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Cationic-perylene-G-quadruplex Complex based Fluorescent Biosensor for Label-free Detection of Pb^{2+}

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Abstract

In this work we use a water-soluble cationic perylene derivative (compound 1) as the G-quadruplex (G4) structure fluorescence indicator to construct a fluorescent biosensor for simple, rapid and label-free detection of Pb^{2+}. In the absence of Pb^{2+}, strong electrostatic interactions between compound 1 and the G-rich DNA probe (pw17) induced the aggregation of compound 1 and resulted in the fluorescence quenching. In the presence of Pb^{2+}, the PW17 formed Pb^{2+}-stabilized G4 structure, which reduced the aggregation of compound 1 and gave rise to high fluorescence. This allowed us to use convenient "mix-and-detect" protocol for quantitative analysis of Pb^{2+}. Since Pb^{2+} can specially induce PW17 to form compact DNA fold, our proposed biosensor displayed high selectivity for Pb^{2+}. It also exhibited a high sensitivity to Pb^{2+}, with a limit of detection of 5.0 nM observed. Furthermore, the

biosensor was applied for the detection of Pb^{2+} in urine and paint samples, and both showed satisfactory results.

Keywords: Perylene derivative; G-quadruplex; Label-free; Fluorescence; Pb^{2+} detection; Biosensor

1. Introduction

It is well-known that Ionic lead is a common environmental and industrial pollutant. It is quite harmful to human body, especially on children, and a very small amount of Pb^{2+} could cause renal malfunction and inhibit brain development [1,2]. Therefore, the development of sensitive and selective methods for the detection of Pb^{2+} is a current research focus in the fields of modern environmental science and life science. A variety of conventional methods have been developed for the detection of Pb^{2+}, including atomic absorption spectrometry [3], inductively coupled plasma-mass spectrometry [4], and inductively coupled plasma-atomic emission spectrometry [5]. However, these techniques are rather expensive, complex, time consuming and not suitable for on-site detection.

In recent years, some functional DNA molecules such as 8-17 DNAzyme and G4 oligonucleotide have been applied for lead ion detection [6-10]. 8-17 DNAzyme is a RNA-cleaving DNAzyme, which displays cleavage activity at the scissile ribo-adenosine position of the substrate strand with Pb^{2+} as the cofactor. Researchers have made use of this unique enzyme-like feature of 8-17 DNAzyme to develop fluorescent, [11,12] colorimetric, [13,14] electrochemical sensors [15,16], which exhibit high sensitivity and selectivity for Pb^{2+} analysis. However, the RNA molecules used in these sensors are unstable and vulnerable in vivo or complex media. G4s are a kind of four-stranded DNA structure, in which G-rich nucleic acid sequences form stacked arrays of G-quartets connected by Hoogsteen-type base pairing, and is stabilized in the presence of coordination cations [17-19]. Recently, some DNA sensors based on Pb^{2+}-induced allosteric G4 structure have been developed for Pb^{2+} detection [20-23]. For example, Liu and co-workers present a new assay for the sensitive detection of Pb^{2+} using a thrombin-binding aptamer (TBA probe) labeled with a fluorophore and a quencher at its 5′ and 3′ termini, respectively

[20]. Li and co-workers developed a sensitive and selective electrochemical sensing platform for lead detection based on a Pb^{2+}-induced G-rich DNA conformational switch with crystal violet as the G4-binding indicator [21]. Nevertheless, in these assays, the DNA probes required an additional DNA tagging process or immobilization technique that are technically demanding, time consuming, and expensive. Therefore, it still remains a challenge to develop a simple, label-free and sensitive method for Pb^{2+} detection.

