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A dual-channel detection of mercuric ions using a label free G-quadruplex-based DNAzyme molecule†

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We have constructed a 'turn-off' and label free bio-sensor using a DNAzyme molecule. This facile bio-sensor is capable of selective detection of mercuric ions with a high sensitivity and satisfactory dynamic range. More importantly, it is able to generate both fluorescent and colourimetric signals for detection. This dual-channel bio-sensor is expected to afford high detection confidence and overcome false-positive readout especially when assaying complex biological samples.

Mercury is a widespread heavy metal pollutant. It is highly toxic and has adverse effects on human beings. Its overdose causes a variety of severe health problems such as nervous system damage, kidney failure, and various cognitive and reproductive disorders. Microbial methylation of solvated Hg^{2+} in aquatic ecosystems is highly hazardous, as it results in neurotoxic and genotoxic methyl mercury.[1] The threat of Hg^{2+} necessitates its routine and facile monitoring in aqueous solutions.[2]

Towards this goal, a few sensors based on polypeptides,[3] oligonucleotides,[4] conjugated polymers,[5] electrochemistry,[6] chemosensors,[7] DNAzyme,[8] and nano-scale materials[9] have been successfully fabricated. These methods largely depend on target-induced changes of fluorescence (FL) or colourimetry (CL), which can be recorded readily at certain wavelengths. Despite the progress, these approaches bear inherent disadvantages for practical use. Firstly, almost all fluorescence methods have background emission in the absence of a target, and this residual fluorescence somehow limits the detection sensitivity.[10] Secondly, these single-channel output methods are likely to produce false-positive results once introduced into complex samples due to non-specific interactions.[11] This might greatly jeopardise the accuracy. Lastly, a single output can be easily affected by other interfering substances which have similar optical profiles especially in complex biological samples.

In this regard, we proposed a label free DNAzyme molecule (named HTD1) to better solve the above-mentioned problems by generating dual-channel outputs in tricolours. It is essentially a DNAzyme molecule capable of being a horseradish peroxidase (HRP) mimic. It is able to form a G-quadruplex–hemin complex and catalyses H_2O_2-mediated oxidation of colourless 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonate disodium salt ($ABTS^{2-}$) to produce a greenish colored radical ion ($ABTS^{\bullet+}$).[12] The latter has a strong absorption at 421 nm. Thereby the activity of the HTD1 molecule can be readily observed using a UV-visible spectrometer or even by the naked eye.

HTD1 has a characteristic G-quadruplex structure in aqueous solution. The DNA G-quadruplex is a secondary structure particularly formed by sequences rich in guanine. The G-quadruplex structure can be further stabilised by cations, especially potassium.[13] Some organic dyes are reported to specifically bind to G-quadruplex and result in enhanced fluorescence. *N*-Methyl mesoporphyrin IX (NMM) is a well known anionic porphyrin characterised by a significant structural selectivity for G-quadruplexes other than duplexes, triplexes, or single-stranded oligonucleotides. NMM is weakly fluorescent and shows pronounced fluorescence enhancement upon binding to a DNA G-quadruplex.[14] This gives rise to specific detection of DNA G-quadruplex assembly and dis-assembly.

The principle of our bio-sensor is depicted in Fig. 1, we used a human telomerase DNA 5′-$(TTAGGG)_4$TTA-3′ and named it HTD1 in this study. HTD1 contains a few thymine bases and is supposed to be well folded into a G-quadruplex in a potassium ion containing buffer, which is termed as 'open' mode. This G-quadruplex plays two roles, when acting as a DNAzyme, it is in complex with hemin to catalyse a colour-generating reaction. While when acting as a G-quadruplex motif, it leads to NMM-binding induced fluorescence. Both readouts can be monitored. With the addition of Hg^{2+} and a 'breaker'

