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Ground-State Elevation Approach To Suppress Side Reactions in Gold-Sensing Systems Based on Alkyne Activation

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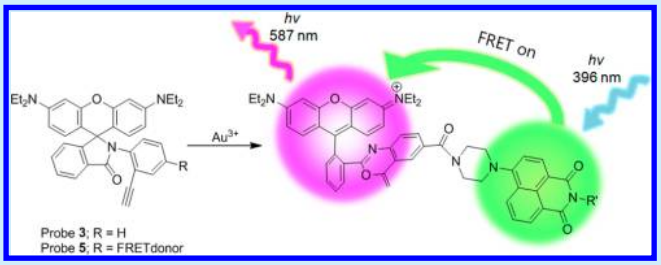
Supporting Information

ABSTRACT: A novel approach to suppress the side reactions observed in the reaction-based gold-sensing systems based on the alkyne activation is disclosed. By elevating steric strain around the reaction site, the gold ion promoted ring-opening process in rhodamine-lactam probes is significantly accelerated, which also leads to suppression of those possible side reactions. As a result, the probes show very high sensitivity in addition to excellent selectivity toward gold species. Furthermore, bioimaging of gold species in live cells was demonstrated with a FRET version.



Gold complexes have been intensively used in various research fields for their unique chemical and biological properties. As a result of relativistic effect, the gold atom has the 6s orbital contracted and the 5d orbital expanded, which makes gold ions strong π-bond acceptors;[1] hence, they are used as mild and efficient catalysts for activating carbon−carbon multiple bonds.[2] Gold complexes also exert cytotoxicity to DNA and enzymes and thus have been investigatged as anticancer and antirheumatoid agents.[3] The rich chemistry and biological activities of gold species demand efficient detection systems; however, it is only in recent years that fluorescent sensing systems appeared.

By following our reaction-based sensing approach to silver ions,[4] we recently reported a novel gold-sensing scheme.[5] Others also concurrently reported related reaction-based probes for gold ions.[6] The initial probes such as **1a** and **1b** (Figure 1) utilize the strong affinity of gold ions to the carbon−carbon triple bond to promote a chemical conversion that brings turn-on fluorescence change. Subsequently, various types of fluorescent probes for gold ions have been developed by changing the alkyne activation mode.[7] Although these probes show high selectivity toward gold ions with turn-on fluorescence response, they pose a serious drawback in the gold ion-promoted chemical conversion: the activation of alkyne by gold ions can involve side reactions including the alkyne hydration,[8] as we raised this issue earlier.[7g] Such side reactions can complicate the quantification process because the degree of side reactions would be dependent on the sensing conditions (media, temperature, concentration, etc). In addition, the nonsignaling processes lower the probe's sensitivity toward gold ions.

One way to alleviate the side reactions is to separate the reaction site from the signaling part, as in the case of probe **2**

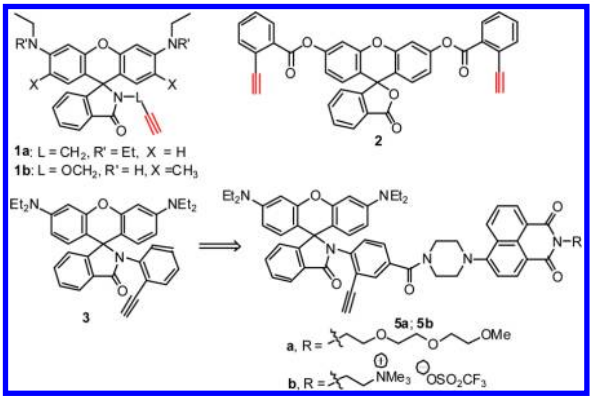


Figure 1. Known fluorescent probes **1** and **2** for gold species and a new probe **3** and its FRET versions **5**.

that was reported by us[7g] and a related one by others.[7f] Soon after, we recognized a potential weakness from such an ester-type probe **2**: the ester linkage is vulnerable to the ubiquitous esterase activity in living systems.[9] Our attempts to develop ester-type probes that resist to esterase activity have not been successful so far. In search of a reaction-based fluorescent probe for gold ions that causes little side reactions and also is stable to esterase activity, we have reinvestigated the initial rhodamine-lactam ring-opening approach.[5] In this paper, we disclose the novel approach that alleviates the side reactions and also improves the slow response rate and low sensitivity of the previous probes. Furthermore, from the new sensing system we can readily construct its fluorescence resonance energy transfer

