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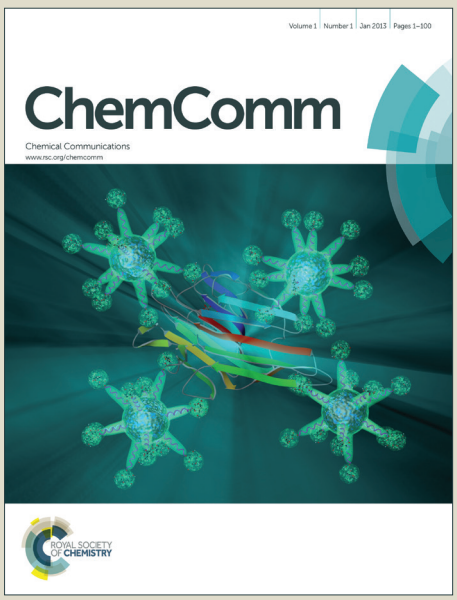

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Cascade Sensing of Gold and Thiol with Imidazole-bearing Functional Porphyrins

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An imidazole-bearing zinc porphyrin (P_{Zn}) has been designed for the selective detection of Au^{3+}, and the porphyrin and gold complex (P_{Zn}▪Au^{3+}) can additionally be used to identify gold-binding functional groups, such as cysteine residues and other mercaptans.

Fluorescence is among one of the most widely utilized readout for molecular recognition,[1] owing to its high sensitivity and straightforward detection with readily available instrumentation.[1c, 2] Many approaches rely on the single-receptor–single-analyte motif that curtails non-specific binding and erroneous readouts. However, the development of a modular platform that allows the detection of various analytes while maintaining biocompatibility and specificity remains a major challenge. Thus, we sought to investigate the chemical and architectural features of functional porphyrins that were designed to be non-cytotoxic and capable of sensing metals and biomolecular building blocks, specifically Au^{3+} and cysteine residues.

Metallic gold nano-objects, such as nanoparticles, are ubiquitous in materials chemistry as well as biomedical devices.[3] Although metallic gold is chemically stable and rather inert, the precursors of gold nano-objects, ionic forms of gold, exhibit strong cellular toxicity due to their strong Lewis acidic nature.[4] Consequently, the detection of ionic gold would be useful for the design of cellular imaging probes and diagnostic tools. To date, limited examples of gold-sensitive molecular probes have been reported and are generally based on the electrophilic attack of alkynes to Au^{3+}.[5] Although effective detection of Au^{3+} has been demonstrated, the development of modular sensors with different molecular architectures is important for understanding fundamental sensing mechanisms and molecular recognition elements.

Since thiol groups generally exhibit high affinity to gold,[6] we envisioned that the same sensors developed for Au^{3+} detection could be used to detect cysteine residues and other mercaptans in biomaterials. Cysteine is an important amino acid that contributes to stereoregulation of the tertiary and quaternary structures of proteins and plays a number of regulatory functions in metabolism, including control of redox environment, intracellular signal transduction, and genetic regulation.[7] Therefore, developing cysteine sensors with new mechanisms of detection is fundamentally important.

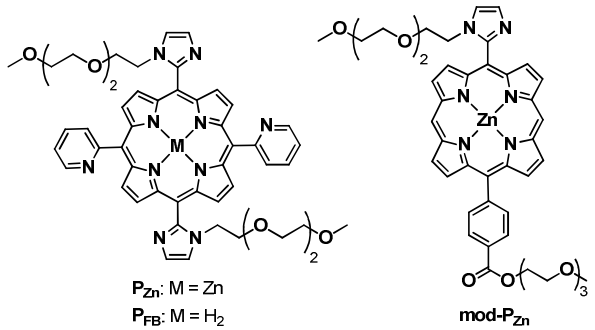

Figure 1. Structures of imidazole-bearing porphyrins.

