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We describe the design, synthesis and spectral behaviour of a fluorescent molecular sensor able to recognize  $\text{Hg}^{2+}$  and  $\text{Au}^{3+}$  ions via different emission modes. The molecular sensor is constructed on a single BODIPY dye appended with a semithio-carbazone functionality as a recognition motif.

In recent years, research on the development of molecular sensors for analysing diverse biologically and environmentally important analyte species has increased.<sup>1</sup> While the vast majority of sensors addressed in such literature are designed to recognize a specific target, less common are sensors capable of differentiating multiple targets. Differential detection of multiple analyte species can be achieved best by recognizing each species through a different signal output (*i.e.*, emission wavelength).<sup>2</sup> Incorporating multiple binding motifs onto a single signal-transducing molecule (chromophore/fluorophore) and, as an alternative, combining different transducing molecules have both appeared as efficient routes for sensors with multiple output modes.<sup>3</sup>

Despite recent advances in the field, it remains a challenge to differentiate metal species with similar chemical natures. For example, the ionic species of gold ( $\text{Au}^{3+}$ ) and mercury ( $\text{Hg}^{2+}$ ) share several similarities in terms of binding properties, since both have strong binding affinities toward sulphur species. When accumulated in the biological system, they thus have great potential to interact with sulphur-bearing biomolecules such as enzymes, proteins, and DNA. As a result, these metal species can disturb a series of cellular processes that cause toxicity in humans.<sup>3,4</sup> Tracking these metal species in a living environment with the aid of a fluorescent molecular sensor is thus crucial to evaluating their roles in certain biological processes.

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## A BODIPY-based fluorescent probe for the differential recognition of $\text{Hg}(\text{II})$ and $\text{Au}(\text{III})$ ions†

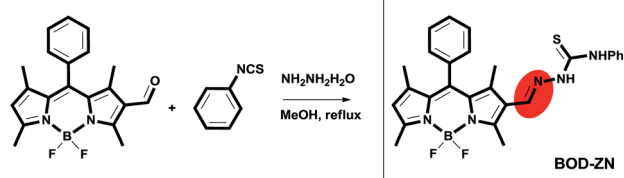
Ceren Cantürk, Muhammed Üçüncü and Mustafa Emrullahoğlu\*

In their structure, most molecular sensors devised for mercury ions use sulphur moieties as a recognition motif.<sup>5</sup> As such, it is always possible that gold and mercury species interfere with each other during their analysis, which could primarily explain why molecular sensors able to differentiate these two metal ions are extremely rare.<sup>6</sup> Molecular tools that can differentiate multiple analytes of a similar chemical nature (*e.g.*, gold<sup>7</sup> and mercury<sup>5,8</sup> ions) are therefore clearly in high demand.

Herein, we designed a molecular sensor with a single fluorophore core appended with semithiocarbazone functionality as the metal ion recognition motif. The fluorophore core, based on a BODIPY dye,<sup>9</sup> is designed to be inactive (*i.e.*, non-emissive) in its initial state yet expected to become active in response to the metal species (Scheme 1). The differential detection of  $\text{Hg}^{2+}$  and  $\text{Au}^{3+}$  relies on different modulation mechanisms and can be realized with two distinct fluorescence changes, based on either an  $\text{Hg}^{2+}$ -ligand coordination event or a gold-mediated chemical transformation.

The title compound, **BOD-ZN**, was prepared (40% overall) by the synthetic route outlined in Scheme 1 and, its structure was unambiguously confirmed by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and HRMS spectroscopy.<sup>10</sup>

The sensing behaviour of **BOD-ZN** toward the addition of a range of metal ion species was studied by UV/Vis and fluorescence spectroscopy. As shown in Fig. 1a, the UV/Vis spectrum of free **BOD-ZN** (phosphate buffer/ethanol 1 : 4, pH 7.0) displays a maximum absorption band at 533 nm, which belongs to the



Scheme 1 Synthesis of BOD-ZN.