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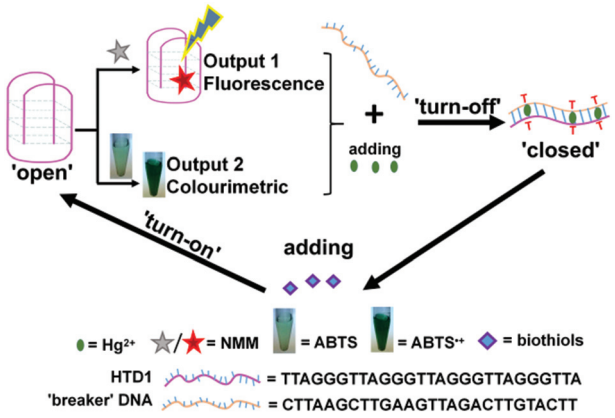

Fig. 1 The working principle and DNA sequences of the dual-channel bio-sensor for Hg^{2+} detection.

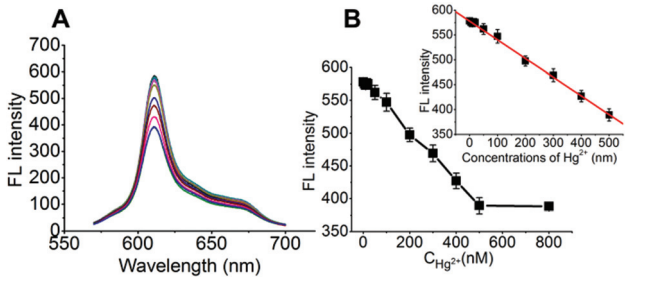

Fig. 2 The FL detection utility of the proposed bio-sensor for sensing Hg^{2+}. (A) Fluorescence spectra for the bio-sensor with different concentrations of Hg^{2+}. (B) The dependence of fluorescence on Hg^{2+} concentrations and the linear relationship of the fluorescence intensity *vs.* the concentrations of Hg^{2+} (inset).

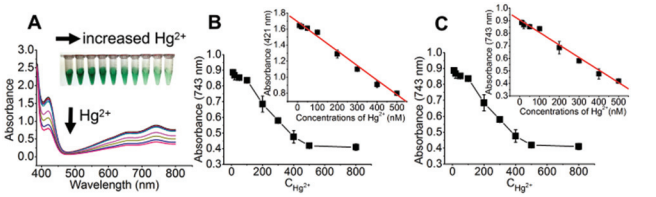

Fig. 3 The CL detection utility of the bio-sensor for sensing Hg^{2+}. (A) UV-Vis spectra for the bio-sensor with different concentrations of Hg^{2+} and the colour changes for samples observed by the naked eye (inset). (B) and (C) are the dependence of fluorescence on Hg^{2+} concentrations, and the linear relationship of the colourimetry intensity *vs.* the concentrations of Hg^{2+} (inset) at 421 and 743 nm respectively.

DNA sequence (5′-CTTAAGCTTGAAGTTAGACTTGTACTT-3′) having a spread of thymine bases, Hg^{2+} mediates the T–Hg^{2+}–T base pairings,[15] which give rise to a double-stranded DNA, breaking the G-quadruplex conformation (termed 'closed' mode). This 'closed' DNA G-quadruplex is nonfunctional in peroxidase-like reactions. Hence it produces a 'turn-off' signal and can be used for selective detection of Hg^{2+}. Biothiols have the ability to chelate Hg^{2+} and this releases the DNA G-quadruplex. The re-generation of the DNA G-quadruplex resets this bio-sensor by restoring the fluorescence and colourimetric signals.

