Supporting information

Title: Defining the copper binding aptamotif and Aptamer Integrated Recovery Platform (AIRP)

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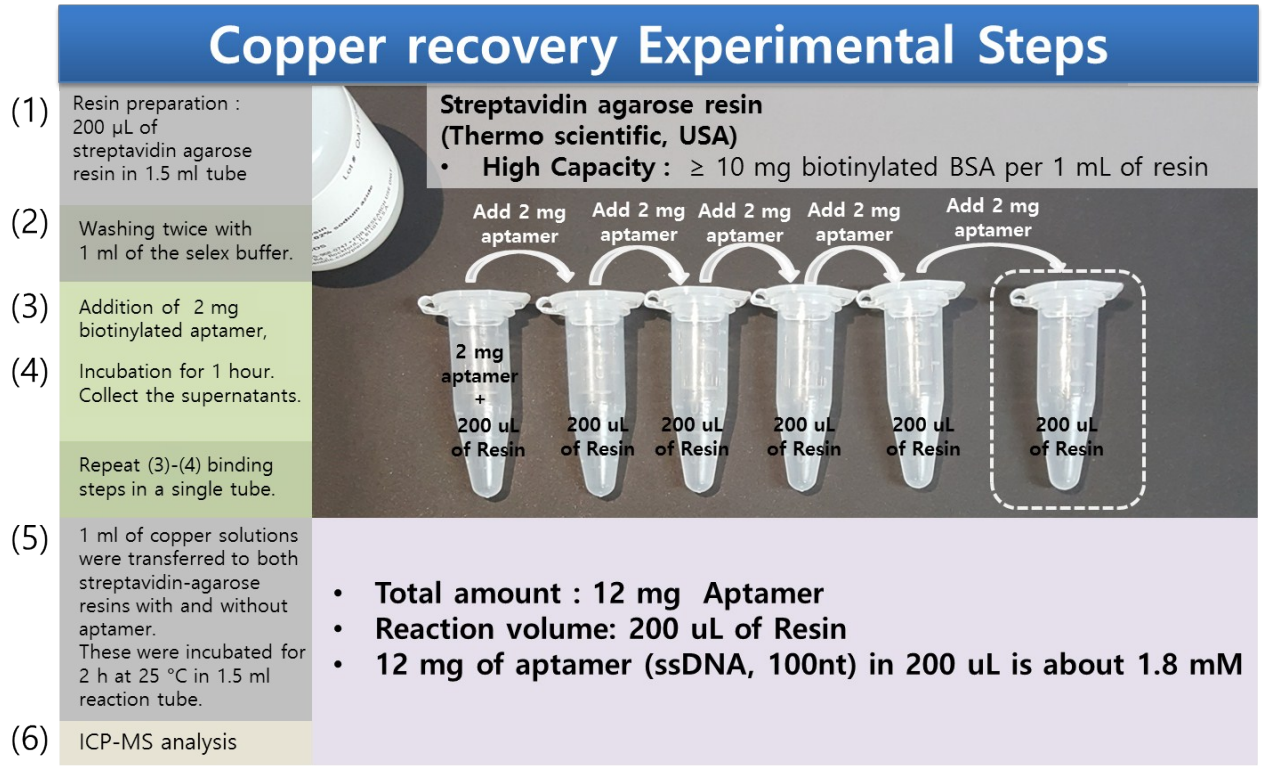
Figures and Table

Figure S1. Copper recovery experimental steps



Experimental steps

(1) Resin preparation:

- Gently mix the streptavidin agarose resin (Thermo scientific, USA)
- Using wide-mouth yellow pipette-tip, transfer 200 µL of agarose resin slurry to 1.5 mL microtubes.

(2) Remove supernatant. The resin was then washed twice with 1 ml of the selex buffer.

(3) Aptamer preparation in 1.5 ml reaction tube:

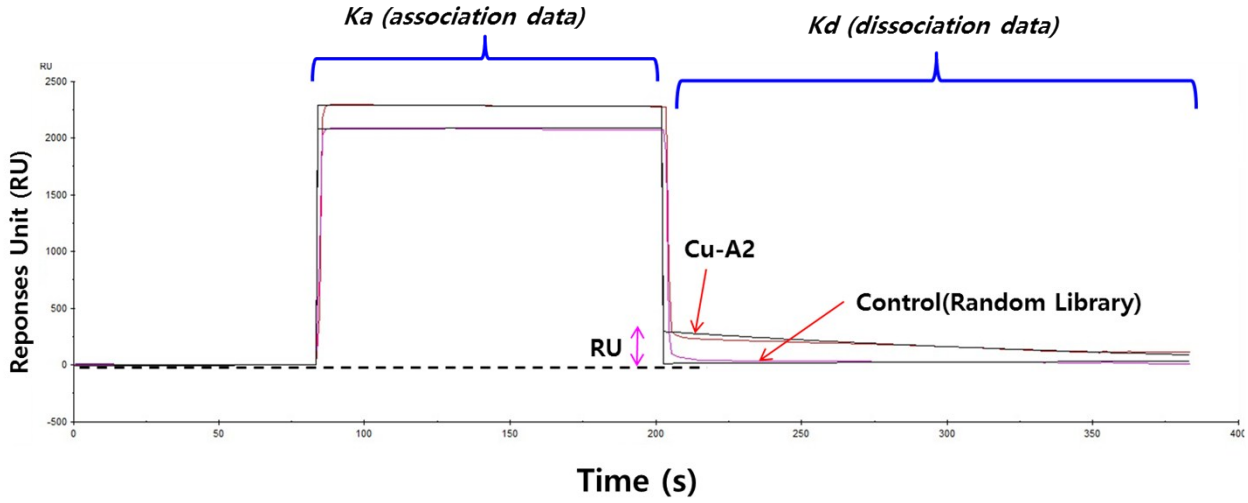
- 2 mg of the biotinylated aptamer in 400 µL of the selex buffer
- Applied drop-by-drop on to the streptavidin agarose resin
- Incubated for 1 h on thermomixer (Eppendorf, Germany) at 1300 rpm.

