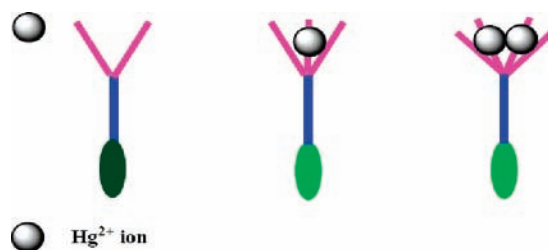# A Series of Polyamide Receptor Based PET Fluorescent Sensor Molecules: Positively Cooperative Hg^{2+} Ion Binding with High Sensitivity

Jiaobing Wang[†] and Xuhong Qian*,[‡,§]

*State Key Laboratory of Fine Chemicals, Dalian University of Technology,
Dalian 116012, China, Shanghai Key Laboratory of Chemical Biology,
East China University of Science and Technology, Shanghai 200237, China, and
State Key Laboratory of Elemento-organic Chemistry, Nankai University, China*

xhqian@ecust.edu.cn

ABSTRACT

A series of PET fluorescent sensor molecules were designed and synthesized based on BODIPY fluorophore and polyamide receptors. Comparison of the photophysical properties of these sensor molecules, equipped with di-, tri-, and tetraamide receptor, provided a deep insight into the polyamide–Hg^{2+} interactions, and an unusual positively cooperative tetraamide–Hg^{2+} complexation was disclosed. In addition, sensor S3 displayed several favorable sensing properties.

Mercury, widely distributed in the air, water, and soil,[1] is considered by the Environmental Protection Agency (EPA) to be a highly dangerous element because of its severe immunotoxic, genotoxic, and neurotoxic effects.[2] As a consequence, mercury-indicating methodologies, which are developed to provide critical information for mercury hazard assessment and mercury pollution management, are in high demand. Among these techniques, fluorescent molecular sensing, which translates molecular recognition into tangible fluorescence signals, has received much attention.[3]

Recently, we disclosed that polyamide receptor based fluorescent molecule sensors could be used to detect Hg^{2+} ions with either fluorescence off–on response or fluorescence color change.[4] These sensors featured high water solubility, unique Hg^{2+} ion selectivity, and significant signal response upon Hg^{2+} ion complexation. However, deep exploration is desired since some basic knowledge about the

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[†] Dalian University of Technology.

[‡] East China University of Science and Technology.

[§] Nankai University.

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actual polyamide−Hg²⁺ interactions remained unclear, which hampered further utilization of these polyamide receptors, such as in the development of functional materials for mercury pollution treatment. In our previous work, we reported that the *o*-phenylenediamine-based tri- and tetraamide sensors, RS2 and RMS, chelated Hg²⁺ ion with a 1:1 stoichiometry, whereas the tetraamide receptor MR bound Hg²⁺ ion in a 1:2 fashion.[4] However, it was not clear (1) whether it was necessary to incorporate all the amide arms to achieve an efficient Hg²⁺ ion complexation, and (2) why the same receptor showed different Hg²⁺ ion chelating stoichiometry and in which way two Hg²⁺ ions were held by one tetraamide receptor. In addition, the performance,
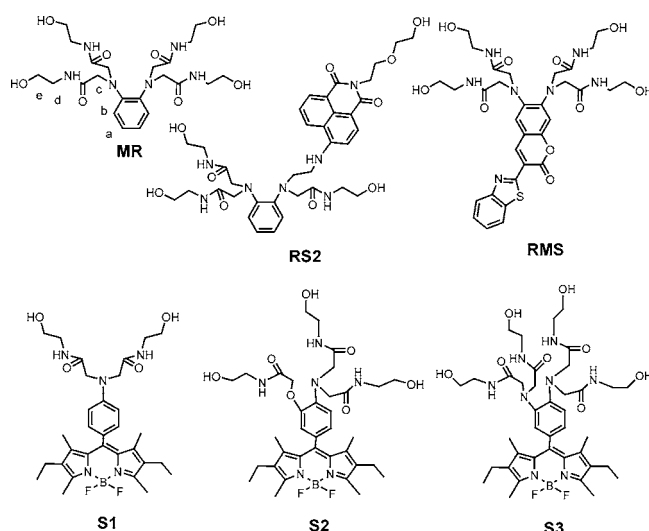
BODIPY fluorophore and polyamide receptor.[5b−d] Before Hg²⁺ ions addition, S1, S2, and S3 all display very weak fluorescence (Table 1), resulting from the efficient photo-

