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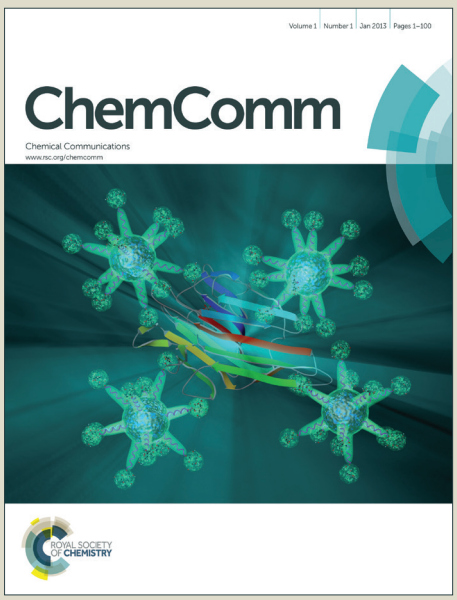





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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

# $K^+$-mediated G-quadruplex formation enhancement fluorescence polarization system based on quantum dot for detection of $Hg^{2+}$ and biothiols

**Juanni zhang, Jianniao Tian,* Yanlong He, Yanchun Zhao* and Shulin Zhao**

***Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX***
**DOI: 10.1039/b000000x**

**Fluorescence polarization homogenous system based on CdTe/CdS QDs that employed $K^+$-mediated G-quadruplex formation as an enhancer was identified for sensitive and selective detection of $Hg^{2+}$ and biothiols in complex samples.**

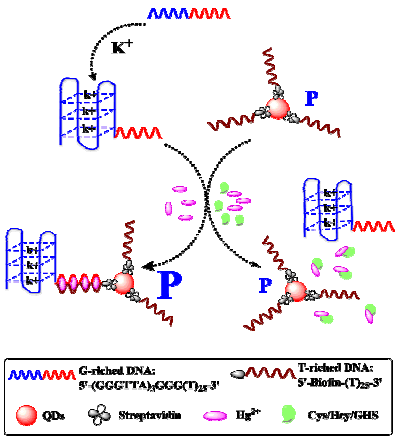
Fluorescence polarization (FP) is a reliable and powerful technique for fluorescence sensing in biochemical homogenous systems.[1] The FP value is sensitive to the changes in the rotational motion of a fluorophore functionalized object, which in turn depends upon a number of parameters including molecular volume and molecular mass at a constant temperature and solution viscosity. Despite the large success of FP immunoassay (FPIA), dye-labelled aptamer based on FP assay has been carried out for analysis of macromolecules and cell.[2] Compared to the macromolecules such as protein, aptamer as a single-stranded oligonucleotide is the relatively small molecule, thus the binding of target can result in significant changes in the rotational motion of fluorophore-labeled objects by changing their molecular mass or volume. However, these FP methods are quite limited to targets, and rarely have been exploited for small molecules because their molecular masses are relatively too small to produce observable signal changes.

Recently, large molecular protein, which can amplify the FP signal by enhanced the molecular volume and molecular mass of fluorophore, were introduced into FP method as mass enhancer for small molecules assay. Eric Peyrin et. al[3] employed single-stranded DNA binding (SSB) protein as a strong FP signal enhancer for a mass amplifying strategy. Moreover, Yang et. al[4] proposed a mass amplifying probe based on a molecular massing amplifying aptamer domain and amplifier protein such as thrombin. These mass amplifying platforms can be universal used for sensitive and selective detection of small molecular, but only several types of protein can be chosen as amplifiers and these candidates are high-cost and delicate for designing FP sensors. Besides, nanometerial, such as gold nanoparticles (AuNPs), silica nanoparticles and graphene oxide(GO), provided an alternative way to develop the mass amplifying FP system for small molecular[5]. Although these FP aptasensors are successful, they require the synthesis of functional nanoparticles which is complex, time-consuming and inconvenient. More recently, based on the unique conformational flexibility and adaptability features of aptamer, novel types of FP aptasensors have been established for detection of metal ion.[6] Although these FP aptasensors are convenience and co-effective, the universal FP method based on the conformation of aptamers is rarely been reported.

