Electronic supplementary information

A general transition metal binding aptamer following the Irving–Williams series

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Chemicals

Calcium(II) chloride, scandium(III) chloride hydrate, chromium(III) chloride hexahydrate, manganese(II) chloride hydrate, iron(II) chloride tetrahydrate, iron(III) chloride hexahydrate, cobalt(II) chloride hexahydrate, nickel(II) chloride, copper(II) chloride dihydrate, zinc(II) chloride, strontium(II) chloride, yttrium(III) chloride hexahydrate, cadmium(II) chloride, barium(II) chloride dihydrate, mercury(II) acetate, lead(II) chloride, thioflavin T (ThT), and amicon ultra-0.5 centrifugal filter unit (with 3k and 10k molecular weight cutoffs) were acquired from Millipore-Sigma (Oakville, ON, Canada). All DNA utilized were synthesized by Integrated DNA Technologies (Coralville, IA).Streptavidin-coated agarose resin possessed a loading capacity of 1-3 mg biotinylated BSA per milliliter was bought from Thermo Scientific (IL, USA). 6% BCL agarose bead standard (50-150 μM) was purchased Agarose Bead Technologies (Spain). The dNTP mix, Taq DNA polymerase with ThermoPol buffer, and a low-molecular-weight DNA ladder were obtained from New England Biolabs (Ipswich, MA). Micro Biospin chromatography columns and SsoFast EvaGreen supermix were procured from Bio-Rad Laboratories (Hercules, CA, American). 2-(N-Morpholino) ethanesulfonic acid (MES) sodium salt, $MgCl_2 \cdot 6H_2O$, NaCl, $EDTA \cdot 2H_2O$, HCl and NaOH were obtained from Bio Basic (Toronto, ON, Canada). Milli-Q water (18.2 $M\Omega\ cm^{-1}$) was used in all experiments.

Table S1. DNA used in this study.

DNA name	Sequences and modifications (5' to 3')
Library	GGAGGCTCTCGGGACGACN_{30}GTCGTCCCGATGCTGCAATCGTAA
Biotin-column	GTCGTCCCGAGAGCCATA/**3BioTEG/**
Forward primer	GGAGGCTCTCGGGACGAC
Reverse primer	TTACGATTGCAGCATCGGGACG
Biotin-reverse primer	**/5Biosg/**TTACGATTGCAGCATCGGGACG
P5-501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCT TTCCCTACACGACGCTCTTCCGATCTTTACGATTGCAGCATCGGG ACG
P7-702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTGACTGGAGTT CAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
P7-704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTGACTGGAGT TCAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
Co-1	GACGACGGAACGGAGGTTCTTAGGTCGGTAGACCGAGTCGTC
Co-1a	GACGACTATGAACGGAGGTTCGGGTCGGTAGACACGGTCGTC
FAM Co-1	/**56-FAM**/CTCTCGACGACGGAACGGAGGTTCTTAGGTCGGTAGAC CGAGTCGTC
Quencher-cDNA	AGTCGTCGAGAG/**3IABkFQ**/
Co-2	GACGACGGAAACGTGTACTTCATTGAGATGGATTGCGTCGTC
Ni-1	GACGACCCAGGAGATTGAGTCGCATGACAGGTTGTGGTCGTC
Ni-4	GACGACCAATGGGGAACCTTAGTGTATACGAAGTAGGTCGTC
Cu-1	GACGACCACGGTAAACGACGCTGTACGGAGTGGTCTGTCGTC
Zn-1	GACGACGCTCCCATTCCAGCTTCGGTGGTAGCAGAAGTCGTC

Table S2. The selection conditions for Co^{2+} and Ni^{2+} binding aptamers.

