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Coupling Selectivity with Sensitivity in an Integrated Chemosensor Framework: Design of a Hg^{2+}-Responsive Probe, Operating above 500 nm

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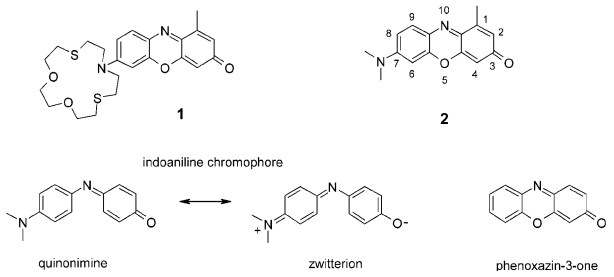
Mercury is a highly toxic element.[1] However, despite its toxicity mercury and mercuric salts are used in a large number of industrial processes and products. For instance, inorganic mercury compounds can be found in electrical equipment, catalysts, paints, or as mining byproducts.[2] Due to its wide use, a high percentage of mercury contamination can be attributed to anthropogenic sources. Apart from intensifying efforts to curtail mercury release into the environment an important aspect in pollutant management is the development of new or improved sensing methods, applicable in a wide range of different sites and environments.

Among newer methodologies for chemical sensing, spectroscopic techniques employing chromo- or fluorogenic molecular sensors are especially appealing.[3] They allow the monitoring of weakly colored or nonfluorescent species such as many heavy- and transition-metal ions with the advantageous features of optical spectroscopy. Besides targeting biologically important cations such as Zn^{2+} and Cu^{2+},[4] considerable effort has been devoted to the design of new chromo-[5] and fluoroionophores[6] for Hg^{2+}. However, many of these molecules display drawbacks in terms of actual applicability such as the lack of water solubility, cross-sensitivities toward other metal ions, or optical signals in wavelength ranges where matrix interference can occur.

To circumvent these problems, we followed a different strategy for the design of title compound **1**, conceived to give a highly selective chromo- and fluorogenic response toward Hg^{2+} in water. The molecular architecture of this simple fluorescent chemosensor is based on the phenoxazinone scaffold, which is also at the heart of widely used polarity probes of the Nile Red family.[7] The use of the phenoxazinone moiety guarantees sufficient water solubility in combination with intense fluorescence in the visible spectral region. In terms of the suitability and performance as a fluoroionophore, however, the π-conjugated and resonant indoaniline electronic structure is essential.[8] In this donor−acceptor ensemble, the carbonyl group acts as acceptor in two important processes, intramolecular charge transfer (CT) and intermolecular hydrogen bonding[9,10] or, when integrated in a metal ion recognition unit, coordinative bond formation.[11] Thus, for instance, upon exchange of an aprotic for an equally polar but protic solvent, a red-shift of the absorption spectrum indicative of an increased CT character is noticed.[10] On the other hand, depriving the chromophore of the amino donor as in phenoxazin-3-one has an opposite effect, i.e, strongly blue-shifted spectra.[12] In equipping **1** with the dithia-dioxa-monoaza crown unit[13] at the 7-position,[14] our aim was to couple improved Hg^{2+} selectivity with efficient signaling via the amino-keto conjugative backbone. To obtain an optimum response toward Hg^{2+}, we avoided the use of polythia or polyaza crowns known to bind most thio- or aminophilic metal ions.[3] Additionally, by offering the charge-dense carbonyl group for coordination, ions such as Fe^{3+} that often interfere due to electrostatic attraction at electron-rich binding sites[3c] should be engaged at this end of the ditopic receptor framework.



The favorable spectroscopic properties of **1** and model compound **2** are directly obvious from Table 1. In highly polar aprotic as well as aqueous media, the absorption and emission bands are centered above 500 and 600 nm, respectively, and are well separated by Stokes shifts >1300 cm^{-1}. The fluorescence quantum yields in acetonitrile are typically high ($\phi_f > 0.6$), and although changing to water reduces the fluorescence yield, $\phi_f = 0.08$ for **1** is still reasonable and substantially higher than for most other fluoroionophores in mixed or neat aqueous media.[3] Furthermore, these properties are accompanied by high molar extinction coefficients, e.g., 4.2×10^4 M^{-1} cm^{-1} at 585 nm for **1** in water.