G-quadruplexes are unique high order structures formed by G-rich nucleic acid sequences based on stacked arrays of G-quartets connected by Hoogsteen-type base pairing.[7] These structures are stabilized by monovalent cations, especially by $K^+$. The formation of G-quadruplex has been employed for developing bioanalysis platforms[8]. $K^+$-mediate G- quadruplex is convenience, low-cost and simple for design the universal platform for detection of small molecules. Also, because of the large three-dimensional structure, it can improve the sensitivity of FP sensor effectively as FP enhancer

In this study, a novel versatile FP platform based on CdTe/CdS quantum dot (QDs) using $K^+$-mediate G-quadruplex as signal enhancer was devised for detecting $Hg^{2+}$, biothiols amino acids or peptides in complex samples. Here the CdTe/CdS QDs fluorescence polarization probe as an ideal candidate could effectively reduce the FP background due to their unique merits and long lifetime.[9] Scheme 1 shows sensing strategy of the utilization of $K^+$-mediated G-quadruplex formation enhanced fluorescence polarization for detection of $Hg^{2+}$ and biothiol. The G-riched DNA consisting of a 21-bp G-quadruplex forming sequence (Hum21) at the 5′-terminus and 25-mer thymine-rich sequence at the 3′-terminus were designed. Firstly, the 5′-guanine-rich sequence of G-riched DNA folds into G-quadruplex formation in the presence of potassium ions which has proved by circular dichroism spectra (Figure S2). In the presence of $Hg^{2+}$, the 3’ thymine-rich sequence of $K^+$-mediated G-quadruplex formation (GQ-DNA) and QDs-T can form large volume complexes though the strong and specific affinity of T-$Hg^{2+}$-T. As a consequence, $K^+$-mediated G-quadruplex formation lead to dramatic changes in the molecular volume of QDs. From the Perrin equation[5a], the FP value of a fluorophore depends on the molecular volume of the rotating molecule, thus, the FP value of the QDs will significantly increase. On the basis of a competition mechanism previously reported,[10] upon addition of biothiols amino acid, the strong reaction of thiols-$Hg^{2+}$ prohibits the formation of the T-$Hg^{2+}$-T complexes and makes the QDs-T and $K^+$-mediated G-quadruplex formation(GQ-DNA) stay in free single state. Subsequently, the FP values of solution greatly

decrease. Based on the decrease of FP value, the detection of biothiols also can be realized in the presented method.




Scheme 1 Schematic illustration of QDs fluorescence polarization enhancement homogenous system based on $K^+$-mediated G-quadruplex formation for the determination of $Hg^{2+}$ and biothiols.

To confirm the feasibility of the present strategy, the FP values of QDs probe under different condition were observed in Figure 1. The FP value of free QDs (column a) was observed as 20mP, after functionalized with T-riched DNA by the affinity of biotin-streptavidin, the FP value(column b) increased as 33mP because of the increased molecule mass on the surfaces of QDs, the result indicated that QDs was successfully functioned by T-riched DNA(see detail in SI). Also, the FP value of background solution (column c) which contains the pre-prepared enhancer G-quadruplex (GQ-DNA) and QDs-T probe was as low as the FP value of QDs-T alone. As expected, in the presence of target ion $Hg^{2+}$, the large volume enhancer G-quadruplex formation (GQ-DNA) integrated with the QDs-T probe by T-$Hg^{2+}$-T complexes, the FP value of the QDs dramatically increased. However, in the presence of biothiols amino acids, a competitive reaction appeared between S-$Hg^{2+}$and T-$Hg^{2+}$-T, thus the form of T-$Hg^{2+}$-T complex was restrained and more G-quadruplex formation (GQ-DNA) were free in solution. Consequently, the small FP value of QDs (column e) was observed in solution. The above results confirmed that the changes of FP in the system were indeed caused by the enhancer G- quadruplex formation (GQ-DNA) due to the different affinity of T-$Hg^{2+}$-T and S-$Hg^{2+}$. Therefore, the proposed method has a good response capability in the identification of the target $Hg^{2+}$ and biothiols.

