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A Single Molecular Light-up Sensor for Quantification of Hg^{2+} and Ag^{+} in Aqueous Medium: High Selectivity toward Hg^{2+} over Ag^{+} in a Mixture

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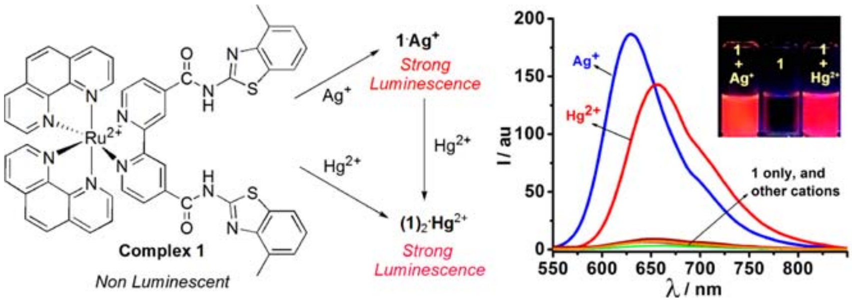
ABSTRACT



A bis-heteroleptic Ru(II) complex (1) is presented that acts as a single molecular dual analyte sensor and quantifies Hg^{2+} and Ag^{+} by luminescence at two different wavelengths. The sensor has stronger binding to Hg^{2+} than to its likely competitor Ag^{+} allowing quantification of Hg^{2+} in a sample with Ag^{+} without a masking agent. 1 also selectively senses Ag^{+} in the absence of Hg^{2+} by enhancing the PL intensity at a different wavelength.

Mercury constitutes a highly toxic heavy metal that is easily absorbed and accumulated in plants, fish, and other living organisms.[1] High exposures to Hg^{2+} may result in damage of the gastrointestinal tract and serious diseases, such as prenatal brain damage, kidney dysfunction, and disorders of the central nervous system (CNS).[2] Consequently, selective quantitative sensing of Hg^{2+} is highly imperative for environmental, biological, and clinical purposes. Recently, various luminescent chemosensors for Hg^{2+} with high sensitivity, versatility, and relatively simple handling have been described.[3] Most of the current Hg^{2+} probes are based on organic or inorganic molecules with sulfur donor centers utilizing the highly thiophilic nature of Hg^{2+}. However, these chemosensors often suffer from selectivity problems, as Ag^{+} and Pb^{2+} strongly interfere. Thus, they are not truly useful for real world applications.[4] In contrast, some reaction-based probes resolve the selectivity issue, but unlike a chemosensor they cannot be reused.[5] Therefore, the development of light-up chemosensors that

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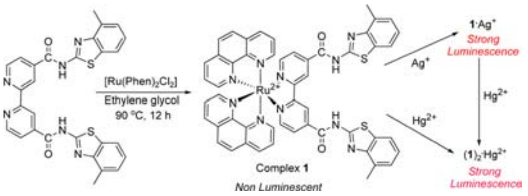
selectively and sensitively detect and quantify Hg^{2+} ions in aqueous solution, in particular in the presence of Ag^+ and Pb^{2+}, is still a topical issue.

Since environmental and biological samples usually contain a variety of ions, the development of competitive molecular probes for real time detection and quantification of multiple analytes is highly demanding.[6] So far, we and others reported various chemosensors with one binding site for the successive detection of multiple analytes using different spectroscopic channels.[7] Although a sensor with multiple binding sites also can serve such a purpose, it is much more tedious from a synthetic viewpoint.[8] On the other hand, using an array of detection methods also suffers from operational problems. As a result, multianalyte sensing with a single molecular probe in one spectroscopic channel is highly desirable. Although there are reports on multianalyte probes, only a few are relevant for the simultaneous detection and quantification of two metal ions in a single channel under aqueous physiological conditions.[9] In most cases, varying conditions are applied in the sensing of different analytes.[10] To the best of our knowledge there is no report on a single molecular probe which senses both Hg^{2+} and Ag^+ by luminescence enhancement at two different wavelengths.

