Small-Molecule Fluorescent Chemosensors for Hg²⁺ Ion

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Fluorometric determination techniques for mercury (Hg²⁺) assays have been very popular in recent years. This review focuses mainly on advances in the field of fluorescent sensors, concerning the detection of Hg²⁺ ion, that have been developed over the last few years. Based on a difference among the bearing functional fluorophores, these works are divided into two major sections. In the first section, rhodamine-derived Hg²⁺ ion sensors have been reviewed according to the essential mechanisms that work together with the spirolactam ring opening reaction. Various fluorescence Hg²⁺ ion sensors based on other functional fluorophores are discussed in the second section, divided by their recognition mechanisms that used for the signal responses.

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1 Introduction

Mercury pollution is a global problem.^{1,2} Owing to the grisly immunotoxic, genotoxic, and neurotoxic effects, mercury is considered to be a highly dangerous element by the United States Environmental Protection Agency.^{3,4} For the purposes of detection and quantitative determination of mercury ions, much effort has been devoted to the development of appropriate methods that are low-cost, rapid, deft and applicable to natural environmental and biological milieus.^{5,6} In contrast with many of the traditional quantitative approaches to Hg²⁺ analysis that require intricate, multistep sample preparation and/or sophisticated instrumentation, fluorometric detections following the changes in the emission of light are more convenient and suitable for monitoring Hg²⁺ in either natural or biological environments.⁶ There are multiple advantages in using fluorescence: (i) its powerful visual effects; (ii) its greater sensitivity than that of absorption spectroscopy; (iii) its simple equipment, commonly available in many scientific laboratories.^{7,8} As a consequence, for fluorometric determination of Hg²⁺, a large number of fluorescent sensors including two components, an ionophore (receptor) for selective binding of the substrate and a fluorophore (transducer) for providing the means of

signaling this binding, have recently been designed and synthesized.⁸

This review focuses mainly on the small-molecular fluorescent chemosensors for Hg²⁺ ion in organic or aqueous solution that were developed by our research laboratories and other groups in recent years. Fluorescent chemosensors in this review are divided into two sections based on the bearing fluorophore. The first section focuses on the development of rhodamine-derived Hg²⁺ ion sensors, while the other section gave a brief introduction about Hg²⁺ ion sensors bearing other functional fluorophores. Mechanisms generally used in explaining the response of a fluorophore to Hg²⁺ binding include photoinduced electron transfer (PET), fluorescence resonance energy transfer (FRET), internal charge transfer (ICT), photoinduced charge transfer (PCT) and excimer/exciple formation or extinction.^{8,9}

2 Rhodamine-derived Hg²⁺ Ion Sensors

Noelting and Dziewonski first reported the preparation of the rhodamine in 1905.¹⁰ However, it was only from the 21th century that the rhodamine-B derivatives and its ring-opening reaction received a great deal of attention and have been applied as fluorescent metal ion sensors in organic and biological researches.¹¹ As schematically shown in Fig. 1, many rhodamine-derived chemical sensors follow the approach of “spirolactam ring opening” to detect the guest metal ions.⁵

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Fig. 1 Spirolactam ring opening process of rhodamine derivative.

Fig. 2 Proposed binding mode of **1** with Hg^{2+} .

Rhodamine derivatives with spirolactam structure are non-fluorescent, whereas ring-opening of the spirolactam will give rise to a strong fluorescence emission. Moreover, the rhodamine fluorophore has a longer emission wavelength (over 550 nm), which is often preferred to serve as a reporting group to avoid the influence of background fluorescence (below 500 nm). Thus many fluorescent chemosensors for Hg^{2+} ion have been developed based on rhodamine modifying.

2-1 Spirolactam ring-opening Hg^{2+} ion sensors

Zheng and Xu *et al.* reported a reversible Hg^{2+} ion sensor **1** based on rhodamine B thiohydrazide, containing an S atom and a $-\text{NH}_2$ group attached to the N-bearing spiro ring (Fig. 2).¹² Because of the spirolactam ring, sensor **1** displayed no absorption in the visible region and gave none fluorescence. Upon addition of Hg^{2+} , colorless free **1** showed a new absorption with a maximum wavelength at 561 nm ($\epsilon = 125000 \text{ M}^{-1} \text{ cm}^{-1}$) and took on a strong pink color which can be seen by unaided human vision. The authors demonstrated that sensor **1** is a highly selective sensor with *ca.* 7-fold fluorescent enhancement ($\lambda_{\text{ex}} = 530 \text{ nm}$, $\lambda_{\text{em}} = 582 \text{ nm}$) only in the case of Hg^{2+} . These results were attributed to the coordination in **1-Hg** of Hg^{2+} center wrapped by two rhodamine **1** using N and S binding sites to open its spirolactam ring.¹²

