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摘要

The development of practical fluorescent probe for detecting toxic mercury ions (Hg²⁺) is desirable for environmental assurance and public health. In this study, a new red emissive fluorescent probe (KJL) was designed and synthesized for monitoring trace Hg²⁺ both in vitro and in vivo with distinct features including ideal response rate (within 4 min), red emission (596 nm), large Stokes shift (162 nm), highly sensitivity (LOD = 4.79 nM) and excellent specificity. KJL also validated the good capability for accurately monitoring trace Hg²⁺ levels in actual samples (faucet water, drinking water, river water, lake water, urine and serum) and possessed the eye-catching ability in visualization of Hg²⁺ under environmental/biological conditions, which revealed the great potential of this red-emitting fluorescent probe for practical applications in complex environmental and biological systems.

关键词

作者关键词: Mercury ion; Fluorescent probe; Red emission; Large Stokes shift; Actual samples

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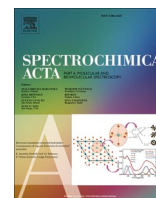
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Monitoring of mercury ion in environmental media and biological systems using a red emissive fluorescent probe with a large Stokes shift

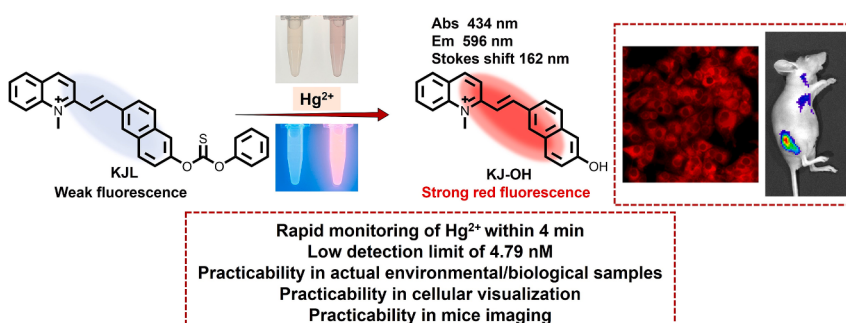
Juan Yin^{*}, Zejie Wu, Heng Li, Bianli Cao, Wanzhi Wang

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HIGHLIGHTS

- A Hg^{2+} -reacted fluorescent probe with red emission and large Stokes shift (162 nm) was developed.
- The probe possesses eye-catching capability, highly selectivity and excellent sensitivity (LOD = 4.79 nM).
- The probe can be applied for the directly detection of Hg^{2+} in environmental/biological samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

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ABSTRACT

The development of practical fluorescent probe for detecting toxic mercury ions (Hg^{2+}) is desirable for environmental assurance and public health. In this study, a new red emissive fluorescent probe (KJL) was designed and synthesized for monitoring trace Hg^{2+} both *in vitro* and *in vivo* with distinct features including ideal response rate (within 4 min), red emission (596 nm), large Stokes shift (162 nm), highly sensitivity (LOD = 4.79 nM) and excellent specificity. KJL also validated the good capability for accurately monitoring trace Hg^{2+} levels in actual samples (faucet water, drinking water, river water, lake water, urine and serum) and possessed the eye-catching ability in visualization of Hg^{2+} under environmental/biological conditions, which revealed the great potential of this red-emitting fluorescent probe for practical applications in complex environmental and biological systems.

1. Introduction

Mercury has been commonly applied in the field of agricultural and chemical industrial processes such as seed treatment, antiseptics in vaccines [1]. However, the highly toxicant mercury ions (Hg^{2+} , the main form of mercury) which primarily originates from various pathways including mining operation, fuel combustion, and municipal/

industrial wastewater discharges, could inevitably circulate into the food chain and eventually accumulated in human bodies, which could harm the organism health to cause some clinical illness (e.g., neurological disorder, liver/kidney injury, bloody diarrhea, endocrine dyscrasia) due to the strong binding affinity of Hg^{2+} with the sulphhydryl groups of bioactive species (metallothionein, erythrocytes, or amino acids) in the body [2–4]. Thus, Hg^{2+} has been identified as a serious

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threat to the environmental system and human health by the Environmental Protection Agency [5,6]. Therefore, the development of an effective method for monitoring Hg^{2+} is very important for the areas of environmental and biological sciences.

Benefiting from the favourable advantages including simplified operation, high temporal resolution, non-invasiveness, the fluorescent sensing technology has been considered as a reasonable tool for detecting various chemical/biological analytes [7–10]. Currently, the exploitation of fluorescent probes for Hg^{2+} has attracted considerable attention and some small-molecule fluorescent probes have been designed for Hg^{2+} detection [11–22]. However, most Hg^{2+} activatable fluorescent probes exhibit some shortcomings such as undesirable short emission wavelengths of less than 550 nm (blue or yellow signal), narrow Stokes shifts, inevitably causing lower detection limit (>2 ppb, 10 nM, the stipulated threshold level in drinking water by the U.S. Environmental Protection Agency), or high self-absorption effect and the interference of biological fluorescent background [11–18]. In addition, the monitoring strategies for Hg^{2+} fluorescent probes are generally based on the coordination categories of Hg^{2+} with heteroatom ligands, which are inevitably subjected to interference from other biological competing ions species, and thus could impair the specificity of fluorescent probes toward Hg^{2+} and induce improper detection signal in actual environmental/biological samples [11,12]. Therefore, it is still urgently demanded to construct a practical Hg^{2+} -reacted probe with long emission wavelength and large Stokes shift for selective/sensitive detection of Hg^{2+} amounts in actual ecology media.

