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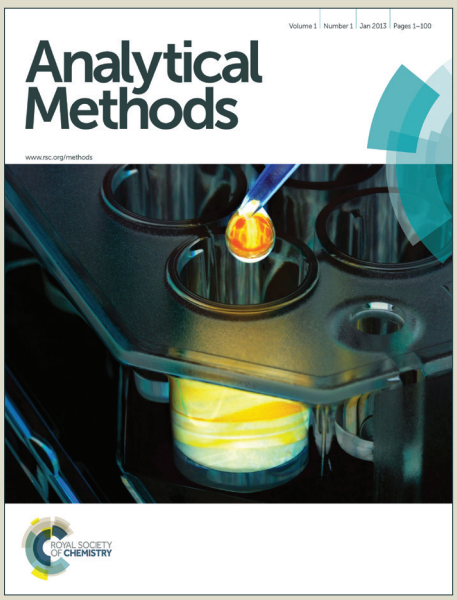

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A Zero-Background Fluorescent Probe for Hg^{2+} Designed via the "Covalent-Assembly" Principle

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We herein report a novel fluorescent probe for Hg^{2+} based on its deprotection of 1,3-dithiolane, via the *covalent assembly* principle. Presence of Hg^{2+} triggers a cascade, which ultimately furnishes the push-pull backbone of a fluorescent dye, rendering a turn-on signal from a zero background and hence high detection sensitivity.

Sensitivity is qualitatively defined as the ability of a probe to detect low level of analyte, and arguably the most important parameter of a chemical probe beside specificity.[1] It is strongly dependent on the signal-to-noise ratio. In the early era of the field of small molecule fluorescent probe, turn-off probes were not uncommon.[2] Turn-on probes, especially off-on probes, shortly dominated the literature for their superior sensitivity because a reduction in fluorescence intensity of unreacted probe, a major contributor of the overall noise level, efficiently promotes detection sensitivity.

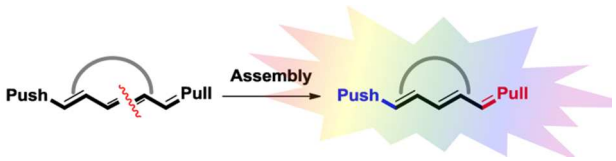

Figure 1. Schematic representation of the "covalent assembly" probe design principle.

We are interested in a new probe design principle, i.e. the "covalent-assembly" approach (Figure 1), which produces chemical probes of zero background signal and therefore is sensitive for detection.[3] Another advantage is that the probe is usually very simple in terms of chemical structure and conveniently synthesized. The probe design involves in chopping the conjugative backbone of a push-pull type fluorophore into two fragments and subsequent "covalent-assembly" of the conjugative backbone by a chemical cascade, specifically triggered by the analyte of interest. For the concept of covalent-assembly at its infancy, we wish to show that it may apply to a broad scope of substrate, for which reason the "dye-linker-receptor" principle is so deeply rooted in the heart of all practitioners of the field. We recently have developed assembly type fluorescent probes for a series of substrates including N_2O_3, $OONO^-$ and Sarin related chemical threats.[3,4] It is the intention of this work to show that particular heavy transition metal ion, i.e. Hg^{2+} is also suitable substrate for probe design via the covalent-assembly principle.

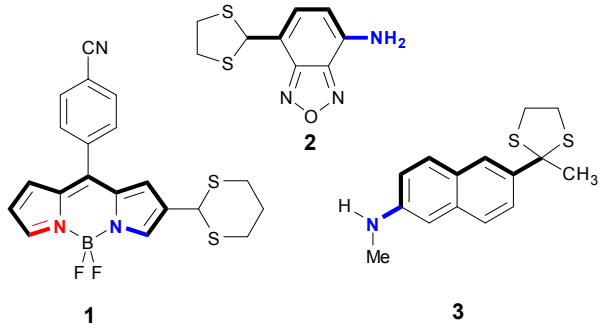

Figure 2. Existing small molecule fluorescent probes for Hg^{2+} based on 1,3-dithiolane deprotection.

