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Giant Catalytic DNA Particles for Simple and Intuitive Detection of Pb^{2+}

Jieun Kim and Jong Bum Lee*

Abstract

DNAzymes have been extensively studied as biosensors because of their unique functionality of cleaving substrate in the presence of metal ion cofactors. However, there are only a few reports on visual detection using gold nanoparticles. Here, we synthesized the DNAzyme microparticle (DzMP) ($\sim 1 \mu\text{m}$) via rolling circle amplification for detection of Pb^{2+} without the help of other materials. Then, the substrate strands were labeled with two different fluorophores (6-carboxyfluorescein and Cy5) to visualize the DzMPs and to monitor the separation of substrate strands. Because of their large size, the decline in the number of fluorescent particles in the presence of Pb^{2+} could be successfully demonstrated by a fluorescence microscopy, presenting a new platform for heavy metal detection.

Keywords: DNAzyme, Heavy metal detection, Biosensor

Background

Nucleic acids have attracted a great attention due to their versatile properties. Especially, various DNA enzymes (termed DNAzyme) have recently been developed by the systematic evolution of ligands by exponential enrichment (SELEX) since Breaker and Joyce presented the in vitro selection of catalytic DNA in 1994 [1]. These DNAzymes have unique properties that are capable of catalyzing bioorganic chemical reactions. For example, DNAzymes catalyzing RNA cleavage [1–3], ligation [4, 5], or modification of nucleic acids [6, 7] have been reported. They have been applied in various fields such as biochemistry, medicine, and analytical chemistry.

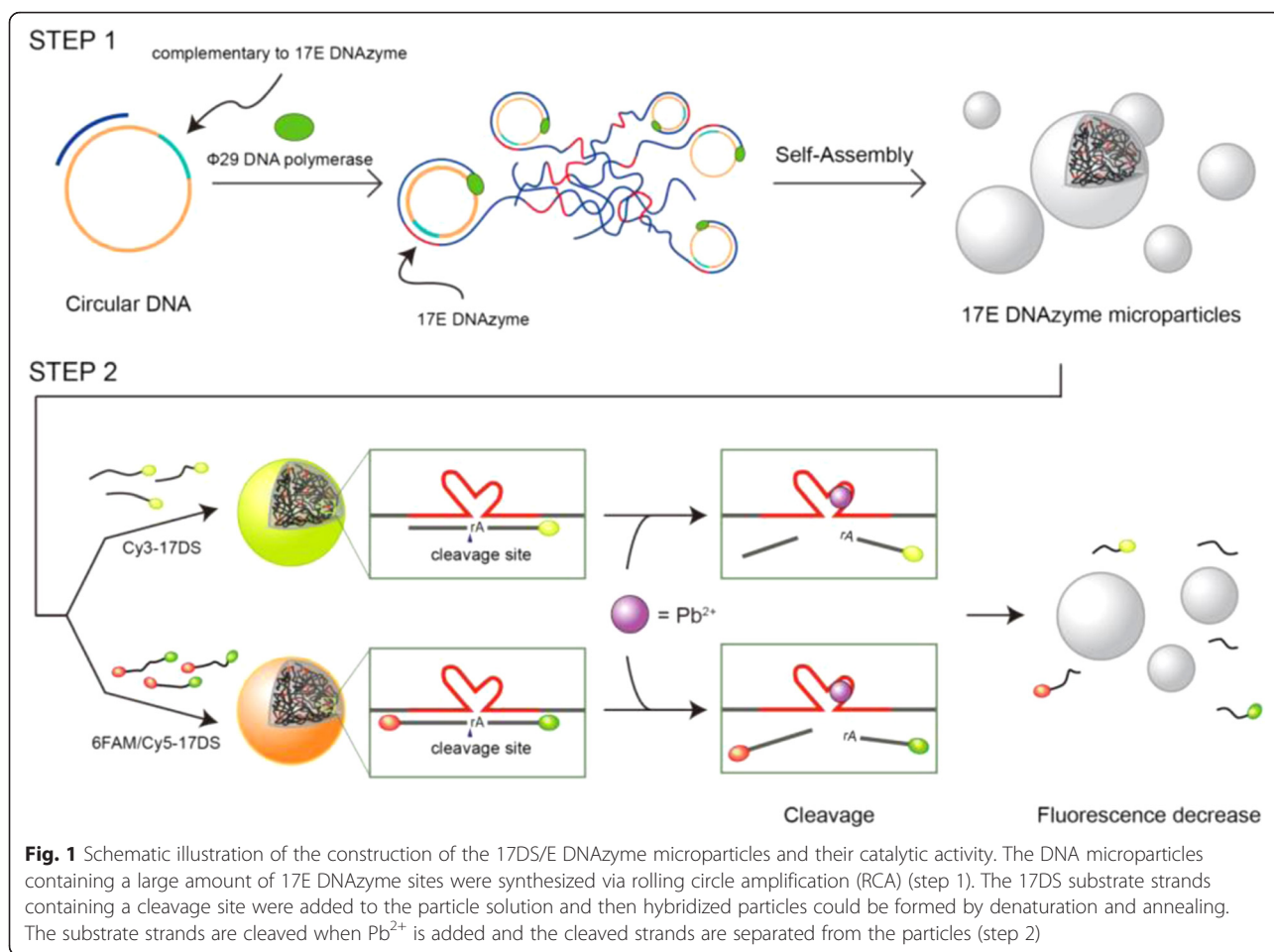
Particularly, DNAzymes cleaving DNA or RNA substrates in the presence of specific metal cofactor such as Pb^{2+} , Mg^{2+} , Cu^{2+} , and UO_2^{2+} have been widely studied [1, 8–10]. Also, Na^+ [11, 12] or lanthanide [13, 14]-dependent DNAzymes have been recently reported. Because the toxicity of heavy metals is well known, these DNAzymes have often been used as a sensor to detect heavy metals. To be specific, fluorescent techniques [15, 16], polymerase chain reaction [17], electrochemical approaches [18], and surface-enhanced Raman scattering (SERS) [19, 20] have been applied for DNAzyme-based sensors. Also, the