Keeping above disadvantage in mind, in this work, a “turn-on” Hg^{2+} -specific red fluorescent probe **KJL** was designed for the rapidly and specifically detecting Hg^{2+} with quinolinium ramification (**KJ-OH**) as fluorescent signal reporter, and the phenyl thiochloroformate group as the Hg^{2+} -responsive unit and the signal quencher (Scheme 1). Probe **KJL** could monitor trace Hg^{2+} rapidly (in less than 4 min) to yield the fluorophore **KJ-OH**, giving a strong red fluorescent turn-on signal at 596 nm with a large Stokes shift of 162 nm, and also accompanying by additional desirable performance including excellent selectivity and sensitivity ($\text{LOD} = 4.79$ nM). Simultaneously, **KJL** possesses the outstanding capability in quantitatively detecting Hg^{2+} levels in various actual samples. More importantly, the great potential applicability of **KJL** was also demonstrated by applying this probe to detect varying amount of Hg^{2+} in cells and mice.

2. Experimental sections

2.1. Materials and methods

All commercial solvents and chemicals were obtained from commercial suppliers and applied without further treatment. The analytical results of nuclear magnetic data (^1H , ^{13}C) were recorded from Bruker spectrometer (500 MHz). High-resolution mass spectra were conducted on Bruker McriOTOF11 mass spectrometer. IR spectra (KBr disks) were obtained on a Bruker Tensor 27 spectrometer. Absorption spectrum and fluorescence emission spectra were measured by using Shimadzu UV-

1700 spectrophotometer and Hitachi F-7000 fluorescence spectrometer, respectively. The cellular fluorescent images were taken by using the Olympus confocal laser scanning microscope (FV3000-IX83). The *in vivo* fluorescent images of mice were obtained via the Caliper IVIS Lumina small animal optical *in vivo* imaging system.

2.2. Synthesis of the probe **KJL**

The synthetic route of the established synthetic procedures and the characterization data were described in Scheme S1 and Figs. S1–S10 in the Supporting Information.

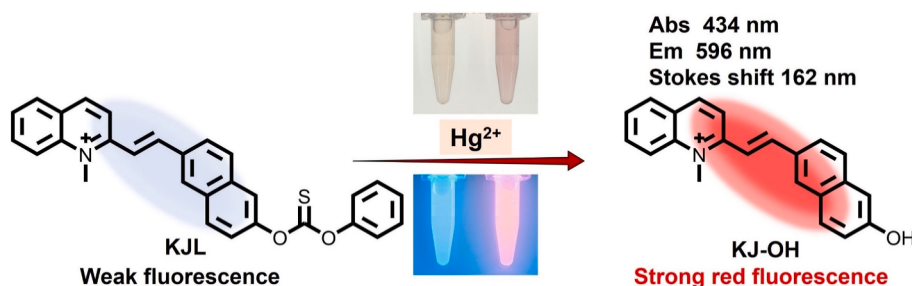
2.3. General procedure for fluorescence detection

The probe stock solution (5 mM) was obtained by dissolving **KJL** into DMSO. The stock solutions of Hg^{2+} (5 mM) and other relevant biological analytes (10 mM) including K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Al^{3+} , Ag^+ , Pd^{2+} , Cd^{2+} , Cl^- , Br^- , NO_3^- , NO_2^- , CO_3^{2-} , HCO_3^- , GSH, Hcy, Cys were dissolved in ultrapure water. Unless otherwise specified, the fluorescence measurements in test solution were performed with probe **KJL** (5 μM) in PBS buffer (5 % DMSO, 10 mM, pH = 7.4) after the treatment with appropriate amount of Hg^{2+} or other relevant species ($\lambda_{\text{ex}} = 441$ nm).

For quantificationally detecting Hg^{2+} in actual samples, the urine and blood samples were initially obtained from the BALB/c nude mice and the blood was centrifuged for 20 min at 3000 rpm in a refrigerated centrifuge to obtain the serum (the resultant supernatant). After that, the sample solutions of Hg^{2+} were afforded by mixing the different amounts of Hg^{2+} and appropriate volume of DMSO into the actual samples including above animal samples (urine and serum) and the collected water samples (faucet water, drinking water, river water) to afford the desired concentration of Hg^{2+} (1.5, 2, 3 μM , respectively). After that, the stock solution of **KJL** (5 mM, 1 μL) was added into the sample solutions (999 μL), and the resulting solution was shaken well for 5 min before monitoring the fluorescence spectra.

2.4. Cell culture and cell viability determination

HepG2 cells were grown at 37 °C in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum and placed in sterile incubator with 5 % CO_2 . The cellular cytotoxicity of probe **KJL** was evaluated by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded in flatbottom 96-well plate (1×10^4 cells/well) and cultured in DMEM for 24 h, before incubating with various concentration **KJL** for 24 h, including 0, 2.5, 5, 10, and 20 μM , respectively. 5.0 mg/mL MTT prepared in PBS (20 μL) was then added to each well and the microplate was incubated for 4 h, before the addition of DMSO (200 μL) for lysing the formazan crystals. After that, the absorbance density for assessing the cell viability was recorded on a microplate reader.



Scheme 1. Sensing mechanism of fluorescent probe **KJL** for monitoring Hg^{2+} .

