Water-compatible fluorescent [2]rotaxanes for Au$^3+$ detection and bioimaging†

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In this study, the synthesis of [2]rotaxanes as fluorescent metal ion sensors has been demonstrated. [2]Rotaxanes, RRA-H-PF$_6$, RRA and RRB, undergo photoelectron transfer resulting in fluorescence quenching. Before the diimine (dynamic covalent bond) reduction on the macrocyclic ring, the dynamic [2]rotaxane RA-H-PF$_6$ can be hydrolyzed and turned fluorescent by trivalent metal ions, giving fluorescence at $\lambda_{\text{max}}$ 424 nm. After reduction of the imines, the reduced [2]rotaxanes RRA and RRB are kinetically stable and highly selective to Au$^3+$ binding among 27 metal ions in a water-compatible (50 vol%) solution with working fluorescence in the range of pH 4–10. 50-Fold and 1.2-fold fluorescence turn-on after addition of Au$^{3+}$ has been observed for RRA and RRB, respectively. Metal interference on Au$^3+$ detection is insignificant, and thereby the fluorescence intensity is linearly proportional to the concentration of Au$^{3+}$ until excess. The solid-state crystal structure of RRA shows the mechanically interlocked structure (mechanical bond). The bioimaging experiment of RRB with HeLa cells demonstrates the potential application of these mechanically interlocked molecules for metal ion detection in aqueous media and biological systems.

Introduction

Simple organic molecules have been used as fluorescent metal ion sensors due to their high selectivity, sensitivity, simplicity, quick response and feasibility of both in vitro and in vivo testing.1–4 In particular, rotaxanes, mechanically interlocked molecules with unique topology,5,6 have been in the spotlight for ion sensing over the past few decades. Compared to conventional sensors, the interlocked system of rotaxanes can provide a dynamic and yet switchable cavity, thus allowing them to bind specific analytes selectively.7 The mechanical bond can also provide unusual coordination geometries and augmented redox activity as a potential ligand.8 It was first reported by Hiratani and co-workers as a selective Li$^+$ fluorescent rotaxane sensor in CH$_2$Cl$_2$/MeCN solution (90 : 10, v/v).9 Previously, Goldup and co-workers reported a [2]rotaxane fluorescent sensor for Zn$^{2+}$ in MeCN/H$_2$O solution (98 : 2, v/v).10 In another study performed by Beer and co-workers, they designed a rotaxane for sensing halide ions in up to 35% H$_2$O in MeCN.11 Lin and co-workers also reported a [2]rotaxane for Fe$^{3+}$ detection and bioimaging.12 However, most of the rotaxane sensors focus on alkalai metal ions,9,13,14 divalent transition metal ions10,15 and anions.11,16–18 In the case of precious metals and other platinum group elements (Pt, Pd, Rh, Ir, Ru, and Os), the application of using rotaxanes as a metal ion sensor in aqueous media is relatively unexplored, and thereby the effect of other metal ions as interference is seldom mentioned.19 Since the detection of metal ions in aqueous media has become crucial in biological systems, the development of water-soluble rotaxane metal ion sensors has become highly desired.

Gold, one of the precious metals, has been utilized in functional nanoparticles.19 In its ion form, Au$^{3+}$, has been applied in biological,20,21 catalysis,22,23 and other industrial applications. For instance, recent studies have shown that an Au(n) complex could be used as an effective anticancer drug due to its tumor growth inhibiting properties.21 Despite the advantages, excess Au$^{3+}$ can damage the organs.25 For example, Au$^{3+}$ can lead to inhibition of the oxygen consumption of liver and kidney slices.26 An Au$^{3+}$ salt solution is over 90% toxic at a concentration of 200 μM.27 Thus, the detection of Au$^{3+}$ has become crucial.

Herein, we report fluorescent [2]rotaxanes as selective metal ion sensors for detecting Au$^{3+}$ and other trivalent metal ions in aqueous media by applying dynamic clipping on R$_2$NH$_3^+$ for [2]rotaxane synthesis. It is part of dynamic covalent chemistry (DCC), which allows self-sorting and self-sorting to give the most thermodynamically stable rotaxane as the major product.28–36

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The rotaxanes reported in this project were obtained in high yield, having pyridine/amine nitrogen and ethylene glycol oxygen donor atom moieties for selective metal ion binding. First, the fluorescent response of the as-synthesized RA-H-PF₆ and RB-H-PF₆ with the trivalent metal ions in 1% H₂O in MeCN was investigated. To increase the selectivity, RA-H-PF₆ and RB-H-PF₆ were further reduced, and the fluorescence response shows much significant improvement for RRA and RRB on detection of Au³⁺ ions in MeCN/H₂O solution. In view of potential bio-application of rotaxanes such as drug delivery, the cell viability of HeLa cells was performed to demonstrate the in vitro imaging of Au³⁺ with both RRA and RRB.