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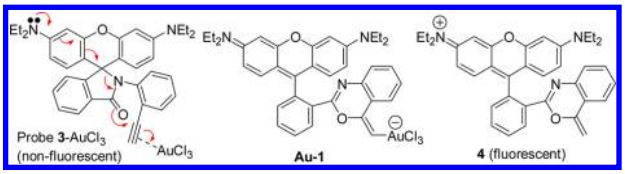
(FRET) system, which allows analyte quantification with minimized interference from the environmental parameters.[10]

We reasoned that enhancing steric strain around the rhodamine-lactam would accelerate the ring-opening process, which might lead to suppression of the side reactions observed in the previous *N*-propargylrhodamine-lactam probe:[5,6] alkyne hydration and unusual hydroarylation. The alkyne hydration can be a serious side reaction for the gold sensing process, as evidenced by our study on a model compound.[11] A designed probe is rhodamine-lactam **3** in which the *N*-(2-ethynlphenyl) group is expected to raise steric strain around the lactam moiety, which would lift the probe's ground state energy and thus accelerate the gold ion-promoted ring-opening process (Scheme 1). To support the ground-state elevation in probe **3**,

Scheme 1. Turn-on Sensing Scheme of $AuCl_3$ by Probe 3



we carried out DFT calculation on the rhodamine ring-opening process by constrained optimization on the Au−C bond and also pH dependent rhodamine ring-opening for probes **1a** and **3** (Figures S1 and S2, Supporting Information). The energy profiles show that the ring-opening reaction is faster in the case of probe **3** than the case of probe **1a**, and the difference between the highest energy barriers in two probes is 12.88 kcal/mol at Au−C bond distance of 2.8 Å and 2.9 Å for probe **1a** and probe **3**, respectively. This rate acceleration in turn would suppress the competing alkyne hydration that is a nonsignaling process. Introduction of phenyl ring between the spirolactam nitrogen and the ethynyl group would also make the unusual cyclization process observed in probe **1a** (hydroarylation involving the diethylaminobenzene ring)[7g] unconceivable because the distance between the two reaction sites becomes so long (4.9 vs 6.0 Å, Figure S3, Supporting Information).

Probe **3** can be synthesized by direct coupling of rhodamine B acid chloride with 2-ethynylaniline in 79% yield (Scheme S1, Supporting Information). Probe **3** alone is nonfluorescent but emits strongly upon addition of gold ions, both Au(I) and Au(III), in aqueous media (pH 6 HEPES containing 30% CH_3CN at 36 °C, Figure 2b, Figure S4, Supporting Information). The turn-on fluorescence response can be explained by gold ion-triggered consecutive spirolactam ring-opening and intramolecular cyclization processes to form

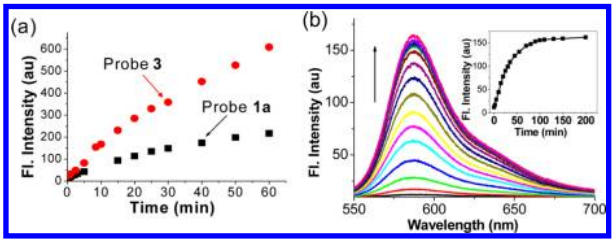


Figure 2. (a) Fluorescence response of probes **1a** and **3** (10 μM each) toward 1 equiv of $AuCl_3$ depending on time at 25 °C. (b) Time course of fluorescence change of probe **3** toward $AuCl_3$ (each at 10 μM) at 36 °C. Both data are obtained in 30% CH_3CN−PBS buffer (pH 6.0, 10 mM).

vinylgold intermediat **Au-1** and subsequent protodeauration to form oxazine **4** that is strongly fluorescent. **Au-1** is also likely to emit strong fluorescence from the fully conjugated xanthene moiety.

In addition, a possible hydration of the ethynyl group in probe **3** promoted by gold ions was carefully followed by TLC and ^1H NMR. Upon addition of $AuCl_3$ to a solution of probe **3** (2.5 mM in CH_3CN containing 30% water), a highly polar spot appeared immediately with no other spots including that of the probe. The simple hydration product, if any, is expected to appear near or below the probe on silica gel TLC plate, but no such spot appeared (Figure S6, Supporting Information). The polar spot is likely to be vinylgold(III) intermediate **Au-1**,[12] which is converted to oxazine **4** through a protodeauration process, as followed by NMR spectral change for the vinylic protons (Figure S7, Supporting Information). Formation of oxazine **4** was supported by its M^+ base peak at 543.29 (Figure S8, Supporting Information). An unusual rhodamine-lactam ring-opening product, as observed in the case of probe **1a**,[7g] seemed to be absent in the present case, plausibly owing to a highly strained transition state expected.

