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A fluorescent metal sensor based on macrocyclic chelation†

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The ethylenediamine functionalized quinacridone derivatives 3a–c display an orange fluorescence ($\lambda_{em\,(max)}$ = 558 nm) which is quenched upon addition of coordinating metal ions by formation of a macrocyclic chelate bringing metal ion and fluorophore in close proximity to one another.

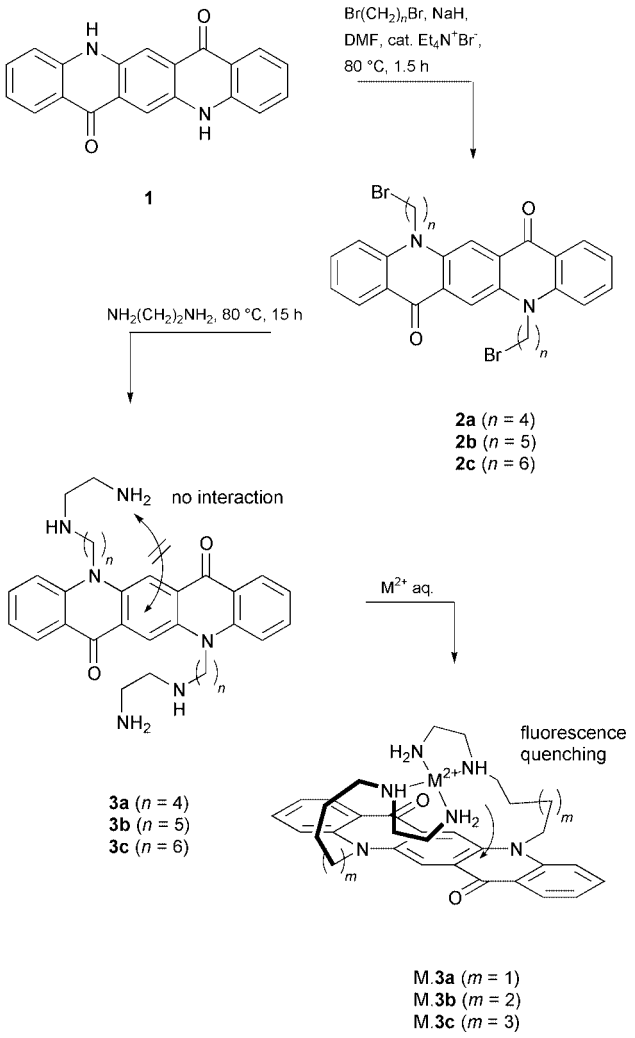
A number of small-molecule fluorescent sensors for metal ions have been developed in recent years, and find applications in trace-ion analysis and imaging. Fluorescence modulation upon metal binding is commonly observed whenever a fluorophore interacts directly with a non-bonding electron pair belonging to a metal-chelating group, typically placed one (phenols and anilines) or two (benzylic heteroatoms) bonds away from the fluorophore. This design forms the basis for the vast majority of fluorescent metal sensors described to date,[1–3] and usually implies that the metal sensors also operate as pH sensors. Herein we report a new type of fluorescent sensor in which a fluorescence quenching effect is obtained by formation of a macrocyclic metal chelate bringing metal ion and fluorophore in close proximity to one another. There is no direct interaction between the chelating group and the fluorophore, which avoids pH sensing.

Our sensor design is based on quinacridone **1**, a red organic pigment used in the dye industry and structurally related to acridone (Scheme 1).[4] A pair of chelating groups, such as ethylenediamines, are attached *via* a linker to each nitrogen atom of the symmetrical quinacridone. The linker is chosen long enough such that the protonation state of the chelating groups does not influence the fluorophore. Most importantly, the long linker now also allows a coordinating metal ion to bind simultaneously at both groups by forming a macrocyclic chelate in which the metal is in close proximity to the quinacridone chromophore. Such an arrangement should result in fluorescence quenching, in particular with energy-transfer quenching metal ions such as copper and nickel.[5]

Double alkylation of quinacridone **1** with dibromoalkanes gives dibromides **2a–c**. Further reaction with excess ethylenediamine then leads to ligands **3a–c** (Scheme 1). All ligands are obtained as trifluoroacetate salts after purification by reverse-phase HPLC.

