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RESEARCH

A Simple Schiff Base as Fluorescent Probe for Detection of Al^{3+} in Aqueous Media and its Application in Cells Imaging

Fuming Sang[1] · Tiedan Xiong[1] · Weijie Wang[1] · Jianxin Pan[1] · Huahua Shi[1] · Yan Zhao[1]

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Abstract

A novel fluorescence probe for the detection of Al^{3+} was developed based on methionine protected gold nanoclusters (Met-AuNCs). A fluorescent Schiff base (an aldimine) is formed between the aldehyde group of salicylaldehyde (SA) and the amino groups of Met on the AuNCs, and developed for selective detection of Al^{3+} in aqueous solution. Al^{3+} can strongly bind with the Schiff base ligands, accompanied by the blue-shift and an obvious fluorescence emission enhancement at 455 nm. The limits of detection (LODs) of the probe are 2 pmol L^{-1} for Al^{3+}. Moreover, the probe can successfully be used in fluorescence imaging of Al^{3+} in living cells (SHSY5Y cells), suggesting that the simple fluorescent probe has great potential use in biological imaging.

Keywords Al^{3+} · Schiff base · AuNCs · Fluorescence · Cell imaging · Methionine

Introduction

Metal ion contamination has induced great threats to the human health and environment, especially when they are present at high concentrations. Among the various metal, aluminum is extensively used in many fields, such as medicines, cookware, food additives and aerospace, automobile and construction industries [1, 2], which results in the increasing of Al^{3+} concentration in the environment. Nevertheless, excess aluminum can induce some diseases due to its toxicity, such as Parkinson's disease, Alzheimer's disease and breast cancer [3–6]. Therefore, selective trace detection of Al^{3+} is of great importance for ecological environment and human health.

Numerous methods have been used to detect Al^{3+}, such as chromatography, atomic absorption spectrometry, fluorescent chemical sensor, colorimetry and electrochemistry [7–11]. Particularly, fluorescent chemical sensor has been paid considerable attention with advantages of superb selectivity, high sensibility, non-invasiveness and low cost [12–16]. Nevertheless, fluorescent chemical sensor has many disadvantages, such as complex synthetic routes, poor water solubility and being unsuitable for bioimaging, which make it difficult to be used easily and conveniently. It is known that Schiff base derivatives fluorescent probes are highly appealing for optical sensing of Al^{3+} due to their strong coordination with metal ions and good water solubility [16–22]. However, the synthesis of Schiff base derivatives generally suffers from sophisticated and tedious preparation process and strict experimental conditions.

Fluorescent gold nanoclusters (AuNCs) have been extensively used in chemo/biosensing and imaging due to their low toxicity, good biocompatibility, easy synthesis, excellent chemical stability and photostability. Despite many small biomolecules, such as glutathione, tyrosine, L-proline and methionine have been applied in the preparation of AuNCs [23–29], a facile and rapid synthesis of fluorescent AuNCs still remains a great challenge.

The aim of the present work is the construction of a fluorescent switching assay for the detection of Al^{3+} via a donor-π conjugation-acceptor (D-π-A) Schiff base as the linker. Firstly, fluorescent AuNCs modified with Met can be rapidly prepared using a domestic microwave oven within 50 s. Further, a Schiff base can be formed between the amino (-NH_2) group of Met on the AuNCs surface and aldehyde (-CHO) group in salicylaldehyde. Al^{3+} is capable of coordination with Schiff base ligands, which leads to a blue shift of the fluorescence peak from 500 to 455 nm and significant fluorescence emission enhancement at 455 nm.

✉ Fuming Sang
sangfuming@hitwh.edu.cn

[1] School of Marine Science and Technology, Harbin Institute of Technology, Weihai 264209, People's Republic of China

Therefore, the new fluorescence assay for monitoring Al^{3+} has the advantages of rapidity, simplicity, high sensitivity, which has been further applied in living cell imaging.

Materials and Methods

Reagents

$HAuCl_4{\cdot}4H_2O$ and L-methionine were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Salicylaldehyde (SA) was bought from Aladdin Ltd. (Shanghai, China). rhodamine 6G was bought from Sangon Biotechnology Company, Ltd. (Shanghai, China). NaOH and other inorganic salts of cations were obtained from Laiyang Fine Chemical Plant (Shandong, China). All aqueous solutions were prepared with high purity deionized water (18.2 $M\Omega\ cm^{-1}$).