Porphyrins play essential roles in nature and may serve as a versatile and biocompatible platform for fluorescent sensors.[4e, 8] Thus, we designed a porphyrin-based sensor ($\mathbf{P_{Zn}}$; Figure 1) selective to Au^{3+}, and the porphyrin and Au^{3+} complex ($\mathbf{P_{Zn}}$▪Au^{3+}) can also be used to detect gold-binding functional groups, such as cysteine residues and other mercaptans. The fluorescent metalloporphyrin, $\mathbf{P_{Zn}}$, features imidazole and pyridine groups to selectively coordinate to Au^{3+}, resulting in fluorescence quenching (Figure 2a). The $\mathbf{P_{Zn}}$▪Au^{3+} complex was used to detect mercaptans by forming a new complex between the existing Au^{3+} ions and mercaptan, thus recovering the fluorescence of $\mathbf{P_{Zn}}$ (Figure 2b).

In the context of porphyrin-based systems, metal ions serve as a handle to tune UV-vis absorption and fluorescence emission, both in terms of intensity and wavelength. Hence, the spectroscopic properties of porphyrin $\mathbf{P_{FB}}$ (Figure 1) were examined in response to the addition of various metal ions (Figure S1). The addition of Au^{3+},

Hg^{2+}, and Cd^{2+} to $\mathbf{P_{FB}}$ elicited spectral changes in the absorption and emission spectrum (Figure S1), possibly due to the heavy atom effect.[8d] For Au^{3+} and Cd^{2+}, the intensity in both the absorption and emission spectrum decreased. Addition of Hg^{2+} resulted in a strong red-shift in the absorption spectrum and fluorescence quenching in the emission spectrum (Figure S1).

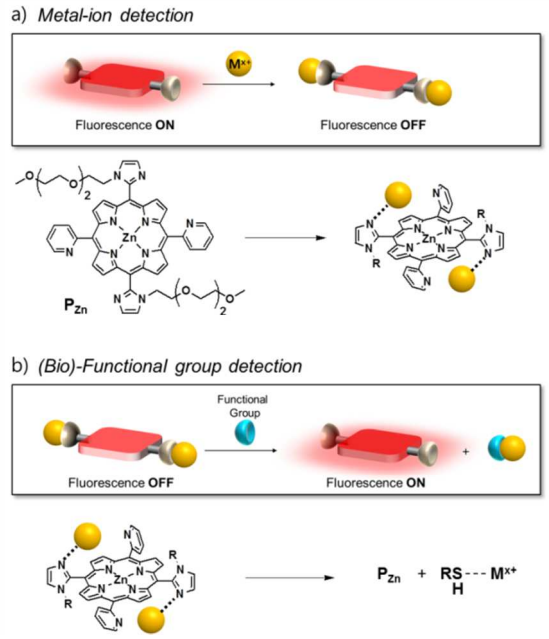

Figure 2. a) Metal-ion sensing with a fluorescent zinc metalloporphyrin $\mathbf{P_{Zn}}$, showing quenching upon binding to a select metal ion. b) Functional group sensing with a biocompatible metalloporphyrin that fluoresces when the supramolecular metal ion is removed in the presence of thiols.

With the aim to design a more sophisticated system for detection of a singular metal ion, $\mathbf{P_{Zn}}$ was prepared and its spectroscopic properties similarly examined in response to the addition of various metal ions, such as Cu^{2+}, Fe^{3+}, Fe^{2+}, Au^{3+}, Hg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mg^{2+}, Cd^{2+}, Ag^{+}, Ru^{3+}, Ni^{2+}, and Pt^{2+} (Figure 3). We found that $\mathbf{P_{Zn}}$ showed a clear red-shift in the UV/vis absorption spectrum and complete fluorescence quenching only with the addition of Au^{3+} (Figure 3b, 3c, and S2a). To validate the selectivity of $\mathbf{P_{Zn}}$ to Au^{3+}, competition experiments were performed, where two equivalents of Au^{3+} were added to a solution of $\mathbf{P_{Zn}}$ and two equivalents of other metal ions. For all metal ions, two equivalents of Au^{3+} was sufficient to induce complete fluorescence quenching, indicative that Au^{3+} has a high binding affinity to $\mathbf{P_{Zn}}$ (Figure S2b).

