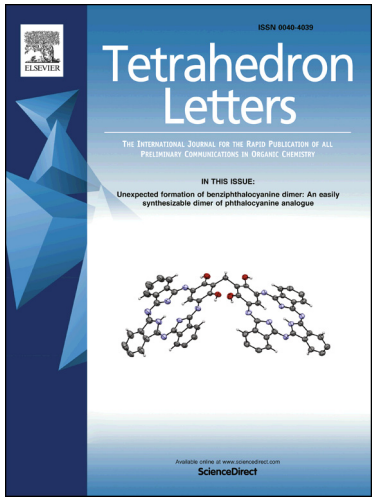

Reversible rhodamine-alkyne Au^{3+}-selective chemosensor and its bioimaging application

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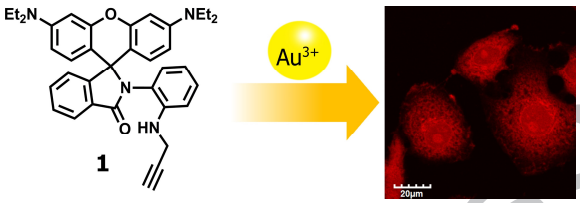
Pailin Srisuratsiri[a,b], Phongthon Kanjanasirirat[c], Arthid Chairongdua[c], and Palangpon Kongsaeree[a,d,*]



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Reversible rhodamine-alkyne Au^{3+}-selective chemosensor and its bioimaging application

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ABSTRACT

A rhodamine-alkyne conjugate has been developed for selective detection of Au^{3+} in a reversible manner. Rhodamine was coupled with *o*-phenylenediamine bearing a propargyl moiety. The probe displayed high selectivity and sensitivity toward Au^{3+} with a 148-fold "turn-on" fluorescence response in EtOH-H_2O (1:1 v/v). The stoichiometry of the chemosensor and Au^{3+} was 1:1 based on the Job plot analysis with the detection limit of 10.5 nM. The chemosensor is membrane permeable and capable of monitoring Au^{3+} in cultured HeLa cells.

Gold-related research is a rapidly growing field in chemistry and materials science due to a number of its advantageous properties. With its strong alkynophilicity, gold-based catalysts have been explored for various chemical transformations such as activation of carbon-carbon triple bonds, selective oxidation, etc.[1-5] In addition, gold nanoparticles with useful chemical and photophysical properties have been demonstrated in diverse areas from basic science to clinical applications and gold complexes have been used as therapeutic agents for rheumatoid arthritis, asthma, and cancer.[6] However, gold species could tightly bind to DNA and enzyme thus causes toxicity in humans.[6-8] The soluble gold salts were reported to cause damage to liver, kidney and nervous system.[9,10] Therefore, it is highly desirable to develop simple, efficient and rapid Au^{3+}-sensing probes that could be applicable to detect Au^{3+} both in vitro and in vivo. Several fluorescent probes for Au^{3+} have been reported with different fluorophores including naphthalimide, BODIPY and xanthene-based dyes including fluorescein, rhodol and rhodamine.[11] In the current study, rhodamine has been chosen due to its excellent photophysical properties such as high absorption coefficient, high fluorescent quantum yield, long absorption and emission wavelength, and high photostability. Development of Au^{3+}-selective fluorescent probes have been designed based on either reaction-based or coordination-based approaches.[11] In continuation of our research interest in chemosensors,[12] we report herein a rhodamine-based fluorescent chemosensor **1** appended with a reactive alkyne moiety for selective detection of Au^{3+} (Fig. 1). Au^{3+}-selective chemical probes with a reactive alkyne unit with a reaction-based design mostly undergo an irreversible transformation,[13] but this rhodamine-alkyne conjugate **1** reacts with Au^{3+} in a reversible sensing manner.