Selection Round	N_{30} library (pmol)	Co^{2+} (μM)	Ni^{2+} (μM)
1	500	10	10
2	100	10	10
3	100	10	10
4	100	10	10
5	100	10	10
6	100	10	10
7	100	10	10
8	100	10	10
9	100	10	10
10	100	10	10
11	100	10	10
12	100	10	10
13	100	10	10
14	100	10	2
15	100	2	2
16	100	2	2
17	100	2	2

Family 1

Co-1	GACGAC	GGAACGGAGGTTCTTAGGTCGGTAGACCGA	GTCGTC	3091	reads, 10.3%
Co-3	GACGAC	GGGCTGGTTTTAAAGACATGGTCATGCTGA	GTCGTC	424	reads, 1.4%
Co-11	GACGAC	GACAGTGTTTATTCCGTTGGGACGCATGTT	GTCGTC	153	reads, 0.5%
Co-12	GACGAC	GGGGAGTGTTTAACGTACTGAGGGGACCGA	GTCGTC	153	reads, 0.5%
Co-14	GACGAC	GGCAGTGCTACAGGCTAGAATACAAGCCGA	GTCGTC	141	reads, 0.5%
Co-15	GACGAC	GGGGCATGAAATGCGAACTTAGGGTACCGA	GTCGTC	139	reads, 0.5%
Co-17	GACGAC	GGCAGTGAAGTTATTGATCTCGCGACTAGA	GTCGTC	135	reads, 0.4%
Co-18	GACGAC	GGGGTGACAATACACCCTTAGGGGATCCGA	GTCGTC	133	reads, 0.4%

Ungrouped

Co-2	GACGAC	GGAAACGTGTACTTCATTGAGATGGATTGC	GTCGTC	589	reads, 2.0%
Co-4	GACGAC	ACGAAGACGGATCTAAGGTAGGTGTCACGC	GTCGTC	274	reads, 0.9%
Co-5	GACGAC	TGGACGTTCGATAGAGGGCCTTGGGTCAAA	GTCGTC	233	reads, 0.8%
Co-6	GACGAC	TGCAGTGTATACTTCGTTGAGACGCAGGTA	GTCGTC	218	reads, 0.7%
Co-7	GACGAC	CGTGTGTGTAAGATCCAATAACGGATACAG	GTCGTC	233	reads, 0.7%
Co-8	GACGAC	GCGACGTTAAGATATAGCTTAGTTCGTGCA	GTCGTC	179	reads, 0.6%
Co-9	GACGAC	CAAAGGGTATATAGCACCTGCGGGGTATGG	GTCGTC	161	reads, 0.5%
Co-10	GACGAC	CGGAAGTTCAACTACGCGTTACTGTGTAAG	GTCGTC	157	reads, 0.5%
Co-13	GACGAC	CGACATGGATCCAAATGGCTTGTGTGCAAG	GTCGTC	149	reads, 0.5%
Co-16	GACGAC	TGGGGTAGTATACTTGTTCCAGATAGAGCA	GTCGTC	138	reads, 0.5%
Co-19	GACGAC	GGCAAGGCTTGTGTCAATGACGGATCTGCA	GTCGTC	132	reads, 0.4%
Co-20	GACGAC	CAGGGCGCATATTATGTCTGGAAGGGGTAG	GTCGTC	132	reads, 0.4%

Figure S1. Alignment of the top 20 sequences of the Co^{2+} selection library. Only one major family was identified.

Family 1

Ni-1	GACGAC	CCAGGAGA----TTGAGTCGCATGACAGGTTGTG-	GTCGTC	588	reads, 1.8%
Ni-2	GACGAC	GGAGTAAGG---TTGAGTCGTATGACAGACGTA--	GTCGTC	197	reads, 0.6%
Ni-5	GACGAC	GCACTAAGAC---TGAGTCGAATGACATGGGGA--	GTCGTC	136	reads, 0.4%
Ni-6	GACGAC	GGCAGAAG----TTGAATGG-ATGAAGTTGAGGCA	GTCGTC	119	reads, 0.4%
Ni-10	GACGAC	GTAAGAGTTCG-TTGAGACGCATGTGCGATA----	GTCGTC	91	reads, 0.3%
Ni-14	GACGAC	GCAGTAAGT---TTGAGTCGGATGACATGCGGA--	GTCGTC	78	reads, 0.2%
Ni-19	GACGAC	CAAAGTTCAGGGTTGAGTGTTGAGTTGAAG-----	GTCGTC	66	reads, 0.2%