Another rhodamine-based off-on fluorescent chemosensor **2** with N-tripodal structure was synthesized and reported by our group in 2007.¹³ Compound **2** (Fig. 3a) was introduced as a triethylenetetramine with tosyl group used for entrapping Hg^{2+} ion. Compound **2** is sensitive to Hg^{2+} ion in CH_3CN solution, showing a clear absorption (a new absorption band centered at 556 nm) and emission (fluorescent enhancement at 575 nm) changes, and is selective to Hg^{2+} ion with smaller/no spectral changes upon addition of various competitive metal ions (Figs. 3b and 3c). The visual color changes from colorless to pink and the fluorescence changes from dark to luminous yellow in the presence of Hg^{2+} ion (inset of Fig. 3). In addition, a similar **3** (with only one tosyl group) was synthesized using diethylenetriamine instead of tren. Upon addition of Hg^{2+} ion, **3** only gave rise to small absorption and fluorescent spectral changes.¹³

Two different rhodamine derivatives bearing urea groups (**4** and **5**) were synthesized by Soh *et al.* in 2007 (Fig. 4).¹⁴

Fig. 3 Structure of chemodosimeters **2** and **3** (a); absorption spectra (b) of **2** (10 μM) and fluorescence spectra (c) of **2** (1 μM) with addition of ClO_4^- salts of Hg^{2+} , Pb^{2+} , Zn^{2+} , Cu^{2+} , Ca^{2+} , Ba^{2+} , Cd^{2+} , Co^{2+} , Mg^{2+} , Ag^+ , Cs^+ , Li^+ , and Na^+ (10 equiv., respectively) in CH_3CN with an excitation at 520 nm. Inset: visual color and fluorescence changes of **2** upon addition of $\text{Hg}(\text{ClO}_4)_2$ in CH_3CN .

Fig. 4 Structure of chemodosimeters **4** and **5**.

Among the various tested metal ions, chemosensor **4** displayed a highly selective colorimetric change and a fluorescent change with Hg^{2+} . Compound **5** displayed a highly selective CHEF (chelation enhanced fluorescence) effect with over 100-fold emission changes upon the addition of Hg^{2+} even though there was a smaller CHEF effect with Zn^{2+} . There was also a large enhancement (~ 120 -fold) in the UV absorption ($\lambda_{\text{max}} = 554 \text{ nm}$) of compound **5** upon the addition of Hg^{2+} . The 1:1 stoichiometry was confirmed by the Job plot using the fluorescence changes.¹⁴

In the same year, Wu *et al.* designed and prepared a highly sensitive fluorescent probe **6** (Fig. 5), containing rhodamine 6G fluorophore and a carbohydrazone binding unit, for selective detection of Hg^{2+} ion in mixed DMF aqueous media.¹⁵ The fluorescent probe **6** can detect the parts per billion level of Hg^{2+} in a mixed aqueous environment and displays a highly selective response of fluorescence enhancement toward Hg^{2+} . X-ray structural analysis of compound **6-Hg}^{2+}** confirmed the formation of a 1:2 metal/ligand complex. As shown in Fig. 5, the Hg^{2+} center was tightly coordinated to two pyridylimine bidentate units from two ligands. The rhodamine groups were present in the form of a ring-opened amide conformation to indicate the delocalized xanthene moiety, which showed long wavelength absorption and fluorescence enhancement.¹⁵

Simultaneously, a highly selective and multisignaling

Fig. 5 Proposed binding mode of **6** with Hg^{2+} . Fig. 6 Proposed binding mode of **7** with Hg^{2+} ion. Fig. 7 Proposed binding mode of **8** with Hg^{2+} ion.

optical-electrochemical chemosensor **7** (Fig. 6) for Hg^{2+} based on a rhodamine dye bearing both a ferrocenyl group and an 8-hydroxyquinoline moiety was designed and prepared by Yang *et al.*¹⁶ Compound **7** displayed a selective fluorescence enhancement, colorimetric change (colorless to pink) and an obvious change in its reversible ferrocene/ferricinium redox cycles in the absence and presence of Hg^{2+} . In the presence of 15 equiv. of Hg^{2+} , **7** showed an intense red fluorescence with a quantum yield of 0.15, and an approximately 105-fold enhancement in the fluorescence intensity at 580 nm was estimated.¹⁶

Further support for the use of spirolactam ring opening approach to detect Hg^{2+} ions has been provided by Shi and Ma and Zhan *et al.* independently.^{17,18} They reported a rhodamine-B thiolactone **8** (Fig. 7) as a highly selective and sensitive sensor for Hg^{2+} in 20 mM phosphate buffer (pH 7) and 10 mM acetate buffer (pH 4), respectively. Compound **8** exhibited prominent absorption and fluorescence enhancements to Hg^{2+} with a particular selectivity and excellent sensitivity and could be used for naked-eye detection in aqueous solutions.^{17,18}