Results and discussion

As shown in Scheme 1, two dynamic rotaxanes, RA-H-PF₆ and RB-H-PF₆, were first synthesized for trivalent metal ion sensing. RA-H-PF₆ was thermodynamically formed via a clipping strategy with equal molar concentrations of tetraethylene glycol bis(2-aminophenyl)ether (diamine 1), 2,6-pyridine dicarbox-aldehyde (dialdehyde 2) and anthracene-based axle 3. On the other hand, RB-H-PF₆ was synthesized with 1, 2, and BODIPY-based axle 4 by using the same clipping strategy. After the [2]rotaxane formation, the original fluorescence from the anthracene-based and BODIPY-based axles could be quenched, which may be caused by the electron transfer from the imine and pyridine moieties within the macrocyclic ring. The fluorescence could be restored when the imine moieties of RA-H-PF₆ hydrolyze back to the aldehyde and amine groups in the presence of acid or water, which leads to the dissociation of the macrocycle and separate components. The breakdown will then inhibit the internal electron transfer and provide a fluorescence band with λmax at 424 nm.

The fluorescence response of RA-H-PF₆ towards cations Li⁺, NH₄⁺, Na⁺, K⁺, Ag⁺, Cs⁺, Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Pd²⁺, Pt²⁺, Hg²⁺, Pb²⁺, Al³⁺, V³⁺, Cr³⁺, Fe³⁺, Ru³⁺, Rh³⁺, Ce³⁺, Ir³⁺ and Au³⁺ was first investigated in a MeCN/H₂O (99:1, v/v) co-solvent system. As shown in Fig. 1, the fluorescence of RA-H-PF₆ was turned on significantly by 5 equivalents...
of common trivalent metal ions like Al\(^{3+}\), V\(^{3+}\), Cr\(^{3+}\), Fe\(^{3+}\), Ru\(^{3+}\), Rh\(^{3+}\), Ir\(^{3+}\) and Au\(^{3+}\), along with moderate fluorescence turn-on by a divalent metal ion (Cu\(^{2+}\)). These results indicate that dynamic [2]rotaxane RA-H-PF\(_6\) is relatively selective to reported trivalent metal ion binding. Al, V, Cr, and Fe are high abundance elements on Earth. Ru, Rh, Ir and Au are commonly used as catalysts for organic reactions. Since those trivalent metal ions are Lewis acids, they could induce acid hydrolysis of the imine moiety of RA-H-PF\(_6\) upon binding to the dynamic [2]rotaxane (Scheme 2). To study the mechanism, NMR spectroscopic titration experiments were conducted. The stacked \(^1\)H NMR spectra (Fig. 2) illustrate that the addition of trivalent metal ions could decrease the amount of imine protons of RA-H-PF\(_6\) while increasing the amount of aldehyde protons. This also supports that the hydrolyzed RA-H-PF\(_6\) could be separated into diamine, dialdehyde and axle components upon the addition of trivalent metal ions. Moreover, the axle 3 could restore its fluorescence because of the absence of PET.

With the aim to investigate another metal sensing mechanism without cleavage of the macrocyclic ring of dynamic [2]rotaxane, the imine moieties on the macrocycle of the dynamic [2]rotaxane RA-H-PF\(_6\) and RB-H-PF\(_6\) were then reduced by BH\(_3\)-THF complex (Scheme 1), yielding diamine-based, kinetically stable [2]rotaxanes RRA and RRB. After the purification by column chromatography, RRA and RRB were obtained in 70% and 75% yield, respectively. The X-ray crystallography analysis of RRA single crystal shows the existence of the interlocking structure (mechanical bond) in the solid-state structure (Fig. 3), confirming successful diimine reduction to form the RRA.