Water-soluble cationic perylene diimide derivative (compound 1, Fig. 1) is attractive because it exhibits high fluorescence quantum yield, good photostability, and excellent chemical inertness [24-27]. Due to the repulsive interactions among the positive charges of the compound 1, it shows strong monomer fluorescence in an aqueous buffer solution. Nevertheless, nucleic acid containing multiple negatively charged phosphate functional groups could induce strong aggregation of compound 1 and result in the fluorescence quenching. By taking advantage of the aggregation-induced emission change of compound 1, some simple, rapid and label-free fluorescent sensing systems were constructed [28-30]. However, no report is focused on the interaction between G4 structure and the water-soluble cationic perylene derivative (compound 1).

In this work, we reported a simple, rapid and label-free approach for fluorescent "turn-on" detection of Pb^{2+} by using a water-soluble cationic perylene derivative (compound 1) as the fluorescence reporter and the G-rich DNA probe (PW17) for the specific binding of Pb^{2+}. PW17 has been found to strongly bind hemin to form DNAzymes with peroxidase-like activity under salt conditions. Moreover, Pb^{2+} could cause K^{+}-stabilized PW17 to undergo a parallel-to-antiparallel conformation transition as a result of its unusually high efficiency at stabilizing G-quadruplexes [19,31,32]. Therefore, the biosensor showed a high selectivity to Pb^{2+}. In addition, our proposed biosensor also exhibited a high sensitivity towards Pb^{2+}, with a limit of detection of 5.0 nM observed. Moreover, it was applied for the detection of Pb^{2+} in urine and paint samples, and both showed satisfactory results.

2. Experimental

2.1Reagents and Apparatus

The G-rich DNA probe (PW17: 5'-GGGTAGGGCGGGTTGGG-3') used in this work was synthesized and purified by Takara Biotechnology Co., LTD. (Dalian, China). 3,4:9,10-Perylenetetracarboxylic dianhydride and N,N'-Dimethyl-1,3-propanediamine were purchased from Alfa Aesar. Methyl iodide was acquired from Shanghai Chemical Reagents (Shanghai). Compound 1 was synthesized as previously reported literature [27]. Lead acetate and all other reagents were of analytical reagent grade, purchased from Sigma-Aldrich Chemical Co., and used without further purification. Milli-Q water (Millipore, 18.2 MΩ cm at 25 °C) was used in all runs.

All fluorescence measurements were carried out on a Hitachi F-4500 Fluorescence Spectrometer (Tokyo, Japan). The instrument settings were chosen as follows: λex = 494 nm (slit 10 nm), PMT detector voltage = 950 V.

2.2 Procedure for fluorescence detection

The solution of PW17 oligonucleotide in Tris-acetate buffer (10 mM, pH 8.0) was heated at 90℃ for 10 min and gradually cooled to room temperature. Then, varying concentrations Pb^{2+} (in the range of 0-10 μM) were added and the mixture was incubated for 40 min. Upon the addition of the compound 1 for 10 min, the fluorescence of the mixture was measured.

3. Results and discussion

3.1. Biosensor design and analytical principle

The design strategy of our proposed sensor is shown in Fig. 1. Compound 1 showed a strong fluorescence emission at 540 nm in aqueous solution, and the addition of Pb^{2+} did not interfere with its fluorescence intensity (see Fig. 2). In the absence of Pb^{2+}, the strong affinity of compound 1 with PW17 induced the aggregation of compound 1, and resulted in the fluorescence quenching. Upon the addition of Pb^{2+}, the conformation of PW17 changed from a random-coil to a Pb^{2+}-stabilized G4 structure. This conformational change weakened the interaction between PW17 and compound 1. As a result, compound 1 monomer molecules were released and fluorescence enhancement was observed, which provided a facile means

for Pb^{2+} quantification.

In addition, the conformational switch of PW17 induced by Pb^{2+} was further analyzed with CD spectra (see Fig. 3). The concentration of PW17 and Pb^{2+} were fixed at 10 μM and 100 μM, respectively. In the absence of Pb^{2+}, CD spectra of PW17 was of relatively low amplitude due to PW17 possessing a random structure. Upon the addition of Pb^{2+} ion, the spectra exhibited a long wavelength maximum near 314 nm,, indicating the formation and stabilization of the G4 structure by Pb^{2+} ion, which is consistent with previously reported result [33].

