Electronic supplementary information

Unexpected enrichment of DNA aptamers for Zn^{2+} ions from an insulin selection

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Materials and Methods

The DNA samples used in this work were purchased from Integrated DNA Technologies (Coralville, IA). All the sequences are listed in **Table S1**. Thermo Scientific Pierce streptavidin agarose resin was purchased from Fisher Scientific (Ottawa, ON, Canada). Insulin was purchased from Millipore-Sigma (Catalog number: 11061-68-0, product number: 91077C). Sodium chloride, magnesium chloride, zinc chloride, cadmium chloride, copper(II) chloride, lead chloride, cobalt nitrate, nickel(II) chloride hexahydrate, chromium(III) chloride hexahydrate, manganese chloride, gadolinium chloride hexahydrate, and barium chloride were purchased from Millipore-Sigma. Ethylenediaminetetraacetic acid (EDTA) disodium salt and 2-(N-morpholino)ethanesulfonic acid (MES) were from Mandel Scientific (Guelph, ON, Canada). Amicon Ultra-0.5 centrifugal filter unit (3K and 10K) were purchased from Millipore-Sigma (Oakville, ON, Canada). Micro bio-spin chromatography columns and SsoFast EvaGreen supermix were from Bio-Rad. Taq DNA polymerase with ThermoPol buffer and dNTP mix were from New England Biolabs. All buffers and solutions were prepared with Milli-Q water.

SELEX

The selection was performed following our previously published procedures.[1-4] Briefly, a 73-nt DNA library with a 30-nt random region was used. Selections were performed in the selection buffer of 10 mM MES at pH 6.3 containing 150 mM NaCl, 5 mM $MgCl_2$ and 10 mM KCl. For the first round of selection, 500 pmol of DNA library was mixed with 2500 pmol of column-binding strand in 200 μL selection buffer (**Table S2**). The mixture was incubated at 95°C for 5 min and cooled to 4°C. After stored in -20 °C for 15 min, the mixture was loaded to a column containing 200 μL streptavidin agarose resin. The column was washed five times with 250 μL selection buffer, and then the DNA library/column-binding strand complex mixture was passed through the column four times. The column was then washed with 10 times of 250 μL selection buffer. Then 250 μL of 25 μM insulin dissolved in the selection buffer was loaded and incubated

for 15 min. After incubation, the column was eluted with an additional of 500 µL of 25 µM insulin and the eluent was collected. The collected target eluents were concentrated using Amico Ultra centrifugal filters (3K MWCO) and washed with 3 times of 500 µL Milli-Q H_2O. Then the DNA was collected, and the final volume was made to 120 µL. This solution was diluted 10-fold for PCR amplification with unlabeled forward primer and biotinylated reverse primer. The PCR amplicons were concentrated and washed using Amico Ultra centrifugal filter (10K MWCO) with 3 times of 500 µL strand separation buffer (1× PBS). Then the DNA was diluted to 500 µL and captured on a streptavidin agarose column. After DNA loading, the resin was washed with 10 aliquots of 500 µL strand separation buffer followed by incubation with 400 µL of 0.2 M NaOH for 15 min. The elution containing the library strand was collected. Additional 200 µL of 0.2 M NaOH was added to the column, and eluent was combined. The eluent was neutralized with 600 µL 0.2 M HCl and concentrated and washed using a centrifugal filter (3K MWCO) with two aliquots of 500 µL of Milli-Q water and one aliquot of 500 µL selection buffer. The DNA was concentrated to 60 µL. The concentration was determined using Nanodrop and used for the next round of selection.

Sample preparation for DNA sequencing

The 10-fold diluted target eluent from round 18 was used as DNA template and was subjected to another PCR reaction. The forward primer (P5-502) and reverse primer (P7-703) containing unique index sequences were used. The PCR product wash with 10 K filter with 3× 500 µL 1× PBS and purified using small DNA fragment extraction kit (IBI Scientific). The concentration of the purified DNA was quantified using Nanodrop and was submitted to McMaster University Genomics Facility for Illumina sequencing.

