A reversible fluorescent Hg^{2+} chemosensor based on a receptor composed of a thiol atom and an alkene moiety for living cell fluorescence imaging†

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A novel reversible fluorescence turn-on chemosensor 1 for Hg^{2+} was constructed based on a new receptor composed of an S atom and an alkene moiety. The sensor showed a large fluorescence enhancement response (1000-fold enhancement), high selectivity, and high sensitivity with a detection limit of 27.5 nM. Significantly, the reversible sensor functioned well in the near pure aqueous medium and could be employed for Hg^{2+} imaging in living cells.

The design and synthesis of reversible fluorescent chemosensors has been a subject of intense interest. From the design point of view, rhodamine is an effective platform for the construction of reversible fluorescent chemosensors for metal ions owing to its favorable photophysical properties (*i.e.* large absorption coefficient, high fluorescence quantum yield, long absorption, and long emission.) and the metal ion-promoted transformation of the non-fluorescent spirocyclic form to the fluorescent opened-ring form.[1] A number of reversible fluorescent chemosensors for Hg^{2+} based on the rhodamine scaffold have been developed.[2] However, some of them only operate in organic solvents[2k–m] and are thus not suitable for fluorescence imaging in living cells. Furthermore, some reversible fluorescent chemosensors were constructed based on known Hg^{2+} receptors. Thus, it is still interesting and challenging to judiciously design reversible fluorescent Hg^{2+} sensors based on new Hg^{2+} receptors. Preferably, the sensors should also function well in bio-compatible conditions such as near pure aqueous medium with neutral pH for fluorescence imaging applications in living cells.

Herein, we present sensor **1** (Scheme 1) as a novel reversible fluorescence turn-on sensor based on a new receptor for Hg^{2+} fluorescence imaging in living cells. The sensor is composed of a rhodamine dye, an S atom, and an alkene moiety. We reasoned that Hg^{2+} could coordinate to the novel receptor formed by the S atom and the alkene unit due to its thiophilic [3] and π-philic[4] character. This coordination may induce the transformation of the rhodamine dye from the non-fluorescent spirocyclic form to the highly fluorescent opened-ring form for a significant fluorescence enhancement signal. Notably, a receptor formed by an S atom and an alkene moiety has not been previously employed in the design of reversible Hg^{2+} fluorescent sensors. In this contribution, we describe the studies of synthesis, fluorescence response, likely sensing mechanism, and living cell imaging for sensor **1**.

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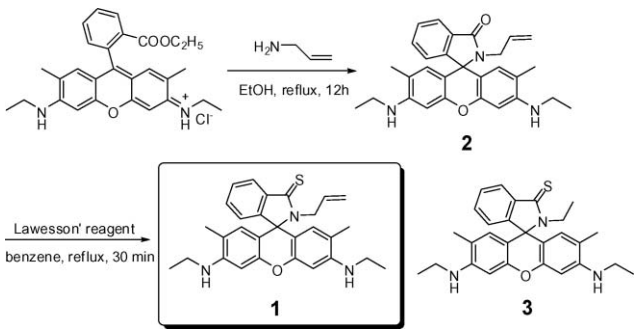


Scheme 1 Synthesis of the model compound **2** and sensor **1**; Structure of the model compound **3**.

The synthesis of chemosensor **1** and the model compound **2** is outlined in Scheme 1. Briefly, treatment of rhodamine 6G with prop-2-en-1-amine in ethanol afforded the key intermediate **2**, which was then reacted with Lawesson's reagent in dry benzene to give compound **1** in 60% yield. The model compound **3** was prepared previously.[5] All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS (see the Supporting Information).

Chemosensor **1** (5 μM) was titrated with Hg^{2+} in a near pure aqueous medium, PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent). As shown in Fig. 1, in the absence of Hg^{2+}, chemosensor **1** exhibited essentially no fluorescence. However, with the addition of increasing concentrations of Hg^{2+}, a remarkable enhancement (up to 1000-fold) in fluorescence intensity at 561 nm was noticed. The large fluorescence increase was substantiated by the observation that the emission color

