

Supporting Information

Desulfurization Activated Phosphorothioate DNAzyme for the Detection of Thallium

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Table S1. DNA sequences used in this work (rA = ribo-adenosine; FAM = carboxyfluorescein; iSp18 = 18-atom hexa-ethyleneglycol spacer; N₅₀ = 50 nucleotide randomized region; * = phosphorothioate). The DNA sequences for in vitro selection are identical to those reported previously.^{S1}

DNA Name	Sequence and modifications
Lib-FAM	5'-GGCGAAACATCTTN ₅₀ TAGTGACGGTAAGCTTGGCAC- FAM
Lib-rA	5'-AATACGAGTCACTATr AGGA AAGAT
splint	5'-AAGATGTTTCGCCATCTTCCTATAGTCCACCACCA
P1 primer	5'-GTGCCAAGCTTACCG
P2 primer	5'-CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACA
P3 primer	5'- FAM -AAATGATCCACTAATACGACTCACTATr AGG
P4 primer	5'-AACAACAACAAC-i Sp18 -GTGCCAAGCTTACCG
PS-sub	5'-GTCACGAGTCACTATr A *GGAAGATGGCGAAA- FAM
TI-sens-sub	5'-GTCACGAGTCACTATr A *GGAAGATGGC- FAM
PO-sub	5'-GTCACGAGTCACTATr AGGA AAGATGGCGAAA- FAM
Tm7	5'-TTTCGCCATCTTCGATACTCTCTTTGACTCGTGAC
Tm7-Q	5'- Iowa Black ® FQ -CGCCATCTTCGATACTCTCTTTGACTCGTGAC
17E	5'-TTT CGCC ATCTT C TCCGAGCCGGTCGAA ATAGTGACTCGT
TI13	TTTCGCCATCATAGCGGAGCGTCTTTTTAGACGAAATAGTGACTCGTGAC
TI22	TTTCGCCATCATAGCAGCGTCTTTTTAGACGAAATAGTGACTCGTGAC
TI27	TTTCGCCATCAGCAGCGTCTTTTTAGACGAAATAGTGACTCGTGAC
cDNA	TTTCGCCATCTTCCTATAGTGACTCGTGAC
MS-DNA	TATr A *GGAAGA

Table S2. In vitro selection conditions and progress.

Ce³⁺ selection	Condition	% cleavage
1st round	50 μ M, 1hr	0.1
2nd round	50 μ M, 1hr	0.1
3rd round	50 μ M, 1hr	4
4th round	50 μ M, 1hr	40.1
5th round	50 μ M, 1hr	41.6
6th round	20 μ M, 1hr	48.2

Tl³⁺ selection (high Tl³⁺ concentrations)	Condition	% cleavage
1st round	1 mM, 1hr	0.1
2nd round	1 mM, 1hr	0.1
3rd round	1 mM, 1hr	0.1
4th round	1 mM, 1hr	0.5
5th round	1 mM, 1hr	1
6th round	1 mM, 1hr	5.4
7th round	20 μ M, 1hr	3.5
8th round	20 μ M, 1hr	2.3
9th round	0.5 mM, 1hr	13.6
10th round	0.5 mM, 1hr	19.7

Tl³⁺ selection (low Tl³⁺ concentrations)	Condition	% cleavage
1st round	50 μ M, 1hr	0
2nd round	50 μ M, 1hr	0
3rd round	50 μ M, 1hr	0.1
4th round	50 μ M, 1hr	0.3
5th round	50 μ M, 1hr	0.1
6th round	100 μ M, 1hr	0.2
7th round	100 μ M, 2hrs	0.1
8th round	100 μ M, 2hrs	0.2
9th round	100 μ M, 2hrs	0.2
10th round	100 μ M, 30min	6.4
11th round	100 μ M, 15min	11

Table S3. Sequence alignment from the Ti^{3+} selection. A few representative sequences were individually tested.