2.5. Fluorescent detection of Hg^{2+} in cells

For evaluating the performance of probe **KJL** in detecting Hg^{2+} in cells. The HepG2 cells were incubated with different amounts of Hg^{2+} (0, 1.25, 2.5, 5 μM) for 15 min, and washed with fresh DMEM to remove extra Hg^{2+} . The cells were then successively treated with probe **KJL** (5 μM) for 15 min, and Hoechst 33342 (5 $\mu\text{g}/\text{mL}$) for 15 min to identify the cell nuclei before washing with PBS two times. Afterwards, the cells imaging was performed under a laser confocal microscope.

For flow cytometry analysis of Hg^{2+} in cells, the cells were cultured in 6-well microplates (5×10^4 cells/well) for 12 h and incubated with Hg^{2+} (0, 1.25, 2.5, 5 μM , respectively) for 15 min before treating with probe **KJL** (5 μM) for 15 min. After collection with trypsin treatment, the cells were centrifuged, diluted with PBS, and analyzed by Attune® NxT Acoustic Focusing Cytometry.

2.6. In vivo fluorescent analysis of Hg^{2+} in mice

All animal experiments of the BALB/c nude mice (5–6 weeks) were performed in accordance with the guidelines issued by The Ethical Committee of Sanquan College of Xinxiang Medical University. All procedures for *in vivo* studies were conformed by the Guide for the Care and Use of Laboratory Animals. The dorsal rear hindquarters of mice were subcutaneously injected with different concentrations of Hg^{2+} (0, 5, 15, 30 μM , respectively), and followed by the injection of probe solution (30 μM) at the same site. Afterwards, the mice were placed into the imaging chamber and the fluorescent imaging experiments were then carried out at the time points of 5 min or 10 min by using the IVIS spectrum imaging system.

3. Results and discussion

3.1. Rational design of probe **KJL** and the reaction mechanism elucidation

As shown in Scheme 1, for constructing the Hg^{2+} -responsive red-emitting fluorescent probe with improved performances, the electron-donating naphthol was connected to electron-accepting quinolinium by carbon-carbon unsaturated bonds to obtain the large conjugated structure of fluorophore **KJ-OH**, which would exhibit desirable properties including strong red fluorescence and large Stokes shift because of the push-pull electron property in the π - π conjugation structure of **KJ-OH**. Furthermore, the electron-withdrawing phenyl thiochloroformate motif (Hg^{2+} recognition site) was grafted to the naphthyl hydroxy group of **KJ-OH** as the responsive moiety of Hg^{2+} to construct probe **KJL** with a weak fluorescence because of the “caged” state of the push-pull electron system by the Hg^{2+} -recognitive moiety. It is expected that strong Hg -S binding property of Hg^{2+} could mediate the cleavage of the phenyl thiochloroformate group of **KJL** to release **KJ-OH**, thus restoring the turn-on red fluorescence output, which can be employed for detecting Hg^{2+} .

To confirm the above reaction mechanism proposed in Scheme 1, the reaction mixture of **KJL** to Hg^{2+} was characterized through HPLC and HRMS analysis. As shown in Fig. 1, the retention time peaks of **KJL** (50 μM) and **KJ-OH** (50 μM) were observed at 21.328 min, respectively. After probe **KJL** (50 μM) was reacted with 30 μM Hg^{2+} , the retention time peak (21.148 min) corresponding to **KJL** decreased, along with the obvious signal at 4.480 min ascribed to fluorophore **KJ-OH** appeared, indicating that the cleavage reaction induced by Hg^{2+} released fluorophore **KJ-OH**. In addition, after the treatment of **KJL** with Hg^{2+} , two major mass spectra peaks (m/z : 448.1046, 312.1452) which were consistent with the peaks of **KJL** and **KJ-OH** were detected clearly, also confirming the generation of fluorophore **KJ-OH** (Fig. S11). Above results demonstrated that Hg^{2+} -mediated cleavage reaction could remove the phenyl thiochloroformate group of **KJL** to release the highly fluorescent **KJ-OH** accompanied by the turn-on fluorescence that can be

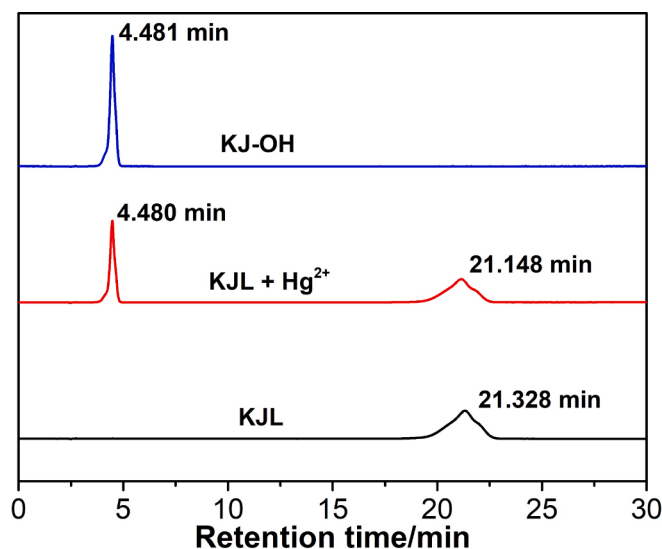


Fig. 1. HPLC chromatogram curve of fluorophore **KJ-OH** (50 μM), probe **KJL** (50 μM) and the reaction product of **KJL** (50 μM) toward Hg^{2+} (30 μM). Mobile phase system: acetonitrile/ H_2O ($v/v = 6/4$).

used to monitoring the Hg^{2+} levels.