A large number of luminescent tris(diimine) Ru(II) complexes with appended pyridine amide sites for sensing anions have been developed. Rigidification of the amide side chain(s) after anion binding is often the key factor for changes in photoluminescence (PL) and thus sensing.[11] So far, amide-functionalized Ru(II) complexes have not been used for cation sensing. In continuation of our research on Ru(II)- and Ir(III)-based chemosensors for cations and anions,[12] we report herein on the selective light-up sensing of Hg^{2+} in the absence and presence of Ag^+ by a single bis-heteroleptic Ru(II) complex (**1**) carrying two benzothiazole amide units linked to a bipyridine ligand (Scheme 1).[13] Furthermore, sensing and quantification of Ag^+ is easily performed in the presence and absence of Hg^{2+} with and without a masking agent.

Scheme 1. Synthesis of **1** and Sensing of Hg^{2+} and Ag^+



To evaluate the selectivity of **1**, various potentially competing cations were tested by UV−vis spectroscopy in a 0.1 M HEPES buffer/CH_3CN (v/v, 1: 1; pH 7.4) at ambient temperature (Figure 1). In the absence of any cations, **1** shows MLCT absorption bands from 400 to 520 nm with λ_{max} at 476 nm. As shown in Figure 1, no significant absorption change is observed after the addition of 10 equiv of various cations, except for Hg^{2+}, Ag^+, and Cu^{2+}.[14] Although the ∼5−10 nm red shift of the absorption band at 476 nm is detected readily by spectroscopic means, sensing by the naked eye is virtualy useless as no distinct color change was observed.

Sensor **1** itself is nonemissive when excited at 476 nm in an aqueous buffer solution (Figure 1b). Supposedly, the structural flexibility at the amide benzothiazole unit in **1** increases nonradiative decay from the MLCT excited state. Upon addition of 30 equiv of Hg^{2+} and Ag^+ ions, however, a significant enhancement of the emission was documented at 656 and 630 nm, respectively. The red and orange-red luminescence may readily be noted by the naked eye using a hand-held UV light (Figure 1b *inset*). The PL intensity was not significantly influenced by the addition of other competitive metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. It is assumed that the coordination of the S-atoms to the highly thiophilic Hg^{2+} and Ag^+ increases the rigidity of the amide functionalized benzothiazole unit at the bipyridine. As a consequence of inhibiting nonradiative decay, PL is enhanced. It is expected that the PL band and the extent of intensity enhancement depend on the mode of binding,

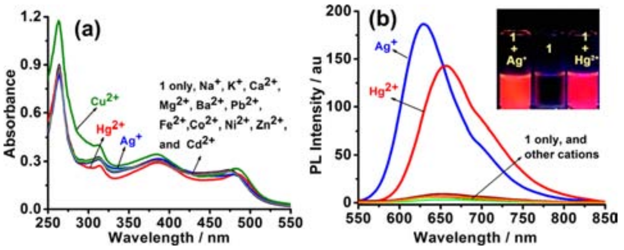


Figure 1. (a) UV−vis and (b) PL spectra of **1** (10 μM) upon addition of various other metal ions (for UV−vis 10 equiv and for PL 30 equiv) in 0.1 M HEPES buffer/CH_3CN (v/v, 1:1; pH 7.4) at 25 °C.

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(13) For the synthesis of **1**, see Supporting Information.

(14) When 30 equiv of cations were used it showed a similar response.

degree of rigidification, and the deformation about the amide residue of the bipyridine ligand.