Family 2

Ni-3	GACGAC	GGAACGGAGGTTCTTAGGTCGGTAGACCGA	GTCGTC	195	reads, 0.6%
Ni-8	GACGAC	GGAACACGTTCTTAGGTCAATTAGGACCGA	GCCGTC	106	reads, 0.3%

Ungrouped

Ni-4	GACGAC	CAATGGGGAACCTTAGTGTATACGAAGTAG	GTCGTC	174	reads, 0.5%
Ni-7	GACGAC	GCAGTTGAAGTTGGTGACAGTTGAAGTTAA	GTCGTC	116	reads, 0.4%
Ni-9	GACGAC	ACGAGTTGAAGTTGGAAGTTGAAGTTCGC-	GTCGTC	99	reads, 0.3%
Ni-15	GACGAC	ACAGTTGATGAATGTNTAAGTTGAAGTTGT	GGCGTC	77	reads, 0.2%
Ni-16	GACGAC	ACACAAAGTTCGAGTCGCATGACATAGTGC	GTCGTC	75	reads, 0.2%
Ni-17	GACGAC	GTTGGTGTGTGTCGTAAGGTTCAGTGGACA	GTCGTC	68	reads, 0.2%
Ni-18	GACGAC	GGTAGTTCAGTGGACTTGGTGTGCGATATA	GTCGTC	68	reads, 0.2%
Ni-20	GACGAC	GGGCTGGTTTTAAAGACATGGTCATGCTGA	GTCGTC	66	reads, 0.2%

Figure S2. Alignment of the top 20 sequences of the Co^{2+} selection library. Two families and some ungrouped sequences were identified. Ni^{2+} selection family 3 and Co^{2+} selection family 1 belong to the same family.

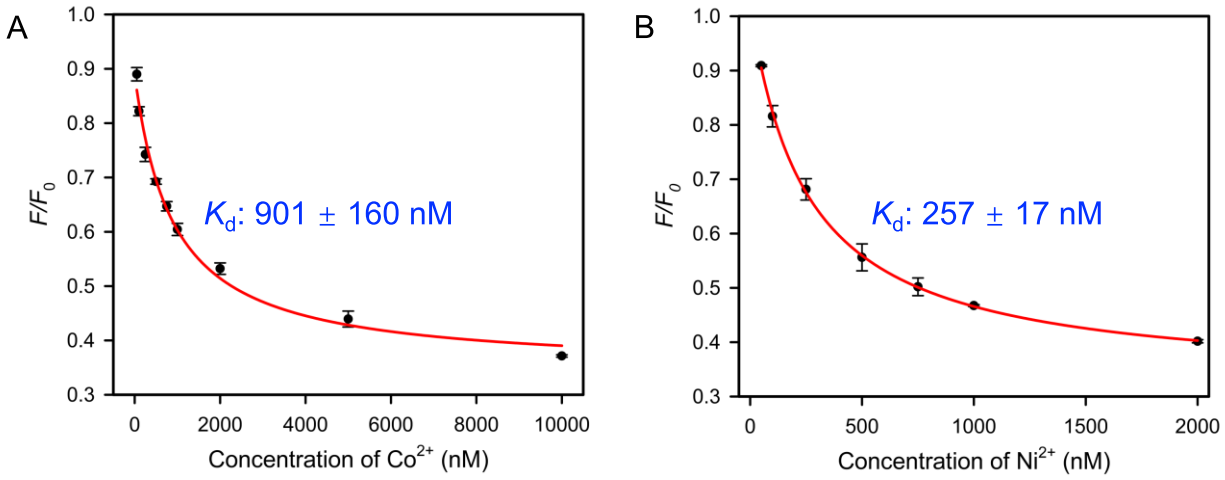


Figure S3. Titration curves of (A) Co^{2+} and (B) Ni^{2+} into Ni-4/ThT mixture in the SELEX buffer (Ni-4: 0.5 μM, ThT: 1 μM).