Based on the extensive literature reporting the stable coordination complexes formed between Au^{3+} and imidazole derivatives, we infer that the fluorescence quenching of $\mathbf{P_{Zn}}$ was induced by Au^{3+} coordination to the nitrogen on the imidazole.[5b, 9] Since $\mathbf{P_{Zn}}$ features two imidazole moieties, the fluorescence quenching correspondingly saturated with two equivalents of Au^{3+} (Figure 3e). Notably, the UV/Vis absorption spectra and fluorescence titration curve exhibited a two-step change (Figure 3d and 3e). There are two discrete linear regimes: zero to one equivalent and one to two equivalents. The linear correlation in the first regime may be potentially used to determine sub-ppm concentration of Au^{3+} in aqueous medium. In the second regime, the negative cooperativity behavior may be attributed to the electrostatic repulsion of cationic guests.

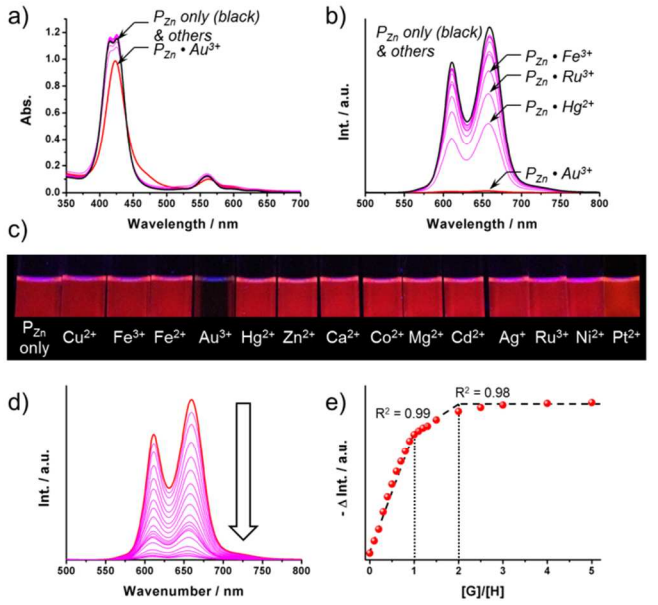

Figure 3. Optical responses of $\mathbf{P_{Zn}}$ (10 μM) upon addition of two equivalents of various metal ions. a) The UV/Vis absorption spectra, b) fluorescence emission spectra with λ_{ex} = 425 nm, and c) visible fluorescence emission with λ_{ex} = 365 nm are shown. d) Fluorescence emission of spectra and e) fluorescence titration curve (λ_{ex} = 425 nm, λ_{em} = 660 nm) of $\mathbf{P_{Zn}}$ (10 μM) upon addition of Au^{3+} in 50% $MeCN/H_2O$.