colorimetric sensors using gold nanoparticles were introduced [21]. However, there have been few studies on visual detection techniques and most of the conventional colorimetric detection methods need the immobilization of DNAzyme on gold nanoparticles [21–23].

Previously, the synthesis of DNA hydrogel via ligase-mediated reaction or rolling circle amplification (RCA) has been reported [24, 25]. Based on these enzymatic approaches, here we synthesized the giant catalytic DNA particles ($\sim 1 \mu\text{m}$) without other inorganic core particles and successfully visualized the detection of Pb^{2+} utilizing a fluorescence microscope as illustrated schematically in Fig. 1. First, the DNAzyme microparticles (DzMPs) comprised of 17E DNAzyme for sensitive Pb^{2+} detection were produced by rolling circle amplification (RCA). Then, the fluorescently labeled 17DS substrate strands were hybridized with the generated particles. In the absence of Pb^{2+} , the 17DS/E DzMPs emit bright fluorescence. However, in the presence of Pb^{2+} , the particles turn off their light since the cleaved substrates are falling apart from the 17E DzMPs. This giant DNAzyme particle-based strategy enables detection of Pb^{2+} by fluorescence microscopy. In addition, DzMPs have numerous catalytic sites, and their sequences can be flexibly changed to have diverse functionalities. Therefore, this DzMP-based approach offers a simple detection method as well as a promise of a potent therapeutic tool for clinical applications.

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Methods

Preparation of Circular DNA

All the oligonucleotides used in the study were purchased from the Integrated DNA Technologies (IDT, USA) (Table 1). To prepare the circular DNA, phosphorylated linear single-stranded DNA (ssDNA) that has complementary sequence to the 17E DNAzyme was designed and the primer ssDNA was designed to contain two complementary regions to each end of the linear ssDNA. For hybridization, equal concentration (3 μM) of the linear ssDNA and primer ssDNA were mixed in nuclease-free water. Next, the mixed solution was heated to 95 °C for 2 min and cooled gradually to 25 °C for 1 h. To connect the nick in the circular DNA, the

solution was incubated overnight at room temperature with T4 DNA ligase (0.03 U/μL, Promega, USA) and ligase buffer (300 mM Tris-HCl (pH 7.8), 100 mM MgCl₂, 100 mM dithiothreitol, and 10 mM adenosine triphosphate).

