

Turn-on fluorescent probe for Cd²⁺ detection by gold nanoclusters/graphene oxide nanocomplex

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This work reported the fabrication of bovine serum albumin stabilised gold nanoclusters (BSA-Au NCs)–graphene oxide nanocomplex (BSA-Au NCs-GO) as a 'turn-on' fluorescent probe for the detection of Cd²⁺. The prepared BSA-Au NCs showed an intense red fluorescence, and the fluorescence of BSA-Au NCs was reduced significantly by graphene oxide due to its excellent quenching properties. However, in the presence of 65 μmol l⁻¹ Cd²⁺, the fluorescence intensity recovered to 82.5% of the intensity of BSA-Au NCs, and the fluorescence intensity increased 1.3-fold relative to the BSA-Au NCs-GO nanocomplex. This proposed probe expands the application of BSA-Au NCs, which can be used as a reversible fluorescence nanoswitch for inexpensive, label free and simple detection of Cd²⁺ in the aqueous samples.

1. Introduction: Among heavy metal ions, Cd²⁺ is one of the most prevalent metal pollutants with persistent bioaccumulation and high toxicity, which causes many serious health problems, including renal dysfunction, reduced lung capacity and some cancers [1]. Numerous methods have been established for determination of Cd²⁺, including inductively coupled plasma mass spectrometry [2], colourimetric detection [3] and fluorescence spectroscopy [4]. Among them, fluorescence spectroscopy techniques offer significant advantages because of their low consumption, simplicity and real-time monitoring [5]. Up to now, the fluorescent probes for metal ions are categorised into two types by fluorescence enhancement or quenching. The 'turn-on' probe is more preferable than the 'turn-off' probe because the latter can produce false positive signals caused by other quenchers in practical samples [6, 7]. Thus, it would be advantageous to design a 'turn-on' fluorescent probe to detect Cd²⁺.

Gold nanoclusters (Au NCs) are emerging fluorescent materials in chemical or biomedical applications. To meet the needs of biomedical application, various Au NCs synthesis processes have been developed using proteins [8]. Among them, the bovine serum albumin stabilised Au NCs (BSA-Au NCs), as a new class of highly attractive fluorescent materials, have been successfully applied to various fields because of their simple, green synthesis, non-toxic, strong red fluorescence emission and bio-compatibility [9, 10]. Graphene oxide (GO) has been found important applications in nanoelectronics due to its unique characteristics such as good water dispersibility, high mechanical strength and the fast electron mobility [11]. Recently, some studies of electron or energy transfer have been carried out, such as, fluorescence quenching of dye by GO [12], energy transfer between quantum dots and GO [13].

Herein, inspiring by the attractive properties of GO as a fluorescent quencher, a novel BSA-Au NCs-GO nanocomplex was constructed as a nanosensor for the development of simple and selective method for Cd²⁺ detection. The method did not need any chemical modification and complicated operations. We found that GO quenched the fluorescence of BSA-Au NCs via multiple contacts such as hydrogen bonding, electrostatic interactions and π–π interactions with aromatic amino acids. Nevertheless, in the presence of 65 μmol l⁻¹ Cd²⁺, the fluorescence of BSA-Au NCs switched to turn-on, and the fluorescence intensity recovered to 82.5% of the intensity of BSA-Au NCs. This method using GO as an effective fluorescence quencher of BSA-Au NCs to investigate Cd²⁺ has not been reported so far.

2. Experimental

2.1. Chemicals and reagents: Chloroauric acid (HAuCl₄·4H₂O, 47.8%) was purchased from Shanghai Chemical Reagent Company (China). BSA was acquired from Beijing Solarbio Science & Technology Co., Ltd (China). CaCl₂, CrCl₃·6H₂O, MgCl₂, FeCl₃·6H₂O, ZnCl₂, Cd(NO₃)₂·4H₂O and PbCl₂ were got from Aladdin Industrial Corporation (China). Other reagents were analytical grade and used without further purification. Standard stock solutions were prepared by dissolving spectral pure grade chemical materials in triply-distilled water and further diluted prior to use.

2.2. Instruments and apparatus: UV–Vis measurement was carried out on a UV-1800 spectrophotometer (SHIMADZU, Japan). Fluorescence spectra were carried out on a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer (Paris, France). Fluorescence microscope images were obtained on a 2X53 Olympus (Japan).

2.3. Preparation of the BSA-Au NCs-GO nanocomplex: All glasswares and magnetic stirrer bars were cleaned with aquaregia, and then rinsed with ethanol and ultrapure water. About 5 ml of 10 mmol l⁻¹ HAuCl₄ aqueous solution was added to BSA solution (5 ml, 50 mg ml⁻¹) under stirring at 37°C. After 2 min, 0.5 ml of 1 mol l⁻¹ NaOH was introduced to adjust the pH. The reaction proceeded at 37°C for 12 h under stirring, and the colour of the solution changed from light yellow to light brown. The purified BSA-Au NCs were stored at 4°C prior to use [9].

