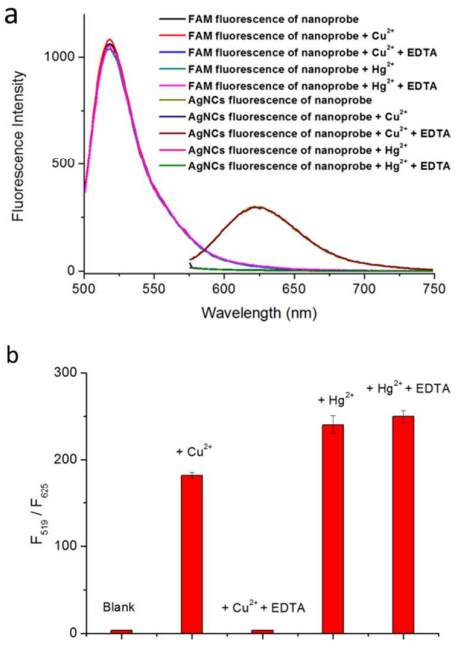


Fig. 4 Fluorescence spectra of FAM and AgNCs (a) and ratio of F_{519}/F_{625} (b) before and after addition of Hg^{2+}, Cu^{2+}, Hg^{2+} + EDTA and Cu^{2+} + EDTA respectively. The concentration of metal ions and EDTA is 2.0 and 2.5 μM.

The analytical performance of the ratiometric fluorescence nanoprobe for individual detection of Hg^{2+} and Cu^{2+} was further studied. The fluorescence intensity ratios of F_{519}/F_{625} against Hg^{2+} concentrations are shown in Fig. 5a. The ratio of F_{519}/F_{625} increased significantly along with the increase of Hg^{2+}, and reached a plateau at a low concentration of 0.5 μM. And Hg^{2+} was detected quantitatively in the range of 0.01 to 0.5 μM with a correlation coefficient of 0.997 (Fig. 5a, inset). The limit of detection was low to 1.03 nM based on 3 times of signal-to-noise ratio. Moreover, this nanoprobe for Cu^{2+} detection at different concentrations was also studied. Similar experimental phenomena compared with Hg^{2+} were obtained (Fig. 5b). But the plateau of F_{519}/F_{625} ratio was reached at the concentration of 1.0 μM for Cu^{2+}. And a good

linearity between the F_{519}/F_{625} ratios and the Cu^{2+} concentrations was obtained in the range of 0 to 1.0 μM (R^2 = 0.9923, Fig. 5b, inset) with a low detection limit of 2.77 nM.

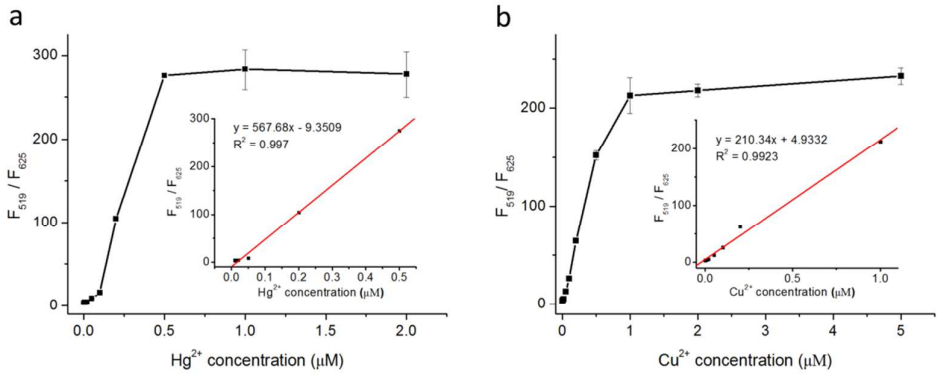


Fig. 5 Fluorescence intensity ratio of F_{519}/F_{625} upon the addition of (a) Hg^{2+} (0, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 nM) and (b) Cu^{2+} (0, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000 nM), (insert: corresponding linear fitting curve).

Real tap water analysis

Furthermore, the application feasibility of the designed nanoprobe in real tap water samples spiked with different concentrations of Hg^{2+} and Cu^{2+} was investigated. The tap water samples were simply filtered through a membrane (0.22 μm) prior to use without any other treatment. The fluorescence ratio of F_{519}/F_{625} was almost not changed after addition of small aliquot tap water, suggesting that the effect of the blank sample can be neglected. Otherwise, after spiked with Hg^{2+} or Cu^{2+}, the fluorescence ratio of these samples increased greatly. The results obtained by the ratiometric fluorescence nanoprobe were shown in Table 1. The good recoveries indicate that the developed method based on DNA-stabilized AgNCs has the potential for the selective and sensitive determination of Hg^{2+} and Cu^{2+} in real water samples.

Table 1. Determination of Hg^{2+} and Cu^{2+} spiked in real tap water samples

Samples	Spiked (μM)	Detected (μM)	Recovery (%)
Hg^{2+}	1.0	0.87±0.03	87%
	2.0	1.66±0.04	83%

	5.0	4.29±0.39	85.8%
	1.0	1.08±0.02	108%
Cu^{2+}	2.0	1.99±0.09	99.5%
	5.0	4.72±0.19	94.4%

Conclusion

In summary, a ratiometric fluorescence nanoprobe for Hg^{2+} and Cu^{2+} was developed using DNA-stabilized AgNCs. The fluorescence of FAM labeled on the DNA kept constant before and after the addition of Hg^{2+} or Cu^{2+}, was used as reference signal. DNA-stabilized AgNCs acted as both recognition element and response signal to Hg^{2+} and Cu^{2+} by the fluorescence quenching. A great increase of fluorescence ratio of F_{519}/F_{625} was obtained upon addition of Hg^{2+} or Cu^{2+}. The designed ratiometric nanoprobe showed good selectivity to Hg^{2+} and Cu^{2+} over other metal ions. And Hg^{2+} and Cu^{2+} can be discriminated and detected exclusively by simple addition of EDTA. The ratiometric nanoprobe was easily prepared by one-pot synthesis method, and the developed ratiometric detection method is simple, highly selective and sensitive, and holds high potential for the determination of Hg^{2+} and Cu^{2+} in real samples.

Acknowledgements

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