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# A novel fluorescent probe for Au(III)/Au(I) ions based on an intramolecular hydroamination of a Bodipy derivative and its application to bioimaging†

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**A novel fluorescent probe for gold ions ($Au^{3+}$/$Au^{+}$) is reported through blocking photoinduced electron transfer, in which a boron dipyrromethene (Bodipy) derivative reveals high selectivity and sensitivity in a gold-catalyzed intramolecular hydroamination, and is successfully applied to fluorescence imaging of $Au^{3+}$ in living cells.**

In the past, gold-catalyzed chemistry has received extensive attention, and gold ions have been known to activate carbon–carbon multiple bonds, especially alkynes, toward nucleophilic addition. Due to the characteristic alkynophilicity of gold ions, many organic transformations have been investigated by employing gold ions.[1] Gold ions also have anti-inflammatory properties and are used as drugs for dealing with diseases such as arthritis, tuberculosis and cancer.[2] However, their salts such as gold chloride are known to cause damage to the liver, kidneys, and the peripheral nervous system.[3] Some gold ions such as Au(III) are so tightly bound to a certain enzyme that they are reported to cause cell toxicity in living organisms.[4] For this reason, it is of urgent necessity to develop fluorescent probes to assess the quantity of environmental gold ions by luminescence methods.

Recently, a few fluorescence probes for gold ions have been reported based on gold-ion-promoted cyclizations[5] from the non-fluorescent spirocyclic rhodamine to the strongly fluorescent rhodamine[6] or from the non-fluorescent apocoumarin to the strongly fluorescent coumarin.[7] Among the former, Lin and co-workers have ingeniously designed a new FRET-based, ratiometric probe through linking a Bodipy unit to the non-fluorescent rhodamine, that is, before the $Au^{3+}$-mediated cyclization, the Bodipy unit gives a short-wave emission, and after, the excited Bodipy unit transfers energy to the fluorescent rhodamine to emit a long-wave emission.[6e] In addition, based on $Au^{3+}$-promoted oxidizing reaction, Wang and Peng *et al.* reported a ratiometric fluorescent sensor for dual-analytes ($Au^{3+}$ and $Hg^{2+}$) through pH tuning in different aqueous solutions, *e.g.* MeOH–$H_2O$ (95 : 5 v/v pH 9.0) for Au(III) ions.[8] However, it is still a challenge to extend a new fluorescent probe with high sensitivity and selectivity for gold ions.

In this communication, we report a Bodipy-based fluorescent probe for highly selective and sensitive detection of gold ions, and its successful application to cell imaging of $Au^{3+}$.

Our strategy is based on the blocking of photoinduced electron transfer (PET) process. The synthesized Bodipy derivative **1** is non-fluorescent resulting from a photoinduced intramolecular electron transfer from the aniline unit to the Bodipy moiety. However, in the presence of $Au^{3+}$/$Au^{+}$ compound **1** can convert to **2**, in which the 2′-ethynyl biphenylamine unit is transformed to the phenanthridine moiety by gold-ion-promoted intramolecular hydroamination.[9] Because of the loss of the electron-donating amino group, the PET process cannot occur, and thus strong green fluorescence of the Bodipy is released. This process is shown in Scheme 1.

The synthetic route to probe **1** is depicted in Scheme 2. A very important building block, diphenylaldehyde derivative **5**, was synthesized by using 2-bromo-1-iodo-4-methylbenzene as starting material undergoing reaction of the Sonogashira coupling, hydroxy methylation, boronization and the Suzuki coupling. The Bodipy derivative **6** was obtained through the TFA catalyzed condensation reaction of intermediate **5** with 3,5-dimethyl-4-(ethoxycarbonyl)pyrrole according to a routine Bodipy formation procedure. At last, the target probe **1** was afforded by reduction reaction of **6** with $SnCl_2$. The detailed experimental procedure and characterization data are provided as the ESI.†

The treatment of probe **1** with a catalytic amount of $AuCl_3$ in ethanol solution results in the appearance of a strong green-fluorescent solution. After the reaction was completed, the fluorescent compound **2** was isolated by column chromatography,


**Scheme 1** Hydroamination of **1** in the presence of $Au^{3+}$/$Au^{+}$ in EtOH.

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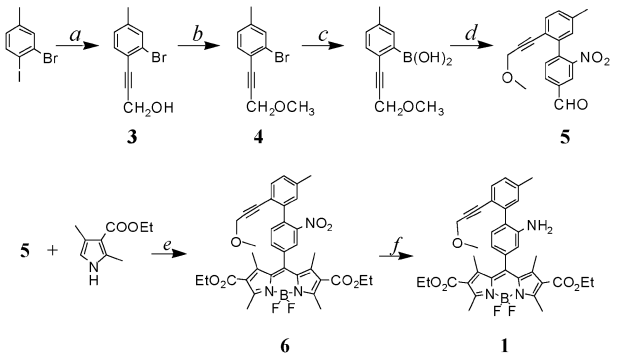


**Scheme 2** Synthetic route to probe **1**. *Reagents and conditions*: (a) $Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$, propargyl alcohol, 40 °C; (b) NaH, MeI, THF; (c) (i) *n*-BuLi, THF, −78 °C; (ii) $B(OCH_3)_3$, −78 °C; (d) $Pd(PPh_3)_4$, $K_2CO_3$, 4-bromo-3-nitrobenzaldehyde, toluene, 80 °C; (e) (i) cat. TFA, DDQ, $CH_2Cl_2$; (ii) $Et_3N$, $BF_3{\cdot}Et_2O$, toluene, r.t.; (f) $SnCl_2$, conc. HCl (aq), THF/EtOH = 1 : 1.

which was confirmed as an intramolecular hydroamination product by $^1H$ NMR and mass spectral analyses.

