Electronic Supplementary Information

DNA aptamer-based surface plasmon resonance sensing of human C-reactive protein

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Experimental details

1. Materials and reagents

Graphene oxide (GO) was obtained from Xianfeng Nanomaterials Technology Co., Ltd (Nanjing, China). C-reactive protein (CRP) was purchased from Biovision, Inc. (Milpitas, CA, USA). Bovine Serum Albumin (BSA) , human serum albumin (HSA) and human immunoglobulin G (IgG) were purchased from Beijing dingguo changsheng biotech Co., Ltd (Beijing, China). Myoglobin was purchased from Abcam, Inc. (Cambridge, MA, USA). Hemoglobin was purchased from Fitzgerald (USA). Avidin and biotinylated bovine serum albumin were purchased from Sigma-Aldrich Corp. (USA). The DNA library was synthesized and purified by Takara Biotechnology (Dalian, China), and all of the other DNA used in the experiment were synthesized by Sangon (Shanghai, China). Power Taq Master Mix was purchased from BioTeke (Beijing, China). GE Streptavidin microbeads were purchased from GE Healthcare Life Sciences (USA) for ssDNA generation experiments. The SYBR Green I 10000× was purchased from MP Biomedicals (USA) for PCR experiments. All of the chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ·cm) was used throughout.

2. Design of a DNA Library

A FAM-labeled ssDNA library consists of a central 40 bp random region flanked by two 20 bp constant regions that function as primer-binding sites for PCR. Furthermore, we also design two short DNA strands to block the primer-binding sites and limit their involvement in the large conformational change in specific response to CRP binding. Prior to each round of SELEX selection, the initial or enriched ssDNA library need to be mixed with the primer site-blocking strands and hybridized in 20 μL 1× binding buffer (BB, 20 mM HEPES containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.35) by heating at 95 ^oC for 10 min. Then the DNA library mixture was gradually cooled down to 25 ^oC over 3 h and used for further selection experiments. Details on the DNA sequences used in this selection are shown in Table S1.

Table S1. Sequences of DNA used in this selection

Name	Sequence	Description
Library	5'-FAM-*AGCAGCACAGAGGTCAGA* TG-N_{40}-*CCTATGCGTGCTACCGTGAA*-3'	Primer sites in italics
FP	5'-AGCAGCACAGAGGTCAGATG-3'	Forward primer used for cloning and sequence
RP	5'-TTCACGGTAGCACGCATAGG-3'	Reverse primer used for cloning and sequence
FP-FAM	5'-FAM-AGCAGCACAGAGGTCAGATG-3'	Forward primer with FAM label at the 5' terminus used in PCR amplification
RP-biotin	5'-biotion-TTCACGGTAGCACGCATAGG-3'	Reverse primer with biotin label at the 5' terminus used for ssDNA generation and blocking the library's 3' terminal primer site
FP-c	5'-CATCTGACCTCTGTGCTGCT-3'	The complementary DNA with forward primer used for blocking the library's 5' terminal primer site

3. Adsorption ratio of DNA library on GO

The purchased GO (20 mg) was dissolved in 10 mL Milli-Q purified water and then sonicated for 2 h (200 W) to give a homogeneous solution and stored at 4 °C for use. 100 pmoles initial DNA library mixture prepared as above described procedures, was mixed with a serious of different concentrations GO solution (from 1.6 ug/ml to 400 ug/ml) at 25 °C for 2 h. The final volume was 1 ml. Subsequently, the supernatant was collected by centrifugation at 30,000 rpm for 10 min. The fluorescence intensity of DNA library mixture after preparation (F_0) was first measured. The fluorescence intensity of the collected supernatant (F) was then measured. As shown in Fig. S1, the quenching efficiency ((F_0–F)/F_0) was nearly saturated when the concentration of GO was 400 ug/ml. Hence, in order to enhance the stringency of the selection, the adsorption ratio of the DNA library on GO was found to be 0.25 nmol $mg^{-1}$of GO.