As expected, the fluorescence spectra of ligands **3a–c** is independent of pH, with < 20% variation in intensity between pH 2 and pH 10. Fluorescence is directly proportional to concentration (0.1–100 μM, pH 7.2 or 9.0), showing that the ligands are not susceptible to auto-quenching. At pH 7.2, addition of Cu^{2+} to ligands **3a–c** induces almost complete quenching of fluorescence (98% quenching, $EC_{50} \approx 0.5$ μM at 1 μM ligand). There is no response with other divalent metal ions such as Hg^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Pb^{2+}, Sr^{2+} (10^{-4} M of the chloride salts). At pH 9.0, quenching is observed for all three ligands with Cu^{2+} (Fig. 1), Ni^{2+} (96% quenching, $EC_{50} \approx 0.8$ μM), Co^{2+} (94% quenching, $EC_{50} \approx 8$ μM), and to a lesser extent with Hg^{2+} (70% quenching, $EC_{50} \approx 0.9$ μM) and Zn^{2+} (45% quenching, $EC_{50} \approx 0.7$ μM). The observed selectivity and pH-dependence of metal complexation with ligands **3a–c** corresponds to the stability constants of ethylenediamine metal complexes.[6] Fluorescence returns to its full intensity upon acidification of the metal–ligand solutions, as well as with high concentrations ($> 10^{-4}$ M) of Zn^{2+} or Hg^{2+}. In all cases the shape of the fluorescence spectrum is unaffected by quenching ($\lambda_{em\,(max)}$ = 558 nm). Remarkably, quenching has little effect on the visible absorbance spetrum or the wavelength of fluorescence emission. Thus solutions of free and complexed ligands **3a–c** show an indistinguishable red color in transparence. Under reflected light, however, the solutions of free ligands **3a–c** shine orange due to their fluorescence, while the metal-complexed ligands remain red (see ESI†).

Fluorescence titration curves at [L] = 1×10^{-5} M show a 1:1 stoichiometry of complexation for L = **3a–c** with Cu^{2+}, suggesting ML, M_2L_2 or M_nL_n modes of complexation.



Scheme 1 Synthesis of ligands **3a–c** from quinacridone **1**. Complexation with coordinating metal ions (M = Cu, Hg, Zn, Ni, Co) leads to a macrocycle in which the metal ion is placed above the aromatic nucleus.

† Electronic supplementary information (ESI) available: electrospray MS data and photographs of solutions of ligand **3c** in the absence and presence of Cu^{2+}. See http://www.rsc.org/suppdata/cc/b1/b100535i/

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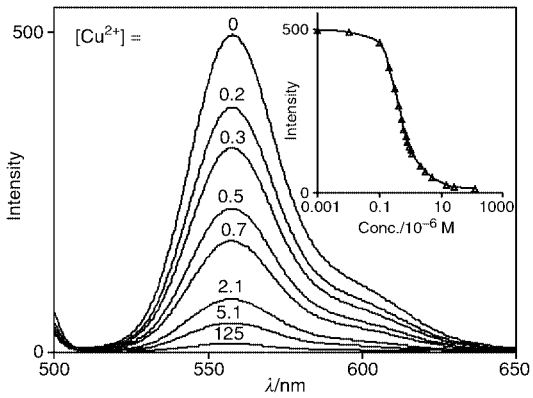

Fig. 1 Fluorescence emmission spectrum of 1×10^{-6} M ligand **3a** in the presence of increasing concentrations of Cu^{2+}. Insert: fluorescence intensity at λ_{em} = 558 nm as a function of copper concentration. Measured at 25 °C in H_2O–DMF 60 : 40, 1 mM Tris, pH 9.0, with λ_{ex} = 485 nm using a Perkin Elmer Luminescence spectrometer LS 50B.