Chen *et al.* also reported a rhodamine 6G thiolactone derivative **9** (Fig. 8) using as a selective fluorescent and colorimetric sensor for Hg^{2+} in neutral aqueous solution in 2008.¹⁹ “Off-on”-type fluorescent and colorimetric changes were observed only for Hg^{2+} , which can be attributed to a

Fig. 8 (a) Binding mode of **9** with Hg^{2+} ; (b) the X-ray crystal structures of **9** and **9-Hg**²⁺; (c) imaging of *C. elegans* previously exposed to HgCl_2 with the mercury chemosensor **9**. A nematode previously not exposed to HgCl_2 showed only weak fluorescence in the intestine in the presence of the chemosensor (left) and a nematode previously exposed to HgCl_2 showed strong fluorescence in the presence of the chemosensor (right).

spirothiolactone ring-opening process. Since sensor **9** can detect Hg^{2+} in the nano-molar range, this sensor has great potential for biological imaging and environmental purposes. The X-ray structure of **9-Hg**²⁺ supported and explained the 2:1 binding mode of sensor **9** with Hg^{2+} . Furthermore, this sensor was applied for *in vivo* imaging of Hg^{2+} using *C. elegans*.¹⁹

Furthermore, a rhodamine-based and cryptand-rhodamine conjugated Hg^{2+} chemodosimeter **10** was reported by Jana *et al.*²⁰ Compound **10** can detect Hg^{2+} ions in aqueous medium selectively at ppb level in the presence of other biologically relevant metal ions and also showed a good viability to the HEK 293 cell line (Fig. 9). Upon addition of Hg^{2+} , **10** exhibited a prominent change in absorbance at 560 nm, which can be ascribed to the spirolactam bond cleavage followed by the formation of a delocalized xanthene moiety of the rhodamine group. Fluorescence spectral studies also corroborated absorption spectral results. Addition of 5 equiv. of Hg^{2+} ion resulted in a significantly enhanced fluorescence (quantum yield $\Phi = 0.51$) at 580 nm ($\lambda_{\text{ex}} = 520$ nm), while other ions were silent.²⁰

Similarly, Huang *et al.* reported a rhodamine derivative **11** (Fig. 10) achieved by combination of a water-compatible sugar group and a rhodamine group into one molecule.²¹ Compound **11** showed the high quantum efficiency for a Hg^{2+} -bound sensor in natural water and sensitivity to environmentally relevant mercury in complex natural samples. The limit of detection of Hg^{2+} of 1 ppb level suggested that **11** was capable of distinguishing between the safe and toxic levels of inorganic mercury in drinking water. Investigation on the fluorescence imaging of Hg^{2+} in living cells (HeLa cells) demonstrated that **11** might be used for monitoring Hg^{2+} within biological samples.²¹

Huang *et al.* reported a rhodamine-based sensor **12** (Fig. 10) synthesized by incorporation the rhodamine fluorophore and ionophore NS_2 .²² Sensor **12**, which could specifically recognize Hg^{2+} ion by the “naked-eye”, UV-vis and fluorescent responses,

Fig. 9 Binding mode of **10** with Hg²⁺ and confocal fluorescence images of Hg²⁺ in HEK 293 cells (Zeiss LSM 510 META confocal microscope, $\times 40$ objective lens). (a) Brightfield transmission image and (b) fluorescence image of HEK 293 cells incubated with CRD-1 (1.0 mM). Further incubation with addition of Hg(ClO₄)₂ gives images (c) – (e): ((c) 1, (d) 3 and (e) 5 equiv., respectively) and (f), an overlay image of (a) and (e).

Fig. 10 Structure of chemodosimeters **11** and **12**.

Fig. 11 Chemodosimeter **13** for Hg²⁺ ion using Hg²⁺-promoted desulfurization and cyclization strategy.

exhibited a high selectivity and an excellent sensitivity. It was also a dual-responsive colorimetric and fluorescent Hg²⁺-specific sensor in aqueous buffer solution. In addition, the spectral response toward Hg²⁺ was established to be reversible by the EDTA-titration experiments.²²

2-2 Spirolactam ring-opening Hg²⁺ ion sensors that include Hg²⁺-induced cyclization

Yang *et al.* first reported in 2005 a predominant fluorescent and colorimetric rhodamine-6G derivative Hg²⁺ chemodosimeter using a Hg²⁺-promoted desulfurization and cyclization strategy.²³

Sensor **13** (Fig. 11) was essentially non-fluorescent because of the spirolactam ring, while **14** showed strong fluorescence with a high quantum yield of 0.52. Accompanying the titration of 1 equiv. of Hg²⁺ ions, a *ca.* 26-fold fluorescence enhancement occurred and the emission maximum shifted from 553 to 557 nm; also, the solution changed from colorless to bright pink. Sensor **13** was a highly sensitive chemodosimeter for Hg²⁺ in aqueous solution because the observed fluorescence intensity was nearly proportional to the Hg²⁺ concentration and these changes reached completion well within 1 min. Simultaneously, sensor **13** was also a highly selective