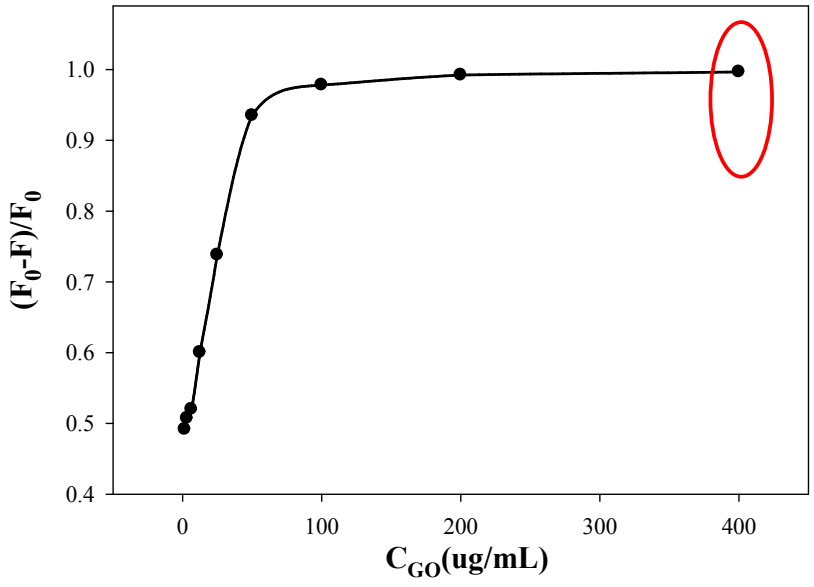


Fig. S1 The absorption ration of the prepared initial ssDNA library mixture on GO

4. Selection of aptamers against CRP

(1) Selection of aptamers

Positive selection: the modified GO-SELEX round was performed by initially heating 1 nmole of ssDNA random library (approximately 10^{14} random molecules) and 1.5 nmoles of each primer site-blocking strand in 1× binding buffer for 10 min at 95 ^{o}C, gradually cooling down to 25 ^{o}C over 3 h. In the first round of selection, 1 nmole of the prepared DNA library mixture was incubated with GO for 2 h. Then 10 nmoles CRP was added to the GO-bound DNA solution and further incubated at 25 ^{o}C for 2 h with mild shaking to competitively bound the DNA molecules with GO. Aptamer molecules which underwent target-binding-induced conformation change were specifically released from GO. Subsequently, the solution was centrifuged for 10 min at 30,000 rpm to discard precipitate and recover the DNA bound CRP from the supernatant. The DNA bound CRP was desalted and then amplified by PCR. The amount of DNA library and CRP was gradually decreased with increasing of the SELEX round.

Counter selection: before the selection rounds 7, 8, 9 and 10, a counter selection was carried out. The purified ssDNA library (60 pmoles) of 6th round of the modified GO-SELEX was mixed with 90 pmoles of each primer site-blocking strand in 1× binding buffer for 10 min at 95 ^{o}C, gradually cooling down to 25 ^{o}C over 3 h. 60 pmoles of counter targets BSA, HSA and myoglobin was incubated with the prepared DNA library for 1 h. The DNA library and counter proteins mixture were added to

GO and further incubated for 2 h. Subsequently the solution was centrifuged for 10 min at 30,000 rpm to collect precipitate. The GO-DNA precipitate was redispersed in 1× binding buffer and directly subjected to the positive selection.

(2) PCR amplification

A PCR mixture containing 25 μL 2× Power Taq Master Mix, 1 μL of 10 μM forward primer, and 1 μL o f 10 μM biotinylated reverse primer was prepared. This PCR mixture was then mixed with 10 μL of DNA sample collected from the selection and nuclease-free water to bring the total volume to 50 μL. The HotStar Taq polymerase was activated prior to PCR by heating reactions to 95 ^{o}C for 10 min, followed by 10 cycles of a rapid three-step PCR (30 s denaturation at 95 ^{o}C, 30 s annealing at 62 ^{o}C, 30 s extension at 72 ^{o}C). During the extension step of each round, 10 μL of PCR mixture were collected and resolved on a 2.5% agarose gel electrophoresis (AGE) to determine the optimal PCR amplification cycle number. Finally, the collected DNA sample was PCR amplified at the optimized cycle number.