Microwave Synthesis of Met-AuNCs

All glasswares were cleaned with aqua regia (HCl/HNO_3 3:1 v/v) and rinsed thoroughly with deionized water. 200 µL $HAuCl_4$ solution (10 g L^{-1}) was firstly mixed with 1 mL methionine solution (0.18 mol L^{-1}). And then, the mixture was heated by microwaves for 50 s at 800 W in a domestic microwave oven and slowly cooled to ambient temperature. Afterwards, the solution was centrifugated at 10,000 rpm for 10 min to remove impurities.

Detection of Al^{3+} Based on SA-Met-AuNCs Complex

Firstly, 94 nmol L^{-1} SA was added into 100 µL Met-AuNCs solution. Then, 0.2 mol L^{-1} NaOH was added into the above mixture solution, which was mixed and incubated for 30 min at room temperature. Then, Al^{3+} solutions with various concentrations were added into the mixture and reacted for 1 min. The fluorescent spectra with an excitation wavelength at 370 nm and an emission wavelength at 455 nm were recorded.

Fluorescence Cellular Imaging

SHSY5Y cells were inoculated a 12-well plate and adhered 24 h at 37 °C and 5% CO_2. Then these cells were incubated with SA-Met-AuNCs complex for 2 h at 37 °C and fixed with methanol, and then washed three times with PBS buffer (0.1 mol L^{-1}, pH 7.4) prior to imaging. And then, Al^{3+} was added into the precultured cells with SA-Met-AuNCs complex, cultured for 30 min at 37 °C, fixed with methanol and washed with PBS buffer for three times, then imaging.

Results and Discussion

Preparation and Characterization of Met-AuNCs

A household microwave furnace is used to prepare Met-AuNCs in order to achieve fast and efficient synthesis [30–32]. Different conditions for microwave-facilitated prepared of Met-AuNCs were optimized (Fig. S1). We found that microwave-assisted synthesis of Met-AuNCs at 800 W took only 50 s. As shown in Fig. 1A, Met-AuNCs prepared with microwave irradiation exhibit much sharper UV/vis absorption band and a sharp emission peak at 565 nm upon 370 nm excitation (Fig. 1B). A luminescence QY of 16.8% is attained (relative to rhodamine 6G in ethanol, Fig. S2). Figure 1C shows the TEM and HRTEM images of microwave-synthesized Met-AuNCs, which reveals that the AuNCs are well-dispersed. The average diameter is about 3.5 nm and the clear lattice fringe is about 2.34 Å, corresponding to the d-spacing of the (111) lattice plane of face-centered cubic Au. XRD is performed to characterize the crystal structure of Met-AuNCs (Fig. 1D). Four peaks at $2\theta = 38.2$, 44.37, 64.62 and 77.68° are observed, corresponding to the diffractions from the (111), (200), (220), and (311) planes of the face-centered cubic Au, which is in accordance with TEM results. XPS of Met-AuNCs is carried out to determine the valence states of Au and the data are shown in Fig. 1E. The binding energy (BE) of the Au $4f_{5/2}$ and Au $4f_{7/2}$ at 87.8 and 84.1 eV is observed in Au 4f XPS spectrum, respectively, demonstrating that both Au^+ and Au^0 exist in Met-AuNCs. Moreover, Met-AuNCs demonstrate a good photostability between pH 4 and 9 and possess high salt stability even in 1 M NaCl (Fig. S3). Therefore, the results demonstrate that Met-AuNCs have been successfully prepared with microwave irradiation.

