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COMMUNICATION

Exploiting the higher alkynophilicity of Au-species: development of a highly selective fluorescent probe for gold ions†

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A new approach, involving the anchoring–unanchoring of a fluorophore, has been developed for the detection of Au-species. The fluorescent probe was found to be highly selective for sensing gold species in the presence of several other metal ions. A successful application to bioimaging has also been demonstrated with A549 lung cancer cells.

During the last decade gold catalysis has emerged as a powerful tool in the area of organic synthesis.[1] Based on the intrinsic π-activation property of gold catalysts, either Au^{1+} or Au^{3+} species,[2] a great number of novel transformations have appeared. The high carbophilicity exhibited by gold salts, unlike other metals in the periodic table, makes them special and unique. Though gold in elemental form is inert, its salt exhibits some biological effects. For instance, gold ions have anti-inflammatory properties and are used as pharmaceuticals in the treatment of tuberculosis, arthritis, and cancer.[3] In addition, it is well established that gold ions are known as inhibitors of macrophages and polymorphonuclear leucocytes.[4] However, gold species may tightly bind to biomolecules such as enzymes and DNA, leading to toxicity to humans.[5] Similarly, certain gold salts such as gold chloride are known to cause damage to the liver, kidneys, and the peripheral nervous system.[6]

Based on the sharp increase in gold catalysis and the toxicity associated with gold ions, it is essential to develop a chemosensor to monitor the presence of gold species both in the environment and under physiological conditions. In general, the development of a chemosensor for the detection of gold is based on two approaches. The first approach (reaction based, Fig. 1A) involves a nonfluorescent probe consisting of a fluorophore and an organic molecule which on reaction with a gold species generates a new structure resulting in a change in the fluorescence intensity (functional group manipulation approach, Fig. 1-a1).[7] Jou *et al.*, for the first time, reported a Au^{3+} selective fluorescent probe based on the cyclization of a rhodamine-based propargylamide.[7a] Almost at the same time, Tae and coworkers disclosed a rhodamine-based probe for the detection of Au^{3+} species based on intramolecular hydroamination.[7b] Ahn and coworkers developed a rhodamine based probe for Au^{1+} and Au^{3+} ions.[7c] Employing a gold-catalyzed cascade reaction, Do *et al.* developed a Au^{3+} selective fluorescence turn-on probe based on the intramolecular hydroarylation of phenyl alkynoates.[7d] Peng and Wang proposed a 1,8-naphthalimide-based probe for the selective recognition of Hg^{2+} and Au^{3+} ions.[7e] Lin and coworkers designed a fast responsive Au^{1+}/Au^{3+} selective fluorescent probe based on the hydrolysis of rhodamine-based acyl-semicarbazides.[7f] In their subsequent paper, the authors have shown that not only gold ions but gold NPs can also be detected.[7g] Chang and coworkers reported a Au^{3+} selective probe based on the desulfurization of thiocoumarin.[7h] Song and coworkers disclosed a fluorescent probe for Au^{1+}/Au^{3+} ions based on the intramolecular hydroamination of Bodipy-derived aminoalkynes.[7i] Another approach is complexation based wherein the nonfluorescent probe, containing a fluorophore and a gold ion receptor, binds with Au ions triggering a change in the fluorescence intensity (Fig. 1B). For instance, Wang *et al.* developed a rhodamine based probe for selective detection of Au^{3+} ions.[8]

In this communication, we report a new approach involving the anchoring–unanchoring of the fluorophore (Fig. 1-a2).

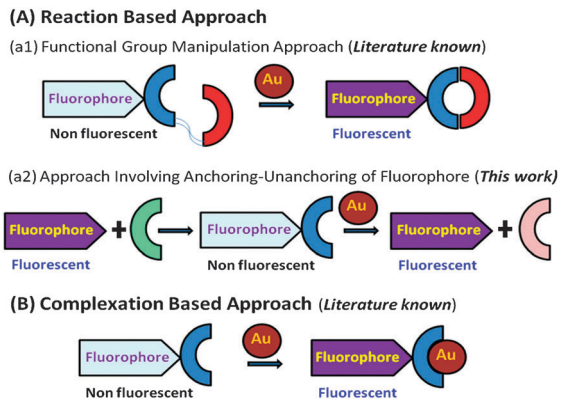

Fig. 1 Various approaches for the detection of gold ions.