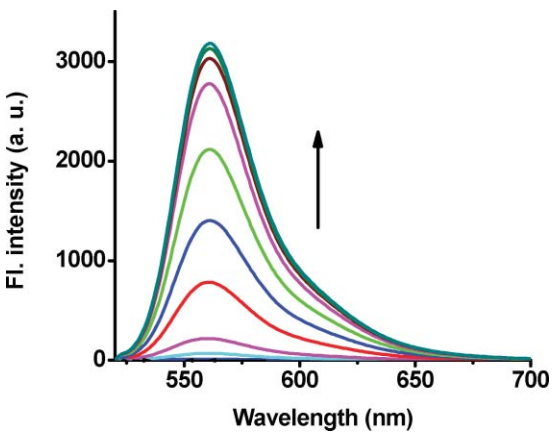


Fig. 1 Fluorescence spectra (excitation at 500 nm) of sensor **1** (5 μM) in PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent) in the presence of (0–2 equiv.) of Hg^{2+}.

turned from dark to bright yellow (Figure S1A). In accordance with the drastic fluorescence enhancement, addition of Hg^{2+} to chemosensor **1** (5 μM) also induced formation of a new strong absorption peak at around 534 nm in the absorption profile of chemosensor **1** (Figure S2), and the solution of the sensor was changed from colorless to pink (Figure S1B). These data indicate that Hg^{2+} ions render the transformation of sensor **1** from the nonfluorescent and colorless spirocyclic form to the highly fluorescent and pink colored ring-opened form.

Indeed, as designed, the binding of sensor **1** with Hg^{2+} is reversible as evidenced by the complete reversal of the fluorescence signal to free sensor **1** when excess EDTA was introduced to sensor **1** (5 μM) pretreated with Hg^{2+} (2 equiv.) (Figure S3). To determine the stoichiometry of sensor **1** and Hg^{2+} in the complex, Job's method was employed by using the emission changes at 561 nm as a function of molar fraction of Hg^{2+}. A maximum emission was observed when the molar fraction of Hg^{2+} reached 0.5 (Figure S4), indicating that Hg^{2+} ions form a 1 : 1 complex with the sensor. The apparent dissociation constant of the complex was then calculated to be 2.5×10^{-5} M with a good linear relationship by a 1 : 1 binding mode (Figure S5, see the Supporting Information).[6] Sensor **1** is highly sensitive to Hg^{2+} with a detection limit [7] of 2.75×10^{-8} M (Figure S6, see the Supporting Information).

We then proceeded to examine the selectivity of the sensor. Fig. 2 displays the fluorescence spectra of sensor **1** (5 μM) in the presence of diverse metal ions represented by K^+, Na^+, Ca^{2+}, Mg^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Ag^+, Au^{3+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, and Pd^{2+} in the PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent). Remarkably, only Hg^{2+} elicited a large fluorescence enhancement (1000-fold). By contrast, alkali metals ions (K^+, Na^+) and alkali-earth metals ions (Ca^{2+}, Mg^{2+}) even in the presence of large excess (200 equiv.) have no observable fluorescence response. Furthermore, sensor **1** gave only a minimal response to transition-metal ions such as Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Ag^+, Au^{3+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, and Fe^{2+}, indicating that the sensor is highly selective. In addition, the obvious visual fluorescence response of sensor **1** to various species (Figure S1A) suggests that the sensor can be employed conveniently for Hg^{2+} detection by simple visual inspection. To investigate the potential interference of other metal ions for the fluorescence detection of Hg^{2+}, sensor **1** was treated with Hg^{2+} in the presence of various metal species in the PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent). As exhibited in Fig. 3, all the metal species tested have virtually no influence on the fluorescence detection of Hg^{2+}. Thereby, sensor **1** appears to be useful for selectively sensing Hg^{2+} even in the presence of other relevant metal ions.

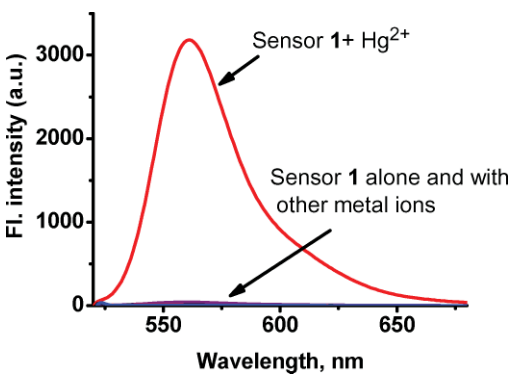


Fig. 2 Fluorescence spectra of sensor **1** (5 μM) with metal ions (200 equiv. for Na^+, K^+, Ca^{2+}, Mg^{2+}, 2 equiv. for other tested metal ions) in the PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent) Excitation at 500 nm.