(3) ssDNA generation

The preparation of ssDNA from double-stranded PCR products was conducted by biotin-streptavidin separations. 100 μL of GE Streptavidin microbeads was preconditioned in 200 mM NaOH 3 h to release some amounts of labile streptavidin from the beads. Subsequently, the beads were precipitated immediately and the supernatant was discarded. The biotinylated, double-stranded PCR products were added to the preconditioning beads and further incubated for 0.5 h at 25 ^{o}C. ssDNAs

were generated by adding 200 mM NaOH and incubating for 10 min, after which the supernatant was collected, followed by a desalting step for the collected ssDNAs. The final concentration of ssDNAs were characterized using UV-visible measurement at 260 nm.

(4) Enrichment Assays

As shown in Fig. S2a, for monitoring enrichment over the progress of selection, all measurements were conducted on an EC-SPR1010 facility (Changchun dingcheng technology, China). Au film was first modified using 200 μg/mL CRP, and then the uncovered sites on the Au surface were blocked by injecting 1% BSA. After an aliquot of ssDNA library from each round was added and incubated for 30 min at 25 ^{o}C, Au film surface was washed repeatedly with 1× binding buffer. The change of resonance angle ($\Delta\theta$) was recorded and analyzed as shown in Fig. S2b. All oligonucleotides were heated at 95 ^{o}C for 5 min and instantly cooled in ice for 5 min, followed by a 5 min incubation at room temperature before use.

(a)

(b)

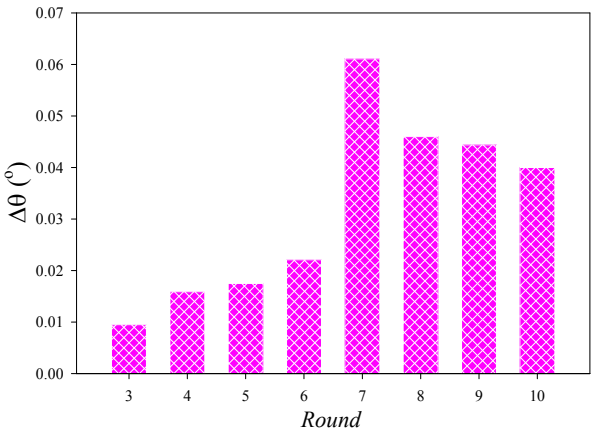


Fig. S2 Schematic mechanism of monitoring the progress of screening aptamers by SPR (a); Results of the evolution of binding affinity between CRP and every round selection library (b).

(5) High-Throughput Sequencing

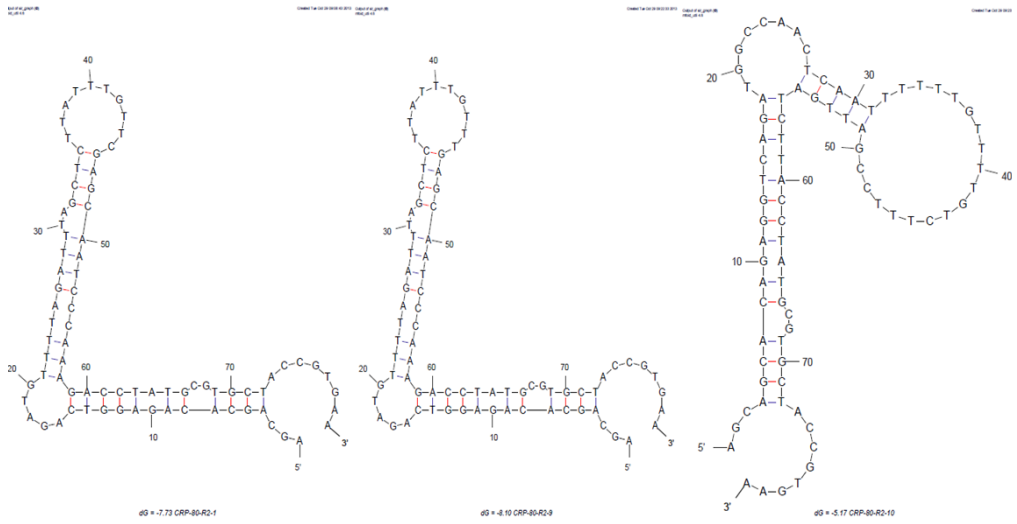
After 10 rounds of selection, a small amount of the purified PCR products from the 7th round of selection was PCR-amplified by use of unlabeled forward and reverse primers at the optimized PCR cycle number determined by the pilot PCR. The generated PCR products were sent for Paliley (Bejing, China) to sequence in Illumina HiSeq 2000 sequencing platform. The secondary structure of selected aptamers was analyzed by the Internet tool Mfold (http://mfold.rna.albany.edu/?q=mfold).

