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# Fluorescent sensor for $Hg^{2+}$ detection in aqueous solution

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ABSTRACT

A new water-soluble fluorescent perylene sensor was synthesized and compared to the previous non-aqueous sensors. The new sensor, MSI-1-9, exhibits sensitive and selective detection of mercuric ion ($Hg^{2+}$) directly in aqueous solution through fluorescence quenching. The detection was not affected by the coexistence of other common divalent metal ions. MSI-1-9 possesses the necessary criteria for use in affordable, real-time measurement of $Hg^{2+}$ in environmental water samples, permitting its incorporation into a portable mercury detection kit.



## 1. Introduction

Mercury pollution is highly hazardous and widespread, resulting in serious environmental [1] and health issues [2], such as severely damage of the human heart, kidney, stomach, and genes [3]. On Oct 10, 2013, more than 90 countries in the world signed "The Minamata Convention on Mercury", a new global treaty to cut mercury emissions and releases and set up controls on products, mines and industrial plants [4]. Mercuric ion ($Hg^{2+}$) is a major and dangerous contaminant in environmental and potable water. The design of improved $Hg^{2+}$ ion sensors that exhibit $Hg^{2+}$ ion sensitivity and selectivity and permit robust, immediate detection of $Hg^{2+}$ ion directly in water sources is an important goal [2b,5].

Since 2004, it has been known that $Hg^{2+}$ ion can bind specifically to two DNA thymine bases (T) to form a stable thymine–$Hg^{2+}$–thymine (T–$Hg^{2+}$–T) complex [6]. A number of methods for the detection of $Hg^{2+}$ ion based on the T–$Hg^{2+}$–T complex chemistry have been explored [1d,7], including the identification of the green fluorescent molecule N,N′-dideoxythymidine-3,4,9,10-perylene-tetracarboxylic diimide (TPT) [8]. When TPT binds $Hg^{2+}$, the fluorescence is efficiently quenched concomitantly with molecular aggregation. This fluorescence quenching can be recovered simply by adding HCl to protonate the thymine moiety and dissociate the aggregate. The reversible sensing makes TPT promising for development into cost-effective assay for trace detection of $Hg^{2+}$ [8,9]. However, TPT sensing of $Hg^{2+}$ required a 70:30 (v:v) ratio of DMF–$H_2O$ for solubility, preventing its facile incorporation into a device for direct mercuric ion detection in environmental water samples in the field. It was critical to develop a water soluble analog of TPT that had the same or better sensitivity and selectivity. Synthesis of such water-soluble analogs is not straightforward. The main challenge involved selection and optimization of side chain modifications, which must afford thermodynamic balance between solubility and p–p stacking aggregation of the molecules.

## 2. Materials and methods

Synthesis of sensor molecules: detailed step-by-step process is provided in the Supporting Information. Briefly, all reactions were carried out with dry, appropriately distilled solvents unless otherwise stated. Unless otherwise noted, all solvents and reagents were available from Aldrich or VWR chemicals and used as supplied or purified by standard laboratory methods as required. To monitor reaction progress and chromatography fractions, thin-layer chromatography (TLC) was performed on precoated silica gel G from VWR. The plates were visualized with a 254 nm UV light, or phosphorimo ethanol solution. Flash chromatography was carried out on silica gel cartridge from Teledyne ISCO. $^1$H NMR was recorded on Varian 400 MHz machine. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and *J*-values are in Hz. Low resolution mass spectrometry was performed by The

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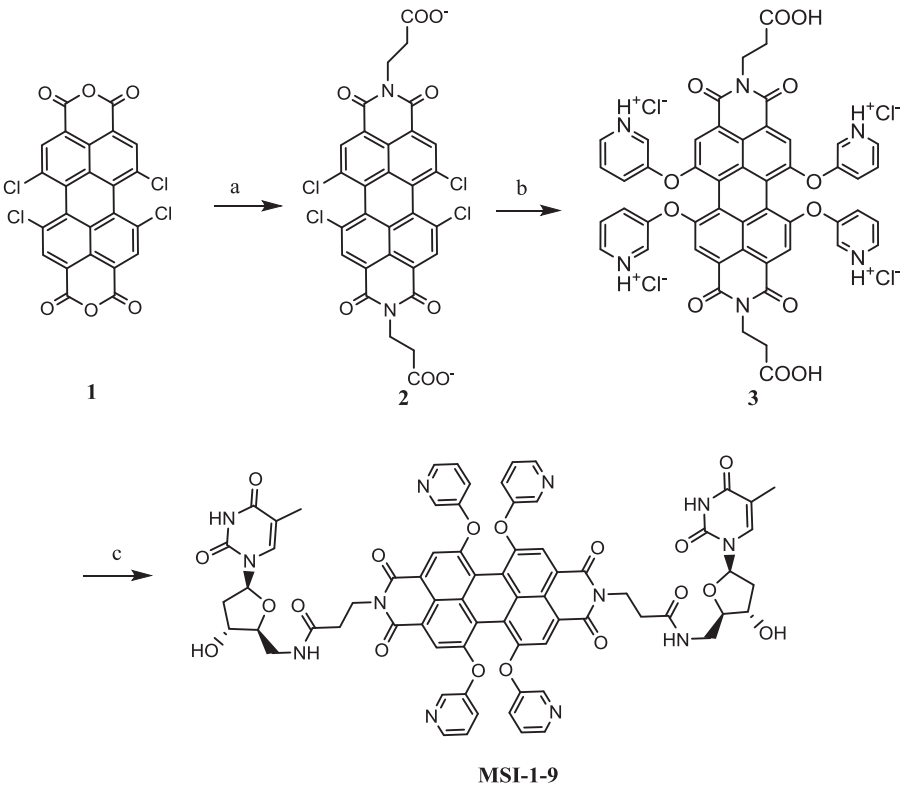


