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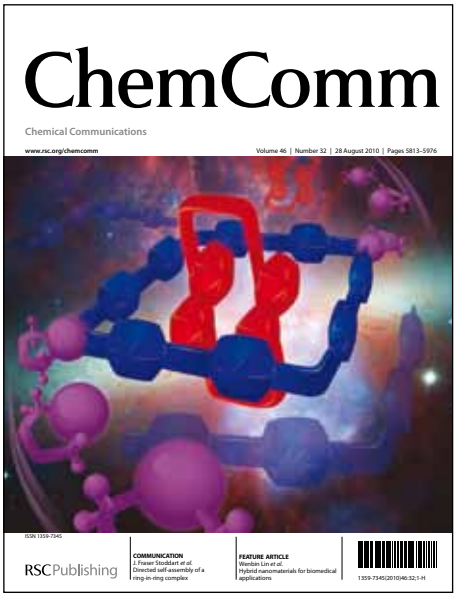

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ARTICLE TYPE

A Rhodamine/BODIPY-based fluorescent probe for the differential detection of Hg(II) and Au(III)††

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We described the design and synthesis of a molecular sensor based on a rhodamine/BODIPY platform that displayed differential fluorescence responses towards Hg^{2+} and Au^{3+} and demonstrated its utility for intracellular ion imaging.

In recent years, the construction of fluorescent molecular sensors for the detection of metal ion species has received a great deal of attention.[1] To date a large number of molecular sensors have been designed and developed, the majority of which are single-ion responsive and present no great challenge to researchers. Compared to single-ion responsive molecular sensors, however, the construction of multi-ion responsive molecular sensors with multiple emission modes are extremely challenging.[2] Molecular sensors displaying differential responses towards multiple ions are indispensable for designing molecular logic gates and molecular keypad lock devices.[3,4]

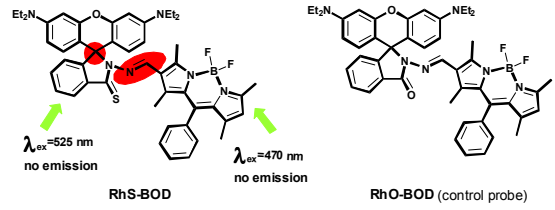
The challenge of multiple analyte recognition presents several detection strategies. Incorporating multiple binding motifs onto a single sensing molecule, or alternatively, combining different transducing units (chromophore/fluorophore), allows for rapid access to molecular sensors with multiple emission modes.[2]

We envisaged that incorporating both a chemosensor and a chemodosimeter onto a single molecule could provide a suitable sensing platform for the differential detection of metal species. On the basis of this hypothesis, we constructed a molecular sensor possessing two different fluorophore units chemically integrated with each other. Both fluorophore units were elegantly designed to be non-emissive (i.e., "off") in their initial states and are expected to turn on respectively in response to the metal species of interest. To the best of our knowledge, molecular sensors based on this novel approach haven't been covered in literature.

Ionic species of mercury (Hg^{2+}) and gold (Au^{3+}) share several similarities in terms of coordination properties. As both metal species show high affinities to thiols, they have the potential to interact with sulfur bearing biomolecules such as enzymes, proteins, and DNA. As a result, these metal species can disturb a series of cellular processes that lead to toxicity in humans.[5,6] In recent years, a variety of well-designed fluorescent probes highly specific for Hg^{2+} and Au^{3+} ions have been developed, most of which are built on the exploitation of the thiophilic and alkynophilic behavior of these metal species.[7,8] Despite impressive advances, many of those probes suffer from cross affinity. Because of their similar coordination properties, it is thus extremely difficult to construct a molecular sensor that differentiates between the Hg^{2+} and Au^{3+} species. To our knowledge, in literature there is only one example of a fluorescent probe that can differentiate between Au^{3+} and Hg^{2+}. That fluorescent probe, reported by Dong et al., operates through a single emission mode and the differentiation is highly dependent on the sensing conditions.[2d]

Obviously, there is a high demand for the development of molecular sensors that can differentiate multiple analytes of a similar chemical nature (e.g. Hg^{2+} and Au^{3+}). In addition, small-molecule fluorescent sensors allowing the intracellular monitoring of multiple-ions via differential responses are of high necessity for real-time cell imaging studies.