5. Analysis of the result of high-Throughput Sequencing

The total number of sequencing reads for this library was about 4.4×10^5. According to the length of sequence, we filtered out sequences that were not 80 bp in length. Ultimately, 2.6×10^5 effective sequences were obtained. The repeated sequences represented about 17.8% of the effective sequences. The top 20 highest repeated sequences were chosen and then classified into nine groups based their similarity of secondary structure as shown in Fig. S3. Nine full-length candidate aptamers (marked in red) were selected out to characterize their affinity toward CRP.

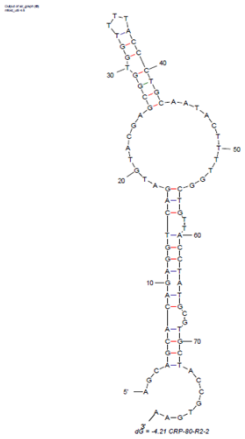
Group 1

CRP-80-1 CRP-80-9 CRP-80-10

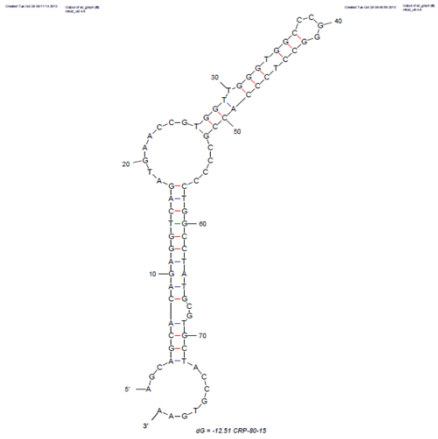


Group 2

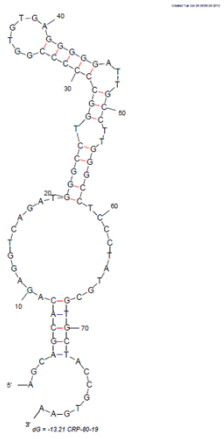
CRP-80-2



CRP-80-15

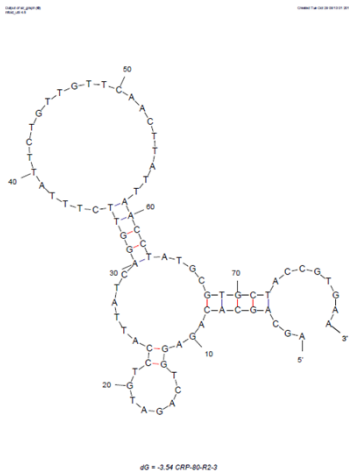


CRP-80-19



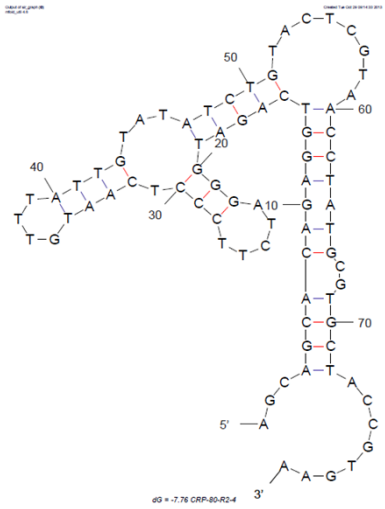
Group 3

CRP-80-3



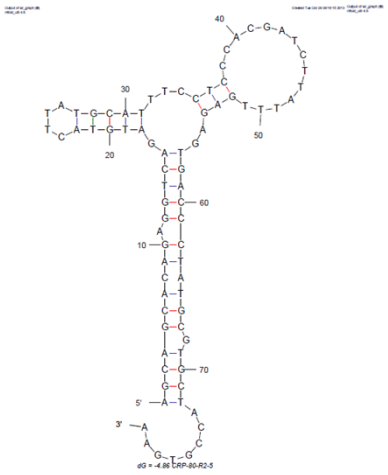
Group 4

CRP-80-4

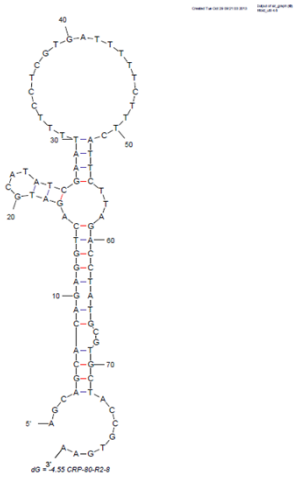


Group 5

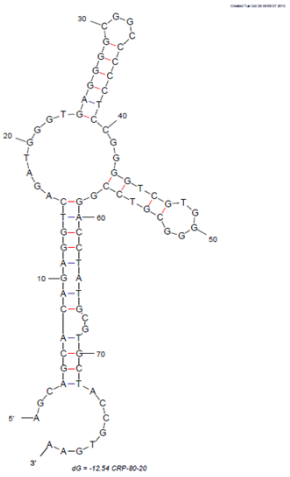
CRP-80-5



