Electronic supplementary information

A high affinity and selective DNA aptamer for copper ions

Jin Wang[1,2], Yibo Liu[2], Xiangmei Li[1], Hongtao Lei[1*], Juewen Liu[2]*

1. Guangdong Provincial Key Laboratory of Food Quality and Safety, College of Food Science, South China Agricultural University, Guangzhou 510642, China;
2. Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

*Corresponding Authors
Hongtao Lei. Email: hongtao@scau.edu.cn
Juewen Liu. Email: liujw@uwaterloo.ca

Materials and Methods

All of the DNA samples utilized in this study were synthesized by Integrated DNA Technologies (Coralville, IA, USA), with their sequences detailed in Table S1. Streptavidin-coated agarose resin possessed a loading capacity of 1-3 mg biotinylated BSA per milliliter was purchased 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). Copper chloride dihydrate, calcium chloride hexahydrate, scandium chloride hydrate, chromium chloride hexahydrate, manganese chloride hydrate, iron(II) chloride tetrahydrate, iron(III) chloride hexahydrate, cobalt chloride hexahydrate, nickel chloride hexahydrate, cadmium chloride hydrate, barium chloride dihydrate, terbium chloride hexahydrate, mercury acetate, lead bromide, ascorbic acid (AA), thioflavin T (ThT), Amicon ultra-0.5 centrifugal filter unit (with 3K and 10K molecular weight cutoffs) were acquired from Millipore-Sigma (Oakville, ON, Canada). MES sodium salt, $MgCl_2 \cdot 6H_2O$, NaCl, HCl and NaOH were obtained from Bio Basic (Toronto, ON, Canada).

Milli-Q water was used in the preparation of all buffers and solutions. The SELEX buffer contained MES (10 mM, pH 6.0) with 2 mM $MgCl_2$ and 100 mM NaCl. The separation buffer was MES (10 mM, pH 6.0) with 100 mM NaCl.

SELEX

The selection process followed our previously established protocol.[1-3] $CuCl_2 \cdot 2H_2O$ was dissolved in Milli-Q water to a final concentration of 100 mM and subsequently diluted with SELEX buffer for the selection process. A total of 12 rounds of selection were performed, with Cu^{2+} concentrations of 10 μM for rounds 1-7 and 2 μM for rounds 8-12. The PCR products from the final round were sequenced. Detailed procedures were as followed.

A 73-nt DNA library containing a 30-nt random region was utilized. In the first round, 500 pmol of DNA library was combined with 1500 pmol of column-binding strand in 250 μL of selection buffer, followed by annealing at 95°C for 3 min, and cool to room temperature for 30 min. A microchromatography column was prepared by packing 100 μL of 6% BCL agarose beads and 100 μL of streptavidin agarose resin, which were pre-washed with 500 μL of SELEX buffer to remove any preservatives. The DNA library/column-binding strand complex mixture was then passed through the column six times to ensure proper binding with the agarose resin. The column was washed 12 times with 500 μL SELEX buffer. After which 750 μL of Cu^{2+} solution in selection buffer was passed through the column, 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 adjusted to 60 μL. This solution was diluted 10-fold and used for PCR amplification with an unlabeled forward primer and a biotinylated reverse primer. The PCR amplicons were captured using 100 μL streptavidin agarose resin. A microchromatography column was prepared by packing 100 μL of 6% BCL agarose beads, after which the PCR amplicons/streptavidin agarose resin mixture was added to the column. Then, the resin was washed with 500 μL of separation buffer for 8 times followed by incubation with 400 μL of 0.2 M NaOH for 15 min. The eluted library strand was collected. An additional 200 μL of 0.2 M NaOH was added to the column, and eluent was combined. The eluent was neutralized with 400 μL 0.2 M HCl and concentrated and washed using a 3K centrifugal filter with 460 μL of Milli-Q water and one aliquot of 460 μL SELEX buffer. For the subsequent rounds, the amount of DNA was adjusted to 100 pmol, while all other steps remained the same as the first round.