Synthesis of 17E DNAzyme Microparticles

To synthesize the 17E DNAzyme microparticle (17E DzMP), the prepared circular DNA was mixed with Φ29 DNA polymerase (1 U/μL, Lucigen, USA), deoxyribonucleotide triphosphate (2 mM), and reaction buffer (40 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM MgCl₂, 5 mM (NH₄)₂SO₄, and 4 mM dithiothreitol). For the RCA process, the mixed solution was incubated at 30 °C

Table 1 DNA sequences for the fabrication of DNAzyme microparticles

Linear DNA	5' - /Phosphate/CAA CTG TAG TGT GTT CAC GGT GCT GTA CTC ACT ATT TCG ACC GGC TCG GAG AAG AGA TGC ACT GAC AAG ACG TCA TAT CAA GTG TAT GGC AA - 3'	
Primer DNA	5' - CAC TAC AGT TGT TGC CAT ACA C - 3'	
Dual-labeled 17DS substrate DNA	5' - /6-FAM/ACT CAC TAT rA* GGA AGA GAT GCA CTG A/Cy5/ - 3'	rA represents the ribonucleotide adenosine
Cy3-labeled 17DS substrate DNA	5' - /Cy3/ACT CAC TAT rA* GGA AGA GAT G - 3'	rA represents the ribonucleotide adenosine

*rA represents the ribonucleotide adenosine

for 20 h. After brief sonication and washing several times using nuclease-free water, the 17E DzMP could be collected. Scanning electron microscopy (Hitachi, Japan, S-4200) was utilized to analyze the morphology of the DzMPs.

Hybridization of 17DS Substrate with 17E DNAzyme Microparticles

To facilitate the confirmation of the hybridization of the 17E DzMP and 17DS substrate strands, the substrate was modified with the Cy3 fluorophore at the end of 5' (Cy3-17DS). To determine the optimum ratio between 17E DzMP and Cy3-17DS, the different concentrations (200 nM, 1 μ M, 5 μ M each) of Cy3-labeled substrate strands was added into the 17E DzMP solutions. The denaturation and annealing processes were performed to produce Cy3-labeled 17DS/17E DNAzyme microparticles (Cy3-17DS/E DzMPs). The solution was heated to 95 °C for 2 min and cooled gradually to 25 °C for 1 h. The substrate residues were removed by washing several times. The fluorescence intensity of the 17DS/E DzMPs were measured using Nucleo Counter (Chemometec, Denmark, NC-3000). The fluorescent images of the particles were obtained by fluorescence microscopy (Nikon, Japan, Eclipse Ti).

Gel Electrophoresis

To confirm the cleavage reaction, the various concentrations of Pb^{2+} solution (100 nM to 1 mM) was added to Cy3-17DS/E DzMP solutions. Then, to avoid the settling of DzMPs, the mixed solution was incubated using rotating oven at room temperature for 1 h. The catalytic activity of the DNAzyme was confirmed using DNA polyacrylamide gel electrophoresis (DNA-PAGE). Gel electrophoresis was carried out on a 10 % DNA-PAGE gel at 95 V at room temperature in Tris-borate-EDTA (TBE) buffer (0.89 M Tris-borate and 0.02 M EDTA, pH 8.3) for 40 min. First, the gel electrophoresis result was analyzed without DNA-specific dyes to monitor the Cy3-17DS substrate strands. Then, the resulting DNAzyme particles were analyzed after staining with GelRed (10^{-4} dilution of the stock solution, Biotium, USA).

Detection of Pb^{2+} Using a Fluorescence Microscope

To observe DzMPs using a fluorescence microscope, dual-labeled substrate was used. The substrate strand which was labeled with a FAM fluorophore (6-carboxy-fluorescein) at the 5'-end and a Cy5 at the 3'-end (6FAM/Cy5-17DS). To verify the optimal condition, different concentrations of 6FAM/Cy5-17DS (10 nM, 100 nM, 1 μ M each) were mixed with 17E DzMPs and the same denaturation and annealing processes as above were performed. The fluorescence intensity of the 6FAM/Cy5-17DS/E DzMP was measured using Nucleo

Counter (Chemometec). For the detection test, various concentrations of Pb^{2+} solution (100 nM to 1 mM) was added to the DzMP solution, and the mixed solution was incubated at room temperature using a rotating oven for 1 h. After cleavage reaction, the separation of the 6FAM/Cy5-17DS was monitored by utilizing fluorescence microscopy (Nikon).

