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COMMUNICATION

A BODIPY-functionalized bimetallic probe for sensitive and selective color-fluorometric chemosensing of Hg²⁺†

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A new BODIPY dye conjugate has demonstrated selective quenching by mercury over other metal ions. Coupling of this probe to Au-Fe₃O₄ nanoparticles as well as platinum electrodes offered sensitive systems for suspension and surface based sensing, respectively.

Accumulation of mercury in the body leads to many serious human afflictions, with exposure resulting in serious damage to the central nervous system and even death.¹ Unfortunately, mercury wastes finding their way into water sources are eventually taken up and collected in higher food chain organisms such as fish.² Standard procedures for environmental assessment of mercury species make use of cold vapor atomic fluorescence spectrometry as a technique for detecting unsafe levels of mercury (<2 ppb).³ An attractive alternative to complex spectroscopic instrumentation is a direct visual detection of Hg²⁺. Chromogenic or fluorogenic based mercury probes, including dithizone derivatives,⁴ diphenylcarbazones⁵ and rhodamine derivatives among others,⁶ have demonstrated sufficient sensitivity. However, depending on the probe, a significant side-response to Ag⁺ and Pb²⁺ may be exhibited. Within the following work, we reveal a new fluoroionophore based Hg²⁺ sensing probe with implementation as a suspension phase system (probes immobilized on Au-Fe₃O₄ nanoparticles) as well as a self-assembled surface based sensor (probes coated onto patterned platinum electrodes). Recent research has made use of the fact that sulphur donor motifs offer higher affinity for Hg²⁺ over other metal ions such as Zn²⁺ and Cd²⁺ in that thioether based metal-binding moieties may be arranged as to confer

selectivity for Hg²⁺.⁷ Thus, sulfur-derivatized chromophores and fluorophores as well as Hg²⁺ induced desulfurization reactions have been widely used in the design of Hg²⁺ sensors.⁸ Although depending on the system in use, multi-specificity to non-mercury species may also be observed. Recent work implementing BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) dyes linked to thiocrown ethers as receptive elements have shown that Hg²⁺ as well as Ag⁺ could occupy the macrocycle to inhibit photo-induced electron transfer from the sulfur atoms to the BODIPY group to increase fluorescence.⁹ Other BODIPY conjugate sensors have utilized multi-dentate receptive motifs in order to demonstrate enhanced selectivity to certain metal ions under physiological conditions.¹⁰ In our previous work, we too utilized structurally similar receptor conjugated BODIPY derivatives with high affinity for Cu²⁺ or Pb²⁺ over other metal ions in aqueous solution.¹¹ In addition to sensing, these probes when immobilized on magnetic nanoparticles provided a means for removal of toxic Pb²⁺ from polluted solutions by repeated binding and isolation with a simple magnet.¹²

As part of our ongoing work to create sensors for toxic metal ions, we reveal a new Hg²⁺ specific BODIPY conjugate capable of immobilization onto nanoparticles as a unique chemosensor/separation system for detection/removal of Hg²⁺ from polluted solutions. In addition, we demonstrate the capability of the probe to self-assemble onto platinum electrodes for use as a surface-sensor platform coating. In our efforts, we came upon a fluoroionophore motif, **2**, which exhibited superb selectivity for Hg²⁺ over other metal cations and operated in a “turn-off” mode for detection. Moreover, this BODIPY-receptor motif demonstrated ppb detection sensitivity, thereby satisfying the applicable detection range for regulated safety levels of Hg²⁺. As evidence of its potential use in sensing applications, we confirmed that immobilization of **2** onto bi-functional Au-Fe₃O₄ nanoparticles and also patterning of **2** onto platinum mini-grids as a sensor coating could be carried out effectively while retaining the inherent selectivity and sensitivity of the probe.

Stable BODIPY derivatives offer strong absorption and fluorescence characteristics making them our preferred reporter for metal ion detection studies. Preparation of the new Hg²⁺ receptive BODIPY conjugate, **2**, and subsequent immobilization onto Au-Fe₃O₄ nanoparticles were carried out as depicted in Scheme 1. The nanoparticles were synthesized by decomposition of Fe(CO)₅ on the surface of the Au nanoparticles followed by oxidation under 1-octadecene solvent reflux as outlined in Scheme S1.†

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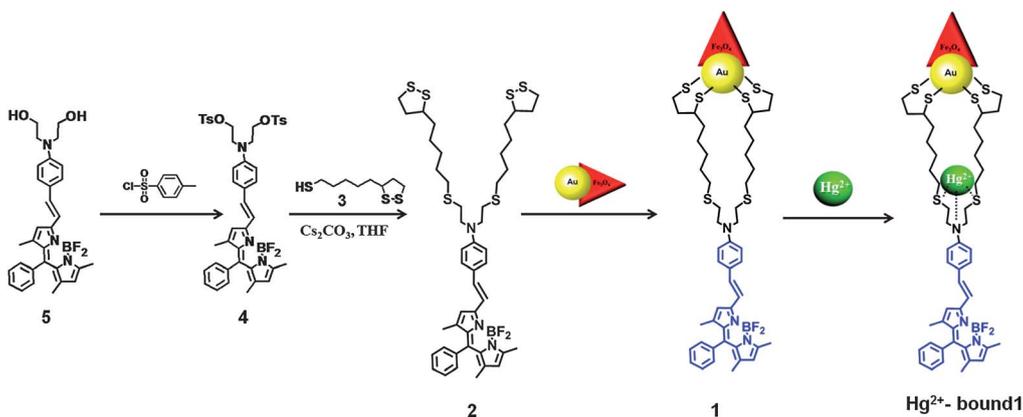
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Scheme 1 Preparation of receptive BODIPY conjugate (**2**) as well as functionalization to a Au–Fe₃O₄ nanoparticle (**1**).