PL titration of **1** (10 μM) with 0−30 equiv of Hg^{2+} shows a gradual red shift and a 24-fold enhancement of the initially weak PL band at λ_{max} 648 to 656 nm (Figure 2a). The Job plot clearly indicates a 2:1 binding stoichiometry of sensor **1** to Hg^{2+} (Figure S17a). The overall association constant of Hg^{2+} with **1** is determined as log $\beta_{[Hg2+]} = 7.8 \pm 0.3$ from a SPECFIT[15] analysis of the PL titration data. During titration the emission intensity of **1** is strongly increased at a lower concentration of Hg^{2+} allowing detection of Hg^{2+} down to 118 nM (Figure S18a) in an aqueous solution. As a result, the sensor meets the industrial wastewater discharge limit according to the U.S. EPA standard.[16]

Equally in the PL channel, **1** is able to sense Ag^+ by a blue shift from λ_{max} 648 to 630 nm (Figure 2b). In addition, the PL titration of **1** in aqueous buffer with Ag^+ (0−30 equiv) revealed an ∼33-fold intensity enhancement and a bright orange-red luminescence. The Job plot clearly indicates a 1:1 binding of **1** and Ag^+ (Figure S17b). Fitting the PL titration data using the nonlinear regression analysis program SPECFIT[15] furnished a good fit with log $K_{[Ag+]} = 4.6 \pm 0.06$ as a binding constant. The detection limit for Ag^+ was calculated to be as low as 112 nM (Figure S18b). It is noteworthy that a lower and higher pH affect the luminescent sensing of the Ag^+ and Hg^{2+} respectively. However the physiological pH is most suitable for the sensing of Hg^{2+} and Ag^+ (Figure S25).

In the ESI-MS spectrum, a major peak is observed at *m/z* 2313.6 corresponding to $[Hg(\mathbf{1})_2(H_2O)(ClO_4)]^+$, upon treatment of **1** with excess Hg^{2+}, confirming the **1**/Hg^{2+} = 2:1 stoichiometry (Figure S19). In contrast, a 1:1 binding stoichiometry of **1** and Ag^+ was supported by the ESI-MS spectrum (Figure S20). Notably in the ^1H NMR spectra, no significant changes in chemical shifts were observed when 5 equiv of Ag^+/Hg^{2+} were added to **1** in acetone-d_6.

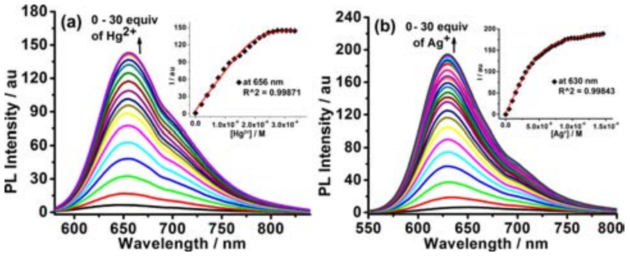

Figure 2. PL titration of **1** (10 μM) with (a) Hg^{2+} (0−300 μM) (b) Ag^+ (0−300 μM) in 0.1 M HEPES buffer/CH_3CN (v/v, 1:1; pH 7.4) at 25 °C. (*Insets*) Plot of PL intensity as a function of (a) Hg^{2+} (λ_{em} = 656 nm; λ_{ex} = 476 nm) and (b) Ag^+ concentration (λ_{em} = 630 nm; λ_{ex} = 476 nm).

The Ru(II) complex may coordinate to the metal ion through N or S centers. A likely interaction mode of **1** with Hg^{2+} and Ag^+ is shown in the Figure 3. Both the Hg^{2+} and Ag^+ are highly thiophilic; hence they may prefer the S center over the N,O chelating site. Hg^{2+} can form a tetrahedral complex, and according to the Job plot the structure of the 2:1 adduct is most likely the one depicted in Figure 3 (left). In contrast, as Ag(I) usually prefers a linear coordination structure, in Figure 3 (right) a 1:1 adduct is presumably assumed.

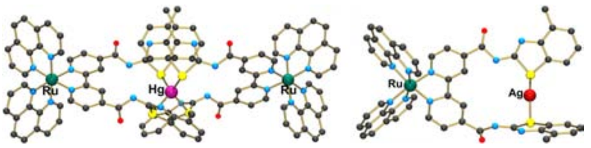

Figure 3. The possible binding mode is showing in the energy minimized structures (see Supporting Inofrmation) of the (left) $(\mathbf{1})_2 \cdot Hg^{2+}$ and (right) $\mathbf{1} \cdot Ag^+$ complex.

