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Specific ratiometric fluorescent sensing of Hg^{2+} *via* the formation of mercury(II) barbiturate coordination polymers†

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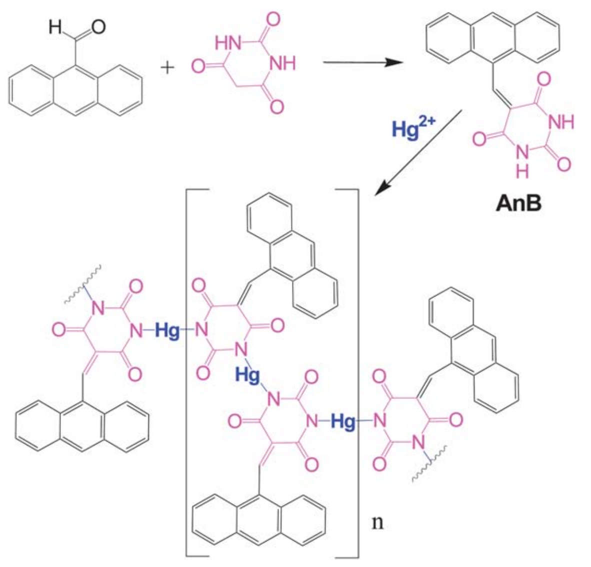
A structurally simple and easily synthesized anthracene derivative bearing a barbituric acid moiety (AnB) was developed as a novel fluorescent chemosensor for Hg^{2+} in terms of specific ratiometric fluorescence response to Hg^{2+} in aqueous solution *via* the formation of Hg^{2+}–AnB coordination polymers.

Mercury is known as a very toxic metal element widespread in the atmosphere, lithosphere and surface water.[1] Because of the deleterious effects of Hg^{2+} on living organisms,[2] convenient and sensitive detection of Hg^{2+} is an important topic attracting continuing environmental and biological concerns. Many fluorescent chemosensors for Hg^{2+} have been developed.[3] However, achieving highly specific and readily available fluorescent chemosensors for Hg^{2+} is still a challenging objective for chemists. Thymine (T) has proven to be one of the most selective ligands for Hg^{2+} to form the unique linear "T–Hg(II)–T" binding motif.[4,5] Highly selective Hg^{2+} sensing systems based on this binding mechanism involve elaborate functionalization of the thymine-containing receptors (*e.g.*, DNA oligomers) with different fluorophores (*e.g.*, energy donor–acceptor pairs) or nanoparticles.[4] It is not hard to imagine that laborious synthesis of the chemosensors is generally needed. Recently, alternative entries were opened by creating high-quantum-yield fluorophores that bears two or more T-moieties or separated imide groups.[5,6] In these sensing systems, coordination polymers with fluorophores in the backbones are formed upon addition of Hg^{2+}, leading to fluorescence quenching because of π–π aggregation of the fluorophores. Herein, we report the first ratiometric fluorescent Hg^{2+} chemosensor containing two imide sites in the same barbituric acid moiety and a fluorophore conjugated into the *meta*-position of the imide sites (Scheme 1). In the presence of Hg^{2+}, the fluorescent receptor is ready to form coordination polymers with fluorophores hanging on the backbone and likely positioned parallel with each other, thus allowing ratiometric fluorescent response instead of the quenching response.

Many fluorescent chemosensors respond to Hg^{2+} by only changes in the emission intensities. Because fluorescence intensity is easy to be influenced by various instrumental or environmental factors, sensing systems based on ratiometric signal measurements, which offer a built-in correction for the instrumental and environmental effects, are generally preferable in practical applications. However, a ratiometric fluorescent chemosensor is usually complex in structure. Our chemosensor, **AnB**, is structurally simple and can be synthesized in one step by reaction of barbituric acid with 9-anthraldehyde (Scheme 1). Upon addition of Hg^{2+}, the excimer emission of anthracene was remarkably enhanced (Fig. 1B)



Scheme 1 Synthesis of **AnB** and formation of the Hg^{2+}–**AnB** coordination polymers.