Herein, we present the design, synthesis, spectral properties, and cell imaging studies of **RhS-BOD**, a new "turn-on" multi-fluorescent probe that allows the Hg^{2+} and Au^{3+} species to be differentiated on the basis of distinct fluorescence responses. **RhS-BOD** constitutes a boron-dipyrromethene dye (BODIPY) and a spirocyclic rhodamine dye covalently attached to each other. **RhS-BOD** was prepared in a reasonable yield (25%, overall) by the synthetic route outlined in Scheme S1 (see ESI†) and the structure of **RhS-BOD** was confirmed by ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy.



Scheme 1 Structure of **RhS-BOD** and **RhO-BOD**

Importantly, the novel molecular sensor, **RhS-BOD,** was designed in such a way that both of the fluorophore units are non-emissive before the addition of any metal species. As can be seen from the structure of the probe shown in Scheme 1, the C=N functionality on the BODIPY core diminishes the BODIPY emission potentially because of a non-radiative deactivation process of the excited state through rapid isomerization of the C=N group. Similarly, the rhodamine fluorophore is non-emissive because the rhodamine dye exists in the ring closed isomeric form.

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The sensing behavior of **RhS-BOD** towards the addition of different metal species was studied using UV-Vis and fluorescence spectroscopy. As shown in Fig.S1 (see ESI†), the UV/Vis spectrum of free **RhS-BOD** (CH_3CN/HEPES 1:1, pH 7.0) exhibits a single absorption band at 527 nm, which belongs to BODIPY core. As the rhodamine core is in the ring closed isomeric form, we expect no absorption bands for the rhodamine derivate. However, the addition of Hg^{2+} (1 equiv.) to **RhS-BOD** led to the appearance of a new strong absorption band at 554 nm, which was assigned to a ring opened rhodamine derivative.

The fluorescence spectra of **RhS-BOD** displayed a similar behavior towards the addition of Hg^{2+} (Fig.1). Initially, when excited at 525 nm there were no emission bands in the fluorescence spectrum of **RhS-BOD**. However, upon the addition of Hg^{2+}, a new emission band with a maximum at 585 nm appeared and the intensity of this band gradually increased with increasing concentration of the Hg^{2+} (Fig.1). The increase in emission intensity showed a linear relationship towards the addition of Hg^{2+} in the range of 0-0.3 μM. The minimum amount of Hg^{2+} was evaluated to be 8.0 nM under these conditions (Fig.S10, ESI†). The response of the probe towards the addition Hg^{2+} was immediate and the emission intensity became saturated when 1 equiv. of Hg^{2+} were added, creating an enhancement factor of over 50-fold.

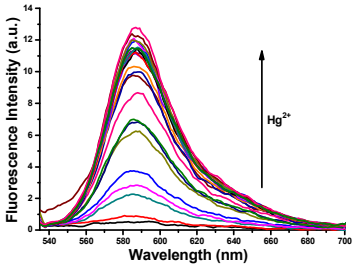

Fig. 1 a) Fluorescence spectra of **RhS-BOD** (5.0 μM) + Hg^{2+} (0.05 to 25.0 μM) in 1:1 CH_3CN/HEPES buffer (pH=7.0), (λ_{ex}: 525 nm).

As expected, in the presence of Hg^{2+}, **RhS-BOD** displayed the optical features of the rhodamine chromophore. During the process of adding Hg^{2+}, no other accompanying emission bands were noticed in the emission spectrum that might belong to the BODIPY dye, indicating that the BODIPY was still in a sleep ("off") mode.

To check the reversibility of the Hg^{2+} sensing process, the highly emissive probe solution pre-treated with Hg^{2+} (**RhS-BOD**/Hg^{2+}) was subsequently treated with a cyanide ion source (KCN or NH_4CN) (Fig.S7, ESI†). The probe solution immediately lost its color and its emission, thus showing the sensing process is based on a reversible metal-ligand coordination process. The binding stoichiometry of the Hg^{2+}/ **RhS-BOD** association was determined by Job's plot from both the UV-Vis absorption and fluorescence data (Fig.S9, ESI†). Both of the plots revealed a 1:4 ratio of the Hg^{2+} ions associating with each molecule of the probe.

