Sensors

DOI: 10.1002/anie.200803069

Highly Sensitive Detection of Mercury(II) ions by Fluorescence Polarization Enhanced by Gold Nanoparticles**

Bang-Ce Ye and Bin-Cheng Yin*

Contamination of the environment with heavy metal ions has been an important worldwide concern for decades. Mercury, which can accumulate in vital organs and tissues, such as the liver, brain, and heart muscle, is highly toxic and can have lethal effects on living systems. Mercury originates mainly from coal-burning power plants, oceanic and volcanic emissions, gold mining, and waste combustion.[1] Futhermore, microbial biomethylation of Hg^{2+} ions yields methyl mercury, a potent neurotoxin that passes through the food chain to the tissues of fish and marine mammals.[2] Therefore, it is highly desirable to develop a sensitive and selective mercury detection method that can provide simple, practical, and high-throughput routine determination of levels of Hg^{2+} ions for both environmental and food samples. Much effort has been devoted towards the design of sensing systems for Hg^{2+} ions, including sensors based on organic chromophores[3] or fluorophores,[4] conjugated polymers,[5] DNAzymes,[6] gold nanoparticles,[7] semiconductor quantum dots,[8] proteins,[9] and genetically engineered bacteria.[10] However, most of these methods have some limitations such as poor selectivity with interference from closely related metals, insufficient sensitivity (limit of detection (LOD) > 100 nM), and in certain cases are nonstable or nonfunctional in aqueous media (because of low water solubility).

Another emerging approach for the detection of Hg^{2+} ions involves the use of oligonucleotides. Hg^{2+} ions can specifically interact with thymine bases to form strong and stable thymine–Hg^{2+}–thymine complexes (T–Hg^{2+}–T). Various Hg^{2+} ion detection assays based on this property of T–Hg^{2+}–T coordination chemistry have been developed in recent years.[11] Ono and Togashi[11a] have described a simple method based on Hg^{2+}-induced DNA folding, which yields an intramolecular fluorescence resonance energy transfer process and allows the detection of Hg^{2+} ions with high selectivity and sensitivity (up to 40 nM). Mirkin and co-workers[11b] recently reported the colorimetric detection of Hg^{2+} ions in aqueous media using DNA-functionalized gold nanoparticle (AuNP) probes with specifically designed T–T mismatches with a sensitivity of up to 100 nM. Furthermore, Liu and co-workers[11c] developed an one-step, room temperature, colorimetric assay of Hg^{2+} ions using DNA–nanoparticle conjugates with a sensitivity of 1 μM.

According to the US Environmental Protection Agency (EPA) standard, the MAL (maximum allowable level) of Hg^{2+} ions in drinking water is 10 nM (2.0 parts per billion (ppb)). This concentration is much lower than the detection limit of most available assays. Thus, the development of a highly sensitive, facile, and practical assay for Hg^{2+} ions remains a challenge. Herein, we present a novel highly sensitive and selective fluorescence polarization assay (FPA) method for the detection of Hg^{2+} ions on the basis of the formation of T–Hg^{2+}–T complexes. The detection sensitivity can be significantly improved to 0.2 ppb (1.0 nM) by using a "gold nanoparticle enhancement" approach. Gold nanoparticle (AuNP) enhancement functions have been employed to substantially improve the performance and sensitivity of various biosensing systems. The enhanced effects were implemented by the use of AuNPs as labels for amplified quartz crystal microbalance detection[12a,b] and electrochemical detection,[12c] by the application of AuNPs as electron relays for the facilitation of interfacial electron transfer on electrodes.[12d] Recently, AuNP-enhanced effects in a responsive polymer gel[12e] and in network field-effect transistors[12f] were also reported.

The fluorescence polarization value P is sensitive to changes in the rotational motion of fluorescently labeled molecules.[13] It can be calculated by the Perrin equation [Eq. (1)], where τ is the fluorescence lifetime, η is the viscosity

$$\frac{1}{P} = \frac{1}{P_0} + \left(\frac{1}{P_0} - \frac{1}{3}\right)\frac{RT\tau}{V\eta} \qquad (1)$$

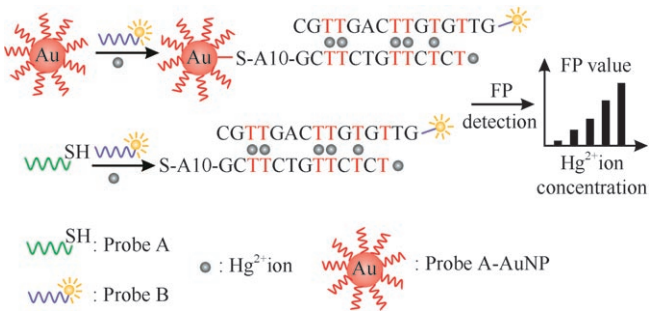
of the solution, T is the temperature in Kelvin, R is the gas constant, V is the volume of the rotating unit, and P_0 is the limiting polarization. The P value of a fluorophore is proportional to its rotational relaxation time, which in turn depends upon its molecular volume (molecular weight). If a molecule is small it will rotate faster and hence will have a smaller P value. Conversely, larger molecules will have larger P values because of their slow rotation.