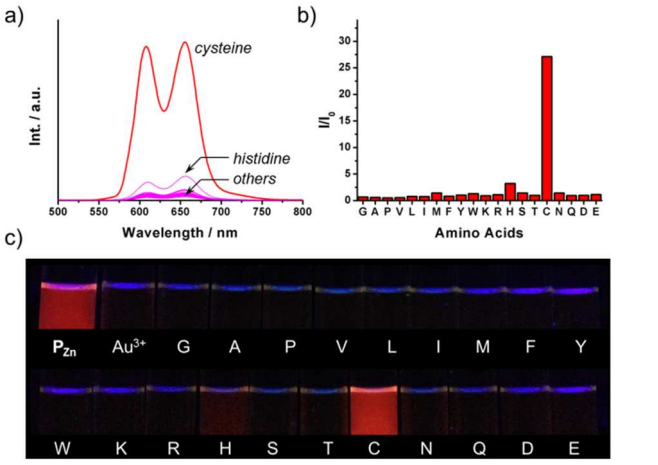

Figure 4. Optical responses of [$\mathbf{P_{Zn}}$·Au^{3+}] (10 μM) by the addition of various amino acids (4 eq.) in 50% $MeCN/H_2O$. a) In the fluorescence emission spectra with λ_{ex} = 425 nm, b) relative fluorescence intensity ratio with λ_{em} = 609 nm, and c) visible fluorescence emission with λ_{ex} = 365 nm, cysteine is the only amino acid which shows significant recovery of fluorescence.

The binding mechanism of Au^{3+} to $\mathbf{P_{Zn}}$ was further investigated by ^1H NMR (Figure S3). Although $\mathbf{P_{Zn}}$ afforded relatively sharp peaks in acetonitrile-d_3, the ^1H NMR spectrum of $\mathbf{P_{Zn}}$ with various equivalents of Au^{3+} was difficult to interpret due to reduced solubility and aggregate formation (Figure S3). To overcome these issues, we synthesized a modified porphyrin, **mod-$\mathbf{P_{Zn}}$**, which is structurally similar to $\mathbf{P_{Zn}}$ but is insoluble in water and only contains one imidazole unit, without pyridine moieties (Figure 1, Figure S4). **mod-$\mathbf{P_{Zn}}$** exhibited similar behavior to $\mathbf{P_{Zn}}$: complete fluorescence quenching was selectively observed upon addition of Au^{3+} (Figure S5). **mod-$\mathbf{P_{Zn}}$** contains one imidazole moiety and correspondingly

saturates with one equivalent of Au^{3+} (Figure S5d). The ^1H NMR spectrum of **mod-P_{Zn}** exhibits sharper peaks and alludes to the formation of a dimeric species through axial coordination of the zinc ion in the porphyrin core to the nitrogen on the imidazole ring of an adjacent **mod-P_{Zn}** (Figure S4b). The proton signals from the imidazole ring appear unexpectedly upfield, which may have resulted from the strong ring current exerted on the imidazole protons located above the porphyrin center in the dimer configuration (Figure S4). Upon addition of Au^{3+} to **mod-P_{Zn}**, the ^1H NMR shows the disassembly of the dimer configuration via a downfield shift of the protons on the imidazole unit and an appearance of a doublet originating from the protons on the phenyl ring.

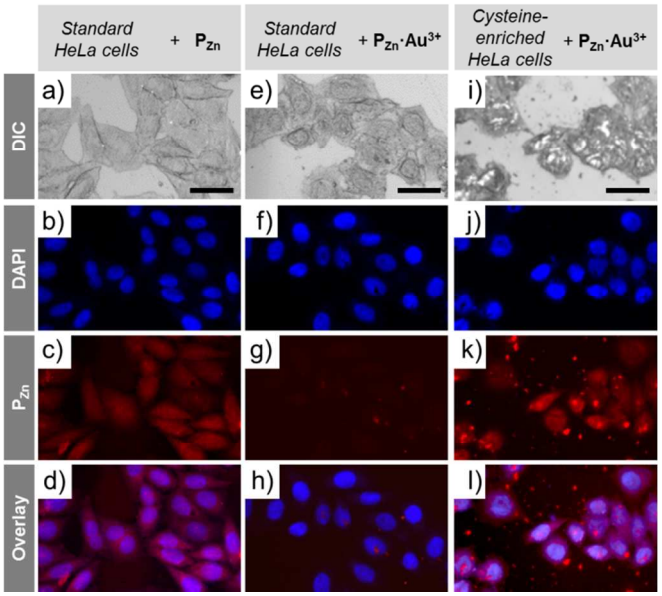

Figure 5. Fluorescence microscope image of HeLa cells. (a - d) Cells incubated with P_{Zn} (160 μM) only; (e - h) cells with [P_{Zn}·Au^{3+}]; (i - l) cysteine-enriched cells (2 mM; incubation time = 24 hrs) with [P_{Zn}·Au^{3+}]; (a, e, and i) differential interference contrast images; (b, f, and j) staining of nuclei by 4',6'-diamidine-2'-phenylindole dihydrochloride (DAPI); (c, g, and k) fluorescence from P_{Zn}; (d, h, and l) overlay image of DAPI and P_{Zn} fluorescence. Scale bar = 50 μm.