Coordination studies with heavy, transition- as well as representative main-group-metal ions and **2** in acetonitrile revealed the importance of the molecular prerequisites for the success of the design concept. Irrespective of the metal ion employed[15]—and including Hg^{2+}—the absorption band of **2** shifts nonselectively to the red (see exemplary data of **2**−Hg^{2+} and **2**−Zn^{2+} in Table 1). A similar effect was observed upon protonation. These bathochromic shifts indicate an increase in acceptor strength due to interaction at the carbonyl group. Furthermore, besides appearing at longer wavelengths, the absorption in the presence of cations is significantly more structured, suggesting that interaction of electrophilic species with the acceptor change the dye's character from less to more polymethinic.[16] When such acceptor complexes of CT chromophores are excited, the increased charge density on the acceptor group of the excited molecule leads to a tightening of the coordinative bond.[17] Thus, if the species bound is a heavy- or transition-metal ion, fluorescence quenching commonly occurs due to electron or energy transfer, or enhanced spin−orbit coupling.[3c] Accordingly, **2**−Hg^{2+} shows virtually no fluorescence.

Similar spectroscopic changes are also found for **1** and the majority of the metal ions[15] and protons in acetonitrile. In contrast, the desired opposite response is obtained when Hg^{2+} is added to an acetonitrile solution of **1** (Table 1). The absorption band shows a drastic hypso- and hypochromic shift, occurring now at 446 nm,

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Table 1: Spectroscopic Data of **1** and **2** in the Absence and Presence of Selected Cations in Acetonitrile and Water

	solvent	λ_{abs}/nm	λ_{em}/nm	ϕ_f	τ_f/ns
1	MeCN	525	610	0.65	3.50
2	MeCN	525	610	0.63	3.80
1$-Hg^{2+}$	MeCN	446	Q[a]	n.d.[b]	n.d.
2$-Hg^{2+}$	MeCN	545 (583)[c]	Q	n.d.	n.d.
1$-Zn^{2+}$	MeCN	546 (599)	613	n.d.	n.d.
2$-Zn^{2+}$	MeCN	538 (588)	610	n.d.	n.d.
1	H_2O	585	634	0.08	0.47
2	H_2O	593	630	0.05	0.34
1$-Hg^{2+}$	H_2O	469	615	0.04	0.24,0.91[d]

[a] Emission is strongly quenched, see text. [b] Not determined. [c] Position of the shoulder of a structured band in brackets. [d] Relative amplitudes = 0.6,0.4; for a detailed discussion of biexponentially decaying complexes, see ref 6b.

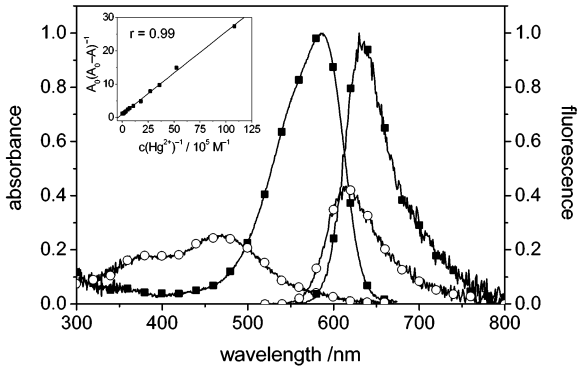

Figure 1. Absorption and fluorescence spectra of **1** (■) and **1**$-Hg^{2+}$ (○) in water ($c_1 = 2 \times 10^{-6}$ M; excitation at isosbestic point at 500 nm). Inset: spectrophotometric titration of **1** with Hg^{2+} under similar conditions.