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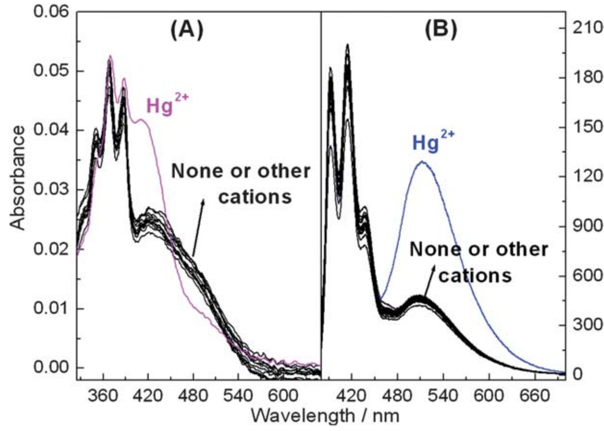

Fig. 1 Absorption (A) and fluorescence (B) spectra of **AnB** (1.00×10^{-5} M) in the presence of 10 equiv. of Hg^{2+}, K^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Al^{3+}, Cd^{2+}, Ag^+, Mn^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+} and Cr^{3+}, respectively. pH: 9.0, buffered by 0.02 M NH_4Cl–NH_3. Excitation wavelength: 367 nm.

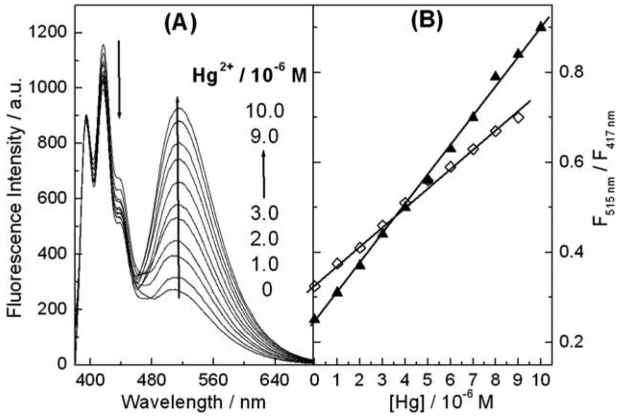

Fig. 3 (A) Fluorescence spectra of **AnB** (1.00×10^{-5} M) in the presence of an increasing amount of Hg^{2+} in aqueous solution at pH 9.0. (B) Ratio of emission intensity (515 nm to 417 nm) of **AnB** (1.00×10^{-5} M) as a function of Hg^{2+} concentration in buffer solutions of pH 9.0 (triangles) and 7.0 (squares). pH 9.0: buffered by 0.02 M NH_4Cl–NH_3; pH 7.4: buffered by 0.02 M NaH_2PO_4–Na_2HPO_4. Excitation wavelength: 367 nm.

because of the formation of Hg^{2+}–**AnB** coordination polymers (Fig. 2). This is different from that of the thymine-bearing anthracene derivative (**An-T**) whose fluorescence was quenched upon interacting with Hg^{2+}.[4r] Ratiometric detection of Hg^{2+} by **AnB** can be carried out by measuring the excimer to monomer fluorescence intensity ratio (Fig. 3).

Aggregation of barbituric acid or its derivatives *via* hydrogen-bonding networks has been well documented in the literature.[7] It was hence assumed that the self-aggregation of **AnB** occurred in a similar pattern under the test conditions. Indeed, the absorption spectrum of **AnB** shows a broad absorption band at long wavelengths between 420 and 540 nm, in addition to the characteristic absorption bands (349 nm, 367 nm and 386 nm) of the anthracene monomer (Fig. 1A), indicating that **AnB** aggregates to some extent in aqueous solution. The appearance of a broad band emission at 515 nm (Fig. 1B), the emission of the anthracene excimer,[8] next to the monomer emission (395 nm, 417 nm and 441 nm), supports the aggregation of **AnB** in solution. This aggregation was found to be pH-dependent (Fig. S4, ESI†), we therefore test the Hg^{2+} sensing performance of **AnB** first in pH 9.0 buffer solution since in which the aggregation of **AnB** is substantially weakened.