**Scheme 1.** Synthesis of MSI-1-9. Key: (a) 3-aminopropanoic acid, Pyridine, 80 °C, 15 h; (b) 3-hydroxypyridine, $K_2CO_3$, DMF, 100 °C, 16 h; (c) 5-aminothymidine, DIEA, PyBOP, DMF.

Mass Spectrometry and Proteomics Core facility at the University of Utah.

Other General Experimental Methods: All UV–visible absorption spectra were recorded on a Perkin–Elmer Lambda 25 spectrophotometer. All Fluorescence spectra were measured using a Perkin–Elmer LS55 fluorometer. Distilled water (18 MΩ) was prepared by Thermo Scientific Barnstead Nanopure ultrapure water purification system. For sensor characterization and testing, all chemicals used were analytical grade or better and dissolved in distilled water. The pH 4 and 5 buffer solutions were prepared by 20 mM HOAc and NaOAc solution, pH 6 buffer solution were prepared by 20 mM $NaH_2PO_4$ and $Na_2HPO_4$ solution. The $Hg^{2+}$ standard solution (1.0 mM) was prepared with $Hg(NO_3)_2$ and other metal ion solutions were prepared from chloride salts.

## 3. Results and discussion

To improve the water solubility, we synthesized fluorescent sensors MSI-1-9 (Scheme 1) and MSI-1-13 (Scheme S1) containing four pyridyl substituents at the perylene backbone [10]. Given that steric hindrance between the pyridyl and 5-aminothymide moieties could interfere with the π–π stacking between perylene conjugation planes (an important component in the mechanism of fluorescence quenching [8]), we incorporated a flexible β-alanine linker in MSI-1-9 to reduce the steric hindrance. Indeed, MSI-1-9 demonstrated higher sensitivity than MSI-1-13 due to its improved intermolecular stacking. The bulk of the translational work focused on optimization of chemical conditions for using MSI-1-9 as the $Hg^{2+}$ ion sensor of choice.

MSI-1-9 is freely soluble in water with maximum solubility of *ca.* 30 μM, making it suitable for direct sensing of $Hg^{2+}$ ion in aqueous environment. MSI-1-9 is stable in 50% glycerol–water solution over 6 months in the dark without any observable change in its UV–visible spectrum. Under UV excitation, MSI-1-9 solutions fluoresce bright red, in contrast to the green fluorescence of TPT (Fig. S1). The maximum absorption wavelength of MSI-1-9 solutions exhibits a 30–40 nm red shift relative to the original TPT sensor due to four pyridyl moieties appended to the PTCDI core. Fig. 1a shows the absorption spectral change of a 1.0 μM MSI-1-9 aqueous solution upon addition of $Hg^{2+}$ ion. With increasing [$Hg^{2+}$], the absorption spectral characteristic of the aggregated state of T–$Hg^{2+}$–T complexes was clearly observed at the longer wavelength above 580 nm. In Fig. 1b, the fluorescence of MSI-1-9 around 600 nm gradually decreased with increasing [$Hg^{2+}$], consistent with the previously observed quenching mechanism [8].

The 1:1 complexation between $Hg^{2+}$ ion and MSI-1-9 was confirmed by a Job's plot (Fig. 2), obtained by measuring the difference in relative fluorescence intensity at 596 nm with the change in molar fraction of MSI-1-9 relative to [$Hg^{2+}$]. The mercury detection limit in water was calculated as 0.4 ppb by linear fitting of fluorescence quenching curve in Fig. S2. This detection threshold is well below the safety level set for drinking water by EPA (2 ppb). Many of the fluorescent sensors reported thus far for $Hg^{2+}$ have detection limit in the range of a few ppb up to a few tens of ppb 1d,2b,3b,7d, about ten times less sensitive than MSI-1-9 described herein.