Probe **3** responds to gold ions faster than the previous probe **1** (Figure 2a):[5] the former showed an ~4.5 times larger pseudo-first-order rate constant than the latter (k_{obs} = 0.13 min^{-1} for **3**, k_{obs} = 0.029 min^{-1} for **1a**; measured in pH 7 PBS buffer containing 50% acetonitrile at 25 °C; Figures S9 and S10, Supporting Information). It is thus proven that the elevation of ground-state energy leads to suppression of the side reactions while enhancing the reaction rate.

Probe **3** shows excellent selectivity toward gold ions over various metal species examined (Mg^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pd^{2+}, Ag^+, Cd^{2+}, Ba^{2+}, Pt^{2+}, Hg^{2+}, and Pb^{2+}; as chloride salts except $AgNO_3$) (Figure 3a).

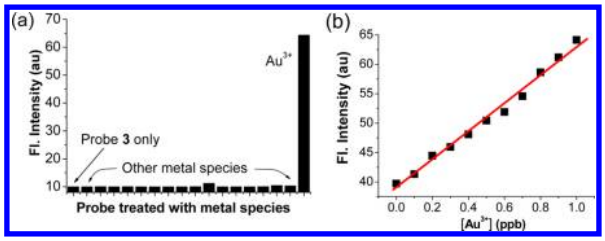


Figure 3. (a) Fluorescence response of probe **3** (10 μM) toward various metal species (Mg^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pd^{2+}, Ag^+, Cd^{2+}, Ba^{2+}, Pt^{2+}, Hg^{2+}, and Pb^{2+} from the left; 10 μM each). (b) Plot of fluorescence enhancement of probe **3** (10 μM) depending on [$AuCl_3$]. Both data were obtained in 30% CH_3CN−HEPES buffer (pH 6, 10 mM) at 36 °C after 60 min of mixing (λ_{ex} = 540 nm).

Furthermore, probe **3** shows a linear response with very high sensitivity, enabling detection of $AuCl_3$ down to 0.5 ppb level, the lowest detection limit so far obtained (Figure S11, Supporting Information, Figure 3b). Probe **3** is thus highly promising for the quantification of gold residues in synthetic products.[7g] Probe **3** showed pH-dependent fluorescent response (Figure S12, Supporting Information), showing pronounced fluorescence as the solution becomes acidic (pH 4−6).

A novel structural feature of probe **3** is that we can readily functionalize the *N*-phenyl ring without significantly affecting sensing properties. Next, we have synthesized two FRET systems. A FRET probe is highly desirable in biological studies

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as it allows more reliable quantification of an analyte compared to the intensity-based probe whose intensity change is affected by environmental parameters such as pH, polarity, temperature, emission collection efficiency, effective cell thickness in the optical beam, and changes in the excitation intensity.[10] So far, only a few ratiometric probes for gold ions have been reported; they are ICT-based sytems[7b,j,l] except for one FRET probe.[7c]

A naphthalimide dye is chosen as the FRET donor, as its emission spectrum overlaps well with the absorption spectrum of rhodamine B.[12] To connect the donor to probe **3**, we introduced a carboxyl group to the *N*-phenyl ring. The resulting FRET probe **5** can be readily synthesized, for example, by coupling rhodamine-derived acid **6** with naphthalimide **7b** that contains an ammonium side group (Figure 1, Supporting Information).

Fluorescent behavior of FRET probes **5a** and **5b** was evaluated separately in pH 7.4 PBS buffer containing 10% acetonitrile at 25 °C. Interestingly, these FRET probes show faster response than probe **3**; the fluorescence change is almost complete within 20 min (Figure S13, Supporting Information). It should be noted that most of the known probes based on alkyne activation show rather slow response toward gold ions. Probe **5a** emitted bright green fluorescence centered at 520 nm when excited at 396 nm (λ_{max} of the naphthalimide moiety).

