Electronic Supplementary Information

Oligonucleotide library and primers

A 20N randomized oligonucleotide sequence was used as the library, which consisted of a randomized 20-mer midsection (5'-AGCAGCACAGAGGTCAGATG-N20-CCTATGCGTGCTACCGTGAA-3'), synthesized at 1 mM scale and PAGE purified (Invitrogen Life Technologies, Grand Island, NY, USA). One set of primers, (Lab-F 5'-AGCAGCACAGAGGTCAGATG-3' and the Lab-R5'-TTCACGGTAGCACGCATAGG-3'), was used to anneal the 5' and 3' degenerating region of the library during the PCR amplification. A 5'-biotin labeled primer, Lab-biotin-R, with the same sequence as was described above, was used to isolate the forward single strand from the double strand PCR product. A set of primers, Lab-FITC-F and Lab-biotin-R, with the same sequence as described above, was used to generate FITC-labeled, single strand aptamers. Biotin-labeled aptamers, coupled with HRP-conjugated streptavidin, was used to synthesize the aptamer-based reagent for an enzyme-linked immunosorbent assay (ELISA). The universal T7 primer was used to sequence the nucleotide of the selected aptamer (T7: 5'-TAATACGACTCACTATAGGG-3').

CRP-coated, bio-functionalized magnetic particles

The streptavidin-coated magnetic reagent (SA reagent) used in this study was purchased from Magqu (Magqu, Taipei, Taiwan). The average hydrodynamic diameter of the streptavidin-coated magnetic nanoparticles (SA-MNPs) in the reagent was 50 nm. The reagent had a concentration of SA-MNPs of 0.3 emu/g. The magnetic nanoparticles were bio-functionalized by coating streptavidin on the outermost surface of nanoparticles and were dispersed in PBS (pH=7.4). The pureness of the human CRP used in this study was greater than 99% and was purchased from MYBIOSOURCE (MYBIOSOURCE, San Diego USA). The biotinylation kit (Biotin Labeling Kit-NH_2) was purchased from Abnova (Abnova, Taipei, Taiwan). The biotinylated CRP protein was prepared according to the manufacturer's instructions. Afterwards, a total of 250 μg biotinylated CRP protein was mixed with 50 μL of SA reagent and incubated overnight at 4°C. The high affinity binding between the streptavidin and biotin ensured the conjugation between the magnetic nanoparticles and the biotinylated CRP protein. Then, the incubated solution was subjected to magnetic separation to remove the unbound biotinylated CRP. The CRP-MNPs were re-dispersed in 50 μL PBS-T (50 mM of K_2HPO_4, pH 7.5, 150 mM NaCl, 0.05% Tween-20) to form a CRP labeled reagent (CRP reagent) and stored at 4°C. The final concentration of the biotinylated CRP in the CRP reagent was 188.47±1.30 ng/μL, as determined by a Bradford assay (Bio-Rad, Hercules, CA. USA). Before being used,

the CRP reagent was washed 3 times with binding buffer (BD: 50 mM of NaH_2PO_4, pH 8.0, 150 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 0.005% (v/v) Tween-20). During each washing step, a magnetic stand (Magqu) was used to collect the CRP-MNPs. Similarly, if the SA-MNPs were needed, then the SA reagent was washed 3 times with BD buffer and the SA-MNPs were collected by using a magnetic stand.

Cloning, Sequencing and Mottif Analysis of Selected Aptamers

The supernatants that were collected from each experimental step were precipitated with 1 mL of 100% cold alcohol and diluted by 100 μL of ddH_2O for subsequent PCR amplification. The collected supernatants were subsequently amplified by PCR, with Lab-F and Lab-R primers. The PCR reaction, which contained 1.25 U of DNA polymerase (Invitrogen), 0.1 mM of dNTPs, 0.5 mM of $MgSO_4$, and 0.5 nM primers, was performed under the following conditions: 5 minutes at 95°C; 35 cycles of 40 seconds at 95°C; 40 seconds at 60°C; 40 seconds at 72°C; and 10 minutes at 72°C. The PCR product was purified by using a DNA Extraction Miniprep System (Viogene, Taipei, Taiwan). The purified product was sub-cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA). The cloning procedure was performed according to the manufacturer's instructions. The plasmids of the picked-up colonies were purified by using a High-Speed Plasmid Mini Kit (Geneaid, Taipei, Taiwan). The plasmids were sequenced by using an Applied Biosystems PRISM 3730 DNA automatic sequencer and a Big Dye terminator cycle sequencing kit (Foster City, CA, USA). The secondary structures of the aptamers were predicted by using Mfold[1] .