We further investigated the selectivity profile of **RhS-BOD** in response to other metal ions. For all other metal cations, such as Cu^{2+}, Ag^{+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Na^{+}, Mg^{2+}, Li^{+}, K^{+}, Pd^{2+}, Fe^{2+}, Co^{2+}, Cd^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+} and Cr^{3+}, no detectable change in the emission intensity for **RhS-BOD** were observed (Fig.S5, ESI†). The probe was highly selective towards Hg^{2+} and showed no spectral response to any other metals ions, except for Au^{3+} ions. Upon the addition of Au^{3+}, the non-emissive probe solution immediately turned to a strong green emissive solution that could be easily monitored by the naked eye under the UV lamp. A green emission was a clear evidence for the existence of an emissive BODIPY derivative. This suggestion was also supported from the outcome of the reaction of **RhS-BOD** mediated by Au^{3+}, as controlled using TLC. The formation of a green emissive compound, **BODIPY-AL**, could be easily monitored from the spots on the TLC plate (Fig. S21, ESI†).

The fluorescence sensing behavior of **RhS-BOD** towards Au^{3+} was comprehensively surveyed upon excitation at 470 nm and 525 nm. As shown in Fig.S11 (ESI†), the fluorescence spectrum of (**RhS-BOD**/Au^{3+}, (1:1)) displays an emission band at 506 nm when excited at 470 nm, a characteristic emission band of a BODIPY fluorophore. On the other hand, the same probe solution (**RhS-BOD**/Au^{3+}) when exited at 525 nm displays a different emission band at 585 nm, which is supposed to belong to the ring opened isomer of the rhodamine core (Fig.S13b, ESI†). The fluorescence emission intensity at both wavelengths increased linearly with an increasing concentration of Au^{3+} over a wide concentration range (Fig.2 and Fig.S14). The response of **RhS-BOD** towards Au^{3+} was fast (<1 min.) and the emission intensity became saturated when 2 equiv. of Au^{3+} was added. In addition, the detection limit measured at both wavelengths was at nM level (65 nM (λ_{em}=585 nm), and 10 nM (λ_{em}=506 nm)).

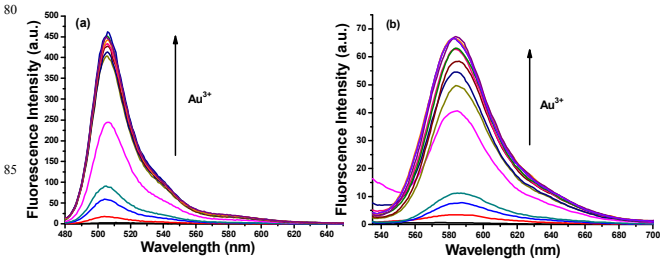

Fig. 2 Fluorescence titration spectra of **RhS-BOD** (5.0 μM) + Au^{3+} (0-10.0 equivalent) in 1:1 CH_3CN/HEPES buffer (pH=7.0) a) λ_{ex}=470 nm, b) λ_{ex}=525 nm.

The fluorescence response of **RhS-BOD** toward Au^{3+} (1 equiv.) in the presence of the other metal ions (10 equiv.) was explored in order to assess the possible interference by other metal ions. As shown in Fig.S16 (see ESI†), the tested metal ions displayed no interference with the detection of Au^{3+} ions.

As discussed before, the addition of Hg^{2+} to **RhS-BOD** triggers a spiro-ring opening reaction and results in the formation of a highly emissive rhodamine derivative. Throughout the addition of Hg^{2+} to **RhS-BOD**, the BODIPY core continues to be non-emissive because the C=N moiety was still preserved. However, the addition of Au^{3+} to the probe solution pre-treated with Hg^{2+} (**RhS-BOD**/Hg^{2+}) resulted in an immediate change in the emission color from orange to green. Evidently, in the presence of Hg^{2+} and Au^{3+}, **RhS-BOD** hydrolyzes to give a green emissive BODIPY derivative, **BODIPY-AL**, which dominates the emission color of the probe solution (Scheme 2).

