Supporting materials

S1 Materials and methods

Chemicals

Lead-specific 8–17 DNAzyme sequences were chosen for investigation of rational design using unmodified AuNPs in the colorimetric assay. All DNA sequences were purchased from Takara Bio Inc. The 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid (HEPES), sodium chloride (NaCl), and sodium hydrooxide (NaOH) were purchased from Sigma-Aldrich. Magnesium chloride ($MgCl_2$) and sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were purchased from Amresco (Ohio, USA). The chloroauric acid ($HAuCl_4$) was purchased from Sinopharm Chemical Reagent Company. Analytical grade standard solutions of metal ions were purchased from AccuStandard, and Macklin Biochemical Co., Ltd. All solutions were prepared using USP sterile purified molecular grade DNase, RNase, and Protease-free water. A UV-Vis spectrophotometer U-3900 (Hitachi, Japan) was used to record the absorption spectra. High-resolution transmission electronic microscopy (TEM) H7650B (Hitachi, Japan) and Zetasizer Nano ZS90 (Malvern Instruments Ltd., England) were used for characterization of AuNPs and zeta potential measurement, respectively (Memon et al. 2017).

Table S1 Description of the DNA sequences for substrate and enzyme part including the truncated sequences, rA indicated adenosine from the RNA base

Name	**Sequence 5′- > 3′**
8–17 DNAzyme	ACA GAC ATC TCT TCT CCG AGC CGG TCG AAA TAG TGA GT
17DS	ACT CAC TAT *rA* GGA AGA GAT GTC TGT
17E-5	CAT CTC TTC T CCG AGC CGG TCG AA ATA GTG AGT
17DS-5	ACT CAC TAT *rA* GGA AGA GAT G
17E-4	ACAT CTC TTC T CCG AGC CGG TCG AA ATA GTG AGT
17S-4	ACT CAC TAT *rA* GGA AGA GAT GT
17DS-9nt	ACT CAC TAT
17DS-10nt	GGA AGA GAT G

S2 Optimization parameters

Reagent / buffer solutions

Table S2 Concentration of reagents used in the bioassay

Name	**Solution**
Buffer	HEPES 50×10^{-3} mol/L pH 7.0 ± 0.2
Hybridization buffer	HEPES 50×10^{-3} mol/L, NaCl 50×10^{-3} mol/L, $MgCl_2$ 5×10^{-3} mol/L
Salt	NaCl 1 mol/L in buffer (HEPES 50×10^{-3} mol/L)

Ionic conditions

Ionic strength for the colorimetric detection plays a vital role in stabilizing the reaction conditions and maintaining pH of the reaction system. The salt concentration for the hybridization process of the substrate and enzyme was set as NaCl 100×10^{-3} mol/L and $MgCl_2$ 10×10^{-3} mol/L in the 50×10^{-3} mol/L HEPES buffer at 7.0 ± 0.2 pH value. Evidently, the salt concentration being higher than 100×10^{-3} mol/L facilitated hybridization and prevented nonspecific dissociation of the complex (Wang et al., 2008). The AuNPs performed differently with changes in the ionic strength of the system.

AuNP:salt ratio

The AuNP:salt ratio was initially adjusted to determine the dispersion to the aggregation state of the AuNPs for colorimetric analysis. The different volumes of the 500×10^{-3} mol/L NaCl was added to 100 µL of freshly synthesized 8.68×10^{-9} mol/L and 13 nm diameter AuNPs. The AuNPs were aggregated, and the color shifted from pink to purple and blue at a 40 µL salt volume. This ratio was consistent with our previous findings for salt-induced aggregation and was used for further analysis (Memon et al., 2017).

Enzyme–Substrate ratio

The enzyme–substrate ratio was needed to be determined to prevent excessive amounts and nonspecific dissociation that could adsorb on the surface of AuNPs and may result in false positive colorimetry. An equimolar ratio of enzyme and the two parts of the substrate 17DS-9nt and 17DS-10nt (which were supposed to be cleaved in two parts from the substrate sequence in the presence of Pb^{2+} ions) were used with the optimized AuNP and salt concentration to determine the optimum ratio for the Pb^{2+} zyme. The final optimized volume of the hybridized substrate to an enzyme (1:1) was 12 μL, and was used in the further quantification.

