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**Desulfurization Activated Phosphorothioate DNAzyme for the Detection of
Thallium**

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Abstract.

Thallium (Tl) is a highly toxic heavy metal situated between mercury and lead in the periodic table. While its neighbors have been thoroughly studied for DNA-based sensing, little is known about thallium detection. In this work, in vitro selection of RNA-cleaving DNAzymes is carried out using Tl^{3+} as the target metal cofactor. Both normal DNA and phosphorothioate (PS) modified DNA are tested for this purpose. While no Tl^{3+} -dependent DNAzymes are obtained, a DNA oligonucleotide containing a single PS-modified RNA nucleotide is found to cleave by ~7% by Tl^{3+} at the RNA position. The remaining 93% are desulfurized. By hybridizing this PS-modified oligonucleotide with the Tm7 DNAzyme, the cleavage yield increases to ~40% in the presence of Tl^{3+} and Er^{3+} . Tm7 is an Er^{3+} -dependent RNA-cleaving DNAzyme. It cleaves only the normal substrate but is completely inactive using the PS-modified substrate. Tl^{3+} desulfurizes the PS substrate to the normal substrate to be cleaved by Tm7 and Er^{3+} . This system is engineered into a catalytic beacon for Tl^{3+} with a detection limit of 1.5 nM, which is below its maximal contamination limit defined by the US Environmental Protection Agency (10 nM).

Introduction

DNA is an excellent platform for developing metal sensors.¹⁻⁴ Some metal ions (e.g. Hg^{2+} , Ag^+ , and Pb^{2+}) interact with specific nucleobases and thus can fold certain DNA sequences.^{5,6} Some (e.g. Na^+ ,⁷ Pb^{2+} ,⁸⁻¹² Zn^{2+} ,¹³ Cu^{2+} ,¹⁴⁻¹⁶ UO_2^{2+} ,^{17,18} Hg^{2+} ,^{19,20} and lanthanide ions,²¹⁻²³) can act as cofactors in DNAzyme-based catalysis. These interactions are the main basis of the current metal sensing strategies using DNA. However, a third type of metal ions also exist; they do not have much interaction with DNA, making it difficult to use the traditional methods. In this work, we found that thallium (Tl) is such an example.

Thallium sits between mercury and lead in the periodic table. Unlike its two neighbors, Tl is often overlooked. Tl exists in very low concentrations in the environment, yet is highly toxic. Tl poisoning causes hair loss even at low exposure and can further damage the nervous system and other organs.²⁴ The maximal contamination level of thallium in water, as defined by the US Environmental Protection Agency (EPA), is 2 $\mu\text{g/L}$ (10 nM),²⁵ which is identical to that of mercury. For comparison, lead is better tolerated (15 $\mu\text{g/L}$) in terms of its toxicity. These numbers confirm Tl's high toxicity. Since most Tl salts are colorless and tasteless, thallium is often called 'the perfect poison' and has indeed been used in a number of murder cases.^{26,27}

Despite its toxicity, only a few electrochemistry-based sensors have been reported for detecting thallium.^{28,29} These sensors often suffer from limited sensitivity and interference from other metals. The standard analytical methods involve atomic absorption, emission, and mass spectrometry, which are costly and cannot achieve onsite detection. In addition, it is more difficult to detect thallium than other heavy metals using these instrument methods.^{25,30}

Following the past success, we are interested in testing whether DNA can also be used for thallium detection. Information on the interaction between Tl salts and DNA is quite limited.

Some Tl containing complexes are reported to cleave DNA.³¹ Tl⁺ interacts with guanine quadruplex,³²⁻³⁶ while little is known about Tl³⁺.³¹ Herein, we present our efforts in searching Tl³⁺-dependent DNazymes. In this process, we discovered that Tl³⁺ has little interaction with DNA. Thus, a new method was developed taking advantage of its strong thiophilicity. A phosphorothioate (PS) modified RNA substrate is used to inhibit the activity of a DNazyme. Tl³⁺ can convert the inactive PS substrate into the active form, thus producing a signal.

Materials and Methods

Chemicals. DNA samples for in vitro selection and fluorophore/quencher-labeled DNA were from Integrated DNA Technologies (IDT, Coralville, IA). Unmodified DNAs were from Eurofins (Huntsville, AL). The details of the DNA names, sequence, and modifications are listed in Table S1. Thallium chloride hydrate and other metal salts at their highest purity were from Sigma-Aldrich. The solutions were made by directly dissolving the metal salts in Milli-Q water. Tris(hydroxymethyl)aminomethane (Tris), 2-(N-morpholino)ethanesulfonic acid (MES), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), EDTA disodium salt dihydrate, NaCl, acetonitrile, methanol, and ammonium acetate were from Mandel Scientific Inc. (Guelph, ON). SsoFast EvaGreen supermix was from Bio-Rad. T4-DNA ligase, dNTP mix, and Taq DNA polymerase, and low molecular weight DNA ladder were from New England Biolabs.

In vitro selection. In vitro selection was carried out as described previously using a library containing 50 random nucleotides (N_{50}) and a single RNA linkage (Figure 1A).²¹⁻²³ The selection buffer was 50 mM MES, pH 6.0, 25 mM NaCl (buffer A). The PCR primer sequences are in Table S1, and the metal concentration and incubation times are listed in Table S2. For each round, the cleaved products were isolated by denaturing gel electrophoresis (dPAGE) and

amplified by PCR following previously described conditions.²³ The amplified double-stranded DNA was separated into two single-stranded DNA by dPAGE and the intended band was extracted from the gel and desalted with a Sep-Pak column for the next round of selection. The last round product of each selection was cloned and sequenced.

Gel-based assays. Gel-based activity assays were performed with a final concentration of 0.7 μM of the DNAzyme complex in the presence of either 10 or 100 μM metal ion in buffer B (25 mM NaCl, 50 mM MOPS, pH 7.5). The reactions were quenched by the 1 \times gel loading buffer containing 8 M urea. The products were then separated on 15% dPAGE gels and analyzed using a Bio-Rad ChemiDoc MP imaging system.