BODIPY chromophore. The fluorescence spectrum of **BOD-ZN** collected upon excitation at 460 nm exhibits a very weak emission band at 601 nm. Reasonably, **BOD-ZN** was nearly non-emissive, since the molecular structure of the probe bears a C=N functionality that diminishes the emission of the BODIPY core caused by a non-radiative deactivation process involving the rapid isomerization of the C=N group.

Our investigation began with the evaluation of the optical behaviour of **BOD-ZN** in response to the addition of  $\text{Au}^{3+}$  ions (e.g.,  $\text{AuCl}_3$ ). The addition of  $\text{Au}^{3+}$  (1 equiv.) to **BOD-ZN** prompted the appearance of a new emission band at 512 nm that was assigned to the formation of a new BODIPY derivative (Fig. 1b). The appearance of this new band was accompanied with a distinct change in the solution's emission colour; the red-emitting probe solution became distinctly green, as was clearly visible to the naked eye (Fig. S19, ESI†).

The compound displaying such green emission was isolated and further characterized as **BOD-AL**, the hydrolysis product of **BOD-ZN** (Scheme 2). Evidently, the recognition of  $\text{Au}^{3+}$  was based on an  $\text{Au}^{3+}$ -mediated hydrolysis reaction that resulted in the formation of a highly emissive BODIPY derivative (**BOD-AL**).

A systematic titration of **BOD-ZN** with  $\text{Au}^{3+}$  reveals that emission band intensity increases linearly with the increase in concentration of  $\text{Au}^{3+}$  in the range of 0.1–100  $\mu\text{M}$  (Fig. S6, ESI†). At the same time, the kinetic study showed that the spectral response toward the addition of  $\text{Au}^{3+}$  was rapid (<1 min) and

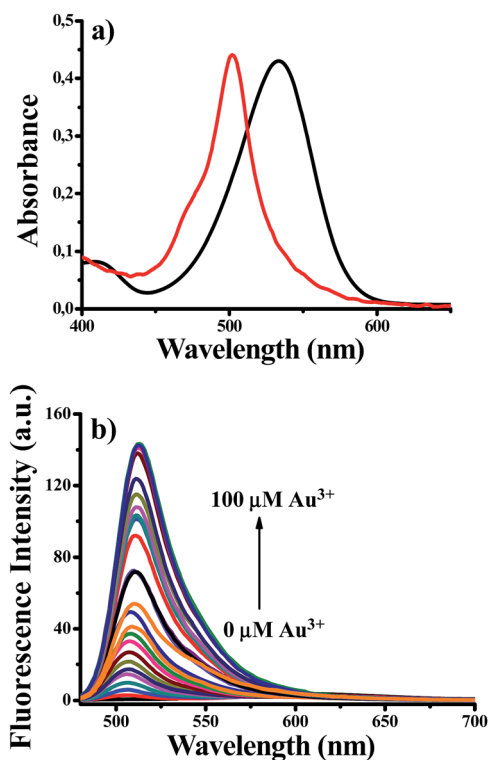
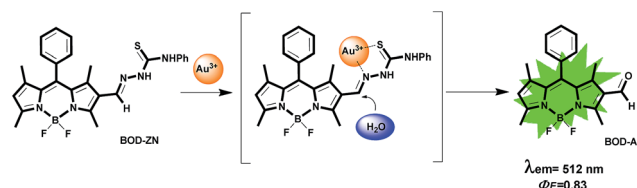


Fig. 1 (a) Absorbance spectra of **BOD-ZN** (10  $\mu\text{M}$ ) in the absence (black line) and presence (red line) of 10 equiv. (100  $\mu\text{M}$ ) of  $\text{Au}^{3+}$ ; (b) fluorescence titration spectra of **BOD-ZN** (10  $\mu\text{M}$ ) +  $\text{Au}^{3+}$  (0.1 to 100  $\mu\text{M}$ , 0.01 to 10 equiv.) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1 : 4) (25  $^{\circ}\text{C}$ ,  $\lambda_{\text{ex}}$  = 460 nm).