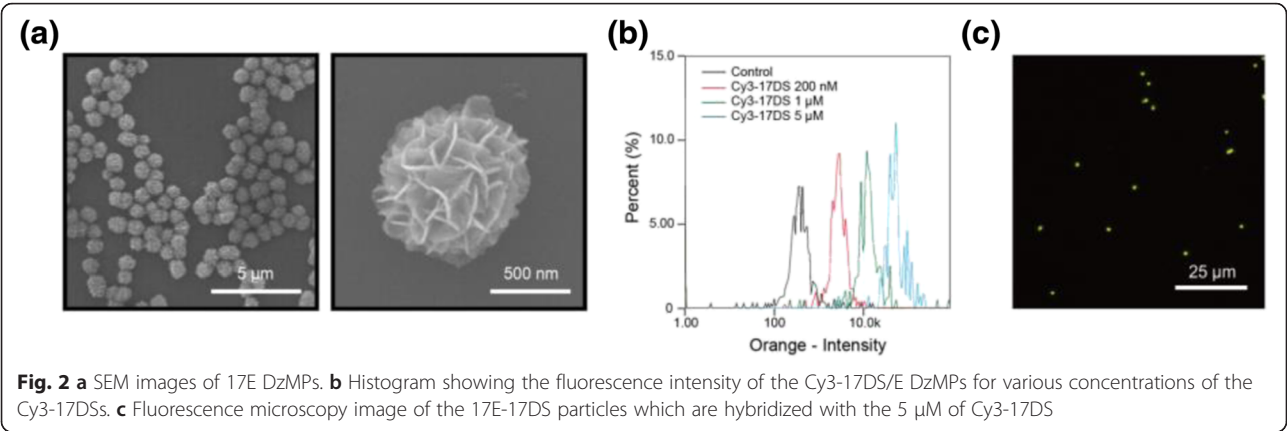
Results and Discussion

Formation of Catalytic DNA Microparticles

As illustrated in Fig. 1, the catalytic DNA particles were prepared from the designed circular template to generate 17E DNAzyme by RCA process. To observe the morphology and structure of the particles, scanning electron microscopy (SEM) was utilized. As seen in Fig. 2a, SEM image showed that the enzymatic particles have flower-like globular shape with uniform size ($\sim 1 \mu$ m). Because of the large surface area of the porous structure, numerous catalytic sites of the particles could participate in the enzymatic reaction. In addition, Cy3-labeled 17DS substrate strands (Cy3-17DS) were added to prepare 17E DzMPs to produce Cy3-17DS/E DzMPs. To determine the optimal ratio between substrates and DzMPs, three different concentrations (200 nM, 1 μ M, and 5 μ M each) of substrate solutions were tested. The hybridization between Cy3-17DS and 17E DzMPs was verified by utilizing image cytometry. As shown in Fig. 2b, fluorescence intensities of the particles increased as the concentration increased from 200 nM to 5 μ M. Especially, there was a significant increase in intensity when the 5 μ M of Cy3-17DS was used, compared to that of control 17E DzMPs. In addition, further study was performed using fluorescence microscopy, and the 17E particles which were mixed with 5 μ M of Cy3-17DS showed bright fluorescence (Fig. 2c). These results indicated that the 17DS substrate strands were hybridized enough with the 17E particles which could catalyze the cleavage reaction. Also, the DzMPs still maintain their structure after hybridization with the 17DS substrate (see Additional file 1: Figure S1).

Confirmation of Catalytic Activity of DzMPs

To confirm the catalytic activities and determine the sensitivity, various concentrations of Pb^{2+} (100 nM to 1 mM) were incubated with the Cy3-17DS/E DzMPs at room temperature for 1 h. After incubation, the solutions were analyzed by gel electrophoresis without purification to monitor the separated strands. Because of the presence of Cy3 fluorophore, the substrate strands could be analyzed without further staining with DNA-specific dyes. As seen in Fig. 3a, some 17DS substrates were detected at untreated control (lane 2). There are no significant changes at concentrations up to 100 μ M. However, the substrate band was disappeared meaning cleavage of the substrate strands when the particles were treated