Desulfurization, isomerization and cleavage assay. The PS-modified DNAzyme complex was prepared by annealing the FAM-labeled PS substrate and the Tm7 enzyme with a molar ratio 1:1.5 in buffer B. The normal PO DNAzyme complex was prepared in the same way. 1 μM PS-modified substrate strand or DNAzyme complex was incubated with either 100 μM Hg^{2+} or Tl^{3+} for 2 h. The samples were then desalted using a Sep-Pak column. Concentrated DNAzyme samples were further separated in 10% dPAGE. Uncleaved substrate strands were extracted and purified using a Sep-Pak column. The purified substrate strands were used to form a duplex with its cDNA, or complexes with the 17E or Tm7 DNAzymes, which were used for gel-based kinetic assays as described above.

Tl^{3+} sensing. The sensing kinetics studies were carried out using a microplate reader (SpectraMax M3). The sensor complex was prepared by annealing 10 μM FAM-labeled PS substrate and 15 μM quencher-labeled enzyme in buffer C (150 mM NaCl, 50 mM HEPES, pH 7.5). In each well, 100 μL of the sensor complex (final concentration: 20 nM) was dissolved in 10 mM HEPES (pH 7.5) in the presence of 300 nM Er^{3+} . 1 μL of metal ion was added after 5

min of background reading and the signaling kinetics were monitored. Most assays were run in triplicates and error bars represent the standard deviation.

Results and Discussion

In vitro selection. To obtain a DNA probe for Tl^{3+} , we first resorted to in vitro DNAzyme selection since thallium's neighbors in the periodic table have been successfully used for DNAzyme catalysis. For example, Hg^{2+} can directly activate RNA-cleaving DNAzymes,²⁰ and it was also used to study ribozymes.³⁷ Pb^{2+} is also an efficient cofactor for RNA cleavage.^{8, 38, 39} Therefore, we suspect that Tl^{3+} might have a similar activity as well. Since little is known about the interaction between DNA and Tl^{3+} , we first carried out an inhibition assay to establish an appropriate metal concentration range. The 17E DNAzyme is a well-characterized system and it can be cleaved by many divalent metals.^{38, 40, 41} We monitored the activity of 17E with Pb^{2+} in the presence of Tl^{3+} (Figure 1E). Cleavage was not affected with up to 100 μM Tl^{3+} , and 50% cleavage was still achieved even with 1 mM Tl^{3+} . This indicates that Tl^{3+} can be used at high concentrations without affecting DNAzyme performance. Such a high tolerance suggests that Tl^{3+} does not interact strongly with DNA, at least for interfering with the RNA cleavage reaction. With its small ionic size and high charge, Tl^{3+} readily forms hydrolysis complexes, which may weaken its electrostatic interaction with DNA. For comparison, Hg^{2+} inhibited the 17E at greater than 10 μM (Figure 1E, the lower panel).

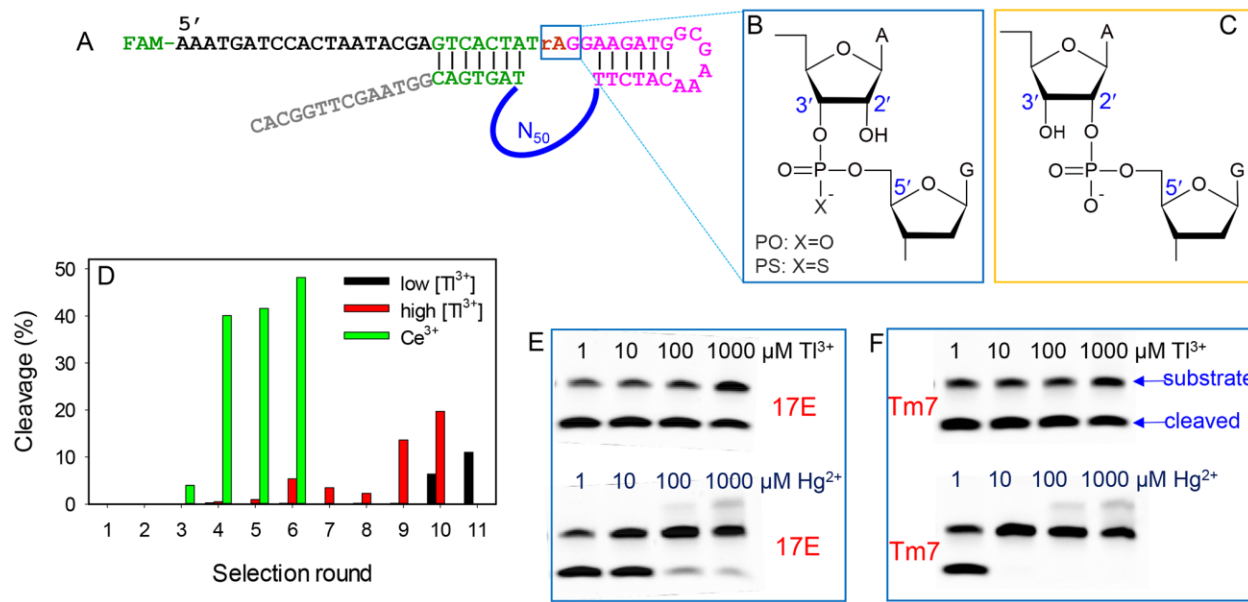


Figure 1. (A) The sequence of the DNA library used for in vitro selection containing 50 random nucleotides (N_{50}). The embedded cleavage junction is rAG. (B) The cleavage site structure. Both the PO and PS linkages site are tested. (C) A scheme of a 2'-5' linkage as a result of the isomerization reaction. (D) Progress of the three in vitro selection experiments. The Ce^{3+} selection was a positive control. Inhibition assay of (E) the 17E DNAzyme with 10 μM Pb^{2+} and (F) the Tm7 DNAzyme with 10 μM Er^{3+} in the presence of increasing concentrations of Tl^{3+} or Hg^{2+} . Hg^{2+} strongly inhibited both DNAzymes, while Tl^{3+} did not.

