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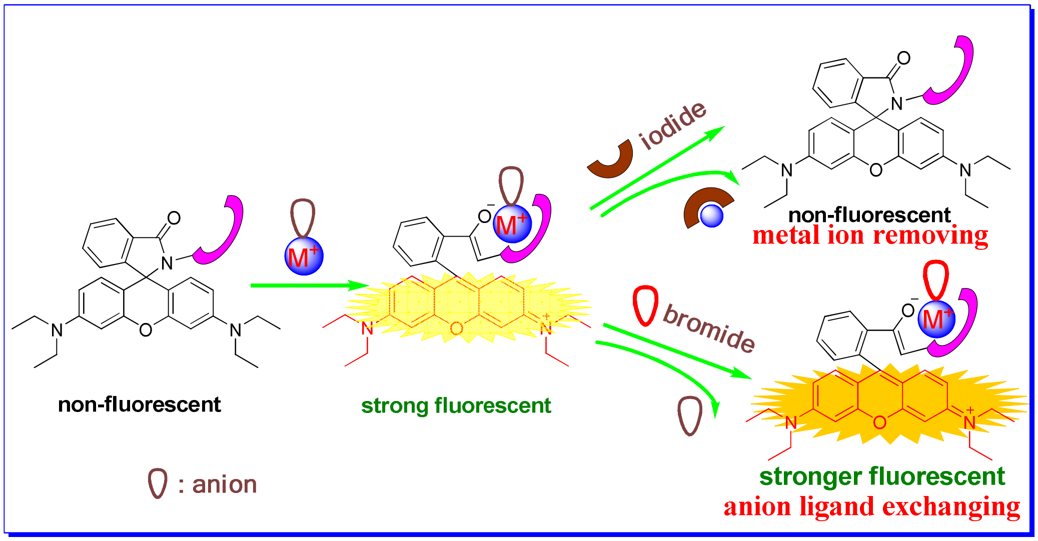

A new rhodamine-based fluorescent probe **RSP** for Hg^{2+} was facilely synthesized. Interesting, **RSP**-Hg-ClO_4 ensemble can serves as a fluorescent probe for iodide and bromide by metal ion removing and anion ligand exchanging, which was a novel rhodamine-based fluorescence sensing mechanism.

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LETTER

Fluorescence sensing of iodide and bromide in aqueous solution: anion ligand exchanging and metal ion removing

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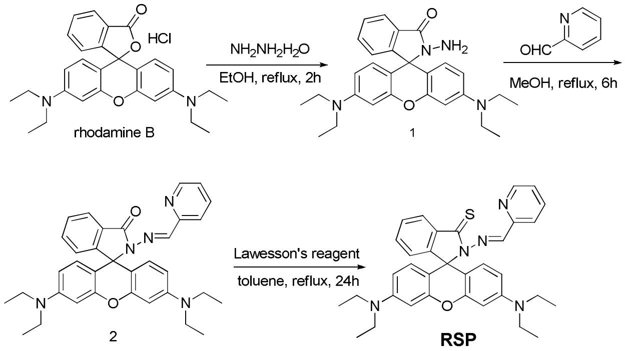
A new rhodamine-based fluorescent probe RSP was facilely synthesized. Interesting, RSP-Hg-ClO$_4$ ensemble can serve as a selective fluorescent probe for iodide and bromide base on metal ion removing and anion ligand exchanging, which was a novel rhodamine-based fluorescence sensing mechanism. Moreover, RSP can recognise Hg^{2+} selectively in 99% aqueous solution.

The specific detection of anions is an active field of research because of their important roles in biological, industrial and environmental processes.[1] Among anions, iodide is of particular interest due to its essential role in normal growth, development and functioning of brain and body.[2] Abnormal iodide level in the thyroid gland can lead to major health concerns. In addition, elemental iodine is frequently involved in organic synthesis, including medicine and dyes.[3]

Along with iodide, bromide is widely present in either natural environment or in biological fluids such as saline, salt well, urine, serum and saliva of organism.[4] Bromide plays important roles in the areas of environmental, biological and chemical systems.[5] However, chronic toxicity from bromide can cause bromism, bromide toxicity can also lead to a type of skin eruption.[6] Therefore, there is a great need of quantification and monitoring iodide and bromide.

To date, fluorescent sensing techniques enabling to the determination of iodide and bromide are of considerable significance owing to their high sensitivity, simplicity and real-time detection. Nevertheless, relatively few examples of fluorescent sensors for iodide and bromide were reported[7] based on two recognition strategies including hydrogen-bonding and metal-coordination. Since iodide and bromide have the large ionic radius, low charge density and low hydrogen bonding ability, it is challenging to develop specific probes for either entities based on hydrogen-bonding. Therefore, we plan to untilize the latter binding strategy for iodide and bromide probe development.