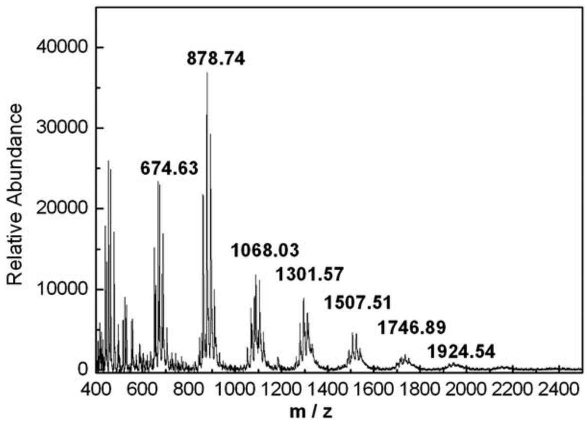

Fig. 2 MALDI-TOF mass spectrum of the Hg(II)–**AnB** adduct.

Fig. 1 shows that both the absorption and emission responses of **AnB** are distinct for Hg^{2+} from the other metal cations tested, demonstrating the high selectivity of the rationally designed **AnB**, which bears two imide sites in a planar six-membered ring. The long-wavelength absorption of **AnB** was found to be narrowed and shifted to blue when bound with Hg^{2+}, suggesting a change in the aggregation of **AnB** (Fig. 1A). This is immediately supported by the Hg^{2+}-induced substantial enhancement in the anthracene-dominated excimer emission (Fig. 1B), instead of the reported fluorescence quenching in the thymine-based fluorescent chemosensors. These spectral changes can be explained by the formation of Hg^{2+}–**AnB** coordination polymers *via* a "T–Hg(II)–T" like binding motif (Scheme 1). Due to the special steric effect of **AnB**, the anthracene fluorophores in the formed coordination polymers are likely to be orderly separated and thus are not able to stack efficiently. As a result, only the excimer emission is enhanced whereas the exciton coupling led quenching is not observed.

The formation of Hg^{2+}–**AnB** coordination polymers was confirmed by detailed spectral studies. Continuous titrations of **AnB** by Hg^{2+} using absorption spectroscopy revealed a 1 : 1 binding stoichiometry of the coordination reaction (Fig. S5, ESI†). The direct evidence for the Hg^{2+} binding mechanism comes from mass spectrometric analysis. As shown in Fig. 2, the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum of the Hg^{2+}–**AnB** adduct shows the presence of a series of polynuclear complexes. The characteristic signals ($m/z > 600$) are spaced regularly and can be assigned to characteristic fragments of the Hg^{2+}–**AnB** 1 : 1 coordination polymers (Fig. S6, ESI†). The dynamic light scattering of the reaction product was also examined. Because of polymerization and the resulting solubility decrease, aggregates with diameters of hundreds of nanometers were formed in the Hg^{2+}-titrated solutions (Fig. S7, ESI†).