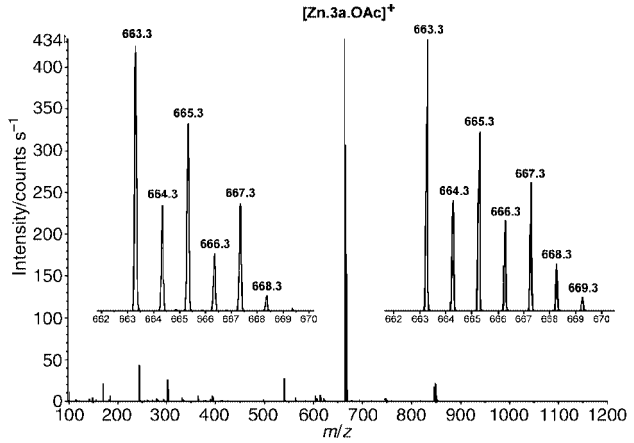

Fig. 2 Electrospray mass spectrum of the Zn.**3a** sample (10^{-3} M equimolar solution of **3a** and $Zn(OAc)_2$ in methanol). The spectrum exhibits an average mass resolving power of 10 000 (FWHM), which allows for full separation of the isotopic patterns. Insets show the measured (left) and calculated (right) pattern of the most abundant complex $[Zn.\mathbf{3a}.OAc]^+$, $C_{34}H_{46}N_6O_4Zn$, *m*/*z* = 663.264. Measured with a Sciex Q-Star Pulsar hybrid quadrupole-time-of-flight mass spectrometer (Applied Biosystems, Rotkreuz, Switzerland) equipped with a nanospray ion source (Protana, Odense, Denmark). A voltage of 1000 V was applied to the nanospray-needle and nitrogen was used as the drying gas.

Electrospray mass spectrometry shows that the ML complex is formed in equimolar solutions of metal ion and ligand **3a–c**. The ML complex disappears in favor of an M_2L species with excess metal [Fig. 2 and Table 1 (ESI)†].

In view of the MS-analysis, the fluorescence data is best interpreted in terms of the formation of an ML complex with a macrocyclic structure as shown in Scheme 1, where fluorescence is quenched by proximity of the coordinating metal.[7] The fact that quenching does not affect markedly either the aborbance (Fig. 2) or the fluorescence emmission spectrum is consistent with an energy-transfer mechanism for quenching. The quenching interaction disappears with the poor energy-transfer quenchers Zn^{2+} and Hg^{2+} when the M_2L complex is formed at high metal concentration, because the metal ions are not close enough to the fluorophore in this complex.[7] The continued quenching observed at high metal concentration with Cu^{2+}, Ni^{2+} or Co^{2+} can be explained by a transition to non-specific quenching by these strong energy-transfer quenchers.

The above experiments demonstrate that efficient fluorescent sensing of metal ions is possible by using a macrocyclic chelation effect. By contrast to standard metal sensors, there is no direct interaction between the metal-chelating groups and the fluorophore, which excludes pH-sensing. The fluorescence modulation obtained is directly visible by the eye since it occurs in the visible range. Remarkably, even weak quenching ions such as Hg^{2+} and Zn^{2+} produce an important fluorescence modulation by macrocyclic chelation. We are currently investigating the construction of macrocyclic chelation sensors with other fluorophores.

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7 Molecular modelling (Spartan 5.1, semi-empirical calculations with PM3(tm)) shows that the metal ions (calculated for Cu^{2+} and Ni^{2+}) are placed at 5.5–6.5 Å above the quinacridone rings in all combinations of ligands **3a**, **3b** or **3c**, with tetrahedral, trigonal bipyramidal (+ 1 H_2O) or octahedral (+ 2 H_2O) coordination. In the M_2L complexes, the metal is placed on the side at 8.2 Å (with **3a**) to 11 Å (with **3c**) from the quinacridone.