CRP-80-8

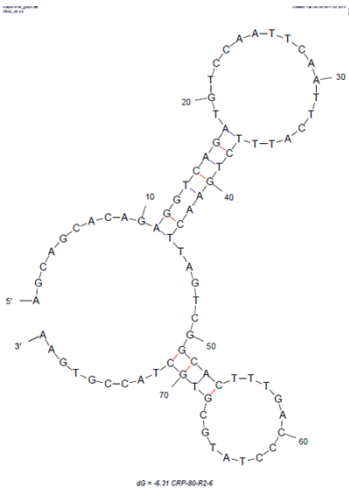


CRP-80-20

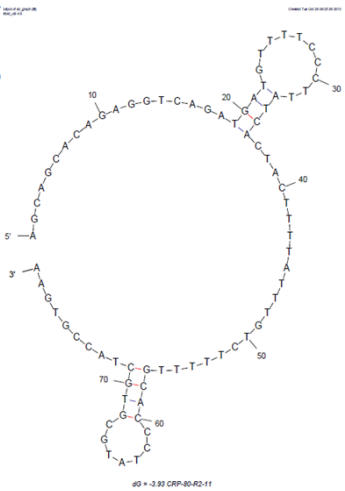


Group 6

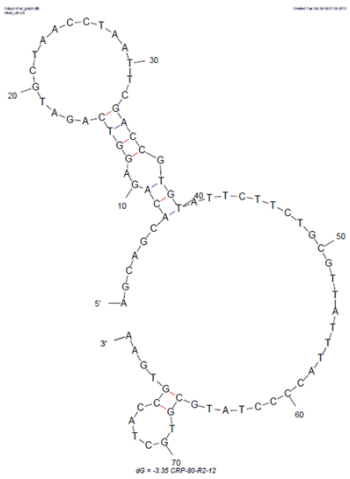
CRP-80-6



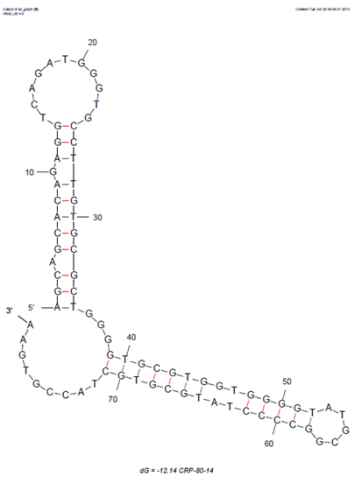
CRP-80-11



CRP-80-12

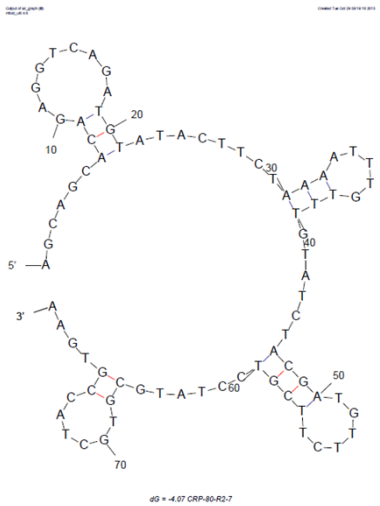


CRP-80-14



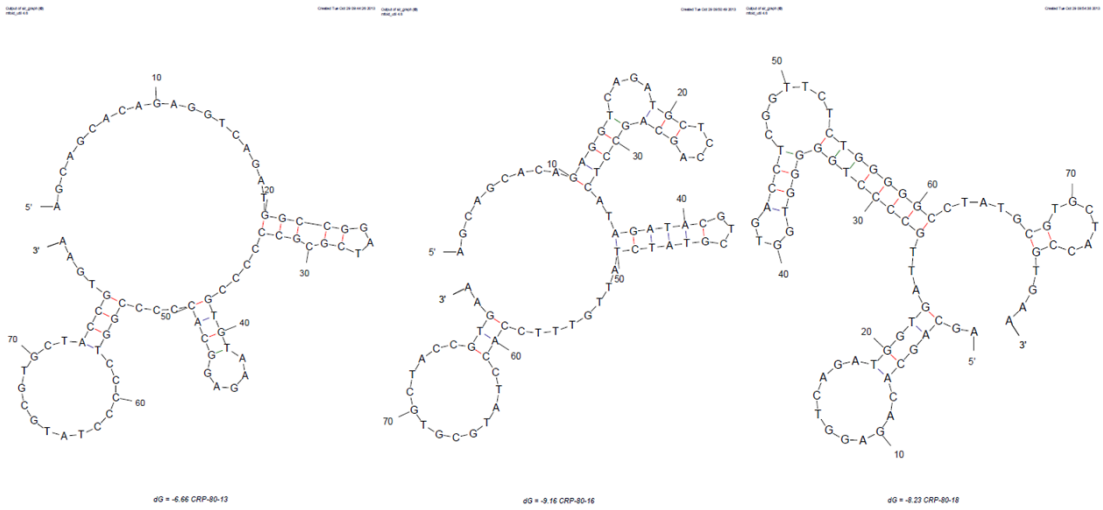
Group 7

CRP-80-7



Group 8

CRP-80-13 CRP-80-16 CRP-80-18



Group 9

CRP-80-17

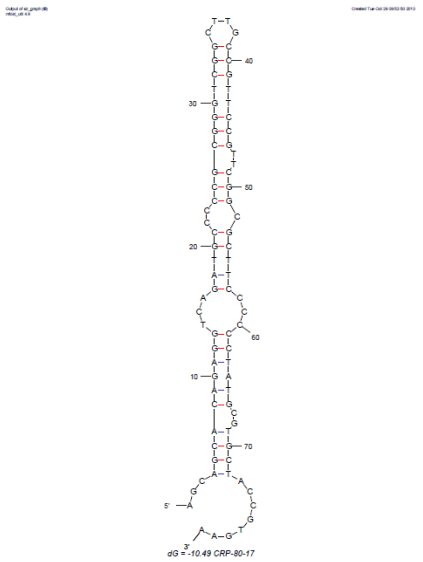


Fig. S3 The secondary structure of nine groups of the top 20 highest repeated sequences.

