Ultraselective fluorescent sensing of Hg^{2+} through metal coordination-induced molecular aggregation†

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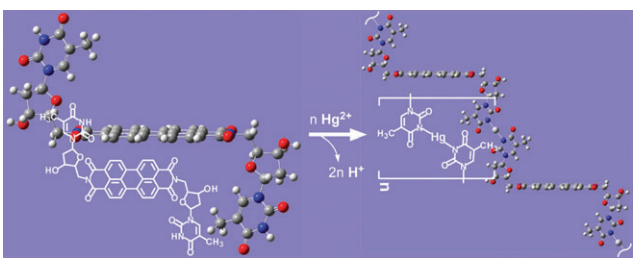
Received (in Austin, TX, USA) 16th December 2007, Accepted 11th January 2008
First published as an Advance Article on the web 29th January 2008
DOI: 10.1039/b719384j

A new type of fluorescent sensor has been developed from a perylene based molecule, *N*,*N*′-dideoxythymidine-3,4,9,10-perylene-tetracarboxylic diimide (TT-PTCDI); the strong, highly selective binding between the thymine ligand (T) and Hg^{2+} ion enables efficient sensing of mercury ions based on a fluorescence quenching mechanism, which is primarily caused by metal-coordination induced molecular aggregation.

As a highly toxic metal ion, Hg^{2+} causes serious health and environmental problems.[1] Many kinds of chemical and physical sensors have been developed for the detection of Hg^{2+}, among which the fluorescence based sensors represent a simple, but sensitive technique providing detection limits of as low as ppb.[2–13] However, improvement of the detection selectivity in the context of interference from coexisting metal ions remains challenging. Indeed, the concentrations of the common coexisting metal ions are usually much higher than the concentration of Hg^{2+}, for which the safety level set for drinking water by the EPA is only 2 ppb (or 10 nM). To detect such a trace amount of Hg^{2+} with minimal false positives requires a sensor technique with extremely high selectivity.

The selectivity of a fluorescent[2–13] or colorimetric[14–19] sensor relies on the design of binding ligands with predominant recognition of Hg^{2+}. Among the binding ligands exploited so far, thymine (T) has proven to be one of the most selective ligands binding to Hg^{2+} in the form of T–Hg–T.[20–23] Recently, the selective complexation between thymine and Hg^{2+} has been employed successfully to develop selective sensors for the detection of Hg^{2+} ions based on fluorescence resonance energy transfer[8] and a colorimetric method.[14] However, both the sensing systems involve the tedious synthesis of the DNA oligomers and chemical functionalization with different fluorophores (energy donor and acceptor) and nanoparticles, thereby presenting a technical hurdle to expedient, cost-effective applications. Moreover, the multiple binding sites within the DNA strands may complicate the chemical process and cause a mismatch in complexation with Hg^{2+}. A DNA strand containing more than four thymine moieties may function as a multidentate ligand that enables effective binding with transition metal ions, such as Zn^{2+}, Cu^{2+}, Ni^{2+}, *etc*. When these metal ions exist in large excess (as they usually do) compared to the concentration of Hg^{2+}, the binding with Hg^{2+} will become less competitive, leading to a decreased selectivity for the sensing.

Herein we report on a new type of fluorescence sensor, as shown in Scheme 1, which involves a simple, easy-to-make fluorophore molecule and provides compelling selectivity in the detection of Hg^{2+}. The mechanism of the sensing relies on fluorescence quenching induced by molecular aggregation, which in turn is caused by the polymerization (*i.e*., intermolecular coordination) of the sensor molecules. The sensor molecule, *N*,*N*′-dideoxythymidine-3,4,9,10-perylene-tetracarboxylic diimide (TT-PTCDI), represents a robust class of fluorophore with extremely high fluorescence yield (close to 100% in organic solvents) as usually observed for other PTCDI molecules.[24] The high photostability of PTCDI molecules provides the sensor with additional credits in terms of sustainability and reproducibility in real applications, where repeated measurements are usually required to avoid false positives. One unique property of PTCDI molecules is that the fluorescence of the individual molecules diminishes dramatically when the molecules are associated into an aggregate state. The cause of such efficient fluorescence quenching is the strong intermolecular π–π interaction between the PTCDI backbones. Particularly, TT-PTCDI possesses a 'Z' structure that favors linear intermolecular coordination with Hg^{2+}, which enables chain polymerization of the molecules, eventually leading to aggregation of the whole molecular system. The molecular aggregation induced fluorescence quenching reported herein is in sharp contrast to the more common fluorescent sensing systems, for which the photoinduced electron transfer (between the fluorophore and the targeted species) usually dominates the quenching mechanism. Indeed, for the sensor molecule shown in Scheme 1 it is unlikely that such



Scheme 1 The molecular structure and optimized configuration of the sensor molecule, TT-PTCDI. The linear coordination of T–Hg–T leads to polymerization and eventually aggregation of the PTCDI molecules. Energy minimization was achieved by DFT calculation (B3LYP/6-31g*) using Gaussian 03.