The last round library was subjected to a PCR with a combination of N501 and N703 sequencing primers (Table S1). These primers contained a unique index sequence to be used for Illumina sequencing. The PCR products were concentrated and washed using a centrifugal filter (10K MWCO) with 500 μL separation buffer, followed by extraction with a small DNA fragment extraction kit (IBI Scientific). The concentration of the eluted DNA was determined using NanoDrop, and sequencing was carried out at the McMaster University Genome Facility. The results were analyzed with Geneious Prime software (Auckland, New Zealand).

ThT-based assay

The fluorescence spectra of ThT-based assay were measured using a Varian Eclipse fluorescence spectrophotometer (CA, American). The assay contained 0.5 μm aptamer and 1 μm ThT dissolved in 500 μL selection buffer. The aptamer/ThT mixture was transferred to a quartz cuvette, where Cu^{2+} was gradually titrated to reach a final concentration of 2000 nM. Excitation at 440 nm was applied, with emission recorded between 460 nm and 520 nm. Fluorescence at 490 nm served as the basis for calculations. The initial fluorescence of the aptamer/ThT mixture is denoted as F_0, and the value after Cu^{2+} addition is F. The ratio F/F_0 was plotted, and the K_d was determined using the equation: $y = y_0 + aK_d / (K_d + [Cu^{2+}])$, where a represents the maximal fluorescence signal change at saturation.

Fluorescence aptasensor based on strand displacement

The fluorescence aptasensor experiments were conducted in a 96-well microplate using a Tecan Spark F200 microplate reader (Switzerland). The excitation and emission wavelengths were set to 485 nm and 525 nm, respectively. A mixture of 1 μL of FAM-labeled aptamer Cu-1 (FAM Cu-1, 100 μM), 2 μL of Quencher-cDNA (100 μM), and 97 μL of SELEX buffer was annealed at 85°C for 1 min, cooled to room temperature for 30 min, followed by cooling at 4°C for 30 min and then at -20°C for 30 min. For the aptasensor, 2 μL of annealed aptamer was added to 98 μL SELEX buffer, and the background fluorescence of the sensor was monitored for 5 min. Then, 2 μL of series of Cu^{2+} solutions were added, and the fluorescence was monitored for an additional 10 min. The data were represented as $(F–F_0)/F_0$, where F_0 denotes the fluorescence prior to Cu^{2+} addition, and F is the fluorescence after adding Cu^{2+}.

Detection of Cu^{2+} in real samples

The tap water from our lab was used to validate the reliability of the aptasensor. Initially, the Cu^{2+} concentration was determined using ICP-MS performed by ALS Canada Ltd. (3.23 mg/mL). To bring the concentration within the detectable range, the tap water was first diluted 20-fold using Mill-Q water. The sample was then prepared by adding 2 μL of the diluted tap water sample to the 20 nM FAM Cu-1 sensor following the same procedure as for the standard Cu^{2+} solution. The concentration was calculated using the standard curve, and the final concentration obtained using the aptasensor was compared to that obtained using ICP-MS.

Table S1. The DNA sequences used in this study. The modifications are based on the format of Integrated DNA Technologies Inc.

DNA name	Sequences and modifications (5' to 3')
Library	GGAGGCTCTCGGGACGAC$\mathbf{N_{30}}$GTCGTCCCGATGCTGCAATCGTAA
Biotin-column	GTCGTCCCGAGAGCCATA/**3BioTEG/**
Forward primer	GGAGGCTCTCGGGACGAC
Reverse primer	TTACGATTGCAGCATCGGGACG
Biotin-reverse primer	/**5Biosg**/TTACGATTGCAGCATCGGGACG
P5-501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACGCTCTTCCGATCTTTACGATTGCAGCATCGGGACG
P7-703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
FAM Cu-1	/**56-FAM**/CTCTCGACGACCACGGTAAACGACGCTGTACGGAGTGGTCTGTCGTC
Quencher-cDNA	AGTCGTCGAGAG/**3IABkFQ**/
Cu-1a	GACGACCACGTGAAACGACGCTGTACGGAGTGTGCTGTCGTC
Zn^{2+} Aptamer	GACGACGCTCCCATTCCAGCTTCGGTGGTAGCAGAAGTCGTC
MNZ-1	GACGACGGGTTTTGTGCTCAAAAGTTCTGGTGGTAAGTCGTC
SSA-CuII	ATCGCGATATTTTCTGTAGCGATTCTTGTTTGAGCGCTCGGTACGAACAGA

