

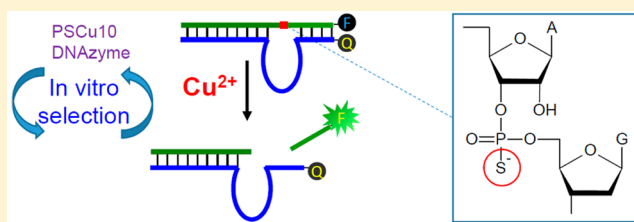
An Ultrasensitive Light-up Cu^{2+} Biosensor Using a New DNAzyme Cleaving a Phosphorothioate-Modified Substrate

Po-Jung Jimmy Huang and Juewen Liu*

Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

S Supporting Information

ABSTRACT: Cu^{2+} is a very important metal ion in biology, environmental science, and industry. Developing biosensors for Cu^{2+} is a key topic in analytical chemistry. DNAzyme-based sensors are highly attractive for their excellent sensitivity, stability, and programmability. In the past decade, a few Cu^{2+} biosensors were reported using DNAzymes with DNA cleavage or DNA ligation activity. However, they require unstable ascorbate or imidazole activation. So far, no RNA-cleaving DNAzymes specific for Cu^{2+} are known. In this work, a phosphorothioate (PS) RNA-containing library was used for in vitro selection, and a few new Cu^{2+} -specific RNA-cleaving DNAzymes were isolated. Among them, a DNAzyme named PSCu10 was studied further. It has only eight nucleotides in the enzyme loop with a cleavage rate of 0.1 min^{-1} in the presence of $1 \mu\text{M}$ Cu^{2+} at pH 6.0 (its optimal pH). Between the two diastereomers of the PS RNA chiral center, the R_p isomer is 37 times more active than the S_p one. Among the other divalent metal ions, only Hg^{2+} can cleave the substrate due to its extremely high thiophilicity. A catalytic beacon sensor was designed with a detection limit of 1.6 nM Cu^{2+} and extremely high selectivity. PSCu10 is specific for Cu^{2+} , and it has no cleavage in the presence of ascorbate, which reduces Cu^{2+} to Cu^+ .



Copper is a very common metal used for numerous applications, which has led to increasing environmental concerns. Although copper is an essential element for life (e.g., found in many protein enzymes), exposure to a high concentration of copper causes liver, gastrointestinal, and kidney damage.¹ To cope with copper contamination, improve industrial waste management, and intracellularly study the metal, effective copper sensors are needed. Many copper sensors were designed based on small molecules,^{2–4} peptides,⁵ proteins,⁶ and nanomaterials.⁷ Although they are responsive to copper ions, some suffer from strong fluorescence quenching by the paramagnetic Cu^{2+} (i.e., signal-off sensors), poor selectivity or sensitivity, or probe denaturation.

In the past two decades, DNAzymes have emerged as a very useful platform for metal sensing.^{8–11} DNAzymes are DNA-based catalysts that often require divalent metal ions for activity.^{12–14} In vitro selection has been intentionally carried out in the presence of specific metal ions to isolate sensor DNAzymes.^{15,16} A Cu^{2+} biosensor was reported using a DNAzyme that performs oxidative DNA cleavage.^{17,18} Although it reached a detection limit of 35 nM Cu^{2+} , this assay required ascorbate, which is an unstable chemical. Another Cu^{2+} -dependent DNA ligating DNAzyme was also made into Cu^{2+} sensors.^{19,20} However, this DNAzyme requires an unstable imidazole-activated substrate. Cu^{2+} was also used as a cofactor to isolate other DNAzymes,^{21,22} but it was not the sole metal in those cases.

To date, the best developed DNAzymes for metal sensing are those cleaving RNA. For example, RNA-cleaving DNAzymes specific for Na^+ ,^{23,24} Pb^{2+} ,^{25,26} Zn^{2+} ,²⁷ UO_2^{2+} ,²⁸ and lanthanide

ions^{29–31} were reported. These DNAzymes are fast, highly selective, and versatile in biosensor design. These metals are either hard or borderline Lewis acids, while it is quite challenging to obtain sensor DNAzymes for soft metals. The natural DNA has only four types of nucleobases connected by phosphates with limited chemical functionality. To isolate DNAzymes that work with thiophilic metals, modified nucleotides containing imidazole and amine groups were tested yielding new Zn^{2+} - and Hg^{2+} -specific DNAzymes.^{32,33} However, these modified nucleotides are not commercially available, and the selection process is quite technically demanding, limiting their general analytical applications.

We reason that an alternative solution is to introduce a phosphorothioate (PS) modification at the cleavage site.³⁴ A PS modification refers to replacing one of the nonbridging oxygen atoms in the phosphate by a sulfur, which is known to recruit thiophilic metals. The PS modification has been used for probing ribozyme mechanisms, designing antisense oligonucleotides, and assembling nanomaterials.^{35–40} Because Cu^{2+} is a thiophilic soft metal, we herein use a PS-containing library for selecting new DNAzymes in the presence of Cu^{2+} and develop a biosensor for Cu^{2+} detection.

