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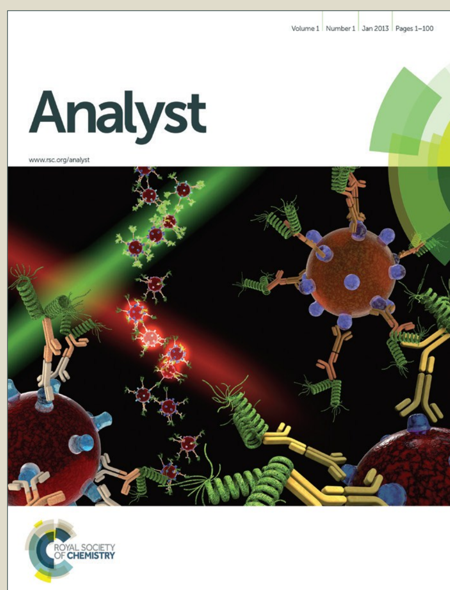

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PAPER

Cu^{2+}-mediated fluorescence switch of gold nanoclusters for the selective detection of clioquinol

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It is of great significance to sense clioquinol (CQ) in a simple and fast way because of its potential application in the treatment of neurodegenerative diseases. In this contribution, we proposed a Cu^{2+}-mediated fluorescence switchable strategy to detect CQ by taking bovine serum albumin (BSA) protected gold nanoclusters (AuNCs) as probes. It was found that the strong red fluorescence of BSA-protected 10 AuNCs at 610 nm could be effectively quenched by Cu^{2+} (off state) and reversibly recovered by CQ (on state) owing to the specific coordination of CQ and Cu^{2+}. Under the optimal conditions, there was a good linear relationship between the off-on efficiency (E_{off-on}) and the amount of CQ in the range of 1-12 μM (R^2=0.9902), with a detection limit of 0.63 μM (3σ). The "turn off-on" mode and the fast and unique complexation of CQ and Cu^{2+} endow AuNCs with high specificity for CQ sensing. The proposed strategy 15 is label-free, fast and selective, which is applicable to the analysis of CQ in cream with satisfactory results.

1. Introduction

New optical and reversible detection modes, most of which based on the off-on switches of fluorescence signals of the luminescent materials or the colorimetric transformations between aggregation 20 and disaggregation of nanomaterials, have received much attention.[1-3] This switchable strategies are capable of improving the selectivity of the "turn-off" detection mode. In a typical off-on stragety, it usually demands a mediator to induce the quenching or aggregation of probe, and then the mediator bind 25 target to recover optical signals.[1, 4, 5] Therefore, the mediator is crucial to the off-on switching process to enhance the off-on efficiency (E_{off-on}).

Clioquinol (5-Cl-7-I-8-hydroxyquinoline, as shown in Fig.S1), a halogenated 8-hydroxyquinoline derivative, is commonly used 30 as antifungal, antibacterial or seborrhea medicine for prevention and treatment of intestinal amebiasis and skin infections. Recently, particular interest has been dedicated to CQ's pharmacodynamics, since CQ is recognized as a potential pharmaceutical against human prostate cancer[6] and 35 neurodegenerative diseases, including Alzheimer,[7, 8] Parkinson[9] and Huntington's diseases.[10] Up to now, assays for CQ detection, including thin-layer chromatography, high performance liquid chromatography and gas chromatography-mass spectrometry,[11-13] have been developed. However, these methods usually require 40 complex operation and time-consuming derivation process. Therefore, it is highly desirable to develop simple, fast and selective methods for CQ detection.

Moreover, it has been reported that by taking the pyridine nitrogen and the phenolate oxygen as metal donors, CQ and Cu^{2+} 45 are able to form 2:1 complex with the stability constant as high as 1.2×10^{10} M^{-2}, which is higher than other metal ions.[14] This unique and strong complexation of CQ and Cu^{2+} contributes to the pharmacodynamics of CQ, which can inhibit the deposition of β-amyloid peptide (Aβ) aggregation induced by Cu^{2+}. It also paves 50 the potential way to detect CQ with Cu^{2+} as a mediator since Cu^{2+} has been demonstrated with paramagnetic property and is able to sensitively quench fluorescence of luminescent materials.[15, 16] For instance, our group developed a off-on strategy for ppGpp sensing by employing Cu^{2+} as a mediator and fluorescent nobel 55 metal nanoclusters as optical probes.[3]