The selectivity of the reduced rotaxanes to cations was investigated. Since the solubility of RRA is relatively low at
MeCN, THF was used to dissolve RRA. After the diimine reduction, the fluorescence response of RRA in THF/MeCN/H_2O solution (2 : 48 : 50, v/v/v) had more than 50-fold enhancement on fluorescence intensity at 417 nm with 10.0 equiv. of Au^{3+}. The fluorescence response has significantly improved and can only be turned on by Au^{3+} (Fig. 4). This suggests that Au^{3+} could inhibit the PET of RRA. In comparison with RRA, the response of RRB has 1.2-fold enhancement upon the addition of 10.0 equiv. of Au^{3+} and 0.6-fold enhancement upon the addition of 10.0 equiv. of Hg^{2+} and Cu^{2+} separately in MeCN/H_2O solution (50 : 50, v/v). Next, the metal interference on Au^{3+} detection was tested. To study the interference, RRA and RRB were added to a mixture of Au^{3+} and one of the metal ions used in the previous experiment. The fluorescence response of rotaxanes to Au^{3+} is similar in the presence of most of the metal ions except for Pd^{2+}, Ru^{3+} and V^{3+}, suggesting that the interference from other metal ions was insignificant (Fig. 4b). The quenching of Pd^{2+}, Ru^{3+} and V^{3+} may be due to the similar size with Au^{3+}, having an effective ionic radius of 86, 68, 64 and 85 pm respectively.

Since both RRA and RRB show high selectivity to Au^{3+}, NMR titration of Au^{3+} was performed and illustrated (Fig. 5). Upon addition of Au^{3+}, the ^1H NMR spectra of RRA and RRB changed dramatically, exhibiting a similar pattern to the acid-equilibrium form of both RRA and RRB. Moreover, the appearance of ammonium protons (H_c) also proved that the dialkylamine of RRA (H_c d 8.89 ppm) and RRB (H_c d 8.99 ppm) was protonated by Au^{3+}.
During the titration, chemical shifts of the pyridyl (H_{a,b}), ammonium proton (H_{c}) and phenyl (H_{d-g}) units which belong to the macrocyclic ring were observed, indicating their participation in the Au^{3+} complexation. Since the proton signal of both the axle and macrocycle has shifted during the complexation, we proposed that the Au^{3+} was bound inside the cavity of the rotaxanes (Scheme 3).
Scheme 3  Plausible mechanism of switch on fluorescence of RRA and RRB upon addition of Au$^{3+}$.

Fig. 6  (a) Fluorescence response of RRA (20 μM, $\lambda_{ex} = 370$ nm) at 417 nm upon the addition of Au$^{3+}$ (0–200 μM) in THF/MeCN/H$_2$O solution (2 : 48 : 50, v/v/v). (b) Fluorescence spectral change of RRA (20 μM, $\lambda_{ex} = 370$ nm) at 417 nm upon addition of Au$^{3+}$ (0–200 μM) in THF/MeCN/H$_2$O solution (2 : 48 : 50, v/v/v). (c) Fluorescence response of RRB (5 μM, $\lambda_{ex} = 500$ nm) at 518 nm upon the addition of Au$^{3+}$ (0–25 μM) in MeCN/H$_2$O solution (50 : 50, v/v). (d) Fluorescence spectral change of RRB (5 μM, $\lambda_{ex} = 500$ nm) at 518 nm upon addition of Au$^{3+}$ (0–25 μM) in MeCN/H$_2$O solution (50 : 50, v/v).
The sensitivity of Au$^{3+}$ detection was studied by a fluorescence titration experiment (Fig. 6). Upon titration, the fluorescence intensity of RRA and RRB is linearly proportional to the increase of Au$^{3+}$ concentration in the range of 0–160 μM and 5–20 μM, respectively. The intensity remains steady after the addition of 160 μM of Au$^{3+}$ to RRA solution and 20 μM of Au$^{3+}$ to RRB. The results indicate that the amount of Au$^{3+}$ can be estimated by both rotaxane sensors. Indeed, the binding constant ($K_a$) with RRA to Au$^{3+}$ is 9.05 × 10$^3$ M$^{-1}$ and that of RRB with Au$^{3+}$ is 2.92 × 10$^4$ M$^{-1}$, calculated by using the equation $K_a = B^{-1} \times \frac{[I_{\text{max}} - I_{\text{min}}]}{C_{\text{Au}^{3+}}}$ from Benesi–Hildebrand analysis (Fig. S2 and S3, ESI†). This reveals that RRB can bind stronger to Au$^{3+}$ than RRA.