Feasibility of Detection of Al^{3+} Based on SA-Met-AuNCs

A fluorescence switch for the determination of Al^{3+} using SA-Met-AuNCs was designed as illustrated in Scheme 1. Upon the addition of SA into Met-AuNCs, SA-Met-AuNCs complex is formed under aqueous solution by a Schiff base reaction (-C=N-) between the amino ($-NH_2$) group of Met on the Met-AuNCs surface and the aldehyde (-CHO) group of SA [33, 34]. And that, the Schiff base (-C=N-) of the SA-Met-AuNCs has a fluorescent emission peak at around 500 nm. When Al^{3+} is further added into SA-Met-AuNCs complex, there is a coordination interaction among Al^{3+}, the imine groups (C=N) of Schiff base, carboxyl group (-COOH) of Met and the hydroxyl groups (-OH) of SA. The coordination interaction reduces charge transfer efficiency and restrains the

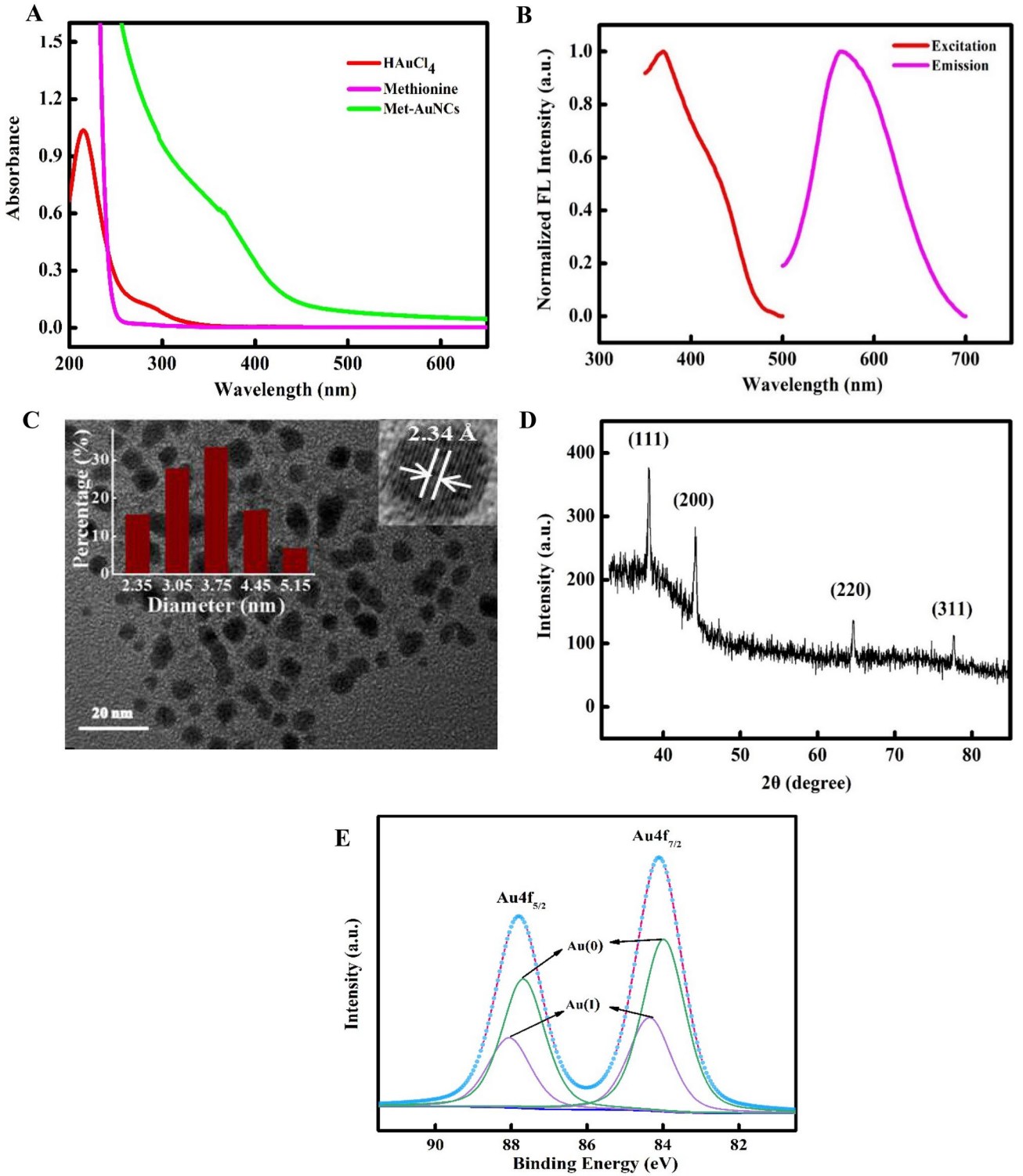
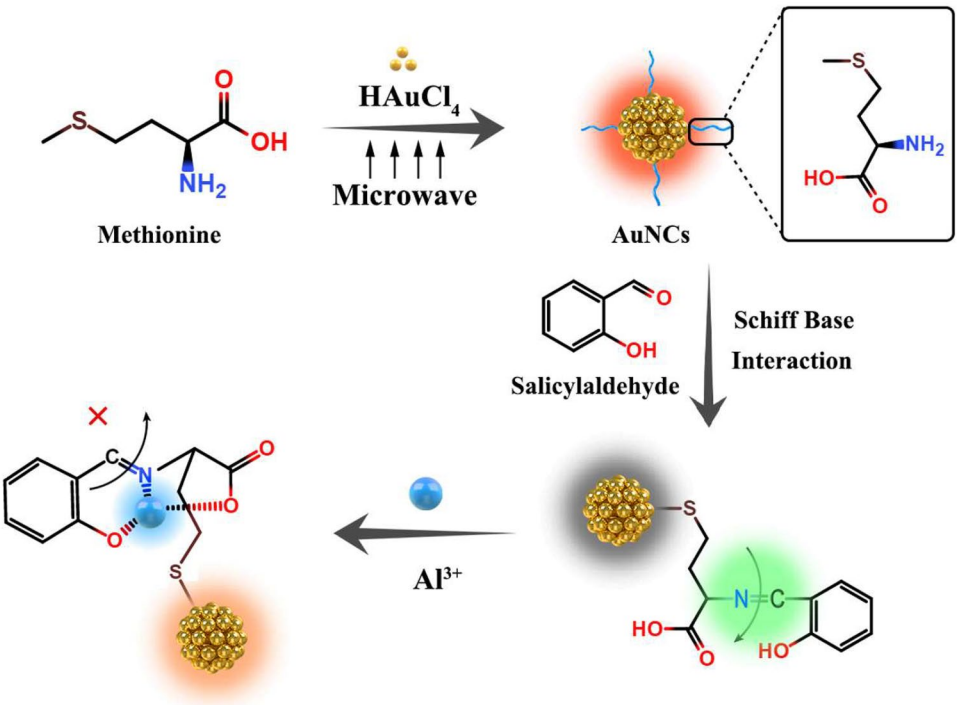