Scheme 2 Hydrolysis mechanism of **BOD-ZN** in the presence of  $\text{Au}^{3+}$  ions.

that the emission intensity plateaued within 20 min. Due to the addition of 10 equiv. of  $\text{Au}^{3+}$ , which thereby enhanced intensity by over 200-fold. Moreover, the minimum amount of  $\text{Au}^{3+}$  detectable was evaluated to be 128.0 nM based on the signal-to-noise ratio ( $S/N = 3$ ) (Fig. S2, ESI†).

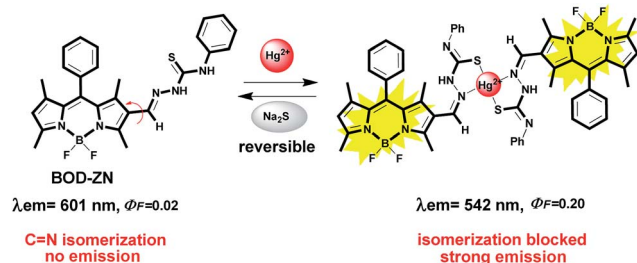
Having established that the detection of  $\text{Au}^{3+}$  ions relies on an irreversible chemical reaction, we investigated the selectivity profile of **BOD-ZN** in response to other metal species. The probe proved to be highly specific for  $\text{Au}^{3+}$  ions, since no change was detected in the spectrum when  $\text{Au}^{+}$  ions were present. Furthermore, **BOD-ZN** showed no spectral response to other metal ions such as  $\text{Cu}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Na}^{+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Li}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Pd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Cr}^{3+}$ , except for  $\text{Hg}^{2+}$ .

We recognized the detection of  $\text{Hg}^{2+}$  with a different emission output. Given the addition of  $\text{Hg}^{2+}$ , the weakly emissive probe solution immediately (<5 s) turned profoundly yellow, possibly due to the blockage of the C=N isomerization *via* strong coordination to the nitrogen electron pair which prevents the electron transfer process, and thus enhances the fluorescence emission (Scheme 3).

Meanwhile, in the fluorescence spectrum of **BOD-ZN**, a new emission band appeared at 542 nm and increased linearly with the increased concentration of  $\text{Hg}^{2+}$  above the range of 0.1–100  $\mu\text{M}$  (Fig. 2b). The detection limit of **BOD-ZN** for detecting  $\text{Hg}^{2+}$  was 160.0 nM.

In sharp contrast to the detection of  $\text{Au}^{3+}$ , detecting  $\text{Hg}^{2+}$  ions proceeded reversibly. The reversibility of the binding event between **BOD-ZN** and  $\text{Hg}^{2+}$  was confirmed by the addition of  $\text{Na}_2\text{S}$  to the solution pre-treated with  $\text{Hg}^{2+}$ , which sharply decreased the emission intensity. The regeneration of fluorescence was again made possible by introducing  $\text{Hg}^{2+}$  ions into the solution, and the off-on switching ability of the system with  $\text{Hg}^{2+}$  proved the reversibility of the process (Fig. S15, ESI†).

At the same time, the binding process of  $\text{Hg}^{2+}$  to **BOD-ZN** could be clearly followed by the aid of  $^1\text{H-NMR}$  spectroscopy.



Scheme 3 Reversible binding of  $\text{Hg}^{2+}$  to **BOD-ZN**.