Fluorescent materials, such as carbon nanodots,[17] graphene quantum dots[18] and noble metal nanoclusters,[19-26] have attracted much interest in the fields of biochemical sensing, imaging and cancer therapy owing to their unique fluorescence properties. 60 Noble metal nanoclusters, consisting of only several to hundreds of metal atoms, have attracted great attention owing to their attractive features such as facile preparation, high fluorescence quantum yield, superior catalytic activity, favorable photostability and excellent biocompatibility.[19-26] Controllable syntheses of gold 65 nanoclusters (AuNCs) with a specific number of gold atoms and tunable optical properties are available. It has been reported that fluorescence emission of AuNCs could be readily adjusted from visible to NIR region by controlling the reaction conditions. For instance, the pH-dependent synthesis of pepsin-mediated AuNCs, 70 including Au_5 (Au_8), Au_{13} and Au_{25}, present blue-, green-, and red-fluorescence emission, respectively.[27, 28] These unique optical characteristics endow AuNCs with a wide range of applications, including chemical and biological sensing,[3, 16, 29] cellular and animal imaging,[20] as well as cancer therapy.[30]

75 Herein, taking the BSA-templated AuNCs as optical probes and Cu^{2+} as the mediator, an 'off-on' strategy was successfully developed for detecting CQ. Based on the strong quenching capability of Cu^{2+} towards AuNCs and the specific and high-affinity chelation between CQ and Cu^{2+},[31, 32] CQ can regulate the

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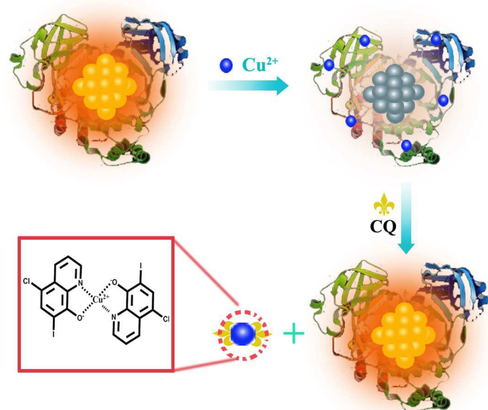

Scheme 1 Schematic representation of the simple, rapid and selective detection of CQ based on Cu^{2+}-mediated BSA-AuNCs.

quenching behavior of Cu^{2+} towards the fluorescence of BSA-AuNCs. Therefore, an 'off-on' fluorescence assay could be developed for highly selective and sensitive detection of CQ (Scheme 1), which provides new and general ways to design 'off-on' probes or sensors through recovery or enhancement of optical signals.

2. Experimental Section

2.1 Reagents

Bovine serum albumin (BSA) was obtained from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4·4H_2O$) was acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Clioquinol was supplied by Sigma-Aldrich Co., Ltd (Missouri, USA). Glucose, sucrose, lactose, starch, dextrin, sodium dodecylsulfate (SDS), $CuSO_4·5H_2O$, amino acids, chloride salts including Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+} and Pb^{2+}, were all analytic grade and used as received without further purification. Milli-Q water obtained from a Millipore water purification system (18.2 MΩ·cm) was used throughout this work.

2.2 Apparatus

The fluorescence and absorption spectra were recorded with a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan) and a Shimadzu UV-3600 spectrophotometer (Tokyo, Japan), respectively. A vortex mixer QL-901 (Haimen, China) was used to blend solutions. The circular dichroism (CD) spectra of BSA molecule and BSA-AuNCs were carried out with a JASCO J-810 spectropolarimeter (Tokyo, Japan). A pH 510 precision pH meter (California, USA) was employed to measure pH.

2.3 Synthesis of BSA-stabilized gold nanoclusters

All glassware for preparing AuNCs were washed with aqua regia (HCl: HNO_3 volume ratio = 3:1), and extensively rinsed with ultrapure water. BSA-stabilized AuNCs were synthesized according to our previous work.[20] The product was dialyzed against ultrapure water for 24 h using a 1 kDa dialysis membrane, then concentrated by freezing (-80 °C) and drying under vacuum. The solid of AuNCs was dissolved to 19.2 mg/mL with water, and stored at 4 °C for further use.