Fig. 12 Color changes (a) and fluorescence changes (b) (A, **15** only; B, **15** + Hg^{2+} ; C, **15** + X + Hg^{2+} ; D, **15** + X; X denotes miscellaneous cations including Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Cd^{2+} , Ca^{2+} , Mg^{2+} , K^{+} , and Na^{+}). The reaction scheme of **15** with Hg^{2+} (c).

chemodosimeter for Hg^{2+} because of no significant changes upon adding of **15** other metal ions.²³ In 2006, this system was successfully applied to a monitor exogenous Hg^{2+} uptake in living cells and vertebrate organisms in real time.²⁴

Based on the typically mercury-promoted desulfurization reaction and spirolactam ring opening approach, a new rhodamine derivative fluorescent chemosensor **15**, with a remarkably high selectivity toward Hg^{2+} ions over miscellaneous competitive cations and a wide pH span (5 – 10), was synthesized and reported by our group in 2007.²⁵ Addition of Hg^{2+} ion caused an increase in absorption intensity at 532 nm, which induced a clear color change from colorless to pink. On the other hand, there was also a new emissive peak at 555 nm in the presence of the Hg^{2+} ion, which was consistent with the results of UV-vis spectra. Among miscellaneous cations, **15** showed significant absorbance increases and fluorescence enhancement (Figs. 12a and 12b) only with Hg^{2+} ion in a 90% methanol aqueous solution (pH ~7). These phenomena were explained by the formation of a stable cyclic product **16** through an irreversible desulfurization reaction (Fig. 12c).²⁵

Xi *et al.* reported a similar rhodamine derivative **17** (Fig. 13) as a highly selective and sensitive chemodosimeter for Hg^{2+} .²⁶ The selectivity of this system for Hg^{2+} over other metal ions was remarkably high, and its sensitivity could be below 2 ppb in methanol solutions. Meanwhile, this system can be used in a wide pH range (pH 5 – 10). Confocal laser scanning microscopy experiments have proven that **17** can be used to monitor Hg^{2+} in living cells (HeLa cells) and to map its subcellular distribution.²⁶

2.3 Spirolactam ring-opening Hg^{2+} ion sensors that include FRET

FRET arises from an interaction between a fluorophore (energy donor) at the excited state and a fluorophore (energy acceptor) at the ground state. This interaction could realize the non-radiative transfer of excitation energy from the donor to the

Fig. 13 Structure of chemosensor **17**.

acceptor only by exciting the energy donor portion. Therefore, how to design and synthesize novel fluorescent Hg^{2+} sensors based on FRET mechanism to achieve unique results have attracted much of the attention of the researchers in recent years.

Calixarenes are well known as inclusion hosts for ions and specific molecules, and also as important building blocks in supramolecular chemistry owing to their unique molecular structures, possible modifications and their “tunable” molecular shapes and conformations. With these conveniences, a calix[4]-arene-rhodamine-based chemosensor **18** (Fig. 14), based on metal-induced FRET, was synthesized and reported by our group and Othman *et al.* in 2007.²⁷ Its sensing behavior toward metal ions was investigated by UV-Vis and fluorescence spectroscopies. Addition of Hg^{2+} ions in a CH_3CN solution of **18** gave a significantly enhanced fluorescence of the rhodamine unit (FRET-ON) by energy transfer from the pyrene excimer to ring-opening rhodamine-B. In contrast, addition of Al^{3+} ions induced a distinct increase of pyrene excimer emission, but no FRET-ON was observed. The addition of Al^{3+} induced strong emission of the pyrenyl excimer but only weak rhodamine emission, suggesting that Al^{3+} preferred the formation of a pyrenyl excimer but not the ring-opening of the spirolactam of the rhodamine (Fig. 14).²⁷ In addition, the related material **19** (without the 2 pyrene groups) was also synthesized for comparative illustration of the FRET phenomenon. Compared to **18**, when excited at 343 nm, **19** showed a smaller change in fluorescence upon addition of 100 equiv. Hg^{2+} , indicating that energy transfer in **18** took place from the pyrene excimer to the rhodamine-B. Energy donor (pyrenyl excimer) and energy acceptor (rhodamine) had some spectral overlap, which made FRET possible, when the pyrenyl moiety was excited. When **18** was excited at 343 nm, the two pyrenyl groups gave rise to not only monomer emission (<400 nm), but also excimer emission (ca. 475 nm). The excited pyrenyl excimer can transfer energy to the ring-opened rhodamine moiety in its ground state, which led to the radiative decay (ca. 575 nm) of the rhodamine moiety.²⁷