To promote cleavage, we started with a high concentration of Tl^{3+} (1 mM) in our selection. The DNA library (Figure 1A) contains a single RNA (rA) as the cleavage site. Sequences that can be cleaved by Tl^{3+} were harvested and amplified by PCR.²³ As a positive control, we also included a Ce^{3+} -dependent selection side-by-side. The Ce^{3+} selection produced saturated cleavage in 6 rounds (Figure 1D, green bars). However, the Tl^{3+} selection did not produce much cleavage until round 9 (red bars). When the round 10 library was treated with the selection buffer alone (no

Tl³⁺), similar cleavage was also observed (data not shown). Therefore, this library evolved in a way independent of Tl³⁺. Next we repeated the selection using a lower concentration of Tl³⁺ (50 μ M, black bars in Figure 1D), but a Tl³⁺-independent library was obtained again. It appears that Tl³⁺ may not be a good cofactor for RNA cleavage. The library was still sequenced (Table S3) and a few sequences were tested. However, none of the tested sequence (see Table S1, Tl13, Tl22, Tl27) was active with Tl³⁺.

Similar to Hg²⁺ and Pb²⁺, Tl³⁺ is also a thiophilic metal. We reason that the chance of success might increase by introducing a sulfur atom to the library. We replaced a non-bridging phosphate oxygen at the cleavage junction by sulfur, forming a phosphorothioate (PS) linkage (Figure 1B). PS modifications have been widely used to probe metal binding in ribozymes,^{37, 42, 43} make antisense DNA,⁴⁴ and assemble nanomaterials.⁴⁵⁻⁴⁷ Using this strategy, we recently isolated a Cd²⁺-specific DNAzyme successfully.⁴⁸ However, when the PS-modified library was used, significant cleavage occurred even in round 1. This non-specific cleavage is due to the extremely strong thiophilicity of Tl³⁺ (similar to Hg²⁺).^{49, 50} Therefore, selection using the PS library cannot proceed either. Although our attempts for selection failed, important DNA/Tl³⁺ interactions were ascertained from this process: 1) Tl³⁺ does not interact strongly with normal DNA (this is different from Hg²⁺), and 2) Tl³⁺ is highly thiophilic (similar to Hg²⁺). With these understandings, we turned to rational sensor design.

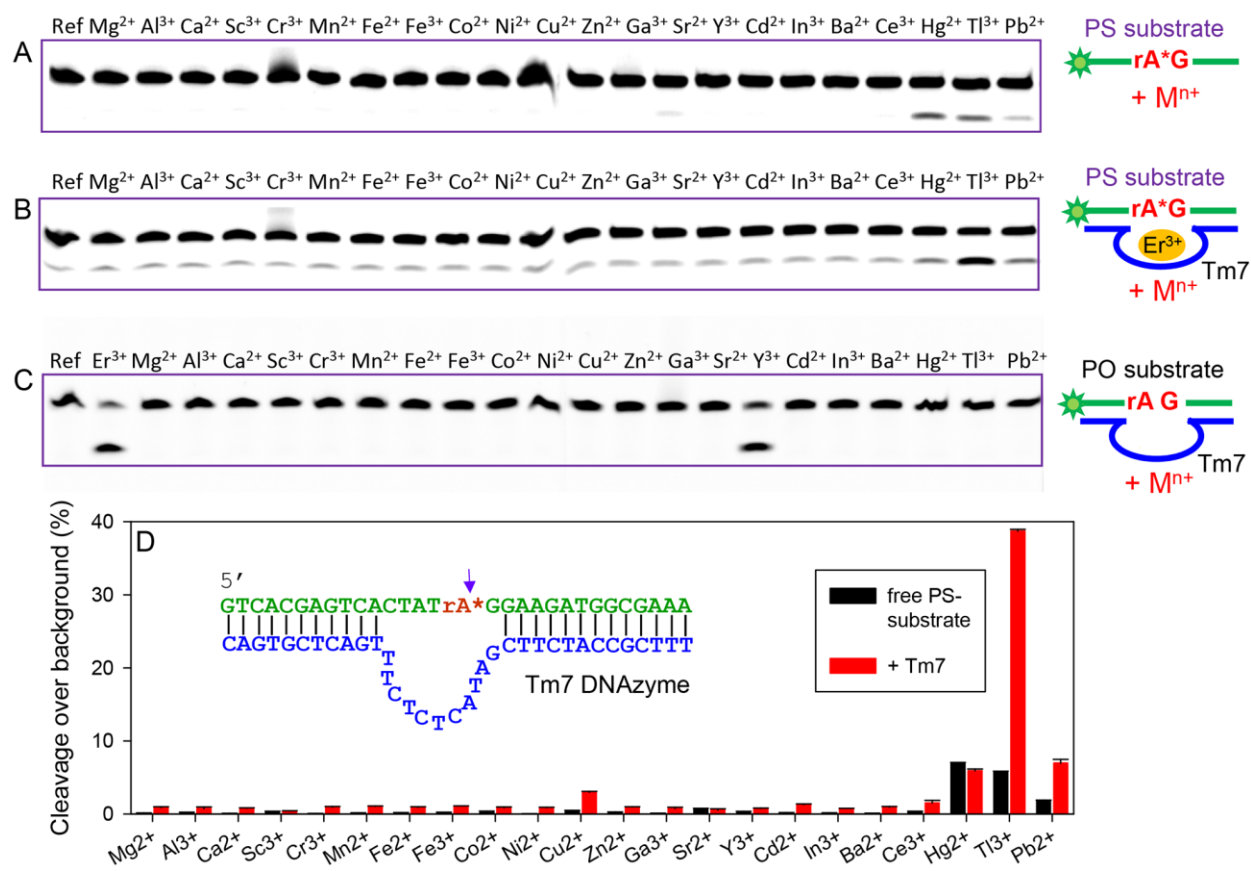


Figure 2. Gel images showing FAM-labeled substrate cleavage in the presence of 10 μM various metal ions with (A) the free PS substrate, (B) the PS substrate/Tm7 complex containing 10 μM Er^{3+} , and (C) the PO substrate/Tm7 complex. (D) Quantification of cleavage in (A) and (B) after background subtraction (reacted with 10 μM of each metal ion). Inset: the secondary structure of the Tm7 DNAzyme, which is active with the normal PO substrate in the presence of Er^{3+} , but inactive with the PS substrate. The reactions were carried out in buffer B.