In this paper, we report a new rhodamine derivative **RSP**, which was facilely synthesized from rhodamine B through three steps (see Scheme 1). Rhodamine B was chosen as fluorophore by virtue of its excellent photophysical properties such as good photostabilty, long-wavelength absorption and emission.[8] Besides, it is a convenient platform to construct colorimetric "naked eye" and/or fluorescence "off-on" probes taking advantage of the opening of the spirolactam ring. The structure of **RSP** was verified by ^{1}H NMR, ^{13}C NMR and HRMS (ESI, Fig. S1).



Scheme 1 Synthesis of **RSP**

As a rhodamine B derivative with a spirolactam group, the free **RSP** remained nearly colorless in ethanol-water (40:60, v/v, 10 mM Tris-HClO$_4$, pH 7.4) system. Upon addition of 1 equiv. of Hg(ClO$_4$)$_2$ to a solution of **RSP**, the absorption at around 563 nm and fluorescence intensity at 592 nm were significantly enhanced, along with an obvious color change from colorless to purple, this indicated the opening of the spirolactam ring (Fig. S3, ESI). We then studied the selectivity of **RSP**-Hg^{2+} ensemble towards various anions. Upon addition of 20 equiv. of various anions to the solutions of **RSP**-Hg^{2+} ensemble, respectively, only iodide caused significantly decreasing of the absorption at around 563 nm and the fluorescence at 592 nm along with the color change from purple to nearly colorless instantaneously (Fig. 1), ATP and PPi induced a slight quenching of fluorescence intensity, however less than 30% of drop caused by iodide. Interesting, bromide caused about 40% increase of the fluorescence at 592 nm. Therefore, **RSP-** Hg^{2+} ensemble is a selective colorimetric "naked eye" and fluorescence "on-off" probe for iodide in aqueous solution, further, RSP-Hg^{2+} ensemble can also be used as a selective fluorescence probe for bromide.

The UV-vis and fluorescence titration was carried out by gradual addition of various concentrations of I$^-$ and Br$^-$ (Fig. 2; Fig. S4, ESI). Upon addition of 0~2 equiv. of I$^-$, caused the increasing of the absorption at around 563 nm and the fluorescence at 592 nm. If additional I$^-$ was added, significantly decreasing of the absorption and the fluorescence were observed. However, upon addition of Br$^-$, the fluorescence at 592 nm was

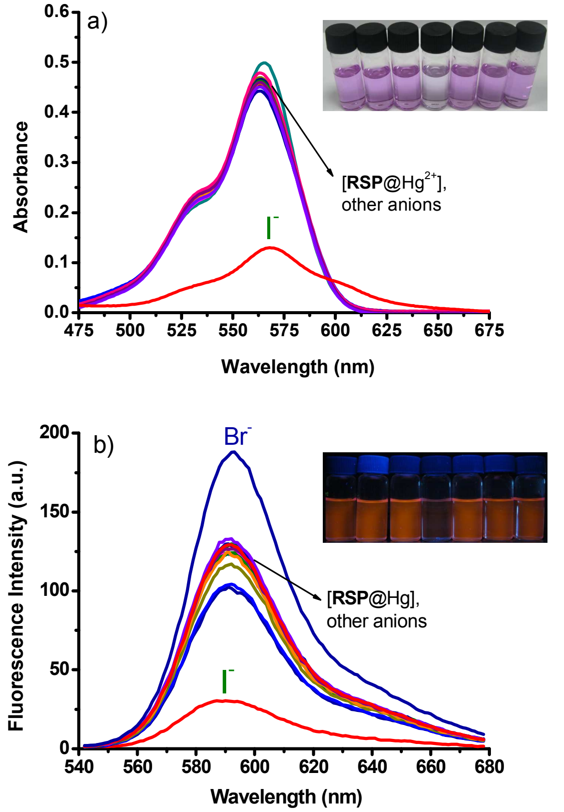


Fig. 1 The absorption (a) and fluorescence spectra (b) of RSP-**Hg^{2+}** (10 μM **RSP** and 10 μM Hg^{2+}) in the presence of 200 μM of various anions as F$^-$, Cl$^-$, Br$^-$, I$^-$, ATP, AMP, PPi, $H_2PO_4^-$, PO_4^{3-}, OH$^-$, AcO$^-$, SO_4^{2-}, HCO_3^-, NO_3^-, HPO_4^{2-} in Tris-HClO$_4$ aqueous buffer (10 mM, pH 7.4, containing 40% ethanol as a co-solvent). (λ_{ex} = 530 nm; slit widths: 2.5 nm, 5 nm). Inset: The photographs showed (a) the color change and (b) the fluorescent change (irradiated at 365 nm) of [**RSP@**Hg^{2+}] (10 μM **RSP** and 10 μM Hg^{2+}) in the presence of 200 μM of various anions. From left to right: RSP-**Hg^{2+}**, ATP, PPi, I$^-$, Br$^-$, OH$^-$, SO_4^{2-}.

increased untill to equilibrium.