Most Au^{3+}-selective alkyne-based chemical probes proceeded to form 5- or 6-membered ring cyclization products,[14-17] while the ring-size limit in gold-catalyzed cyclization reactions were mostly 7- and 8-membered rings with 7-exo-dig or 8-endo-dig mechanisms.[17-20] For rhodamine-*o*-phenylenediamine-propargyl conjugate **1,** if Au^{3+}-induced cyclization occurs, it would proceed through 8- or 9-membered ring intermediates with 8-exo-dig or 9- endo-dig pathways. Compound **1** was prepared by appending a propargyl moiety to rhodamine with *o*-phenylenediamine as a

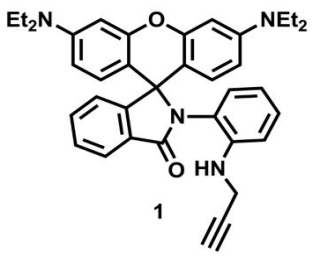
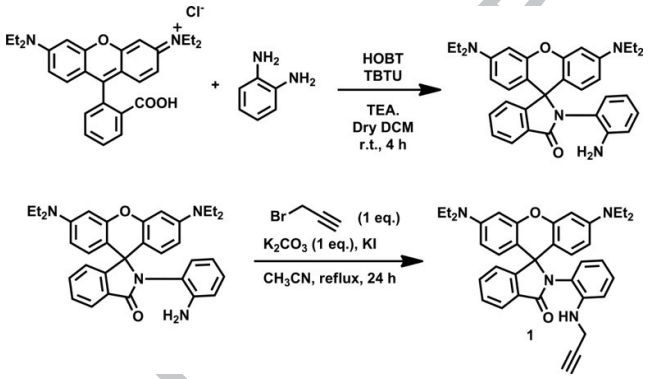


Fig. 1. Rhodamine-propargyl fluorogenic probe **1** with an *o*-phenylenediamine linker for Au^{3+} detection.



Scheme 1. Synthesis of rhodamine-alkyne chemosensor **1**.

spacer. The coupling reaction between rhodamine with *o*-phenylenediamine was achieved by TBTU. Rhodamine-*o*-phenylenediamine conjugate was subsequently reacted with propargyl bromide and K_2CO_3 in CH_3CN to give chemosensor **1** in 61.7 %yield (Scheme 1). The structure of chemosensor **1** was confirmed by ^1H NMR ($CDCl_3$). The spectra displayed the terminal alkyne proton at δ1.9 ppm and the CH_2 proton of the propargyl group at δ 3.4 ppm, respectively (Fig. S4). Preliminarily, the probe solutions were tested with various metal ions including main group and transition metals (Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^+, Cu^{2+}, Pd^{2+}, Au^{3+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Ce^{3+}) in EtOH-H_2O (1:1, v/v).

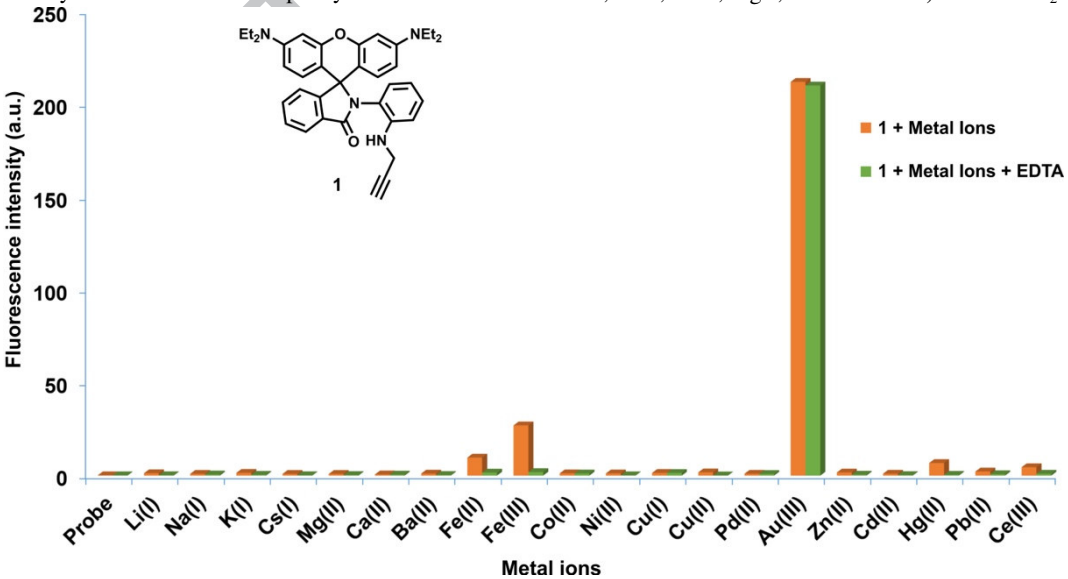


Fig. 2. Selectivity of **1** toward Au^{3+} among various metal ions. Solutions of **1** (60 μM, orange bars) with metal ions (1 equiv.) in EtOH-H_2O (1:1, v/v). Solutions of **1** (60 μM, green bars) with various metal ions (1 equiv.) in EtOH-H_2O (1:1, v/v) and EDTA (1 equiv.). Upon the addition of Au^{3+}, the colorless solution of **1** changes to pink and a strong fluorescence emission is observed. (λ_{ex} 520 nm, λ_{em} 583 nm).