Cleavage of the PS substrate. Since Tl^{3+} cleaves the PS library, we systematically studied this reaction with a FAM-labeled DNA containing a single PS RNA linkage (named PS-Sub). This

substrate was respectively incubated with 10 μM of various metal ions at pH 7.5 (Figure 2A). A small fraction of cleavage was observed with Hg^{2+} (~8%), Tl^{3+} (~7%) and Pb^{2+} (~2%, Figure 2D, black bars). The effect of pH on this reaction was also studied, and Hg^{2+} achieved more cleavage at lower pH (Figure S1), which is consistent with our previous study.⁵⁰ While Tl^{3+} can cleave this DNA, it is not the only metal capable of performing this reaction. It results in a low cleavage yield. Therefore, sensors directly based on this simple cleavage reaction may suffer from interference and also low signal.

To characterize the reaction product, a shortened and non-labeled PS RNA substrate was treated with Tl^{3+} and then analyzed by mass spectrometry (Figure S2). After the treatment, the peaks from the original PS substrate completely disappeared. We have identified cleaved fragments as well as the full-length desulfurized products, where the PS linkage was converted to a normal phosphate (PO) linkage.

Based on this observation, we reason that the yield of Tl^{3+} -induced cleavage can be increased by hybridizing the PS substrate to a DNAzyme that cleaves the normal PO substrate. After reacting with Tl^{3+} , a small fraction of the PS substrate is directly cleaved, and those converted to PO can still be cleaved by the DNAzyme. The candidate DNAzyme needs to have no activity with the PS substrate in the absence of Tl^{3+} , but highly active once the PS substrate is converted to PO. Most known DNAzymes, however, do not meet this requirement. A PS substrate is a mixture of two diastereomers (R_p and S_p). For most DNAzymes that cleave PO substrates, the R_p form of the PS substrate is usually inactive, but the S_p form can still be effectively cleaved (only with slightly decreased activity).^{42, 51, 52} Such cleavage causes background signal in the sensor. To avoid chiral separation, an ideal DNAzyme needs to be inactive with both isomers.

Fortunately, we recently discovered the Tm7 DNAzyme (see the inset of Figure 2D for structure).²¹ Tm7 cleaves the normal PO substrate only in the presence of heavy lanthanide ions. For example, Figure 2C shows that the PO substrate is cleaved only by Er^{3+} and Y^{3+} . The cleavage activity is completely lost with the PS-modified substrate (either R_p or S_p),²¹ which is attributed to the multiple lanthanide ions needed to simultaneously bind both non-bridging oxygen atoms. Therefore, it might be possible to combine Tm7 and the PS substrate to achieve Tl^{3+} detection.

To test this idea, we hybridized the Tm7 DNAzyme with the PS substrate in the presence of 10 μM Er^{3+} (Figure 2B), since Er^{3+} is the most efficient metal cofactor for Tm7.²¹ The cleavage was then measured after adding various metal ions. All the samples, including the control, showed ~5% cleavage (Figure 2B). This is attributed to the PO impurity in the PS substrate which has been previously characterized.²¹ Indeed, the cleavage yield in the presence of Tl^{3+} has increased to ~40% (Figure 2D, red bars). Surprisingly, the cleavage in the presence of Hg^{2+} remained unchanged, although Hg^{2+} can also desulfurize the PS RNA. Therefore, the Tm7 DNAzyme might provide a solution for Tl^{3+} detection.

Mechanistic studies. While selectivity for Tl^{3+} and cleavage yield are drastically improved using Tm7, a few fundamental questions need to be answered: 1) Why did Tl^{3+} only show 40% cleavage instead of full cleavage? 2) Why did Hg^{2+} fail to show more activity, and what caused the difference between Tl^{3+} and Hg^{2+} in this system? When a PS RNA is treated with a thiophilic metal, three reactions may take place.⁴⁹ The first possibility is cleavage, which only occurs by ~7% for Hg^{2+} or Tl^{3+} . The remaining 93% are desulfurized as indicated by mass spectrometry. In addition to the normal 3'-5' linkage, a fraction of the desulfurized products undergo isomerization to form the 2'-5' linkage (Figure 1C), which may not be cleaved by the DNAzyme.

Since mass spectrometry cannot tell the difference between these two types of linkages, a chemical method was designed (Figure 3A). We treated the free PS substrate, and the PS substrate/Tm7 complex respectively with Tl^{3+} or Hg^{2+} . The uncleaved products were isolated using gel electrophoresis, and then hybridized with its complementary DNA (cDNA, pH 9 with Mg^{2+}), or 17E (pH 7.5 with Mg^{2+}) or Tm7 (pH 7.5 with Er^{3+}). The cDNA treatment only cleaves the 2'-5' linkage,⁵³ while 17E only cleaves the 3'-5' linkage.^{40, 54}

After treating the free PS substrate with Hg^{2+} or Tl^{3+} , for the remaining uncleaved substrate, ~40% cleavage was observed after reacting with 17E (Figure 3B, red and green dots). This yield is about half of that from the pure 3'-5' PO substrate (black dots). Therefore, about half of the uncleaved substrates are in the 3'-5' linkage while the other half might become 2'-5' linkages. There is no selectivity for Hg^{2+} or Tl^{3+} for this reaction. If the Tm7 DNAzyme was used (Figure 3C) instead of 17E, a very similar reaction pattern was still obtained, suggesting that Tm7 also only cleaves the 3'-5' linkage. To confirm that the remaining ones are indeed 2'-5' linkages, we employed a cDNA reaction (Figure 3D). Indeed cleavage was observed for the Hg^{2+} or Tl^{3+} treated samples, but the normal 3'-5' PO substrate failed to cleave. Therefore, for the free substrate, both standard desulfurization and isomerization can take place.