ThT assay

In a typical experiment, the target solution was gradually titration to the mixture containing 1 µM aptamer and 2 µM ThT in selection buffer. The fluorescence spectra were recorded using a Varian Eclipse fluorometer (Ex: 420 nm; Em: 440-550 nm, the peak values at 490 nm were used for calculation). The apparent K_d was fitted using $y=B_{max}\cdot x/(K_d+x)$, whereas y is $(F_0-F)/F_0$ and x is the target concentration. The experiments were run in triplicate and the error bars indicate the standard deviations.

Strand displacement assay

One micromolar sensor (aptamer/quencher complex) was prepared by annealing 1 µM FAM-Zn-1 aptamer with 2 µM quencher-cDNA in the selection buffer at 80°C for 1 min, followed by cooling to room temperature over 20 min and then at 4°C for 1 h. Afterwards, the sensor was stored at -20°C prior to use. In a typical experiment, 100 µL of 20 nM sensor in selection buffer was used. The background fluorescence

of the sensor was monitored for 5 min. Then 2 μL of target solution was added. The fluorescence signal was continuously monitored for another 25 min (Ex: 485 nm, Em: 535 nm) with 20 s intervals.

Detection of Zn^{2+} in tap water

For sensing the Zn^{2+} concentration in tap water, tap water with a volume of 10 μL, 20 μL and 50 μL was added to a final volume of 100 μL sensor in selection buffer. Thus, these samples had a dilution factor of 10, 5 and 2, respectively. The final sensor concentration was 20 nM FAM-Zn-1 in selection buffer with a final volume of 100 μL. The fluorescence was monitored for 30 min, the signal at 20 min (F) was used for calculation. The FAM-Zn-1 sensor without tap water was used for background (F_0). $(F\text{-}F_0)/F_0$ was calculated and the zinc concentration in sensor solution was calculated using the standard curve fitting equation $(F\text{-}F_0)/F_0 = 6.02 \times [Zn^{2+}]/(8.44 + [Zn^{2+}])$ (Figure 4D, pink trace). And the obtained $[Zn^{2+}]$ was corrected with the dilution factor to obtain the total $[Zn^{2+}]$ in tap water. The zinc concentration obtained using the FAM-Zn-1 sensor was compared to the zinc concentration obtained using ICP-MS performed by ALS Canada Ltd. (0.212 mg/L).

Table S1. DNA sequences used in the in vitro selection and sensors.

DNA Name	Sequence and modification (From 5′ to 3′)
Library	GGA GGC TCT CGG GAC GAC (N_{30})-GTC GTC CCG CCT TTA GGA TTT ACA G
Column-binding strand	GTCGTCCCGAGAGCCATA-/3Biosg/
Forward Primer	GGA GGC TCT CGG GAC GAC
Reverse Primer	CTG TAA ATC CTA AAG GCG GGA CG
Biotin-Reverse Primer	/5Biosg/CTG TAA ATC CTA AAG GCG GGA CG-3
P5-502	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACC TCT CTA TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TCT GTA AAT CCT AAA GGC GGG ACG
P7-703	CAA GCA GAA GAC GGC ATA CGA GAT TTC TGC CTG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT GGA GGC TCT CGG GAC GAC
Q-cDNA	AGT CGT CGA GAG/3lABkFQ/
FAM-Zn-1	/56-FAM/CTC TCG ACG ACG CTC CCA TTC CAG CTT CGG TGG TAG CAG AAG TCG TC

Table S2. In vitro selection conditions used for the selection of insulin aptamer.

Round No.	Library (pmol)	Column-binding strand (pmol)	Insulin (μM)
1	500	2500	25
2	100	250	25
3	100	250	25
4	100	250	25
5	100	250	25
6	100	250	25
7	100	250	25
8	100	250	25
9	100	250	25
10	100	250	25
11	100	250	25
12	100	250	25
13	100	250	25
14	100	250	25
15	100	250	25
16	100	250	25
17	100	250	25
18	100	250	25

Table S3. Top 20 sequences of Round 18 library for insulin selection.