Based on the FRET mechanism, Shang *et al.* reported a new fluorescence probe **20** (Fig. 15) for Hg^{2+} with a high selectivity in 2008.²⁸ The color of this probe changed from yellow to magenta when reacted with Hg^{2+} , which made **20** available to detect Hg^{2+} either by ratiometric fluorometry or by rapid “naked-eye” detection.²⁸

Zhang *et al.* developed another FRET-based ratiometric probe **21** that can selectively detect amounts of Hg^{2+} ions on the ppb scale under physiological conditions (Fig. 16).²⁹ Compound **21** exhibits a clear Hg^{2+} -induced change in the intensity ratio of the two well-separated and comparably strong emission bands of BODIPY (boron-dipyrromethene) and rhodamine. The significant changes in the fluorescence color can be observed by eye. Preliminary confocal laser scanning microscopy experiments show that **21** has practical application in living cells.²⁹

Fig. 14 Structures of calix[4]arene-rhodamine-based chemosensors **18** and **19** (above) and proposed different complexation behaviors of **18** with Hg^{2+} and Al^{3+} (below).

Fig. 15 Rhodamine-based chemodosimeter **20** and its FRET-based detection mechanism of Hg^{2+} .

Fig. 16 FRET rhodamine-based chemodosimeter **21** for Hg^{2+} ion.

3 Hg^{2+} Ion Sensors with Other Fluorophores

Besides rhodamine-derived chemical sensors that following the approach of “spirolactam ring opening” to detect the Hg^{2+} ions, many new Hg^{2+} ion sensors with other fluorophores using different strategies have been reported in recent years.

3.1 Hg^{2+} ion sensors using metal ion replacement strategy

Wu *et al.* reported a turn-off Hg^{2+} chemosensor **22** (Fig. 17) employing a metal ion replacement strategy.³⁰ The ligand **22** could self-assemble to form a molecular square in the presence

of Zn^{2+} . **22**- Zn^{2+} complex showed strong emission in solution. The fluorescence of the **22**- Zn^{2+} complex dropped dramatically with the addition of Hg^{2+} . After examination of the sensitivity and selectivity, researchers deduced that **22** could be a good Zn^{2+} sensor candidate and that **22**- Zn^{2+} could be a Hg^{2+} sensor candidate based on the mechanisms of the CHEF (chelation-enhanced fluorescence effect) and the replacement of central metal ion induced CHEQ (chelation-enhanced fluorescence quenching effect), respectively.³⁰

Our group reported a new fluorescent agent 5-(4-nitrobenzene-

Fig. 17 Sensor **22** and the metal-ion replacement process. Fig. 18 Complexation modes of **23** with Zn^{2+} and Hg^{2+} and the crystal structure of **23-Hg** $^{2+}$.

diazo)-8-benzene-sulfonamidoquinoline (**23**), employing a metal ion replacement strategy, to detect Hg^{2+} in living cells.³¹ Figure 18 summarizes the binding mechanism of **23** with Zn^{2+} and Hg^{2+} based on fluorescence on-off switching. **23** exhibited a weak fluorescence but emitted strong fluorescence upon Zn^{2+} complexation. The metal ion was believed to coordinate with the nitrogens of the sulfonamide unit and the heterocyclic ring in a 2:1 complexation mode. Upon addition of Hg^{2+} ions to the $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ solution of **23-Zn** $^{2+}$, fluorescence quenching was observed. This was consistent with a greater binding affinity of **23** for Hg^{2+} than for Zn^{2+} . Hg^{2+} binding induced Zn^{2+} to be released from the **23-Zn** $^{2+}$ complex and induced the formation of a new complex **23-Hg** $^{2+}$ that lacked fluorescence. Interestingly, the exchange of Hg^{2+} for Zn^{2+} in **23-Zn** $^{2+}$ complexes could also be detected in LLC-MK2, AMC-HN3, Hep-2, and MDCK cell lines.³¹

Different from the “turn-off” Hg^{2+} sensors reported by Yang and our group, He *et al.* reported a “turn-on” fluorescent sensor **24** (Fig. 19) for Hg^{2+} via a displacement approach in aqueous media.³² Compound **24** exhibited an emission band centered about 530 nm (quantum yield $\Phi \approx 0.080$) in $\text{DMSO}/\text{H}_2\text{O}$ (4:1 v/v) when excited at 430 nm. Upon the addition of 1 equiv. of Cu^{2+} ions, the fluorescence was quenched significantly. The fluorescent spectra of **24** using some other divalent metal ions instead of Cu^{2+} were recorded. The results showed that the fluorescent intensity of **24** was hardly affected by these ions even up to the 10-fold amounts of the ligand. When excited at 430 nm, **24-Cu** $^{2+}$ exhibited a very weak

Fig. 19 Potential displacement signaling mechanism of sensor **24** for sensing Hg^{2+} . Fig. 20 Sensor **25** and Hg^{2+} -promoted desulfurization cyclization. Fig. 21 Sensors **27** – **29** and Hg^{2+} -promoted desulfurization cyclization based on benzoylthiourea derivatives.