The probe **1** in EtOH/0.01 M PBS buffer (1 : 1, v/v, pH 7.4) (5 μM) exhibits almost no fluorescence due to the effective photoinduced electron transfer (PET) quenching process. Upon addition of 5.0 equiv. of $Au^{3+}$ ions to the solution, the fluorescence intensity increased rapidly and an approximately 116-fold fluorescence enhancement after 60 min was observed (Fig. 1). This shows that the gold-ion-mediated intramolecular hydroamination occurs. Similar fluorescence enhancements at 511 nm were observed in the presence of $Au^+$ ions under the same conditions (Fig. S1, ESI†), however, a longer time was required to reach fluorescence intensity maximum relative to $Au^{3+}$. A pseudo-first-order rate constant of probe **1** (5 μM) conversion to **2** in the aqueous solution was measured in the presence of $Au^{3+}$ (5 equiv.), and the observed rate constant ($k_{obs}$) is obtained to be $9.2 \times 10^{-4}$ $s^{-1}$. The $k_{obs}$ value of $Au^+$ is $6.0 \times 10^{-4}$ $s^{-1}$, which is lower than that of $Au^{3+}$ (Fig. S2, ESI†), and this may be because of a stronger interaction between highly charged $Au^{3+}$ and the alkyne than that of $Au^+$, in a protic solvent.[6c] These rate constants are faster than those for propargylamine-derived rhodamine ($4.5 \times 10^{-4}$ $s^{-1}$)[6a] and apocoumarin ($3.31 \times 10^{-5}$ $s^{-1}$),[7] and show that probe **1** can respond rapidly to gold ions.

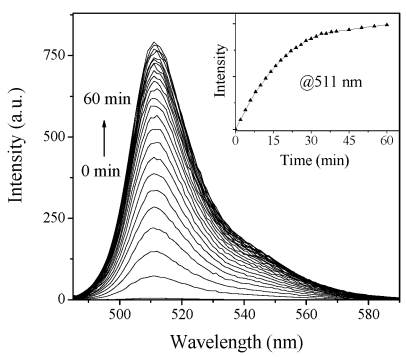


**Fig. 1** Time-dependent fluorescence spectral changes of probe **1** (5 μM) with addition of 5.0 equiv. of $Au^{3+}$ in EtOH/0.01 M PBS buffer (1 : 1, v/v, pH 7.4), excitation at 480 nm. Inset: time dependent fluorescence intensity changes (at 511 nm) of probe **1** with $Au^{3+}$.

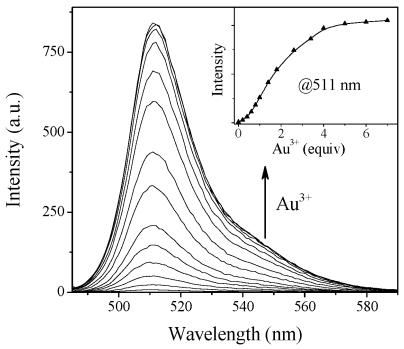


**Fig. 2** Fluorescence changes of probe **1** (5 μM) with various amounts of $Au^{3+}$ (0, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2.6, 3.4, 4, 5, 6, 7 equiv.) in EtOH/0.01 M PBS buffer (1 : 1, v/v, pH 7.4), recorded after 30 min, excitation at 480 nm. Inset: fluorescence intensity changes (at 511 nm) of probe **1** with various amounts of $Au^{3+}$.

The fluorescence response of probe **1** in the aqueous solution toward various amounts of $Au^{3+}$ was examined. The fluorescence intensity was measured after 30 min for each addition of $Au^{3+}$. As shown in Fig. 2, the fluorescence intensity at 511 nm increases rapidly with the concentration of $Au^{3+}$, and reaches the saturation of intensity at about 4 equiv. $Au^{3+}$. It is a linear relationship between the fluorescent intensity and the concentration of $Au^{3+}$ in the range from 0.1 μM to 0.6 μM (Fig. S3, ESI†). Based on those data, the detection limit of probe **1** was estimated to be 320 nM (63 ppb) on the basis of the signal-to-noise ratio of 3. This shows that probe **1** has a high sensitivity for detecting gold ions.