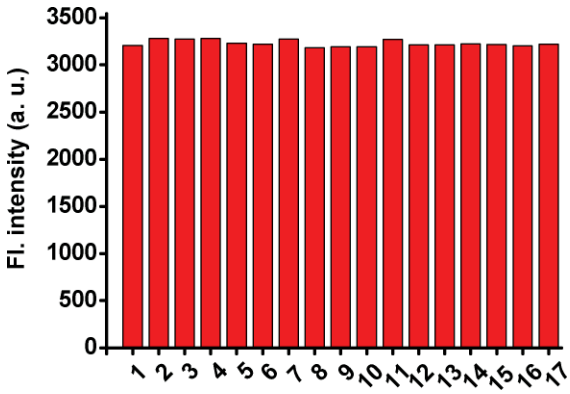


Fig. 3 Fluorescence response of sensor **1** (5 μM) to the various species (200 equiv. for Na^+, K^+, Ca^{2+}, Mg^{2+}, 2 equiv. for other tested metal ions) in the PBS buffer (25 mM, pH 7.0, containing 2.5% CH_3CN as a co-solvent) 1: Ca^{2+} + Hg^{2+}; 2: Ag^+ + Hg^{2+}; 3: Au^{3+} + Hg^{2+}; 4: Zn^{2+} + Hg^{2+}; 5: Mg^{2+} + Hg^{2+}; 6: Pd^{2+} + Hg^{2+}; 7: Cu^{2+} + Hg^{2+}; 8: Fe^{3+} + Hg^{2+}; 9: Fe^{2+} + Hg^{2+}; 10: Pb^{2+} + Hg^{2+}; 11: Cd^{2+} + Hg^{2+}; 12: K^+ + Hg^{2+}; 13: Na^+ + Hg^{2+}; 14: Ni^{2+} + Hg^{2+}; 15: Co^{2+} + Hg^{2+}; 16: Mn^{2+} + Hg^{2+}; 17: Hg^2.

To understand the function of the S atom and the alkene moiety of the sensor in the binding mode, we studied the sensing response of the model compounds **2** and **3** (the structures are shown in Scheme 1) to Hg^{2+}. The model compound **2** which contains the alkene moiety but lacks the S atom exhibited no fluorescence response toward Hg^{2+} (Figure S7a) indicating that the S atom in sensor **1** plays an important role in the interaction with Hg^{2+}. Furthermore, the model compound **3** which contains the S atom but lacks of the alkene moiety did not show observable sensing response to Hg^{2+} (Figure S7b) suggesting that the alkene unit in sensor **1** also plays a key role in the sensing mechanism.

To get insight into the binding mode, sensor **1** was treated with different concentrations of Hg^{2+} in CD_3CN/D_2O, and the ^1H NMR spectra were recorded. As shown in Fig. 4, addition of Hg^{2+} induced marked changes in the spectra. In particular, the resonance signals corresponding to H_1, H_2, and H_3 on the allyl group essentially disappeared when the sensor was treated with

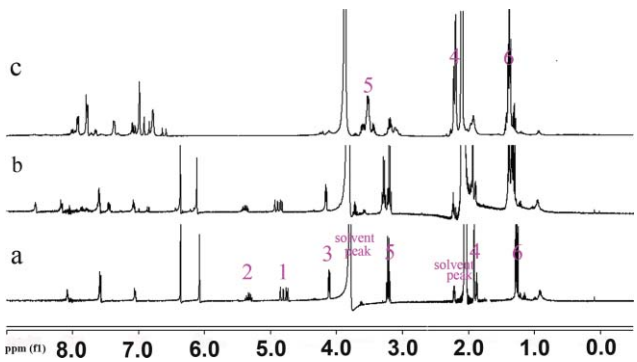
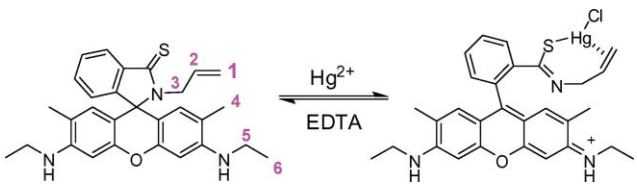


Fig. 4 ^1H NMR spectra of (a) free sensor **1**, (b) sensor **1** + Hg^{2+} (0.5 equiv.), and (c) sensor **1** + Hg^{2+} (1.0 equiv.) in CD_3CN and D_2O (v : v = 1 : 1).