Table 1. Summarized Key Parameters of the Reported Sensors

entry	Φ_0	Φ_1	ϵ (M⁻¹ cm⁻¹)	Log Ks
RMS	0.051	0.051	24000	4.41 ± 0.02
RS2	0.007	0.24	11000	7.10 ± 0.05
S1	0.013	0.013	60000	[a]NA
S2	0.013	0.19	60000	4.72 ± 0.02
S3	0.012	0.61	60000	12.4 ± 0.25

[a] Not available.

induced electron transfer (PET) quenching process from the electron-donating receptor moiety to the excited BODIPY fluorophore.[5d] However, when 2 equiv of Hg²⁺ ions are added, the quantum yield of sensor S3 increases immediately (within a few seconds) and dramatically from 0.012 to 0.61 (Figure 2),[6] and a maximum fluorescence enhancement factor

Figure 1. Chemical structures of the reported sensor molecules.

especially in terms of sensitivity, of the early reported sensor molecules needs to be improved to enhance their practical utility. To address these issues, we report herein three boron dipyrromethene (BODIPY) based fluorescent Hg²⁺ ion sensor molecules, S1, S2, and S3. We incorporated two, three, and four amide arms into the aniline, *o*-hydroxyaniline, and *o*-phenylenediamine based receptors to probe the effects of the convergent amide arms on polyamide−Hg²⁺ interactions. The BODIPY fluorophore was chosen because of its outstanding photophysical properties such as high absorption coefficient (ϵ > 50000 M⁻¹ cm⁻¹), high fluorescence quantum yield (Φ > 0.5), and high photostability.[5]

Absorption spectra of S1, S2, and S3, in the absence or presence of Hg²⁺ ions, show identical shape and absorption coefficient typical of a BODIPY fluorophore (λ_{abs} = 523 nm, ϵ = 60000 M⁻¹ cm⁻¹), indicating that there are no ground-state interactions between the virtually decoupled
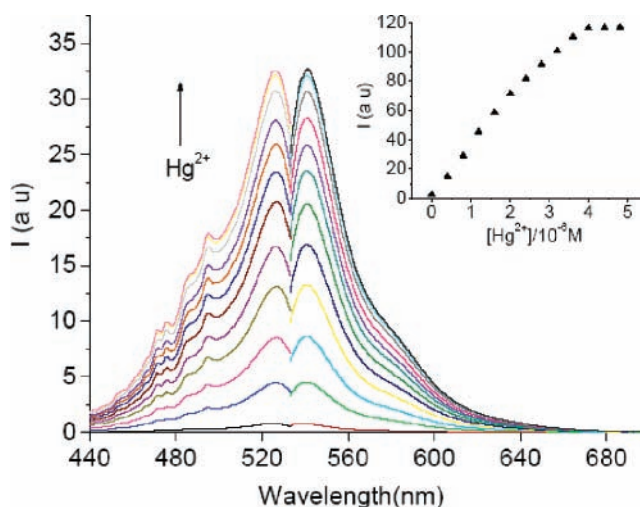

Figure 2. Excitation spectra (emission at 541 nm) and emission spectra (excitation at 527 nm) of S3 (2 μM) in phosphate (0.1 M) solution (pH = 7.5) in the presence of different concentrations of Hg²⁺ ions. The up-arrow indicates the increase of [Hg²⁺] from 0 to 4.2 μM. Inset: integrated fluorescence intensity from 533 to 700 nm as a function of Hg²⁺ ion concentration.