Competition experiments with other metal ions were performed in the PL channel to check for possible interferences in sensing Hg^{2+}. Except for Ag^+, the addition of other metal ions (50 equiv) to **1** (10 mM) did not influence the PL intensity. As a result, Hg^{2+} (10 equiv) in a 1:50 mixture of **1** and other competitive cations is readily detected by a clear PL enhancement at 656 nm (Figure 4a). In the absence of Hg^{2+}, the PL intensity of chemosensor **1** is enhanced only substantially when Ag^+ (10 equiv) is added to a 1:50 mixture of **1** and other competitive cations. However, in this case Fe^{2+} and Cu^{2+} interfere to some extent (Figure 4b).[17]

In competition experiments of Ag^+ and Hg^{2+}, 50 equiv of Ag^+ enhance the PL intensity at 630 nm by ∼33-fold, while addition of only 10 equiv of Hg^{2+} significantly shifts the PL to λ_{max} = 642 nm along with a small decrease of the PL intensity (∼26-fold referenced to plain **1**) (Figure S21).

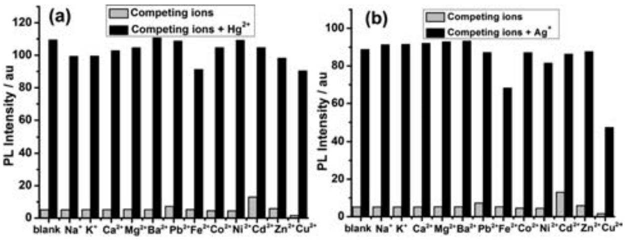

Figure 4. The PL responses of **1** (10 μM) to various competing metal ions (50 equiv) in the absence and presence of (a) Hg^{2+} (10 equiv) and (b) Ag^+ (10 equiv) in 0.1 M HEPES buffer/CH_3CN (pH 7.4) at 25 °C. The λ_{ex} at 476 nm and λ_{em} (a) at 656 nm and (b) at 630 nm.

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(15) *SPECFIT*, Global Analysis system, Version 3.0.32.

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(17) See Figures S21−S22 in the Supporting Information where both Ag^+ and Hg^{2+} were used.

Clearly, a red shift of the PL is only possible if the Hg^{2+} displaces the Ag^+ from the $\mathbf{1}\cdot Ag^+$ complex. Such replacement is possible because the overall association constant of $(\mathbf{1})_2\cdot Hg^{2+}$ is ~1586 times higher than that of $\mathbf{1}\cdot Ag^+$. According to the energy minimized structure, the linear $\mathbf{1}\cdot Ag^+$ complex is quite strained as compared to the tetrahedral $(\mathbf{1})_2\cdot Hg^{2+}$ complex. This result suggested that **1** is Hg^{2+} selective, even in the presence of a small excess of Ag^+. To study the displacement, an ensemble of **1** and Ag^+ (1:30) was titrated with Hg^{2+} in aqueous buffer solution (Figure 5a). With incremental addition of Hg^{2+} (0–30 equiv), the initial PL band (λ_{em} = 630 nm) was decreased gradually and shifted to 656 nm, i.e. to the PL band position of a $\mathbf{1}/Hg^{2+}$ = 1:30 mixture (Figure 2a). A plot of the PL intensity ratio at 656 and 630 nm against the concentration of Hg^{2+} shows good linearity at lower concentrations of Hg^{2+} (0 to 100 μM). Hence, the Hg^{2+} can be quantified in presence of preformed $\mathbf{1}\cdot Ag^+$ (Figure 5b). Inversely, a PL titration was executed to evaluate whether the $(\mathbf{1})_2\cdot Hg^{2+}$ complex is susceptible for Hg^{2+} displacement by equimolar or a large excess of Ag^+. Figure S23 shows that the addition of 0–210 equiv of Ag^+ to $(\mathbf{1})_2\cdot Hg^{2+}$ (1:30 mixture) does not change the band at 656 nm supporting the stronger binding of **1** to Hg^{2+} than to Ag^+. Accordingly in an unknown mixture containing Hg^{2+} and its frequent competitor Ag^+, selective sensing and quantification are possible without use of any external masking agent. It is notable that there are many reports on Hg^{2+} selective sensors/probes, but sensors/probes that quantify Hg^{2+} in a mixture with Ag^+ without using a masking agent are hardly reported.