The complex formation of the MSI-1-9 with $Hg^{2+}$ ion is affected by the acidity of the solution, but plateaus in the pH value for most environmental samples. A solution of 1.0 μM $Hg(NO_3)_2$ was added to 1.0 μM MSI-1-9 in 20.0 mM HOAc/NaOAc solutions buffered to pH 4–7. Fig. S3 shows the relationship between the value of fluorescence quenched $(1 - I/I_0)$ and pH from 4.0 to 7.0. The quenching increased with increasing pH, reaching a plateau at pH 5.0–7.0, the optimal range for the best $(1 - I/I_0)$ values.

High selectivity is crucial for a robust sensor. Pyridine is well known for weak binding of metal ions [11]. Since there are four pyridyl substituents in MSI-1-9, pH variation could protonate these pyridyl nitrogens, resulting in slight fluorescence quenching from interfering metal ions. In an assay that allows 11 other commonly

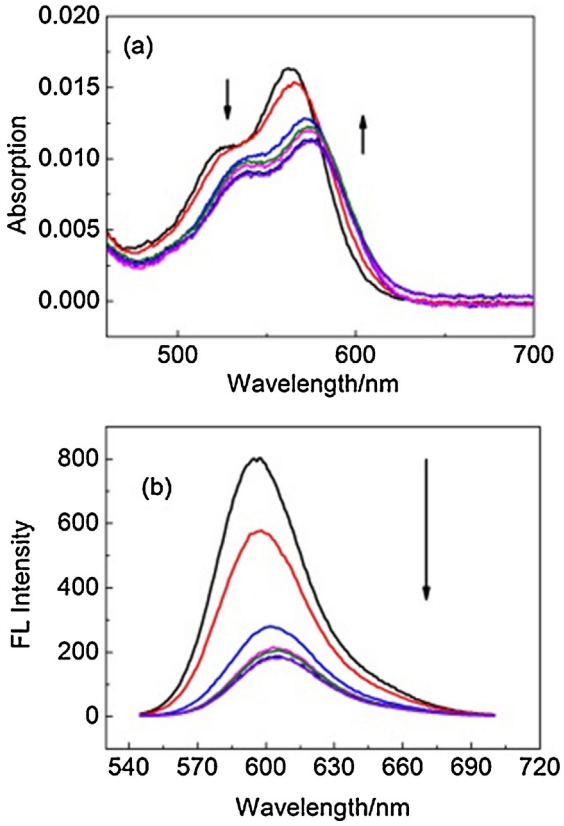


**Fig. 1.** Absorption (a) and fluorescence spectra (b) of 1.0 μM MSI-1-9 in aqueous solution in the presence of various concentrations of $Hg^{2+}$ ion (0, 0.2, 0.6, 1.0, 1.2, 2.0, and 2.6 μM)

co-occurring metal ions to compete with $Hg^{2+}$ for binding ($Cu^{2+}$, $Ni^{2+}$, $Fe^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $K^{+}$, $Na^{+}$), MSI-1-9 showed highly selective detection of $Hg^{2+}$ with quenching efficiency in pH 5 buffer (Fig. 3a). At pH 6 and pH 7, the fluorescence quenching from interfering metal ions was significant (Fig. S4), limiting the selectivity. Moreover, the quenching efficiency by $Hg^{2+}$ at pH 4 was low due to protonation of the thymine moiety (Fig. S3), dramatically reducing the sensitivity. This resulted in a pH optimum for the MSI-1-9 of pH 5.0. These results were achievable due to protonation of the pyridine ($pK_a = 5.2$) at low pH, which also improved aqueous solubility. At pH 5, the sensor not only has high selectivity but also has the best quenching $(1 - I/I_0)$ ratio to

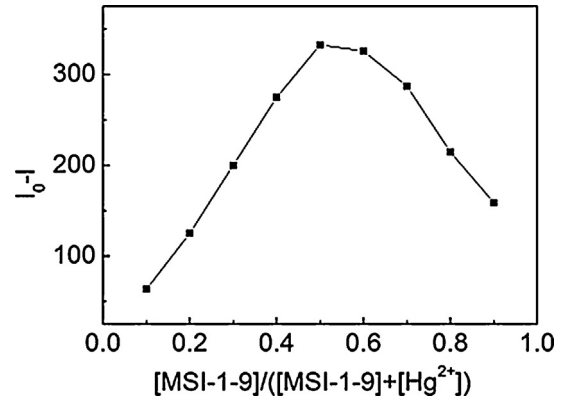


**Fig. 2.** Job's plot of the complexation between MSI-1-9 and $Hg^{2+}$. The total concentration of MSI-1-9 and $Hg^{2+}$ was kept constant at 5 μM in aqueous solution. $I_0$ and $I$ are fluorescence intensity at 596 nm in the absence and presence of $Hg^{2+}$, respectively.