GO was obtained according to the improved method with slight modification [14]. The 9:1 mixture of concentrated H₃PO₄/H₂SO₄ (20: 180 ml) was added into flask which had 1.5 g graphite fakes and 9.0 g KMnO₄. The reaction was heated to 50°C and stirred for 12 h. Then, the product was cooled and placed onto ice following by H₂O₂ until the colour varied to brilliant yellow, and the mixture was washed with 5% HCl and ultrapure water. To obtain homogeneous yellow solution, the product was dialysed for 1 week, and then exfoliated under sonication for 2 h prior to use.

The BSA-Au NCs-GO nanocomplex was fabricated by incubating BSA-Au NCs and different aliquots of GO (1 mg ml⁻¹) in phosphate-buffered saline (PBS) buffer (500 μl, 10 mmol l⁻¹, pH 7.8) for 2 min at room temperature. The fluorescence spectra were recorded with an excitation wavelength of 470 nm.

2.4. Procedures for Cd²⁺ determination: About 20 μl of BSA-Au NCs and 150 μl of GO (1 mg ml⁻¹) were added into 96-well

solid black plate for fluorescent reading, and then various amounts of Cd^{2+} solution were added. The final volume was adjusted to 500 μl with PBS buffer (10 mmol l^{-1} , pH 7.8). The mixture was incubated for 2 min at room temperature with gentle shaking before the fluorescence spectra were measured. All of the experiments were repeated three times. The process for the synthesis and the application of BSA-Au NCs-GO nanocomplex in Cd^{2+} detection was illustrated in Fig. 1. To evaluate the selectivity of BSA-Au NCs-GO nanocomplex towards Cd^{2+} , other cations such as Ca^{2+} , Cr^{3+} , Mg^{2+} , Fe^{3+} , Zn^{2+} and Pb^{2+} were tested under the same conditions.

3. Results and discussion

3.1. Characterisation of BSA-Au NCs: The optical properties of BSA-Au NCs were displayed in Fig. 2. The UV-Vis spectrum of BSA-Au NCs exhibited a distinctive surface plasmon resonance peak at 520 nm (blue line), which was assigned to the characteristic absorption band of Au NCs [9]. The fluorescent spectrum of BSA-Au NCs showed an emission peak at 642 nm (black line, $\lambda_{\text{ex}}=470$ nm). The suspension was red-brown colour in visible light, and emitted a very bright red luminescence under fluorescence microscope (Fig. 2, inset picture).

3.2. Fluorescence determination procedure: Quenching effect of GO on BSA-Au NCs fluorescence was investigated by adding increasing amounts of GO into BSA-Au NCs. Finally, 20 μl BSA-Au NCs and 150 μl of a GO stock solution (1 mg ml^{-1}) were added to colourimetric tube, and adjusted to 500 μl with PBS buffer solution (pH 7.8). The results showed that the intensity of BSA-Au NCs-GO (Fig. 3e) was much less than that

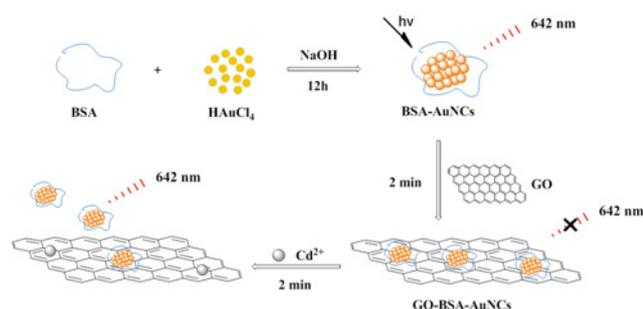


Fig. 1 Schematic representation of the fabrication of BSA-Au NCs-GO nanocomplex and the application for the detection of Cd^{2+}

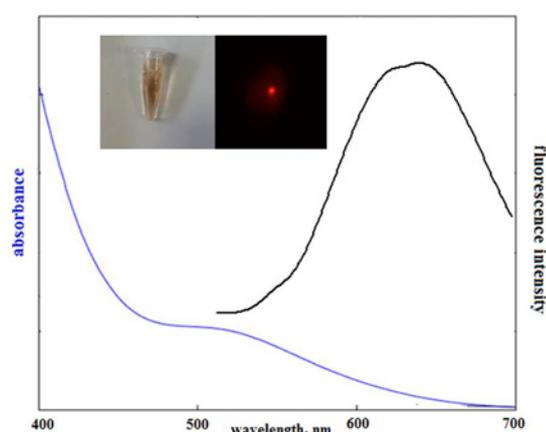


Fig. 2 UV-Vis absorbance (blue line) and emission spectrum (black line) of BSA-Au NCs; BSA-Au NCs in ambient light (the left image of inset picture); fluorescence microscope image of BSA-Au NCs (the right image of inset picture)