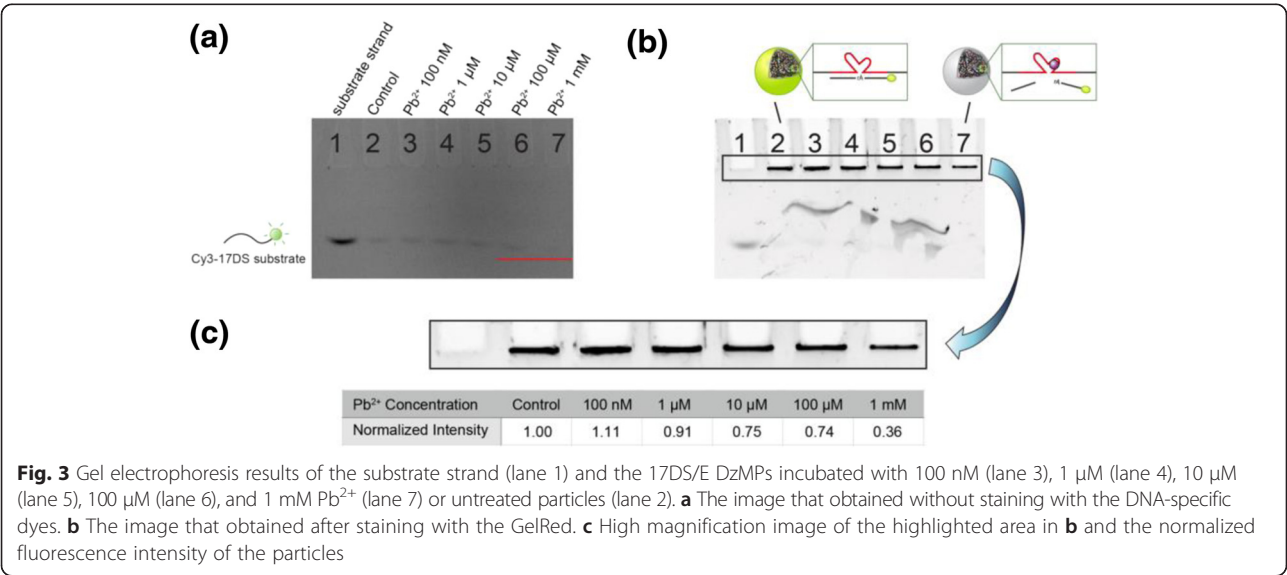


with 1 mM Pb^{2+} (lane 7) and the cleaved substrate band was also observed (see Additional file 1: Figure S2). In addition, the previous gel was stained with GelRed to confirm the particles. Since the DzMPs have a size larger than the pore size, the particles were found within the wells (Fig. 3b). The intensities of the bands from lane 2 to lane 6 reveal little difference. On the other hand, in lane 7, the intensity of the band significantly decreased. This is probably due to the fact that the bulk of cleaved substrate strands were separated from the 17E DzMPs because of the catalytic activity of DNAzyme in the presence of Pb^{2+} . For a quantitative study, the normalized intensities have shown in Fig. 3c. Under the concentration of 1 μ M Pb^{2+} , the differences between the intensities of untreated and Pb^{2+} -treated samples were not substantial. However, the intensities were reduced up to about 25 % in the case of the concentration of 10 and 100 μ M. In addition, the 1 mM treated particles showed an appreciable decrease of 64 % in the gel electrophoresis band intensity. Also, a gradual decrease in intensity of the

DzMPs was demonstrated by additional experiment between 20 and 200 μ M Pb^{2+} (see Additional file 1: Figure S3, Tables S1 and S2). These results suggest that the detection of Pb^{2+} was successfully achieved in the presence of Pb^{2+} .

Simple Detection of Pb^{2+} Using a Fluorescence Microscope

In order to develop a more convenient detection system, we designed the dual-labeled 17DS substrate. The substrate strand was labeled with a 6-carboxyfluorescein fluorophore (6-FAM) at the 5'-end and a Cy5 at the 3'-end (6FAM/Cy5-17DS). Therefore, more precise observations are available since the particles emit two different colors at once. To determine the proper concentration, different concentrations (10 nM, 100 nM, 1 μ M) of 6FAM/Cy5-17DS were hybridized with the 17E DzMPs. The fluorescence intensities of the 6FAM/Cy5-17DS/E DzMPs were also measured by image cytometry. The results show that the number of the particles in the right upper quadrant



