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Sensors

A Ratiometric Fluorescent Probe for Gold and Mercury Ions

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Abstract: A fluorescent probe that displays a ratiometric fluorescence response towards gold and mercury ions has been devised. Emitting at a relatively longer wavelength, the conjugated form of the fluorescent dye transforms in the presence of the gold or mercury ions into a new dye, the molecular structure of which lacks the conjugation and consequently emits at a distinctly shorter wavelength.

Gold catalysis has recently become a highly popular subject in synthetic chemistry. Its popularity stems from the unique catalytic properties of certain gold ion species, which have been incrementally exploited in the synthesis of complex molecular structures.[1] Though the unparalleled contribution of gold catalysis to synthetic chemistry remains unquestionable, employing metal species such as gold as catalysts in chemical processes raises important health issues concerning the toxicity of gold ion species.[2]

In contrast to gold's elemental form, its ionic forms (i.e., Au^{+} and Au^{3+}) are sensitive, extremely reactive, and are able to interact and bind with biomolecules, such as enzymes, proteins, and DNA, thereby disturbing a series of cellular processes and precipitating serious health problems. For instance, it has been documented that the intake of $AuCl_3$ causes damage to vital human organs, including the kidney and liver, as well as the peripheral nervous system.[2]

Considering the deleterious effects of gold species on living organisms and their increasing role as catalysts in the chemical industry, it is crucial for researchers to be able to assess the levels of gold species in certain chemical, environmental, and biological samples.

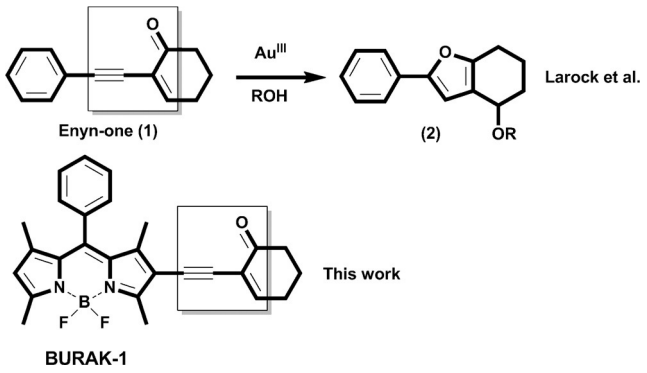
Recently, fluorescence-based techniques for sensing and monitoring target species in solutions as well as in living environments have received a great deal of attention.[3] In this context, several types of fluorescent probes have been devised for analysing gold species.[4] By extension, a variety of fluorophore core units such as rhodamine,[5] boron-dipyrromethene (BODIPY),[6] fluorescein,[7] and naphthalimide[8] have been judiciously modified with specific molecular motifs to recognize gold species through a distinct optical output: colorimetric and/or fluorometric change.

Notably, most existing gold ion sensors are based on specific chemical reactions that exploit the exceptional catalytic behaviour of gold species. In general, the optical signal is recognized as either an increase ("turn-on") or a decrease ("turn-off") in emission intensity without any noticeable change in emission wavelength. Importantly, measurements based on intensity changes are easily influenced by a host of environmental factors, including concentration variations and intensity of excitation.

By contrast, measuring optical signals as intensity ratios at two different wavelengths provides a built-in correction for the environmental effects and may assuage many of the problems associated with intensity-based sensors. Interestingly, the ratiometric recognition of gold ions by a single fluorescent probe structure is currently uncommon.[7a,8b] Two of those examples in recent literature are smart extensions of intensity-based sensors that benefit from the FRET (i.e., fluorescence resonance energy transfer) principle to achieve a ratiometric response.[9]

In response, we herein present the design, synthesis, spectral behaviour, and living cell application of a fluorescent probe, **BURAK-1**, which displays a sensitive, highly selective ratiometric response to gold ions and, surprisingly, Hg^{2+} ions as well.

In our sensing approach, we drew inspiration from a gold-catalyzed intramolecular cyclization reaction presented years ago by Larock et al. (Scheme 1).[10] It was reported that enyn-one (**1**) in the presence of a catalytic amount of $AuCl_3$ transforms rapidly into a new furan (**2**) derivative. By extension, our attention focused chiefly on exploiting this unique chemical transformation as a signal-transducing event for the recognition of gold ions.



Scheme 1. Work of both our and the Larock group.