3.2. Optimization of assay conditions

In order to achieve the best sensing performance, we investigated the effect of the concentration of compound 1 on the response of the biosensor to Pb^{2+}, in the presence of 200 nM of PW17 and 5 μM of Pb^{2+}. As shown in Fig. 4, F/F_0 increased significantly with the increase of the concentration of compound 1. However, when the concentration was higher than 2.0 μM, F/F_0 decreased with a further increasing concentration of compound 1, which might be caused by the existence of free compound 1 to result in high background fluorescence. Therefore, a concentration of 2.0 μM was chosen for compound 1 for further experiments.

3.3. The sensitivity of the sensing system

Fig. 5(A) illustrates the fluorescence spectra of the sensing system upon the addition of different concentrations of Pb^{2+} under the optimal experiment conditions (200 nM DNA, 2.0 μM compound 1, 10 mM Tris-acetate, pH 8.0). In the absence of Pb^{2+}, the fluorescence intensity of compound 1 is relatively high. However, it was decreased obviously upon the addition of PW17 due to the aggregation of compound 1 (Fig. 5A inset). In the presence of Pb^{2+}, the fluorescence intensity of the sensing system increased notably. It is because that the formation of G4 structure reduced the aggregation ability of compound 1 and resulted in the fluorescence increasing. Fig.5(B) describes the relationship between the fluorescence intensity and the different concentration of Pb^{2+}. According to the linear response toward Pb^{2+}, a practically usable range from 10 nM to 1 μM for quantitative determination was observed. The response can be expressed by the following equation (1) of the

calibration line:

$$\log F = 1.0956 + 0.2553 \log C_{Pb}^{2+} \quad (R = 0.9942) \qquad (1)$$

Here F denotes the fluorescence emission intensity of the sensor in the presence of Pb^{2+} and C_{Pb}^{2+} represents the concentration of Pb^{2+} added. The limit of quantification (LOQ) for Pb^{2+} was 4.25 nM (10δ). The detection limit (LOD) for Pb^{2+} analysis was 5 nM (3δ/slope), which was lower than previously reported DNAzyme fluorescence sensors for Pb^{2+} detection [34]. Table 1 lists some common G4 structure-based sensors for the detection of lead ions. Comparing to the other sensors, our biosensor exhibits comparable sensitivity, and is fairly simple, inexpensive and rapid.

3.4. The specificity of the sensing system

To test the specificity of this sensing system, metal ion selectivity assays were investigated by fixing the concentration of Pb^{2+} and Hg^{2+} at 1 μM and other metal ions at 10 μM under the optimum conditions. As shown in Fig. 6, only Pb^{2+} induced the significant fluorescence enhancement, while all other metal ions resulted in weak signal changes. This is mainly attributed to the excellent efficiency of Pb^{2+} for stabilizing PW17 [34]. Moreover, we further investigated the fluorescent intensity change of the sensing system toward 1 μM of Pb^{2+} in the presence of 1 mM K^+ (see the Fig. 7). Experimental results indicated that 1 mM K^+ did not give obvious fluorescence interference for Pb^{2+} detection. The main reason is that Pb^{2+}-stabilized G4 structure generally have shorter M-O and O-O bonds than those stabilized by K^+. [35,36] Therefore, Pb^{2+} has higher efficiency at stabilizing G4 structure in comparison with K^+, which lead to the releasing of more compound 1 free monomer molecules and larger fluorescence enhancement. All these results indicate that the specificity of this biosensor toward Pb^{2+} is satisfactory.