2.4 Procedure of clioquinol detection

The stock solution of clioquinol (1 mM) was prepared with ethanol, and then diluted to work concentration. In a typical test, 30 μL Tris-HCl buffer (50 mM, pH 7.4), 30 μL 1.92 mg/mL BSA-AuNCs solution, 30 μL 60 μM Cu^{2+}, and different amount of CQ were successively added into a 1.5 mL vial, then diluted to 300 μL with water and mixed thoroughly. The mixture was incubated at room temperature for 10 min and then determined with an excitation wavelength at 370 nm.

2.5 The reusability of AuNCs for sensing CQ

The reusability of AuNCs for sensing CQ was carried out in a cuvette by continuous addition of AuNCs, Cu^{2+} and CQ, which was mixed with a pipette. After detection of fluorescence of AuNCs, Cu^{2+} was added and mixed to measure the fluorescence intensity. Followingly, CQ was dropped into the AuNCs-Cu^{2+} mixture to check the fluorescence recovery. This was the first cycle. Then, Cu^{2+} and CQ were introduced into the above mixture alternately, and fluorescence was detected after every addition of Cu^{2+} or CQ. Importantly, the concentration of Cu^{2+} was 6 μM and CQ was 10 μM for each cycle.

2.6 Detection of CQ in cream

This proposed assay is available to analyze the content of CQ in cream. The cream was diluted with ethanol, and then filtered with 0.22 μm filter membranes. The supernatant was diluted with ethanol and stored at 4 °C for further analysis. The process of CQ detection in cream was according to the procedure in 2.4.

3 Results and Discussion

3.1 The characterization of gold nanoclusters

The BSA-stabilized AuNCs with relatively high fluorescence quantum yield were facilely synthesized by manipulating the reaction kinetics.[20] The TEM image and statistics datum showed that AuNCs were well dispersed with the uniform sizes of 1.15-2.12 nm (Fig. 1A). This narrow distribution and small size endow AuNCs with unique optical property.[20] Correspondingly, the as-prepared BSA-AuNCs showed the typical absorbance of tryptophan at 275 nm. When excited at 370 nm, the AuNCs presented strong fluorescence at 610 nm. The obtained BSA-AuNCs were highly dispersed in aqueous solution and emitted the intensely red fluorescence under a 365 nm UV lamp (inset in Fig. 1B). Furthermore, the quantum yield of these red AuNCs was up to ~15%, suggesting the potential applications in a wide ranges.

3.2 The mechanism of the off-on fluorescent detection of CQ

Scheme 1 displays the mechanism of the 'off-on' fluorescence assay of CQ with the Cu^{2+}-mediated BSA-AuNCs as optical probe. According to the previous investigation, Cu^{2+} is able to coordinate to the amino acid residues on BSA surface to induce the excited state of AuNCs to lose its energy by facilitating intersystem crossing (ISC) process, resulting in the quenching of BSA-AuNCs fluorescence.[16] What's different from the metal-metal interaction such as Au^+-Hg^{2+}, this quenching mechanism is normally reversible.[33]

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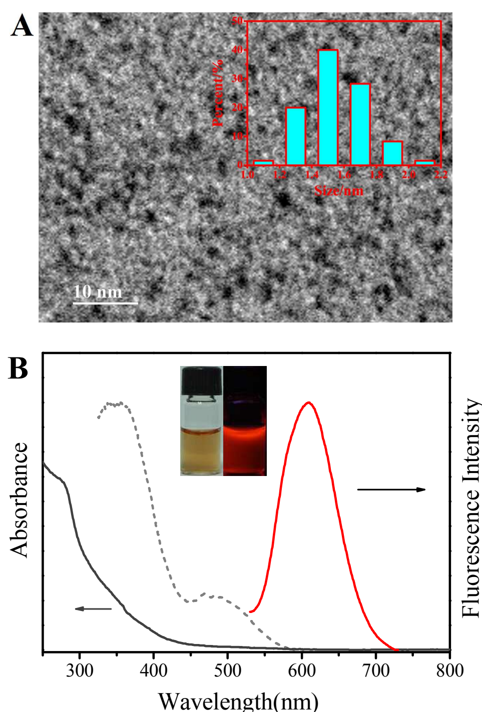
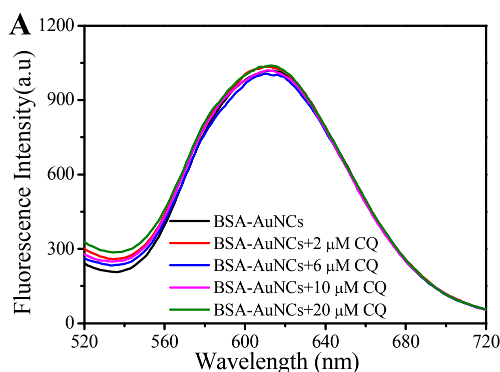