3.2. Spectral response properties, sensitivity and selectivity of **KJL** to Hg^{2+}

The optical sensing behavior of probe **KJL** towards Hg^{2+} was evaluated in 10 mM PBS buffer (mixed with 5 % DMSO) at physiological pH. As shown in Fig. 2A–B, probe **KJL** solution exhibited the maximum absorption at 401 nm, and a weak fluorescence emission signal ($\Phi_F = 0.011$ in PBS solution). The response of the probe to Hg^{2+} significantly redshift the absorption peak to 434 nm and the fluorescence signal intensity of **KJL** at 596 nm increased significantly after the reaction toward Hg^{2+} ($\Phi_F = 0.089$ in PBS solution), indicating that Hg^{2+} induced Hg -S reaction effect enables the cleavage of the responsive moiety of **KJL** to produce fluorophore **KJ-OH** with obvious red fluorescent signal. Interestingly, the color of the probe solution obviously changed from faint yellow to pink under daylight, and also transformed from light blue to bright red under 365 nm light irradiation (Fig. 2C), clearly demonstrating the applicability of **KJL** for readily colorimetric detecting Hg^{2+} by the naked-eye. Furthermore, the turn-on red emission of **KJL** towards Hg^{2+} with large Stokes shift (162 nm) could enhance the detection accuracy of **KJL** because of the substantially reduction of the measuring error induced by self-absorption effect and auto-fluorescence [23–25]. Subsequently, the response time of probe **KJL** was investigated by recording the time-dependent fluorescence signal changes of **KJL** with Hg^{2+} . As shown in Fig. 2D, the addition of Hg^{2+} induced the rapid fluorescent response, the emission intensity enhancement at 596 nm saturated nearly within 4 min, indicating the superior sensing speed for fast visual monitoring Hg^{2+} .

Subsequently, the sensitivity of probe **KJL** for Hg^{2+} was investigated by the treatment with various concentrations of Hg^{2+} (0–5 μM). As shown in Fig. 2E, the addition of increasing concentration of Hg^{2+} caused the dose-dependent emission signal enhancement of probe solution. Notably, the fluorescence intensity at 596 nm showed a good linearity ($R^2 = 0.9922$) toward the rise of Hg^{2+} concentration. The estimated detection limitation (LOD) was calculated to be as low as 4.79 nM based on the formula $\text{LOD} = 3\delta/k$ [26,27], which demonstrated that **KJL** could detect Hg^{2+} quantitatively with higher sensitivity compared with most reported Hg^{2+} -activated probes [11–16].

The influence of pH on probe **KJL** before and after reacting with Hg^{2+} was then evaluated through monitoring the sensing emission intensity of