Received: December 28, 2015

Accepted: February 9, 2016

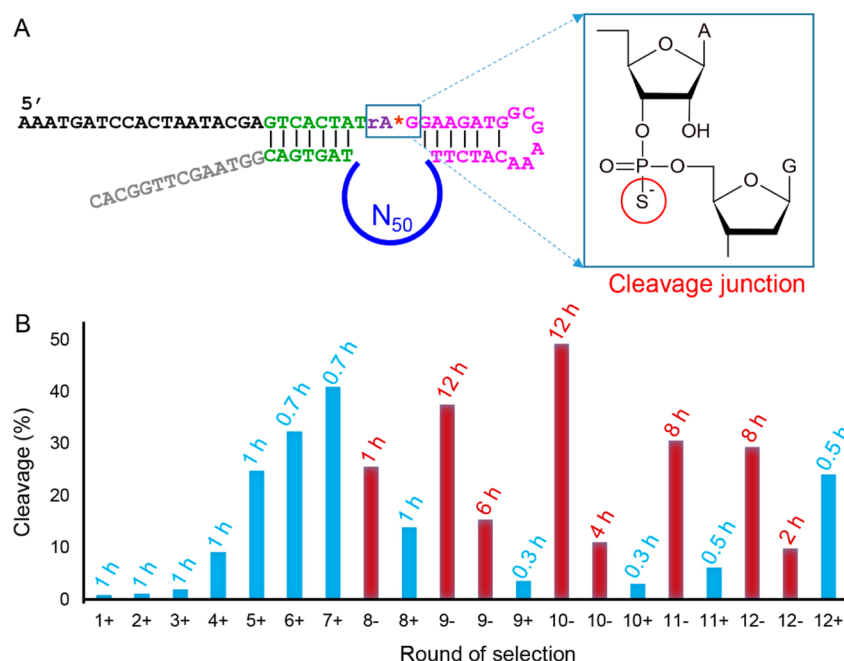


Figure 1. (A) The sequence of the library used for the in vitro selection of Cu²⁺-specific DNazymes cleaving the PS-RNA linkage (denoted by an asterisk, its structure is also given). (B) Progress of the selection. The selection rounds are marked with a plus sign and a blue bar (indicating positive selection using Cu²⁺; 50 μ M Cu²⁺ for the first 8 rounds, 20 μ M Cu²⁺ for round 9 and 10 rounds, and 10 μ M Cu²⁺ for the last 2 rounds) or with a minus sign and a red bar (indicating negative selection using a mixture of Cd²⁺, Pb²⁺, and Zn²⁺ (20 μ M each) for rounds 8–10 and Cd²⁺ alone (10 μ M) for rounds 11 and 12). In some rounds, two negative selections were performed in tandem to improve metal specificity for Cu²⁺. The incubation times of each round are also marked.

MATERIALS AND METHODS

Chemicals. The DNAs used in this work were from Integrated DNA Technologies (Coralville, IA) and Eurofins (Huntsville, AL). Note that the DNA sequences related to in vitro selection are listed in Table S1, and the other DNAs are shown in Table S2. Tris(hydroxymethyl)aminomethane (Tris), 2-(*N*-morpholino)ethanesulfonic acid (MES), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), ethylenediaminetetraacetic acid disodium salt (EDTA), sodium chloride, and ammonium acetate were from Mandel Scientific (Guelph, Ontario, Canada). 3-(*N*-Morpholino)propanesulfonic acid (MOPS), agarose M, urea, 40% acryl/bis solution (29:1), and 10 $\times$ TBE were from Bio Basic (Markham, Ontario, Canada). SsoFast EvaGreen supermix was from Bio-Rad. T4-DNA ligase, dNTP mix, Taq DNA polymerase, gel loading dye (6 $\times$), and DNA ladder were from New England Biolabs. Barium(II) chloride dihydrate, cadmium(II) chloride hydrate, calcium chloride, cobalt(II) chloride hexahydrate, copper(II) chloride dihydrate, iron(III) chloride hexahydrate, lead acetate, magnesium chloride, manganese(II) chloride tetrahydrate, mercury(II) perchlorate hydrate, nickel(II) chloride hexahydrate, tetrakis(acetonitrile)copper(I) hexafluorophosphate, and zinc chloride were from Sigma-Aldrich at the highest available purity.

In Vitro Selection. The selection has largely followed a previously reported method³⁴ except that the metal cofactor was changed to Cu²⁺. Briefly, the initial library was prepared by ligating Lib-FAM (0.2 nmol) and Lib-rA* (0.3 nmol) with a splint DNA (0.3 nmol) using T4 ligase following the vendor's protocol. The ligated DNA was purified with 10% denaturing polyacrylamide gel electrophoresis (dPAGE) and extracted from the gel with buffer A (1 mM EDTA, 10 mM Tris-HCl, pH 7.0). The full length DNA library was desalted with a Sep-Pak

C18 column (Waters) and lyophilized overnight. The library was resuspended in 60 μ L of buffer B (50 mM MES, pH 6.0, 25 mM NaCl), and 5 μ L was used for the first round of selection. For each subsequent round, the DNA library was generated from polymerase chain reaction (PCR). The selection condition for each round is listed in Figure 1B.

Biochemical Assays. In a typical assay, the DNzyme complex was formed with carboxyfluorescein (FAM)-labeled PS substrate (5 μ M) and enzyme (7.5 μ M) in buffer B. For a gel-based assay, each sample contains a final of 5 μ L of 0.7 μ M DNzyme complex. Metal salts were diluted with Milli-Q water, and 2 μ L of metal solution was added to initiate the cleavage reaction. The reaction was quenched by 8 μ L of 1 $\times$ gel loading dye containing 8 M urea at a designated time. The products were then separated on 15% dPAGE gels and analyzed using a ChemiDoc MP imaging system (Bio-Rad). All assays were run at least in duplicate. The cleavage kinetics were fit using the following equation: $y = y_0 + a(1 - \exp(-kx))$, where k is the cleavage rate.