Mercury (Hg) and its divalent ion (Hg^{2+}) are widely present in the environment mainly as a result of volcanic eruptions or industrial discharge.[5] Due to the high toxicity of this heavy transition metal to central nervous system[6] and endocrine system[7], the use of mercury has been curtailed in many industries. The Hg^{2+} level in water bodies is important to be monitored for public health concern.[8] Various methods based on colorimetry, atomic spectroscopy, ICP-MS, and electrochemistry are available.[9] Fluorescence based techniques are suitable for direct detection of analyte of interest in complex matrices and therefore fluorescent probes for Hg^{2+} has been actively pursued.[10] Hg^{2+} efficiently deprotects a dithioacetal or dithioketal to the corresponding carbonyl derivatives.[11] This reaction has been frequently harnessed in designing probes for Hg^{2+}. For example, Qian et al reported the probe **1**[10x], in which in a 1,3-dithiane is

attached as an auxochrome, while the 1,3-dithiolane in probe **2**[10y] by He and Guo et al, or in probe **3**[10z] by Zhou et al is the masked "pull" of the corresponding push-pull chromophore (Figure 2). Herein, we have designed an assembly-type probe (**Hg570**) for Hg^{2+} by using this old Hg^{2+} detection chemistry in a new way.

Hg570 was obtained near quantitatively in one synthetic step by condensing 1,2-dithioethane with **NA570**[3b], which we previously reported as an assembly type probe for nerve agent mimics (Figure S1 in the supplementary information). In the presence of Hg^{2+}, a highly absorbing and fluorescing dye, **Pyronin B**, is expected to be generated via the following proposed mechanism (Figure 3): 1) a sulfur atom of the 1,3-dithiolane moiety coordinates to the Hg^{2+}; 2) the carbon-sulfur bond is subsequently cleaved with the assistance of the other sulfur atom; 3) the resulted alkyl sulfonium ion is highly electrophilic and should react with the nucleophilic N,N-diethylaniline moiety in close proximity; 4) re-aromatization followed by elimination of $Hg(S\text{-}CH_2CH_2\text{-}S)$ complex furnishes the fluorescent Pyronin B scaffold.

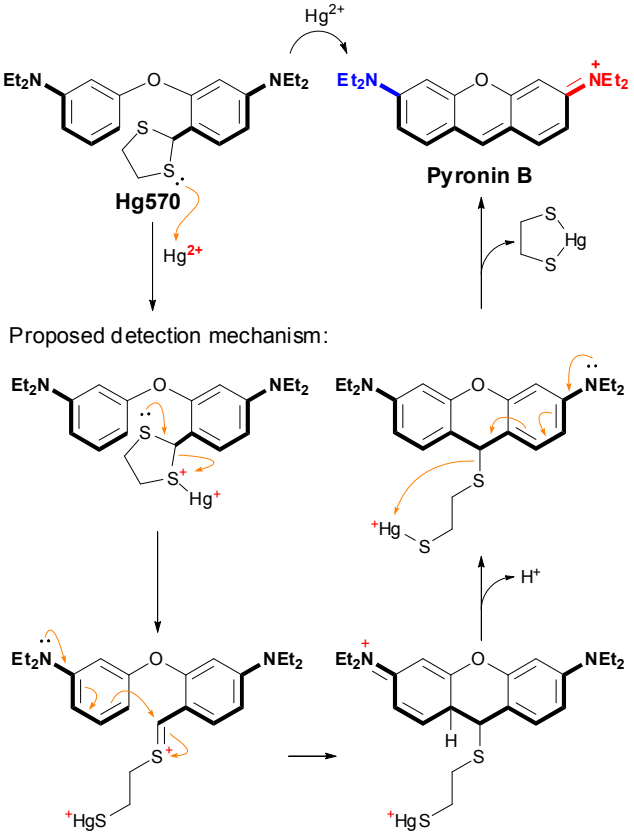

Figure 3. The chemical structure of **Hg570** and proposed Hg^{2+} detection mechanism.