Family 1

Cu-01	GACGAC	CACGGTAAACGACGCTGTACGGAGTGGTCT	GTCGTC	**114 Reads**
Cu-02	GACGAC	CACGGTAAGGTGATGATGGATCACCGGTCT	GTCGTC	**93 Reads**
Cu-03	GACGAC	CACGGTAAAGGACGTATGGAGTCCTGGTCT	GTCGTC	**65 Reads**
Cu-04	GACGAC	CACGGTAAGGTCATGTGAAGTGACCGGTCT	GTCGTC	**60 Reads**
Cu-05	GACGAC	CACGGTAAGAAGGTCAGGGACATTCGGTCT	GTCGTC	**54 Reads**
Cu-06	GACGAC	CACGGTAACCGGATCGAAGATACGGGGTCT	GTCGTC	**49 Reads**
Cu-08	GACGAC	CACGGTAAGACGAGAGTTACGAGGCGGTCT	GTCGTC	**39 Reads**
Cu-09	GACGAC	CACGGTAAGCCTTGTGTCAAAAGGCGGTCT	GTCGTC	**38 Reads**
Cu-12	GACGAC	CACGGTAAGNWTGTNNTAACTNNAANCGGTCT	GTCGTC	**36 Reads**
Cu-13	GACGAC	CACGGTAAAGGTNTGTGCATNAAACCTGGTCT	GTCGTC	**36 Reads**
Cu-15	GACGAC	CACGGTAAGTGAKRCWRTGSSWRACGGTCT	GTCGTC	**35 Reads**
Cu-16	GACGAC	CACGGTAAGCTGTACATTGTAGAGCGGTCT	GTCGTC	**35 Reads**
Cu-17	GACGAC	CACGGTAAGAGAGGCAACGCCAATCGGTCT	GTCGTC	**35 Reads**
Cu-20	GACGAC	CACGGTAAGTAATCGGATGGATTACGGTCT	GTCGTC	**34 Reads**
Cu-21	GACGAC	CACGGTAAAYGAGCGTAANCGACTGGTCT	GTCGTC	**32 Reads**
Cu-22	GACGAC	CACGGTAATGCCGACTAAGTTGGCAGGTCT	GTCGTC	**31 Reads**
Cu-24	GACGAC	CACGGTAAGCTGTAGGTACTATAGCGGTCA	GTCGTC	**30 Reads**
Cu-25	GACGAC	CACGGTAAGTGTGCGCTCTACACACGGTCT	GTCGTC	**30 Reads**
Cu-27	GACGAC	CACGGTAAACGAAGAGTATTCGAGTGGTCT	GTCGTC	**29 Reads**
Cu-28	GACGAC	CACGGTAAGGTCCGATACTAGGACCGGTCA	GTCGTC	**29 Reads**
Cu-29	GACGAC	CACGGTAAGCCATCGTAAGAATGGCGGTCA	GTCGTC	**29 Reads**

Family 2

Cu-07	GACGAC	CAAGTGTCAANGNTGCATAGNNTCGTNNNGATGG	GTCGTC	**40 Reads**
Cu-10	GACGAC	CAAGTGTCANGANWNGCNNTATATGATGG	GTCGTC	**36 Reads**
Cu-11	GACGAC	CAAGTGTCACWNNNGTGCNAGWWNGTNGATGG	GTCGTC	**36 Reads**
Cu-23	GACGAC	GGGCAAGTGTCAACAGNATGTGGATAGACA	GTCGTC	**30 Reads**
Cu-26	GACGAC	CAAGTGTCATAAGKCNTGKNNTSNNTGATGG	GTCGTC	**29 Reads**
Cu-30	GACGAC	CAAGTKGGTATANAGTNTATGNNATGGTAT	GTCGTC	**28 Reads**

Ungrouped

Cu-14	GACGAC	GYAGATGCAAGTGTCTCYGATAGMTTCGGA	GTCGTC	**35 Reads**
Cu-18	GACGAC	CGGAAGTGTCGTAATTGGATTACGAAGAG	GGCGTC	**34 Reads**
Cu-19	GACGAC	GGCATGGAAAGTGACTTTGTATTAGATGCA	GTCGTC	**34 Reads**

Figure S1. Alignment of the top 30 sequences of the Cu^{2+} selection library. Two families were identified. The nucleotides in the primer-binding regions are in black and underlined, the conserved nucleotides are marked in red. This is consistent with that presented in the main paper, where the top 10 sequences were aligned.