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† Electronic supplementary information (ESI) available: All experimental procedures, analytical data, and copies of ^{1}H and ^{13}C NMR spectra of all newly synthesized products. See DOI: 10.1039/c2cc35083a

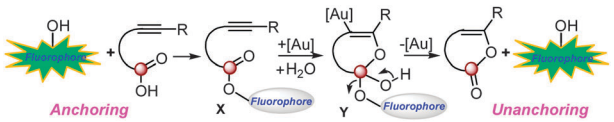


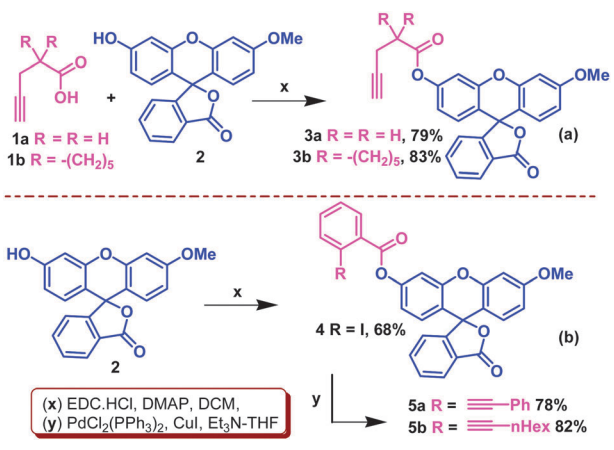
Fig. 2 Approach involving the anchoring–unanchoring of the fluorophore.

It can be seen from Fig. 1-a2, that the fluorescence of fluorophore is turned off by anchoring with an organic substrate. Once the gold ions have been sensed, the probe liberates the highly fluorescent fluorophore with formation of the organic product.

As a part of our ongoing interest in gold catalysis,[9] we envisioned that an organic molecule of type **X** would be activated by gold ions which, in turn, would trigger a cascade as depicted in Fig. 2 (*cf.* **Y**).[10] The reaction, thus envisaged, would liberate the highly fluorescent fluorophore[11] and therefore the designed molecules of type **X** would serve as a probe for sensing gold ions.

Scheme 1 outlines the synthesis of probes **3a–b** and **5a–b**. The probes **3a–b** were obtained by the reaction of methoxyfluorescein (**2**) with alkynoic acids **1a** and **1b**, respectively. The synthesis of probes **5a–b** was achieved in two steps from fluorescein (**2**). The esterification of 2-iodobenzoic acid with **2** was achieved by a conventional procedure to obtain halo-ester **4** in 68% yield. The halo-ester **4**, thus obtained, reacted with phenyl acetylene and 1-octyne under Pd–Cu catalysis to give the desired probes **5a** and **5b** in 78 and 82% yields, respectively. As expected, all the probes **3a–b** and **5a–b** show negligible fluorescence and are colorless in H_2O–CH_3CN-buffer (pH = 7.4). The probes **5a–b** (30 μM) on treatment with 100 μM solution of $HAuCl_4$ exerted strong fluorescence at 515 nm. In addition, the solution changes from colorless to a yellow color. Since the probes **3a–b** did not give promising results (ESI†), we considered **5a** and **5b**; out of which the former was chosen for further studies because of its superiority (ESI†).

Probe **5a** in CH_3CN/PBS buffer (1 : 1, pH = 7.4) has an absorption maxima centered at 313 nm with no absorption in the visible region (ESI†). However, the addition of Au^{1+} or Au^{3+} ions to probe **5a** induces a colorimetric change from colorless to yellow which is visible to the naked eye (ESI†).



Scheme 1 Synthesis of fluorescein-based probes.

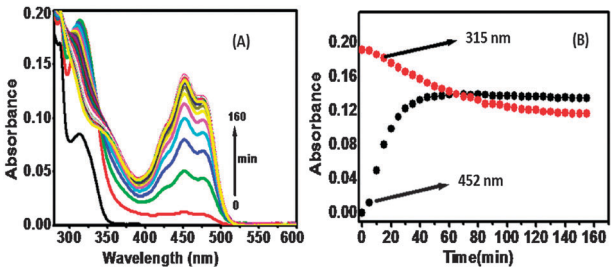


Fig. 3 (A) Time dependent absorption change of **5a** (10 μM) upon addition of 10 equiv. of Au^{1+} in CH_3CN : PBS buffer (1 : 1, 0.1 M, pH = 7.4). (B) Absorption kinetics change upon the addition of 10 equiv. of Au^{1+} to **5a** (10 μM) in CH_3CN : PBS buffer (1 : 1, 0.1 M, pH = 7.4).

Fig. 3 shows the time dependent absorption change of **5a** (10 μM) upon addition of 10 equiv. of Au^{1+} ion. A new band appeared in the visible region (λ_{max} = 452 nm) immediately after addition of the Au^{1+} ion and a large enhancement in absorption is observed with time. The increase in absorbance (at λ_{max} = 452 nm) is rapid and it saturates after 40 min, indicating the reaction is very fast. Similar spectral behaviour has been observed to take place between **5a** with the Au^{3+} ion with much less enhancement in absorption (at λ_{max} = 452 nm) (ESI†).