The absorption and emission spectra of both **Hg570** and **Pyronin B** were collected in a HEPES buffer solution (10 mM with pH = 7.4) with 5% DMSO as a co-solvent (Figure 4a). The absorption band of the **Hg570** showed a peak at 265 nm ($\varepsilon = 8.20 \times 10^3$ $cm^{-1}M^{-1}$). Its solution was colorless as the absorption was restricted to the ultraviolet region. Excitation of the **Hg570** solution at its maximum absorption wavelength did not yield a noticeable emission. In comparison, a solution of **Pyronin B** in the same solvent was intensely pink-colored with a maximum absorption wavelength at 552 nm ($\varepsilon = 8.17 \times 10^4$ $cm^{-1}M^{-1}$). Upon excitation, an emission band with a peak at 570 nm was observed. Its fluorescence quantum yield was measured to be 0.36 with rhodamine B as a reference, consistent with literature value[12]. The probe **Hg570** and the detection product **Pyronin B** are optically active at completely separated spectral region. Therefore, unreacted probe **Hg570** will not pose any background signal to compromise the detection sensitivity of **Pyronin B**.

The detection kinetics of **Hg570** toward Hg^{2+} was studied by addition of an aliquot of Hg^{2+} stock solution (0.5 equiv.) into a solution of **Hg570** (10 μM) in HEPES buffer (10 mM with pH = 7.4) with 5% DMSO (Figure 4b). The emission intensity at 570 nm was continuously monitored for a duration of 1,200 s with an excitation at 530 nm. Fluorescence intensity appeared abruptly from a zero background. Most of the signal enhancement was achieved in the first *ca*. 300 s. A slow but steady signal enhancement followed afterwards. This is largely in agreement with the existing probes based on dithioacetal deprotection. A fluorescence titration was performed and a dose-dependent fluorescence emission was recorded (Figure 4c and 4d). Based on the titration data, a lower detection limit (LDL) of *ca*. 90 nM was estimated based on (LDL = 3σ). Admittedly, this LDL is above the upper limit of mercury level in drinking water set by EPA, which is 1 ppb (≈ 5 nM).

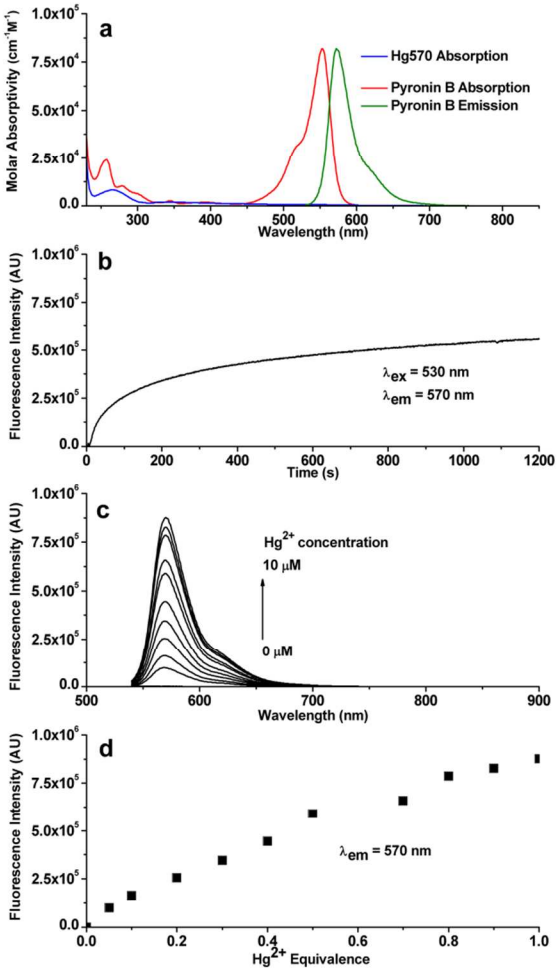

Figure 4. (a) Absorption and emission spectra of both **Hg570** and **Pyronin B**. (b) Kinetic trace monitoring the formation of **Pyronin B**

upon addition of 0.5 equiv. of Hg^{2+} into a solution of **Hg570**. (c) A fluorescence titration of **Hg570** by addition of various amount of Hg^{2+} into a solution of **Hg570** (10 μM). (d) Fluorescence emission intensity of **Pyronin B** at 570 nm with respect to the added Hg^{2+} equivalence.