Due to its severe toxicity and role in biological systems the selective sensing and quantification of Ag^+ in biological and environmental systems is highly important.[18] Probe **1** can act as selective dual-analyte chemosensor because it can sense and quantify Ag^+ in absence (Figure 2b) of Hg^{2+}, while in presence of Hg^{2+} a masking agent is required. EDTA is well-known to form strong complexes and to sequester Hg^{2+} in solution. In a typical PL experiment, the intensity of the PL band at 656 nm decreases ~5 fold while shifting to 648 nm when the 1:30 ensemble of compound **1** and Hg^{2+} was treated with 30 equiv of EDTA. Such finding clearly indicates that EDTA snatches Hg^{2+} from the $(\mathbf{1})_2\cdot Hg^{2+}$ complex. PL titration of the resulting mixture with 0–30 equiv of Ag^+ reveals a gradual blue shift of the emission band to 630 nm and a concomitant intensity enhancement (Figure 5c). The plot of the PL intensity as a function of the Ag^+ concentration shows linearity at low concentration of Ag^+ and clearly supports that Ag^+ quantification is possible in an unknown mixture with Hg^{2+} by the use of a masking agent (Figure 5d).

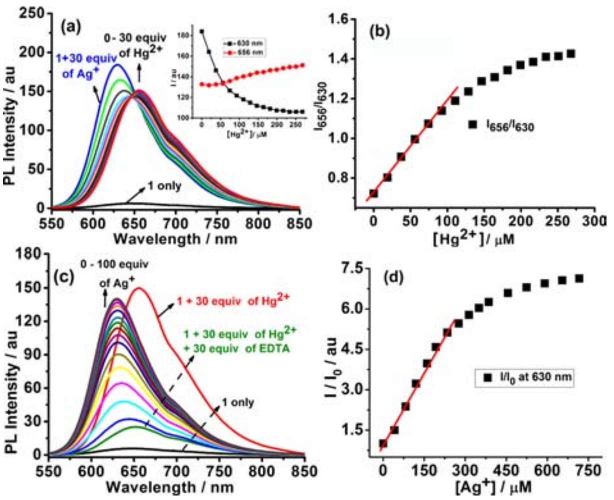

Figure 5. (a) The PL titration of **1** (10 μM) + Ag^+ (30 equiv) mixture with 0–30 equiv of Hg^{2+} in aqueous buffer at 25 °C. (b) The plot of I_{656}/I_{630} as a function of $[Hg^{2+}]$. (c) The PL titration of **1** (10 μM) + Hg^{2+} (30 equiv) + EDTA (30 equiv) mixture with 0–100 equiv of Ag^+ in similar conditions as (a). (d) The plot of I/I_0 at 630 nm as a function of $[Ag^+]$.

In conclusion, we have designed and synthesized a bis-heteroleptic Ru(II) complex as asingle molecular dual analyte sensor that is highly selective for Hg^{2+} and Ag^+ in an aqueous buffer solution. Most importantly, the sensor has stronger binding to Hg^{2+} than to its natural competitor Ag^+ allowing quantification of Hg^{2+} in a sample with Ag^+ without a masking agent. **1** also selectively senses Ag^+ in the absence of Hg^{2+} by enhancing the PL intensity at different wavelengths. By using a masking agent, sensing and quantification of Ag^+ are possible in a sample containing unknown amounts of Hg^{2+}.

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Supporting Information Available. Synthesis and characterization details, UV–vis, PL analysis, CV, ESI-MS, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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