3.5. The practical application of the sensing system

In order to evaluate the practical application of our sensor for detection of Pb^{2+}, the recovery experiments with spiked Pb^{2+} in urine samples were carried out. The urine samples were obtained from three healthy volunteers and filtered through 0.2 mm membranes. Then, the urine samples were diluted 100-fold using 10 mM Tris-acetate

buffer and showed that no Pb^{2+} was present. Using standard addition method, Pb^{2+} in the urine sample was added into the sensor solution to the final concentration of 1 μM. Table 2 lists the analytical results. One can see that the recovery in the range of 97% -106% was achieved. In addition, we tested the capability of the biosensor to detect and quantify lead in leaded paint (NIST SRM2582). The lead concentrations determined using our biosensor and the atomic absorption spectrometry were 199 ppm (±2.4) and 204 ppm (±3.1), respectively, with relative deviation less than 3%. These results clearly indicate that the sensing system could be used for Pb^{2+} detection in real samples.

Conclusions

In summary, we have developed a G-quadruplex-based biosensor for fluorescent "turn-on" detection of Pb^{2+} by using a water-soluble cationic perylene derivative (compound 1) as the fluorescence reporter. Because Pb^{2+} can form more compact DNA folds than other ions, the selectivity of this system for Pb^{2+} is high. In addition, because it is a label-free and "mix-and-detect" protocol, the assay is fairly simple, inexpensive and rapid. Moreover, the sensing system was used for the detection of Pb^{2+} in urine and paint samples with satisfying results.

Acknowledgement

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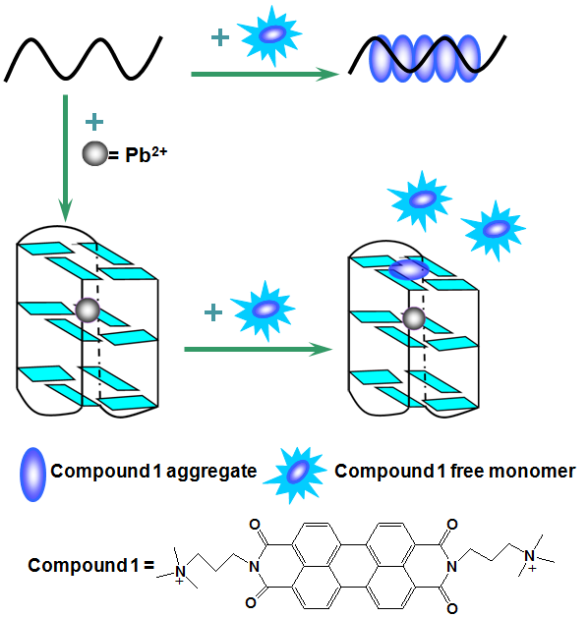


Fig. 1 Schematic illustration of the selective sensing of Pb^{2+}

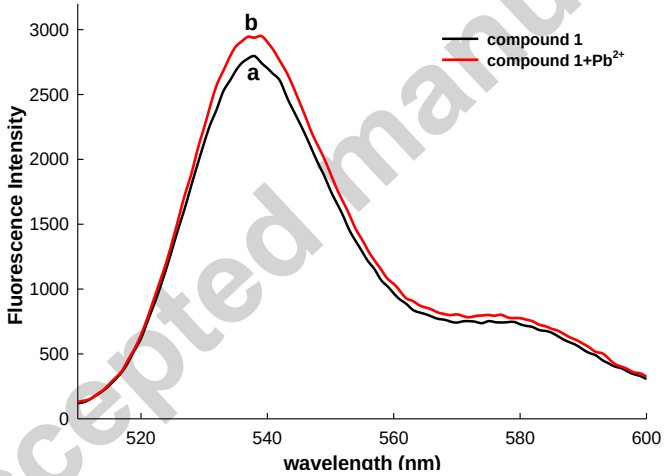


Fig. 2 Fluorescence emission spectra of compound 1 in the presence and absence of Pb^{2+}. The concentrations of compound 1 and Pb^{2+} were 2 μM and 10 μM, respectively.

