This article is part of the

**Nucleic acids: new life, new materials**

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An invasive DNA approach toward a general method for portable quantification of metal ions using a personal glucose meter†‡

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We report herein a general methodology for metal ion detection using low-cost, simple, and widely accessible personal glucose meters through an invasive DNA approach.

Metal ions can be beneficial or hazardous to human health and the environment. Of particular concern are toxic metal ions such as lead (Pb$^{2+}$), which can interfere with a variety of physiological processes and cause damage to many organs, leading to several disorders in permanent learning and behavior in children, and uranium (UO$_2^{2+}$), which is a radioactive and potentially carcinogenic species. To prevent potential human exposure to these hazardous metal ions, methods to quantify them are in high demand. Traditional techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), are sensitive but require sophisticated instruments and skilled personnel. In contrast, methods based on chemo sensors and biosensors are simpler in instrumentation and operation, and are therefore more suitable for on site and real time detection with limited resources.

One challenge in developing such sensors is the establishment of a general platform for designing sensors for a broad range of target metal ions, while avoiding the time-consuming trials and errors in designing sensors for each type of metal ion. One way to meet this challenge is to employ DNAzymes that are specific to a variety of metal ions, such as Mg$^{2+}$, Pb$^{2+}$, UO$_2^{2+}$, Cu$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$. Many analytical techniques have been utilized to transform the target-specific activity of DNAzymes into physically detectable signals for the detection of metal ions, including colorimetry, fluorescence, and electrochemistry. Most of these methods, however, require laboratory-based or customized instruments that are not cost effective or commercially available to the general public. Although some colorimetric or fluorescent tests, including dipsticks, can detect metal ions without any instruments, they can only achieve qualitative or semi-quantitative detection based on color observation, whose accuracy may vary among different observers or can change under different light conditions.

To meet this challenge, we propose the use of low-cost, simple, and widely accessible personal glucose meters (PGMs) to quantify hazardous metal ions. PGM is currently one of the most widely used diagnostic devices in the world, as a result of the more than 30 years of development. The recent integration of PGMs into mobile phones can further facilitate their use by the public. However, the state-of-art PGMs are currently only used for blood glucose monitoring by diabetic patients. Recently, we have successfully developed a method to use PGMs to detect a broad range of non-glucose targets. The method was applied to UO$_2^{2+}$ detection using a UO$_2^{2+}$-specific DNAAzyme and showed promising results. However, we found that the performance of the above method, such as the sensitivity, significantly decreased when the method was applied to detect other heavy metal ions, such as Pb$^{2+}$, probably because the proteins used in the design (including streptavidin, invertase and blocking proteins on the magnetic beads (MBI)) could compete with DNAzymes in binding metal ions through accessible amino acid residues such as on those proteins. To make the methodology more generally applicable to different metal ions, including thiophillic ones like Pb$^{2+}$, we report here a new approach to separate the metal-ion-induced cleavage of DNAzymes’ substrates from the target-induced release of invertase-DNA conjugates.

The schematic diagram of the methodology is shown in Fig. 1. To detect Pb$^{2+}$, the DNA sequence of the original 8–17 DNAzyme was extended by 9 nucleotides (nt) in the 5’ arm to reduce the amount of non-hybridized substrates (Fig. 1a). To demonstrate the generality of the method, we also chose a UO$_2^{2+}$-specific DNAzyme and made similar minor changes to its sequences (Fig. 1b). One remarkable feature of DNAzymes is...
that the sequences in the substrate binding arms at both ends can be varied with little effect on the enzymatic activity or metal ion selectivity.\textsuperscript{27,28,51} Upon addition of the respective metal ions, i.e., Pb\textsuperscript{2+} for the 8–17 DNAzyme, or UO\textsubscript{2+} for the 39E DNAzyme, the DNAzymes catalyzed the cleavage of the DNA substrates at the 3’ phosphoester bond of the ribonucleotide A (rA). The right arm (red) of the cleaved substrates could no longer form a stable duplex with the DNAzymes due to the decrease of base pair number from 23 to 9, and thus was released as a 19-nt oligonucleotide (called invasive DNA, Fig. 1c) into the solution. In a separate solution, the invertase was conjugated to a 24-nt DNA (in green, containing a A\textsubscript{12} linker, as in Fig. 1c)\textsuperscript{47} that could hybridize to a biotinylated DNA (in black) that bound to streptavidin-coated MBs. Because the released 19-nt oligonucleotide from the cleaved substrate is longer in matched invasive DNA. (d) The invasion and release steps, followed by the conversion of sucrose into glucose by the released DNA–invertase conjugates for PGM measurement.

In 100 µL buffer A containing 50 mM HEPES (pH 7.1) and 0.15 M NaCl, 2 µM DNA substrate and 3 µM DNAzyme for Pb\textsuperscript{2+} detection were mixed with different concentrations of Pb\textsuperscript{2+} to initiate the cleavage reaction (Fig. 1a). After quenching the reaction with 2 µL 100 mM EDTA, the solution was mixed with 100 µL buffer B containing 0.1 M sodium phosphate (pH 7.2), 0.2 M NaCl, and 0.05% Tween-20. An aliquot of 150 µL of the resulting solution was then transferred to the MB residues containing DNA–invertase conjugates to induce release. After removal of the MBs by a magnet, 40 µL of the supernatant solution was mixed with 40 µL of 1 M sucrose in buffer B to generate glucose. Finally, the solution was tested by a PGM and the signal readouts were used to calculate the Pb\textsuperscript{2+} concentration in the samples.