Next, the same experiment was repeated but the PS substrate was hybridized to the Tm7 DNAzyme first before adding Hg^{2+} or Tl^{3+} (no Er^{3+} added). The cleavage probed by Tm7 (Figure 3F) was ~10% higher for the Tl^{3+} treated substrate than the Hg^{2+} treated one. When this sample was treated with the cDNA, the cleavage was ~10% more for Hg^{2+} (Figure 3G). Therefore, Tm7 has indeed favored Tl^{3+} by forming more uncleavable 2'-5' linkages in the presence of Hg^{2+} . However, this difference is not large enough to account for the excellent selectivity for Tl^{3+} in Figure 2B.

We reason that the remaining difference might be from the stronger inhibition effect of Hg^{2+} , which was already observed for its inhibition of 17E (Figure 1E). We then carried out an inhibition assay of Tm7 in the presence of various concentrations of Tl^{3+} and Hg^{2+} (Figure 1F). Tm7 was hybridized with the normal PO substrate and then incubated with various concentrations of Hg^{2+} or Tl^{3+} before adding Er^{3+} .²¹ The inhibition effect of Hg^{2+} was indeed very strong for Tm7, and no cleavage was observed even with 10 μM Hg^{2+} . On the other hand, the activity was not significantly affected by the addition of Tl^{3+} . To further confirm this, we added mercaptohexanol (MCH) into the mixture of PS substrate/Tm7/ Er^{3+} with Hg^{2+} or Tl^{3+} . In this case, the cleavage in the Tl^{3+} sample remained constant (Figure 3E, triangles), while the Hg^{2+} sample cleavage increased to nearly 40% (squares). MCH strongly binds Hg^{2+} but it does not affect Tm7 cleaving the PO substrate (dots). Therefore, the lack of cleavage of the PS substrate by Tm7 and Er^{3+} in the presence of Hg^{2+} is attributed to both the inhibition effect of Hg^{2+} and its tendency to form the inactive 2'-5' linkage.