was gradually increased as the concentration of 6FAM/Cy5-17DS increased (Fig. 4a). Especially, the scatterplot of 1 μM of substrate showed a clear linear form, and thus the DzMPs hybridized with 1 μM of 6FAM/Cy5-17DS (6FAM/Cy5-17DS/E DzMPs) were selected for the detection experiment. For the detection using a fluorescence microscope, 1 μM and 1 mM Pb^{2+} solution were investigated. As shown in Fig. 4b, it is confirmed that the untreated 6FAM/Cy5-17DS/E DzMPs emit both green and red fluorescence. There was not a particular change in the case of 1 μM Pb^{2+} ; however, when the 1 mM Pb^{2+} ions were treated, the number of fluorescent particles remarkably decreased. This means

that the 6FAM/Cy5-17DS substrates were cleaved in the presence of Pb^{2+} and the cleaved substrates were separated from the DzMPs, leading to the reduction in fluorescence intensity. In addition, there were several fluorescent particles which have larger size than untreated control. It was probably caused by aggregation of particles with high concentration of positive charge ion. From these changes, a simple detection of lead ions could be achieved by the DzMP-based detection system.

Conclusions

In conclusion, the DNzyme microparticle having numerous DNzyme sites was successfully synthesized.

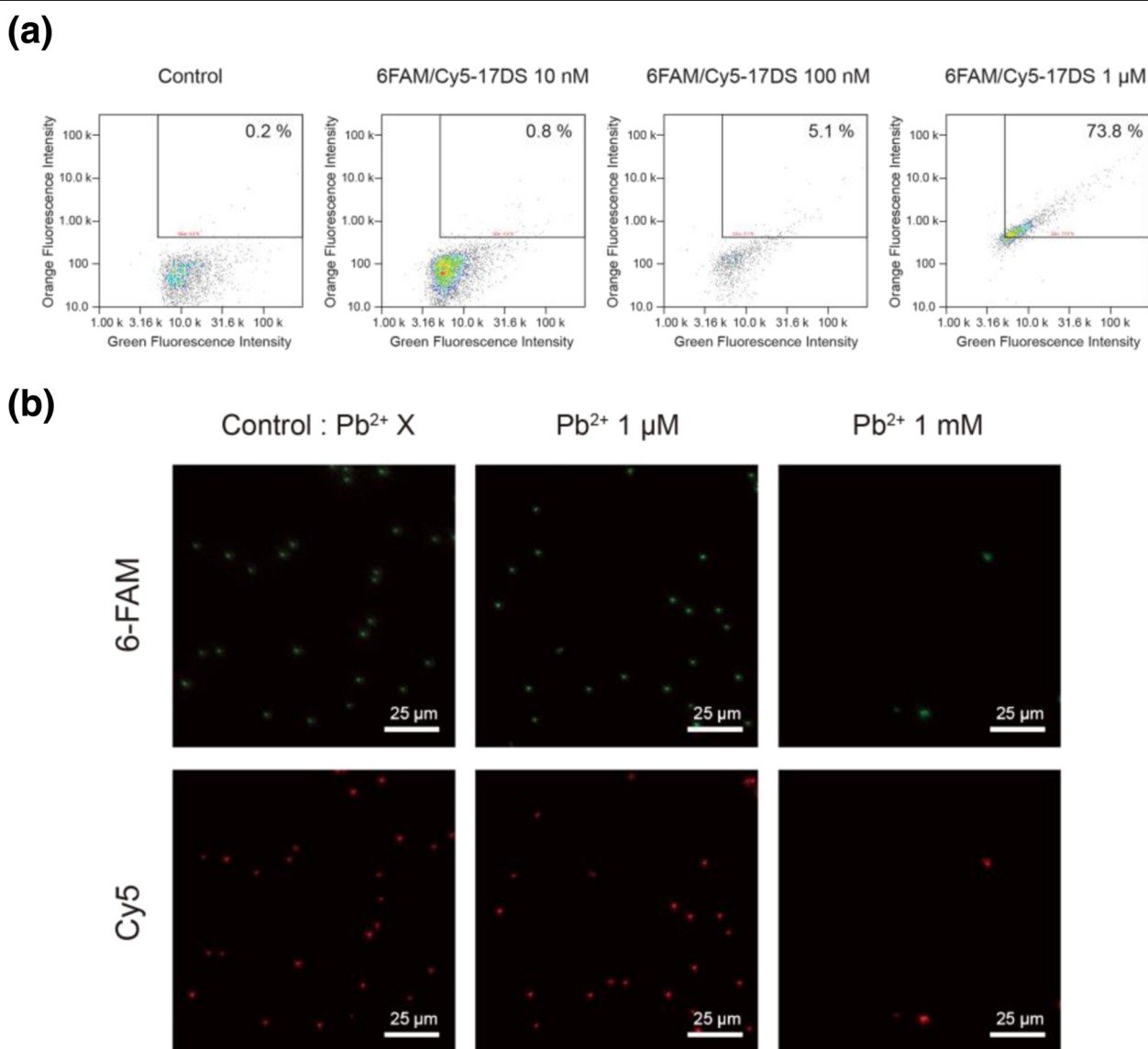


Fig. 4 **a** Image acquisition cytometry plots of the 17E-17DS particles for the different concentrations of the 6FAM/Cy5-17DS substrates. **b** Fluorescence microscopy images of the 6FAM/Cy5-17DS/E DzMPs in the presence of different concentrations of Pb^{2+} . The particles which are hybridized with the 1 μM of 6FAM/Cy5-17DS were incubated with lead ions at room temperature for 1 h