To further test the interference for other anions on the determination of I$^-$, the competition experiments were carried out. Fig. S5 indicated that all other anions show no obvious interference for I$^-$ detection.

A tentative detection mechanism was proposed to explain the aforementioned results and shown in Fig. 3. Upon addition of 1 equiv. of Hg(ClO$_4$)$_2$ to the solution of **RSP**, significant enhancement in fluorescence intensity was observed (Fig. 4). Clearly, coordination of the **RSP** to the Hg^{2+} induced the opening of the five-membered spirolactam ring. Hg^{2+} is a heavy metal ion and known to quench the fluorescence. However, it is obvious that this quenching effect[9] is overwhelmed by the drastic fluorescence enhancement by the formation of a strongly fluorescent xanthene moiety. The fourth coordination site of Hg^{2+} was occupied by ClO_4^- as evidenced by the ESI-MS (ESI, Fig. S11). Addition of the Br$^-$ to the solution of **RSP**-Hg(ClO$_4$)$_2$ leads to further enhancement in fluorescence intensity and the ligand exchanges from ClO_4^- to Br$^-$ was proved by ESI-MS (ESI, Fig. S11). Similar to Br$^-$, upon introduction of 2 equiv. of I$^-$ to the solution of **RSP**-Hg(ClO$_4$)$_2$, further enhancement in fluorescence intensity was also observed, it might be also attributed to the ligand exchanges from ClO_4^- to I$^-$. Addition of another 2 equiv. of I$^-$ to the solution of **RSP**-Hg(ClO$_4$)$_2$, further enhancement of fluorescence was observed. It is likely that the heavy-atom effect of Hg^{2+} was partly inhibited due to Hg^{2+}→ I$^-$ back-bonding, which is not present between Hg^{2+} and ClO_4^-. Recently, Wong[9] reported a probe, upon Hg^{2+} ion binding, the fluorescence increased but also little quenching due to the heavy-atom effect of Hg^{2+}. Further addtion of I$^-$ beyond 2 equiv. leads to the extrapolation of Hg^{2+} from the RSP-**Hg^{2+}** complex and hence fluorescence intensity of the solution decreases due to the restoration of the non-fluorescent lactam form, whose formation was confirmed by ESI-MS. However, addition of excess Br$^-$ up to 20 equiv. was not able to remove Hg^{2+} from the RSP-**Hg^{2+}** ligand, this is in agreement with the more poor affinity of Br$^-$ to Hg^{2+} than I$^-$. This is also verified by the obersvation that addition of Br$^-$ up to 5 equiv. to the solution of [**RSP**+Hg^{2+}+I$^-$] nearly did not result in any fluorescence change (ESI, Fig. S7). This is an interesting reslut that addition of anion to the sensor-metal ion-anion ensemble lead to the further increasing of fluorescence intensity based on anion exchanging mechanism (Scheme 2).