Biosensor Assays. The sensor complex was formed by annealing the FAM-labeled PS substrate (5 μ M, after 17E treatment) and the quencher-labeled enzyme (7.5 μ M) in buffer B. The sensing kinetics were performed in a 96-well plate. For each well, 100 μ L of the sensor (final 50 nM) in buffer C (50 mM MES, pH 6.0, 5 mM NaCl) was used. One microliter of metal ion was added to initiate cleavage, and the signaling kinetics were monitored ($\text{Ex} = 485 \text{ nm}$; $\text{Em} = 520 \text{ nm}$) using a microplate reader (M3, SpectraMax).

Cu²⁺ Detection in Lake Water. Water samples from Lake Ontario were passed through a 0.2 μ m filter before analysis. The filtered water was mixed 1:1 with 50 mM MES buffer (pH 6.0). The sensing kinetics were measured in a 96-well plate with a final of 100 μ L of sample containing 50 nM sensor probe. The

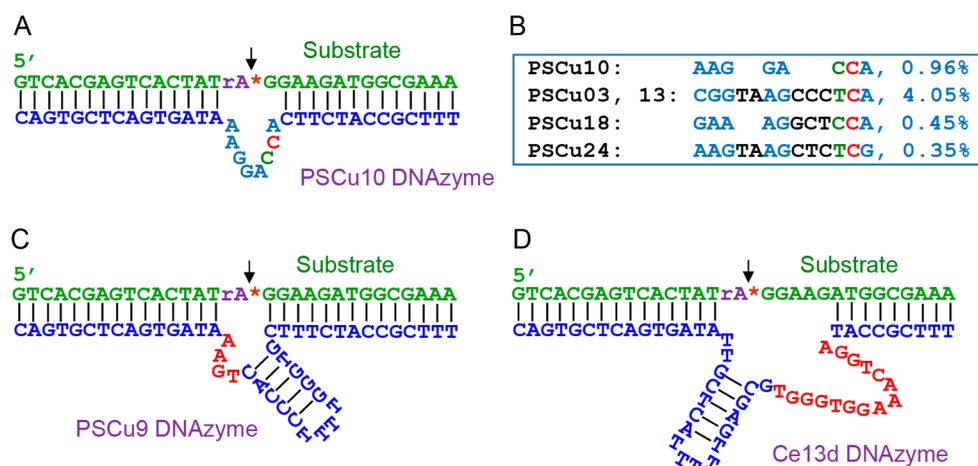


Figure 2. Secondary structures of (A) the PSCu10, (C) the PSCu9, and (D) the Ce13d DNAzymes. They all cleave the PS-RNA substrate in the presence of Cu^{2+} . (B) Alignment of the catalytic core sequences of three active DNAzymes based on PSCu10. The color code matches with that in (A). The percentage of each sequence in the final library is also shown.

sensor fluorescence background was monitored for 10 min before spiking the samples with various concentrations of Cu^{2+} .

RESULTS AND DISCUSSION

In Vitro Selection. To isolate a Cu^{2+} -specific DNAzyme, in vitro selection was carried out using a library containing 50 random nucleotides (N_{50} , Figure 1A). The embedded cleavage junction is denoted by rA*G, where rA is an RNA adenine nucleotide and the asterisk represents a phosphorothioate (PS) modification. Because RNA is approximately one-million-fold less stable compared to DNA,⁴¹ cleavage is most likely to occur at this single RNA linkage site. This PS modification is for recruiting the thiophilic Cu^{2+} to the scissile phosphate for the cleavage reaction. Because this PS modification was at the fixed region of the library, the selection method was the same as normal selections. The initial library contained $\sim 10^{13}$ random DNA sequences, and we hoped to isolate a subpopulation that can selectively use Cu^{2+} for cleavage.

We previously reported a DNAzyme named Ce13d that cleaves the normal phosphodiester RNA linkage in the presence of trivalent lanthanides.³¹ With the PS modification at the cleavage site, Ce13d becomes active with all thiophilic metals, including Cu^{2+} .⁴² The sequence of Ce13d is shown in Figure 2D, and it contains a stretch of 16 highly conserved nucleotides (in red).^{31,43,44} Because Ce13d lacks specificity for Cu^{2+} , this sequence needed to be eliminated during the selection. Therefore, prior to the metal addition step, the library was hybridized to a short piece of DNA complementary to the conserved region in all of our selections. In this way, the Ce13d sequence is deactivated and can later be removed from the library. The detailed selection protocols have been described previously.³⁴

After adding Cu^{2+} to the library, a fraction of the library could have been cleaved, resulting in a shorter sequence. These cleaved sequences were readily harvested after performing a denaturing gel electrophoresis and amplified using two rounds of PCR to reconstruct the library. The progress of the selection is presented in Figure 1B. At the end of round 7, the library achieved $\sim 40\%$ cleavage yield with $50 \mu\text{M}$ Cu^{2+} . However, at the same time, the library was also active with other thiophilic metals. For example, after 1 h incubation with a metal mixture of Cd^{2+} , Zn^{2+} , and Pb^{2+} , 25% cleavage was observed (the first

red bar in Figure 1B). To achieve a high metal specificity, we then carried out extensive negative selections, where the population active with Cd^{2+} and Pb^{2+} and Zn^{2+} were removed, and the remaining uncleaved population were harvested for positive Cu^{2+} selection. To push the selectivity limit, negative selections were incubated for a long time (up to 12 h and sometimes performed consecutively). To push for high Cu^{2+} activity, we incubated the positive selections for only 0.5 h or less.