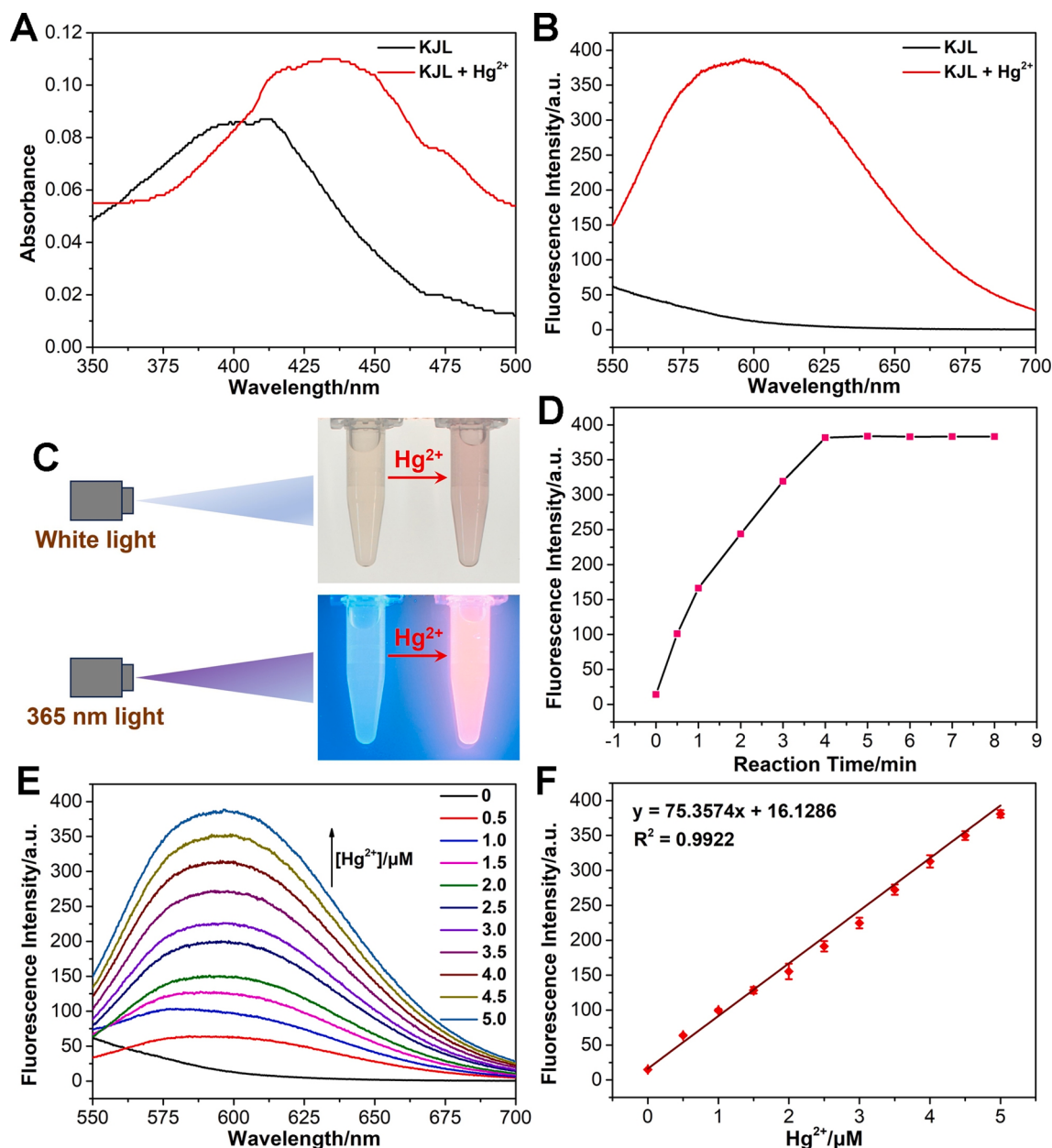


Fig. 2. Spectral profiles of probe **KJL** toward Hg^{2+} in PBS buffer (10 mM, 5 % DMSO, pH 7.4). (A, B) UV-vis and fluorescence spectral changes of **KJL** (5 μM) in the absence and presence of Hg^{2+} (5 μM). (C) The corresponding color images of probe solution under visible light or UV light (365 nm). (D) Fluorescent emission intensity (596 nm) of **KJL** over time (0–8 min) upon incubation with Hg^{2+} (5 μM), excitation = 441 nm. (E) Fluorescent titration spectra of **KJL** (5 μM) after treating with different concentration of Hg^{2+} (0–5 μM), excitation = 441 nm. (F) Linear emission intensity (596 nm) changes of **KJL** (5 μM) responding to different concentration of Hg^{2+} from 0 to 5 μM . Error bars represent $\pm$ S.D. ($n = 3$).

test solutions at different pH conditions (4–9). As shown in Fig. S12, **KJL** possessed feeble fluorescence at wide pH range, while the fluorescent intensity of **KJL** increased sharply and achieved the excellent signal at physiological pH range after the treatment with Hg^{2+} , suggesting the good potential of probe **KJL** for tracking Hg^{2+} in biological system.

The specificity of Hg^{2+} detection in complicated environment was further confirmed by evaluating the selectivity of **KJL** toward Hg^{2+} . As shown in Fig. 3A, after treating **KJL** with various biologically relevant analytes (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Al^{3+} , Ag^+ , Pd^{2+} , Cd^{2+} , Cl^- , Br^- , NO_3^- , NO_2^- , CO_3^{2-} , HCO_3^- , GSH, Hcy, Cys), negligible fluorescent intensity change of **KJL** can be caused by the other relevant species, only Hg^{2+} induced obvious enhancement of emission intensity of **KJL**, accompanied by a remarkable color change via naked eye detection (Fig. 3C). The results revealed that **KJL** holds superior selectivity for

Hg^{2+} detection and could also qualitatively distinguish Hg^{2+} by the naked-eye in the complicated environmental system. In addition, the competition assessment of **KJL** toward Hg^{2+} also displayed that the presence of other analytes was not affect the fluorescent response of probe **KJL** for Hg^{2+} (Fig. 3B), indicating the probe's good anti-interference ability. The above results indicated that **KJL** possesses the excellent specificity toward Hg^{2+} with the good applicability for tracking Hg^{2+} in environmental/biological samples.

3.3. Practical application of **KJL** in actual samples

The highly toxic Hg^{2+} residues inevitably cause severe environmental pollution, along with a seriously threaten to human health. Therefore, we further evaluated the practical capability of probe **KJL**