(4) Correct the supernatants by extracting supernatant and repeat aptamer binding in a single tube.

(5) After washing, 1 ml of copper solutions were transferred to both streptavidin-agarose resins with and without aptamer and incubated for 2 h at 25 °C in 1.5 ml reaction tube (final concentrations, 1, 3, 5, 7, 10, 30, 50, 70, 100, 150 and 300 mg/L).

(6) After centrifugation at 13000 rpm for 1 min, the copper content in supernatant fraction was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent 7500ce, Agilent, USA).

Figure S2. The binding affinity test by SPR



The binding affinity of Cu-A2 was analyzed by SPR using Biacore 3000 Instrument. All data sets were listed in **Supporting information Table 2**. Random initial library was also introduced as a control.

Table S1. Sequence analysis of the copper-aptamer candidates and their copper-binding affinities, as determined from the direct coupling SPR assay.

Name	Aptamer sequences (N40)	Affinity ($K_{D,}$ M)	Random sequences size (bp)	Identical sequences
Cu _A1	GGTGTGACTGAGTAACTCACTCCAACCCCTGGAGTGGGAT	$7.88 \pm 1.41 \times 10^{-11}$	40	2
Cu _A2	CCCAGGCTTGGATAGCACATTCATGGCAGCGTTCTTTACG	$1.83 \pm 4.503 \times 10^{-11}$	40	6
Cu _A3	ATTTGACCATCGTCTCCGGCTCACGGAATTATCAGCAGCG	$8.95 \pm 2.5 \times 10^{-10}$	40	3
Cu _A4	TTCTTAGGGAAGTAATCACGGAAAACCGTCTCCGTTCTCA	$1.73 \pm 1.03 \times 10^{-10}$	40	1
Cu _A5	ACCCACCGTACTAGTGACGGATACAATGGCCGCCTTGTCG	$8.16 \pm 1.93 \times 10^{-8}$	40	1
Cu _A6	CCAGGCAAGAAGTAATCACGGAAAACCGTCTCCGTTCTCA	$8.01 \pm 0.11 \times 10^{-8}$	40	1
Cu _A7	AACGTTTAGCATTTAGAGTCATTTGCGATGATCCACGTTG	$1.10 \pm 0.02 \times 10^{-8}$	40	1
Cu _A8	CGGGGCACTGTTGAGGGTCTGGCACGGTCACAAGCTAG	$9.42 \pm 1.18 \times 10^{-7}$	38	1
Cu _A9	AAGAGGCTTGAAGACGCCATCATGATGAGGAACTTGATTA	$7.02 \pm 0.08 \times 10^{-8}$	40	1
Cu _A10	AGGACAATCAGTAACGGCGAGCCACATAACGTTCGAGTG	$1.09 \pm 1.77 \times 10^{-8}$	39	1
Cu _A11	CGTATTCCGAATATTGGGTCACTCATGTGTCAGTCGCCTG	$1.39 \pm 1.52 \times 10^{-8}$	40	1

Table S2. The binding affinity test of Cu-A2

Cu-A2	K_a (1/Ms)	K_d (1/s)	K_D (M)	RU
1st Test	7.01E+07	1.33E-03	1.90E-11	305
2nd Test	8.04E+07	2.67E-03	3.32E-11	305
3rd Test	5.89E+05	6.10E-06	1.04E-11	119
4th Test	7.01E+07	1.49E-03	2.13E-11	305
5th Test	7.89E+05	6.10E-06	7.73E-12	119
Average	**3.38E+07**	**2.62E-03**	**1.83E-11**	**213**

Table S3. The binding affinity values of Cu-A1, Cu-A2, Cu-A3, and Cu-A4

Name	$K_{D,}$ (M)	SD (M)
Cu-A1 Nat	7.88E-11	±1.4176E-11
Cu-A1 Mut	7.65E-10	±1.3429E-10
Cu-A1 Del	3.97E-09	±1.6411E-9

Name	$K_{D,}$ (M)	SD (M)
Cu-A2 Nat	1.83E-11	±1.3304E-11
Cu-A2 Mut	8.34E-09	±1.5194E-9
Cu-A2 Del	4.84E-08	±1.1768E-8

Name	$K_{D,}$ (M)	SD (M)
Cu-A3 Nat	8.95E-10	±2.5202E-10
Cu-A3 Mut	8.5E-11	±5.0082E-11
Cu-A3 Del	1.69E-08	±1.1460E-8

Name	$K_{D,}$ (M)	SD (M)
Cu-A4 Nat	1.73E-10	±1.0279E-10
Cu-A4 Mut	7.73E-11	±1.3405E-11
Cu-A4 Del	1.92E-10	±2.1575E-10

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