1 equiv. of Hg^{2+}, indicating that the allyl group participates in the binding.[8] Furthermore, the resonance signals assigned to H_4, H_5, and H_6 have a marked downshift in the presence of Hg^{2+}, consistent with the deshielding effect due to the formation of the ring-opened form of rhodamine.[2g] The apparent downshift of the signals around δ 6.0 ppm may also be attributed to the same reason.

The 1 : 1 binding mode of the sensor with Hg^{2+} was confirmed by the ESI-MS mass spectrum of the complex (Figure S8), which showed an intense peak at m/z = 706.0, assigned to the 1 : 1 complex [Sensor **1** + Hg + Cl]$^+$.

Thus, based on the above results of the EDTA-reversible experiment, the model compound studies, ^{1}H NMR titration, and ESI-MS mass spectrometry analysis, we proposed a likely binding mode of the sensor with Hg^{2+} as shown in Scheme 2. Clearly, the reversible sensing mechanism of sensor **1** is drastically distinct from the irreversible nature of a fluorescent Hg^{2+} chemodosimeter based on a thiol atom and alkyne moiety equipped in a rhodamine scaffold.[5] The distinction in the sensing mechanisms could be ascribed to the different physicochemical properties of alkenes and alkynes, indicating the profound effect of functional group variations on the underlying mechanism of the sensing systems. This renders the rational design of the fluorescence sensing systems intriguing and challenging.



Scheme 2 Proposed binding mode of sensor **1** with Hg^{2+}.

As sensor **1** is highly sensitive and selective in the near pure aqueous medium (97.5% PBS buffer + 2.5% CH_3CN) at neutral conditions (pH 7.0), we then examined the suitability of the sensor for Hg^{2+} fluorescence imaging in living cells due to its biocompatible nature. When Hela cells were incubated with the sensor, and then with Hg^{2+}, bright red fluorescence was noted (Fig. 5b). By contrast, When Hela cells were treated with sensor **1** only, no fluorescence was detected (Fig. 5c). In another control experiment, Hela cells were incubated sequentially with the sensor, Hg^{2+}, and TPEN (a metal chelator). As expected, essentially no fluorescence was observed (Fig. 5d). These results indicate that sensor **1** is cell membrane permeable and able to response to Hg^{2+} in the living cells.

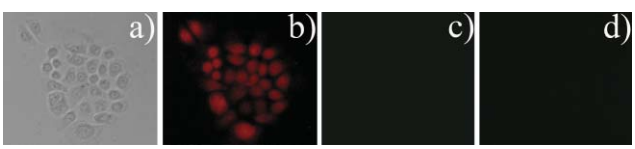


Fig. 5 (a) Bright-field image of Hela cells pre-incubated with sensor **1** (5 μM) for 30 min and then treated with Hg^{2+} (2 equiv.). (b) Fluorescence image of (a). (c) Fluorescence image of Hela cells treated with only sensor **1** for 30 min. (d) Fluorescence image of Hela cells pre-incubated with sensor **1** (5 μM) for 30 min, then treated with Hg^{2+} (2 equiv.) for 30 min, and finally incubated with TPEN (1 mM) for 10 min.

In conclusion, we have constructed a novel reversible fluorescence turn-on Hg^{2+} sensor based on a new receptor composed of an S atom and an alkene moiety. Sensor **1** exhibited a large fluorescence turn-on signal to Hg^{2+} (1000-fold enhancement). In addition, the sensor is highly selective and sensitive to Hg^{2+} with a detection limit of 27.5 nM. Importantly, the reversible sensor operated well in the near pure aqueous medium at neutral conditions, which renders the sensor suitable for Hg^{2+} imaging in living cells. We expect that the reversible nature of the new sensor will find interesting biological applications and the novel Hg^{2+} receptor presented herein will be useful for construction of other types of reversible fluorescent Hg^{2+} sensors.

Acknowledgements

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