RhO-BOD, the oxygen bearing derivative of **RhS-BOD**, was used as the control probe to clarify the nature of the sensing

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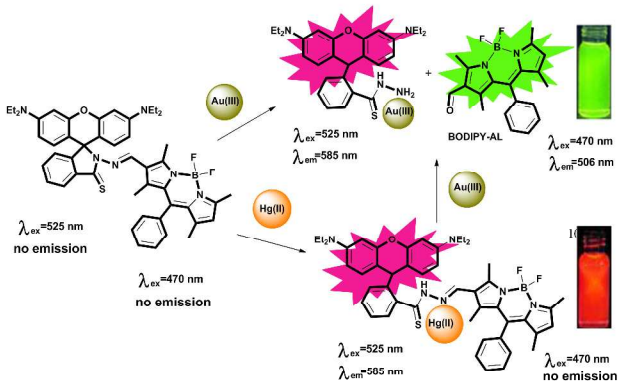
process. Under the same sensing conditions, **RhO-BOD** displayed no response towards any metal species, indicating the indispensable role of the sulfur functionality in the detection of both metal species.



Scheme 2 Response of **RhS-BOD** towards the addition of Au^{3+} and Hg^{2+}

We next assessed the ability of **RhS-BOD** to operate within living organisms. To our delight, **RhS-BOD** showed the same sensing behavior in living cells. Human A549 lung adenocarcinoma cell lines were incubated with the probe (5 μM) for 40 min. and then followed by the addition of metal species. With the aid of fluorescence microscopy, the differential turn-on response towards Au^{3+} and Hg^{2+} was clearly monitored in the cells (Fig.3). The images taken before and after the addition of the metal species displayed a distinct fluorescence change consistent with the results observed in the solution.

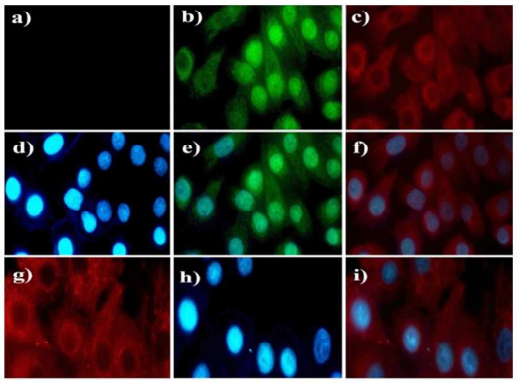


Fig. 3 a) Fluorescence image of A549 cells treated with only **RhS-BOD** (5μM); b) image of cells treated with probe (5μM) and Au^{3+} (5μM) (λ_{ex}=470 nm); c) image of cells treated with probe (5μM) and Au^{3+} (5μM) (λ_{ex}=525 nm); d and h) Image of cells treated with DAPI for 15 min (control); d) merged image of frame b and d; f) merged image of frame c and d; g) image of cells treated with probe (5μM) and Hg^{2+} (5μM) (λ_{ex}=525 nm); i) merged image of frame g and h.

To close, we have presented the synthesis, spectral properties, and biological application of **RhS-BOD**, a new type of fluorescent probe for the differential detection of Hg^{2+} and Au^{3+}. This novel probe features excellent selectivity for Hg^{2+} and Au^{3+}. Detection of Hg^{2+} and Au^{3+} is realized through two distinct fluorescence changes resulting from Hg^{2+}/ligand coordination or from the hydrolysis of the C=N moiety catalyzed by Au^{3+}. **RhS-BOD** exhibits a dual emission mode for the detection of Au^{3+} ions, and a single emission mode for the detection of Hg^{2+} ions.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis and characterization of all compounds, and all data for UV-Vis and fluorescence titrations. See DOI: 10.1039/b000000x/

‡ These authors contributed equally to this work

†† Dedicated to the memory of Prof. Dr. Ayhan S. Demir (1950-2012)

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