13	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCGGAGC	56
20	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCGGAGC	56
7	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
22	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GC--AGC	54
2	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
6	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
21	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
4	CTGCAGA-TTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	55
9	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
26	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCATA----	GCAGAGC	56
27	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATC-TA----	GCAG--C	53
29	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCGTA----	GCAGAGC	56
25	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGGCGAGGC	59
30	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGGCGAGGC	59
28	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	AGGGGCGAGGC	60
23	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGSGAGGC	59
5	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGGCGAGGC	59
19	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGGCGAGGC	59
14	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	GGGCGAGGC	59
8	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	AGGGGCGAGGC	60
15	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	AGGGGCGAGGC	60
24	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	AGGGGCGAGGC	60
10	CTGCAGAATTCTATTACGAGTCACTATAGGAAGATGGCGAAACATCTTGA-	AGGGGCGAGGC	60
1	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACAT---G---	AGCCATAG	54
3	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACAT---GG---	AGCCATAG	55
18	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCCTGG--	AGCCATAG	58
31	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACAT---GG---	AGCCATAG	55
16	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACAT---GG---	AGCCATAG	55
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13	GTCTA-GAGATGTAAGTAGA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		112
20	GTCTA-GAGATGTAAGTAGA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		112
7	GCCTA-GAGATGTAAGTA-A--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		111
22	GTCTA-GAGATGTAAGTAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		110
2	GTCTA-GA--TGTAAGTAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		110
6	GTCTA-GA--TGTAAGTAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		110
21	GTCTA-GA--TGTAAGTAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		110
4	GTCTA-GAGATGTAAGTAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		111
9	GTCTA-GAGATGTA-GCAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		111
26	GTCTA-GAGATGTA-GCAAA--TCTTTTCTCAGCG-AGACGAAATAGTGACGGTAAGCTT		111
27	GTCTAAGAGATGTAAGTAAAA-TCTTCTCTAGCA-AGACGAAATAGTGACGGTAAGCTT		111
29	GTCTA-GAGATG--AAGTAAA--TCTTCTCTAGCA-AGACGAAATAGTGACGGTAAGCTT		111
25	-TCAATGAG----AAGTGCAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
30	-TCAATGAG----AAGTGCAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
28	-TCARTGAG----AAGTACRA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		112
23	-TCAATGAG----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
5	-TCAATGAG----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
19	-TCAATGAG----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
14	-TCAATGAG----GAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
8	-TCAATGAG----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		112
15	-TCAATGAT----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		112
24	-TCAATGAT----AAGTACAA-CGTGCTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		112
10	-TCAATGAG----AAGTACRA-CRT-CTTGAATTG-CGG-AAAATAGTGACGGTAAGCTT		111
1	GTCAAAGGT----GGGTGCGAGTCGTATCATATCGACGAAATAATAGTGACGGTAAGCTT		110
3	GTCAAAGGT----GGGTGCG-GTCGTATCATATCGGCCAG-TAATAGTGACGGTAAGCTT		109
18	GTCAAAGGT----GGGTGCGTGTGTCGTAT--ATCGACTAAGTA-TAGTGACGGTAAGCTT		110
31	GTCAAAGGT----GGGTGCGTGTGTCGTATCTTATCGACTAAGTA-TAGTGACGGTAAGCTT		110
16	GTCAAAGGT----GGGTGAGAGTCGTATCATAACGACTAG-CAATAGTGACGGTAAGCTT		110
* * * * *			

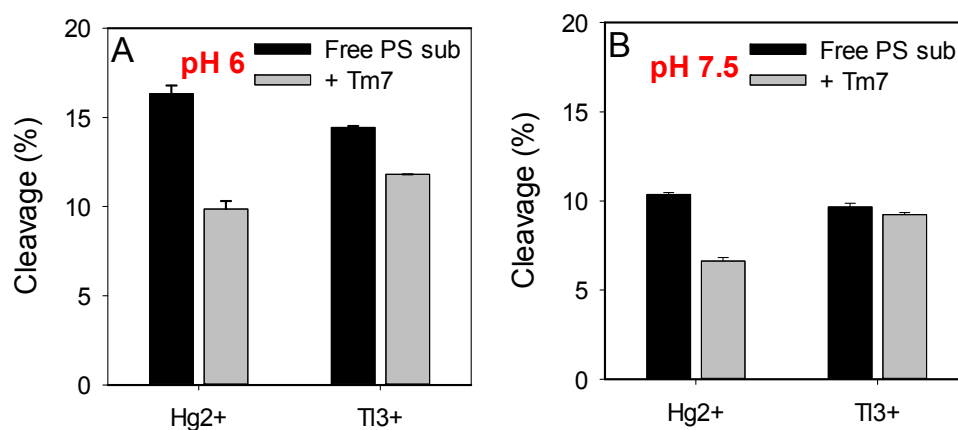


Figure S1. Cleavage of the PS-modified substrate (PS-sub) by Hg^{2+} or Tl^{3+} as a free substrate or when it is hybridized with Tm7 at (A) pH 6 and (B) pH 7.5. Cleavage by Hg^{2+} is significantly suppressed at higher pH and is further suppressed by hybridizing with Tm7. On the other hand, cleavage by Tl^{3+} is less affected by Tm7 hybridization.