On the basis of the optimized assay conditions (Figure S3), various concentrations of $Hg^{2+}$ were introduced to evaluate the sensitivity of the fluorescent polarization sensor. As shown in Figure 2, the FP value of QDs in solution increased with the increasing of the target concentration. The FP value was found to be linear with the concentration of $Hg^{2+}$ in the range from 10 to 800nM (insert of Figure 2) and the detection limit was found to be 8.6nM based on three times signal-to- noise level of the blank sample (3σ), which was below the maximum level permitted by the US Environmental Protection Agency (2ppb). The sensitivity of this method is better than that of reported previously using a FAM-labelled fluorescent probe (40nM) [11a] and shows the same performance as the sensitive electrochemical method or colorimetric method (10nM).[11b, c] To determine the specificity of

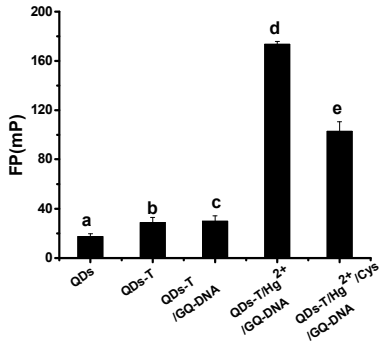



Figure 1 Fluorescence polarization values of QDs probe upon different conditions. The concentration of $Hg^{2+}$and Cys were 800nM and1000nM, respectively. A blank (QDs-T/ GQ-DNA) was used as a control reference for detection of $Hg^{2+}$, another blank (QDs-T/GQ-DNA/$Hg^{2+}$) was used as a control reference for detection of biothiols.

this system, the FP value in presence of various environmentally relevant metal ions (2.5-fold higher concentration) was tested under the same condition as in the case of 400nM $Hg^{2+}$. As shown in Figure 2(B), the FP changes of sensor were clearly observed only upon addition of $Hg^{2+}$, whereas no obvious FP signal changes were induced by other relevant metal ions, the results shows the excellent selectivity of this method. The lake water and spiked lake samples, at two different concentrations (50nM, 400nM), were employed for further examining the practicality of the $Hg^{2+}$ assay. The results were listed in the Table S1. The method reveals the good recovery from 96.5 to 99.5% and great accuracy and reliability (RSD ≤9.8% even at low concentration of $Hg^{2+}$).

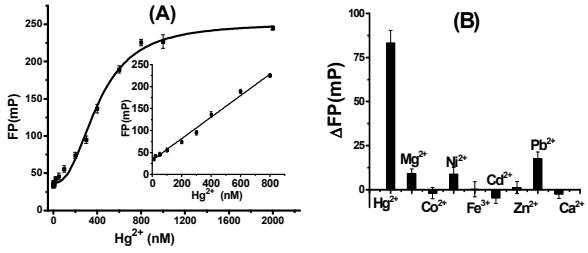



Figure 2 (A) Plot of fluorescence polarization of QDs solution as a function of the $Hg^{2+}$ concentration. Insert: linear relationship between the fluorescence polarization values and the concentration of $Hg^{2+}$. (B) Selectivity of the $Hg^{2+}$ ion assay method over other metal ions. The concentration of $Hg^{2+}$ and other metal ions were 400nM, 1μM, respectively. Δ FP=$FP_1$-$FP_0$ ($FP_1$ and $FP_0$ are the FP values in the presence and absence of mental ion)

In consideration of the ability of Cys to grab $Hg^{2+}$ from T-$Hg^{2+}$-T complexes, we further estimated another sensing platform of the QDs-T/G-quadruplex containing $Hg^{2+}$ for quantitative detection of biothiols. To evaluate the sensitivity of biothiols detection, Cys was chosen as model thiol compounds in the current strategy. Figure 3A illustrated that upon addition of Cys with increasing concentrations, a gradual decrease of QDs FP value was observed. As indicated in insert of Figure 3A, the decrease FP value of QDs in solution showed a linear relationship with the logarithmic concentration of Cys in the range of 50 nM to 2000 nM. The detection limit of Cys was calculated to be 9.9

nM (3σ), which was much lower than the fluorescence system(0.1μM)[12] or colorimetric sensing platform(19nM).[11c] Apart from Cys, the FP changes of QDs was monitored to test the specificity of the assay for biothiols in presence of other amino acids or other bithiols (Homocysteine (Hcy), Reduced L-glutathione (GSH)) at a concentration of 2μM (Figure 3B). The current system was also studied to detect biothiols in human urine samples. The results were listed in Table S2. The recoveries of different known amounts of Cys spiked were obtained from 91.0 % to 103.5% with a satisfying analytical precision (RSD ≤5.0%).