The fluorescence polarization method is outlined in Scheme 1. We first prepared two complementary probes (Probe A: AuNP-5′S-A_{10}-GCTTCTGTTCTCT3′; and Probe B: 5′FAM-GTTGTGTTCAGTTGC3′) that contain

[*] Prof. B. C. Ye, B. C. Yin
Laboratory of Biosystems and Microanalysis
State Key Laboratory of Bioreactor Engineering
East China University of Science & Technology
Meilong RD 130, Shanghai, 200237 (P.R. China)
Fax: (+86) 2164252094
E-mail: bcye@ecust.edu.cn
Homepage: http://sklbe.ecust.edu.cn/English/group/yebc.html

[**] This work was financially supported by the National Natural Science Foundation (20627005 and 20776039), the Shanghai Municipal Commission for Science and Technology (06DZ19151), the 863 project (2007AA02Z331), the Program for New Century Excellent Talents (NCET-07-0287), and the Shanghai Shuguang Program (06SG32).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200803069.



Scheme 1. Schematic illustration of the strategy of Hg^{2+} ion detection using fluorescence polarization enhancement by gold nanoparticles.

six strategically placed thymine–thymine mismatches complexed with Hg^{2+} ions. Probe A was modified with AuNPs (13 nm). Probe B was labeled with the fluorescence dye FAM (6-fluorescein-CE phosphoramidite). As shown in Scheme 1, the stable hybridization between Probe A and Probe B occurs only when Hg^{2+} ions are present. From the Perrin equation [Eq. (1)], the P value will increase significantly when the fluorophore-labeled Probe B binds to the AuNP surface in the presence of Hg^{2+} ions.

As a proof-of-principle experiment, a mixture of Probe A and Probe B (300 μL, both 2.5 nM) in aqueous $NaNO_3$ (0.1M) and 3-(4-morpholinyl)-1-propanesulfonic acid (MOPS) buffer (10 mM) was placed in the wells of a black 96-well microtiter plate. An aqueous solution of Hg^{2+} ions (1.0 μM) was added to each well, and plate was preincubated at room temperature for ten minutes. The fluorescence polarization value (mP; 1 P = 1000 mP) was calculated using Equation 2, in which I_P

$$\mathrm{m}P = 1000\ \frac{I_P - I_S}{I_P + I_S} \qquad (2)$$

and I_S are the fluorescence intensity parallel to and perpendicular to the excitation plane, respectively. A significant increase of mP indicates the formation of stable Hg^{2+}-mediated complex between Probes A and B (Figure 1). The polarization of the solution was examined both with and without modification of DNA probes with AuNPs. The mP value is approximately six times higher in the system employing the AuNP-functionalized DNA probe (AuNP–DNA) compared with the system without AuNP enhancement (DNA–DNA). The enlargement of the molecular volume V of the AuNP-functionalized T–Hg^{2+}–T complexes results in a substantial increase in the mP value upon the rotational correlation time.

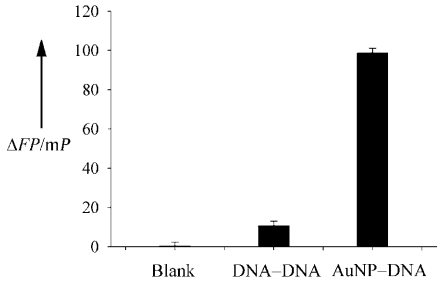


Figure 1. Fluorescence polarization changes upon addition of Hg^{2+} ions at a concentration of 1.0 μM. A blank (probes A and B without Hg^{2+} ions) was used as a control reference.