Search for Active DNAzymes. At the end of round 12, the library was analyzed using deep sequencing. The resulting 23,505 sequences were clustered into different families using the Geneious program. The representative sequences from the 22 most populated families are aligned in Table S3 in which each sequence was individually folded using Mfold.⁴⁵ Among them, 12 can be truncated into reasonable secondary structures. These were then used to construct trans-cleaving DNAzymes and individually tested (see Figure S2 for their trans-cleaving structures). All these DNAzymes share the same substrate sequence, which is labeled with a FAM fluorophore.

Our goal was to identify the best candidate for Cu^{2+} detection. First, the activity of these sequences were tested with Cu^{2+} . The gel analysis showed that the PSCu3, 6, 9, 10, and 18 DNAzymes have high activity (nearly 50% cleavage) in all of the tested conditions (Figure S3), indicating the success of our in vitro selection. To further narrow the candidates for Cu^{2+} sensing, we next screened for Cu^{2+} selectivity. These five active DNAzymes were then tested against 1 or $10 \mu\text{M}$ of Cu^{2+} , Cd^{2+} , Pb^{2+} , or Hg^{2+} for 10 min (Figure S4). From this experiment, PSCu9 and PSCu10 were studied further because of their highest selectivity for Cu^{2+} . Although PSCu3 is also highly active with Cu^{2+} , it has lower selectivity and was thus abandoned.

The secondary structures of PSCu10 and PSCu9 are shown in Figure 2A and C, respectively. An interesting feature is the very small enzyme loop of PSCu10, containing only 8 nucleotides. A few other active DNAzymes also have some sequence similarity, and they are aligned in Figure 2B for comparison of these core sequences (color coding matches that in Figure 2A). PSCu9 has a hairpin structure, which is also typically seen for many RNA-cleaving DNAzymes.^{28,31} Aside from this hairpin, PSCu9 has only four nucleotides in the enzyme loop, and it is also a very small DNAzyme.

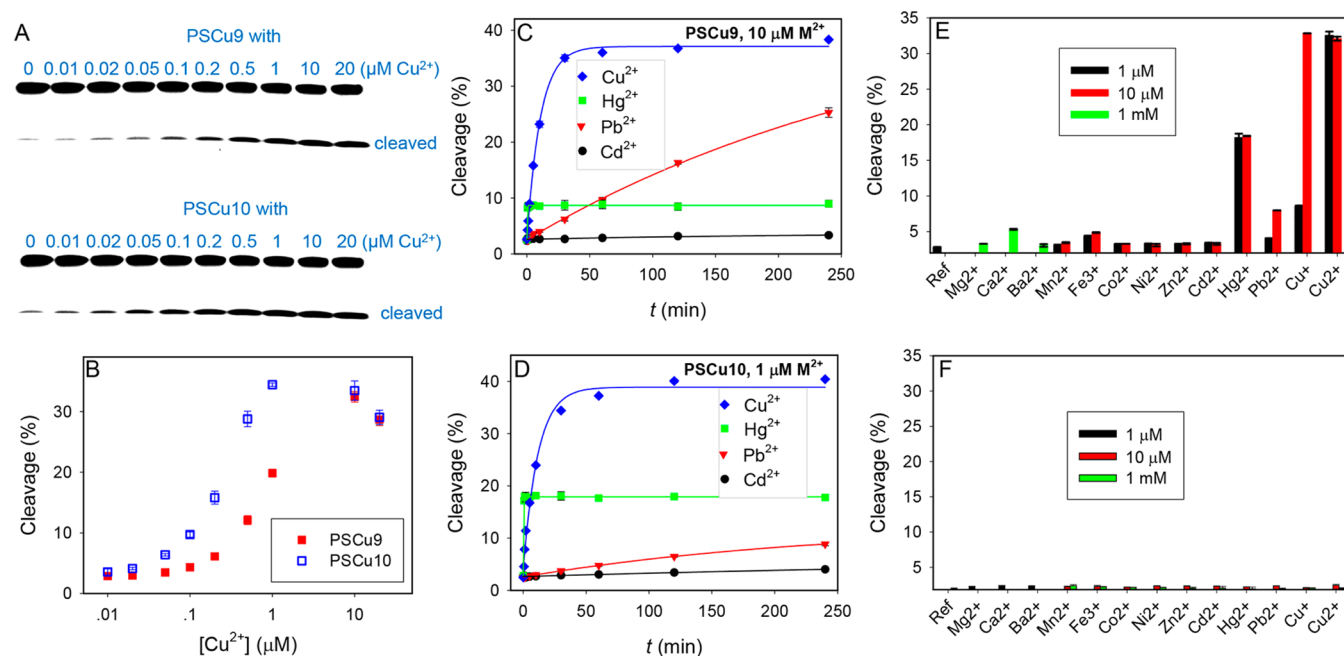


Figure 3. (A) Gel images of PSCu9 and PSCu10 cleaving the PS-RNA substrate in the presence of various concentrations of Cu^{2+} . (B) Quantification of the cleavage yield as a function of Cu^{2+} concentration for these two DNAzymes. Cleavage kinetics of (C) PSCu9 and (D) PSCu10 in the presence of four thiophilic metals. Cleavage yield of PSCu10 in the presence of various metal ions at a few different concentrations with (E) the PS substrate and (F) the PO substrate.