S3 Thermodynamic properties

Energy calculations for the left part of the substrate binding region of the sequence 17DS using the online tool of DNA thermodynamics and hybridization of Integrated DNA Technologies (http://biophysics.idtdna.com/cgi-bin/meltCalculator.cgi).

Table S3 Thermodynamic calculations of the substrate binding region

17DS (excluding nucleotides at 3′)	T_m (℃)	**Gibbs Energy (ΔG_{37})** (kcal/mol)	**Enthalpy (ΔH)** (kcal/mol)	**Entropy (ΔS)** (cal/(K·mol))
Actual	43.86	−11.76	−110.70	−319.00
−1 T @ 3′	41.26	−10.78	−104.50	−302.17
−2 TG @3′-	37.71	−9.58	−93.80	−271.55
−3 TGT@3′-	34.80	−8.74	−88.20	−256.21
−5 TGT CT@3′-	25.79	−6.68	−71.60	−209.31

S4 PAGE analysis for the lead-based cleavage of the truncated sequence against the concentration of Pb^{2+} ions

The substrate and enzyme of the truncated Pb^{2+} dependent sequence, small fragments of substrate of 9 and 10 bases, and hybridized sequence were utilized with a concentration of Pb^{2+} ions in the sample to determine the cleavage of the sequence with the addition of lead ions. The gradient concentration of the PAGE was increased to 20%, 21%, 22%, and 25% in the native non-denaturing gel to visualize the small ssDNA fragments of 9 and 10 bases. A total of 20 mL

gel solution was prepared for each duplicate casting. The gradient preparation was made as stated in Table S4.

Table S4 Native PAGE preparation with various gradient concentration

Gradient concentration for native PAGE Volume	Material				
	H_2O	40% PAM	5X TBE	10% APS	TEMED
15%	8.5 mL	7.5 mL	4 mL	200 μL	20 μL
20%	6 mL	10 mL	4 mL	200 μL	20 μL
21%	5.5 mL	10.5 mL	4 mL	200 μL	20 μL
22%	5 mL	11 mL	4 mL	200 μL	20 μL
25%	3.5 mL	12.5 mL	4 mL	200 μL	20 μL

After the reaction, the samples were prepared for electrophoresis. Each sample was added with 2 × loading buffer (LB) as a final concentration and were loaded in the separate lane of the prepared gel. The controlled sample was assayed in parallel with the other samples that contained 20 bp ladders (markers). The gel was assayed for 80 min at 120 V in 1 × TBE buffer. Moreover, the temperature was controlled using iced water. Subsequently, gel staining was conducted with 3 × SYBR gold prepared in 1 × TBE for 20 min in the dark. Finally, the image was captured with gel-doc equipment of Biorad Inc.

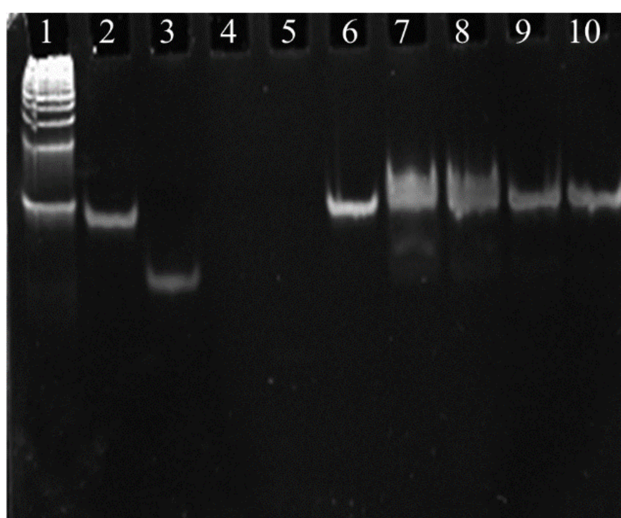

Fig. S1 Native PAGE at 21% containing samples in each lane as (1) marker, (2) DNAzyme, (3) substrate, (4) substrate of 9 bases fragment, (5) substrate of 10 base fragment, (6) DNAzyme, substrate fragment of 9 and 10 bases in 1:1:1, (7) DNAzyme and substrate, (8) Hybridized DNAzyme-substrate without Pb^{2+} ions, (9) Hybridized DNAzyme-substrate with 1×10^{-6} mol/L Pb^{2+} ions and (10) Hybridized DNAzyme-substrate with 5×10^{-6} mol/L Pb^{2+} ions