	Sequences (5'-3')	Counts
Family 1		24.5%
Insulin 1	GACGACGCTCCCATTCCAGCTTCGGTGGTAGCAGAAGTCGTC	1533
Insulin 17	GACGACGTAGCAGAAGGTCGATCTCCGCTCCCATTCGTCGTC	35
Family 2		24.8%
Insulin 2	GACGACC-GTAAGGATTTAATCTATCTCGCGATTATGGTCGTC	1481
Insulin 19	GACGACAGGAAAGGTACCTACTTAGGACTCG-TTGCAGTCGTC	102
Family 3		19.4 %
Insulin 3	GACGACACAGAAAGCCTCGTCGCAGAATTCCTCTATGTCGTC	1063
Insulin 8	GACGACGGATAGAACCGTAGAATTATACTGCAGAACGTCGTC	180
Family 4		5.9 %
Insulin 5	GACGACGCAGGGTTTCCATTCGTTCCTCGGTTAAACGTCGTC	280
Insulin 20	GACGACTAGAGGGTTTCAGTTATTCCACGGTTAGTAGTCGTC	32
Insulin 19	GACGACAGGGTTGCAAAGTTCAAACTGAGAGGTTCCGTCGTC	33
Insulin 18	GACGACGCTTCAAGGAGTACTTACGGGTTATGTTACGTCGTC	34
Family 5		2.7%
Insulin 10	GACGACACTGAGAGGTGCCATTTGGGGTTGCAAGGGGTCGTC	86
Insulin 16	GACGACTTGAGAGGATCCAGACGATGGGTTGCAAAAGTCGTC	36
Insulin 13	GACGACGAGAGAGGCTCATGTTTAGGATGGTTGCAAGTCGTC	50
Others		17.7%
Insulin 4	GACGACTACCCGTCTGAGGTACAAGTACCGCACATAGTCGTC	497
Insulin 6	GACGACGACAGCACTGCCGGTAGAGAAAACGGTATCGTCGTC	240
Insulin 7	GACGACGCGCGTCTTGTCGCCTGACAAAGCTTCGTGGTCGTC	200
Insulin 11	GACGACGGGGTGTCCTACCTTAGCGAGGTCCAGCGAGTCGTC	61
Insulin 12	GACGACGGTGCCGTGCCCGGGTTAGCGCTTGTGTACGTCGTC	53
Insulin 14	GACGACGGGAGGCGAAGCGCGAGTAACGTGCATCCTGTCGTC	43
Insulin 15	GACGACCAGGCTAGTGTAGCTTTCTCGTGTGCGAGTGTCGTC	39

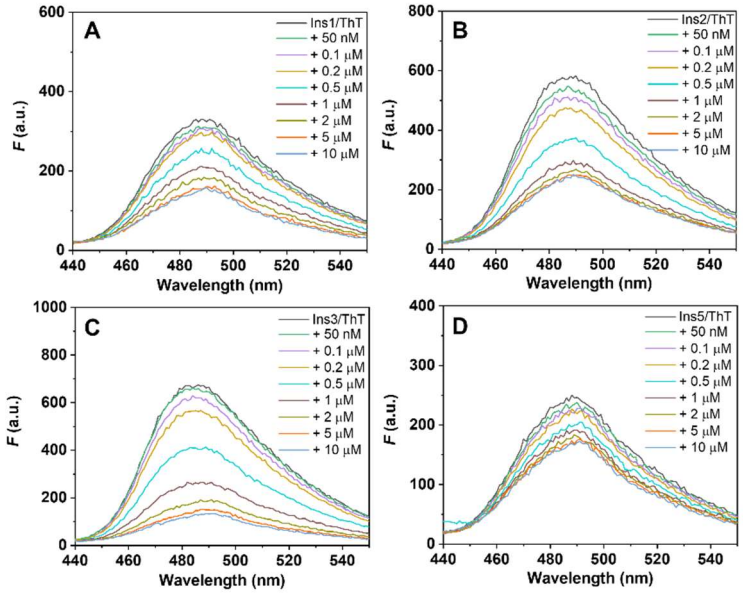

Figure S1. Fluorescence spectra of titrating Zn^{2+} to (A) Insulin-1/ThT, (B) Insulin-2/ThT, (C) Insulin-3/ThT, and (D) Insulin-5/ThT.