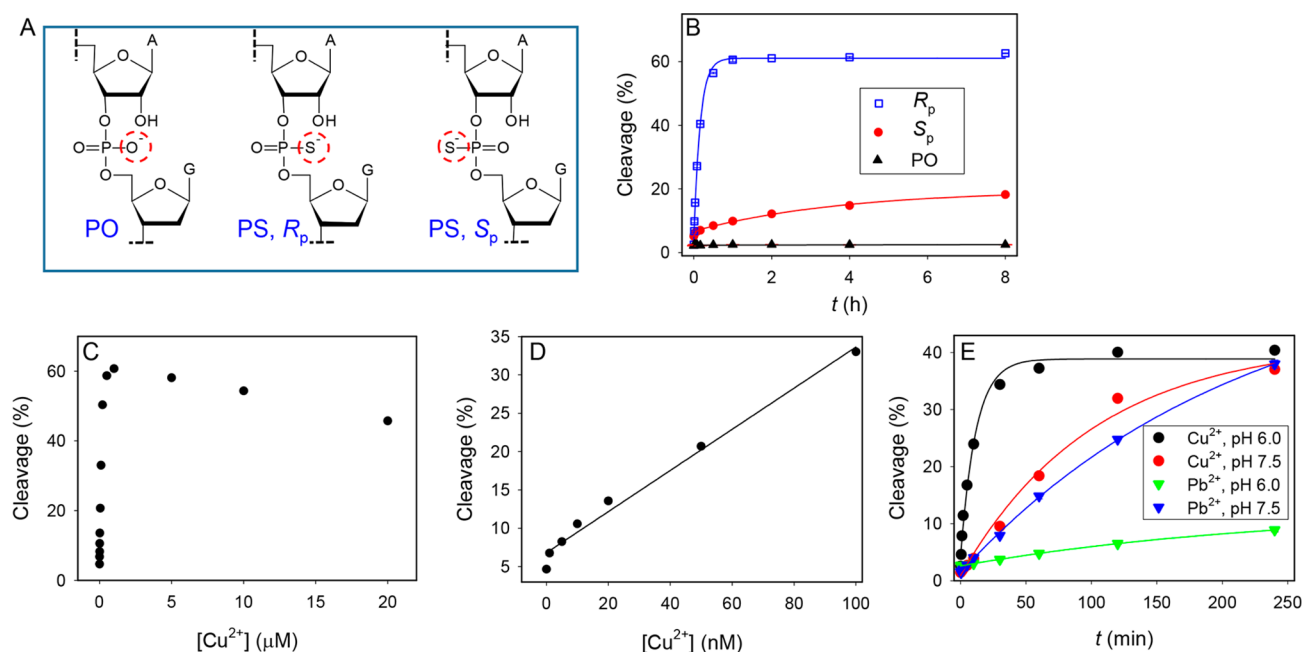


Figure 4. (A) The structures of the three types of cleavage junctions. The normal phosphodiester junction is called PO, and the PS junction contains two diastereomers. (B) Kinetics of PSCu10 with 1 μM Cu^{2+} cleaving these three types of junctions. Cleavage yield of PSCu10 for the purified R_p substrate as a function of Cu^{2+} concentration (C) and the low Cu^{2+} concentration region (D). (E) Kinetics of PSCu10 cleaving the PS substrate (mixture of R_p and S_p) at two pH values with 1 μM Cu^{2+} or 1 μM Pb^{2+} .

Biochemical Characterizations. So far, two DNAzyme candidates that work efficiently in the presence of Cu^{2+} were identified. Next, biochemical studies were performed to further understand them. First, these two DNAzymes were tested in the presence of various concentrations of Cu^{2+} (Figure 3A). Both DNAzymes show Cu^{2+} concentration dependent cleavage, and PSCu10 is more sensitive, yielding saturated cleavage with just 1 μM Cu^{2+} (Figure 3C). On the other hand, PSCu9

produces maximal cleavage with 10 μM Cu^{2+} . At even higher Cu^{2+} concentrations, a slight inhibition effect is observed for both DNAzymes. That is likely due to nonspecific Cu^{2+} binding to DNA bases to disrupt DNAzyme folding. Subsequently, the DNAzyme kinetics at their respectively optimal Cu^{2+} concentrations were measured. PSCu9 has a cleavage rate of 0.093 min^{-1} with 10 μM Cu^{2+} (Figure 3C, blue dots), and this