AnB was applied to fluorescent detection of Hg^{2+} in aqueous solution. Fig. 3A shows the fluorescence evolution of **AnB** upon titration with $HgCl_2$ at pH 9.0. A remarkable increase in the excimer emission at the slight expense of the monomer emission is observed with increasing Hg^{2+} concentration. A stable fluorescence response can be achieved in 15 minutes (Fig. S8, ESI†). There is a good linearity between the fluorescence intensity ratio of excimer (515 nm) to monomer (417 nm) and the Hg^{2+} concentration at the micromolar level, allowing Hg^{2+} to be monitored ratiometrically. Meanwhile, the detection limit was calculated to be 31 nM. It is worthy indicating that ratiometric fluorescent sensing of Hg^{2+} with considerable sensitivities can be carried out by **AnB** in aqueous solution over a wide pH range between 6.0 and 10.0, although the optimal performance is obtained at pH 9.0 (Fig. S9, ESI†). For example, ratiometric fluorescent sensing of Hg^{2+} at the micromolar level can be well established in aqueous solution at physiological pH (Fig. 3B). Compared with that at pH 9.0, a slightly lower sensitivity was attributed to an increased tendency of self-aggregation of **AnB** *via* hydrogen-bonding networks at lower pH.

The Hg^{2+} sensing performance of **AnB** was further evaluated in the presence of other metal species (Fig. 4). The coexistence of alkali, alkaline-earth or transition metal ions at a high concentration (20 equiv.) caused minor or no interference. The puny shift of the ratiometric response was attributed to the slight quenching caused by excess coexisting cations, which may form different ion pairs with the deprotonated **AnB** monomers (Fig. 1B). It was also observed that fluorescence responses resulting from the addition of these coexisting species followed by the addition of Hg^{2+} were similar to those obtained when Hg^{2+} was added first. These results indicate that the binding affinity of Hg^{2+} is far higher than that of the investigated interfering species, accounting for the observed high selectivity of the designed chemosensor. Obviously, the uniquely high Hg(II) affinity of imide is maintained in **AnB**, although the two imide groups are integrated into a small ring.

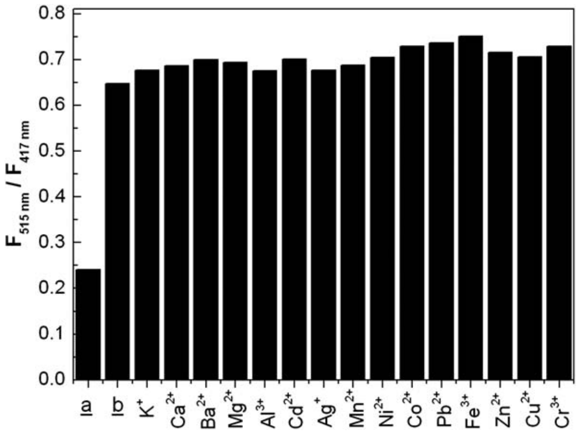

Fig. 4 Influence of coexisting metal cations (1.20×10^{-4} M) on the Hg^{2+} sensing response (*a*: 1.00×10^{-5} M **AnB**; *b*: *a* + 6.00×10^{-6} M Hg^{2+}) in aqueous solution. pH: 9.0, buffered by 0.02 M NH_4Cl–NH_3. Excitation wavelength: 367 nm. High concentration of Na^+ can be tolerated as shown in Fig. 3B for the data obtained in 0.02 M NaH_2PO_4–Na_2HPO_4 buffer of pH 7.0.

Standard addition assays confirmed that Hg^{2+} in synthetic samples could be determined with satisfactory recoveries (96–105%) at the micromolar level.

In summary, a specific ratiometric fluorescent chemosensor for Hg^{2+} was developed using a barbituric acid structural framework in which two Hg^{2+} binding imide sites exist *meta* to each other in a six-membered ring. The rationally designed chemosensor, **AnB**, is structurally simple and easily synthesized. Addition of Hg^{2+} into the solution of **AnB** results in a sensitive ratiometric fluorescence response, which can be measured to determine a trace amount of Hg^{2+}. Our study proves that the barbituric acid group is ready to form ordered 1 : 1 coordination polymer frameworks with Hg^{2+}. This unique reactivity of barbituric acid may lead to its applications in the construction of cost-effective Hg^{2+} chemosensors. The important role of barbituric acid in supramolecular chemistry may afford opportunity for functional Hg(II)-mediated supramolecular assemblies as well.

Acknowledgements

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