Fig. 1 **A** UV–vis absorption spectra of $HAuCl_4$, Met and Met-AuNCs. **B** Fluorescence spectra of Met-AuNCs. **C** TEM image of Met-AuNCs. Inset: size distribution. **D** XRD spectra of Met-AuNCs. **E** XPS spectra of Au(4f) of Met-AuNCs

isomerization of C = N, which can cause blue-shift of fluorescent peak wavelength of Schiff base from 500 to 455 nm, accompanied by a remarkable fluorescence enhancement around 455 nm. Thereby, an ultrasensitive fluorescent assay is proposed for Al^{3+} detection based on the change in fluorescent intensity at 455 nm.



Scheme 1 Illustration for the microwave-assisted synthesis Met-AuNCs and the Schiff base mediated fluorescence platform for Al^{3+} detection

UV–vis and fluorescence spectroscopy are carried out to further investigate the feasibility of the fluorescent method. As displayed in Fig. 2A, a new absorption peak at 392 nm in a range of 300–500 nm is observed after the addition of SA into Met-AuNCs, which can be attributed to the C = N absorption band of SA-Met-AuNCs complex [35]. Upon successive addition of Al^{3+} ions, the band at 392 nm was blue-shifted to 342 nm, demonstrating the formation of a new compound between Al^{3+} and SA-Met-AuNCs complex. Fluorescence investigation results (Fig. 2B) demonstrate a new characteristic peak of Schiff base centered at 500 nm after adding SA into Met-AuNCs [36], accompanied by an obvious fluorescence color change from pink into green under UV lamp (Fig. 2A). After the addition of Al^{3+} to the SA-Met-AuNCs complex solution, a blue-shift in the emission peak of Schiff base from 500 to 455 nm is also observed (Fig. 2B). Concomitantly, the fluorescence intensity around 455 nm enhances dramatically, along with a noticeable fluorescence color change from green into blue (Fig. 2A), which is attributed to the formation of a Al^{3+} and SA-Met-AuNCs complex. Hence, all above results affirm that SA-Met-AuNCs complex fluorescent sensor can detect Al^{3+} with high sensitivity and high selectivity.