DNAzyme activity of the particles was confirmed by gel electrophoresis and quantification analysis and as low as 20 μM Pb^{2+} could be detected. Furthermore, dual-labeled substrate was designed for the simple detection platform. By utilizing the DNAzyme microparticles, the detection of lead ions was demonstrated based on the Pb^{2+} -dependent DNAzyme chemistry. This strategy offers a simple and intuitive detection method with a detection limit of 1 mM Pb^{2+} and it could be applied in environmental applications [26, 27]. Although the sensitivity of this platform should be improved, the DNAzyme microparticles have a lot of advantages such as a large surface area, a high density of catalytic sites, and stability against nuclease [28]. Moreover, the functionality of DzMPs can be modified to contain diverse DNAzymes against a target in cancer cells by controlling sequences. Therefore, this catalytic DNA microparticles may well be useful not only as a detector but as a therapeutic medicine.

Additional file

Additional file 1: Figure S1. SEM images of 17DS/E DzMPs (left) and high magnification image (right). The 17E DzMPs were annealed with the 5 μM of Cy3-17DS. **Figure S2.** gel image showing cleavage activity of DNAzyme microparticles with different concentrations of Pb^{2+} after 1 h reaction. Lanes 1 and 2 indicate 17DS substrate strand and DzMPs without Pb^{2+} treatment. Lanes 3–7 correspond to 100 nM, 1 μM , 10 μM , 100 μM , and 1 mM Pb^{2+} , respectively. Gel electrophoresis was carried out on a 3 % agarose gel at 95 V at 4 °C in 1× TAE buffer (40 mM Tris, 1 mM EDTA, 40 mM acetic acid) for 80 min. The gel image was obtained without DNA-specific dyes. **Figure S3.** fluorescence intensities of the Pb^{2+} -treated DzMPs. Note: the slopes of the fitted linear plots were given as 1. **Table S1.** the slope and intercept of the linear fitting graphs. **Table S2.** the slope and intercept of the linear fitting graphs. (PDF 1278 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JK and JBL designed and analyzed the data. JK performed the experiments. JK and JBL wrote the manuscript. Both authors read and approved the final manuscript.