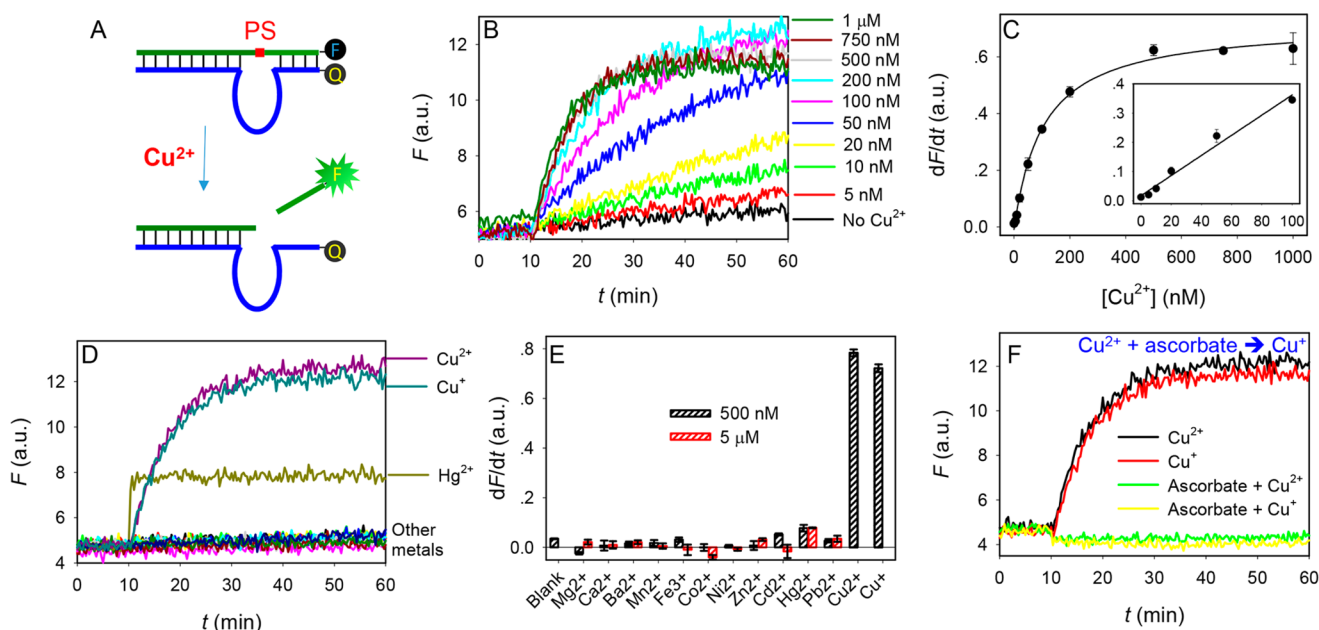


Figure 5. (A) Schematic representation of the fluorescent DNAzyme beacon for Cu^{2+} detection. (B) Kinetics of sensor fluorescence enhancement with various concentrations of Cu^{2+} . (C) Quantification of Cu^{2+} based on the initial rate of sensor fluorescence enhancement (from 1 to 6 min after Cu^{2+} addition). Inset: the initial linear response at low Cu^{2+} concentrations. (D) Sensor response to 500 nM divalent and trivalent metal ions. The list of the other metal ions tested can be found in Figure 2E. (E) Sensor selectivity quantification at two metal ion concentrations. (F) Sensor response to 500 nM of Cu^+ or Cu^{2+} in the presence of 50 μM ascorbate.

rate is similar to that of PSCu10 with 1 μM Cu^{2+} (Figure 3D, blue dots, 0.085 min^{-1}).

Next, the kinetics of the other thiophilic metal ions were also measured. Both DNAzymes have essentially no activity in the presence of Cd^{2+} . For PSCu9, Pb^{2+} has a significant amount of cleavage (0.003 min^{-1} , ~ 30 -fold slower than Cu^{2+}), whereas PSCu10 is nearly inactive with Pb^{2+} . Both DNAzymes cleave very quickly in the presence of Hg^{2+} , which is expected because Hg^{2+} can cleave the PS-RNA substrate even without the enzyme strand present.^{46,47} Because the Hg^{2+} cleavage is finished almost instantaneously, this kinetic signature can be used to distinguish Hg^{2+} from Cu^{2+} (vide infra). Overall, it appears that PSCu10 is the best candidate in terms of sensitivity and selectivity for Cu^{2+} detection, and it was chosen for further studies. The cleavage yield with other common metal ions are also compared, including Cu^+ (Figure 3E). Although Cu^+ has lower cleavage yield than Cu^{2+} at 1 μM , they reached a similar cleavage at 10 μM . The Cu^+ salt used in this work was tetrakis(acetonitrile)copper(I) hexafluorophosphate, which is a commonly used source for Cu^+ . We suspected that, at a low salt concentration, this Cu^+ salt would be quickly oxidized to Cu^{2+} by the oxygen in water. For testing this hypothesis, Cu^+ was generated in situ by reacting Cu^{2+} with ascorbate (Figure S5). In the presence of ascorbate, neither Cu^{2+} nor this Cu^+ salt produced any cleavage, thus confirming the selectivity of PSCu10 to Cu^{2+} . This is understandable because PSCu10 is a transesterification DNAzyme that does not perform oxidative cleavage. This is in sharp contrast to the previously reported Cu^{2+} sensor, which used an oxidative DNA-cleaving DNAzyme requiring ascorbate.^{17,18} Most other metals do not promote cleavage (except Hg^{2+} and high concentrations of Pb^{2+}). If the PS substrate was replaced with the PO analogue, none of the metals produced any cleavage (Figure 3F).

PS Diastereomer Preference. In all the above assays, the cleavage yield never exceeded 50%, and this is likely due to the

chiral phosphorus center at the PS modification site (Figure 4A). In our selection and all the above assays, a mixture of the two diastereomers was used. To study the activity of each individual isomer, we prepared the substrate after HPLC separation. The assignment of each isomer was published previously.³⁴ The R_p substrate has a rate of 0.10 min^{-1} with PSCu10, whereas the S_p has a rate of 0.0028 min^{-1} (37-fold slower, see Figure S6 for its full kinetic trace measured over 1 day). The R_p rate measured here is slightly faster than that for the mixture (0.085 min^{-1}) because the latter is an averaged value. Therefore, PSCu10 uses the R_p sulfur to bind Cu^{2+} , which is similar to most known RNA-cleaving DNAzymes and ribozymes.^{34,48,49} Using the separated R_p substrate, PSCu10 indeed achieved over 50% cleavage yield (Figure 4C), and the cleavage at low Cu^{2+} concentrations is shown in Figure 4D (linear regression equation: $y = 6.80 + 0.268x$). Even 10 nM Cu^{2+} produced a significant amount of cleavage.