Significantly, upon addition of 1 equiv of $AuCl_3$ to the probe (10 μM), **5a** for instance, an absorption band centered at 560 nm rose immediately with a concurrent color change from light yellow to pink (Figure S14, Supporting Information). When the solution was excited at 396 nm, a new fluorescence band at 587 appeared with a sudden fall of the band at 520 nm, with corresponding emission color change from green to amber (Figure 4, Figure S15, Supporting Information). These spectral

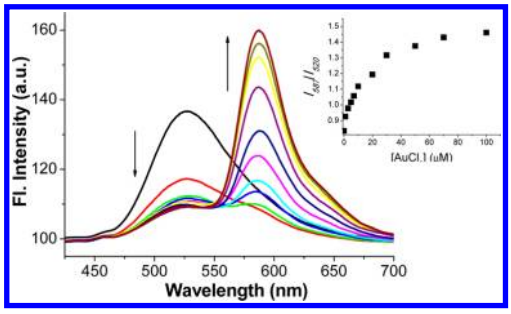

Figure 4. Fluorescence change of probe **5a** (10 μM) upon addition of $AuCl_3$ (0–100 μM), measured in 10% CH_3CN–PBS buffer (5 mM, pH 7.4) at 25 °C (λ_{ex} = 396 nm). Inset: a plot of the fluorescence intensity vs [$AuCl_3$]. All data were taken after 30 min of mixing at rt.

changes indicate that the spirolactam ring-opening by gold ions activates FRET from the naphthalimide to the rhodamine moiety. The FRET sensing system shows good ratiometric behavior from pH 4–9 (Figure S16, Supporting Information). As expected from the sensing characteristics shown by probe **3**, its FRET system **5a** again shows excellent selectivity toward gold ions among various metal ions (Figure S17, Supporting Information). Also, in addition to AuCl and $AuCl_3$, a stabilized gold(I) species $(CH_3CN)Au[P(t\text{-}Bu)_2(2\text{-biphenyl})]SbF_6$ can also be sensed with a linear ratiometric change depending on the concentration of gold ions (Figures S18 and S19, Supporting Information).

Finally, the FRET probes were applied for fluorescent imaging of gold species in cells. An attempt to use probe **5a** for imaging of $AuCl_3$ in cells met with failure, owing to its low cell permeability (Figure S20, Supporting Information). To overcome this problem, we synthesized probe **5b** that contained a quaternary ammonium group, which showed good cell permeability. Fluorescent imaging data for N2A cells incubated with probe **5b** were obtained in the absence and presence of $AuCl_3$. Probe **5b** alone in cells emits green fluorescence in cytosol when excited at a fixed laser wavelength of 405 nm (Figure 5a). When cells preincubated with probe **5b** were

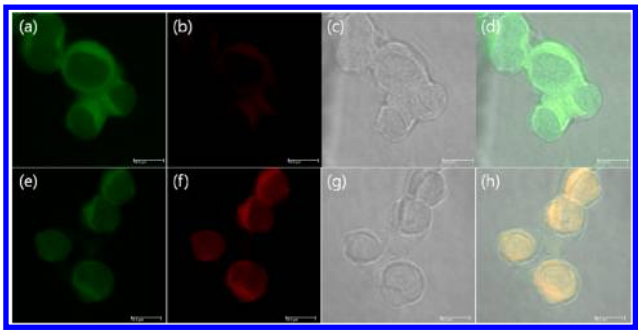

Figure 5. Fluorescent images of N2A cells treated with probe **5b** (50 μM) only (the upper row) and the probe for 30 min followed by $AuCl_3$ (250 μM) for 1 h at 37 °C (the lower row): images observed through (a, e) green channel (500–575 nm) and (b, f) red channel (576–700 nm); (c, g) bright field images; (d, h) merged images. Scale bar = 10 μm.

treated with $AuCl_3$, red fluorescence emitted under excitation at 405 nm (Figure 5f, Figure S21, Supporting Information) through FRET from the naphthalimide to the ring-opened rhodamine dye. An MTT assay shows that probe **5b** has low cytotoxicity toward cells (Figure S22, Supporting Information).

In conclusion, we have disclosed a novel approach to suppress side reactions observed in the reaction-based gold-sensing systems based on alkyne activation. By elevating steric strain around rhodamine-lactam nitrogen, the gold ion-promoted ring-opening process is significantly enhanced. As a result, the gold-sensing process proceeds as an exclusive chemical conversion. As a result, the new sensing system shows much higher sensitivity as well as faster response compared with the previous ones, in addition to show excellent selectivity toward Au(I) and Au(III) species. Furthermore, the sensing system allows straightforward attachment of a donor dye, leading to a novel FRET probe that enables fluorescent imaging of gold species in cells. The ground-state elevation approach demonstrated here holds a promise for the development of other reactive sensing systems.

■ ASSOCIATED CONTENT

Supporting Information

Fluorescence data and synthesis of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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