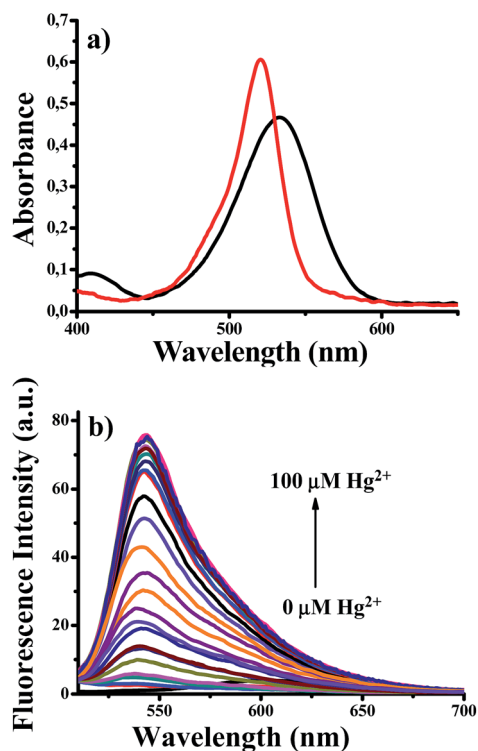


Fig. 2 (a) Absorbance spectra of **BOD-ZN** (10  $\mu\text{M}$ ) in the absence (black line) and presence (red line) of 10 equiv. (100  $\mu\text{M}$ ) of  $\text{Hg}^{2+}$ ; (b) fluorescence titration spectra of **BOD-ZN** (10  $\mu\text{M}$ ) +  $\text{Hg}^{2+}$  (0.1 to 100  $\mu\text{M}$ , 0.01 to 10 equiv.) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1 : 4) (25  $^{\circ}\text{C}$ ,  $\lambda_{\text{ex}}$  = 460 nm).

During  $\text{Hg}^{2+}$  incubation lasting 5 min, we observed pronounced differences in the  $^1\text{H-NMR}$  spectrum of **BOD-ZN**. For one, the resonance of the  $\text{H}_a$  proton signal at 8.01 ppm belonging to the hydrogen atom of the aldimine shifted to a higher frequency, while the resonance of the methyl protons ( $\text{H}_b$  and  $\text{H}_c$ ) in close proximity to the recognition motif shifted to a lower frequency. Furthermore, a reorganization of the phenyl ring proton signals strongly suggests the structural modification in the phenyl thiourea motif (Fig. 3).

The stoichiometry of the sensing event was established by following the Benesi–Hildebrand method and, accordingly, the related binding constant was determined as  $4.2 \times 10^4 \text{ M}^{-2}$ .<sup>11</sup> With HRMS analysis, we also confirmed the binding of  $\text{Hg}^{2+}$  ions to **BOD-ZN**. HRMS data of the solution ( $\text{Hg}^{2+}/\text{BOD-ZN}$ ) indicated the formation of an  $\text{Hg}^{2+}$  ion complex, ( $m/z$  = 1204.36841 found; 1204.36354 calc.), with a binding stoichiometry of 1 : 2.<sup>10</sup> Given all of the above, the structure of the binding complex is most likely that shown in Scheme 3.

Having clarified the detection of both metal species, we assessed the interference of other metal ions in the detection of  $\text{Au}^{3+}$  and  $\text{Hg}^{2+}$ . Although the spectral response of **BOD-ZN** induced by  $\text{Au}^{3+}$  ions showed no interference with other metal ions, the detection of  $\text{Hg}^{2+}$  was disturbed in the presence of  $\text{Au}^{3+}$  ions. More specifically, in the presence of  $\text{Au}^{3+}$  and  $\text{Hg}^{2+}$ , the fluorescence spectrum initially displayed an emission band at 542 nm for the  $\text{Hg}^{2+}/\text{BOD-ZN}$  binding complex. However, the band disappeared within 10 min, as a new band appeared at