This information is useful for biosensor development because the S_p half of the substrate does not contribute much to the signal but does to the background. The PO substrate (Figure 4B, black triangles) was also tested, and it is completely inactive. Therefore, the presence of the PS modification is critical for PSCu10.

A Biosensor for Cu^{2+} . With the PSCu10 DNAzyme, a biosensor for Cu^{2+} detection was designed using the catalytic beacon strategy.⁵⁰ The substrate strand was labeled with a FAM fluorophore, and the corresponding end of the enzyme was labeled with a dark quencher (Figure 5A). In the presence of Cu^{2+} , the cleavage reaction takes place, and the signal is increased after release of the cleavage fragment. Because FAM is a pH-sensitive fluorophore and has the highest fluorescence at pH above 7.5, we first tested the sensor at pH 7.5. Although a Cu^{2+} -dependent signal increase was observed, strong interference from Pb^{2+} was found (Figure S7), which is inconsistent with the gel-based activity assay at pH 6.0. Therefore, we

compared the activity of Cu^{2+} and Pb^{2+} at pH 6.0 and pH 7.5 (Figure 4E). Interestingly, the Cu^{2+} activity dropped at a higher pH, whereas the Pb^{2+} activity increased. Therefore, for Cu^{2+} detection, pH 6.0 is the optimal condition.

The sensor signaling kinetics were measured at pH 6.0 at various Cu^{2+} concentrations (Figure 5B). Even 5 nM Cu^{2+} produced an obvious signal increase, and a saturated response occurred beyond 200 nM Cu^{2+} . The initial signaling rate after Cu^{2+} addition was plotted (Figure 5C), and the response can be fitted to a single Cu^{2+} binding with an apparent K_d of 105 nM Cu^{2+} . At low Cu^{2+} concentrations, the response was linear (inset of Figure 5C; linear regression equation: $y = 0.0175 + 0.00345x$). The detection limit was calculated to be 1.6 nM Cu^{2+} based on $3\sigma/\text{slope}$, where σ is the background variation of the sensor in the absence of Cu^{2+} . This is over 20× more sensitive compared to the previously reported DNA-cleaving DNzyme Cu^{2+} sensor.¹⁷ We compared our sensor with many representative Cu^{2+} optical sensors reported in the literature (Table S4), and our sensor has the best detection limit. The relative standard deviation is typically below 10%. The noisy kinetic traces in Figure 5B are due to the low pH condition. If the FAM label is replaced by a pH-insensitive fluorophore, the fluorescence signal should be stronger. We did not try higher sensor concentrations to improve signal for cost reasons. The US EPA-defined maximal contamination level for copper in water is 20 μM (1.3 ppm), which is much higher than the detection limit of our sensor. Such high sensitivity allows sufficient sample dilution to remove the sample matrix effect.

Next, the sensor response to 500 nM of various competing metal ions was measured (Figure 5D). Cu^{2+} and Cu^+ produced a similar response. As explained above, this Cu^+ response should be due to its oxidation to Cu^{2+} at low metal concentrations. With 50 μM ascorbate, neither Cu^{2+} nor Cu^+ produced any signal (Figure 5F). Hg^{2+} also produced a high signal, but it has a kinetic signature of an instant rise followed by a flat response. When fitting the slope, the initial 20 s was filtered out, and the Hg^{2+} interference was removed (Figure 5E). The response of this sensor to Cu^{2+} in Lake Ontario water was also tested, and a high sensitivity was achieved (Figure S8).

CONCLUSIONS

In summary, in vitro selection was performed to search for a DNzyme cleaving a PS-RNA substrate in the presence of Cu^{2+} . A new DNzyme named PSCu10 was identified with excellent affinity and specificity for Cu^{2+} . PSCu10 works optimally with the R_p diastereomer of the PS substrate, which is 37× faster than the S_p form, and the PO form is inactive. Using this DNzyme, a Cu^{2+} sensor was designed with a detection limit of 1.6 nM Cu^{2+} . It has no activity in the presence of ascorbate, suggesting that it is specific for Cu^{2+} instead of Cu^+ . This is in sharp contrast to a previous sensor based on oxidative DNA cleavage, which requires ascorbate. This DNzyme is useful as a probe for measuring Cu^{2+} in water and also for understanding Cu^{2+} binding to DNA. This study indicates that PS modification at the cleavage site is a valuable method for isolating DNzymes for detecting thiophilic metal ions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b04904.

DNA sequences, additional DNzyme characterization data, sensing in Lake Ontario water, and other literature-reported Cu^{2+} sensor performances (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: liujw@uwaterloo.ca.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Funding for this work is from the Ontario Ministry of Research & Innovation and the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant and Strategic Project Grant).