Fig. 1 (A) TEM image of BSA-AuNCs. (B) Optical absorption and fluorescence spectra of BSA-AuNCs. The inset shows the images of BSA-AuNCs under visible (left) and 365 nm UV light irradiation (right).



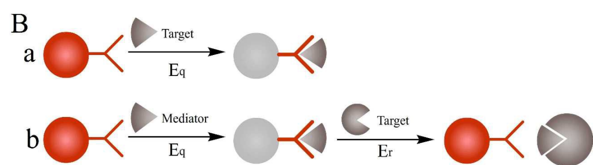


Fig. 2 The effect of CQ on the fluorescence of BSA-AuNCs. Conditions: BSA-AuNCs, 0.192mg/mL; pH 7.0, Tris-HCl buffer. (B) The traditional detection strategy based on the fluorescent probes. a, the direct off strategy; b, the mediator regulated off-on strategy.

To achieve the simple analysis of CQ, AuNCs were directly mixed with CQ. However, the mixture could not result in any change of optical signals (Fig. 2A), which is possibly attributed to the prevention effect of BSA to CQ.[34, 35] Namely, the direct analysis strategy in Fig.2B (a) for CQ seemed not work. Therefore, to analyze CQ with BSA-AuNCs as optical probe, it is necessary to consider a mediator to regulate the off-on or quenching-recovery strategy as presented in Fig.2 B (b).

To achieve the effective detection of CQ, many kinds of metal ions were taken to act as the mediator. Fig. 3A represents the off-on efficiency ($E_{off\text{-}on}$) with the different ions. Herein, $E_{off\text{-}on}$ is calculated by the equations below, F_0 and F_q are the fluorescence intensity of AuNCs at 610 nm in the absence and presence of Cu^{2+}, and F_r is the fluorescence of AuNCs when CQ is present, respectively. Metal ions, including Co^{2+}, Ni^{2+}, Cr^{3+}, especially Hg^{2+}, could effectively quench the fluorescence of BSA-AuNCs, while in the presence of these metal ions, CQ could not lead to the fluorescence recovery of AuNCs. Fortunately, Cu^{2+}-quenched AuNCs fluorescence would be recovered, indicating the specific binding between Cu^{2+} and CQ.[31, 32]

$$E_{\text{off-on}} = E_{\text{q}} \times E_{\text{r}} \quad \text{(Eq. 1)}$$

$$E_{\text{q}} = \frac{F_{\mathbf{0}} - F_{\text{q}}}{F_{\mathbf{0}}} \quad \text{(Eq. 2)}$$

$$E_{\text{r}} = \frac{F_{\text{r}} - F_{\text{q}}}{F_{\mathbf{0}}} \quad \text{(Eq. 3)}$$

Taking Cu^{2+} as a mediator, it was able to quench the fluorescence of AuNCs, which was then gradually recovered with increasing concentrations of CQ. In details, 6 μM Cu^{2+} could cause the quenching of AuNCs fluorescence to 47.5%, however, the subsequent addition of 10 μM CQ led to the intensity recovery to 89.6% (Fig. 3B). Thus, the "off-on" mode was available to construct for CQ detection with BSA-AuNCs as the optical probes and Cu^{2+} as the mediator.