luminescence band at about 530 nm, corresponding to the typical emission of the naphthol rings. The low luminescent intensity was likely to result from the quenching of emission by Cu^{2+} through a PET mechanism and/or a paramagnetic quenching mechanism. The addition of $\text{Hg}(\text{ClO}_4)_2$ caused a dramatic immediate increase in emission intensity up to the maximum ($\Phi \approx 0.081$), suggesting that the Cu^{2+} ion in the **24-Cu** $^{2+}$ complex was substituted by Hg^{2+} .³²

3-2 Hg^{2+} -induced desulfurization cyclization for Hg^{2+} ion sensors with other fluorophores

Zhao *et al.* reported a thiocarbazon Hg^{2+} sensor **25** (Fig. 20) employing a Hg^{2+} -promoted desulfurization and cyclization strategy.³³ **25** showed a very weak emission with $\Phi < 0.001$ ($\lambda_{\text{em}} = 530$ nm) when it was excited at 380 nm. Upon addition of one equiv of Hg^{2+} ions, approximately 100-fold fluorescence enhancement occurs at 635 nm ($\Phi \sim 0.022$) because of the formation of the emissive triazanaphthalene **26**. This system exhibited a high selectivity for fluorescent detection of Hg^{2+} in an aqueous solution. Neither the new emission band nor the enhancement in the fluorescence intensity associated with the addition of Hg^{2+} was obviously influenced by addition of other competing metal ions (5-fold excess). Reaction of **25** with HgCl_2 yielded a mononuclear Hg^{2+} coordination complex (**26**: $\text{Hg} = 1:1$) with the thiocarbazon group being desulfurized.³³

A new series of benzoylthiourea derivatives **27** – **29** (Fig. 21) were prepared to detect Hg^{2+} , employing Hg^{2+} -promoted

Fig. 22 Hg^{2+} -induced cyclization of receptors **30** and **31**. Fig. 23 Sensor **32** and Hg^{2+} -promoted desulfurization cyclization.

desulfurization and cyclization strategy.³⁴ Among various metal cations tested, exclusively Hg^{2+} ion responded to irreversible color changes of receptors, along with distinctive blue shifts in UV-vis spectra. The receptors can be applicable for the monitoring of Hg^{2+} ion in aqueous solution with a pH span from 4 to 9. In the solvent system of DMSO/ H_2O (4:1, v/v), the detection limit within visual color changes was allowable to 1.0 μM level of Hg^{2+} ion in 1.0 μM solution of **27**. In addition, the reaction responsible for these changes reached completion within the time frame (<1 min) of these measurements. **28** and **29** showed similar photochemical changes to **27** upon Hg^{2+} ion introduction, but exhibited different wavelengths from **27**. Compound **28** showed absorption spectral changes from 444 to 384 nm with one isosbestic point at 401 nm. The titration profile and Job's plot analysis indicated 1:1 stoichiometry between **28** and the Hg^{2+} ion. The visual color changed from deep yellow to colorless in the presence of the Hg^{2+} ion. Regarding the Hg^{2+} detection by a naked eye, **27** seemed to be the best among the three receptors.³⁴

Wu *et al.* reported another two sensors **30** and **31** (Fig. 22) of Hg^{2+} -induced cyclization.³⁵ The presence of 1 equiv. Hg^{2+} gave a 73-fold enhancement in fluorescence intensity with respect to **30** metal-free solution. Meanwhile, the emission peak shifted from 383 to 388 nm. The enhancement in fluorescence intensity was probably associated with Hg^{2+} promoted desulfurization reaction, as shown in Fig. 22. The limit of detection was $3.1 \times 10^{-8} \text{ mol l}^{-1}$, indicating a promising approach for simple and rapid tracking of trace mercury ions in biological or environmental systems. Addition of Hg^{2+} into a solution of **31** also resulted in an enhancement of fluorescence intensity. However, upon the initial addition of Hg^{2+} , the enhancement in emission intensity was very weak. A dramatic increase of intensity was observed with increasing amounts of Hg^{2+} because the desulfurization reaction can more easily occur for **30**- Hg^{2+} than for **31**- Hg^{2+} .³⁵

Our group reported a Nile blue-based chemodosimeter **32** (Fig. 23) and its application for detection of the Hg^{2+} ion in 100% aqueous solution.³⁶ Compound **32** revealed two absorption maxima at 630 nm ($\epsilon = 1.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and 592 nm ($\epsilon = 1.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). Upon addition of the Hg^{2+} ion, the maxima were shifted to 583 nm ($\epsilon = 1.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and 546 nm ($\epsilon = 1.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), respectively. In the fluorescence spectra of **32**, an obvious blue shift from 652 nm ($\Phi_F = 0.20$) to 626 nm ($\Phi_F = 0.35$), with an intensity increase, was observed only in the presence of Hg^{2+} ions. **32** was a highly efficient colorimetric and fluorometric sensor for Hg^{2+} ion within a pH