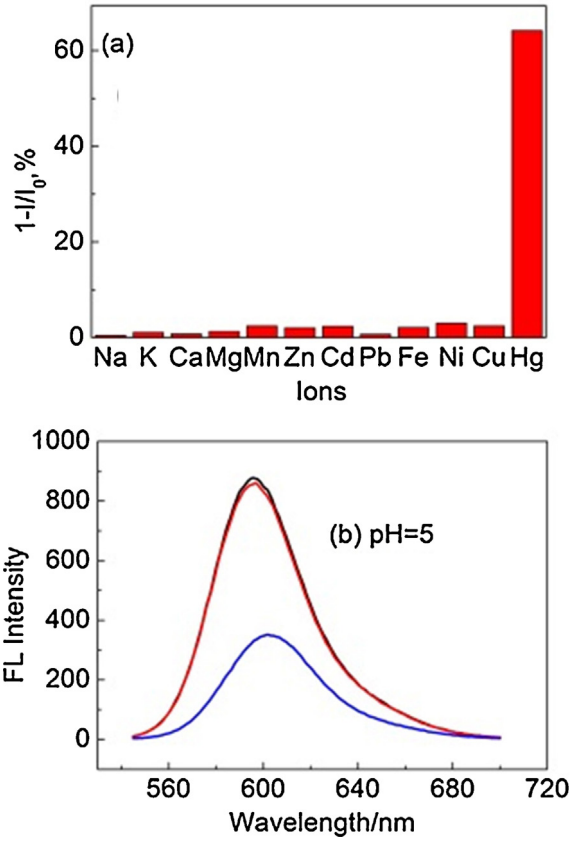


**Fig. 3.** (a) Fluorescence response of MSI-1-9 (1.0 μM) to $Hg^{2+}$ ion (2.0 μM) and various other metal ions (5.0 μM) in aqueous solutions. The bars represent the percentage of fluorescence quenched $(1 - I/I_0)$. (b) Fluorescence spectra of a 1.0 μM MSI-1-9 solution in the absence (black) and presence (red) of a mixture of all 11 metal ions (each 5 μM). Addition of 2.0 μM $Hg^{2+}$ ion to the mixed solution resulted in a dramatic fluorescence quenching (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

pH (Fig. S3). Fig. 3b indicates that only $Hg^{2+}$ ion induced dramatic decrease (64%) in fluorescence intensity, while other metal ions did not cause obvious changes under identical conditions in pH 5 buffered solution.

Finally, many fluorescent sensors are plagued by long response times. The effect of the reaction time on the binding process of $Hg^{2+}$ ion to MSI-1-9 was studied as shown in Fig. 4. Following the addition of 2.0 μM $Hg^{2+}$ ion to 1.0 μM MSI-1-9, the fluorescence intensity of MSI-1-9 was quenched rapidly, reaching a stable value

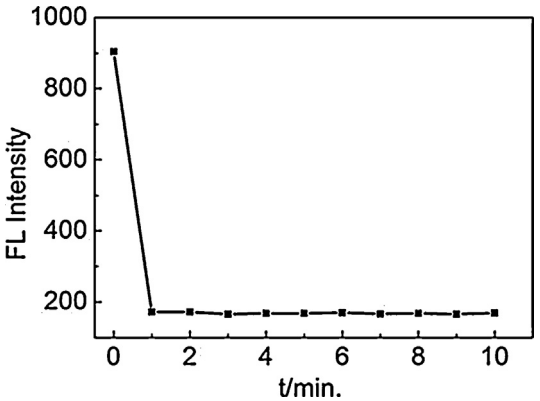


**Fig. 4.** Fluorescence quenching profile of addition $Hg^{2+}$ (2.0 μM) to MSI-1-9 (1.0 μM) in aqueous solution from 1 to 10 min.

within 1 min and remaining constant from 1 to 10 min. The rapid, stable complexation of $Hg^{2+}$ ion by MSI-1-9 and the resulting quick response profile are important features for robust, real time detection of $Hg^{2+}$ by portable device in field. In contrast, many previously reported fluorescent sensors showed responses to $Hg^{2+}$ in the time range of tens of minutes, generally due to slower sensor reaction processes [2a, 6b, 7b].

## 4. Conclusion

We have developed a novel water-soluble fluorescent sensor which displays sensitive, selective and rapid fluorescence quenching by $Hg^{2+}$ ion in a pH 5 buffer solution. This sensor can detect $Hg^{2+}$ on-line and in real time, permitting its incorporation into a portable mercury detection kit.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.03.033.

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