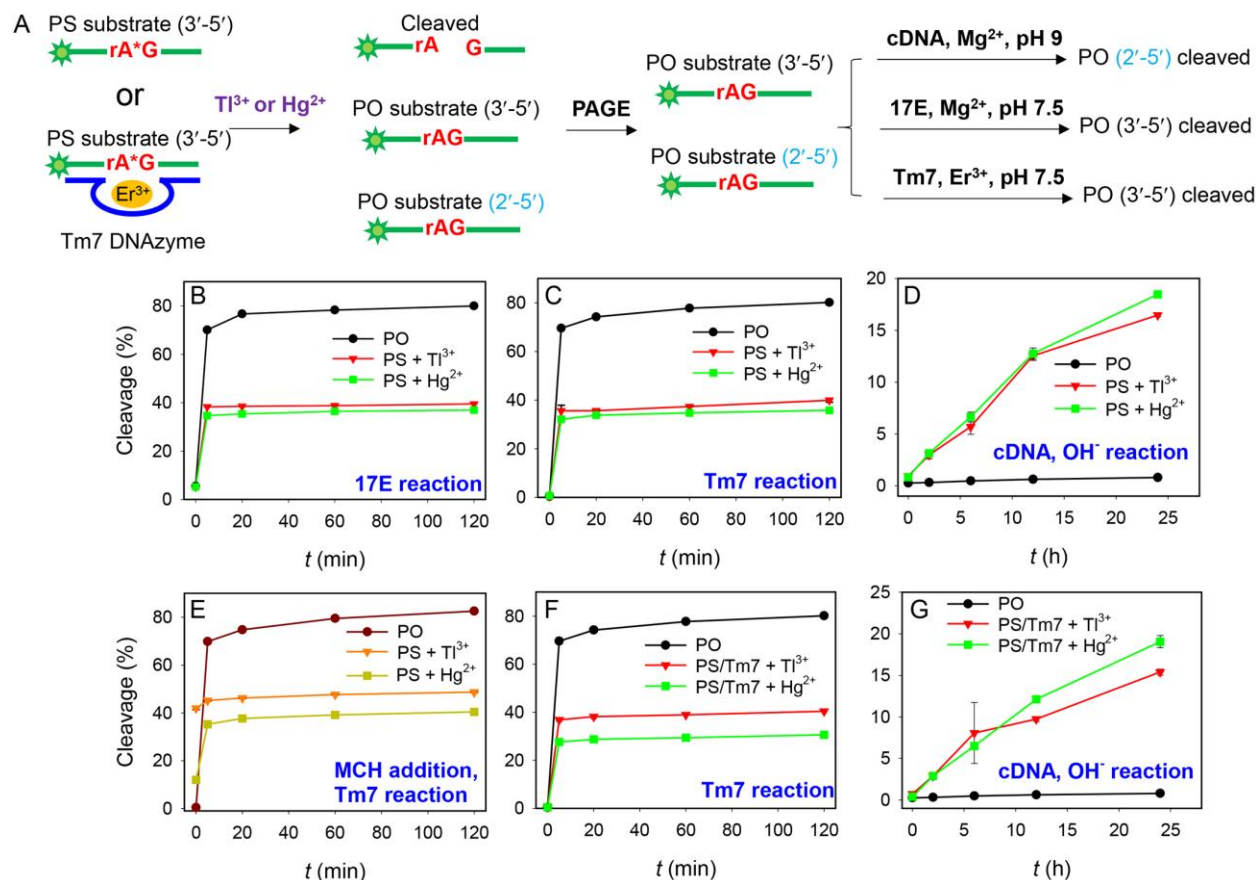


Figure 3. (A) Experiment design of probing the cleavage, desulfurization, and isomerization reactions. After treating the free PS substrate with (100 μM) Hg^{2+} or Ti^{3+} , the uncleaved substrate is harvested and then reacted with (B) 17E (10 mM Mg^{2+} , pH 7.5), (C) Tm7 (10 μM Er^{3+} , pH 7.5), or (D) cDNA (100 mM Mg^{2+} , pH 9.0 at 37 $^{\circ}\text{C}$). After treating the PS substrate/Tm7 complex with (100 μM) Hg^{2+} or Ti^{3+} , the uncleaved substrate is harvested and then reacted with (F) Tm7 (10 μM Er^{3+} , pH 7.5), or (G) cDNA (100 mM Mg^{2+} , pH 9.0 at 37 $^{\circ}\text{C}$). (E) To the reaction mixture containing the PS substrate/Tm7/ Er^{3+} and (100 μM) Hg^{2+} or Ti^{3+} , 250 μM MCH was added and the cleavage kinetics was followed. MCH masks Hg^{2+} and cleavage was observed. The normal PO substrate/Tm7 in the presence of the same concentration of MCH was also followed.

DNAzyme characterization. Based on the above results, it appears that the PS substrate/Tm7 complex forms a good probe for Tl^{3+} . We then measured its cleavage rate (Figure 4A). This rate is a combined kinetics involving desulfurization followed by Tm7 cleavage of the resulting PO substrate. The resulting rate was a value of $\sim 0.9 \text{ min}^{-1}$ with 10 or 100 μM Tl^{3+} (10 μM Er^{3+} included). This is similar to cleavage of the PO substrate with the same concentration of Er^{3+} ($\sim 1.4 \text{ min}^{-1}$).²¹ For comparison, the same reaction in the presence of Hg^{2+} yielded only $\sim 11\%$ cleavage (Figure 4B), which also included $\sim 5\%$ background cleavage due to the PO impurity.

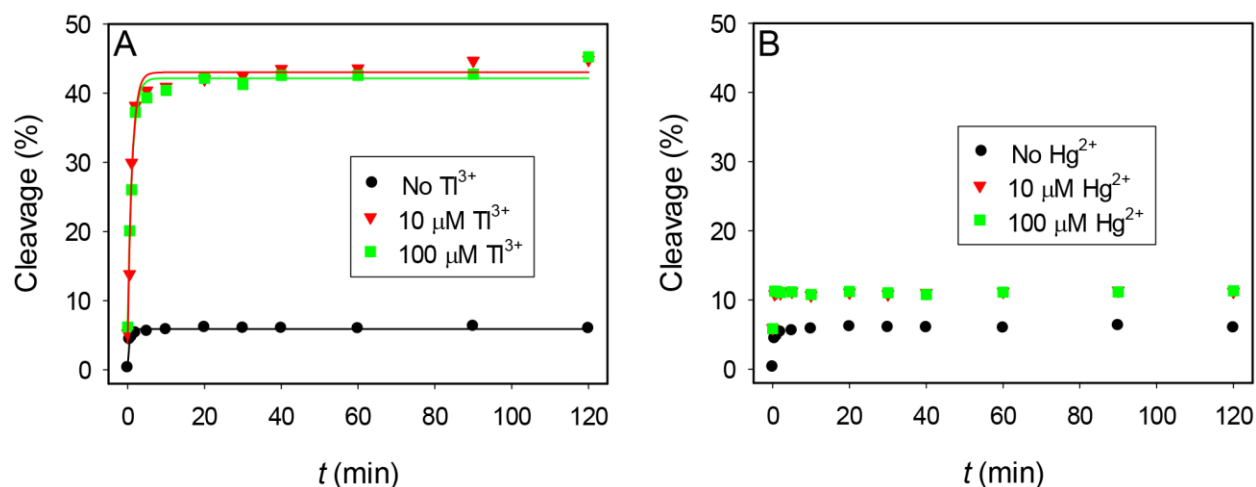


Figure 4. Cleavage kinetics of the PS substrate/Tm7 complex with 10 μM Er^{3+} and various concentrations of (A) Tl^{3+} or (B) Hg^{2+} . The cleavage without Tl^{3+} or Hg^{2+} was due to the PO impurities (black dots).

Tl^{3+} detection. Based on the above results, we designed a sensor for Tl^{3+} detection. The FAM-labeled PS substrate was hybridized to the Tm7 DNAzyme bearing a dark quencher (Figure 5A), resulting in a low fluorescence complex. See Figure S3 for DNA sequence. Er^{3+} was also included in the sensing system. Since Tm7 cannot cleave the PS substrate, the fluorescence

signal remained stable. In the presence of Tl^{3+} , the cleavage reaction is initiated (either by direct cleavage of the PS substrate or by converting to PO), leading to a Tl^{3+} -concentration dependent fluorescence enhancement (Figure 5B). The intensity of fluorescence at 10 min after adding Tl^{3+} was plotted in Figure 5C to obtain the calibration curve, where the apparent dissociation constant is calculated to be only 20 nM Tl^{3+} . The detection limit was calculated to be 1.5 nM Tl^{3+} based on the signal greater than three times of background variation. This is much lower than the EPA defined contamination level in water (10 nM),²⁵ and might be useful for monitoring Tl^{3+} in water.