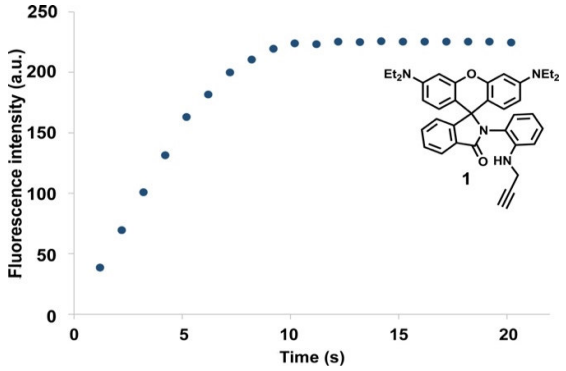


Fig. 3. Enhanced fluorescence profiles were investigated by addition of Au^{3+} (1 equiv.) to probe **1** (60 μM) in EtOH-H_2O (1:1, v/v) from 1 to 20 sec.

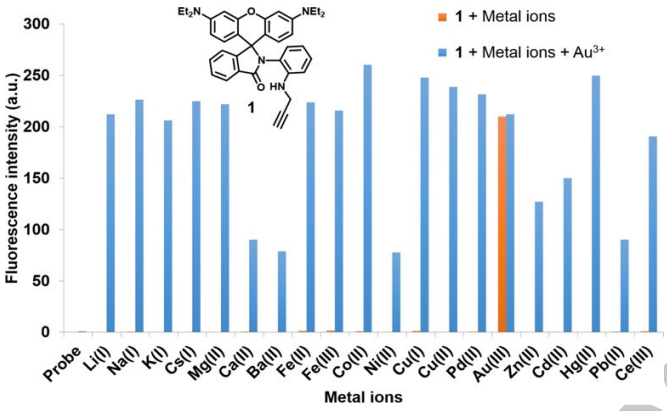


Fig. 4. Interference of other metal ions to Au^{3+} selectivity of the chemosensor **1**. Orange bars: fluorescence response of probe **1** (60 μM) to various metal ions (10 equiv.). Blue bars: fluorescence response of probe **1** (60 μM) in the presence of Au^{3+} (1 equiv.) with different metal ions (10 equiv.). The experiments were carried out by mixing probe **1** and metal ions in 1:1 (v/v) EtOH-H_2O. (λ_{ex} 520 nm, λ_{em} 583 nm).

In the presence of Au^{3+}, the colorless solution of **1** rapidly changed to pink and gave the characteristic fluorescence emission of rhodamine (λ = 583 nm) with a 148-fold enhancement. However, minor signals from Fe^{2+} and Fe^{3+} (7.4 and 20.7 folds) were also observed. EDTA was then employed as a masking agent and the enhanced selectivity of the probe was accomplished without losing the sensitivity of the sensor toward Au^{3+}.[13] Evidently, EDTA could eliminate the interferences from other metal ions and thus rendered the probe highly selective to Au^{3+} (Fig. 2). The reaction of the probe with Au^{3+} proceeded rapidly to completion within 10 sec (Fig. 3). A competitive experiment was carried out in the presence of various metal ions showed that the sensing of Au^{3+} by **1** exhibited a satisfactory selectivity toward Au^{3+} without being interfered by other metal ions. Notably, the fluorescence signal of probe **1** was marginally quenched by Ca^{2+}, Ba^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} (Fig. 4). A fluorescence titration experiment was performed by adding increasing Au^{3+} concentrations to the solution of **1** as shown in Fig. 5. In the absence of Au^{3+}, the probe solution was non-emissive until the addition of Au^{3+}. This result suggested that a ring-opening process of the spirolactam could be triggered by Au^{3+}.[21,22] The sensitivity of the rhodamine-alkyne probe **1** was studied with the limit-of-detection (LOD) value of 10.5 nM (S/N = 3), somewhat lower or comparable with previously reported Au^{3+}-selective fluorescent probes (Table S1).[11,14-16,21-46] Based on a Job plot analysis, a series of solutions containing **1** and Au^{3+} were prepared such that the sum of the total metal ion and the