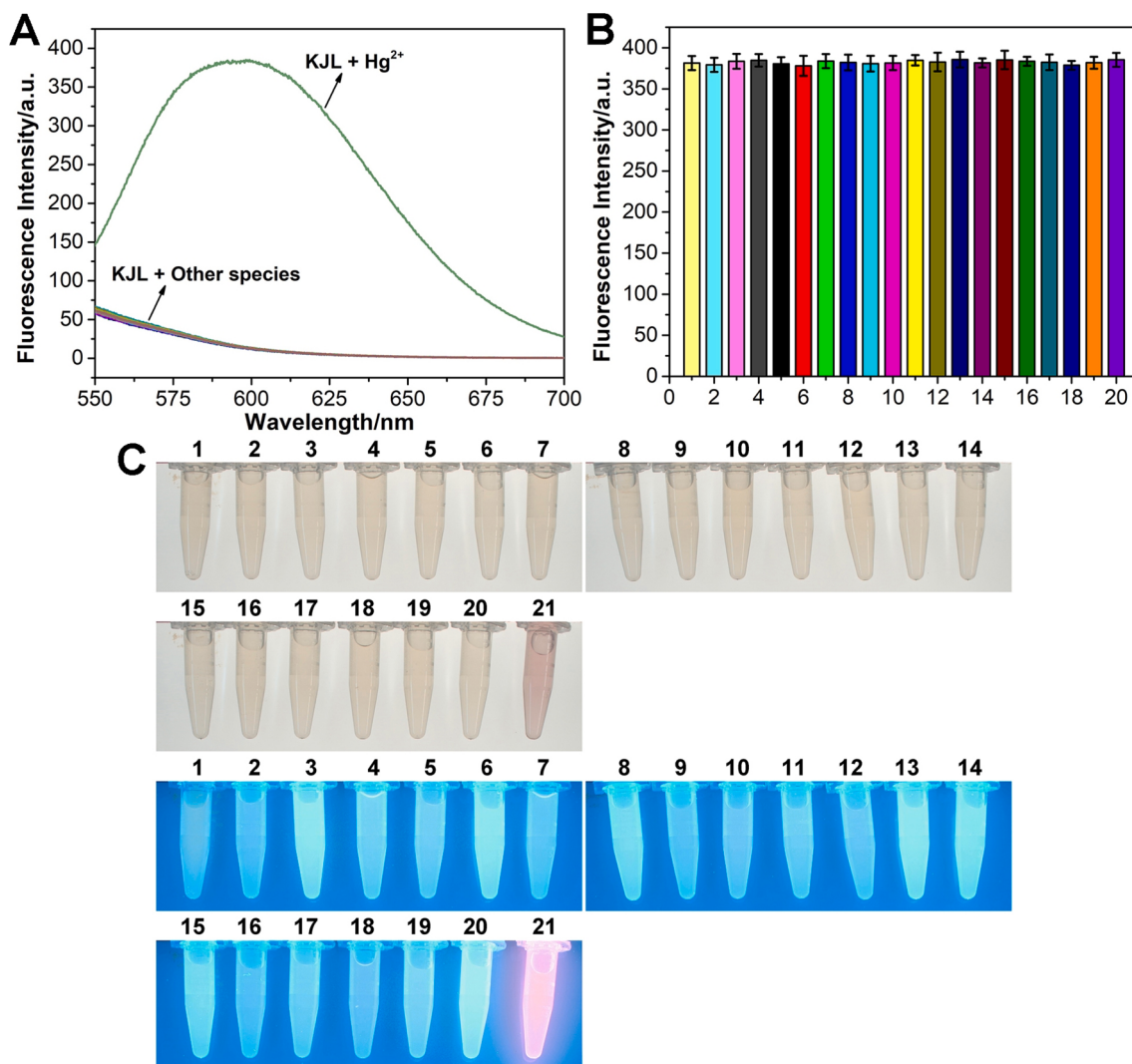


Fig. 3. (A) Fluorescent spectra responses of probe **KJL** (5 μM) responding to Hg^{2+} (5 μM) or other biologically relevant species (10 μM) in PBS buffer (10 mM, pH 7.4) with 5 % DMSO. (B) Fluorescence emission intensity changes of **KJL** (5 μM) at 596 nm upon incubation with Hg^{2+} (5 μM) and various species (10 μM). 1: Blank; 2–20: K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Al^{3+} , Ag^+ , Pd^{2+} , Cd^{2+} , Cl^- , Br^- , NO_3^- , NO_2^- , CO_3^{2-} , HCO_3^- , GSH, Hcy, Cys. Error bars represent $\pm$ S.D. ($n = 3$). (C) The corresponding photographs of probe solution after treating with Hg^{2+} (5 μM) or various analytes (10 μM) as noted in image A under the excitation at ambient light or 365 nm UV light, 1: Blank; 2–20: K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Zn^{2+} , Al^{3+} , Ag^+ , Pd^{2+} , Cd^{2+} , Cl^- , Br^- , NO_3^- , NO_2^- , CO_3^{2-} , HCO_3^- , GSH, Hcy, Cys; 21: Hg^{2+} .

toward Hg^{2+} by measuring the amounts of Hg^{2+} in environmental/biological samples including water samples (faucet water, drinking water, river water), and animal samples (urine and serum of mice) which were individually spiked with varying concentration of Hg^{2+} (1.5, 2, and 3 μM). As shown in Table 1 and Fig. S12, the detection results were consistent with the actual quantities of Hg^{2+} , and the corresponding recovery rate was found to be the acceptable range of 97.99–101.90 %. These results demonstrated that probe **KJL** could be applied as a suitable tool for quantitative detecting trace Hg^{2+} in actual environmental and biological samples with satisfying accuracy.

3.4. Fluorescent detection of Hg^{2+} in cells and in vivo

Encouraged by the good spectral performance of probe **KJL** toward Hg^{2+} in actual samples. The potential application of **KJL** for detecting Hg^{2+} was further assessed in living biological systems (cells and mice). Prior to cell imaging, the MTT assays toward HepG2 cells were initially applied to determine the biocompatibility and cytotoxicity of **KJL**. As shown in Fig. S13, the viability rate of HepG2 cells still exceeded 85 % after the 24 h treatment with the concentration of **KJL** up to 20 μM , indicating the good biocompatibility and low cytotoxicity of **KJL**.

Table 1

Detecting of Hg^{2+} amounts in actual water and mice urine/serum samples by using probe **KJL**.