To evaluate the sensitivity of the AuNP-enhanced FPA method, the assay was tested on different concentrations of Hg^{2+} ions from the same stock solution. The mP value increased as the concentration of Hg^{2+} ions increased (Figure 2). The present limit of detection for this method is

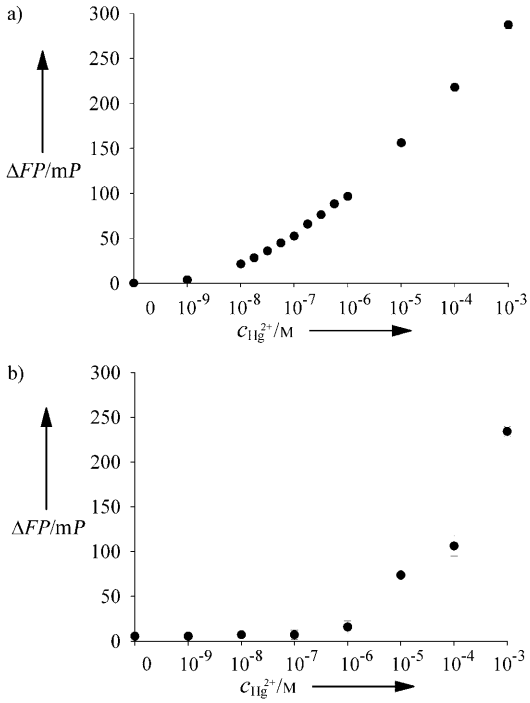


Figure 2. Plots of fluorescence polarization changes as a function of Hg^{2+} ion concentrations for a) AuNP–DNA and b) DNA–DNA).

approximately 1.0 nM (0.2 ppb) Hg^{2+} ions, which, to the best of our knowledge, is the lowest ever reported for Hg^{2+} ion sensing systems without signal and PCR amplification using enzymes. The result also illustrates that the method has a wide detection range of Hg^{2+} ions from 1.0 nM to 1.0 mM.

When the FPA method is used without AuNP enhancement, a sensitivity that corresponds to a Hg^{2+} ion concentration of 1.0 μM is observed (Figure 2). The substantial sensitivity improvement of the AuNP-enhanced method is mainly attributed to the slower rotation of fluorescent unit when dye-labeled oligonucleotides were hybridized to the gold nanoparticle surface. In addition, the surface loading of the oligonucleotides on the AuNPs (approximately (98 ± 3.2) Probe A oligonucleotides per nanoparticle) also contributes to its high sensitivity. The sensitivity of method may be further improved by using larger Au nanoparticles (30 nm).

The selectivity of the method has also been investigated by testing the response of the assay to other metal ions, including Mg^{2+}, Pb^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}, Zn^{2+}, and Fe^{2+} at a concentration of 10.0 μM—1000 times greater than that of the Hg^{2+} ions (Figure 3). The results demonstrate excellent selectivity over alkali, alkaline earth, and heavy

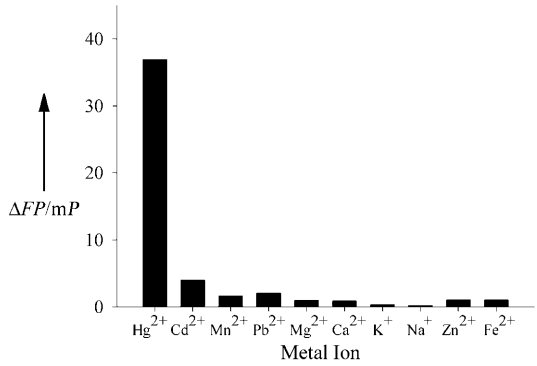


Figure 3. Selectivity of the Hg^{2+} ion assay method over other metal ions. The concentration of Hg^{2+} ions was 10.0 nM, the other metal ions were tested at 10.0 μM.

transition-metal ions. There was very little increase of the observed m*P* value in the presence of other metal ions. The specific detection for Hg^{2+} ions is attributed mainly to its ability to chelate T–T mismatches, which results in the formation of stable T–Hg^{2+}–T complexes.

To investigate if the FPA method developed here was applicable to natural systems, the real river samples and the spiked river samples, at two different concentrations (0.4 μM and 4 μM), were analyzed using the AuNP–DNA system. The suspected interfering materials in the river samples did not influence the Hg^{2+} ion detection with the described method. Moreover the methd reveals the good recovery rates of standard addition from 97.6 to 102.5%.

As AuNPs can act as fluorescence quenchers, we considered the possibility that AuNPs could be quenching the fluorescence of FAM-labeled oligonucleotides, which could result in the nonuniformity of the assay. The effects of AuNPs and a molecular quencher (5-carboxytetramethylrhodamine, TAMRA) on fluorescence intensity and m*P* values in this biosensing system were investigated (see the Supporting Information). The results demonstrated that the fluorescence quenching properties of AuNPs have no effect on the FPA assay of Hg^{2+} ions.