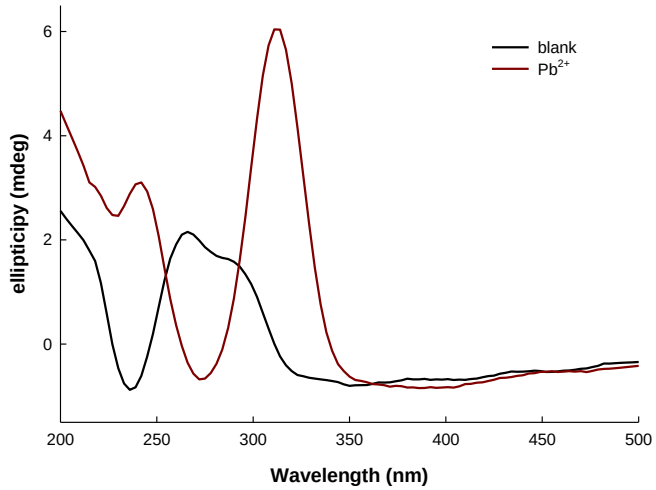


Fig. 3. CD spectra of PW17 in the presence and absence of Pb^{2+}. The concentration of PW17 and Pb^{2+} were fixed at 10 μM and 100 μM, respectively

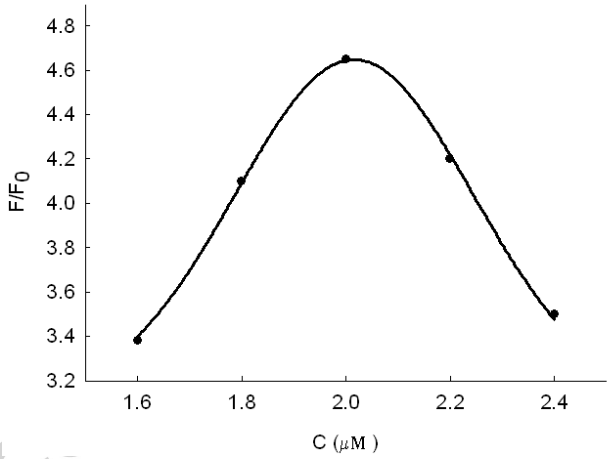


Fig. 4. The effect of compound 1 concentration. The concentration of PW17 and Pb^{2+} were fixed at 200 nM and 5 μM, respectively