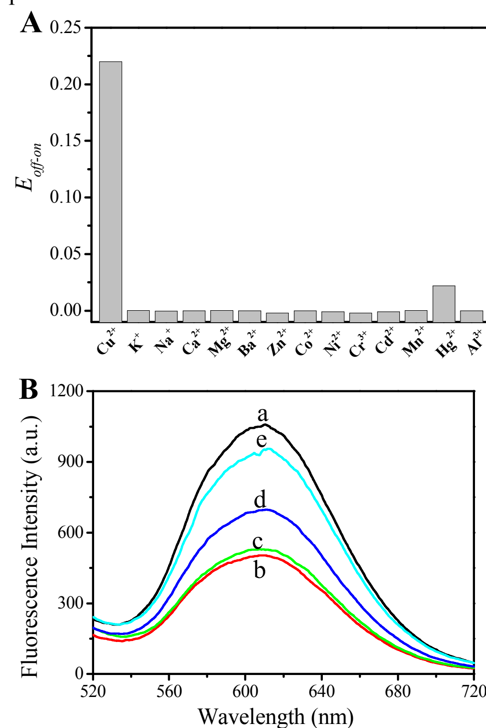


Fig. 3 (A) The off-on efficiency in the presence of different metal ions. Conditions: BSA-AuNCs, 0.192mg/mL; metal ions, 6 μM; CQ, 10 μM; pH 7.0, Tris-HCl buffer. (B) Fluorescence emission spectra of BSA-AuNCs. a, BSA-AuNCs; b, BSA-AuNCs+Cu^{2+}; c-e, BSA-AuNCs+Cu^{2+}+ CQ. Conditions: BSA-AuNCs, 0.192mg/mL; Cu^{2+}, 6 μM; CQ, 2μM for c, 6μM for d, 10μM for e; pH 7.0, Tris-HCl buffer.

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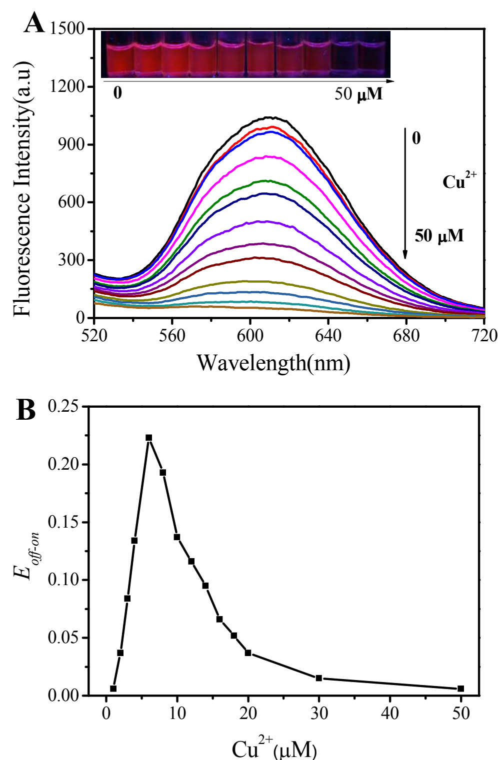

Fig. 4 Evolutions of the fluorescence spectra of BSA-AuNCs with the increasing amount of Cu^{2+} (from top to bottom: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 15, 20, 30, 50 μM). The inset shows the image of BSA-AuNCs with various concentrations of Cu^{2+} under the UV lamp (365 nm). (B) The off-on efficiency versus the concentration of Cu^{2+}. Conditions: BSA-AuNCs, 0.192mg/mL; CQ, 10 μM; pH 7.0, Tris-HCl buffer.

To further rule out the possible effect of ethanol on AuNCs fluorescence, ethanol was introduced into AuNCs and the AuNCs-Cu^{2+} mixture as a control. The result suggested that ethanol presented negligible influence on BSA-AuNCs fluorescence, and could not recover Cu^{2+} quenched fluorescence (Fig. S2, ESI†), suggesting that the restoration of AuNCs fluorescence was contributed to the binding of Cu^{2+} and CQ.