Immobilization onto the gold domain of the Au–Fe₃O₄ nanoparticles *via* the thiol groups of **2** facilitated the formation of receptive BODIPY functionalized Au–Fe₃O₄ nanoparticles, herein referred to as **1**. FT-IR spectra (Fig. S1†) revealing enhancement of aromatic peak signals at 1463 cm⁻¹ and methyl group peaks at 1639 cm⁻¹ suggest evidence for **2** on the nanoparticle surface. Fig. S2† provides a TEM image of the formed nanoparticle conjugates of **1**, where the coupled Au and Fe₃O₄ domains can be clearly observed to have size ranges of 6–9 nm and 16–24 nm respectively.

Examination of the recognition capabilities of **1** for Hg²⁺ was carried out in a series of UV-Vis and fluorescence spectroscopy studies. The first observation of the mercury ion detection capability was revealed by a correlation between the absorption properties of **1** and

the concentration of Hg²⁺ in aqueous solution (Fig. 1A). A distinct and instantaneous decrease in absorbance of **1** at *ca.* 591 nm with a concomitant increase at *ca.* 510 nm was revealed for increasing concentrations of Hg²⁺. The strong emission of **1** in the absence of metal ions is due to the blocking of the photoinduced electron transfer (PET). On the other hand, in the presence of Hg²⁺, the fluorescence quenching can be explained as reverse photoinduced electron transfer (PET) when Hg²⁺ is bound to nitrogen atoms of the benzoyl unit (which behaves as a PET donor). It is well known that heavy metal ions such as Cu²⁺, Hg²⁺, and Cd²⁺ tend to quench luminescence through electron- and/or energy-transfer processes.¹³ Accordingly, Hg²⁺ addition caused a decrease in the 657 nm fluorescence emission peak of **1** (excitation at 591 nm) (Fig. 1B). This “turn-off” mechanism of the sensor operates by a one to one complex formation of Hg²⁺ with **1** resulting in a large blue shift in the absorption spectra thereby reducing the absorption intensity at the excitation wavelength. The binding specificity of **1** to other metal ions was assessed for the chromogenic and fluorogenic probe by exposure to Na⁺, Ca²⁺, Mg²⁺, Cd²⁺, Ag⁺, Cu²⁺, Fe²⁺, Pb²⁺, Co²⁺, Cd²⁺ and Ni²⁺; however, no significant spectral changes were observed upon addition of any of these metal ions (Fig. 2 and S4†), indicating that the nanoparticle complex **1** is a highly selective chemosensor for the detection of Hg²⁺.

Comparison of this response across a large pH range revealed that this characteristic fluorescence quenching by Hg²⁺ exposure was

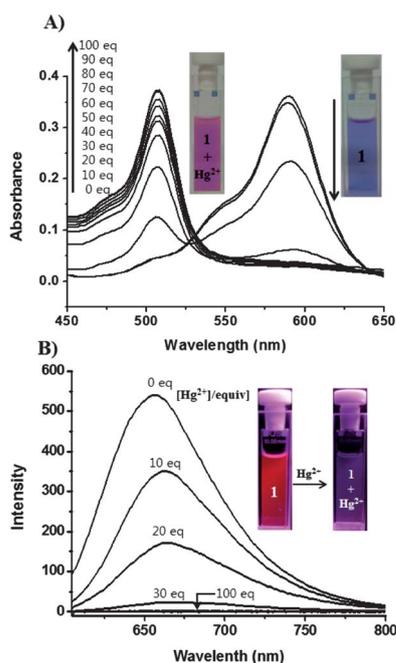


Fig. 1 (A) Absorption spectra of **1** (10 μM with respect to the Au–Fe₃O₄ nanoparticle concentration) upon addition of increasing Hg²⁺ concentrations (0–100 equiv. as indicated by the direction of the black arrows) in 20 mM HEPES aqueous solution at pH 7.4. (B) Fluorescence responses of **1** (10 μM) upon addition of increasing Hg²⁺ concentrations (0–100 equiv.) in 20 mM HEPES aqueous solution at pH 7.4 ($\lambda_{\text{ex}} = 591 \text{ nm}$).

