Non-immobilized GO-SELEX screening of the CsA aptamer and its application in AuNPs colorimetric aptasensor

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1. Materials and Instrumentation

1.1. Materials

The DNA library (5'-TGAGAGGAGCAGATGGACAT-(N44)-ACAGCATGA CACACCCGTAA-3'), primers for long- and short-strand polymerase chain react ion (forward primer: 5'-TGAGAGGAGCAGATGGACAT-3', polyA-reverse prime r: 5'-AAAAAAAAAAAAAAAAAAAAA/spacer18/TTACGGGTGTGTCATGCTG T-3'), DNA Marker A (25-500 bp) and CsA candidate aptamers were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). PrimeSTAR Max Pre mix (2×) was purchased from TaKaRa Bio Group (Tokyo, Japan). Cyclosporin A, rapamycin, hydrocortisone, tetracycline and carbamazepine were purchased fr om Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Ammonium persulfate, tetramethylethylenediamine (TEMED), n-butanol, Tris, and urea were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Tetracycline, a nhydrous ethanol, acrylamide, N,N'-methylenebis(acrylamide), sodium hydroxide, hydrochloric acid, sodium chloride, anhydrous magnesium chloride, ethylenedia minetetraacetic acid (EDTA), boric acid, and methanol were purchased from Si nopharm Chemical Reagent Co., Ltd. (Shanghai, China). AuNPs were purcha sed from XFNANO Co., Ltd. (Nanjing, China). Micro Nucleic Acid Dia lysis Membrane (3.5 KD) were purchased from Amptamer Co., Ltd. (Anhui, China). All other chemicals and reagents used in the study were of analytical grade.

1.2. Instrumentation

Polymerase chain reaction was performed by a WD-9402D non-medical gene amplification instrument (Beijing Liuyi Biotechnology Co., Ltd., China.). A Synerg LX multifunctional microplate reader (BioTek Instruments, Inc.) for DNA concentration determination in experiments. All vessels were sterilized by a BXM-30R Vertical Pressure Steam Sterilizer (shang hai bo xun Co., Ltd., China.). Incubation shaking was performed by a ZQPW-70A full-temperature oscillation incubator (Tianjin Leibo Terry Equipment Co., Ltd., China.). Gel electrophoresis was performed by a DYCZ-24DN mini double vertical electrophoresis tank (Beijing Liuyi Biotechnology Co., Ltd., China) and a DYY-6C electrophoresis instrument (Beijing Liuyi Biotechnology Co., Ltd., China). A Q-LAB20-DV ultrapure water meter (>18.25 MΩ, Qiqin, China) provided Milli-Q grade water for the experiment.

2. PCR amplification and preparation of secondary library

The essence of the long and short-strand PCR method for ssDNA preparation is to perform PCR amplification by specially designed primer structures, resulting in differences in the length of PCR products. Then, after separation by urea-denaturing polyacrylamide gel electrophoresis, the cut gels were recovered to obtain the desired single-stranded nucleic acids. The design principle of the unique primer structure is as follows: the reverse primer adds the blocking strand motif spacer 18 after the complementary sequence with the template to prevent the extension of the forward strand; at the same time, a polyA of approximately 20 bases is added after the inter-arm to increase the length difference between the reverse strand and the forward strand (as shown in Fig. S1A).

The preparation of long and short-strand PCR amplification solution is shown in Table S1. Recovery of PCR amplification products. By denaturing PAGE electrophoresis with 12% urea (7 M), the two DNA product strands are separated due to their different lengths, and the secondary library is obtained by cutting and recovering the (+) ssDNA strand without lengthening modifications (e.g., Fig. S1B).