S5 Fluorescence measurements to determine Pb^{2+} -dependent cleavage of the DNAzyme

The DNA sequences of the substrate and enzyme labeled with fluorescence and quencher molecules as FAM and BHQ-1 were purchased from Takara Inc. The substrate and enzyme were hybridized in 1:1 concentration in hybridization buffer at 95℃ for 5 min and later allowed to cool slowly at RT for approximately 90 min. The two salt concentrations were tested for the preparation of hybridization buffer as 300 and 100×10^{-3} mol/L NaCl together with 10×10^{-3} mol/L $MgCl_2$ in 50×10^{-3} mol/L HEPES at 7.0 ± 0.2 pH value. The hybridized solution was then allowed to react with the lead solution of various concentrations and incubated for 25 min at RT. Subsequently, the samples were analyzed for fluorescence intensity using a spectrophotometer.

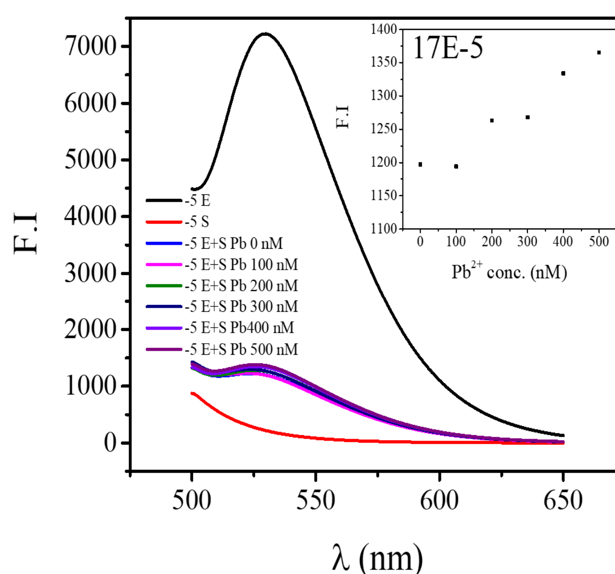

Fig. S2 Fluorescence absorption spectra of Pbzyme sequence 17–5 with hybridization buffer containing 100×10^{-3} mol/L as NaCl and 10×10^{-3} mol/L mol/L $MgCl_2$. The –5E represent the enzyme part, and –5S represents the substrate part of the Pbzyme truncated for 5 nucleotides from the original 8–17 DNAzyme. (Inset: represent peak intensity for each of the samples)

S6 Temperature influence on the stability of the DNAzyme

Thermodynamic properties are significant in the hybridization and stable structure of the substrate to the enzyme in the unmodified nonspecific adsorption mechanism of AuNP and ssDNA

sequences. The melting properties for the substrate binding region were carefully studied. Hybridization of the substrate binding region at 3′ site was stable when cleaved at ambient temperature (~26℃) by utilizing the standard reported Pb^{2+} zyme sequence of 8–17 (Lan et al., 2010; Li and Rothberg, 2004; Li et al., 2009). The thermodynamic properties suggested a 44℃ melting temperature for the same region. Previous studies have focused on the 9-nt ssDNA substrate binding region, and have worked efficiently with labeled or modified AuNP-based colorimetric detection. In our proposed system, the sequence performed differently. A certain volume/concentration of the hybridized -zyme was required to produce sufficient quantities of the cleaved substrate parts, which were adsorbed on the AuNP surface to protect against salt-induced aggregation. Accordingly, the Pb^{2+} concentration increased due to saturation. Hence, the melting properties, including thermodynamics for the substrate binding region, was recalculated using the online tool DNA thermodynamics and hybridization of Integrated DNA Technologies (http://biophysics.idtdna.com/cgi-bin/meltCalculator.cgi). The modified sequences were utilized for substrate–enzyme hybridization at 95℃, and the reaction temperature was maintained in accordance with the suggested calculations. Significant changes in dissociation and hybridization were observed for Pb^{2+} quantification.