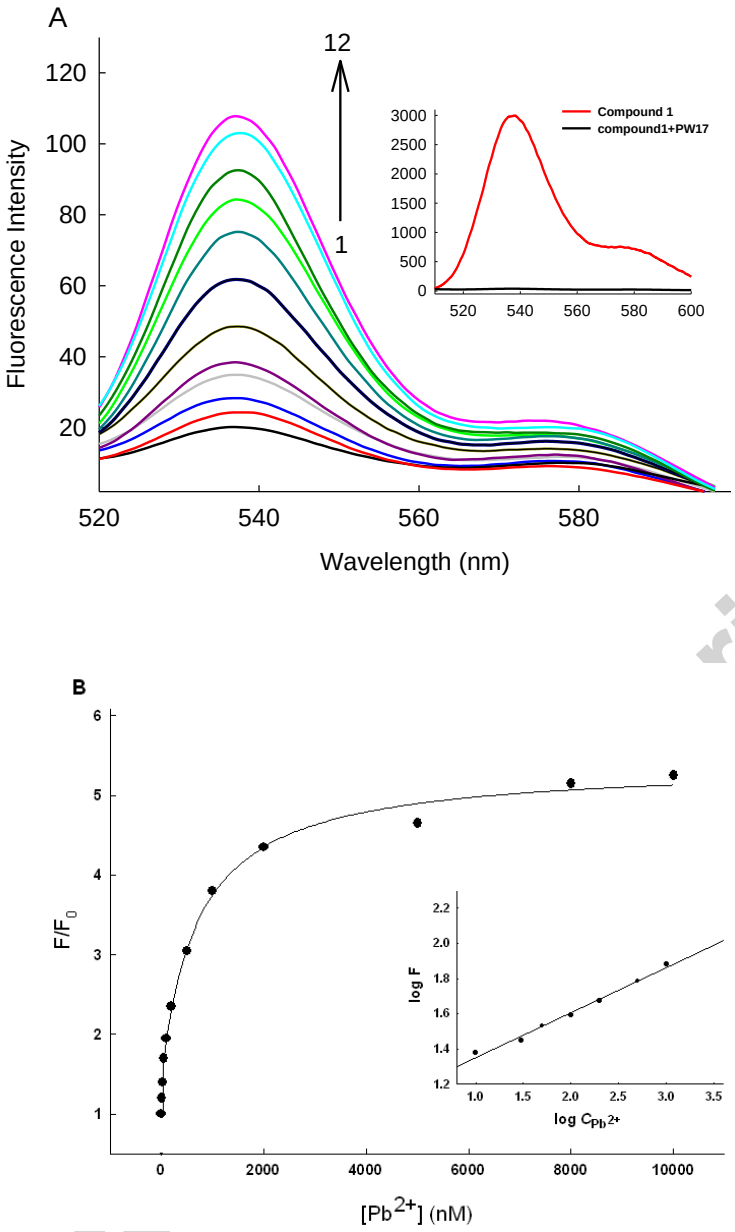


Fig. 5. (A) The fluorescence emission spectra of the sensing system upon the addition of different Pb^{2+} concentrations: (1) 0; (2) 10 nM; (3) 30 nM; (4) 50 nM; (5) 100 nM; (6) 200 nM; (7) 500 nM; (8) 1 μM; (9) 2 μM; (10) 5 μM; (11) 8 μM; (12) 10 μM. (Inset) Fluorescence emission spectra of compound 1 in the presence and absence of PW17. The concentrations of compound 1 and PW17 were 2 μM and 200 nM, respectively. (B) Fluorescence increase over background fluorescence at varying concentrations of Pb^{2+}. (Inset) The probe responses to low concentrations of Pb^{2+}. F and F_0 are the fluorescence intensity in the presence and absence of Pb^{2+}.

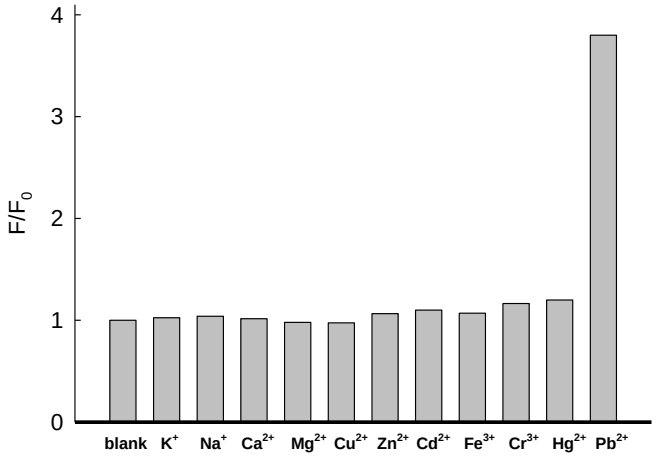


Fig. 6. Selectivity of the Pb^{2+} sensing system against other metal ions. The concentration of Pb^{2+} and Hg^{2+} were fixed at 1 μM, and other tested metal ions were kept at 10 μM.