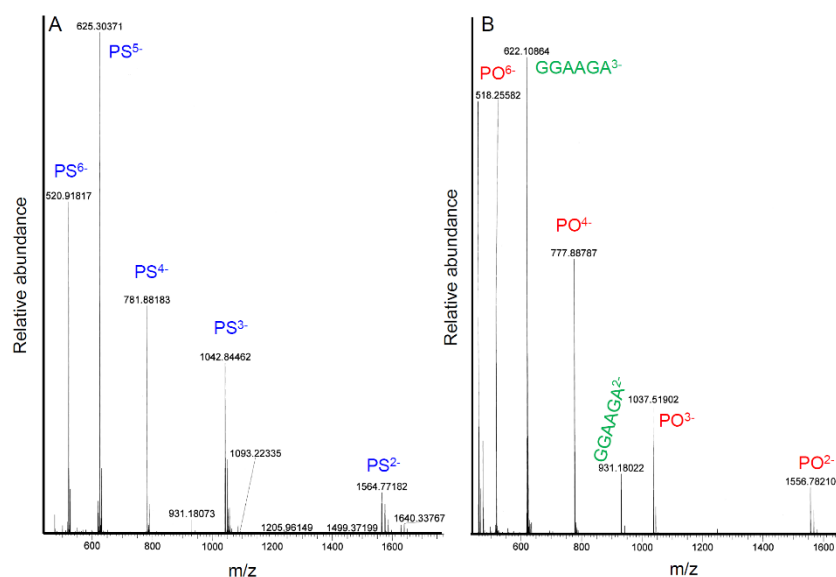


Figure S2. Mass spectrometry of (A) the original PS substrate (named MS-DNA, TATrA*GGAAGA), and all the peaks correspond to the same molecule with different number of negative charges; (B) the PS substrate after Tl^{3+} treatment. Cleaved fragments are marked in green and the remaining peaks are from the desulfurized full-length substrate (marked by PO). For the preparation of samples for mass spectrometry, MS-DNA (1 μM , 2 mL) was incubated with 10 μM Tl^{3+} ion in buffer A (25 mM NaCl, 50 mM MES pH 6) for 2 h. The sample was desalted using a

Sep-Pak C18 column (Waters) and then lyophilized overnight. The dried samples were dissolved in Mill-Q water without any additional buffer. 40 μ M sample was mixed with water-methanol (1:1) containing 0.1% NH_4OH to facilitate ionization. Mass spectra were obtained with a Micromass Q-TOF Ultima Global mass spectrometer using electrospray ionization (ESI).

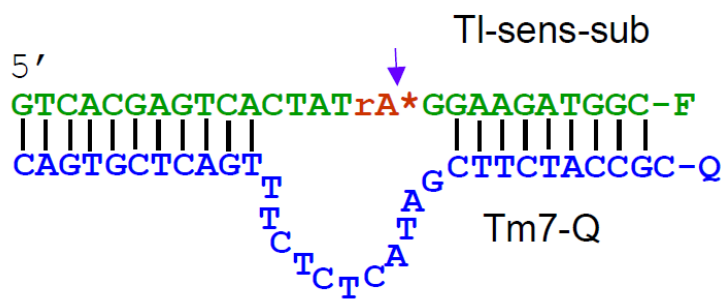


Figure S3. The DNA sequences and DNAzyme secondary structure of the sensor for TI^{3+} detection. The substrate contains a PS modification denoted by the red asterisk. F = FAM. Q = Iowa Black ® FQ dark quencher.

Additional References

(S1) Huang, P.-J. J.; Lin, J.; Cao, J.; Vazin, M.; Liu, J. *Anal. Chem.* **2014**, *86*, 1816-1821.