Potential interferences from other transition metal ions were tested (Figure S2). No interferences were observed with Mn^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, and Fe^{2+} which were tested up to 10 equiv. (Figure S2). Ag^{+} was reported to give minor interferences to other probes based on desulfurization.[10c,f,j,s,x] However, up to 10 equiv. of Ag^{+} did not induce a noticeable signal.

The potentials of **Hg570** for in vitro cell imaging applications were studied with HeLa cells. The 561 nm laser line of the Nikon A1R confocal microscope is close to the maximum absorption wavelength of **Pyronin B**, which is at 552 nm, and was used for excitation in imaging. Emission in the range of 570 nm to 620 nm was collected with a red fluorescent protein (RFP) emission filter. **Hg570** does not carry any charged functional group and therefore was found to readily diffuse through the cell membrane. HeLa cells were incubated with **Hg570** (5 μM) for 30 min at room temperature. Imaging of cells at this moment yielded a dark background (Figure 5a and 5b). This is expected since the **Hg570** does not give any background signal. Such a dark background is highly desirable for any imaging applications since even a weak signal can be sensitively captured over. In contrast, the cells in a different well, which were also treated with 5 μM of $HgCl_2$ yielded an intense signal (Figure 5c and 5d). This experiment exemplified the capability of an assembly type probe to yield a fluorescence signal over a dark background and also the potential of **Hg570** for in vitro studies. Cytotoxicity of **Hg570** was checked by incubating HeLa cells with 200 μM of **Hg570** for 24 hrs in dark. Cell survival rate was over 95% by MTT assay and this suggested that **Hg570** was minimally cytotoxic.

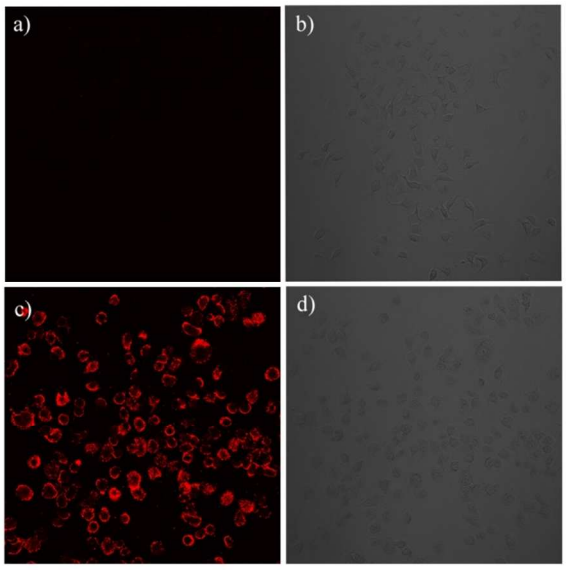

Figure 5. (a) Confocal fluorescence image of HeLa cells treated with only **Hg570** (5 μM) for 30 min at room temperature. (b) Phase contrast for figure 6a. (c) Fluorescence image of cells treated with **Hg570** (5 μM) and Hg^{2+} (5 μM). (d) Phase contrast for figure 6c. The laser line at 561 nm was used for excitation and emission wavelengths from 570 nm to 620 nm were collected.

In conclusion, we have rationally designed a fluorescent probe (**Hg570**) for detection of Hg^{2+} based on the reactivity of a dithioacetal toward Hg^{2+} via a novel "covalent-assembly" approach. Upon Hg^{2+} recognition, a drastic color change from colorless to pink was observed with a concomitant sensitive fluorescent turn-on signal from a zero background. The feasibility of **Hg570** for Hg^{2+} detection in various applications was exemplified.

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Notes and references

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Electronic Supplementary Information (ESI) available: The general experimental, detailed synthetic procedures for **Hg550** and characterization including ^{1}H- and ^{13}C-NMR and HRMS were provided. See DOI: 10.1039/c000000x/

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