(EF, I/I_0) of 50 is accomplished, indicating that the PET quenching pathway is efficiently blocked by Hg²⁺ ion complexation. For sensor S2, more Hg²⁺ ions (40 equiv) are needed to achieve a lower maximum EF of 15.[7] In a sharp contrast, sensor S1 does not exhibit any measurable EF even in the presence of 70 equiv of Hg²⁺ ions; obviously, sensor

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(7) See Supporting Information.

S1 does not form a complex with Hg^{2+} ion. This could be attributed to the fewer chelating sites available as well as the more conformational flexibility of the two appended amide arms compared with S2 and S3.

A Job's plot suggests that S2 forms a 1:1 complex with Hg^{2+} ion. The association constant is determined to be 5.1 × 10^4 M^{-1} from the Hg^{2+} ions titration curve.[7] In contrast, a Job's plot indicates that S3 chelates Hg^{2+} ions with an 1:2 stoichiometry (Figure 3), different from that of sensor S2,
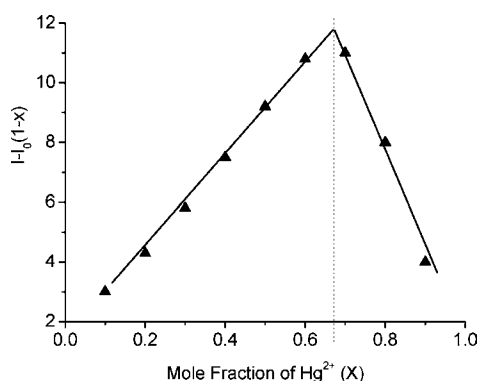
Figure 3. Job's plot of sensor S3 in 0.1 M phosphate buffered water solution (pH = 7.5). The total concentration of sensor and Hg^{2+} ion is 4 μM.

as well as the early reported RS2 and RMS. To give a clear receptor−Hg^{2+} complexation structure, we recorded the ^{1}H NMR spectra (in D_2O, Figure 4) of the tetraamide mercury receptor (MR) in the presence of different concentrations of Hg^{2+} ions. MR is chosen in the NMR studies for its higher water solubility and more simplified NMR signals compared with those of sensor S3. A distinct change occurs at the peak centered at 6.94 ppm (assigned to the four aromatic protons showing overlapped signals), which progressively shifts downfield and splits into two peaks (7.54, 7.66 ppm) with the stepwise addition of Hg^{2+} ions. This shift approximately reaches its limit after the addition of 2 equiv of Hg^{2+} ions, reminiscent of the 1:2 stoichiometry shown in the S3−Hg^{2+} complex and consistent with Job's plot analysis, using NMR data, which indicates a 1:2 MR−Hg^{2+} coordination mode.[4b] In contrast, the single peak assigned for the eight H_c (Figure 1) protons experiences a slight net upfield shift from 4.11 to 3.98 ppm, which could be ascribed to the shielding effect induced by the negatively charged deprotonated amide groups arising from −N−Hg^{2+} complexation (−N, deprotonated amide nitrogen, Figure 5).[8]

Determination of the association constants K_{11} and K_{21}, in the two equations Hg^{2+} + S3 ⇌ S3·Hg^{2+} and S3·Hg^{2+} + Hg^{2+} ⇌ S3·$Hg^{2+}{}_2$, provides substantial information on S3−Hg^{2+} interactions. Because the inserted Hg^{2+} ions titration curve (Figure 2) is too steep to be used for the determination

Figure 4. ^{1}H NMR spectra of MR (in D_2O, 23 mM) in the presence of different concentrations of Hg^{2+} ions. The up-arrow indicates the increase of Hg^{2+} ion concentrations, and the final mole ratio of [Hg^{2+}] to [MR] is 0, 0.5, 1.0, 1.5, 2.0 and 4.0, respectively.

of reliable association constants, we carried out a titration experiment with a distinctly more dilute (1 × 10^{-7} M)

Figure 5. Proposed tetramide−Hg^{2+} complex structures and the energy-minimized conformations by using Hyperchem software with the molecular mechanics subroutine.