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† Electronic supplementary information (ESI) available: Experimental and spectroscopy data. See DOI: 10.1039/b719384j

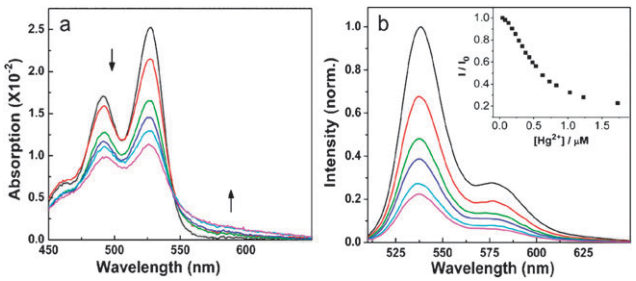

Fig. 1 Absorption (a) and fluorescence (b) spectra of 1.0 μM TT-PTCDI solution in DMF–H_2O (70 : 30, vol.) in the presence of various concentrations of Hg^{2+}, 0, 0.38, 0.63, 0.83, 1.23 and 1.73 μM. Inset of (b): relative intensity (λ_{max} 538 nm) *vs.* the concentration of Hg^{2+}.

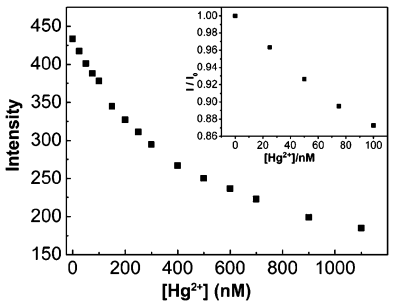

Fig. 2 Fluorescence quenching of a 0.1 μM solution of TT-PTCDI in DMF–H_2O (70 : 30, vol.) by various concentrations of Hg^{2+}: fluorescence intensity (at λ_{max} = 538 nm) as a function of the concentration of Hg^{2+}. Inset: relative intensity (I/I_0) *vs.* the concentration of Hg^{2+} in the low concentration region up to 100 nM, showing the detection limit of Hg^{2+} around 5 nM, corresponding to a 1% decrease in the fluorescence intensity.

an electron transfer will occur, mainly because of the large spatial distance between the bound mercury and the PTCDI backbone, where the linker is primarily composed of σ-bonds.

Fig. 1(a) shows the absorption spectral change of a 1.0 μM solution of TT-PTCDI dissolved in 70 : 30 (vol.) DMF–H_2O upon addition of Hg^{2+} ions. With an increase in the concentration of Hg^{2+} the absorption bands due to the individual molecules decrease, while a new band emerges and increases at the longer wavelength, which is characteristic of the aggregation state of PTCDI molecules.[25–28] An isosbestic point was clearly observed around 545 nm, indicating the stoichiometric conversion of the free molecules into the aggregation state. Indeed, with addition of about two-fold excess of Hg^{2+} ions all the TT-PTCDI molecules were eventually precipitated out and turned out to be dark red flocs (see Fig. S1†), leaving the rest of the solution totally colorless. Moreover, these flocs can be redissolved back to the solution simply by addition of acid, which breaks up the T–Hg–T coordination by reprotonation of the thymine moiety, *i.e.*, shifting the reaction equilibrium shown in Scheme 1 to the left. The 1 : 1 complexation between Hg^{2+} and TT-PTCDI was confirmed by the Job's plot (Fig. S2†), which was obtained by measuring the difference in absorption at 527 nm with the change in molar fraction of TT-PTCDI.

The effective molecular aggregation resulted in a dramatic decrease in fluorescence intensity as depicted in Fig. 1(b), mainly owing to the strong π–π interaction between the tightly packed PTCDI molecules within the aggregate.[25,26,29] The fluorescence titration shown in Fig. 1(b) was also conducted in DMF solutions with varying volume fractions of water to investigate the effect of solvent property on the fluorescence quenching efficiency. Since the binding affinity between Hg^{2+} and TT-PTCDI is determined by the solubility of both species (Hg^{2+} is more soluble in water, whereas TT-PTCDI is predominantly soluble in DMF), there must be an optimal volume fraction of water in DMF that provides the maximal fluorescence quenching. Indeed, upon examination for various volume fractions ranging from 0% to 50% the optimal fluorescence quenching was observed for the solvent containing 30% water, the binary solvent used in Fig. 1. Such a water-containing sensor system should be highly desirable for the future practical application of the sensor in aqueous environments, where Hg^{2+} ions usually exist.