Optimization of the Conditions

The main experimental parameters including pH, incubation time between SA and Met-AuNCs and SA concentration are optimized for Al^{3+} detection. pH is very important for the formation of a Schiff base between the Met-AuNCs and SA. As shown in Fig. S4A, the maximum fluorescence intensity is observed when 0.2 mol L^{-1} NaOH is added, which

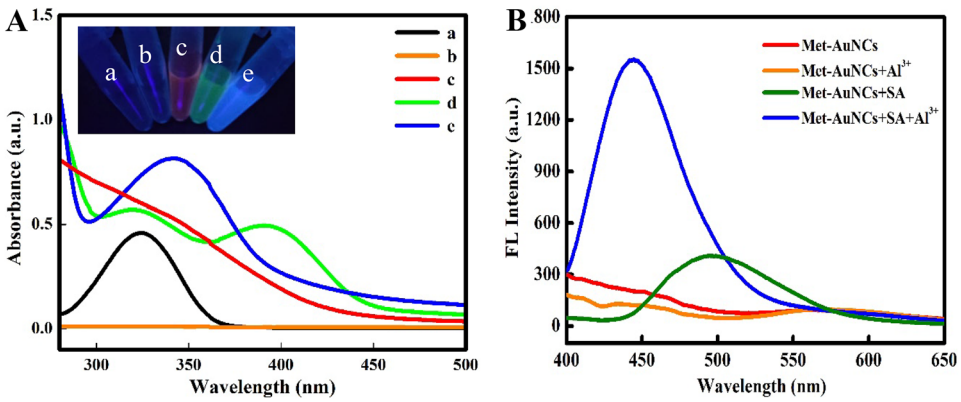


Fig. 2 **A** UV–vis absorption spectra of SA (**a**), Al^{3+} (**b**), Met-AuNCs (**c**), Met-AuNCs + SA (**d**) and Met-AuNCs + SA + Al^{3+} (**e**). Inset: corresponding photographs image under UV light (λex = 365 nm); **B** Fluorescence emission spectra of Met-AuNCs, Met-AuNCs + Al^{3+}, Met-AuNCs + SA, Met-AuNCs + SA + Al^{3+}, SA, SA + Al^{3+}

is therefore used in all subsequent experiments. The fluorescence intensity at 500 nm increases with the increase of SA concentration (Fig. S4B). Simultaneously, the solution color under UV lamp change from red to orange, and then to green (Fig S4C). When 94 nmol L^{-1} SA is added, the obvious fluorescence solution color changes can be observed, implying that SA is excessive. Therefore, 94 nmol L^{-1} SA is used for selective and sensitive detection of Al^{3+}. The results in Fig. S4D demonstrate that the fluorescence intensity at 500 nm decreases gradually as an increase of the incubation time between SA and Met-AuNCs, and then tends to level off after 30 min. Accordingly, 30 min incubation time is chosen for the next experiments.

Sensitive Detection of Al^{3+}

At the optimal conditions, the sensing performance of SA-Met-AuNCs complex probe toward Al^{3+} was investigated. As shown in Fig. 3A, the fluorescence intensity of the probe at 455 nm enhances obviously with increase of Al^{3+} concentration. As demonstrated in Fig. 3B, a good linear relationship is obtained between the fluorescence intensity at 455 nm and Al^{3+} concentration over the 0.002 to 1.6 µmol L^{-1} range. The limit of detection (LOD) was estimated to be 2 pmol L^{-1} (3α/S, where α and S represent the standard deviation of blank and slope of the linear plot), which is far below the permitted level in drinking water permitted by the World Health Organization (WHO) (7.41 µmol L^{-1}). Moreover, Table S1 summarizes some other reported sensors for the analysis of Al^{3+}, which demonstrates that the assay is comparable or superior to the previous approaches. Hence, all the results suggest that the developed fluorescent probe is sensitive enough to monitor Al^{3+} in the drinking water.