It is well-known that extending the conjugation within a fluorophore–chromophore structure dramatically affects the

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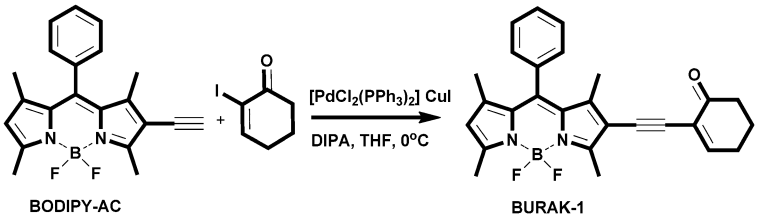
HOMO/LUMO levels of molecules and results in a dramatic red-shift of its absorption and emission band.[11] With this in mind, we integrated the enynone scaffold to a BODIPY-based fluorophore dye with the expectation of generating a highly conjugated BODIPY derivative emitting at a wavelength distinctly longer than its unmodified form (Scheme 1). We envisioned that only in the presence of gold species would the sensor structure transform itself into another BODIPY derivate lacking the extended conjugation. Gold-ion-triggered structural modification of the fluorescent dye was then anticipated to enable us to recognize the presence of gold species as a ratio of two distinct wavelengths, which is a primary principle of ratiometric sensing.

The title compound **BURAK-1** investigated in this study was prepared according to the synthetic route outlined in Scheme 2. Following a Sonogashira coupling protocol, the acetylene derivative of BODIPY (**BODIPY-AC**) prepared in three individual steps[12] was coupled with 2-iodocyclohex-2-enone[10] to give the desired probe structure in a moderate yield. The structure of the probe was clearly confirmed by NMR spectroscopic and HRMS analysis.[13]



Scheme 2. Synthesis of **BURAK-1**.

The probe's spectroscopic behaviour toward the added metal species was systematically investigated with the aid of ultraviolet (UV) and fluorescence spectroscopy. As depicted in Figure 1a, the UV/Vis spectrum of free **BURAK-1** (phosphate buffer/ethanol 6:4, pH 7.0) displays a maximum absorption band at 526 nm, while its fluorescence spectrum collected upon excitation at 460 nm exhibits an intense emission band at 562 nm, which belongs to the BODIPY chromophore.

Our investigation resumed with an evaluation of the optical behaviour of **BURAK-1** in response to the addition of Au^{3+} ions (e.g., $AuCl_3$). The spectral changes of the probe in the absence and presence of Au^{3+} ions appear in Figure 1. As shown, the addition of Au^{3+} (2 equiv) to **BURAK-1** prompted the appearance of a new emission band at 516 nm, with a concomitant decrease in the emission band at 562 nm. As anticipated, when the probe solution was treated with $AuCl_3$, the orange-emitting probe solution became distinctly green, as was clearly visible to the naked eye (see Figure S28 in the Supporting Information). The dramatic change in colour of the solution was attributed to a change of the BODIPY dye structure. Moreover, green emission was evidence of the existence of a nonconjugated BODIPY derivative.

This suggestion was supported by the outcome of the reaction of **BURAK-1** mediated by Au^{3+}, controlled by using TLC analysis. The green emissive compound, clearly followed on the TLC plate, was isolated and further characterized by NMR spectroscopy and HRMS as **BOD-FUR**, the cyclization product of **BURAK-1** (Scheme 3).[13] Evidently, the recognition of Au^{3+} was based on an Au^{3+}-mediated cyclization reaction that resulted in the formation of a highly emissive BODIPY-furane derivative (**BOD-FUR**).

The systematic titration of **BURAK-1** with Au^{3+} revealed that the ratiometric change of emission intensities at both wavelengths linearly correlated with the increased concentration of Au^{3+} in the range of 0.005–10 μM. At the same time, our kinetic study showed that the spectral response toward the addition of Au^{3+} was rapid (<1 min) and that emission intensity at 516 nm plateaued within 40 min due to the addition of 2 equiv of Au^{3+}, which thereby enhanced intensity at 516 nm by more than 70-fold. Moreover, the minimum amount of Au^{3+} detectable was evaluated to be 8 nM,[13] one of the lowest detection limits reported in fluorescence-based gold ion sensing.[4a] Meanwhile, a similar spectral trend was observed in the presence of Au^{+} species, revealing that the probe operates efficiently for both oxidation states of gold in a nondiscriminative manner.