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(8) Consistent with our early work, an amide deprotonation process occurred when Hg^{2+} ion was grasped by the polyamide receptor, as indicated by the pH titration experiment. See ref 4 and Supporting Information.
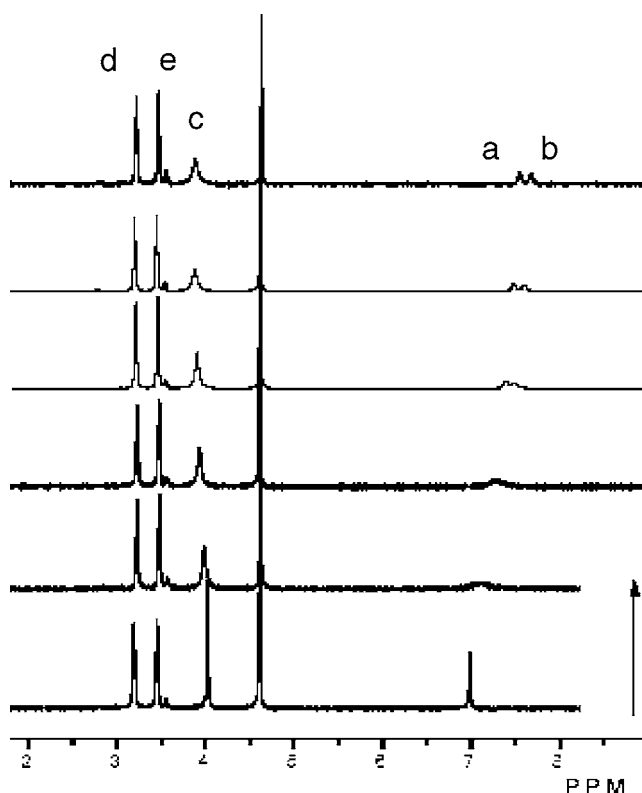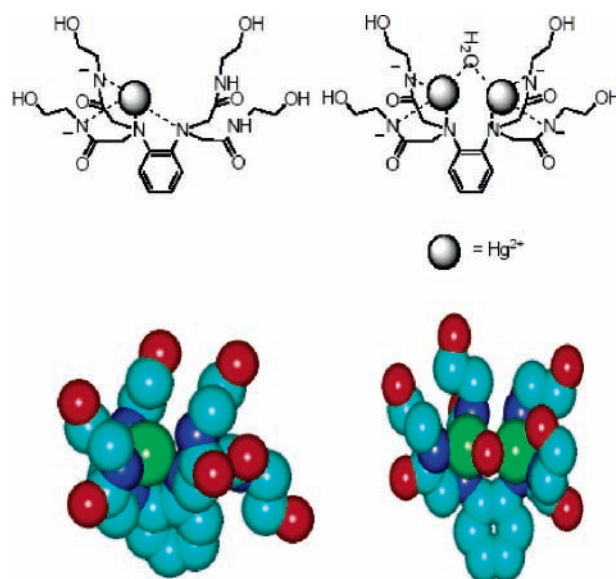
solution and get a smoother titration curve.[7] K_{11} and K_{21} are determined by a nonlinear least-squares analysis of fluorescence intensity I versus Hg^{2+} ion concentration to be 1.1×10^6 and 2.4×10^6 M^{-1}, respectively. Notably, an unusual positively cooperative $S3-Hg^{2+}$ complexation effect is observed because the ratio of K_{21} to K_{11}, amounting to 2.2, is significantly larger than the statistical value 0.25.[9]