Samples	Hg^{2+} added (μM)	Hg^{2+} detected (μM)	Recovery (%)
Faucet water	1.50	1.47 ± 0.07	97.99
	2.00	1.98 ± 0.12	99.05
	3.00	3.02 ± 0.10	100.58
Drinking water	1.50	1.53 ± 0.05	101.90
	2.00	2.02 ± 0.12	100.99
	3.00	3.02 ± 0.14	100.83
Lake water	1.50	1.48 ± 0.09	98.78
	2.00	1.97 ± 0.13	98.42
	3.00	2.98 ± 0.12	99.30
Urine	1.50	1.53 ± 0.07	101.88
	2.00	1.92 ± 0.15	95.84
	3.00	2.97 ± 0.08	99.20
serum	1.50	1.47 ± 0.11	98.36
	2.00	1.98 ± 0.14	98.86
	3.00	2.96 ± 0.09	98.78

Subsequently, the cellular imaging was explored after the HepG2 cells were treated with different concentrations of Hg^{2+} (0, 1.25, 2.5, 10 μM) for 15 min, and probe **KJL** (5 μM) for 15 min. As shown in Fig. 4, the fluorescent signal intensity of the cells was dose-dependent enhanced with the increasing Hg^{2+} concentrations, demonstrating the feasibility of **KJL** for visualizing Hg^{2+} in cells. Moreover, similar fluorescent test results were also observed in flow cytometry analysis (Fig. 5), the cells pretreated with increasing concentration of Hg^{2+} also showed a gradually increased signal intensity, also demonstrating that **KJL** could be used for detecting Hg^{2+} in cells by monitoring the fluctuation of Hg^{2+} -induced turn-on fluorescent signal.

We continued to investigate the capability of probe **KJL** for *in vivo* fluorescent imaging Hg^{2+} in mice. After the administration of different concentration of Hg^{2+} (15 min, 0, 5, 15, and 30 μM , respectively) in PBS (100 μL) and probe **KJL** (100 μL , 30 μM) into the rear legs of the mice through subcutaneous injection, the fluorescent images were obtained and quantified by the IVIS spectral imaging system. As shown in Fig. 6, weak fluorescence signal was detected in the control mice treated with **KJL** only, while the signal intensity of the mice injected with Hg^{2+} and **KJL** demonstrate a dose-dependent and time-dependent manner enhancement,

suggesting the good ability of **KJL** for detecting Hg^{2+} *in vivo*. Therefore, these results collectively indicate that probe **KJL** is an effective probe for visualizing and monitoring Hg^{2+} in biological systems.

4. Conclusions

In summary, a new Hg^{2+} -responsive fluorescent probe (**KJL**) was reasonable designed for detecting Hg^{2+} in environmental samples and biological contexts. **KJL** can be activated by Hg^{2+} to exhibit obvious red fluorescence signal (596 nm) and a large Stokes shift (162 nm), as well as fast response time (with 4 min), highly sensitivity (LOD = 4.79 nM) and excellent selectivity toward Hg^{2+} over other potential biological analytes. Additionally, **KJL** is well suitable for naked-eye detecting Hg^{2+} with obvious color change under the administration of daylight or UV light (365 nm). Furthermore, **KJL** could serve as an excellent tool to quantitatively monitor Hg^{2+} levels in environmental samples (faucet water, drinking water, river water), and biological samples (mice urine and serum). More importantly, the successful application of probe **KJL** for the visualization of varying amounts of Hg^{2+} in living cells and mice also confirms the potential practicability of **KJL** in biological/