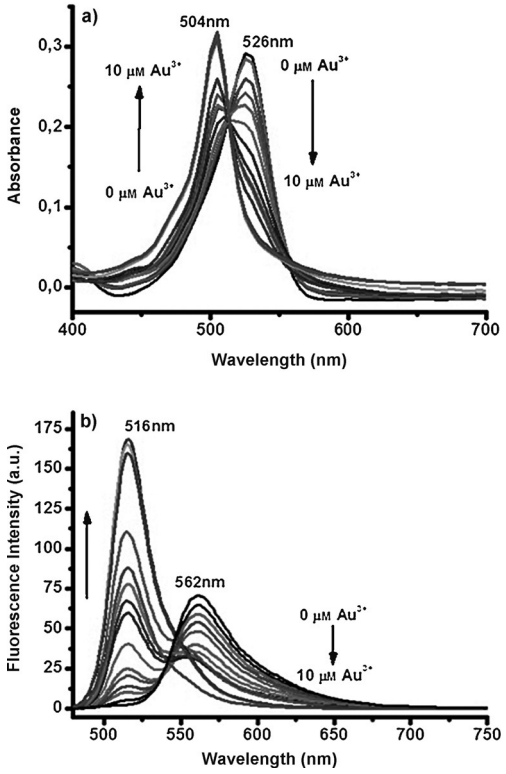
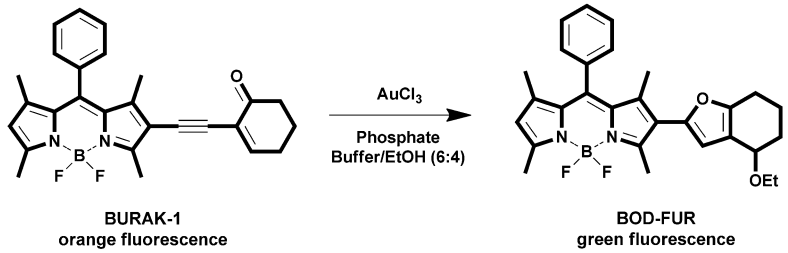


Figure 1. a) Absorbance and b) fluorescence titration spectra of **BURAK-1** (5 μM) + Au^{3+} (0.005 to 10 μM) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 6:4).



Scheme 3. Gold-mediated cyclization of **BURAK-1**.

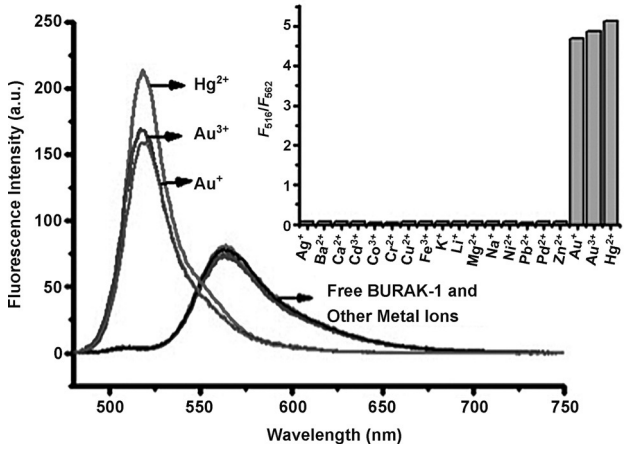


Figure 2. Fluorescence spectra of **BURAK-1** (5 μM), **BURAK-1** (5 μM) + Au^{3+}/Au^{+}/Hg^{2+} (25 μM, 5 equiv), **BURAK-1** (5 μM) + other metal ions (50 μM, 10 equiv) in 0.1 M potassium phosphate buffer, pH 7.0/EtOH (v/v, 6:4) (λ_{ex} = 460 nm, at 25 °C). Inset: Bar graph notation.

