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Biosensors

A Metal–Organic Framework/DNA Hybrid System as a Novel Fluorescent Biosensor for Mercury(II) Ion Detection

Lan-Lan Wu,[a, b] Zhuo Wang,[a, b] Shu-Na Zhao,[a, b] Xing Meng,[a, b] Xue-Zhi Song,[c] Jing Feng,[a] Shu-Yan Song,*[a] and Hong-Jie Zhang*[a]

Abstract: Mercury(II) ions have emerged as a widespread environmental hazard in recent decades. Despite different kinds of detection methods reported to sense Hg^{2+}, it still remains a challenging task to develop new sensing molecules to replenish the fluorescence-based apparatus for Hg^{2+} detection. This communication demonstrates a novel fluorescent sensor using UiO-66-NH_2 and a T-rich FAM-labeled ssDNA as a hybrid system to detect Hg^{2+} sensitively and selectively. To the best of our knowledge, it has rarely been reported that a MOF is utilized as the biosensing platform for Hg^{2+} assay.

Mercury, one of the most highly toxic heavy metals, has attracted much attention in recent years, due to its severe harmfulness[1,2] to both human health and the environment.[3,4] Even at a low concentration, mercury compounds can cause adverse effects on the endocrine system, nervous system, and kidneys in human bodies.[5] The Hg^{2+} ion, the most stable inorganic form of mercury, is known to be more dangerous owing to its good solubility in water.[6,7] Therefore, multiple attempts have been made by researchers to find effective methods for the detection of Hg^{2+}. Generally, the traditional Hg^{2+} detection methods include atomic absorption/emission spectrometry, gas chromatography, ultrasensitive stripping voltammetry, inductively coupled plasma mass spectrometry, and so on.[7–9] However, all these methods have various limitations because they not only require sophisticated instrumentations but are also time-consuming. As a consequence, it is still a great challenge to develop simple, fast, and low-cost methods to detect Hg^{2+} sensitively and selectively.

Over the past few years, metal–organic frameworks (MOFs), which are constructed by the self-assembly process of metal cations or metal clusters and flexible organic ligands in one pot, play an important role in the field of multifunctional materials for their diversified topological structures, tunable pore sizes, high surface areas, and robust thermal stability.[10] Given these fascinating features, they have emerged as potential applications in gas storage/separation,[11] catalysis,[12] proton conducting,[13] and sensing.[14] The organic linkers usually contain a conjugated π-electron system, thus permitting MOFs to bind small molecules.[10d] Most notably, the π-electron-rich ligands endow the MOF materials with the feature to be able to adsorb single-stranded DNA (ssDNA) through π–π stacking interactions, thus quenching the labeled fluorophores, which are considered to be a rising application for MOFs.[15,16] Meanwhile, the flexibility of the organic ligands is a promising peculiarity to enhance the adsorbing ability of MOFs, since the formed flexible frameworks could adjust their configurations to adapt to the nucleic acid molecules. To date, there are some reports about MOFs utilized as biosensors to detect specific DNA effectively.[16] For instance, a copper-based MOF, reported by Zhu and co-workers, was successfully used as a sensing platform to detect HIV DNA and thrombin.[16a] Inspired by this, we believe that there must be further applications of MOFs in biological analysis still waiting to be exploited.

Recently, the formation of a duplex structure between nucleic acids and different metals has proven to be a versatile routine for metal-ion detection.[17] It is known that mercury(II) ions can specifically bind to thymine–thymine (T–T) mismatched base pairs in oligonucleotides to form thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) base pairs, which are usually called T–Hg^{2+}–T sandwich complexes.[18] Many studies have been conducted to test and verify this T–Hg^{2+}–T interaction, suggesting that Hg^{2+} ions have high affinity for T–T base pairs instead of other metal ions.[18c] So far, a number of approaches have been proposed to set up Hg^{2+} sensing strategies based on this unique Hg^{2+}-mediated coordination chemistry, since it is well established.[19] Specifically, the employed materials traditionally include gold nanoparticles,[2,20] silver nanoclusters,[19a,21] carbon nanomaterials,[22] silica nanoparticles,[23] semiconductor quantum dots,[24] graphene oxide (GO),[25] proteins,[26] and DNAzymes.[23a] However, the preparations of the materials are often elaborate, difficult, or costly, whereas MOF production is cost-effective, time-saving, and can be carried out on a large scale,