Unlike many reaction-based chemosensors for the detection of Au^{3+} ions, the fluorescence quenching of P_{Zn} relies on the coordination of Au^{3+} to the imidazole moieties and offers the opportunity for reversibility. A variety of compounds were introduced to compete with binding of Au^{3+} and P_{Zn} (P_{Zn}▪Au^{3+}) in an effort to disrupt the complex and therefore recover the fluorescence emission. In one study, the optical responses of P_{Zn}▪Au^{3+} to twenty standard amino acids were measured. Besides cysteine, the solutions did not show appreciable recovery of fluorescence (Figure 4). The continuous variation method (*i.e.* Job's method)[10] indicated that more than three equivalents for cysteine are required for full dissociation of the P_{Zn}▪Au^{3+} complex and full recovery was achieved with ten equivalents of cysteine (Figure S6). The fluorescence recovery of P_{Zn}▪Au^{3+} may be extended to cysteine containing peptides, which are generally used to functionalize hydrogels and nanostructured surfaces (Figure S7).[11] The optical response of P_{Zn}▪Au^{3+} with CRGDS, a cysteine containing peptide, mirrors the optical response of P_{Zn}▪Au^{3+} with cysteine. Alternatively, the absence of cysteine in GRGDS yields minor differences from P_{Zn}▪Au^{3+} (Figure S7c). In another study, a variety of thiol-containing species was subjected to a similar experiment and exhibited different levels of fluorescence recovery (Figure S8). The signature fluorescence level relative to P_{Zn} may be applicable for distinguishing between different thiol-containing species. A thiol-containing hydrogel, which is formed via thiol-ene crosslinking of polyethylene glycol diacrylate (PEGDA) and pentaerythritol tetrakis (3-mercaptopropionate) (PTMP), displays bright red fluorescence in the presence of P_{Zn} and P_{Zn}▪Au^{3+} (Figure S9). Alternatively, a thiol-free hydrogel of PEGDA did not show significant fluorescence (Figure S9).

Finally, the detection of cysteine with P_{Zn}▪Au^{3+} was applied to in vitro bioimaging of cysteine enriched HeLa cells (Figure 5). Standard HeLa cells showed expected fluorescence behavior with **PZn** and P_{Zn}▪Au^{3+} (Figure 5a-h). Whereas P_{Zn} showed red fluorescence localized in the HeLa cells (Figure 5c), little fluorescence was detected for cells incubated with P_{Zn}▪Au^{3+} (Figure 5g), further confirming the stability of the P_{Zn}▪Au^{3+} complex in physiological conditions. Cysteine enriched HeLa cells incubated with P_{Zn}·Au^{3+} displayed red fluorescence (Figure 5k). The fluorescence was localized on the cells, which confirms that P_{Zn}▪Au^{3+} was active with cysteine residues from these HeLa cells, thus serving as an in vitro fluorescent probe for the detection of cysteine in biological systems.

In summary, we have designed an imidazole-bearing zinc porphyrin for the selective detection of Au^{3+} by the coordination of Au^{3+} onto imidazole groups. The zinc porphyrin and Au^{3+} complex further showed response to the addition of thiol-bearing compounds. This unique characteristic of the imidazole-bearing zinc porphyrin was exploited for the in vitro bioimaging as well as detection of residual thiols in thiol-ene crosslinking hydrogels.

Notes and references

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