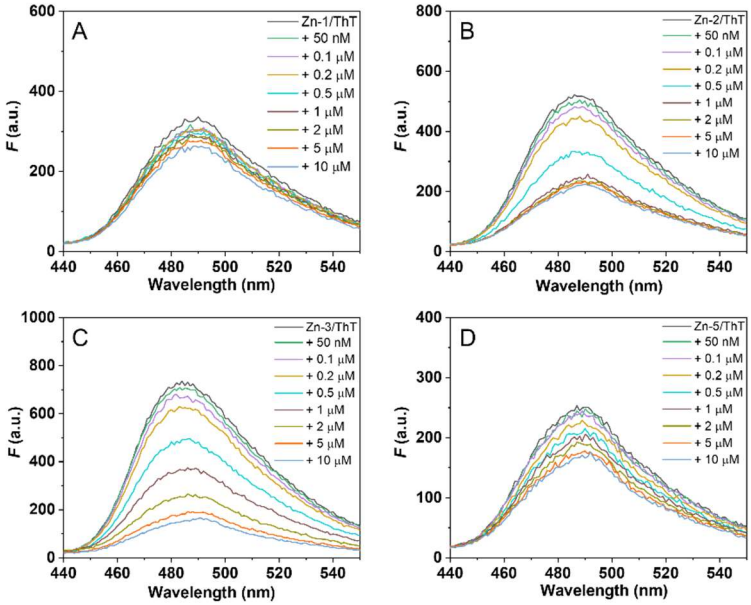

Figure S2. Fluorescence spectra of titrating Cd^{2+} to (A) Zn-1/ThT, (B) Zn-2/ThT, (C) Zn-3/ThT, and (D) Zn-5/ThT.

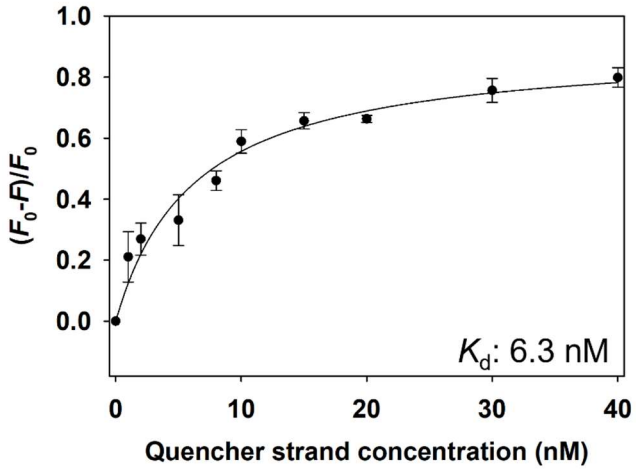

Figure S3. Fluorescence quenching of 10 nM of FAM-Zn-1 with increasing concentration of Quencher-cDNA in selection buffer. The K_d for the reaction of FAM-Zn-1 (F) and Quencher-cDNA (Q) can be defined as K_{d1} = [F][Q]/[FQ]. The K_d for the reaction of FAM-Zn-1 sensor (FQ) and Zn^{2+} (L) can be defined as K_{d2} = [FQ][L]/[FL][Q]. Therefore, the K_d for the reaction of aptamer binding can be written as $K_{d3} = K_{d1}K_{d2}$ = [F][Q]/[FQ] × [FQ][L]/[FL][Q] = [F][L]/[FL]. The K_{d1} was obtained from the fitting of Figure S3, and the K_{d2} was obtained from the sensor calibration curve in Figure 4C. [FQ] and [FL] had the same concentration when Zn^{2+} at the apparent K_d value, thus, $K_{d2}=[K_{d2\ apparent}]/[Q]$. Since 20 nM of the FAM-Zn-1 and 40 nM Quencher-cDNA were used for the sensor, [Q] was 30 nM at the Zn^{2+} concentration equal to the apparent K_d. Thus, $K_{d2} = [K_{d2\ apparent}]/[Q]$ = 8.4 μM/30 nM = 280, and $K_{d3} = K_{d1}K_{d2}$ = 280 × 6.3 nM = 1.8 μM.