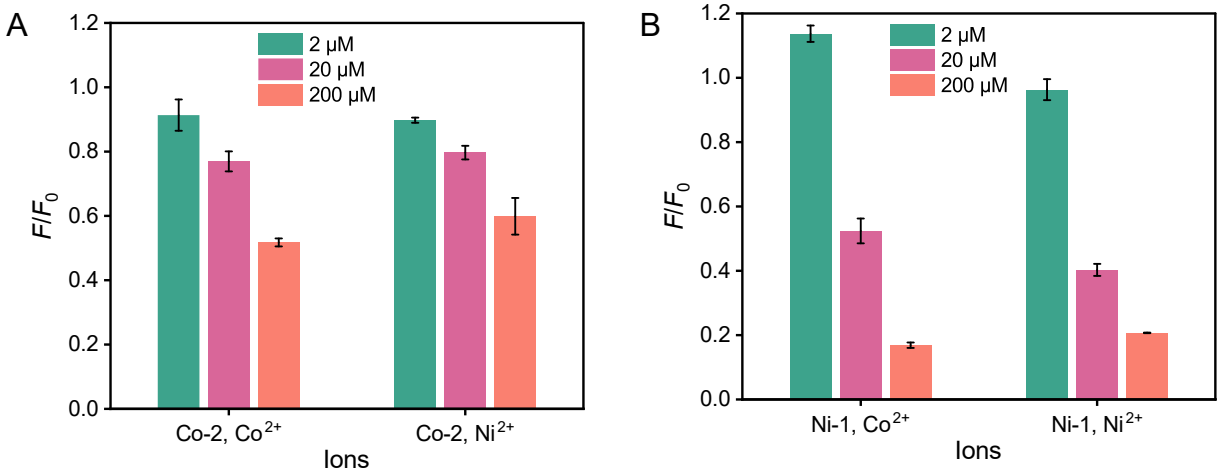


Figure S4. The response of aptamers (A) Co-2 and (B) Ni-1 to 2 μM, 20 μM and 200 μM Co^{2+} or Ni^{2+} (Aptamers: 0.5 μM, ThT: 1 μM, buffer: 10 mM MES pH 6, 100 mM NaCl, 2 mM $MgCl_2$). F_0 and F denote the fluorescence before and after adding ions, respectively.

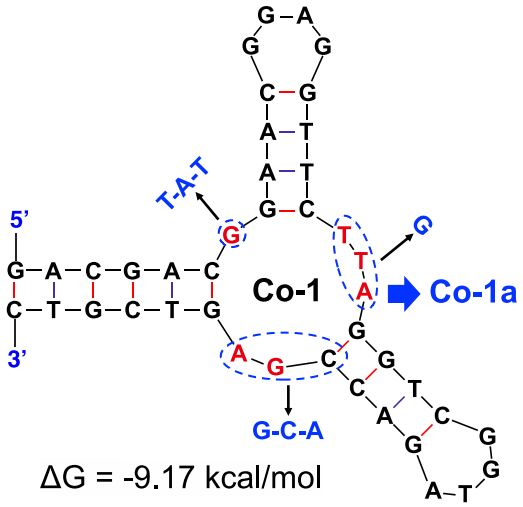


Figure S5. Secondary structures of Co-1a mutant. Co-1a has the same nucleotide composition as Co-1 but exhibits a completely different secondary structure.