We next thought of testing the utility of this bio-sensor for Hg^{2+} detection. In order to do so, some key factors, which are in close relationship with the performance of optical measurements, have been optimised in detail. At first, we focused on the FL assay, the concentrations of NMM and K^+ were tested, as shown in Fig. S1 and 2.† When increasing the NMM concentration from 0 to 5 μM, the FL output of our bio-sensor enhanced accordingly. While this tendency was maintained up to 5 μM NMM, it seemed to reach a plateau afterwards. Hence we used 5 μM NMM for the FL assay. Similarly, we found that a suitable concentration for K^+ was 10 mM. For the CL assay, the dependence of the substrate $ABTS^{2-}$ and H_2O_2 on the absorption intensity was studied. A discontinuous assay was used, as shown in Fig. S3 and 4,† and we decided to choose an optimal absorbance around 1.5, which was suitable for 'turn-off' changes on addition of Hg^{2+}. Also an absorbance around 1.5 is in the linear region of a common UV-Vis spectrometer when using the Beer–Lambert law for measurements. Under this assumption, the concentrations of $ABTS^{2-}$ and H_2O_2 used in the assay were 3.2 mM and 2.5 mM respectively. Lastly, we observed that the temperature around 37 °C was ideal as shown in Fig. S5 and 6,† since at this temperature it gave the largest difference for both FL and CL measurements when monitoring Hg^{2+}.

Upon the completion of the optimisation of experimental conditions, the relationship between FL or CL intensities and added Hg^{2+} was tested respectively as shown in Fig. 2 and 3. With incremental amounts of Hg^{2+}, more T–Hg^{2+}–T structures formed, which resulted in more global structural changes in HTD1. This led to a gradual decrease in the fluorescence intensity (Fig. 2A). There was a linear relationship (R^2 = 0.999) between FL intensity and Hg^{2+} concentrations over the range from 5 nM to 500 nM, and a 5.0 nM limit of detection (LOD) for Hg^{2+} analysis was achieved, based on a signal-to-noise ratio of 3. For the CL detection, the linear relationship between the CL and Hg^{2+} concentration was from 10 nM to 500 nM for both 421 (R^2 = 0.990) and 743 nm (R^2 = 0.988) wavelengths with a LOD of 10 nM for both.

Next, we would like to test the selectivity of the Hg^{2+}-sensing system. Some other metal ions were individually added to the sensing solution, and both of the FL and CL signals were measured. Fig. 4 presents the histograms of the FL and CL intensity in solutions with Hg^{2+} (100 μM) and other metal ions (500 μM). It can be observed that compared with Hg^{2+}, even at a five-fold higher concentration, the other metal ions do not largely interfere with the system. It is interesting to see that the selectivity for FL is arguably better than the CL channel in general. We propose the reason was probably because a DNAzyme molecule requires high structure rigidity to display peroxidase-like activity. It can be concluded that these competing metal ions have only a negligible effect on our detection system. This approach exhibits high selectivity for Hg^{2+}, which enables its scope for future applications. This

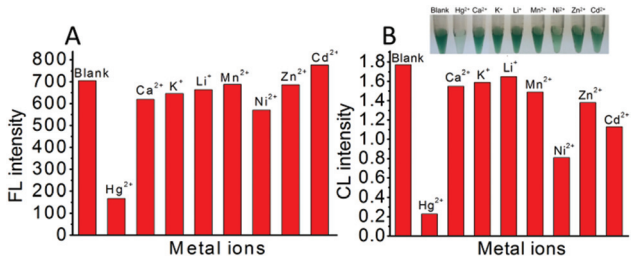

Fig. 4 The selectivity of the proposed bio-sensor for Hg^{2+} over other common metal ions. (A) and (B) are FL and CL signals respectively.

difference in selectivity when sensing Hg^{2+} for this dual-channel bio-sensor highlights the importance of using diverse outputs to enhance confidence.