Thus, a full picture of the receptor−Hg^{2+} complexation structure could be abstracted from the above-mentioned evidence: as shown in Figure 5, the *o*-phenylenediamine-derived tetraamide receptor catches the first Hg^{2+} ion by two *o*-phenylenediamine nitrogens and two deprotonated amide groups to form a favored tetrahedral Hg^{2+}-ligand structure.[10] Once the first Hg^{2+} ion is caught, the other two unbound amide arms are further restricted and fixed into a more rigid conformation, which facilitates the complexation of the second Hg^{2+} ion. Consequently, we observed a positively cooperative $S3-Hg^{2+}$ complexation effect. When the tetraamide receptor accommodates two Hg^{2+} ions, each Hg^{2+} ion coordinates with one *o*-phenylenediamine nitrogen atom and two negative deprotonated amide groups. In that way, the electrostatic repulsion between the two divalent cations is significantly weakened as a result of the electrostatic complementary action. Other chelating sites may be occupied by H_2O to fulfill the usual tetrahedral Hg^{2+}−ligand structure.

In sensors S2, S3, RS2, and RMS, both the steric and the electronic reasons are speculated to affect the sensor−Hg^{2+} binding stoichiometry. Although RS2, like S3, could also provide six coordinate sites (the 4-amino nitrogen atom of the naphthalimide fluorophore is involved in Hg^{2+} ion binding),[4a] steric repulsion, derived from the bulky fluorophore, may prevent it from adopting a comformation that could accommodate two Hg^{2+} ions. For sensor RMS, the same tetramide receptor possesses a significantly lower Hg^{2+} ion binding strength since the two *o*-phenylenediamine

nitrogen atoms are electronically conjugated with two electron withdrawing groups, and thus, it could also grasp only one Hg^{2+} ion.

Benefiting from so many favorable parameters (ϵ, Φ, EF, Ks), sensor S3 displays a super sensitivity unparalleled by our early reported polyamide fluorescent sensor molecules (Table 1). A clear emission turn-on response is observed when as low as 2 ppb (content limit in drinking water set by EPA) of Hg^{2+} ions are present.[7] Importantly, the unique selectivity of the polyamide receptors is still maintained (Figure 6). These results endow sensor S3 an immediate
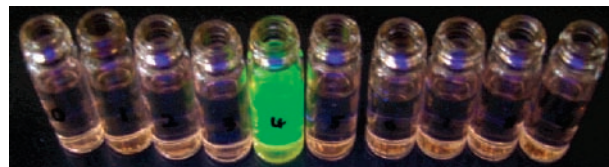

Figure 6. Visual fluorescence changes of S3 after the addition of 2 equiv of different metal ions.[11] The photos were taken under a handheld UV (365 nm) lamp 2 min after the addition of metal ions. Conditions: 2 μM of S3 in 10 mM phosphate buffered water solution (pH = 7.5). From left: 0, control; 1, Cu^{2+}; 2, Zn^{2+}; 3, Fe^{3+}; 4, Hg^{2+}; 5, Pb^{2+}; 6, Ni^{2+}; 7, Cd^{2+}; 8, Co^{2+}; 9, Ag^+.

practical utilization as a highly sensitive Hg^{2+}-ion "annunciator" for drinking water. Overall, comparison of these sensor molecules highlights the potential and the versatility of this polyamide receptor based fluorescence Hg^{2+} ion sensing methodology. These sensor molecules cover a wide dynamic window, which means that the requirement of monitoring different Hg^{2+} ions concentrations, from millimole to sub-nanomole range, could be met just by using an appropriately designed polyamide fluorescent sensor molecule.

Supporting Information Available: Synthetic details, characterization, and spectrascopic data of sensors S1−S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) In Figure 6, except for Hg^{2+} ion, the addition of other detected metal ions did not induce any detectable spectral change. However, we noticed that phosphate buffer might mask the real sensor-ion interactions because some metal ions such as Pb^{2+} formed precipitate in phosphate solution. Thus, to decisively rule out such possible interference, an analogous experiment was also performed in neat water solution. Preserved was the high selectivity of S3 for Hg^{2+} ion (EF = 50) in addition to negligible fluorescence enhancements by Pb^{2+} (EF = 1.4) and Ag^+ (EF = 0.8), respectively. See Supporting Information.