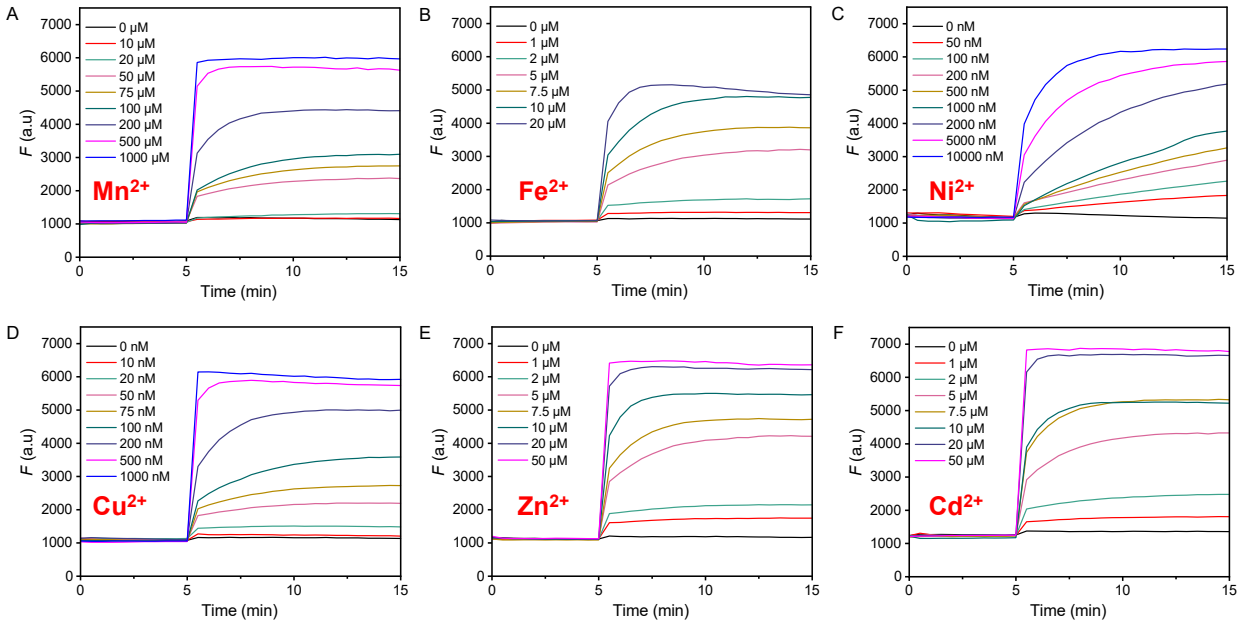


Figure S6. Kinetics traces of the 20 nM FAM Co-1 and 40 nM Quencher-cDNA sensor in the presence of various concentrations of (A) Mn^{2+}, (B) Fe^{2+}, (C) Ni^{2+}, (D) Cu^{2+}, (B) Zn^{2+}, and (E) Cd^{2+} in SELEX buffer. Metal ions were added at 5 min.