[a] L.-L. Wu, Z. Wang, S.-N. Zhao, X. Meng, Prof. J. Feng, Prof. S.-Y. Song, Prof. H.-J. Zhang
State Key Laboratory of Rare Earth Resource Utilization
Changchun Institute of Applied Chemistry
Chinese Academy of Sciences
5625 Renmin Street, Changchun, 130022 (P. R. China)
E-mail: songsy@ciac.ac.cn
hongjie@ciac.ac.cn

[b] L.-L. Wu, Z. Wang, S.-N. Zhao, X. Meng
University of Chinese Academy of Sciences
Beijing, 100049 (P. R. China)

[c] X.-Z. Song
School of Petroleum and Chemical Engineering
Dalian University of Technology
2 Dagong Road, Liaodongwan New District, Panjin 124221, Liaoning (P. R. China)

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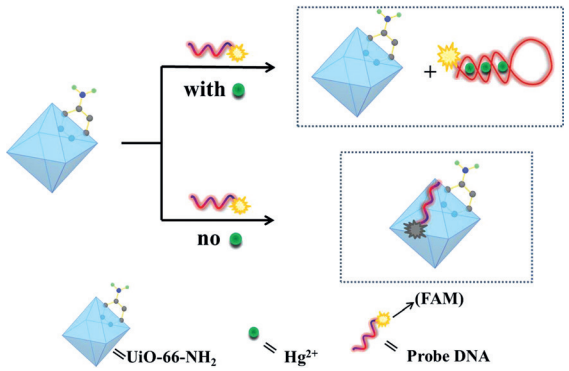
which has an outstanding superiority in practical applications. Up to now, there are few examples applying MOFs to fulfill Hg^{2+} detection accompanying the T–Hg^{2+}–T interactions. Herein, the known UiO-66-NH_2 was used for the preparation of a MOF/DNA hybrid system as an efficient fluorescent sensing platform to detect Hg^{2+} sensitively and selectively. The concept of this assay is shown in Scheme 1. We preferred a T-rich



Scheme 1. The schematic concept of this fluorescent sensing system.

single-stranded DNA labeled with a fluorophore (FAM) at its 3′ end as the probe (P) according to the previous report.[22a] Free P has strong fluorescence at 518 nm under excitation at 480 nm. In the absence of Hg^{2+} ions, P would bind to UiO-66-NH_2 on account of π–π stacking and hydrogen-bonding interactions between the DNA nucleotide bases and the aromatic organic linker anchored with an amino functional group in UiO-66-NH_2, bringing the dye moiety close to the UiO-66-NH_2 surface. As a result, the fluorescence from FAM is quenched due to the photoinduced energy transfer (PET).[16a] In the presence of targets, Hg^{2+} ions, hybrids induced by hairpin-like T–Hg^{2+}–T structures come into being. The structural alteration can isolate P from UiO-66-NH_2 and recover the dye fluorescence. Hence, the detection of Hg^{2+} is realized by monitoring the fluorescence intensity.

To verify the feasibility of this strategy, we carried out a series of experiments. Figure 1 demonstrates the fluorescence emission spectra of P under different conditions in Tris-HCl buffer (pH 7.4). In the case without UiO-66-NH_2, the free P has strong fluorescence emission upon exciting at 480 nm (curve a), whereas the addition of UiO-66-NH_2 gives rise to a striking decline of the fluorescence emission (curve d). It was found that the fluorescence quenching efficiency (Q_E) is nearly 75%, indicating that UiO-66-NH_2 can interact with P and quench the fluorescence emitting from FAM effectively.