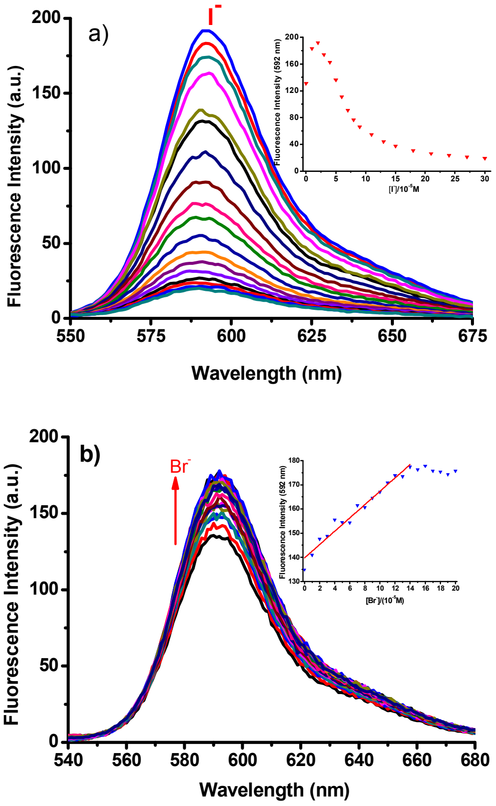


Fig. 2 The fluorescence spectra of (a) RSP-**Hg^{2+}** (10 μM **RSP** and 10 μM Hg^{2+}) upon addition of I$^-$ (10~300 μM) and (b) fluorescence spectra of RSP-**Hg^{2+}** (10 μM **RSP** and 10 μM Hg^{2+}) upon addition of Br$^-$ (10~200 μM) in Tris-HClO$_4$ aqueous buffer (10 mM, pH 7.4, containing 40% ethanol as a co-solvent). (λ_{ex} = 530 nm; slit widths: 2.5 nm, 5 nm). Inset: Fluorescence intensity at 592 nm of RSP-**Hg^{2+}** as a function of (a) [I$^-$] and (b) [Br-].

Generally, addition of metal ion to the solution of free rhodamine-based probe, the fluorescence intensity was significantly enhanced, further additon of anion or small molecular which has stronger binding capacity of the metal ion to the ensemble solution induced the metal ion removal from the rhodamine-metal ion ensemble, concomitantly, fluorescent intensity of the solution return to be weak. However, in this work, addition anion to the rhodamine-metal ion ensemble induced stronger fluorescent base on anion exchanging, it may be could serve as a novel approach for designing of rhodamine-based fluorescent probe for anion (Scheme 2).

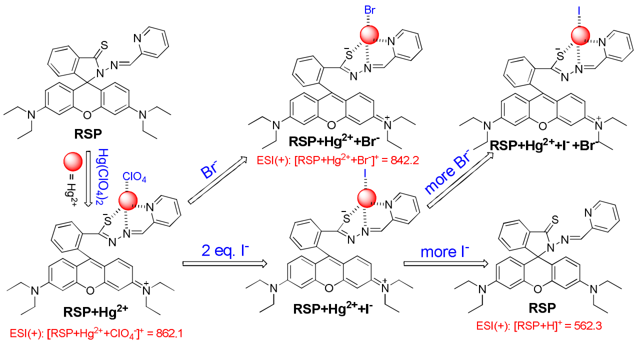


Fig. 3 Proposed binding mode of [**RSP**@Hg^{2+}] with Br$^-$ and I$^-$.

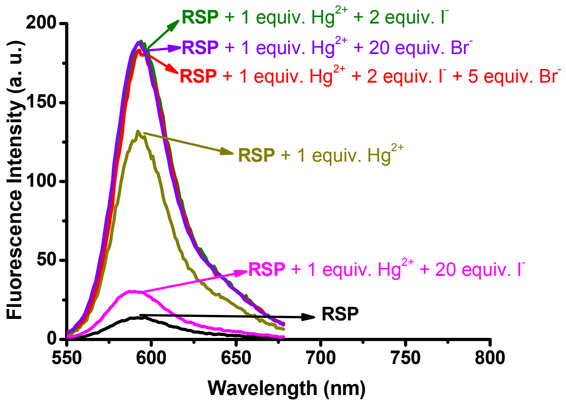
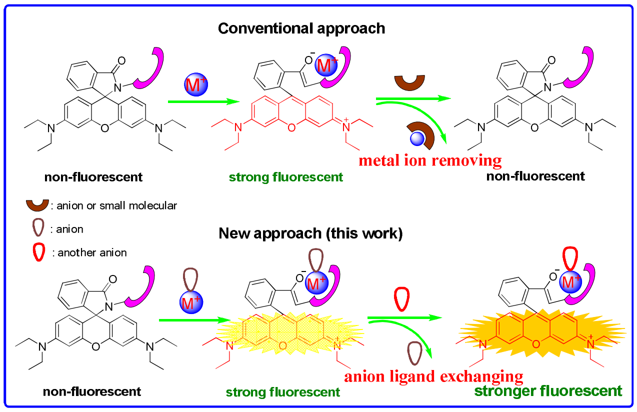


Fig. 4 The fluorescence spectra of **RSP** (10 μM) in the presence of various ions in Tris-HClO$_4$ aqueous buffer (10 mM, pH 7.4, containing 40% ethanol as a co-solvent). (λ_{ex} = 530 nm; slit widths: 2.5 nm, 5 nm).

To develop more wider practice of **RSP**, the selectivity of **RSP** to metal ions in water (10 mM HEPES, pH 7.4) was also measured. As shown in Fig. S8, upon addition of Hg^{2+} to a solution of **RSP**, the absorption at around 563 nm and the fluorescence at 592 nm were significantly enhanced along with the color change from nearly colorless to pink instantaneously, presumably as a result of the spirolactam ring-opened **RSP**. By contrast, the other ions (even excess) had almost no influence on the fluorescence spectra. Therefore, **RSP** was a highly selective fluorescence "off-on" probe for Hg^{2+} in 99% aqueous solution.