As shown in Fig. 2a, when the concentrations of Pb\textsuperscript{2+} present in the samples increased from 0 to 1000 nM, the final readout in the PGM increased accordingly until reaching a plateau. The detection limit was found to be 16 nM, as defined by 3σ\textsubscript{b}/slope (σ\textsubscript{b} is the standard deviation of five blank samples), with a dynamic range of at least 16–1000 nM. This detection limit is more than 4-fold lower than the US EPA regulated level (72 nM) in drinking water, and comparable or even better than some other reported methods based on similar DNAzymes.\textsuperscript{18,29,32,37} In addition, other metal ions showed very low interference to the Pb\textsuperscript{2+} detection (Fig. 2b), suggesting that the high selectivity of the DNAzyme\textsuperscript{17,18} was well preserved in the sensor design. The effect of pH and ionic strength on the performance of the method was also investigated and shown in Fig. S1 in ESI.\textsuperscript{1} The method was successfully applied to the detection of Pb\textsuperscript{2+} in drinking water and table dust, where the Pb\textsuperscript{2+} concentrations were dependent on the glucose signal readout via the PGM measurements (Fig. S2 in ESI).\textsuperscript{1} The Pb\textsuperscript{2+} detection in drinking water, using two different batches of the DNA–invertase immobilized MBs, gave similar calibration curves (Fig. S3 in ESI), suggesting that the batch-to-batch difference is small.

To demonstrate that our methodology is generally applicable to other metal ions, we have also employed a similar design using a UO\textsubscript{2+}-dependent DNAzyme in buffer C containing 50 mM MES (pH 5.5) and 0.3 M NaCl (Fig. 1b).\textsuperscript{19,31} Because the invasive DNA here (Fig. 1b) has an identical sequence to that in the Pb\textsuperscript{2+} detection (Fig. 1a), the same DNA–invertase conjugate and MBs can be used for the detection of both Pb\textsuperscript{2+} and UO\textsubscript{2+} in order to avoid the complicated procedures of preparing new DNA–invertase conjugates and MBs for detecting each metal ion. With a similar mechanism, the invasive DNA was produced only in the presence of UO\textsubscript{2+} (Fig. 1b), and caused the release of

![Fig. 1](a) Pb\textsuperscript{2+}-induced cleavage of the DNA substrate by the Pb\textsuperscript{2+}-dependent 8–17 DNAzyme. (b) UO\textsubscript{2+}-induced cleavage of the DNA substrate by the UO\textsubscript{2+}-dependent 39E DNAzyme. Both reactions yield the cleaved ssDNA product (red) as the invasive DNA. (c) Release of DNA–invertase conjugates from MBs by the invasive DNA. (d) The invasion and release steps, followed by the conversion of sucrose into glucose by the released DNA–invertase conjugates for PGM measurement.

![Fig. 2](a) Detection of Pb\textsuperscript{2+} using a PGM. (b) The selectivity of the Pb\textsuperscript{2+} detection over other divalent metal ions using a PGM.
DNA-invertase conjugates from the MBs via DNA invasion (Fig. 1c). Finally, the released DNA–invertase conjugates catalyzed the hydrolysis of sucrose into glucose, which was quantified by a PGM. More glucose production was detected by the PGM when an increasing amount of UO$_2^{2+}$ was present in the samples until signal saturation, where all the DNA–invertase conjugates were released by the invasive ssDNA (Fig. 3a). The detection limit, according to the definition of 3σ/slope, was estimated to be 5.0 nM, which is more than 20-fold lower than the US EPA regulated level (126 nM). Like the Pb$_2^+$ detection above, the UO$_2^{2+}$ detection here was also very selective to UO$_2^{2+}$ over other metal ions because of the high selectivity of the DNAzyme (Fig. 3b).

In summary, we have developed a general methodology using PGMs to detect metal ions, including thiophillic and non-thiophillic heavy metal ions, by separating the metal-dependent DNAzyme reactions from the release of DNA–invertase conjugates. The invasive DNA yielded from the DNAzyme-catalyzed reactions could release the DNA–invertase conjugates from the MBs, resulting in hydrolysis of sucrose to produce glucose for PGM measurement. Using this method, both Pb$_2^+$ and UO$_2^{2+}$ were successfully quantified by PGMs, with detection limits (16 and 5.0 nM for Pb$_2^+$ and UO$_2^{2+}$, respectively) below the maximum contamination levels defined by the US EPA in drinking water (72 and 126 nM for Pb$_2^+$ and UO$_2^{2+}$, respectively), and with excellent selectivity. The methodology should also be applicable for the quantification of other metal ions, if the proper metal-specific DNAzymes and substrates are used. Because of the simple operation, low cost, and wide availability of PGMs, the method demonstrated in this work can be used by the public for quantitative detection of hazardous metal ions in drinking water and other samples at any point of interest.

Notes and references