It was found that when CQ was mixed with AuNCs and Cu^{2+}, the quenched fluorescence of AuNCs could be restored gradually, indicating that the chelation between CQ and Cu^{2+} is stronger than that between Cu^{2+} and BSA molecule, leading to the formation of unique and stable Cu(II)-CQ complex.[31, 32] In this case, the ISC process was hampered, resulting in the recovery of AuNCs emission. The reversible change of fluorescence suggested that the bound Cu^{2+} was responsible for the quenching. The circular dichroism spectra of BSA-AuNCs were further confirmed that both Cu^{2+} and CQ did not induce obvious structure change of BSA-AuNCs (Fig. S3, ESI†), suggesting that CQ bound Cu^{2+} specifically and strongly to form complex to drive Cu^{2+} far away from BSA-AuNCs.

3.3 Effect of Cu^{2+} concentration

Fig. 4A displays the fluorescence spectra of BSA-AuNCs in the presence of Cu^{2+} at different concentrations. The increasing concentrations of Cu^{2+} led to the gradual reduce of AuNCs fluorescence intensity at 610 nm, which presented a quenching efficiency as high as 92.3 % when in the presence of 30 μM Cu^{2+}. Visually, the introduction of Cu^{2+} resulted in a gradual decrease of AuNCs fluorescence intensity under the UV lamp (the inset of Fig. 4A), further confirming the quenching of AuNCs fluorescence by Cu^{2+}.

The optimal concentration of Cu^{2+} was evaluated by E_{off-on}, which should reach the high efficiency between 'off' state and 'on' state. For example, if the concentration of Cu^{2+} ions was too low, it resulted in a poor 'off' efficiency. However, when the concentration of Cu^{2+} ions was too high, there would be much free Cu^{2+} ions in the solution to bind CQ directly, which could not lead to the effective fluorescence recovery. Namely, it supplied a low 'on' efficiency. As shown in Fig. 4, when Cu^{2+} ions concentration was 6 μM, the E_{off-on} achieve the optimal state.

3.4 Other optimum conditions

To choose the optimal conditions for CQ detection based on the Cu^{2+}-mediated BSA-AuNCs fluorescence, some key factors including pH, reaction temperature, reaction time and ion strength, should also be carefully considered.

This 'off-on' fluorescence process was pH-dependent due to the coordination of Cu^{2+} with BSA and CQ, so the effect of various pH of Tris-HCl buffer on E_{off-on} was first evaluated (Fig. S4A, ESI†). At pH 5.0, BSA-AuNCs were easy to aggregate, since the pH of buffer was close to the isoelectric point of BSA (pI=4.7), causing a significant enhancement of light scattering to interference fluorescence signal. What's more, in an acid solution, it was more difficult for Cu^{2+} to access the surface of BSA-AuNCs, owing to the electrostatic repulsion between Cu^{2+} and the positively charged BSA-AuNCs or CQ. When the pH was higher than 8, the recovery became weaker than at pH 7, which was ascribed to the formation of $Cu(OH)_2$ precipitate, resulting in a higher background.[36] Thus, Tris-HCl buffer at pH 7 was optimal for detecting CQ. In contrast, the temperature had no obvious influence on the BSA-AuNCs fluorescence intensity (Fig. S4B, ESI†), since the synthesis of BSA-AuNCs was conducted at high temperature (100 °C) to endow AuNCs with the great thermal stability. Thus, the procedures were performed at room temperature.

To understand the response rate, the incubation time was also tested (Fig. S4C, ESI†). The fluorescence was immediately recorded after the addition of 10 μM CQ to the mixture of BSA-AuNCs and Cu^{2+}. This rapid response could remain stable in ten minutes later, which is highly desirable and will be a candidate for rapid CQ detection. To detect CQ in real samples such as cream, ionic strength effect was investigated. Here, NaCl was chosen to adjust the ionic strength, which had no influence on the detection of CQ (Fig. S4D, ESI†). Therefore, the proposed method allowed detecting CQ in samples with high ion strength.