Selectivity of Al^{3+} Detection

Selectivity is another important characteristic of a sensor for metal ions. To evaluate the high selectivity of the fluorescent probe for Al^{3+}, the fluorescence responses of the assay were carried out in the presence of various metal cations under the same experimental condition. As shown in Fig. 4, for the detection of Al^{3+}, no fluorescence response at 455 nm is observed upon addition of other metal ions even with high concentration (50 µmol L^{-1}). Moreover, the mixture of Met-AuNCs, SA and Al^{3+} demonstrated almost no fluorescence variation against other metal cations. These results demonstrated that the simple D-π-A Schiff base probe exhibited a strong selectivity and could be as a fluorescent probe for sensing Al^{3+} over all other metal ions.

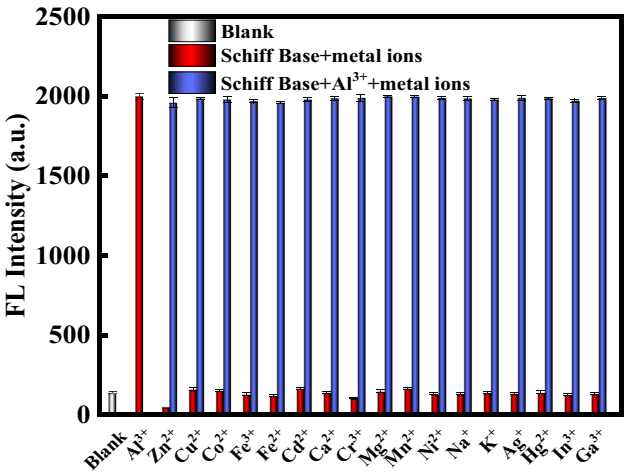


Fig. 4 Selectivity of the sensing assay for Al^{3+}. 100 µL Met-AuNCs and 94 nmol L^{-1} SA were used. Al^{3+} (10 µmol L^{-1}) and other interfering metal ions (50 µmol L^{-1}) were used

Real Sample Analysis

To evaluate the accuracy of the developed method, we applied it to the detection of Al^{3+} in lake water. The lake water was filtered through a 0.45 µm filter prior to measurement. Al^{3+} was spiked into the lake water at different concentrations. The results have been shown in Table 1. Satisfactory recoveries of the assay are 98–106% for Al^{3+}, which further indicate the suitability of the assay for the determination of Al^{3+} in real samples.

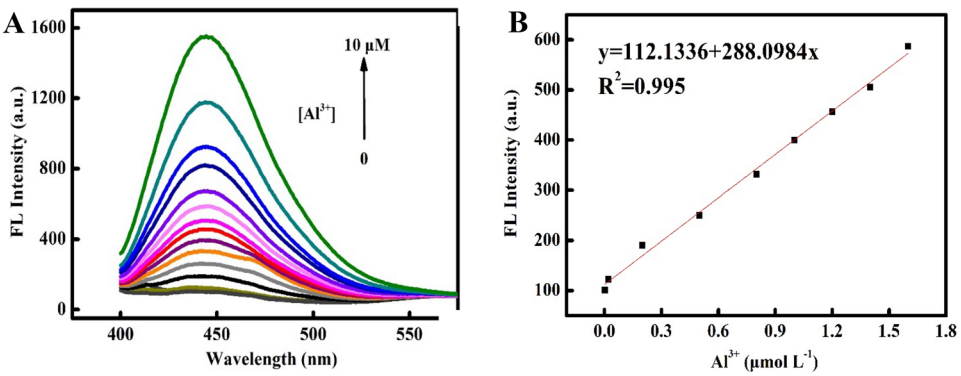


Fig. 3 **A** Fluorescence emission spectra of SA-Met-AuNCs upon the addition of Al^{3+} at various concentrations from 0 to 10 µmol L^{-1}. **B** Fluorescence intensity at 455 nm as a function of Al^{3+} concentration. 100 µL Met- AuNCs and 94 nmol L^{-1} SA were used