6. Affinity determination

The affinity of the selected candidate aptamers for CRP was determined by SPR. Au film was first modified using 200 μg/mL CRP, and then the uncovered sites on the Au surface were blocked by injecting 1% BSA. Different concentrations of candidate aptamers (from 0 nM to 500 nM) were added and incubated for 30 min at 25 °C, Au film surface was washed repeatedly with 1× binding buffer. The change of resonance angle (Δθ) was recorded and analyzed. Dissociation constants (Kd) described the affinity of C-reactive protein/aptamer interaction. To calculate Kd, the change of resonance angle versus candidate aptamer concentrations was plotted, and the data points were fitted by the non-linear regression analysis.

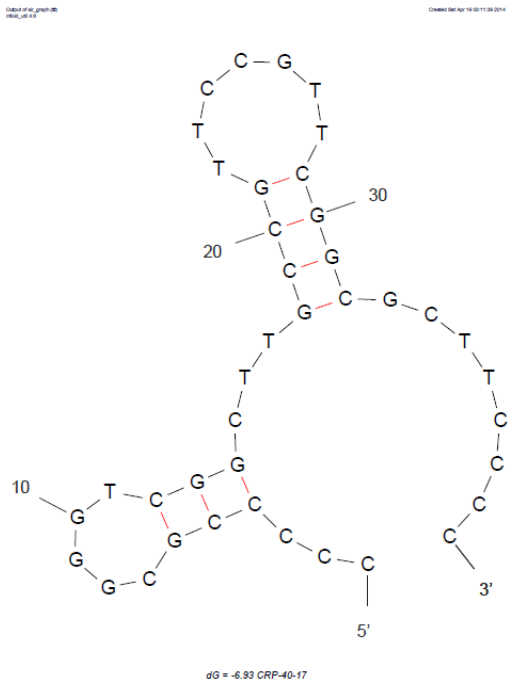


Fig. S4 The secondary structure of aptamer CRP-40-17.

7. Ability of immobilized aptamer to bind CRP using aptamer-based SPR analysis

Au film was first modified using 1 mg/ml biotin-BSA and avidin, respectively. Then 1 μM biotin functionalized aptamer was immobilized by biotin-avidin interaction. After different concentrations of CRP (from 0 nM to 100 nM) were added and incubated for 30 min at 25 ^{o}C, Au surface was washed repeatedly with 1× binding buffer. The change of resonance angle was recorded and analyzed. The sequences of used in this part were listed in Table S2.

Table S2. Sequences of DNA used in this part.

Name	**Sequence**
CRP-80-17-5' biotin	5'-biotin-AGCAGCACAGAGGTCAGATGCCCCCGCGGGTCGGCTT GCCGTTCCGTTCGGCGCTTCCCCCCTATGCGTGCTACCGTGAA-3'
CRP-80-17-3' biotin	5'-AGCAGCACAGAGGTCAGATGCCCCCGCGGGTCGGCTT GCCGTTCCGTTCGGCGCTTCCCCCCTATGCGTGCTACCGTGAA-biotin-3'
CRP-40-17-5' biotin	5'-biotin-*TTTT TTTT TTTT* CCCCCGCGGGTCGGCTTGCCGTTCCGTTCGG CGCTTCCCC-3'
CRP-40-17-3' biotin	5'-CCCCCGCGGGTCGGCTTGCCGTTCCGTTCGGCGCTTCCCC *TTTT TT TT TTTT*-biotin- 3'

8. Specificity determination

For identifying the specificity of the selected aptamers, 50 nM five control proteins, i.e. IgG, HSA, hemoglobin, BSA and myoglobin were respectively reacted with aptamers immobilized on Au surface for 30 min at 25 ^{o}C, then washed with 1× binding buffer thoroughly. The changes of resonance angle were recorded.