Next, $Ag^+$, $Hg^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Cr^{3+}$, $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Pd^{2+}$ ions were used to measure the selectivity of probe **1** toward gold ions in EtOH/0.01 M PBS buffer (1 : 1, v/v, pH 7.4) and fluorescence spectra were recorded after 60 min upon the addition of 5 equiv. of those metal ions. As shown in Fig. 3a, those ions display almost no fluorescence except $Pd^{2+}$. Hence, probe **1** displays excellent selectivity toward gold ions.

The interference experiments were also carried out for the selectivity of probe **1** toward $Au^{3+}$ ions in the presence of those metal ions. The fluorescence was turned on again when the $Au^{3+}$ ions were added to the solutions of probe **1** and those metal ions, which indicated that those ions have little effect on the fluorescent probe **1** except $Hg^{2+}$ and $Pd^{2+}$ (Fig. 3b). Both $Hg^{2+}$ and $Pd^{2+}$ can activate alkynes especially terminal alkynes,[1d,e,10] so we had hoped to avoid their interference by designing the probe molecule **1** as a non-terminal alkyne. However, it is surprising that probe **1** is still interfered by the two ions. The detail mechanism remains to be investigated.

Fluorescence microscopic imaging for the $Au^{3+}$ mediated probe **1** in HeLa cells was performed. HeLa cells were seeded in a 24-well size plastic-bottom dish at a density of $5 \times 10^4$ cells per well in a culture medium overnight. The cells were treated with 10 μM of probe **1** for 1 h and washed 3 times with PBS buffer solution. Then cells were incubated with 20 μM $AuCl_3$ for 1 h and the cell cultures were washed with PBS to remove the residue $Au^{3+}$ ions. The images of the cells were obtained by a confocal laser scanning microscope, shown in Fig. 4. The cells exposed to both **1** and $Au^{3+}$ display a green emission, while those treated only with **1** are dark.

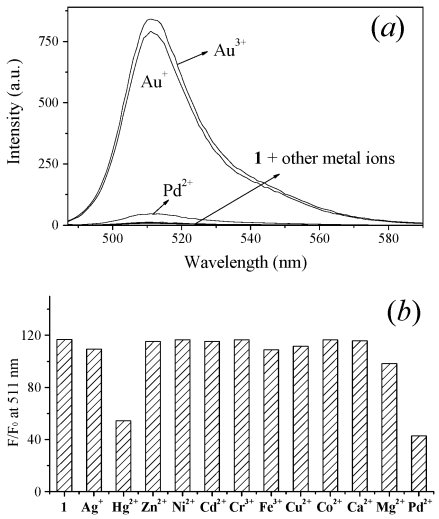


**Fig. 3** (a) Fluorescence spectra of probe **1** (5 μM) with $Au^{3+}$/$Au^{+}$ ions and other metal ions (5 equiv.) including $Ag^{+}$, $Hg^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Cr^{3+}$, $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Pd^{2+}$ in EtOH/0.01 M PBS buffer (1 : 1, v/v, pH 7.4). (b) Relative fluorescence responses at 511 nm of **1** in the presence of $Au^{3+}$ (5 equiv.) and various metal species (5 equiv.), where $F_0$ is the intensity in the absence of $Au^{3+}$.

In summary, we have designed a novel fluorescent probe for gold ions based on the gold-ion-mediated intramolecular hydroamination, which converts the electron donor, the aniline moiety, to phenanthridine, resulting in a loss of the electron-donating ability, thus blocking the intramolecular PET process to emit a strong green fluorescence. This is the first time a fluorescent probe for gold ions has been constructed in this way. It provides a new idea for designing fluorescent probes for gold ions. The fluorescent probe with a Bodipy chromophore possesses a short response time and a low detection limit.

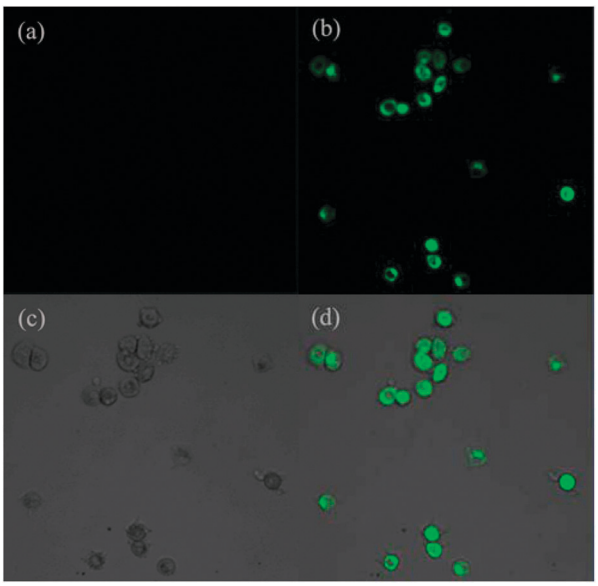


**Fig. 4** Bright-field and fluorescence images of HeLa cells: (a) fluorescence image of HeLa cells treated with **1**; (b) fluorescence image of HeLa cells treated with **1** and $Au^{3+}$; (c) brightfield image of HeLa cells treated with **1** and $Au^{3+}$; (d) merged image of (b) and (c).

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