The solution of probe **5a** in CH_3CN/PBS buffer (1 : 1, pH = 7.4) is colorless and exhibits negligible fluorescence. However, the addition of Au^{1+} ions to probe **5a** triggers a large enhancement in fluorescence (λ_{max} = 515 nm). The time dependent fluorescence response of probe **5a** (10 μM) with Au^{1+} (100 μM) showed a rapid increase in intensity up to 30 min and then saturates (Fig. 4). Similar behaviour has been found with Au^{3+}, but the rate at which the intensity increases is less compared with Au^{1+} (ESI†).

Next, we have examined the selectivity of probe **5a** towards various other metal species. The study reveals that probe **5a** shows a special selectivity towards Au^{1+} and Au^{3+} metal ions only. The fluorescence response of other metals such as Ag^{1+}, Ba^{2+}, Bi^{3+}, Fe^{3+}, Hg^{2+}, Ir^{3+}, K^{1+}, La^{3+}, Mn^{2+}, Ni^{2+}, Pd^{2+}, Pt^{2+}, Pt^{4+}, Ru^{3+}, Sc^{3+}, Yb^{3+}, Zn^{2+}, Cd^{2+} and Cr^{3+} is negligible. Further the selectivity of probe **5a** towards Au^{1+} in the presence of other competing metal species has been tested (ESI†). It is evident that the interference of other metal species is minimal, so this probe can be used as a Au^{1+} sensor in the presence of other metal species. The fluorescence

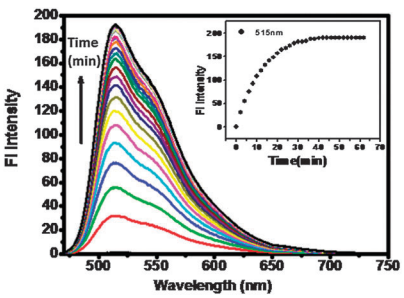


Fig. 4 Time dependent fluorescence change obtained for a mixture of **5a** (10 μM) and Au^{1+} (100 μM) in CH_3CN : PBS buffer (1 : 1, 0.1 M, pH = 7.4). λ_{excit} = 452 nm. Inset: plot of fluorescence intensity at 515 nm against time.

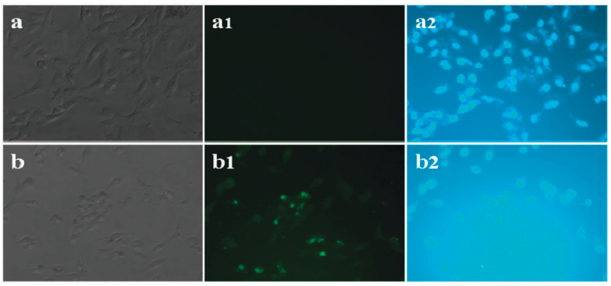


Fig. 5 Fluorescence images of A549 cells: (a–b) bright field images, (a1–b1) fluorescence images and (a2–b2) DAPI stained images of A549 cells. The figures a, a1 and a2 indicate the bright field, fluorescent and DAPI stained images of control untreated A549 cells. Similarly, b, b1 and b2 indicate the bright field, fluorescent and DAPI stained images of A549 cells treated with 20 μM of Au^{1+} and 50 μM of probe **5a**.

response of the probe shows an excellent linear relationship towards Au^{1+} in the range of 5–80 μM, indicating that the probe can be used for the quantitative determination of Au^{1+}.

The favourable features of **5a** such as a fast response, high selectivity and fluorescence under physiological pH encouraged us to further examine the potential of the sensor for imaging Au^{1+} in living cells. The lung cancer cell A549 and Au^{1+} were chosen for studies. The bright field images of control untreated A549 cells are depicted in Fig. 5a. The corresponding fluorescence image and the blue color DAPI stained image of untreated control A549 cells are shown in Fig. 5a1 and a2, respectively. As can be seen, the green fluorescence of untreated A549 cells was not observed in the absence of **5a** and Au^{1+} (Fig. 5a1). However, the presence of green fluorescence was noticed when the **5a** was treated with Au^{1+} (Fig. 5b1) even after extensive washing with DPBS. These observations clearly indicate that the probe **5a** can sense Au^{1+} ion in living cells.

In summary, we have developed a new approach involving the anchoring–unanchoring of a fluorophore, for the detection of gold ions.[12] We believe that this new strategy will attract the attention of the scientific community and therefore many probes based on the present approach will appear in the future. Bioimaging studies have also been successfully demonstrated with A549 lung cancer cells. We anticipate that this study could provide the basis for the future development of a biodegradable fluorescent probe for cancer diagnostics.

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