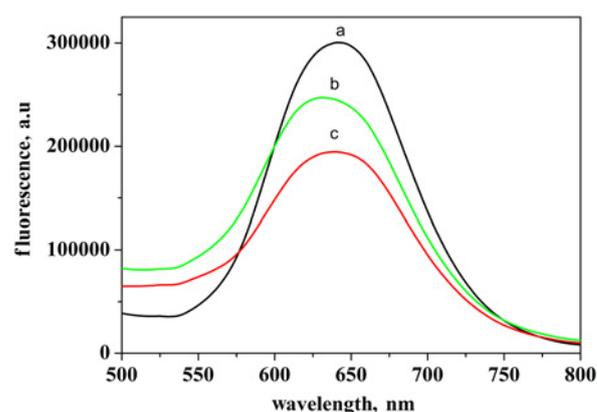


Fig. 3 Fluorescence spectra

a BSA-Au NCs

b BSA-Au NCs- Cd^{2+} -GO

c BSA-Au NCs-GO

$\lambda_{\text{ex}}=470$ nm; GO: 300 $\mu\text{g mL}^{-1}$; Cd^{2+} : 65 $\mu\text{mol L}^{-1}$

of BSA-Au NCs (Fig. 3a), whereas its maximum emission peak did not show obvious shift. According to previous reports [8, 15], a strong interaction between BSA-Au NCs and GO can be generated via multiple contacts such as hydrogen bonding, electrostatic interactions and π - π interactions with aromatic amino acids.

As shown in Fig. 3b, the interaction between BSA-Au NCs and GO was disturbed in the presence of Cd^{2+} , resulting in restored fluorescence of BSA-Au NCs, whereas the spectral width and maximum emission wavelength did not show obvious change. When the concentration of Cd^{2+} was 65 $\mu\text{mol l}^{-1}$, the fluorescence intensity recovered to the intensity of 82.5% of the BSA-Au NCs. At the same time, the fluorescence intensity increased 1.3-fold relative to the BSA-Au NCs-GO nanocomplex. Utilising the metal ions adsorption capacity of GO [16], the fluorescence-enhanced mechanism was presumably due to the chelation of Cd^{2+} with the oxygen atoms of GO, with which Cd^{2+} formed more stable complex than BSA-Au NCs with GO surface. Cd^{2+} displaced the BSA-Au NCs from the surfaces of GO, leading to the restoration of fluorescence signal of BSA-Au NCs. The detection limit for Cd^{2+} ions was 1.5 $\mu\text{mol l}^{-1}$. These results showed that the BSA-Au NCs-GO nanocomplex can be used to detect Cd^{2+} in a ‘turn-on’ response manner.

To assess the selectivity of the BSA-Au NCs-GO nanocomplex for Cd^{2+} , other metal ions, including Ca^{2+} , Cr^{3+} , Mg^{2+} , Fe^{3+} , Zn^{2+} and Pb^{2+} , were examined under the same conditions. Fig. 4 showed the fluorescence intensity changes $((F-F_0)/(F'-F_0))$ of the BSA-Au NCs-GO upon the addition of different metal ions,

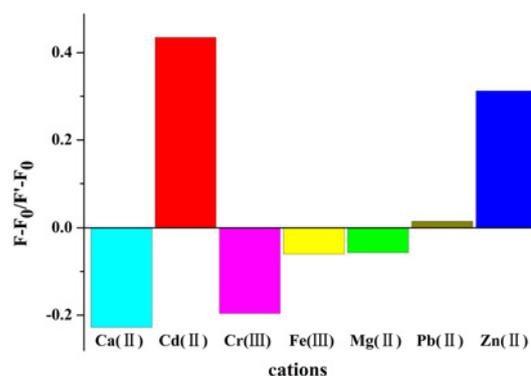


Fig. 4 Fluorescence intensity changes $((F-F_0)/(F'-F_0))$ of the BSA-Au NCs-GO nanocomplex towards different metal ions

where F_0 and F were the fluorescence intensities of BSA-Au NCs-GO in the absence and presence of metal ions, and F' was the fluorescence intensity of BSA-Au NCs. Clearly, these ions except Zn^{2+} did not cause any significant increase in the fluorescence intensity of BSA-Au NCs-GO nanocomplex. These results indicated that the present system exhibited favourable selectivity for the detection of Cd^{2+} .

4. Conclusions: In summary, we designed a 'turn-on' fluorescent probe for the determination Cd^{2+} based on the BSA-Au NCs-GO nanocomplex. The results showed that the BSA-Au NCs-GO nanocomplex showed favourable selectivity for Cd^{2+} . This method may act as a platform for the rapid detection of Cd^{2+} in biological and environmental samples.

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6 References

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