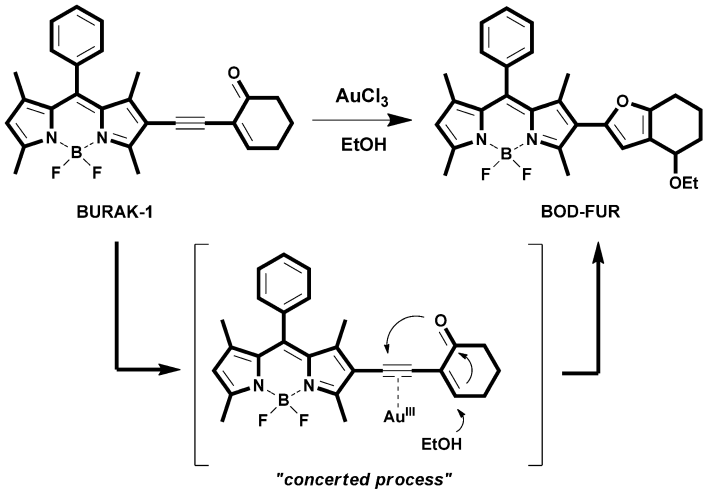
The selectivity profile of **BURAK-1** was surveyed by screening the spectral response toward metal species, including Zn^{2+}, Cd^{2+}, Ba^{2+}, Cu^{2+}, Li^{+}, K^{+}, Ni^{2+}, Cr^{2+}, Mg^{2+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Co^{2+}, and Ag^{+} (Figure 2). Surprisingly, in the presence of Hg^{2+} ions the probe displayed the exact sensing behaviour as in the detection of gold species. Similar to the gold ion sensing event, with the addition of Hg^{2+} a new peak band appeared at 516 nm, while the band at 562 nm decreased with an increased concentration of Hg^{2+} ions. This unexpected observation was reasonable, for Hg^{2+} ions similar to Au^{3+} species are known to have high affinities to alkynes. Notably, the detection limit for Hg^{2+} was measured to be slightly higher than that for Au^{3+} ions (60 nM) yet still at nanomolar levels.[13] From the competition experiment, **BURAK-1** was ultimately shown to display a dual nature and to efficiently operate for two metal species: Au^{+}/Au^{3+} and Hg^{2+} ions.

Having clarified the nature of detecting both metal species, we next assessed the possible interference of other metal species in the detection of Au^{3+} and Hg^{2+}. As shown in Figures S7 and S14 (Supporting Information), the response of **BURAK-1** toward both species remained unaffected in the presence of other competitive metal species. These results established that **BURAK-1** can also properly detect Au^{3+} and Hg^{2+} ions in mixtures of other related species.

Further experiments on improving the selectivity of the probe toward one distinct metal species, that is, either Au^{3+} or Hg^{2+}, revealed that the selectivity toward Hg^{2+} could be significantly improved by adjusting the nature of the sensing media. Remarkably, in an alternative solvent system, namely, a HEPES/acetonitrile (pH 7.0) buffer (6:4 (v/v)), the probe displayed exceptional selectivity toward Hg^{2+} ions. Importantly, in HEPES/acetonitrile, particularly at high contents of water (e.g. 6/4; v/v), Au^{3+} ions were rapidly reduced to gold black (aggregates of elemental gold), which indicates considerable loss of catalytic activity and accounts for the selectivity towards Hg^{2+}. Under these sensing conditions, the detection limit for Hg^{2+} was evaluated to be 250 nM.[13] Furthermore, no cross-talk with other metal species in the detection of Hg^{2+} was detected. Lastly, **BURAK-1** shows condition-dependent selectivity toward Hg^{2+} species and can be used on-demand as a sensitive, selective fluorescent probe for Hg^{2+} ions.

As consistent with findings reported in the literature, the recognition of Au^{3+} or Hg^{2+} is suggested to proceed by a concerted process initiated by the activation of the triple bond by the metal species (Scheme 4). This process follows a conjugate addition of an oxygen nucleophile that promotes intramolecular cyclization and yields a new BODIPY structure appended with a furan motive, which displays the distinct colour and emission of the solution.

Since **BURAK-1** constitutes all of the desirable features necessary for tracking species in a living milieu, we assessed its sensing capacity in living cells. To this end, human colon carcinoma cells (A-549) were incubated first with the probe (10 μM), to which was added Au^{3+} or Hg^{2+} (10 μM) to be incubated for another 60 min. The cells were also stained with a nucleus-staining dye (DAPI) for another 10 min. With the aid of



Scheme 4. Proposed reaction mechanism for the detection of gold ions.

fluorescence microscopy, the fluorescence images of the cells were taken before and after the addition of the metal species. As Figure 3 clearly shows, the cells incubated with **BURAK-1** emitted a red fluorescence in the absence of the metal species, while following Hg^{2+} or Au^{3+} accumulation they emitted