Estimation of Aptamer Binding Affinity by Real-Time Quantitative PCR

The affinity of the aptamers for the CRP target was described by the equilibrium dissociation constant (Kd), which was measured by a real-time PCR[2]. For each plasmid picked from the cloning experiments, 10 ng of aptamer clone plasmid was used as a PCR template to generate double strand DNA (dsDNA) with Lab-biotin-R and Lab-F primers. The PCR condition and procedure were as described above. After the completion of the PCR amplification, the PCR product was mixed with SA-MNPs, obtained by magnetic separation from 5 μL of SA reagent. Forward single strand aptamers (non-biotinylated strand) were separated from the immobilized complementary strand, by being incubated with 0.15 N of fresh NaOH for 5 minutes. The bound SA-MNPs were removed with a magnetic stand. An equal amount of 0.15 N of HCl was added to the collected supernatant to adjust the final pH to 7.0, after which the forward ssDNA was precipitated with 1 mL of 100% ice-cold alcohol. The concentration of the single strand aptamers was determined with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). A series of progressively diluted aptamers (200 nM to 1.5625 nM) in 20 μL of BD buffer were

heated to 95°C for 5 minutes and cooled at 4°C for the formation of secondary structures. Partial diluted aptamers were retained as an input control (input). CRP-MNPs, obtained by magnetic separation from 5 μL of CRP reagent, were added into each micro-tube containing diluted aptamers and incubated for 30 minutes at room temperature. The bound CRP-MNPs were washed twice with 100 μL of BD buffer. The bound aptamers were then eluted from the CRP-MNPs by heating the aptamers at 94°C for 10 minutes in 20 μL of ddH_2O. The CRP-MNPs in the solution were removed with a magnetic stand, and the supernatants were collected. Both the input control and eluted aptamers were precipitated with 1 mL of 100% ice-cold alcohol. The input control and eluted aptamers were individually dissolved in test tubes filled with 100 μL of ddH_2O. The quantities of the aptamers in each test tube, including input control tube and eluted aptamer tubes, were calculated by real-time quantitative PCR (q-PCR). q-PCR was performed with MicroAmp optical 96-well reaction plates, and the threshold cycle (ct) value was calculated automatically using the maximum correlation coefficient approach with the StepOnePlus Real-Time PCR Systems software, version 2.0 (Applied Biosystems). The mixture for each q-PCR run was 10 μL containing 2.5 μL of SYBR Green PCR master mix (Applied Biosystems) and 0.5 nM of primer Lab forward and Lab reverse. The reaction condition was as follows: 95°C for 3 minutes; 40 cycles at 94°C for 30 seconds; 60°C for 30 seconds; and 72°C for 30 seconds. The concentrations of the aptamers in the input control and of the eluted aptamers were calculated, using a 200 nM concentration of aptamers as indicative of maximum binding. The Kd value of the selected aptamer was then determined by fitting a saturation binding curve based on the experimental data via a curve fitting program, CurveExpert1.3 (curveexpert.webhop.net).

FITC-Labeling Aptamer Preparation, Selectivity and Specificity Analyses of Selected Aptamers

The plasmids of the aptamers were used as templates and a set of primers, Lab-FITC-F and Lab-biotin-R, was used for PCR amplification. The PCR condition and purification procedure of the FITC-labeled, single strand aptamers were the same as those for the forward single strand aptamers described above, except for the usage of the Lab-FITC-F primer during PCR process instead of the Lab-F primer. For the selectivity analysis of the aptamers via MARAS, 10 nM FITC-labeled, single strand aptamers were heated to 95°C for 5 minutes to disrupt the formation of secondary structures, snap cooled at 4°C for 10 minutes, and incubated with 5 μL of CRP reagent for 30 minutes at room temperature. The bound compounds were washed 3 times with BD buffer to remove the unbound FITC-labeled aptamers by magnetic separation. The bound mixtures of the FITC-labeled aptamers with CRP reagent were

re-dispersed in 20 μL of BD buffer and were observed by a fluorescence microscopy (DP72; Olympus, Center Valley, PA). Sequentially, the re-dispersed bound mixtures were subjected to an alternating magnetic field (25 gauss/200 KHz was used for AC-MARAS and 400 gauss/100 KHz was used for HAC-MARAS) for 10 minutes, and stirred every 2.5 minutes by pipetting, via the setup depicted in Figure 2. The bound compounds were washed 3 times with BD buffer to remove detached aptamers by magnetic separation. The resultant bound mixture was re-dispersed in 20 μL of BD buffer and was analyzed with a fluorescence microscopy. For the specificity analysis of the aptamers, 10 nM of FITC-labeled aptamers were heated to 95°C for 2 minutes to disrupt the formation of secondary structures, snap cooled at 4°C for 10 minutes, and incubated with 5 μL of CRP reagent and 5 μL of SA reagent for 30 minutes at room temperature, separately. The bound compounds were washed 3 times with BD buffer to remove unbound aptamers by magnetic separation. After washing, the CRP reagent and SA reagent bound with/without aptamers were re-dispersed with 20 μL of BD buffer and examined by using a fluorescence microscopy.