Upon the addition of Hg^{2+} ions, the fluorescence emission intensity of the P/UiO-66-NH_2 composite exhibits a remarkable enhancement (Figure 1, curve c). In particular, the fluorescence intensity in the presence of 10.0 μM Hg^{2+} is more than twofold higher than that without Hg^{2+}. The fluorescence recovery could be attributed to the formation of a helical structure induced by the Hg^{2+} ion from ssDNA that protects the ssDNA from binding to UiO-66-NH_2 and obstructs the energy transfer

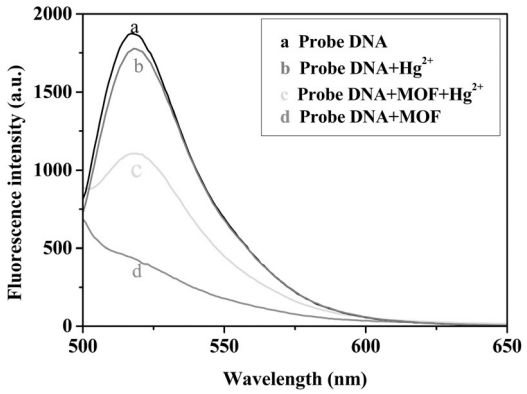


Figure 1. Fluorescence emission spectra of P under different conditions (λ_{ex} = 480 nm): a) P; b) P + Hg^{2+}; c) P + UiO-66-NH_2 + Hg^{2+}; and d) P + UiO-66-NH_2. All measurements were done in Tris-HCl buffer (pH 7.4); concentrations were P = 100 nM, UiO-66-NH_2 = 0.15 μg μL^{-1}, and Hg^{2+} = 10.0 μM.

between the dye species and UiO-66-NH_2. It is worth mentioning that the fluorescence of P is almost not influenced by Hg^{2+} without UiO-66-NH_2 (curve b). Thus, the fluorescence intensity changes are the foundation of fluorescent detection of Hg^{2+} in this communication.

Additionally, the UV/Vis diffuse reflectance spectrum (DRS) of UiO-66-NH_2 was measured. It indicated that UiO-66-NH_2 had no absorption at the fluorescence emission wavelength of FAM (see the Supporting Information, Figure S3). That is to say, there was no spectral overlap between the emission band of P and the absorption of UiO-66-NH_2, so fluorescence resonance energy transfer (FRET) could not occur between the two. The reasonable quenching mechanism should be PET, which is different from that found in carbon nanostructures.[16]

The amount of UiO-66-NH_2 adjusted with P was also investigated. The fluorescence intensity decreased rapidly along with the UiO-66-NH_2 concentration ranging from 0–0.15 μg μL^{-1}, but the continued increase in the amount of UiO-66-NH_2 did not make a big difference, because the adsorption and desorption reached equilibrium (see the Supporting Information, Figure S6). Therefore, the experimental concentration of UiO-66-NH_2 in this study was 0.15 μg μL^{-1}, unless otherwise stated.

We have evaluated the sensitivity of our novel sensing system. Various concentrations of Hg^{2+} ranging 0–14.0 μM were added in a series of suspensions containing P (100 nM) and UiO-66-NH_2 (0.15 μg μL^{-1}). Figure 2A shows the fluorescence change of P/UiO-66-NH_2 in the presence of Hg^{2+} with different concentrations in Tris-HCl buffer (pH 7.4) at $\lambda_{ex}/\lambda_{em}$ = 480/518 nm. The inset shows the corresponding fluorescence intensity plotted against Hg^{2+} concentration ranging from 0–14.0 μM. It is evident that the fluorescence intensity was enhanced by increasing the concentration of Hg^{2+}, initially by a fast rate and more slowly thereafter. The linear relationship between the fluorescence intensity and Hg^{2+} concentration in the range of 0.1–10.0 μM (R^2 = 0.972) is shown in Figure 2B. It can be clearly seen that the fluorescence intensity has different enhancements corresponding to the Hg^{2+} concentration range, even at a low concentration. The detection limit 17.6 nM, which was calculated from the signal that was equiva-

