A "turn-on" coumarin-based fluorescent sensor with high selectivity for mercury ions in aqueous media[†]

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The synthesis and spectral profile of a "turn-on" fluorescent sensor selective for Hg^{2+} ions in aqueous media is described.

Mercury contamination, bioaccumulation, and the resulting alarming consequences on human health are problems of global dimensions.¹ The Environmental Protection Agency of the USA has already set the upper limit of Hg²⁺ levels in drinking water as low as 2 ppb (10 nM).² Human health risks, springing from mercury exposure have been the subject of extensive investigations.³ Ethylmercury in the form of a preservative is added to certain vaccines.⁴ Methyl mercurial species are known to be involved in CNS dysfunction.⁵ Inorganic mercury is also neurotoxic, and attacks the immune system.⁶ Endocrine-related mechanisms of mercury action across the thyroid and adrenal systems have been detected.⁷ A number of hypotheses were raised about potential molecular mechanisms for the metal genotoxicity.8 The presence of Hg²⁺-Se²⁺ compounds in biological systems has been suggested and their potentially key roles in alleviating the toxicity of Hg²⁺ has been studied.⁹ Detecting and monitoring of mercury ions in environmental and biological systems today present a great challenge for the scientific community, triggering a large number of related investigations that have been recently reviewed.1,10

 Hg^{2+} belongs to the so-called "silent ions" since, unlike some biological metal ions (such as Fe²⁺, Mn²⁺ or Cu²⁺), it does not have an intrinsic spectroscopic or magnetic signal because of its 5d¹⁰ 6s⁰ electronic configuration. The fluorometric detection of such ions is based in the use of small-molecule fluorescent sensors that consist of an ionophore– chromophore system. These probes may coordinate selectively to the target ion and respond to changes in ion levels with changes in their fluorescence profile that may be translated to accurate measurements of the ion concentrations.

Depending on the impact of Hg^{2+} levels to the spectral changes of the free probe, mercury fluorescent probes have been subdivided into "turn-on" (enhancement of fluorescence in the presence of Hg^{2+}), "turn-off" (quenching of fluorescence by Hg^{2+}), and ratiometric probes, the latter group exhibiting a mercury-induced shift in the excitation and/or emission

maxima. Moreover, depending of the degree of their hydrophilicity, mercury probes have been used in organic solvents, aqueous–organic systems (with the organic solvent comprising $\geq 5\%$ of the mixture), and in aqueous solutions. One of the best combinations is that of turn-on/aqueous sensors since their use has a number of advantages such as (a) it reduces the chance of a false positive, observed in some turn-off probes, (b) allows for the use of multiple probes, selective for different ions, and (c) is applicable in the analysis of both, aqueous environmental and biological samples.¹

In this report we describe the synthesis and fluorescence spectral profile studies of a new ratiometric fluorescent ion probe. Structure-wise the compound consists of a monoaza-15-crown-5 moiety, acting as an ionophore, connected via a thiourea linker to 7-amino-4-methylcoumarin, which functions as the chromophore of the system (Scheme 1). The choice of the ionophore was based in previous literature reports using similar crown ether systems for the coordination of heavy metals.^{1,10,11} The choice of the aminocoumarin presented a challenge. Aminocoumarins incorporate an electron donor (amino group) conjugated to an electron acceptor (carbonyl group). This system is expected to follow a photoinduced charge transfer (PCT) mechanism. Upon ion binding, a blue or a red shift is observed in the excitation spectrum, provided that the target ion, in this case Hg^{2+} , coordinates with the donor or the acceptor moiety, respectively. Studies on N-substituted and N,N-disubstituted 7-aminocoumarins indicated that excitation of the system leads to a twisted intramolecular charge transfer (TICT) state and therefore, to radiationless decay.¹²⁻¹⁵ Alternatively, the system is expected to follow a photoinduced electron transfer (PET) mechanism where, upon excitation of the free probe, an electron would be transferred from the crown ether to the fluorophore diminishing fluorescence.¹⁶ Coordination of the mercury ion with the heteroatoms of the crown ether would cancel the electron transfer, increasing considerably the fluorescence signal, a process followed in a turn-on sensor system.

Probe **3** was synthesized in only two steps, *via* an isothiocyanate intermediate, in 97% overall yield (Scheme 1). The synthesis of 4-methyl-7-[(2-thioxoethenylidene)amino]-1*H*isochromen-1-one (**2**) was accomplished easily by using two equivalents of base, excess of CSCl₂, dichloromethane as the solvent, and heating at 35–40 °C. It is important that the excess of thiophosgene is fully removed (caution!), after the reaction is completed, to avoid reaction with the monoazamacrocycle in the next step. Nucleophilic attack of the

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Scheme 1 Synthesis of fluorescent Hg^{2+} sensors 3.

ionophore at intermediate **2** was carried out very rapidly, at room temperature, and almost quantitatively (see ESI[†]).