3.5 Sensitivity for CQ detection

Under the optimal conditions, the sensitivity for CQ analysis was investigated with the Cu^{2+}-mediated BSA-AuNCs as optical probes. Fig. 5A displays the fluorescence spectra of BSA-AuNCs-Cu^{2+} upon the addition of various concentrations of CQ, which increased gradually with the increasing concentration of CQ from 1 μM to 12 μM and almost kept constant when concentrations higher than 12 μM. Fig. 5B reveals the linear relationship between the concentration of CQ and E_{off-on}, which could be expressed as E_{off-on} = -0.02358 + 0.02368c_{CQ} (μM), with the correlation coefficient R^2=0.9902. The concentrations of CQ

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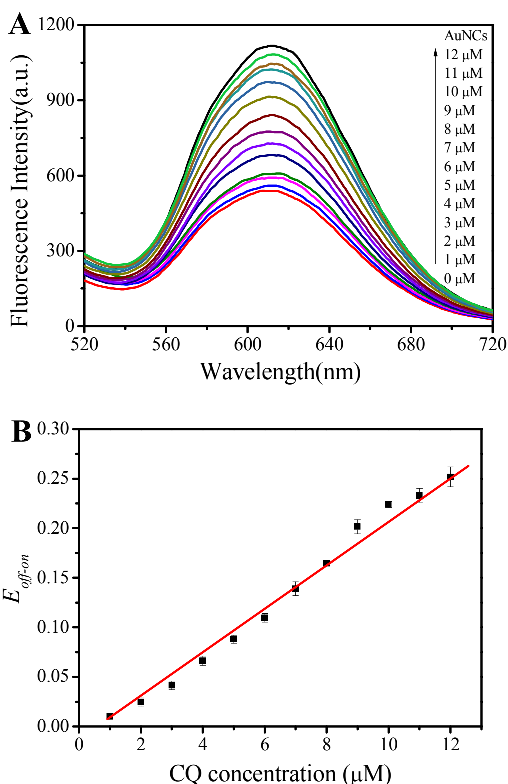

Fig. 5 (A) CQ induced recovery of the Cu^{2+}–mediated AuNCs fluorescence. (B) The CQ-dependent linear relationship of relative fluorescence intensity. Conditions: BSA-AuNCs, 0.192mg/mL; Cu^{2+}, 6 μM; pH 7.0, Tris-HCl buffer. The error bars represent the relative standard deviation (RSD) for three measurements.

could be analyzed in the range of 1–12 μM with the limit of detection (LOD) of 0.63 μM (3σ).

3.6 The reusability of AuNCs for sensing CQ

The reusability of AuNCs for sensing CQ was feasible. That is, the fluorescence quenching of AuNCs could be induced by Cu^{2+} successively, which was then recovered by CQ (Fig. S5, ESI†). Herein, the concentration of CQ was 10 μM with the intensity recovery less than 90%, thus the fluorescence of AuNCs presented the downward trend during the reusability test. The above result was summarized in Fig. 6, which confirmed that the reusability for sensing CQ was feasible at least for four cycles. And $E_{\text{off-on}}$ kept stable during the reusability test, wherein, the F_0, F_q and F_r were dependent on the cycles. For example, in the second cycle, F_0 was the fluorescence of AuNCs-Cu^{2+}-CQ mixture (step 3 in Fig. 6A), F_q was the fluorescence of AuNCs-Cu^{2+}-CQ-Cu^{2+} mixture (step 4 in Fig. 6A), and F_r was the fluorescence of step 5 in Fig. 6A. Then, $E_{\text{off-on}}$ in Fig. 6B in could be calculated in accordance with Eq. 1-3. The rest results may be deduced by analogy.

3.7 Selectivity towards CQ detection

To evaluate the selectivity of the proposed assay, the responses of Cu^{2+}–mediated BSA-AuNCs probe to other potential coexisting substances in cream were investigated under the optimal conditions. No distinct fluorescence restoration was observed for the potential coexisting substances, such as some saccharine, common metal ions and amino acids, which suggested that Cu^{2+}-mediated BSA-AuNCs were specific to the target (Fig. S6, ESI†).