In conclusion, we have reported a fluorescent probe **RSP**, which could sensitively and selectively recognize Hg^{2+} in 99% aqueous solution. Interestingly, Based on the RSP-Hg-ClO$_4$ ensemble, addition of iodide less than 2.0 equiv. induced the fluorescence further increased and more iodide induced the fluorescence quenching selectively by removing of Hg^{2+} from the RSP-Hg-ClO$_4$ ligand, however, addition of bromide induced the fluorescence further increased by exchanging of anion ligand from ClO$_4$$^-$ to Br$^-$. Therefore, RSP-Hg-ClO$_4$ ensemble can serves as a selective fluorescent probe for iodide and bromide in aqueous solution. Rational analysis of the sensing mechanism of **RSP**-Hg^{2+} ensemble to iodide and bromide based on anion exchanging and metal ion removal was given. This sensing mechanism may be could serve as a novel approach for designing of rhodamine-based fluorescent probe for anion.



Scheme 2 Conventional approach and new approach in this work

Experimental

Synthesis of chemosensor RSP

Rhodamine B hydrozide (1): In this work, **1** was synthesized according to the reference.[10] To a 50 mL flask, rhodamine B (480 mg, 1.0 mmol) was dissolved in 12 mL ethanol. 1.2 mL (excess) hydrazine hydrate (85%) was then added. After the addition, the mixture was heated to reflux for 2 h. The solution changed from dark purple to light orange and became clear. Then the mixture was cooled and solvent was removed under redured pressure. 1 M HCl (about 20 mL) was added to the solid in the flask to generate a clear red solution. After that, 1 M NaOH (about 28 mL) was added slowly with stirring until the pH of the solution reached 9~10. The resulting precipatate was filtered and washed 3 times with 6 mL water, and then dired in air. The product was then chromatographed on silica gel using dichloromethane/methanol 30: 1 (v/v) as eluant to afford 370 mg (81%) **1** as pink solid.

2: Compound **1** (0.64 mmol, 300 mg) was dissolved in 10 mL absolute methanol, then 2-pyridinecarbaldehyde (0.64 mmol, 68 mg) was added. And then the mixture was heated to reflux and stirred for 6 h. After that, white precipitates obtained were filtered off, washed with methanol to give of compound **2** (333 mg, 93%) as a white solid. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.15 (t, J = 6.4 Hz, 12H), 3.29-3.34 (m, 8H), 6.24-6.26 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 6.47 (d, J = 2.4 Hz, 2H), 6.57 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 6.8 Hz, 2H), 7.43-7.51 (m, 2H), 7.58-7.61 (m, 1H), 8.02 (d, J = 7.2 Hz, 2H), 8.44 (s, 1H), 8.46 (s, 1H). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 12.64, 44.30, 65.86, 98.32, 105.65, 108.03, 120.53, 123.54, 123.59, 123.72, 127.59, 128.10, 128.24, 133.70, 136.04, 146.11, 148.95, 149.00, 152.49, 152.95, 154.68, 165.42.

RSP: Compound **2** (0.55 mmol, 300 mg) and Lawesson's reagent (0.55 mmol, 222 mg) were dissolved in dry benzene (15 mL), and the reaction mixture was refluxed for 24 h under N$_2$ atmosphere. After removal of benzene under reduced pressure, the product was then chromatographed on silica gel using dichloromethane/methanol 60: 1 (v/v) as eluant to afford 99 mg

(32%) **RSP** as orange solid. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.15 (t, J = 6.4 Hz, 12H), 3.29-3.34 (m, 8H), 6.24-6.26 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 7.16 (t, J = 5.2 Hz, 1H), 7.28 (m, 1H), 7.47 (t, J = 4.8 Hz, 2H), 7.68 (t, J = 7.6 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.19 (t, J = 5.2 Hz, 1H), 8.65 (s, 1H), 8.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 12.61, 44.44, 63.46, 97.56, 108.36, 110.36, 121.75, 122.63, 124.53, 127.08, 127.95, 130.18, 132.51, 135.17, 136.25, 148.21, 149.55, 151.88, 153.73, 155.36, 158.96, 173.36. HRMS (ES+): Calcd for ([M+H])$^+$, 562.2641, found, 562.2636.

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Notes and references

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† Electronic supplementary information (ESI) available: Synthetic details and spectroscopic data. See DOI:

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