Furthermore, because the NMR titration indicates that RRA and RRB were acidified during the binding to Au$^{3+}$, the fluorescence response of RRA and RRB to Au$^{3+}$ can be changed according to the pH equilibrium between the rotaxanes and their protonated form. Therefore, the fluorescence responses of

![Fig. 7](image_url)

(a) Fluorescence response of RRA (20 μM, $\lambda_{\text{ex}} = 370$ nm) at 417 nm upon addition of 10 equiv. of Au$^{3+}$ in THF/MeCN/H$_2$O solution (2 : 48 : 50, v/v/v) (pH 1–13) indicated by the black line; fluorescence of RRA at 417 nm in THF/MeCN/H$_2$O solution (2 : 48 : 50, v/v/v) (pH 1–13) indicated by the red line. (b) Fluorescence response of RRB (5 μM, $\lambda_{\text{ex}} = 500$ nm) at 518 nm upon addition of 10 equiv. of Au$^{3+}$ in MeCN/H$_2$O solution (50 : 50, v/v) (pH 1–13) indicated by the black line; fluorescence of RRB at 518 nm in MeCN/H$_2$O solution (50 : 50, v/v) (pH 1–13) indicated by the red line.

![Fig. 8](image_url)

Fig. 8 Fluorescence images of HeLa cells pretreated with Au$^{3+}$ (0, 0.5, 1.0 and 2.0 mM) for 24 h and then incubated with RRB for 2 h. The cell nuclei were stained with Hoechst 33342 (blue) (scale bar: 50 μm).
RRA and RRB were tested at different pH values (Fig. 7). Without Au$^{3+}$, RRA has weak fluorescence in the pH range of 1.0–13.0. While the fluorescence of RRA was turned on significantly from pH 4.0–10.0 upon addition of Au$^{3+}$. The results show that the Au$^{3+}$ detection is partially pH independent with RRA when the pH is in the range of 4.0–10.0. For RRB, it gives weak fluorescence in the pH range of 3.0–13.0 without Au$^{3+}$. Upon addition of Au$^{3+}$, fluorescence was turned on significantly from pH 4.0–11.0. Under extreme acidic conditions, the pyridyl and amine units were protonated, which affects the binding to Au$^{3+}$. Moreover, BODIPY was unstable and could not give reliable sensing results based on fluorescence signals at extremely acidic conditions (pH < 2). Under extreme alkali conditions, the environment facilitates the formation of gold hydroxide, which has a low water solubility ($K_{sp} = 5 \times 10^{-46}$). Without dissolving gold ions in the solution, the formation of a sensor-metal complex is slow, so the fluorescence response of the rotaxanes is greatly reduced.

In addition to the titration experiment, the response time experiment was also conducted to investigate the sensitivity of RRA and RRB to Au$^{3+}$ (Fig. S4, ESI††). The results show that the fluorescence intensity became steady around 15 min and 5 min for RRA and RRB respectively, suggesting that both sensors can be used for quick recognition. Moreover, the limit of detection (LOD) of RRA for Au$^{3+}$ is 3.23 μM and RRB for Au$^{3+}$ is 0.35 μM according to equation: LOD = 3σ/m.

The cell viability of HeLa cells treated with RRA and RRB for 24 h was assessed using CCK-8 (Fig. S5, ESI††). As a result, the two sensors presented different toxic effects on HeLa cells. For RRA treated cells, the viability showed a remarkable decline at 0.2 μM, while the viability in RRB treated cells only displayed a slight suppression at 2.0 μM.

Since RRA was toxic to the cells even at low concentration (0.2 μM), we only examined the ion detection ability using RRB in HeLa cells. It was found that RRB could stain the cells and show weak green fluorescence without Au$^{3+}$ treatment (Fig. 8). After the pre-treatment of Au$^{3+}$, the green fluorescence in the cells was obviously enhanced along with the increase of Au$^{3+}$ concentration. Interestingly, the green fluorescence was mostly found in the vacuole-like organelles appearing as green light spots under a fluorescent microscope. This result suggests that RRB could detect Au$^{3+}$ and present an ion concentration-dependent sensing capacity in HeLa cells.

### Conclusion

In conclusion, we have synthesized new fluorescent [2]rotaxane sensors. The fluorescence properties of rotaxanes in metal ion detection have been demonstrated. Dynamic rotaxane RA-H-PFs can be hydrolyzed and its fluorescence switched on by common trivalent metal ions. For the reduced form of dynamic rotaxanes, RRA and RRB have been applied to detect Au$^{3+}$ in 50% H$_2$O in MeCN as a turn-on fluorescent sensor, and the metal interference on Au$^{3+}$ detection is insignificant. Moreover, the fluorescence intensity of rotaxanes is linearly proportional to the concentration of Au$^{3+}$ until excess. RRB can be used for Au$^{3+}$ imaging in HeLa cells, demonstrating the application of using mechanically interlocked molecules as imaging probes in biological systems.

### Conflicts of interest

There are no conflicts to declare.

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