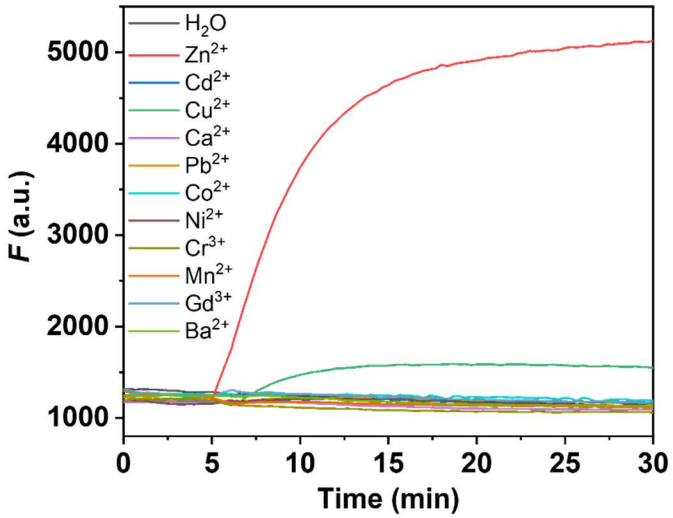

Figure S4. Kinetic response of the FAM-Zn-1 strand displacement sensor to 10 μM various metal ions.

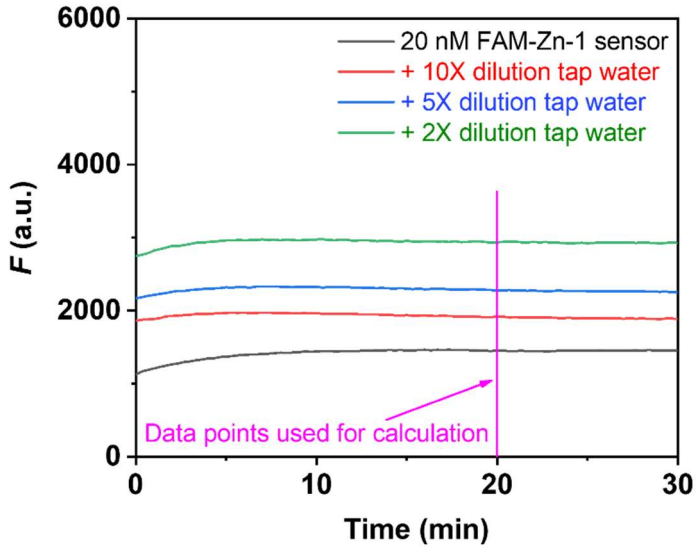

Figure S5. Kinetic response of FAM-Zn-1 sensor to 10-, 5- and 2- times dilution of tap water. The data points at 20 min were used for calculation.

References

1. P.-J. J. Huang and J. Liu, *Anal. Chem.*, 2022, **94**, 3142-3149.
2. Y. Liu and J. Liu, *Analysis & Sensing*, 2022, **2**, e202200010.
3. P.-J. J. Huang and J. Liu, *Angew. Chem. Int. Ed.*, 2023, **62**, e202212879.
4. L. Gu, Y. Ding, Y. Zhou, Y. Zhang, D. Wang and J. Liu, *Angew. Chem. Int. Ed.*, 2023, **63**, e202314450.