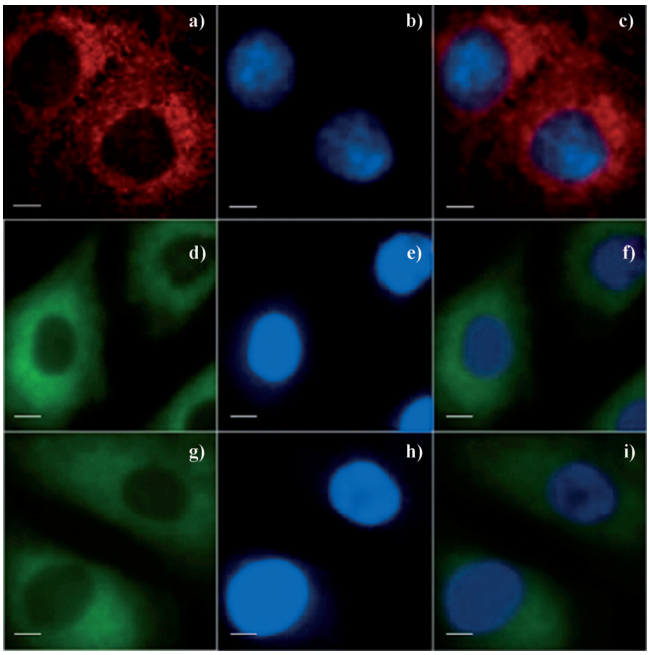


Figure 3. Fluorescence images of Human Lung Adenocarcinoma cells (A549). a) Fluorescence image of A549 cells treated with only **BURAK-1** (10 μM); b,e,h) Fluorescence image of cells treated with DAPI (control); d,g) fluorescence image of cells treated with **BURAK-1** (10 μM) and Au^{3+} (10 μM), Hg^{2+} (10 μM); c,f,i) merged images of frames a–b, d–e, and g–h (λ_{ex} = 460 nm). Scale bar represents 10 μm.

a characteristic green BODIPY emission, which agrees well with the spectral response obtained in the solution phase. Based on the nucleus counter-stain experiment and from the distinct change in cell fluorescence emission, we conclude that the probe passes through the cell membrane and detects Au^{3+} and Hg^{2+} from within the cell, particularly in the cytosol.

In summary, we have devised a unique BODIPY-based ratiometric fluorescent probe that shows a remarkable change in fluorescence emission toward Au^{3+} and Hg^{2+} ions with high sensitivity and selectivity over other metal ions. Notably, this probe displays a dual character and can detect both of the metal species with an extremely low detection limit. By adjusting sensing conditions and simply switching the solvent combination, it was also possible to selectively sense Hg^{2+} ions. Apart from the rapid and specific response to both metal ions in the solution, this probe proved highly successful in imaging gold and Hg^{2+} species in living cells.

Experimental Section

General method

$[PdCl_2(PPh_3)_2]$ (3.51 mg, 0.05 equiv), CuI (1.9 mg, 0.1equiv), and **BODIPY-AC** (69.6 mg, 2.0 equiv) were added to a mixture of 2-iodo-2-cyclohexen-1-one (22.1 mg, 0,1 mmol) in THF at 0 °C. Then, diisopropylamine (42 μL, 3.0 equiv) was added and the resulting mixture was stirred at 0 °C for 1 h. After completion of the reaction, THF was removed under vacuum and the resulting residue extracted three times with CH_2Cl_2 (3×30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resultant residue was purified by column chromatography (4:1 (hexane/ethyl acetate)) to afford **BURAK-1** as a red solid (31 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49–7.48 (m, 3H), 7.27–7.22 (m, 2H), 6.02 (s, 1H), 2.66 (s, 3H), 2.56 (s, 3H), 2.52–2.45 (m, 4H), 2.04 (quint. J = 6.4 Hz, 2H), 1.47 (s, 3H), 1.18 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.1, 157.2, 156.5, 152.2, 144.4, 142.9, 141.9, 134.4, 132.1, 130.0, 129.0, 127.6, 125.3, 121.8, 114.7, 90.0, 84.7, 37.9, 26.2, 22.2, 14.5, 14.2, 13.2, 12.9 ppm.

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Keywords: BODIPY · fluorescent sensors · gold · mercury · ratiometric

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[13] See the Supporting Information for more details.

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