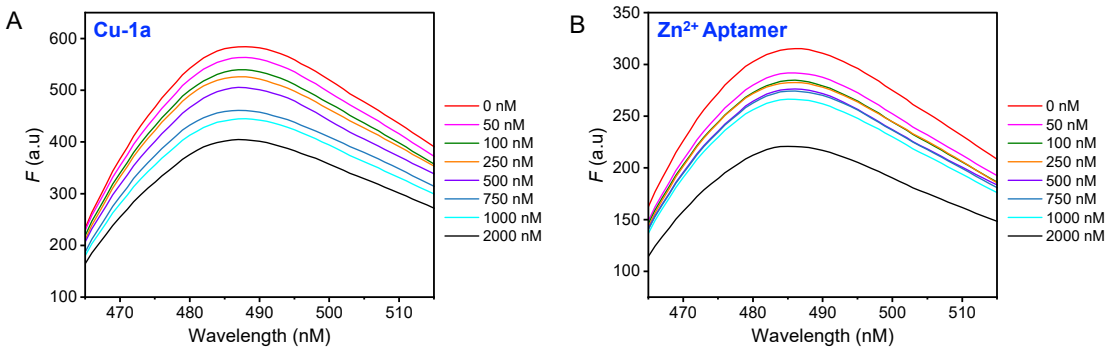

Figure S2. The fluorescence spectra of (A) ThT/Cu-1a and (B) ThT/Zn^{2+} aptamer mixtures in the presence of different concentrations of Cu^{2+} (Buffer: 10 mM MES pH 6, 100 mM NaCl, 2 mM $MgCl_2$, Cu-1: 0.5 μM, ThT: 1 μM).

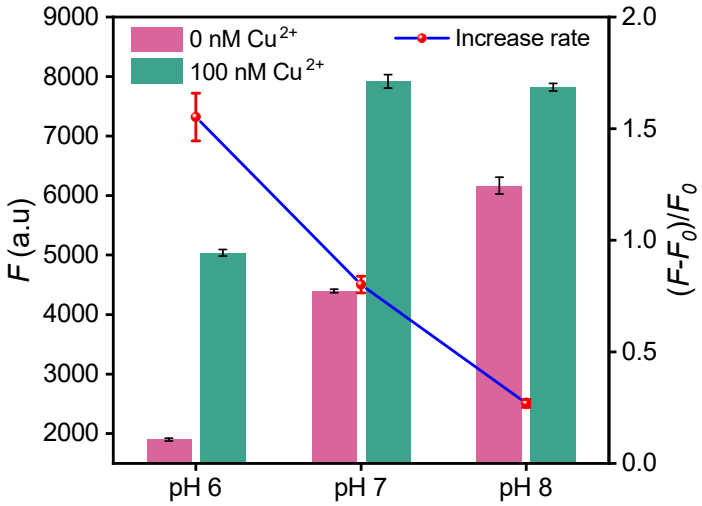

Figure S3. Effect of pH on aptamer Cu-1 Binding. pH 6, 10 mM MES (100 mM NaCl, 2 mM $MgCl_2$). pH 7, 10 mM HEPES (100 mM NaCl, 2 mM $MgCl_2$). pH 8, 10 mM HEPES (100 mM NaCl, 2 mM $MgCl_2$). The best performance was achieved at pH 6.