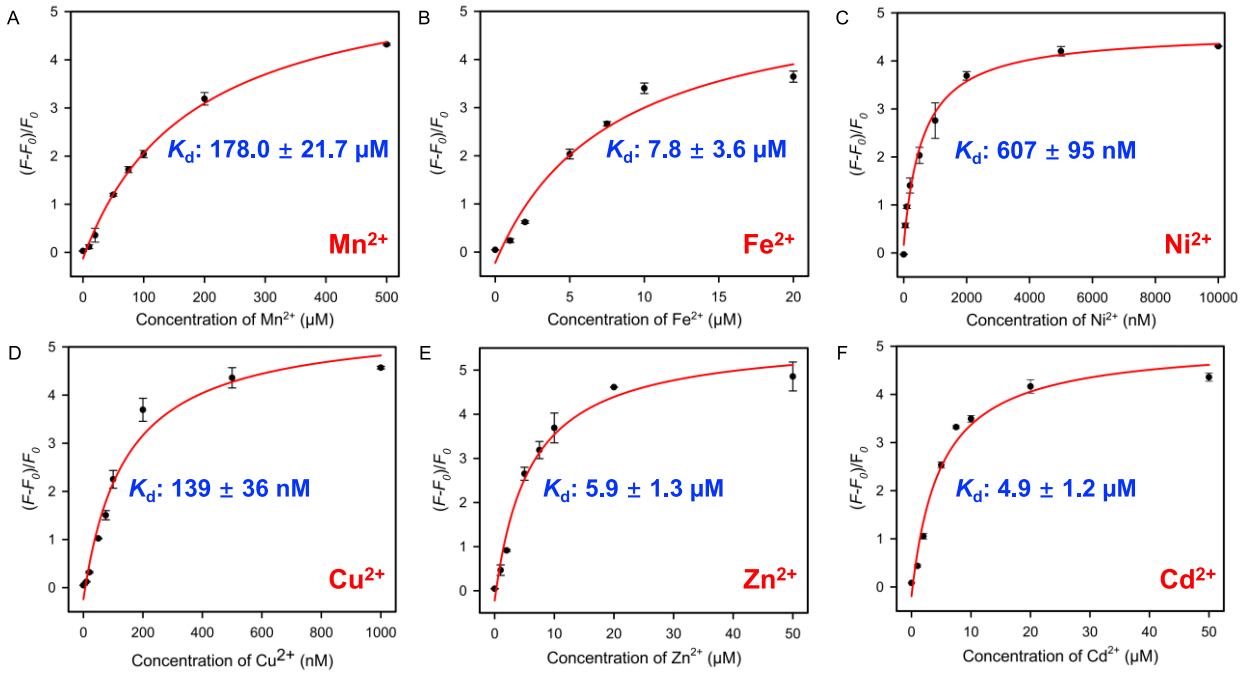


Figure S7. Calibration curves of the sensor for (A) Mn^{2+}, (B) Fe^{2+}, (C) Ni^{2+}, (D) Cu^{2+}, (B) Zn^{2+}, and (E) Cd^{2+} based on the data in Figure S6. The apparent K_d values are also listed in the figure.

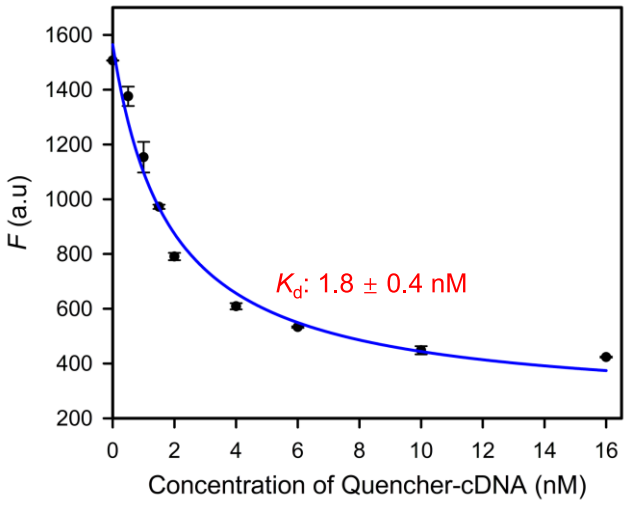


Figure S8. Fluorescence quenching of 5 nM of FAM Co-1 by increasing concentration of Quencher-cDNA in SELEX buffer (10 mM MES, pH 7.4, 100 mM NaCl, 2 mM $MgCl_2$). This experiment allows us to obtain the true K_d of the aptamer based on Easley's work.[1]

The reaction between FAM Co-1 (F) and Quencher-cDNA (Q) is defined as Reaction 1. The reaction between FQ and Co^{2+} is defined as Reaction 2. The binding of the aptamer (FQ) to Co^{2+} is defined as Reaction 3.

Reaction 1: F + Q =FQ, $K_{d1} = \frac{[F][Q]}{[FQ]}$

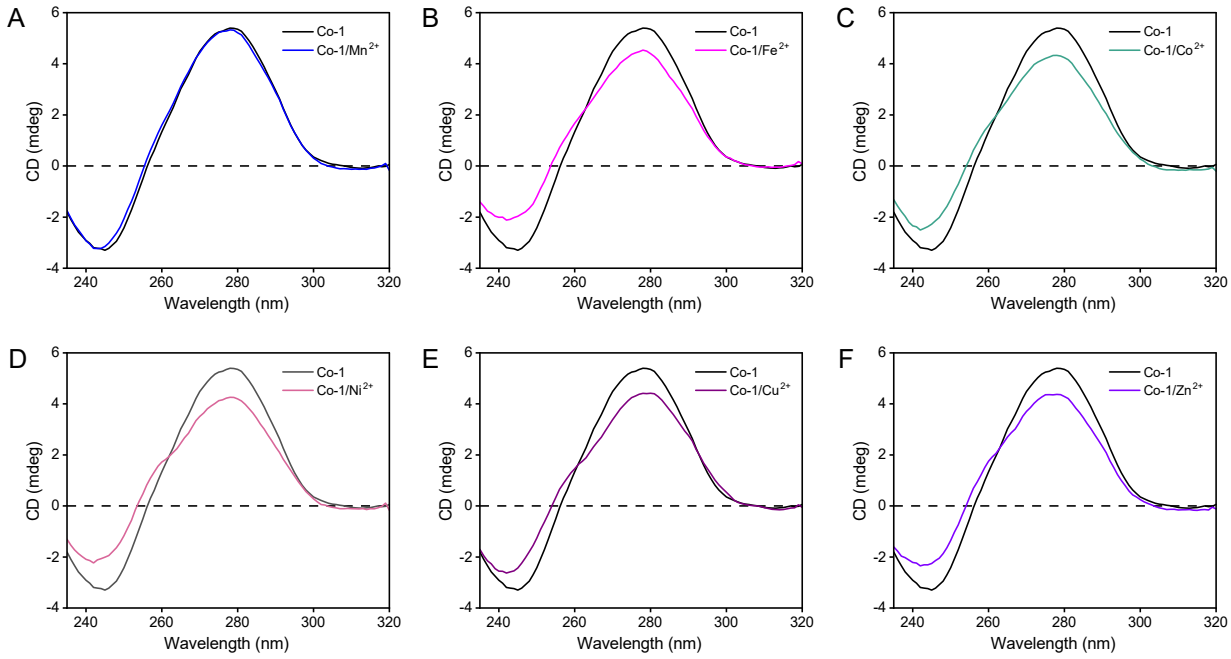
Reaction 2: FQ + Co^{2+} =F-Co + Q, $K_{d2} = \frac{[FQ][Co]}{[F-Co][Q]}$