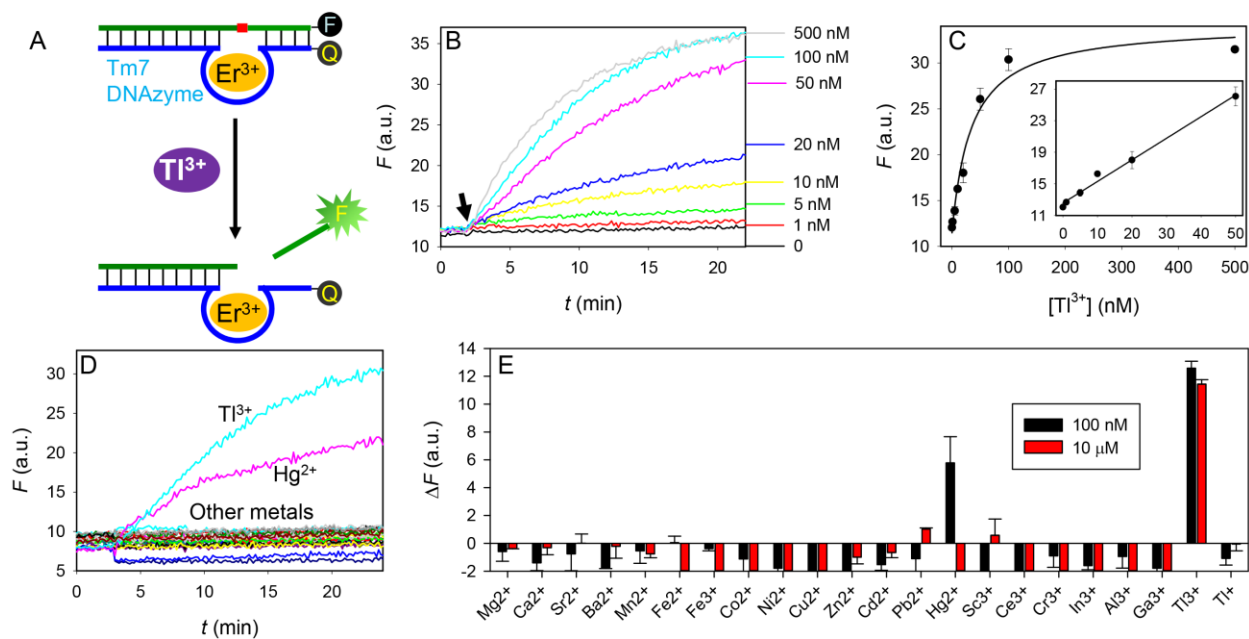


Figure 5. (A) The catalytic beacon strategy for detecting Tl^{3+} . The Tm7 DNAzyme is hybridized to the PS substrate. Er^{3+} is added as a metal cofactor for Tm7. Activity is produced in the presence of Tl^{3+} to convert the PS substrate to the PO form. (B) Sensor signaling kinetics in the presence of various concentrations of Tl^{3+} . The arrowhead points the time of Tl^{3+} addition. (C) Sensor response at 10 min after adding Tl^{3+} . Inset: sensor response at low Tl^{3+} concentrations

fitting to a straight line. (D) Sensor response to 100 nM of various metal ions (see (E) for other metals tested). (E) Fluorescence change of the sensor (10 min reaction time) after adding various metal ions at two metal concentrations.

For selectivity test, we incubated the sensor with various metals. At 100 nM metal concentration, only Hg^{2+} produced some signal other than Tl^{3+} , while at 10 μM , Tl^{3+} is the only active metal (Figure 5E). It is likely that the inhibition effect of Hg^{2+} is weak at 100 nM concentration, and Tm7 still shows cleavage activity. Hg^{2+} is an equally toxic metal, and detecting Hg^{2+} at the same time might also be analytically useful. If the distinction between Tl^{3+} and Hg^{2+} needs to be made, there are a suite of DNA-based sensors for Hg^{2+} detection that are unlikely to respond to Tl^{3+} .² Making a sensor array for detecting multiple analytes will be a topic of follow-up studies. It is interesting to note that Tl^+ did not produce any signal at both concentrations. Therefore, this sensor is selective for Tl^{3+} .

Conclusions. In summary, we carried out both in vitro selections and rational design to understand the interaction between DNA and Tl^{3+} . While in vitro selection failed to produce active DNAzymes, a PS RNA linkage was found to be cleaved by ~7% by Tl^{3+} due to its strong thiophilicity, and the remaining 93% were desulfurized. Based on this observation, we designed a highly specific probe using the Tm7 DNAzyme. Compared to Hg^{2+} , Tl^{3+} yields relatively more cleavable 3'-5' desulfurized product. At the same time, Tl^{3+} does not inhibit the Tm7 DNAzyme, while Hg^{2+} is a strong inhibitor. These differences allow us to separate these two very similar metals. Among all the DNAzymes, we chose to use Tm7 since it has no activity with the PS substrate, and cannot be rescued by thiophilic metals. This study represents the first effort to use

DNA to detect Tl^{3+} and has suggested an important role of DNAzymes in directing the outcome of metal/DNA interactions.

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Supporting Information Available: DNA sequences and selection conditions, effect of pH and sensor sequences. This information is available free of charge via the Internet on <http://pubs.acs.org/> at DOI:10.1021/acs.anal-chem.5b02568

References

- (1) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948–1998.
- (2) Zhang, X.-B.; Kong, R.-M.; Lu, Y. *Annu. Rev. Anal. Chem.* **2011**, *4*, 105-128.
- (3) Ma, D. L.; Chan, D. S. H.; Man, B. Y. W.; Leung, C. H. *Chem.-Asian J.* **2011**, *6*, 986-1003.
- (4) Li, D.; Song, S. P.; Fan, C. H. *Acc. Chem. Res.* **2010**, *43*, 631-641.
- (5) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300-4302.
- (6) Ono, A.; Cao, S.; Togashi, H.; Tashiro, M.; Fujimoto, T.; Machinami, T.; Oda, S.; Miyake, Y.; Okamoto, I.; Tanaka, Y. *Chem. Commun.* **2008**, 4825-4827.