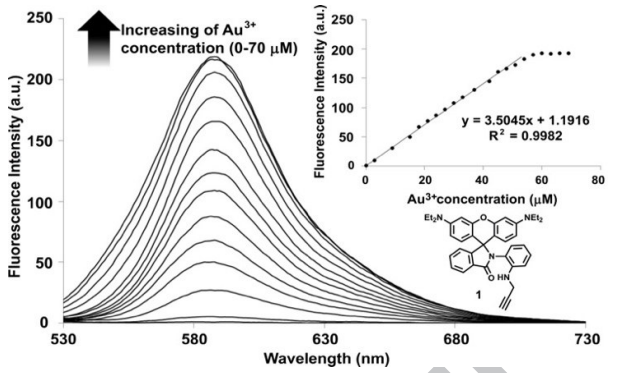


Fig. 5. Fluorescence spectra of **1** (60 μM) titrated with increasing concentrations of Au^{3+}: (0 - 60 μM) in EtOH-H_2O (1:1, v/v). (λ_{ex} 520 nm, λ_{em} 530-800 nm).

probe concentration remained constant (50 μM). The mole

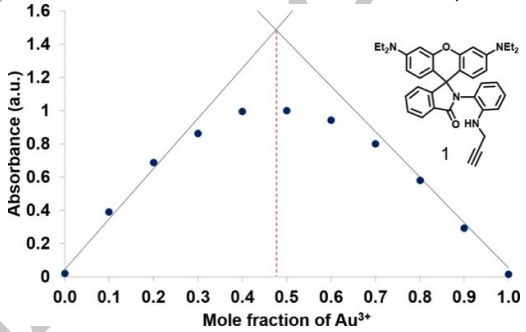
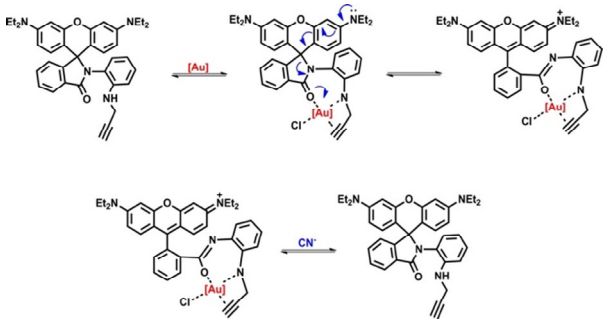


Fig. 6. A Job plot of probe 1 and Au^{3+} reveals a 1:1

fraction of Au^{3+} (x = 0 to 1.0) was then plotted against the corrected absorbance at 565 nm. The Job plot indicated a 1:1 stoichiometric ratio between Au^{3+} and the probe (Fig. 6). The detection mechanism of probe **1** for Au^{3+} was then investigated by addition of KCN (1.2 equiv.) to the probe-Au^{3+} complex solution. Interestingly, KCN could completely quenched the fluorescence of the complex solution, indicating the reversible complexation of Au^{3+} and probe **1**. Further HR-ESI-MS analysis revealed the unchanged molecular ion of the probe at *m/z* 609.2549 $[\mathbf{1} + K]^+$, suggesting the plausibly intact structure of **1** (Fig. S9). The ^1H NMR analysis of **1** in the presence of Au^{3+} (1.0 equiv.) was carried out in MeOH-d_4, due to the insolubility of Au^{3+} in $CDCl_3$. After the addition of Au^{3+}, the alkyne proton was downfield shifted from δ 2.5 to δ 3.6 ppm, indicating that Au^{3+} might chelated at the alkyne unit to form Au^{3+}-alkyne intermediate.[47] Additionally, the nonequivalent signals of the methyl groups of rhodamine, in the ring-opening form, were observed at δ 1.25 and δ 1.35 ppm, respectively [46]. Evidently, the chelation of Au^{3+} to **1** occurred without leading to any detectable cyclization products (Fig. S10). A reversible binding mechanism for Au^{3+} detection of the chemical probe **1** was therefore proposed as shown in Scheme 2. In the beginning, the probe exhibited no fluorescence due to the spirolactam of rhodamine in closed form. The addition of Au^{3+} promoted ring opening of the rhodamine spirolactam, resulting in a strong fluorescence enhancement. Upon the addition of KCN to remove Au^{3+} from the complex, the color of the solution was reversed from pink to colorless (Fig. S11) and the fluorescence emission was quenched. Apparently, the rhodamine-alkyne conjugate **1** could react with Au^{3+} in a reversible manner without formation of the intramolecular ring-closure. A 10-membered ring complex could be formed without leading to the cyclization via 8-exo-dig or 9-