Seasonal effect

Despite maintainging ionic strength of the reaction system, designed Pb^{2+} zyme will not work at variable temperature. Ambient temperture of the winter will influence on the Pb^{2+} cleavage compared to seasonal summer temperature. This phenoma can be found by revisiting melting properties. We designed an experiment using identical parameters at a controlled temperature for the modified sequences and observed stable hybridization/cleavage at a temperature of 28℃ and 20℃. Clear aggregation and dispersion of unmodified AuNPs can be visually obsereved in blue to pink color respectively (Fig. S3).

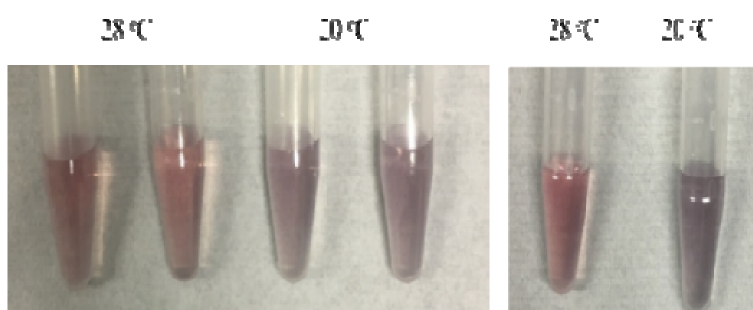

Fig. S3 samples containing AuNPs with DNAzyme sequence without Pb^{2+} concentration at two controlled temperature sets

S7 Table of comparison for DNAzyme-based optical assays for the detection of lead

Table S5 Summary table of DNAzyme based assays for the detection of lead

Technique	**Method**	**LOD**	**Reference**
Fluorescent	Label-free DNAzyme-based fluorescing molecular switch for lead ion detection	10×10^{-9} mol/L	(Zhang, et al., 2011)
Fluorescent	A homogeneous catalytic beacon sensor (fluorescein and quencher-labeled)	7.8×10^{-9} mol/L	(Lan et al., 2010)
Fluorescent	Abasic site-containing DNAzyme (homogeneous phase, label free)	4×10^{-9} mol/L	(Xiang et al., 2009)
Fluorescent	A catalytic DNA molecular beacon on Au (surface-based, fluorescein and quencher-labeled)	1×10^{-9} mol/L	(Swearingen et al., 2005)
Fluorescent	DNA–protein conjugates as signal probes using EWIE sensing platform	1×10^{-9} mol/L	(Wang et al., 2015)
Fluorescent	Graphene-DNAzyme based biosensor (homogeneous phase, FAM-labeled)	0.3×10^{-9} mol/L	(Zhao et al., 2011)
Colorimetric	Colorimetric Detection of Lead Ion Based on Gold Nanoparticles and Lead-Stabilized G-Quartet Formation	5×10^{-6} mol/L	(Chen, et al., 2015)
Colorimetric	DNAzyme-directed assembly of gold nanoparticles (homogeneous phase)	1×10^{-9} mol/L	(Jiang et al., 2010)
Colorimetric	Target induced assembly of DNAzyme modified gold nanoparticle and graphene oxide	0.1×10^{-9} mol/L	(Li, et al., 2014)
Colorimetric	Lead ions detection with a tunable dynamic range using DNAzyme and gold nanoparticles.	3×10^{-9} mol/L	(Wang et al., 2008)
Colorimetric	DNAzyme based lead sensing using unmodified gold nanoparticle probes	500×10^{-9} mol/L	(Wei et al., 2008)
Colorimetric	Label-free colorimetric nanosensor for Pb^{2+} in water by using a truncated 8-17 DNAzyme	**0.2**$\times10^{-9}$ mol/L	**This study**

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