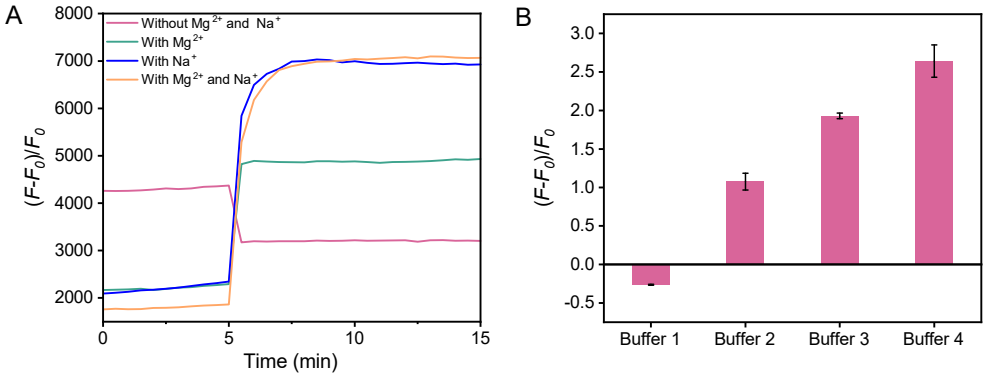

Figure S4. (A) Kinetics traces of the FAM-based sensor at a Cu^{2+} concentration of 500 nM in the presence of different binding buffer: 10 mM MES (pH 6.0, Buffer 1), 10 mM MES with 2 mM Mg^{2+} (pH 6.0, Buffer 2), 10 mM MES with 100 mM Na^{+} (pH 6.0, Buffer 3), and 10 mM MES with 2 mM Mg^{2+} and 100 mM Na^{+} (pH 6.0, Buffer 4). (B) Fluorescence enhancement of the sensor under the four different buffers. Buffer 4 has the best sensor response and was chosen for sensing studies.

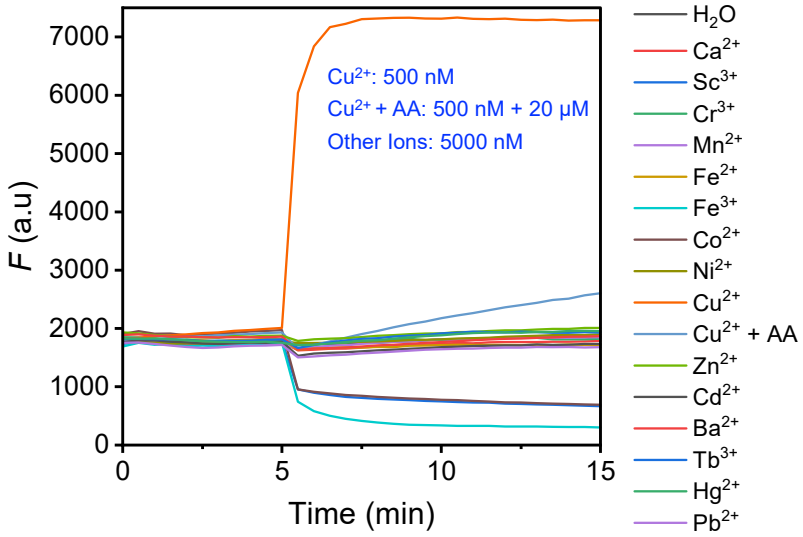

Figure S5. Kinetics traces of 20 nM FAM and 40 nM Quencher-cDNA Cu-1 sensor in the presence of different metal ions in Buffer 4 (10 mM MES pH 7.4, 100 mM NaCl, 2 mM $MgCl_2$)

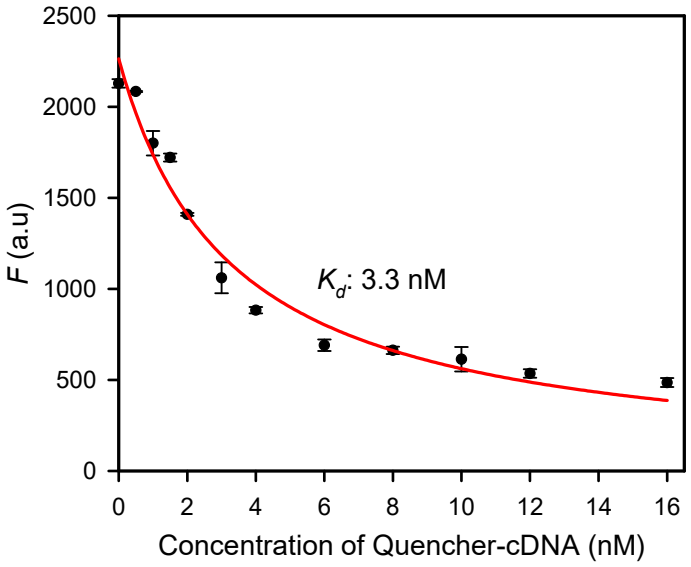

Figure S6. Fluorescence quenching of 5 nM of FAM Cu-1 by increasing concentration of Quencher-cDNA in Buffer 4 (10 mM MES pH 6, 100 mM NaCl, 2 mM $MgCl_2$). From this experiment, the K_d value of the Quencher-cDNA and FAM-labeled Cu-1 aptamer can be calculated.