REFERENCES

- (1) Georgopoulos, P. G.; Roy, A.; Yonone-Lioy, M. J.; Opiekun, R. E.; Lioy, P. J. *J. Toxicol. Environ. Health, Part B* **2001**, *4*, 341.
- (2) Zhao, Y.; Zhang, X.-B.; Han, Z.-X.; Qiao, L.; Li, C.-Y.; Jian, L.-X.; Shen, G.-L.; Yu, R.-Q. *Anal. Chem.* **2009**, *81*, 7022–7030.
- (3) Zhou, Y.; Wang, F.; Kim, Y.; Kim, S.-J.; Yoon, J. *Org. Lett.* **2009**, *11*, 4442–4445.
- (4) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 10.
- (5) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 609–610.
- (6) Wegner, S. V.; Arslan, H.; Sunbul, M.; Yin, J.; He, C. *J. Am. Chem. Soc.* **2010**, *132*, 2567.
- (7) Lan, G. Y.; Huang, C. C.; Chang, H. T. *Chem. Commun.* **2010**, *46*, 1257–1259.
- (8) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948–1998.
- (9) Zhang, X.-B.; Kong, R.-M.; Lu, Y. *Annu. Rev. Anal. Chem.* **2011**, *4*, 105–128.
- (10) Willner, I.; Shlyahovsky, B.; Zayats, M.; Willner, B. *Chem. Soc. Rev.* **2008**, *37*, 1153–1165.
- (11) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272–281.
- (12) Sigel, R. K. O.; Sigel, H. *Acc. Chem. Res.* **2010**, *43*, 974–984.
- (13) Sigel, R. K. O.; Pyle, A. M. *Chem. Rev.* **2007**, *107*, 97–113.
- (14) Ward, W. L.; Plakos, K.; DeRose, V. J. *Chem. Rev.* **2014**, *114*, 4318–4342.
- (15) Lu, Y. *Chem. - Eur. J.* **2002**, *8*, 4588–4596.
- (16) Liu, J. *Can. J. Chem.* **2015**, *93*, 273–278.
- (17) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2007**, *129*, 9838–9839.
- (18) Carmi, N.; Balkhi, H. R.; Breaker, R. R. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 2233–2237.
- (19) Liu, J.; Lu, Y. *Chem. Commun.* **2007**, 4872–4874.
- (20) Cuenoud, B.; Szostak, J. W. *Nature* **1995**, *375*, 611–614.
- (21) Sigel, R. K. O.; Li, Y. *Biochemistry* **2004**, *43*, 9695–9707.
- (22) Wang, M.; Zhang, H.; Zhang, W.; Zhao, Y.; Yasmeen, A.; Zhou, L.; Yu, X.; Tang, Z. *Nucleic Acids Res.* **2014**, *42*, 9262–9269.
- (23) 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.
- (24) Zhou, W.; Saran, R.; Chen, Q.; Ding, J.; Liu, J. *ChemBioChem* **2016**, *17*, 159.
- (25) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223–229.
- (26) Lan, T.; Furuya, K.; Lu, Y. *Chem. Commun.* **2010**, *46*, 3896–3898.
- (27) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481–488.
- (28) Liu, J.; Brown, A. K.; Meng, X.; Croke, D. M.; Istok, J. D.; Watson, D. B.; Lu, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2056–2061.
- (29) Huang, P.-J. J.; Vazin, M.; Matuszek, Z.; Liu, J. *Nucleic Acids Res.* **2015**, *43*, 461–469.

- (30) Huang, P.-J. J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 9993–9999.
- (31) Huang, P.-J. J.; Lin, J.; Cao, J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 1816–1821.
- (32) Hollenstein, M.; Hipolito, C.; Lam, C.; Dietrich, D.; Perrin, D. *M. Angew. Chem., Int. Ed.* **2008**, *47*, 4346–4350.
- (33) Santoro, S. W.; Joyce, G. F.; Sakthivel, K.; Gramatikova, S.; Barbas, C. F., III *J. Am. Chem. Soc.* **2000**, *122*, 2433–2439.
- (34) Huang, P.-J. J.; Liu, J. *Nucleic Acids Res.* **2015**, *43*, 6125–6133.
- (35) Deleavey, G. F.; Damha, M. J. *Chem. Biol.* **2012**, *19*, 937–954.
- (36) Thaplyal, P.; Ganguly, A.; Hammes-Schiffer, S.; Bevilacqua, P. C. *Biochemistry* **2015**, *54*, 2160–2175.
- (37) Wang, S.; Karbstein, K.; Peracchi, A.; Beigelman, L.; Herschlag, D. *Biochemistry* **1999**, *38*, 14363–14378.
- (38) Lee, J. H.; Wernette, D. P.; Yigit, M. V.; Liu, J.; Wang, Z.; Lu, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 9006–9010.
- (39) Farlow, J.; Seo, D.; Broaders, K. E.; Taylor, M. J.; Gartner, Z. J.; Jun, Y.-w. *Nat. Methods* **2013**, *10*, 1203–1205.
- (40) Ma, N.; Sargent, E. H.; Kelley, S. O. *Nat. Nanotechnol.* **2009**, *4*, 121–125.
- (41) Li, Y.; Breaker, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 5364–5372.
- (42) Huang, P.-J. J.; Liu, J. *Anal. Chem.* **2014**, *86*, 5999–6005.
- (43) Vazin, M.; Huang, P.-J. J.; Matuszek, Z.; Liu, J. *Biochemistry* **2015**, *54*, 6132–6138.
- (44) Zhou, W.; Zhang, Y.; Huang, P.-J. J.; Ding, J.; Liu, J. *Nucleic Acids Res.* **2016**, *44*, 354.
- (45) Zuker, M. *Nucleic Acids Res.* **2003**, *31*, 3406–3415.
- (46) Huang, P.-J. J.; Wang, F.; Liu, J. *Anal. Chem.* **2015**, *87*, 6890–6895.
- (47) Huang, P.-J. J.; Vazin, M.; Liu, J. *Anal. Chem.* **2015**, *87*, 10443–10449.
- (48) Thaplyal, P.; Ganguly, A.; Golden, B. L.; Hammes-Schiffer, S.; Bevilacqua, P. C. *Biochemistry* **2013**, *52*, 6499–6514.
- (49) Nawrot, B.; Widera, K.; Sobczak, M.; Wojcik, M.; Stec, W. J. *Curr. Org. Chem.* **2008**, *12*, 1004–1009.
- (50) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466–10467.