respectively. This color change from pink to yellow clearly indicates coordination of Hg^{2+} at its designated receptor unit.[18] The loss of conjugation due to lone electron pair abstraction at the 7-position is consistent with a step from **1** to phenoxazin-3-one. Obviously, whereas Hg^{2+} now binds to the crown, all the potentially interfering species[15] still prefer the carbonyl group as in **2** and can be trapped at this end of the π-system.[19]

Most remarkable is the fact that the selective and reversible[20] response toward Hg^{2+} is preserved in water (Table 1, Figure 1). Exclusive binding of Hg^{2+} to the crown is now also indicated by two other features: (i) the complex is still emissive in water, conceivable with excited-state cation decoordination often found for probes with a donor binding site,[17] and (ii) the binding kinetics follow a strict 1:1 model with $K_S = 1.20 \times 10^6$ M^{-1} (Figure 1). In the presence of 2 mM Na^+, K^+, Mg^{2+}, and Ca^{2+} as well as 20 μM of all the other metal ions,[15] no significant variation of the absorption or emission band of **1** was found.[21] Furthermore, with the probe concentrations employed in our studies, Hg^{2+} could be detected down to 10^{-7} M, i.e, at concentrations in the ppb range.

In conclusion, **1** takes advantage of an integrated design concept where the selectivity of the Hg^{2+} binding site is amplified by electronic properties of the chromophore, while maintaining the favorable spectroscopic features of the binding reaction. Fluorescent sensor molecules showing strong absorption and weak emission changes are suitable reporters in dual excitation wavelength ratiometric measurements as employed in imaging-based fluorometry.[22] In this respect, due to its intense absorption and emission bands centered above 500 nm, **1** is a particularly attractive candidate for such applications. Finally, to the best of our knowledge, **1** is the first molecular chemosensor able to selectively sense Hg^{2+} in the ppb range in water by using either absorption or emission measurements.

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Supporting Information Available: Details on synthesis and competition studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Note that, as compared to phenoxazinones, indoaniline dyes are nonfluorescent due to the lack of the rigidifying bridge in the central ring.
(9) Although the impact of the quinonimine−zwitterion equilibrium on the spectroscopic behavior in protic solvents is still controversial (Morley, J. O.; Fitton, A. L. *J. Phys. Chem. A* **1999**, *103*, 11442−11450), previous works generally agree on the underlying process of the spectroscopic response upon hydrogen bonding.
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(14) For synthetic details, see Supporting Information. Detailed photophysical and NMR studies of the functional dyes will be published separately, Descalzo, A. B.; Martínez-Máñez, R.; Radeglia, R.; Rurack, K.; Soto, J. Manuscript in preparation.
(15) Metal ions (as NO_3^-, SO_4^-, or ClO_4^- salts) besides Hg^{2+} used for the studies in MeCN were Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+}, Ca^{2+}, and Na^+; Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, La^{3+}, K^+, Mg^{2+}, and Al^{3+} were additionally checked for potential interference in H_2O (for competition studies, see Supporting Information).
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(18) In fact, coordination of Hg^{2+} to **1** in acetonitrile appears to be more complicated as photometric titrations in this solvent could not be fitted to a clear 1:1 model. This suggests that, although most of Hg^{2+} binds to the macrocycle as indicated by the colour changes, it may also partially coordinate to the carbonyl group. Such a behavior was not observed in water where Hg^{2+} exclusively coordinates to the macrocycle. Mechanistic coordination studies will be published separately, see ref 14.
(19) These results are supported by ^1H NMR studies carried out for **1** and **2** with Hg^{2+} and H^+ in acetonitrile, and which will be reported separately, see ref 14.
(20) Complex formation can reversibly be switched by acidification/ neutralization employing HCl and NaOH. Proton-induced spectroscopic changes occur only at pH <3.5.
(21) The only exception was a 50% decrease of the absorption band at 585 nm upon addition of 10 μM Ag^+. However, no new blue-shifted band appeared so that Ag^+ does not interfere with the detection of Hg^{2+}.
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