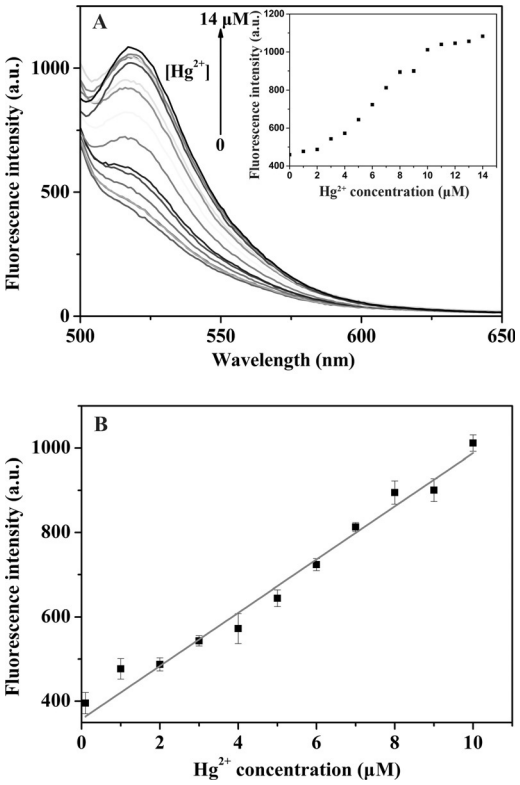


Figure 2. A) Fluorescence spectra of P/UiO-66-NH_2 in the presence of different concentrations of Hg^{2+} in Tris-HCl buffer (pH 7.4) (from bottom to top: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, and 14.0 μM); excitation was at 480 nm and the emission intensity was recorded at 518 nm; inset: the corresponding fluorescence intensity plotted against Hg^{2+} concentration. B) The linear relationship between the fluorescence intensity at 518 nm and the Hg^{2+} concentration range (0.1–10.0 μM).

lent to three times the standard deviation of the blanks, is lower than the toxicity level of Hg^{2+} in drinking water (30 nM) defined by the World Health Organization (WHO). All the above descriptions demonstrate the outstanding sensitivity of this detection system.

Next, the selectivity of our system for metal ions was also estimated. Suspensions containing metal ions (Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, and Pb^{2+}) were tested under the same conditions as in the case of Hg^{2+}. The fluorescence intensity gained a significant enhancement in the presence of Hg^{2+}, whereas the other metal ions only showed a slight response (Figure 3), indicating that the sensor possesses an excellent selective signal towards Hg^{2+} with respect to other metal ions.

In summary, we have developed a novel fluorescent sensor for the Hg^{2+} detection in aqueous solutions using UiO-66-NH_2 and a T-rich FAM-labeled ssDNA. The fluorescence emission of ssDNA was quenched effectively by UiO-66-NH_2, while it could be recovered with Hg^{2+} presence due to T–Hg^{2+}–T interactions. Through monitoring the change of fluorescence intensity, we achieved the sensitive and selective detection of Hg^{2+}. Our present work is significant for the MOF-based biosensors utilizing T–Hg^{2+}–T specific interactions for Hg^{2+} assay, which are unusual and somewhat rare. The research on this MOF-based type of hybrid system is still in the preliminary stage by comparison. Hence, it is state of the art research to apply a MOF in a biosensor system to detect Hg^{2+}, potentially opening a new area in MOF applications at the same time. We hope that this will engage other researchers in further studies.

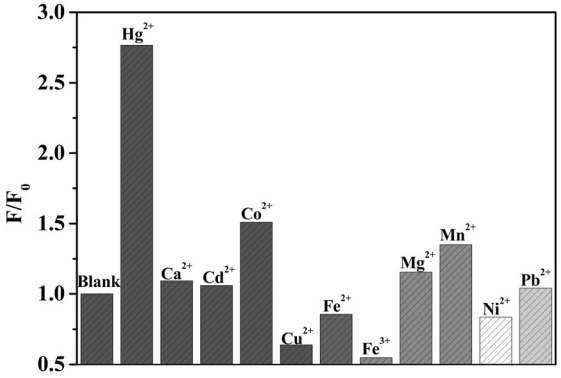


Figure 3. Selectivity of the sensing system against other metals. F and F_0 represents the fluorescence intensity of P/UiO-66-NH_2 with and without metal ions (10.0 μM), respectively; $\lambda_{ex}/\lambda_{em}$ = 480/518 nm.

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