With the 70 : 30 (vol.) DMF–H_2O solvent we attempted to explore the detection limit by decreasing the concentration of TT-PTCDI. Within a certain concentration range (where effective binding between Hg^{2+} and the thymine ligand still exists as determined by the binding affinity), it is generally true that the lower the concentration of the fluorophore, the less quencher is required for the same percentage of fluorescence quenching. For a 0.1 μM solution of TT-PTCDI as low as 5 nM (or below) of Hg^{2+} can be feasibly detected considering the fact that a well-calibrated photodetector can reliably measure an intensity change down to 1% or lower (Fig. 2). Such a low detection limit is indeed competitive with most of the fluorescence or colorimetric sensors previously reported. The high sensitivity thus obtained is consistent with the strong

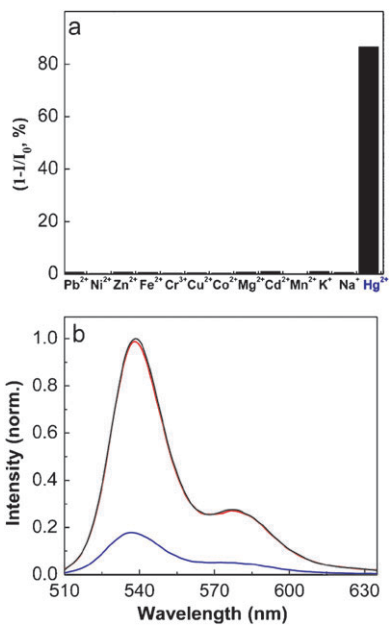

Fig. 3 (a) Fluorescence response of TT-PTCDI (1.0 μM) to Hg^{2+} (3.5 μM) and various other metal ions (12.5 μM) in DMF–H_2O (70 : 30, vol.) solutions. The bars represent the percentage of fluorescence quenched ($1 - I/I_0$). (b) Fluorescence spectra of a 1.0 μM TT-PTCDI solution in the absence (black) and presence (red) of a mixture of all 12 metal ions (each 12.5 μM). Addition of 3.5 μM Hg^{2+} to the mixed solution resulted in a dramatic fluorescence quenching (blue).

complexation of T–Hg–T, for which Hg^{2+} binds to the thymine by replacing the proton at the secondary amine (Scheme 1),[20–23] in a manner similar to metallic coordination within a porphyrin.

Since the linear coordination of T–Hg–T is extremely selective for Hg^{2+}, the presence of other metal ions should not produce fluorescence quenching similar to that observed for Hg^{2+}. Indeed, as tested for the environmentally relevant metal ions including Cu^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, Cr^{3+}, Mg^{2+}, Ca^{2+}, K^{+} and Na^{+}, none of these ions demonstrated a positive response to the same sensing system depicted in Fig. 1. As shown in Fig. 3(a), even at relatively higher concentration (*e.g.*, 12.5 μM) the fluorescence decrease observed for all these background ions was around only 1% or below, whereas *ca.* 87% fluorescence quenching was observed in the presence of only 3.5 μM Hg^{2+}. Such a high selectivity was further tested in an extreme case as presented in Fig. 3(b), where a mixture of all the metal ions mentioned above (each at 12.5 μM) was added to the sensing system, and resulted in almost no change in the fluorescence intensity. However, addition of 3.5 μM Hg^{2+} to the mixed solution led to *ca.* 83% quenching of the fluorescence. Consistent with these fluorescence observations, no significant change was observed for the absorption spectra of the TT-PTCDI solution upon addition of the background ions (Fig. S3†). Indeed, no precipitation was observed for the TT-PTCDI solution in the presence of any of the interfering ions. The ultrahigh selectivity thus obtained for the sensor may help avoid the false positives in real applications, where detection of Hg^{2+} is often interfered with by other transition metal ions (particularly when they are present in much higher concentrations).

In conclusion, we have developed a new type of molecular sensor for the detection of Hg^{2+}. The sensing mechanism is primarily based on fluorescence quenching induced by molecular association and aggregation, which in turn is caused by linear complexation with Hg^{2+}. The ultrahigh selectivity and sensitivity thus obtained may enable future development of a sensor assay, which can find broad application in both environmental monitoring and clinical diagnostics.

This work was supported by NSF (CAREER CHE 0641353, CBET 730667), ACS-PRF (45732-G10) and NSFC (20520120221).

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