K_{d2} can be calculated based on the apparent Kd2 from the sensor calibration curve in Figure 3 of the main paper. When the lactate concentration is at the apparent Kd value, [FQ] and [F-Co] had the same concentration. Thus, K_{d2} = [K_{d2} apparent]/[Q]. Since we used 20 nM of the FAM Co-1 and 40 nM Quencher-cDNA for the sensor, [Q] was 30 nM when lactate was equal to the apparent

Reaction 3: F + Co^{2+} = F-Co, $K_{d3} = K_{d1}K_{d2} = \frac{[F][Q]}{[FQ]} \frac{[FQ][Co]}{[F-Co][Q]} = \frac{[F][Co]}{[F-Co]}$

For Co^{2+}, $K_d = K_{d1}\ K_{d2}$ = 1.8 nM × $K_{d2\ apparent}$ /30 nM = 1.8 nM = 76 nM.

Similarly, the true Kd values for Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} are 10,680 nM, 468 nM, 37 nM, 8 nM, 352 nM, and 295 nM, respectively.



Figures S9. The CD spectra of 5 μM Co-1with 20 μM (A) Mn^{2+}, (B) Fe^{2+}, (C) Co^{2+}, (D) Ni^{2+}, (E) Cu^{2+}, and (F) Zn^{2+}. Buffer: 10 mM MES (pH 6.0) with 100 mM NaCl and 2 mM $MgCl_2$.

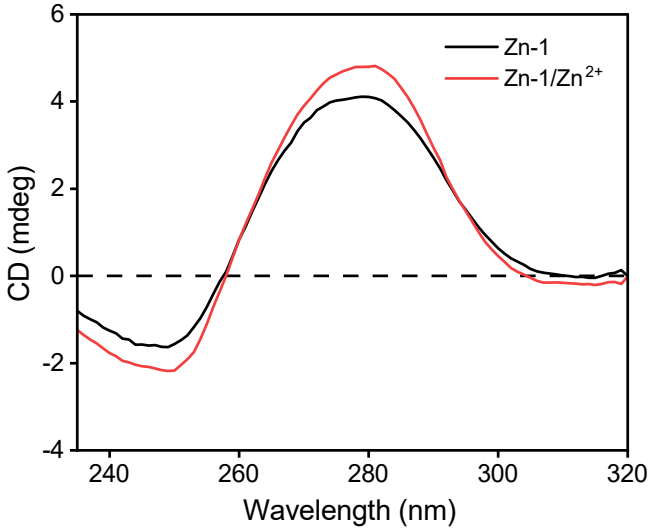


Figure S10. The CD spectra of 5 μM Zn-1with 20 μM Zn^{2+}. Buffer: 10 mM MES (pH 6.0) with 100 mM NaCl and 2 mM $MgCl_2$.

References

1. J. Hu and C. J. Easley, *Analyst*, 2011, **136**, 3461-3468.