Preparation of the Aptamer-Based ELISA Assay

The assessment of the aptamer-based assay with aptamers selected via the AC-MARAS process was performed for an enzyme-linked immunosorbent assay (ELISA). In brief, an ELISA microplate was coated with a series of progressively diluted CRP protein with concentration from 2 to 0.0625 μg/mL in PBS and was left overnight at 4°C. The wells were subsequently blocked with 100 μL PBS containing 10% (w/v) bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) for 2 hours at 37°C. The wells were then washed 3 times with 200 μL of washing buffer (PBS containing 0.05%, Tween-20: PBS-T). The preparation of the biotin-labeled single strand is described briefly, as follows. A set of primers, Lab-biotin-F and Lab-R, was used to generate biotin-labeled PCR product. The biotin-labeled PCR product was incubated with 5 μL of SA reagent. Reversed single strand aptamers (non-biotinylated strand) were separated from the immobilized complementary strand, by being incubated with 0.15 N of fresh NaOH for 5 minutes and were removed by magnetic separation. The retained bound mixture was dispersed with 20 μL of ddH_2O and heated at 70°C for 30 seconds to disrupt the biotin-streptavidin conjugation[3]. Immediately, the biotin-labeled single strand aptamers were separated from the SA-MNPs by magnetic separation and collected for the following analysis. The concentrations of collected biotin-labeled single strand aptamer were 8.5 ng/μL (OS-AC-20N-1) and 9.4 ng/μL (OS-AC-20N-3) determined with a NanoDrop 2000c spectrophotometer. Total 150 ng biotin-labeled single strand aptamer was air-dried and re-dissolved with 1.5 mL BD buffer. Aptamers were heated and cooled to form secondary structures. Then, the 100

μL biotin-labeled aptamer (5 nM) was added per well to ensure saturation of all binding sites, and the mixture was incubated for 2 hours at room temperature. The plate was washed 3 times with 200 μL washing buffer and a 100 μL of streptavidin-HRP (Sigma-Aldrich, Missouri, USA) was added to the plate and incubated for 1 hour at room temperature. The plate was washed 3 times with 200 μL PBS-T. The color was developed by adding 100 μL 3,3′,5,5′-tetramethyl-benzidine (TMB, Sigma-Aldrich) substrate solution and by having the mixture stand at room temperature for 20 minutes. The reaction was terminated with the addition of 100 μL of 2N HCL, and the absorbance was measured at 405 nm by using EMax precision microplate reader (Molecular Devices, CA, USA). The measured result was compared to the standard curve of the CRP concentration, which was adopted from the data sheet of ELISA kit (eBioscience, San Diego, CA, USA).

Table S1. The sequences of 20N region of the aptamers screening by AC-MARAS for frequency dependent experiment

Clone name	5'-sequence-3'
20N AC F2-2	ATTGTTCGTTATTGCGTTCG
20N AC F2-4	ATACTCGCCGCTTTTGAATT
20N AC F20-2	TATGCGGGTGTTTGCGACTG
20N AC F20-4	CTTTCTTTCTAGGTGTGTG
20N AC F200-1	TGACTTACATGTACTACTTT
20N AC F200-2	TGTCAGTTTGATCTGTAATT
20N AC F2K-1	CTCCTTTTGATTTGCGGGTA
20N AC F2K-4	TTGGCTTTAACTATATGGAG
20N AC F20K-1	TCTACGCGATATTCTCCCCC
20N AC F20K-3	TCTTCCGCCGTACCGTTCCC
20N AC F200K-1	GCATTGTATCACAGGTACTG
20N AC F200K-2	TTGTCTCTGGGGATCTAAAC
20N AC >200K-1	TCCTGTTGATGTGTGTTATATG
20N AC >200K-2	AATTACAAATTTGGACTGTT

AC: alternating magnetic field

MARAS: magnetic-assisted rapid aptamer selection

MARAS condition: 25 Gauss

N: random oligonucleotide library

Table S2. The sequences of 20N region of the aptamers screening by AC-MARAS for field strength dependent experiment