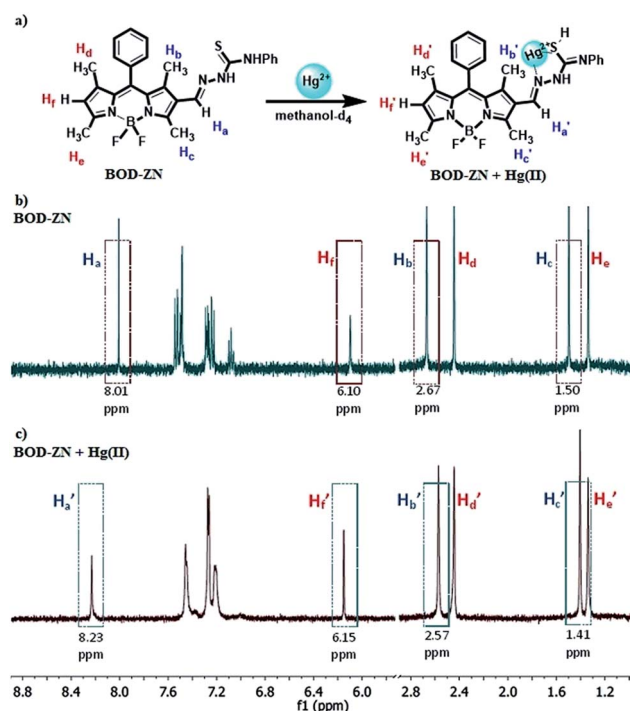


Fig. 3 (a) Proposed coordination mechanism of  $\text{Hg}^{2+}$  to **BOD-ZN**; (b)  $^1\text{H-NMR}$  of **BOD-ZN** in methanol- $\text{d}_4$ ; (c)  $^1\text{H-NMR}$  of **BOD-ZN** +  $\text{Hg}^{2+}$  (1 equiv.) in methanol- $\text{d}_4$ .

512 nm, which indicates that  $\text{Au}^{3+}$  ions also mediate the hydrolysis of the  $\text{Hg}^{2+}$  binding complex (Fig. S18, ESI<sup>†</sup>). Notably, the same sensing behaviour was also observed by adding  $\text{Au}^{3+}$  ions to a solution pre-treated with  $\text{Hg}^{2+}$ .

Relying on the impressive sensing properties of **BOD-ZN**, we next investigated its capacity for imaging  $\text{Hg}^{2+}$  and  $\text{Au}^{3+}$  ions in living cells. As Fig. 4a and a' show, the images of human lung adenocarcinoma (A549) cells incubated with **BOD-ZN** did not display any fluorescence until the addition of the metal species. However, upon incubation with  $\text{Au}^{3+}$  or  $\text{Hg}^{2+}$ , the cells started to emit a distinct fluorescence emission consistent with results obtained in the solution. Based on the nucleus staining experiment using DAPI as the staining dye, we concluded that the probe passes through the cell membrane and detects both

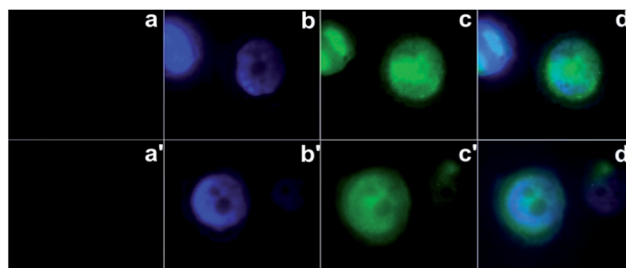


Fig. 4 Fluorescence images of human lung adenocarcinoma cells (A549). (a and a') Fluorescence image of A549 cells treated with only **BOD-ZN** (10  $\mu\text{M}$ ); (b and b') fluorescence image of cells treated with DAPI (control); (c and c') fluorescence image of cells treated with **BOD-ZN** (10  $\mu\text{M}$ ) and  $\text{Au}^{3+}$  (10  $\mu\text{M}$ ) or  $\text{Hg}^{2+}$  (10  $\mu\text{M}$ ) ( $\lambda_{\text{ex}}$  = 460 nm); (d and d') merged images of frames b–c or b'–c'.



metal species from within the cell. This preliminary cell imaging study suggested that **BOD-ZN** can be used efficiently for the *in vitro* imaging of Au<sup>3+</sup> and Hg<sup>2+</sup> species in living cells.

## Conclusions

To close, we have studied the design, spectral behaviour, and cell-imaging capacity of a unique fluorescent molecular structure that can efficiently differentiate Hg<sup>2+</sup> and Au<sup>3+</sup> ions. The differential detection of Hg<sup>2+</sup> and Au<sup>3+</sup> was recognized in two distinct fluorescence changes: one resulting from a reversible Hg<sup>2+</sup>/sensor complex formation, the other an irreversible Au<sup>3+</sup>-mediated hydrolysis reaction. This novel molecular structure displayed the ability to recognize both metal species at nanomolar levels.

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