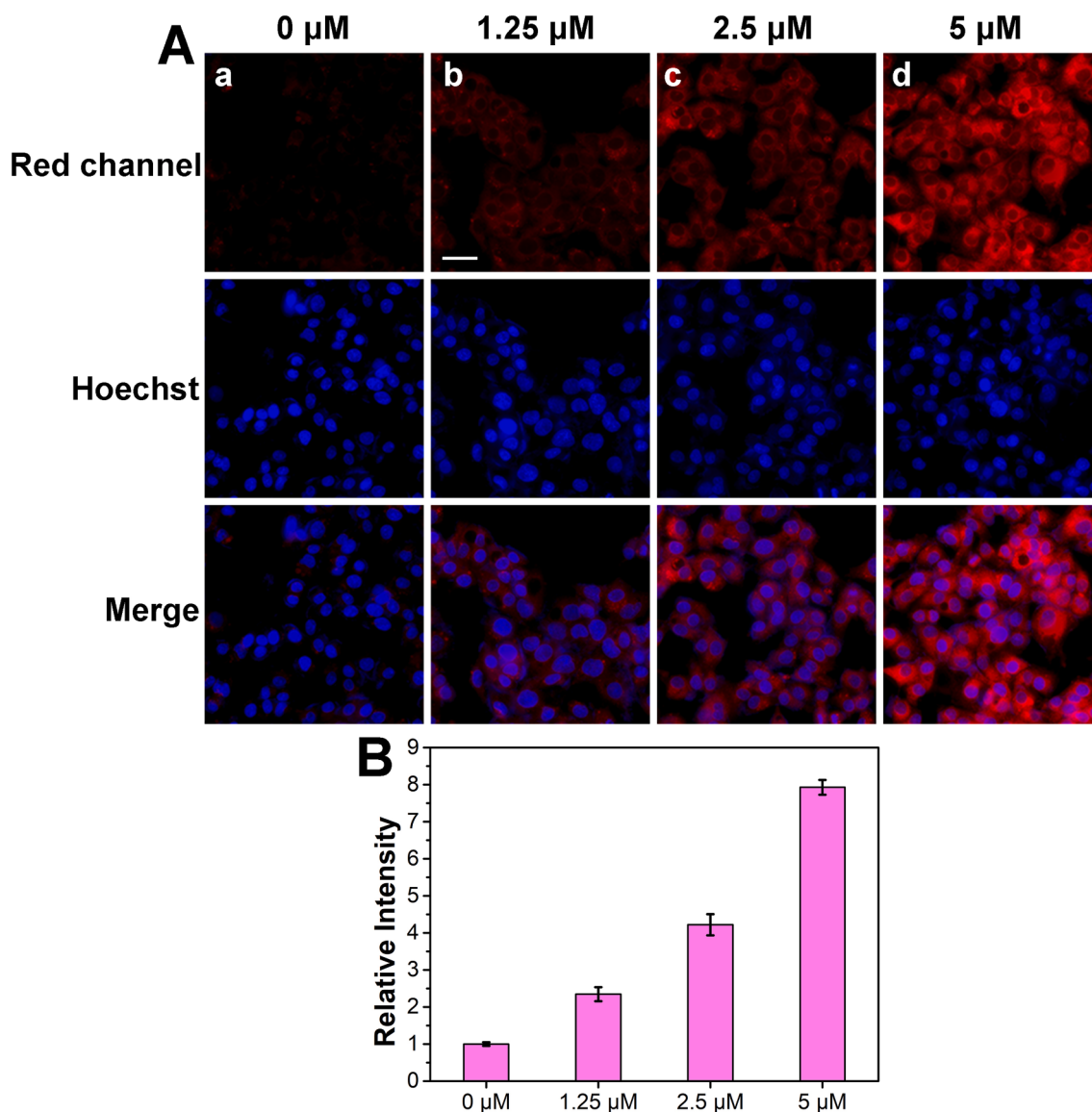


Fig. 4. Fluorescent imaging of probe **KJL** responding to Hg^{2+} in HepG2 cells. (A) The cells were stained with different concentration of Hg^{2+} (0, 1.25, 2.5, 5 μM) for 15 min, and then incubated with **KJL** (5 μM) and hoechst 33342 (5 $\mu\text{g/mL}$, for identifying the cell nuclei) for 15 min before imaging. Scale bar = 40 μm . (B) Quantified fluorescence intensity of cells as described in image A. Data represent average values of three independent measurements.

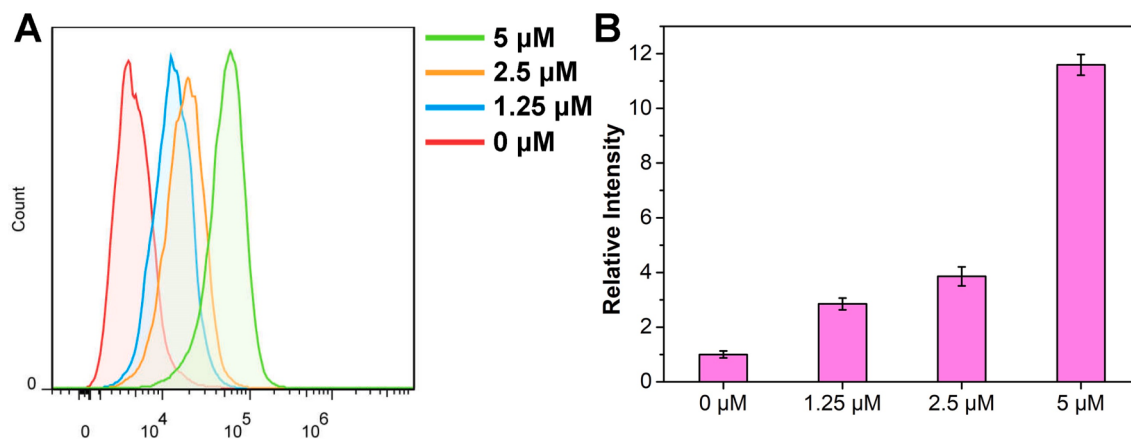


Fig. 5. Flow cytometry analysis of HepG2 cells that were pretreated respectively with different concentration of Hg^{2+} (0, 1.25, 2.5, 5 μM) for 15 min, and subsequently treated with 5 μM probe KJL for 15 min. (B) The relative intensity of the cells treated under the conditions as noted in image A. Data represent the standard deviation from three experiments.

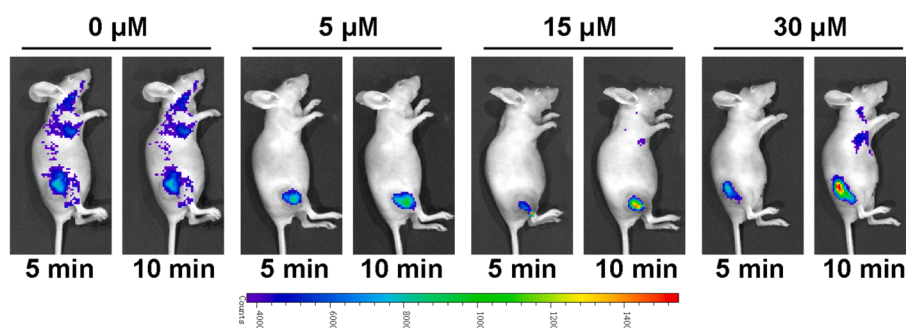


Fig. 6. *In vivo* fluorescent imaging of living mice at different time points (5 min, 10 min) upon subcutaneous injection of Hg^{2+} (0, 5, 15, 30 μM , respectively) and followed by infection of probe KJL (30 μM).

environmental areas.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.saa.2024.125272>.

Data availability

Data will be made available on request.

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