Fluorescence studies of probe **3** revealed the profile of a photoinduced electron transfer (PET) indicator with a high response to the presence of Hg^{2+} ions. The ion-free form of this PET probe exhibited close to zero fluorescence intensity, while addition of increasing concentrations of Hg^{2+} ions up to 80 μ M resulted in a twenty nine-fold increase in fluorescence intensity, with no shifting in the excitation and emission maxima (Fig. 1). Spectral studies of probe **3** were performed in nanopure water at pH = 7.0 and its K_d value for Hg^{2+} ions was calculated¹⁷ to be 13.1 μ M, while λ_{max} of excitation and emission spectra were detected at 341 nm and 407 nm, respectively (Table 1).

Ion competition study of probe **3** revealed its selectivity for Hg^{2+} in the presence of a host of competitive ions, including heavy metals (Fig. 2). Specifically, in the first set of bars, the first bar (indicated as: free) represents the ratio of fluorescence intensities ($F_{bound} - F_{free}$)/ F_{free} , which is zero, while the second bar (indicated as Hg(II)) represents the fluorescence ratio of the probe after the addition of 60 $\mu M Hg^{2+}$.

The degree of selectivity of indicator **3** for Hg^{2+} is depicted in the next set of measurements. The third bar (Zn(II)) shows the probe's fluorescence ratio in the presence of Zn²⁺ ions. It has a very low value, indicating that Zn²⁺ ion binding is very week, as compared to that of Hg^{2+} . The fourth bar (Zn–Hg) represents the probe's fluorescence ratio in the presence of both Zn²⁺ and Hg^{2+} ions: mercury ions do bind to **3** with the same affinity as before, independently of the presence of zinc or other competitive ions.

As mentioned before, the mode of binding of Hg^{2+} ions onto the probe is a crucial factor determining the spectral profile of the mercury sensor that was synthesized. Indeed, N-substituted and N,N-disubstituted 7-aminocoumarins undergo charge transfer upon excitation leading to a TICT state and therefore, to radiationless decay. The question here is if the nitrogen of a thioamide (such as the 7-aminocoumarin nitrogen in **3**) is able to participate in a charge transfer process given that its free electron pair is delocalized within the thiocarbonyl system of the thiourea linker. As indicated in Fig. 1, the excitation spectrum does not show the wavelength



Fig. 1 Excitation (top) and emission (bottom) spectra of sensor 3 in solutions of increasing Hg^{2+} concentrations in nanopure water. The emission and excitation wavelengths were set at 407 and 341 nm, respectively.

Table 1 Spectral properties of fluorescent indicator 3, in Hg^{2+} solutions





Fig. 3 DFT geometry optimization for the $3-Hg^{2+}$ complex. Atoms are represented in colours; mercury: blue, sulfur: yellow, nitrogen: pink, oxygen: red, carbon: grey, hydrogen: white.

shift expected in a PCT process. In our systems, containing an ionophore with heteroatoms, it is known that an alternative mechanism involves a photoinduced electron transfer (PET) from the ionophore to the excited chromophore, thus quenching the fluorescence process in the free probe. When the metal binds onto the ionophore this process is cancelled and an enhancement of fluorescence intensity is observed with increasing metal concentrations. Literature data¹⁹ on compounds containing (a) the 7-aminocoumarin nitrogen as amide, and (b) a monoaza crown ether, show that upon photoexcitation the predominant effect is that of the electron transfer (PET) from the crown ether to the excited fluorophore. In another study²⁰ the authors indicated that this electron transfer may take place from a 15-crown-5 to a chromophore group. To verify our proposed interaction of Hg^{2+} ions with the heteroatoms in sensor **3** we performed first principles calculations in the DFT level of theory for the Hg^{2+} -3 complex.‡

The structure obtained is illustrated in Fig. 3. Apparently, mercury, being a thiophile, coordinates with the sulfur atom interacting at the same time with elements of the macrocycle system. This spatial arrangement prevents the Hg^{2+} ion from coordinating with the aminocoumarin nitrogen, therefore depleting the PCT effect and initiating the PET process.

In conclusion, a water soluble, fluorescent ion probe with high selectivity for Hg^{2+} ions was designed and synthesized *via* a short and high yield synthetic procedure. The probe belongs to the turn-on class of sensors, functioning *via* the photoinduced electron transfer process, thus providing a dye that is a potential tool for the detection and quantification of mercury in environmental and biological samples.

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Notes and references

[‡] DFT calculations were carried out using Becke-Perdew86 (BP86) functional,^{21,22} the Resolution-of-the-Identity (RI) approximation²³ for computing the electronic Coulombic energy and the def2-SVP^{24,25} basis set for all atoms (default ECPs were included for Hg) and the corresponding auxiliary basis sets²⁶ for the RI method as implemented in the Turbomole 5.9.0²⁷ Program Package. The molecular system was optimized without any constraints during the optimization procedure.

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