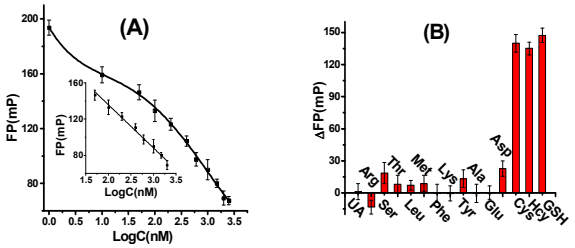



Figure 3 (A) Plot of fluorescence polarization values of QDs solution as a function of the Cys concentration upon addition of $Hg^{2+}$ at a concentration of 800nM. Insert: linear relationship between the fluorescence polarization values and the concentration of Cys. (B) Selectivity of the biothiols assay method over other amino acid. The concentration of all amino acids was 2μM. $\Delta FP=FP_1-FP_0$ ($FP_1$ and $FP_0$ are the FP values in the presence and absence of amino acids)

To evaluate the enhancement of $K^+$-mediated G-quadruplex formation for the QDs fluorescence polarization system, the FP changes of system were recorded at the series concentration of $Hg^{2+}$ upon addition of $K^+$-mediated G-quadruplex formation, G-riched DNA, random DNA, only QDs-T, respectively. As shown in Figure S4, the enormous FP changes was observed in $K^+$-mediated G-quadruplex formation system due to the large volume of G-quadruplex formation compared with that in G-riched DNA , random DNA system or self-association of QDs-T. The FP changes of QDs probe in the $K^+$-mediated G-quadruplex formation system can be observed at low concentration of 10nM $Hg^{2+}$, while in the G-riched DNA system, double-stranded system (random DNA) and self-association of QDs-T system, it can be observed at the concentration of 100nM $Hg^{2+}$ or even higher concentration of 1000nM $Hg^{2+}$. Notablely, in contrast to random DNA system, mild FP changes were also observed in G-Riched DNA system because a part of free coil G-Riched DNA could fold into unstable G-quadruplex without $K^+$ in solution. These results means that $K^+$-mediated G-quadruplex formation as an enhancer contribute to the sensitivity of the proposed method and the effect of self-association of QDs-T could be ignored. The detection limited of present system could be improved at least approximately 10 times compare with the system of G-riched DNA or others.

In summary, a novel $K^+$-mediated G-quadruplex formation enhancement fluorescent polarization sensor based on CdS/CdTe core-shell QDs was constructed for detection of $Hg^{2+}$ and biothiols. This approach has several advantages. First of all, due to the introduction of the steric conformation of $K^+$-mediated G-quadruplex, the proposed strategy lead to a significant molecular volume change and sensitive FP detection. The detection limits of two probes designed in this study against $Hg^{2+}$ and Cys were 8.6nM and 9.9nM, respectively, with excellent selectivity against other coexistent matter. More importantly, because the FP is less affected by environmental interferences, the target can be detected in complex real samples with simple sample pre-treatment. Finally, the current method provides a simple, rapid, inexpensive and universal way for detection of small molecular in complex samples only by simply replacing the aptamer domain with another aptamer.

This work has been supported by National Natural Science Foundation of China (No. 21165004, 21163002), the Guangxi Natural Science Foundation of China ( 2012GXNSFBA053022, 2013GXNSFBA019044), and the project of Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources(Guangxi Normal University), Ministry of Education of China(CMEMR2011-14).

## Notes and references

*Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Ministry of Education of China), School of Chemistry and Pharmaceutical Science of Guangxi Normal University, Guilin, 541004, China Fax: +86-7735832294; Tel: +86-7735846279; E-mail: birdtjn@sina.com; yanchunzhao@aliyun.com*

† Electronic Supplementary Information (ESI) available: [Experimental procedure and the tables]. See DOI: 10.1039/b000000x/

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