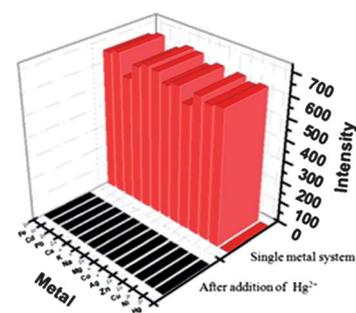


Fig. 2 Fluorescence responses of **1** to various metal ions. Red bars represent the addition of selected metal ions (100 equiv.) to a 10 μM solution of **1**. Black bars represent subsequent addition of Hg²⁺ (100 equiv.) to the mixture. For all measurements, the pH value was adjusted by using 20 mM HEPES in pure aqueous solution, pH 7.4. Excitation was provided at 591 nm, and the emission was monitored at 657 nm.

unaffected by environmental pH changes (Fig. S5[†]), a sign of the strong complex formation between receptive motif **1** and Hg²⁺. Interestingly, the non-fluorescent complex of **1** with Hg²⁺ could be disrupted by the addition of ethylene diamine tetra acetic acid (EDTA), as this revealed an immediate and full return in fluorescence intensity by sequestration of Hg²⁺ by EDTA (Fig. S6[†]). The reversibility of fluorescence quenching of **1** by Hg²⁺ exposure and reappearance by EDTA exposure provide support for the potential of a reusable system. Aside from reusable sensors, an easily regenerated separation and purification system for removing toxic Hg²⁺ from polluted solutions may prove even more valuable. It may be reasonable that **1** has potential for simultaneous detection and separation such as in the analogous system which we previously reported for toxic Pb²⁺.¹²

To further assess the sensing capabilities of **1**, we examined the fluorescence response at low Hg²⁺ concentrations as well as in the presence of various interfering metal ions (Fig. 2 and S7[†]). From Fig. 2 and S7[†] we can see the full capability of **1** to be preferentially quenched in the presence of Hg²⁺ over other metal ions at equivalent concentrations. For the most part, exposure to non-mercury species resulted in only marginal reduction in fluorescence. Secondary exposure of each sample to the same amount of Hg²⁺ confirmed that the presence of the other metal ions did not inhibit the binding ability of Hg²⁺. We found significant reduction in the fluorescence intensity of **1** upon ppb range exposure to Hg²⁺ in pure aqueous solution with a detection limit of approximately 5 ppb for the given instrumentation (Fig. S8[†]).

From Fig. 3, we can see the capability of **2** to self-assemble on the platinum surface in order to provide a fluorescent chemosensor surface for Hg²⁺. The surface patterned sensor operates in a “turn-off” mode of detection by Hg²⁺ binding induced quenching analogous to **1**. In comparison to the nanoparticles suspension system, this surface patterned chemosensor may be more easily reset or regenerated since there is no need for magnetic isolation.

After direct self-assembled patterning of **2** onto the platinum mini-grid surface, we could observe the corresponding fluorescence image pattern attributed to the preferential immobilization of **2** onto platinum as opposed to the glass surface (Fig. 3B). After exposure of the patterned sensor to Hg²⁺ containing solution, the fluorescence

effectively vanished. In doing so, we revealed that surface patterned BODIPY-receptor conjugates may be implemented as coatings for platinum electrode sensor platforms.^{13,14}

In summary, the new BODIPY-conjugate probe **2** was incorporated onto nanoparticles as well as patterned surfaces to provide “turn-off” sensors for the instantaneous detection of Hg²⁺ in room temperature aqueous environments. A very significant shift of the absorption spectra induced a corresponding reduction in fluorescence emission that was observed down to the ppb range of Hg²⁺ exposure, a meaningful sensitivity that is relevant to regulatory limits. In addition, assessment across a range of metal ions established that our system was selective for Hg²⁺ detection. The potential of this BODIPY-conjugate warrants further integration and testing with deployable sensing platforms in order to appropriately meet the needs of a practical Hg²⁺ sensor.

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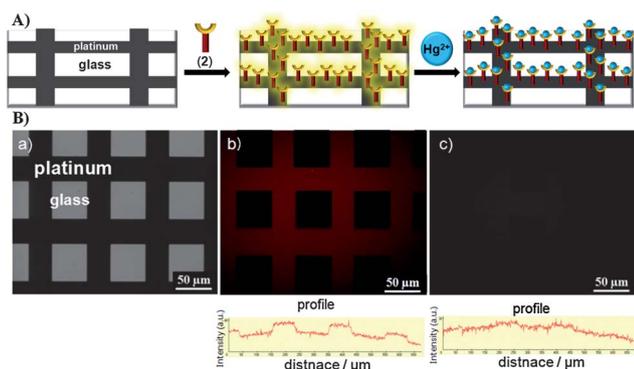


Fig. 3 (A) Schematic of a **2**-immobilized platinum mini-grid electrode. (B) (a) Optical image of the platinum-mini-grid electrode. (B) (b) Fluorescence image of the BODIPY-modified platinum mini-grid electrode and fluorescence intensity profile. (B) (c) Fluorescence image of the BODIPY-modified platinum mini-grid electrode after immersion of Hg²⁺ (10 μM) solution and fluorescence intensity profile.