Fig. 24 Proposed binding mode of **33** with Hg^{2+} ion. Fig. 25 Structures of turn-on Hg^{2+} sensors with polyamide receptors **34**–**39**.

range of 2–9 at room temperature. In particular, **32** exhibited a high selectivity toward Hg^{2+} and was not disrupted by the presence of other metal ions, with a response to the Hg^{2+} ions that was immediate and highly sensitive (less than 1.0 ppb). Furthermore, the selectivity and sensitivity of **32** toward the Hg^{2+} ion were also confirmed with blood plasma and albumin samples.³⁶

3.3 Hg^{2+} ion sensors with other fluorophores using other strategies

By incorporating the well-known fluorophore quinoline group and a water-soluble D-glucosamine group within one molecule, a selective and sensitive fluorescent sensor **33** (Fig. 24) for detection of Hg^{2+} in natural water, with the low concentration limit being 0.5 mM, was achieved by Ou *et al.* in 2006. Compound **33** responds to Hg^{2+} through a combined signaling of both PET and PCT mechanisms.³⁷

Wang *et al.* reported a series of turn-on Hg^{2+} sensors with polyamide receptors (Fig. 25).^{38–40} These sensors featured high water solubility, unique Hg^{2+} ion selectivity, and significant signal response upon Hg^{2+} ion complexation. The *o*-phenylenediamine-based tri- and tetraamide sensors, **35** and

Fig. 26 Ratiometric chemosensors **40** and **41** based on BODIPY-dithiazacrown derivatives.

Fig. 27 Suggested complexation mechanism of **42** for Hg^{2+} ion.

36, chelated Hg^{2+} ion with a 1:1 stoichiometry, whereas the tetraamide receptor **34** bound Hg^{2+} ion in a 1:2 fashion.^{38,39} Compounds **37** – **39** with BODIPY fluorophore were synthesized, since BODIPY has outstanding photophysical properties such as high absorption coefficient, high fluorescence quantum yield, and high photostability. Before Hg^{2+} ions addition, **37** – **39** all displayed very weak fluorescence, resulting from the efficient photoinduced electron transfer (PET) quenching process from the electron-donating receptor moiety to the excited BODIPY fluorophore. However, when 2 equiv. of Hg^{2+} ions were added, the quantum yield of sensor **39** increased immediately and dramatically, indicating that the PET quenching pathway was efficiently blocked by Hg^{2+} ion complexation. In a sharp contrast, **37** did not form a complex with Hg^{2+} ions, while a Job's plot suggested that **38** formed a 1:1 complex with Hg^{2+} ions and **39** chelated Hg^{2+} ions with a 1:2 stoichiometry. Sensor **39** provided 50-fold fluorescence enhancement immediately following Hg^{2+} addition and the quantum yield increases from 0.012 to 0.61. The Hg^{2+} -induced fluorescence response was observable in the presence of two ppb Hg^{2+} . Because of its brightness, Hg^{2+} selectivity and sensitivity, this probe has high potential for use in application-based contexts.⁴⁰

Coskun *et al.* reported two ratiometric chemosensors (Fig. 26) based on BODIPY derivatives **40** and **41**.⁴¹ The specific dithiazacrown selected was reportedly selective for Hg²⁺ ions in THF solvent. The emission ratio changes 35-fold on binding to Hg²⁺. This is a remarkable value for a ratiometric fluorescent chemosensor. Upon addition of Hg²⁺ ions to **41**, a spectral shift in the absorbance of the longer wavelength dye increased the spectral overlap between the energy donor and the acceptor, and an enhanced emission peak was observed. More efficient energy transfer was found together with the changes at the 540 nm.⁴¹

Our group reported a Hg^{2+} sensor **42** (Fig. 27) based on a fluorometric BODIPY appended thiacycrown.⁴² Free **42** showed a

Fig. 28 Structure of chemodosimeter **43**.

Fig. 29 Structures of turn-on Hg^{2+} sensors **44** and **45** with amine-thioether polyamide receptors.

sharp and strong absorption band at 498.0 nm in H₂O/CH₃CN (6:4, v/v). When Hg²⁺ was bound to **42**, the absorption band of **42** at 498 nm was slightly red-shifted to 499.5 nm ($\Delta\lambda = 1.5$ nm). However, other ions (Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺, Ag⁺, Cd²⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Zn²⁺, Pb²⁺, Co²⁺, Cu²⁺ and Al³⁺) did not cause any detectable changes with **42**. Compound **42** showed a pronounced selectivity and sensitivity for Hg²⁺ when it was irradiated at 499 nm. The fluorescence enhancement phenomena of **42** upon Hg²⁺ binding were ascribable to the CHEF (chelation-induced enhanced fluorescence) mechanism. When the Hg²⁺ ion interacted with sulfur atoms of the thiacyclopentadiene part, the PET (photo-induced electron transfer) from sulfur atoms to fluorescence BODIPY group was inhibited.⁴²