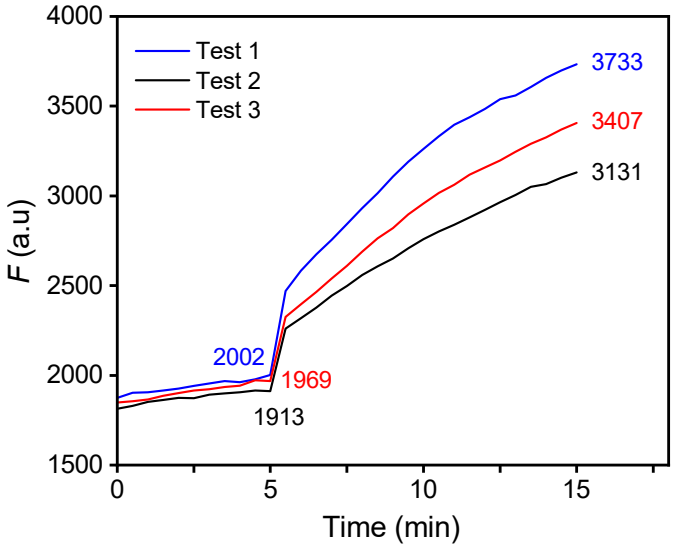

Figure S7. Kinetic response of FAM Cu-1 sensor to 20 times dilution of tap water. The data points at 15 min of this graph were used for calculation.

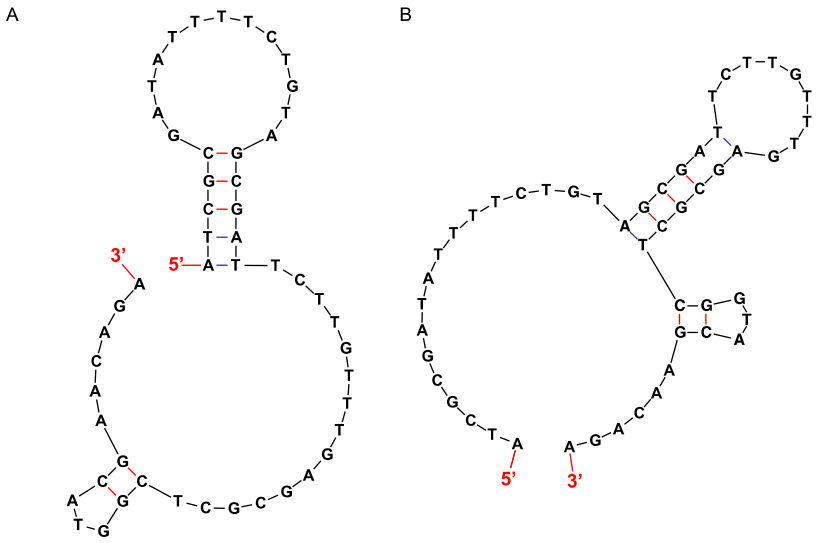

Figure S8. The predicted secondary structures of the SSA-CuII aptamer by mflod. These two structures (A and B) were from the same sequence. Compared to these two aptamers, the newly selected Cu-1 aptamer is shorter and has a better defined secondary structure allowing rational sequence modification for sensing applications.

References

1. X. Li, Z. Yang, M. Waniss, X. Liu, X. Wang, Z. Xu, H. Lei and J. Liu, *Anal. Chem.*, 2023, **95**, 16366-16373.
2. L. Gu, Y. Ding, Y. Zhou, Y. Zhang, D. Wang and J. Liu, *Angew. Chem., Int. Ed.*, 2024, **63**, e202314450.
3. Y. Ding and J. Liu, *J. Am. Chem. Soc.*, 2023, **145**, 7540-7547.