It is noted that the fluorescence of zinc(II)-protoporphyrin IX (ZnPPIX) has also been tested for Hg^{2+} detection; however, its fluorescence was not proportional to the concentrations of Hg^{2+} (data not shown). The possible reason would be that the fluorescence of ZnPPIX is only highly increased for parallel over antiparallel G-quadruplexes and duplexes.[16] The sequence (HTD1) we used has been reported to likely have multiple conformations including the basket-type, propeller-type, chair-type or mixed-type polymorphisms.[17] We also used CD (circular dichroism) to probe the conformational changes of HTD1, but we did not have CD readouts which corresponded well with the characteristics of either parallel or anti-parallel G-quadruplexes (data not shown). This was indicative of the multiplicity of HTD1 in conformations. For CL detection based on the peroxidase-like activity of the DNAzyme molecule, the 'turn-on' detection approaches have been previously reported.[18] It is noticeable that when the concentration of Hg^{2+} exceeds a certain level, the absorption signal decreases continuously with the addition of Hg^{2+}. This is probably caused by the fact that high concentrations of Hg^{2+} inhibit the peroxidase activity of DNAzymes.[19] Therefore, as a Hg^{2+} bio-sensor, our 'turn-off' strategy seems to precede the 'turn-on' counterparts.

The reversibility of this bio-sensor was investigated by the repetitive addition of Hg^{2+} and cysteine (Cys) in turn as shown in Fig. S7.† Hg^{2+} demonstrates a thiophilic nature, and hence it is reasonable to use biothiols such as cysteine to chelate Hg^{2+} and reset this bio-sensor to its original state.[20] Thus the on/off behavior of this bio-sensor can be clearly seen. These results suggest that our bio-sensor can be operated reversibly, promising applications in practical scenarios.

Lastly, the applicability of this system was further verified by testing a real sample. A tap water sample from the Tianjin University of Science and Technology was acquired. The collected sample was filtered. Then the filtered water was spiked with a series of concentrations of Hg^{2+} (10, 50, 100, 200 and 400 nM as the final concentrations). The analytical results are listed in Table S1.† The recovery values ranged from 95.0 to 115.0% and standard deviations of the triplicate measurements for all samples were all less than 5.5%. These indicate that this proposed bio-sensor is able to detect Hg^{2+} in aqueous-derived samples.

Naturally-derived or inspired products (small molecules, proteins and nucleic acids) have been recognised to be of high importance in sensing, diagnosis and treatment.[21] In this study, we have fabricated a prototype of a reversible bio-sensor with high sensitivity and selectivity. It is based on a peroxidase-like G-quadruplex DNAzyme molecule which enables FL and CL two types of detection by generating tri-colours. It is independent of labeled oligonucleotides and expensive instruments, which considerably benefits the users. The greatest advantage of this bio-sensor over previously reported ones would be that its tri-colour outputs can be detected using two different types of spectrometers, which easily avoids interferences in a certain wavelength. Hence this bio-sensor is helpful in overcoming false positive signals. To the best of our knowledge, our sensor is the first reported G-quadruplex DNAzyme based sensor integrated with two-channel (both FL and CL) outputs for Hg^{2+} detection. Our study also validates that the DNA G-quadruplex molecule can be fabricated with a constructed dual-channel output sensor thus enriching the repertoire of nucleic acids for detection purposes.[22] Different channels would help to enhance confidence especially when one channel turns to be less selective. Hg^{2+} is environmentally relevant. Our device integrates high sensitivity and selectivity with good reversibility for sensing it. The LOD of this bio-sensor for Hg^{2+} is 5.0 and 10.0 nM for FL and CL measurements, lower than the guideline value of Hg^{2+} (0.006 mg L^{-1}, equivalent to 30 nM) in drinking water recommended by the World Health Organization (WHO).[23] This LOD and dynamic range for Hg^{2+} (up to 500 nM) are comparable or better than most reported methods, particularly for fluorescence methods (a comparison of this approach with others for Hg^{2+} sensing is listed in Table S2).† Integrating the excellencies of dual analytical modalities, our proposed strategy well balances the confidence, sensitivity, and dynamic range, making it an ideal platform and should be placed in the toolkit for detection. Surely, with the development of studies on DNA–metal base pairs,[24] the Hg^{2+}-sensing method can be reasonably adapted to the analysis of other metal ions.

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