Lu *et al.* reported a fluorescent turn-on chemosensor **43** (Fig. 28) for Hg^{2+} based on BODIPY derivative containing a tridentate diaza-oxa ligand. This chemosensor showed a very high sensitivity (detection limit ≤ 2 ppb), a rapid response time (≤ 5 s), and high selectivity for Hg^{2+} over other metal cations in aqueous media.⁴³ Upon addition of Hg^{2+} , the fluorescence intensity of **43** increased remarkably and a fluorescence enhancement factor at 509 nm of approximately 27-fold was estimated. Such significantly enhanced fluorescence of **43** upon addition of Hg^{2+} could probably be attributed to the formation of a 2:1 complex of **43**- Hg^{2+} in which the PET quenching process was efficiently suppressed by coordination with the Hg^{2+} ion. Moreover, confocal fluorescence microscopy experiments had established the utility of **43** in monitoring Hg^{2+} within living cells, indicating its potential application for studying the effect of Hg^{2+} in biological systems.⁴³

Nolan *et al.* reported two fluorescent turn-on chemosensors **44** and **45** (Fig. 29) for Hg^{2+} based on an amine-thioether or a pyridyl-amine-thioether receptors.^{44,45} **44** exhibited an emission maximum at 524 nm and a quantum yield of 0.04, resulting from photoinduced electron transfer (PET) quenching of the fluorescent emission by the lone pair of the aniline nitrogen atom. Upon disruption of this quenching pathway by Hg^{2+} coordination, the emission maximum red-shifts slightly to

Fig. 30 Structures of Hg^{2+} sensors **46** and **47** based on metal complexes.

528 nm and the quantum yield increases *ca.* 2.75-fold to 0.11. The absorption spectrum exhibited a blue shift from 505 to 501 nm upon Hg^{2+} binding, resulting in a *ca.* 3.3-fold increase in brightness. A *ca.* 5-fold increase in integrated emission was observed upon addition of Hg^{2+} . Chemosensor **45** that gives a ~ 4 -fold ratiometric ($\lambda_{624}/\lambda_{524}$) response upon Hg^{2+} coordination in aqueous chloride-containing media at pH 8, was selective for Hg^{2+} over a range of environmentally relevant alkali and alkaline earth metals, a number of divalent first-row transition metals, and Zn(II) and Cd(II) .^{44,45}

Zhao *et al.* reported a highly selective phosphorescent chemosensor for Hg^{2+} based on an iridium(III) complex **46** (Fig. 30).⁴⁶ Upon addition of Hg^{2+} , the absorbance of **46** at 480 nm decreased gradually, whereas a new band at 420 nm appeared, corresponding to a $\lambda_{\text{max}}(\text{abs})$ blue-shift of 60 nm with two isobestic points at 440 and 385 nm, which induced a color change from orange to yellow-green. Metal-binding titrations indicate that **46** forms a 1:1 complex with Hg^{2+} . In CH_3CN solution, **46** showed an intense emission band at 610 nm with a shoulder band at 660 nm; the photoluminescent color was deep red. Upon addition of Hg^{2+} , the intensity of these two bands decreased and two new bands at 585 and 630 nm appeared gradually, corresponding to a blue-shift of about 30 nm with three isoemissive points at 647, 632, and 598 nm. The emission color was changed from deep red to orange, which could be observed by the naked eye. By the changes in the fluorometric response of **46** in the presence of varying concentrations of Hg^{2+} , the stoichiometry of the complex system was also determined to be 1:1, indicating the formation of a 1:1 complex, which was in agreement with the result obtained from the UV-vis absorption technique.⁴⁶

In the same year, Li *et al.* reported another selective chemosensor **47** (Fig. 30) for Hg^{2+} based on a ruthenium(II) complex. Among a series of surveyed metal ions, the complex showed a unique naked-eye selectivity to Hg^{2+} , owing to the existence of an interaction between the mercury(II) ion and NCS group, indicating that it can be used as a selective colorimetric sensor for Hg^{2+} .⁴⁷

4 Conclusions

Well-designed Hg sensors with a wide range of fluorophores have been extensively explored and shown to be highly efficient and extremely sensitive. The studies described in the current review demonstrate that the distinct fluorescent and color changes make these sensors convenient and suitable for monitoring Hg^{2+} ion through fluorescence or naked-eye detection. It was thus found that the fluorescent Hg^{2+} sensors are great candidates for analytical reagents. Since the applications of Hg^{2+} sensors are limited at the moment, further efforts are still required to improve the detection sensitivities

and selectivities. We really hope that the applications of well-performed fluorescent Hg^{2+} sensors will be extended to the wider areas in the future.

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6 References

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