Scheme 2. A proposed reaction mechanism of **1** with Au^{3+}.

endo-dig pathways. The binding constant (K_a) of Au^{3+} to the sensor **1** was calculated to be 6.67×10^5 M^{-1} (Fig. S12) based on the modified Benesi-Hildebrand equation.[48]

Supported by the effectiveness of the probe to detect Au^{3+}, the chemosensor **1** was further applied to monitor intracellular Au^{3+}in HeLa cells. The cells were cultured in MEM (modified Eagle's medium), supplemented with 10% FBS (fetal bovine serum) with 1% antibiotics and 5% CO_2 in humidified environment. The cells were plated in a 12-mm cover slip in culture media at a density of 2.5×10^4 cells per well and incubated with Au^{3+} (100 μM) for 1 h at room temperature and then washed with Dulbecco's phosphate-buffered saline buffer (DPBS, pH 7.4) to remove the remaining Au^{3+}. The treated cells were incubated with probe **1** for 10 min and mounted with a DAPI nuclear staining solution. HeLa cells were subsequently visualized using a confocal laser scanning microscope (Fig. 7). The fluorescence emission, observed by excitation at 559 nm, indicated that probe **1** was membrane permeable and could be utilized for monitoring Au^{3+} in the cellular environment. In the control experiment, cells treated with probe **1** without Au^{3+} showed no fluorescence emission. This implied that probe **1** was not activated by cellular endogenous metal ions (Fig. 7a-7d). Hence, the fluorescence signal obtained from these images occurred selectively from Au^{3+}. Cell viability was performed to evaluate cytotoxicity of chemosensor **1** by MTT assays. The cells were exposed with sensor **1** in 1% EtOH at various

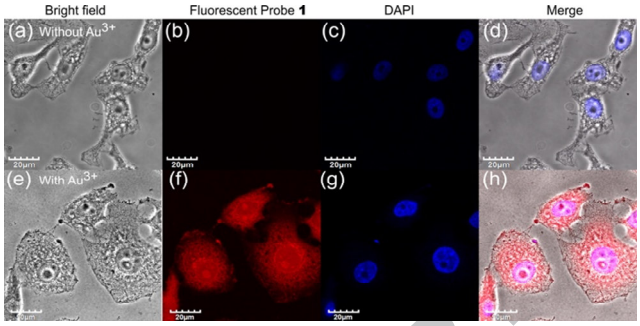


Fig. 7. Confocal fluorescence images of probe **1** in HeLa cells. The cells were incubated with probe **1** (60 μM) in DPBS buffer (pH 7.4) for 1 h without Au^{3+} (a-d). The cells were incubated with Au^{3+} (100 μM) for 1 h at RT then with probe **1** (60 μM) in DPBS buffer (pH 7.4) for 10 min (e-h) and (a) and (e) bright-field images; (b) and (f) fluorescence images; (c) and (g) fluorescence images of nuclei counterstained with DAPI; (d) and (h) merged images of (a-c) and (e-g), respectively.

concentrations for 24 h. At 60 μM of sensor **1**, ~90% of the HeLa cells remained viable (Fig. S13).

In conclusion, the rhodamine-alkyne fluorogenic probe **1** is selective to Au^{3+} with the detection limit of 10.5 nM (2.1 ppb). The sensing mechanism was based on the alkynophilicity of Au^{3+} and the reaction was found to be reversible. The probe could be employed for bioimaging of Au^{3+} in cultured HeLa cells.

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at .....

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Highlights

- A rhodamine-propargyl conjugate was synthesized and characterized by ^{1}H NMR, ^{13}C NMR and HRMS.
- The rhodamine-based probe is highly selective toward Au^{3+} among other metal ions and the sensing mechanism is reversible.
- The rhodamine-propargyl sensor was used in bioimaging of Au^{3+} in HeLa cells.