In summary, we have developed a highly sensitive and selective Hg^{2+} ion determination method at room temperature using thymine–Hg^{2+}–thymine coordination chemistry and fluorescence polarization enhanced by gold nanoparticles. This method demonstrates several analytical advantages. Firstly, it has high sensitivity with a detection limit of 0.2 ppb, which can be two to three orders of magnitude more sensitive than many other techniques. Secondly, it is highly selective, which allows detection of Hg^{2+} ions in the presence of an excess (1000-fold) of other metal ions in samples. Thirdly, it takes only approximately ten minutes to determine the concentration of mercury in aqueous media. Finally, the assay can be carried out in 96- or 384-well plates, rendering it suitable for routine high-throughput applications. The method has enormous potential for the application of Hg^{2+} ion monitoring in environment, water, and food samples.

Experimental Section

Preparation of DNA-functionalized AuNPs: 13 nm gold nanoparticles were prepared by the citrate reduction of $HAuCl_4$ according to a reported method.[14] AuNPs were functionalized[15] with Probe A. The surface coverage of oligonucleotides on the AuNPs was determined to be approximately (98 ± 3.2) strands per as-prepared particle by using fluorescence spectroscopy[16] (see the Supporting Information).

FPA detection: Solutions of Probe A-modified AuNPs (1.0 μL, 25 nM particle concentration, corresponding to 2.5 μM of Probe A) and Probe B (1.0 μL, 2.5 μM) were added to a 1.5 mL eppendorf tube containing $NaNO_3$ (997 μL, 0.1M) and MOPS buffer (10 mM, pH 7.5). The mixture was then incubated for 5 min at room temperature. Subsequently, 1 μL of $Hg(NO_3)_2$ solution (at different concentrations) or solutions of other metal ions were added, and the solution was incubated in wells (300 μL per well) for an additional 10 min at room temperature before fluorescence polarization measurements. The control experiments using SH-Probe A and FAM-Probe B were carried out under identical conditions. All experiments were repeated twice. Each sample was measured four times.

Received: June 26, 2008
Revised: September 5, 2008
Published online: ■■ ■■, 2008

Keywords: DNA · fluorescence polarization · mercury · nanoparticles · sensors

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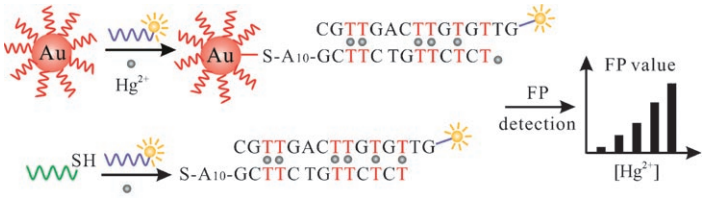

Going for gold! Thymine–Hg^{2+}–thymine coordination is used as the basis for a selective assay that detects Hg^{2+} ions with a limit of up to 0.2 parts per billion. The detection is based on the enhancement of fluorescence polarization (FP) by gold nanoparticles (see picture). The overall assay can be carried out at room temperature within only ten minutes, making it suitable for high-throughput routine applications in environment and food samples.

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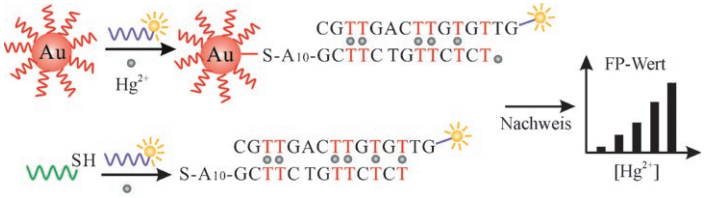

Durch Gold veredelt: Ein selektiver Nachweis für Hg^{2+}-Ionen in Konzentrationen bis hinab zu 0.2 ppb beruht auf der Thymin-Hg^{2+}-Thymin-Koordination sowie auf dem Umstand, dass die Fluoreszenzpolarisation (FP) durch Goldnanopartikel verstärkt wird (siehe Bild). Der Nachweis gelingt bei Raumtemperatur binnen zehn Minuten und eignet sich somit für Hochdurchsatz-Routinemessungen an Umwelt- und Nahrungsmittelproben.

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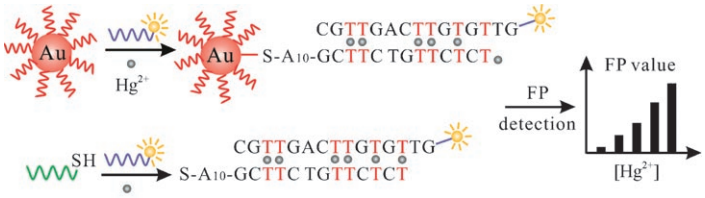

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