- (7) Torabi, S.-F.; Wu, P.; McGhee, C. E.; Chen, L.; Hwang, K.; Zheng, N.; Cheng, J.; Lu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 5903-5908.
- (8) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223-229.
- (9) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466-10467.
- (10) Lan, T.; Furuya, K.; Lu, Y. *Chem. Commun.* **2010**, *46*, 3896-3898.
- (11) Wang, H.; Kim, Y.; Liu, H.; Zhu, Z.; Bamrungsap, S.; Tan, W. *J. Am. Chem. Soc.* **2009**, *131*, 8221-8226.
- (12) Xiao, Y.; Rowe, A. A.; Plaxco, K. W. *J. Am. Chem. Soc.* **2007**, *129*, 262.
- (13) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481-488.
- (14) Cuenoud, B.; Szostak, J. W. *Nature* **1995**, *375*, 611-614.
- (15) Carmi, N.; Balkhi, H. R.; Breaker, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2233-2237.
- (16) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2007**, *129*, 9838-9839.
- (17) Liu, J.; Brown, A. K.; Meng, X.; Crokek, D. M.; Istok, J. D.; Watson, D. B.; Lu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2056-2061.
- (18) Brown, A. K.; Liu, J. W.; He, Y.; Lu, Y. *ChemBioChem* **2009**, *10*, 486-492.
- (19) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 7587-7590.
- (20) Hollenstein, M.; Hipolito, C.; Lam, C.; Dietrich, D.; Perrin, D. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4346 - 4350.
- (21) Huang, P.-J. J.; Vazin, M.; Matuszek, Ż.; Liu, J. *Nucleic Acids Res.* **2015**, *43*, 461-469.
- (22) Huang, P.-J. J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 9993-9999.
- (23) Huang, P.-J. J.; Lin, J.; Cao, J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 1816-1821.

- (24) Rodriguez-Mercado, J. J.; Altamirano-Lozano, M. A. *Drug Chem. Toxicol.* **2013**, *36*, 369-383.
- (25) Peter, A. L. J.; Viraraghavan, T. *Environ. Int.* **2005**, *31*, 493-501.
- (26) Li, S.; Huang, W.; Duan, Y.; Xing, J.; Zhou, Y. *J. Forensic Sci.* **2015**, *60*, 247-251.
- (27) Tsai, Y.-T.; Huang, C.-C.; Kuo, H.-C.; Wang, H.-M.; Shen, W.-S.; Shih, T.-S.; Chu, N.-S. *Neurotoxicology* **2006**, *27*, 291-295.
- (28) Jorge, E. O.; Neto, M. M. M.; Rocha, M. M. *Talanta* **2007**, *72*, 1392-1399.
- (29) Singh, A. K.; Saxena, P. *Talanta* **2005**, *66*, 993-998.
- (30) Galvan-Arzate, S.; Santamaria, A. *Toxicol. Lett.* **1998**, *99*, 1-13.
- (31) Nowicka, A. M.; Krasnodebska-Ostrega, B.; Wrzosek, B.; Jastrzebska, M.; Sadowska, M.; Mackiewicz, M.; Stojek, Z. *Electroanalysis* **2014**, *26*, 340-350.
- (32) Ouameur, A. A.; Nafisi, S.; Mohajerani, N.; Tajmir-Riahi, H. A. *J. Biomol. Struct. Dyn.* **2003**, *20*, 561-565.
- (33) Caceres, C.; Wright, G.; Gouyette, C.; Parkinson, G.; Subirana, J. A. *Nucleic Acids Res.* **2004**, *32*, 1097-1102.
- (34) Gharib, F.; Monajjemi, M.; Ketabi, S. *Main Group Metal Chemistry* **2004**, *27*, 71-79.
- (35) Gill, M. L.; Strobel, S. A.; Loria, J. P. *J. Am. Chem. Soc.* **2005**, *127*, 16723-16732.
- (36) Gill, M. L.; Strobel, S. A.; Loria, J. P. *Nucleic Acids Res.* **2006**, *34*, 4506-4514.
- (37) Cunningham, L. A.; Li, J.; Lu, Y. *J. Am. Chem. Soc.* **1998**, *120*, 4518-4519.
- (38) Brown, A. K.; Li, J.; Pavot, C. M. B.; Lu, Y. *Biochemistry* **2003**, *42*, 7152-7161.
- (39) Brown, R. S.; Hingerty, B. E.; Dewan, J. C.; Klug, A. *Nature* **1983**, *303*, 543-546.
- (40) Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262-4266.
- (41) Schlosser, K.; Li, Y. *ChemBioChem* **2010**, *11*, 866-879.

- (42) Wang, S.; Karbstein, K.; Peracchi, A.; Beigelman, L.; Herschlag, D. *Biochemistry* **1999**, *38*, 14363-14378.
- (43) Nawrot, B.; Widera, K.; Wojcik, M.; Rebowska, B.; Nowak, G.; Stec, W. J. *FEBS J* **2007**, *274*, 1062-1072.
- (44) Deleavey, Glen F.; Damha, M. J. *Chem. Biol.* **2012**, *19*, 937-954.
- (45) Lee, J. H.; Wernette, D. P.; Yigit, M. V.; Liu, J.; Wang, Z.; Lu, Y. *Angew. Chem. Int. Ed.* **2007**, *46*, 9006-9010.
- (46) Farlow, J.; Seo, D.; Broaders, K. E.; Taylor, M. J.; Gartner, Z. J.; Jun, Y.-w. *Nat. Methods* **2013**, *10*, 1203-1205.
- (47) Ma, N.; Sargent, E. H.; Kelley, S. O. *Nat. Nanotechnol.* **2009**, *4*, 121-125.
- (48) Huang, P.-J. J.; Liu, J. *Nucleic Acids Res.* **2015**, *43*, 6125-6133.
- (49) Ora, M.; Peltomaki, M.; Oivanen, M.; Lonnberg, H. *J. Org. Chem.* **1998**, *63*, 2939-2947.
- (50) Huang, P.-J. J.; Wang, F.; Liu, J. *Anal. Chem.* **2015**, *87*, 6890-6895.
- (51) Thaplyal, P.; Ganguly, A.; Golden, B. L.; Hammes-Schiffer, S.; Bevilacqua, P. C. *Biochemistry* **2013**, *52*, 6499-6514.
- (52) Warnecke, J. M.; Fuerste, J. P.; Hardt, W.-D.; Erdmann, V. A.; Hartmann, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8924-8928.
- (53) Rohatgi, R.; Bartel, D. P.; Szostak, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 3340-3344.
- (54) Pyle, A. M.; Chu, V. T.; Jankowsky, E.; Boudvillain, M. *Meth. Enzymol.* **2000**, *317*, 140-146.