Clone name	5'-sequence-3'
20N HAC 12.5g-1	TTGCATAGTTCGGTTCTATG
20N HAC 12.5g-2	TGTATGGTGGTTCCATCTTC
20N HAC 25g-1	TATGTATGGAATCACAATCC
20N HAC 25g-2	TTGCGGGATGAAGTGTCTAA
20N HAC 50g-2	TTGGTATTCCTCGCGTTTTT
20N HAC 50g-9	GCGTTTGGCATCCCTCGGTC
20N HAC 100g-1	CTGTGGTTGGCGCTTGGCAT
20N HAC 100g-2	TGGACTTCATTTTAAGTATG
20N HAC 200g-2	GTTTACTGTTTTATTGCGCT
20N HAC 200g-3	TATGATAAGGTGATAACATG
20N HAC 400g-1	TTGGCACAAAGCGGTGTTAC
20N HAC 400g-5	GTGTCGTTCGGTGCATCATG
20N HAC >400g-1	TGCTATTCAGGTGGACTATG
20N HAC >400g-4	TGGCAACTTTTGAACGCGTT

AC: alternating magnetic field

MARAS: magnetic-assisted rapid aptamer selection

MARAS condition: 100 KHz

N: random oligonucleotide library

Table S3. The sequences of 20N region in the aptamers from one-shot AC-MARAS

Clone name	5'-sequence-3'
OS-AC-20N-1	GATACCAAGGTCCGCTGGTT
OS-AC-20N-3	CGCTTGTGATGGGTGATGGG

OS: one shot

AC: alternating magnetic field

MARAS: magnetic-assisted rapid aptamer selection

MARAS condition: 400 Gauss, 200 KHz

N: random oligonucleotide library

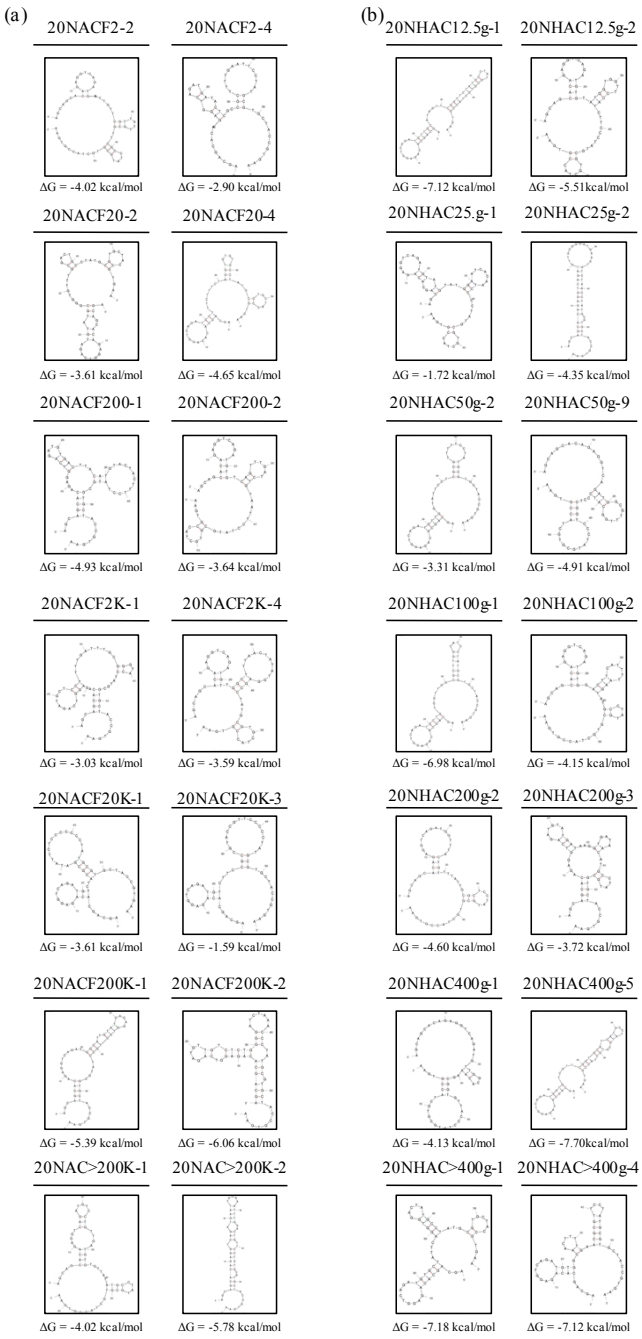

Figure S1. The representative predicted secondary structures of selected aptamer sequences form each step of MARAS using MFold analysis.

Reference

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3. Holmberg A., Blomstergren A., Nord O., Lukacs M., Lundeberg J., Uhlén M., 2005. The biotin-streptavidin interaction can be reversibly broken using water at elevated temperatures. *Electrophoresis*. **26**, 501-10.