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References

- Breaker RR, Joyce GF (1994) A DNA enzyme that cleaves RNA. *Chem Biol* 1(4):223–229
- Schubert S, Guél DC, Grunert HP, Zeichhardt H, Erdmann VA, Kurreck J (2003) RNA cleaving '10-23' DNAzymes with enhanced stability and activity. *Nucleic Acids Res* 31(20):5982–5992
- Silverman SK (2005) In vitro selection, characterization, and application of deoxyribozymes that cleave RNA. *Nucleic Acids Res* 33(19):6151–6163
- Cuenoud B, Szostak JW (1995) A DNA metalloenzyme with DNA ligase activity. *Nature* 375(6532):611–614
- Flynn-Charlebois A, Wang Y, Prior TK, Rashid I, Hoadley KA, Coppins RL et al (2003) Deoxyribozymes with 2'-5' RNA ligase activity. *J Am Chem Soc* 125(9):2444–2454
- Li Y, Breaker RR (1999) Phosphorylating DNA with DNA. *Proc Natl Acad Sci* 96(6):2746–2751
- Sheppard TL, Ordoukhanian P, Joyce GF (2000) A DNA enzyme with N-glycosylase activity. *Proc Natl Acad Sci* 97(14):7802–7807
- Breaker RR, Joyce GF (1995) A DNA enzyme with Mg^{2+} -dependent RNA phosphoesterase activity. *Chem Biol* 2(10):655–660
- Carmi N, Breaker RR (2001) Characterization of a DNA-cleaving deoxyribozyme. *Bioorgan Med Chem* 9(10):2589–2600
- Moshe M, Elbaz J, Willner I (2009) Sensing of UO_2^{2+} and design of logic gates by the application of supramolecular constructs of ion-dependent DNAzymes. *Nano Lett* 9(3):1196–1200
- Torabi S-F, Wu P, McGhee CE, Chen L, Hwang K, Zheng N et al (2015) In vitro selection of a sodium-specific DNAzyme and its application in intracellular sensing. *Proc Natl Acad Sci* 112(19):5903–5908
- Zhou W, Saran R, Chen Q, Ding J, Liu J (2016) A new Na^+ -dependent RNA-cleaving DNAzyme with over 1000-fold rate acceleration by ethanol. *ChemBioChem* 17(2):159–163
- Huang P-JJ, Lin J, Cao J, Vazin M, Liu J (2014) Ultrasensitive DNAzyme beacon for lanthanides and metal speciation. *Anal Chem* 86(3):1816–1821
- Huang P-JJ, Vazin M, Matuszek Z, Liu J (2014) A new heavy lanthanide-dependent DNAzyme displaying strong metal cooperativity and unrescuable phosphorothioate effect. *Nucleic Acids Res*. doi:10.1093/nar/gku1296
- Zhang X-B, Wang Z, Xing H, Xiang Y, Lu Y (2010) Catalytic and molecular beacons for amplified detection of metal ions and organic molecules with high sensitivity. *Anal Chem* 82(12):5005–5011
- Wu P, Hwang K, Lan T, Lu Y (2013) A DNAzyme-gold nanoparticle probe for uranyl ion in living cells. *J Am Chem Soc* 135(14):5254–5257
- Wang F, Wu Z, Lu Y, Wang J, Jiang J-H, Yu R-Q (2010) A label-free DNAzyme sensor for lead (II) detection by quantitative polymerase chain reaction. *Anal Biochem* 405(2):168–173
- Shen L, Chen Z, Li Y, He S, Xie S, Xu X et al (2008) Electrochemical DNAzyme sensor for lead based on amplification of DNA—Au Bio-Bar codes. *Anal Chem* 80(16):6323–6328
- Wang Y, Irudayaraj J (2011) A SERS DNAzyme biosensor for lead ion detection. *Chem Commun* 47(15):4394–4396
- Ye S, Guo Y, Xiao J, Zhang S (2013) A sensitive SERS assay of L-histidine via a DNAzyme-activated target recycling cascade amplification strategy. *Chem Commun* 49(35):3643–3645
- Liu J, Lu Y (2004) Accelerated color change of gold nanoparticles assembled by DNAzymes for simple and fast colorimetric Pb^{2+} detection. *J Am Chem Soc* 126(39):12298–12305
- Liu J, Lu Y (2004) Colorimetric biosensors based on DNAzyme-assembled gold nanoparticles. *J Fluoresc* 14(4):343–354
- Zhang H, Lin L, Zeng X, Ruan Y, Wu Y, Lin M et al (2016) Magnetic beads-based DNAzyme recognition and AuNPs-based enzymatic catalysis amplification for visual detection of trace uranyl ion in aqueous environment. *Biosens Bioelectron* 78:73–79
- Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D (2006) Enzyme-catalysed assembly of DNA hydrogel. *Nat Mater* 5(10):797–801
- Lee JB, Peng S, Yang D, Roh YH, Funabashi H, Park N et al (2012) A mechanical metamaterial made from a DNA hydrogel. *Nat Nanotechnol* 7(12):816–820
- Gichner T, Žnidar I, Száková J (2008) Evaluation of DNA damage and mutagenicity induced by lead in tobacco plants. *Mutat Res Genet Toxicol Environ Mutagen* 652(2):186–190
- Prasad M, Strzalka K (2002) Physiology and biochemistry of metal toxicity and tolerance in plants. 1st ed. Dordrecht: Kluwer Academic
- Zhu G, Hu R, Zhao Z, Chen Z, Zhang X, Tan W (2013) Noncanonical self-assembly of multifunctional DNA nanoflowers for biomedical applications. *J Am Chem Soc* 135(44):16438–16445