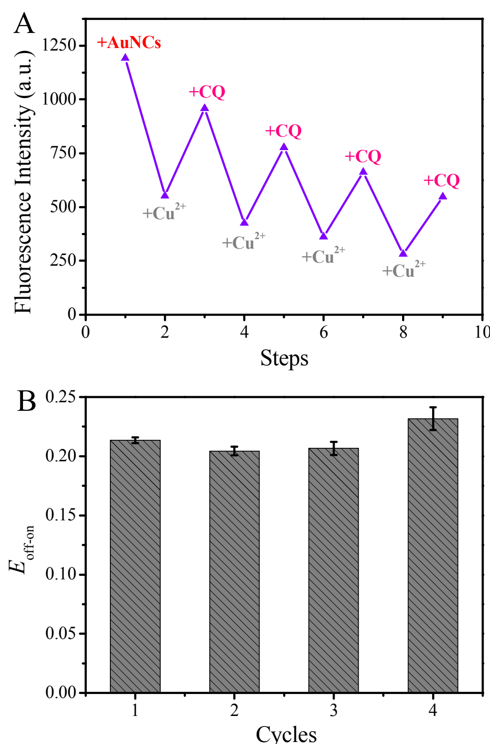

Fig. 6 The reusability of AuNCs for sensing CQ. A, the fluorescence change of AuNCs during reusability test; B. the $E_{\text{off-on}}$ for during reusability test. Conditions: BSA-AuNCs, 0.192 mg/mL; Cu^{2+}, 6 μM for each cycle; CQ, 10 μM for each cycle; pH 7.0, Tris-HCl buffer.

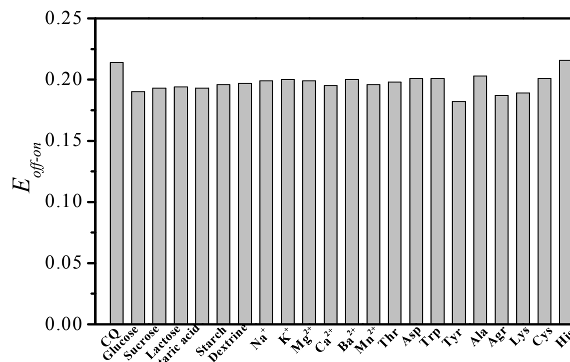

Fig. 7 The selectivity towards CQ detection. Conditions: BSA-AuNCs, 0.192mg/mL; Cu^{2+}, 6 μM; CQ, 10 μM; glucose, sucrose, lactose, and tartaric acid, 100 μM; starch and dextrin, 100 μg/mL; His, 10 μM; other metal ions, 6 μM; other amino acids, 100 μM; pH 7.0, Tris-HCl buffer.

It is reported that Cu^{2+} could quench fluorescence emission of nanoclusters *via* electron or energy transfer. When in the presence of L-histidine (His), it results in a dramatic fluorescence enhancement of nanoclusters because of the chelation between Cu^{2+} and the imidazole group of His, leaving Cu^{2+} far away from nanoclusters to recover the fluorescence. However, His requires longer incubation time (more than 1h)[37,38] than CQ (within 10 min), thus the kinetic difference in the fluorescence recovery enables the proposed assay for CQ with high specificity.

Moreover, the responses of Cu^{2+}-mediated BSA-AuNCs in the presence of the mixtures that containing these potential coexisting substances and CQ were investigated. The result showed the

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Table 1 Determination results of CQ in cream

Batch No.	Sample	Labeled amount (g/10g)	Found average amount (g/10g)	Recovery (n=3, %)
13102201	cream	0.300	0.298	99.23
13102203	cream	0.300	0.310	103.3
13102206	cream	0.300	0.302	100.5

Conditions: BSA-AuNCs, 0.192mg/mL; Cu^{2+}, 6 μM; pH 7.0, Tris-HCl buffer.

proposed sensing method did not suffer from interfering by these substances (Fig. 7). Thus, this new method is potentially capable of analyzing CQ in cream real samples, which was in good agreement with the labeled amount (Table 1).

Conclusions

In summary, a label-free and simple fluorescence 'off-on' mode has been developed for rapid and selective detection of CQ, which utilizes highly fluorescent BSA-AuNCs as optical probes and Cu^{2+} as a mediator. CQ could effectively remove Cu^{2+} from the surface of BSA-AuNCs, leading to the fluorescence recovery of AuNCs that quenched by Cu^{2+} with facilitated ISC process. Both the unique coordination of CQ and Cu^{2+}, as well as the 'off-on' fluorescence switch endow the strategy with excellent selectivity. This strategy is expected to be further generalized for other targets, which could bind the mediator specifically.

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Notes and references

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