Table 1 Determination of Al^{3+} in lake water samples by the developed assay

Sample	Spiked (μM)	ICP (μM)	found (μM)	Recovery (%)	RSD (%) (n = 3)
Lake water	1.0	1.04	0.98	98	5.03
	0.5	0.51	0.53	106	3.75
Tap water	1.0	1.01	1.03	103	4.24
	0.5	0.49	0.48	96.0	3.85

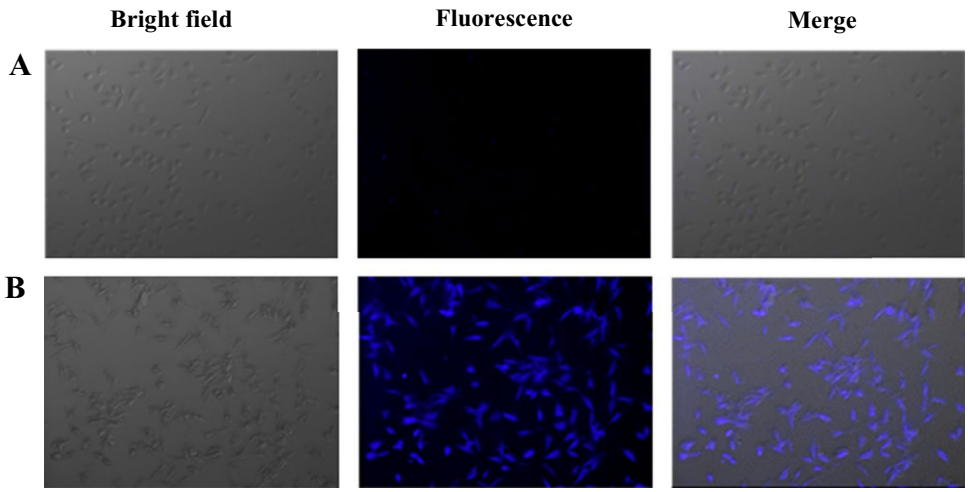


Fig. 5 Fluorescence images of SHSY5Y cells. **A** Control cells. **B** Cells treated with Met-AuNCs + SA + Al^{3+}. 100 μL AuNCs, 94 nmol L^{-1} SA and 1 μmol L^{-1} Al^{3+} were used

Cell Imaging

To investigate the biological applications of the strategy, cellular uptake and fluorescence imaging of SA-Met-AuNCs to detect Al^{3+} were conducted using SHSY5Y cells as a model. As displayed in Fig. 5A, the intracellular imaging of SHSY5Y cells display no fluorescence signals. However, the cells display a pronounced blue fluorescence image inside the cells (Fig. 5B), when SHSY5Y cells were pretreated with SA-Met-AuNCs and Al^{3+} (1 μmol L^{-1}) for 30 min under the same conditions. Hence, the results verify the internalization of the probe and its binding to Al^{3+}. Moreover, no morphological changes can be seen in cells after pretreatment with probe, which demonstrates that the probe has a good membrane permeability and can be applied to detection of Al^{3+} in living cells.

Conclusions

In summary, a microwave-assisted method was applied in the synthesis of Met-AuNCs, which dramatically improved the QY of AuNCs and shortened the reaction time. Remarkably, a new Schiff base was formed between the amino groups of Met on the as-prepared AuNCs surface and aldehyde groups of SA, which exhibited a highly selective fluorescence response to Al^{3+} in aqueous media. Moreover, it had a good cell permeability and low cytotoxicity, which made it possible to monitor intracellular Al^{3+} in the living cells with high selectivity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10895-022-03047-5.

Author Contribution All authors contributed to the study conception and design. Writing-original draft preparation, data collection and analysis were performed by [Fuming Sang]. Writing-original draft preparation, data collection and editing: [Tiedan Xiong]. Material preparation, data collection and analysis: [Weijie Wang]. Data collection and analysis: [Jianxin Pan]. Material preparation: [Huahua Shi]. Writing-review and editing: [Yan Zhao]. All authors read and approved the final manuscript.

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Data Availability All data generated or analyzed of this study are available within the article.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare that they have no conflicts of interests.

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