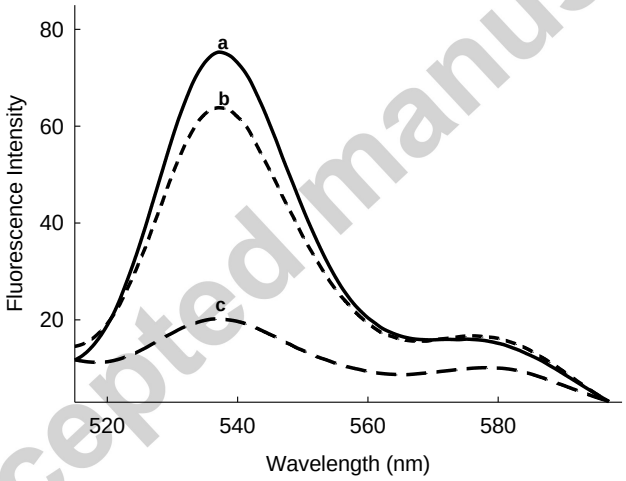


Fig. 7. The fluorescence emission spectra of the sensing system in the presence of Pb^{2+} (curve a), K^+ (curve c), Pb^{2+} and K^+ (curve b). The concentration of Pb^{2+} and K^+ were 1 μM and 1 mM, respectively.

Table 1

G4 structure-based sensors for the detection of lead ions

Technique	Method	Reaction time	LOD; linear	ref
Electrochemistry	G4/CV[a]	2.5h	0.4nM;1 nM-1μM	21
Chemiluminescence	G4/ABTS or	2h	32nM/1nM;10^{-5}-10^{-7}	30
Colorimetric/	luminol		M /$10^{-6.5}$-10^{-9} M	
Fluorescence	TBA [b] probe; labeled/turn-off	15min	0.3nM;0.5nM-30nM	20
Fluorescence	G4/NMM [c]; label-free/turn-off	30min	1nM;5 nM -1μM	22
Fluorescence	G4/ZnPPIX [d] label-free/turn-on	5h	5nM;20 nM -1μM	23
Fluorescence	G4/Perylene derivative; label-free/turn-on	50min	5nM;10 nM -1μM	this work

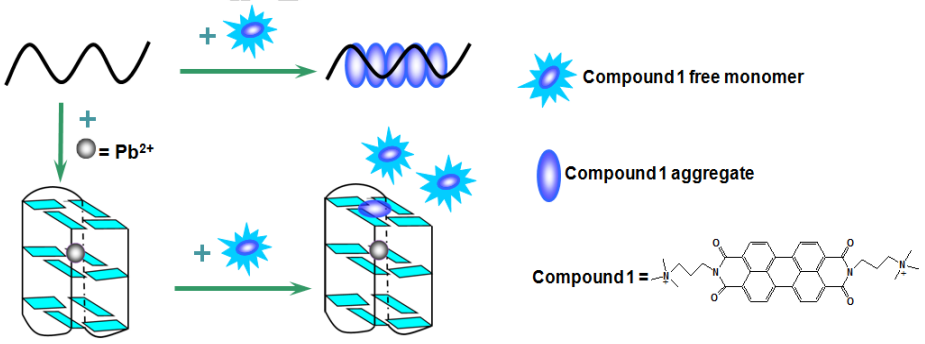
[a]Crystal violet; [b]thrombin-binding aptamer; [c]N-methyl mesoporphyrin IX; [d]zinc protoporphyrin IX

Table 2

Recovery experiments of Pb^{2+} in urine

Sample	Pb^{2+} spiked (μM)	Pb^{2+} recovered (μM)	Recovery (%)
urine 1	1.0	1.03[a]±0.04[b]	103
urine 2	1.0	0.97[a]±0.02[b]	97
urine 3	1.0	1.06[a]±0.05[b]	106

[a] Mean of three determinations. [b] SD: standard deviation.



Schematic illustration of the selective sensing of Pb^{2+}

Highlights

- This assay is a simple, rapid and label-free protocol.

- The sensing system displayed high sensitivity and selectivity
- The sensing system can be used for the detection of Pb^{2+} in urine and paint samples