Reagent	Dosage（μL）
PrimeSTAR Max Premix (2×)	25
Forward primer (10 μM)	2
PolyA-Reverse prime r(10 μM)	2
Template (2 μM)	1
ddH$_2$O	20

Table S1 Conditions for long- and short-chain PCR amplification

Amplification conditions: 95℃ for 5 min; 95℃ for30 s; 55℃ for30 s; 72℃ for 15 s; 29 rounds of cycles; 72℃ for another 5 min.
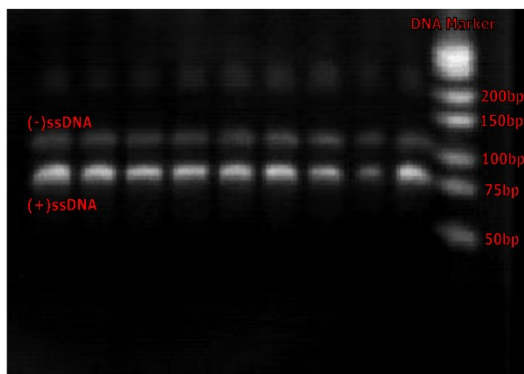
(a)

(b)

Fig. S1 Long and short-chain PCR amplification. (a) Schematic diagram of long- and short-chain PCR amplification; (b) is a 12% urea (7 M) denaturing PAGE gel graph, the picture shows the running results of long- and short-chain PCR amplification products in 12% urea (7 M) denaturing PAGE gel.

3. Optimization of SELEX conditions

Table S2 lists the incubation conditions for the entire SELEX process. The amount of ssDNA input, amount of CsA input and GO incubation time were adjusted for each SELEX round as the screening progressed. Stronger competition was generated by reducing the amount of ssDNA and CsA, and counter target was added in round 7 to exclude the non-specific binding of aptamers. Additionally, we reduced the incubation time of ssDNA with CsA and increased the incubation time of ssDNA with GO to provide a more stringent screening environment to obtain CsA aptamers with higher sensitivity and better binding ability.

Table S2 The screening conditions of GO-SELEX in each round,

Selection round	CsA (pmol)	ssDNA (pmol)	Incubation time of ssDNA with CsA (h)	Incubation time of ssDNA with GO (h)	Counter-target
1	5000	1000	2.0	1.0	N.A.
2	1500	300	2.0	1.0	N.A.
3	1500	300	2.0	1.5	N.A.
4	1500	300	1.5	1.5	N.A.
5	1000	200	1.5	2.0	N.A.
6	1000	200	1.0	2.0	N.A.
7	1000	200	1.0	2.0	[a] HC/ [b] TCN/ [c] CBZ/ [d] RAPA

| 8 | 1000 | 200 | 1.0 | 2.0 | N.A. |

N.A.：Not action, this project was not conducted. [a] Hydrocortisone; [b] Tetracycline;

[c] Carbamazepine; [d] Rapamycin.

4. Cloning, sequencing, and selection of optimal aptamers

Table S3 Round 8 selecting ssDNA enrichment library sequencing results

| Number	Sequence Information (5'-3')
Seq1	TGAGAGGAGCAGATGGACATCGTAGGGTGAGATGAGACTAACCGCTACCCTGAAGCCGTTGCAGACAGCATGACACACCCGTAA
Seq2	TGAGAGGAGCAGATGGACATAAACGCGTTAAGCTGCGGGATCCGGGGAGGCTGACGGCTGTATCACAGCATGACACACCCGTAA
Seq3	TGAGAGGAGCAGATGGACATTGCCCTTGTGGATGGGTGCGTAAGACTTGCCAGAAGGCGCATTTACAGCATGACACACCCGTAA
Seq4	TGAGAGGAGCAGATGGACATGGCCAAAGGCAGTAACCATCACGACCTAGGGATGGCTGTCAAGTACAGCATGACACACCCGTAA
Seq5	TGAGAGGAGCAGATGGACATACTGCAGTGCAGCCCATGACCGCGAAACTACAAGAGAGTAGGTGACAGCATGACACACCCGTAA
Seq6	TGAGAGGAGCAGATGGACATAACCTTTAAGCGATATGGCGATCGGGCACCGAGTACGTATTAGTACAGCATGACACACCCGTAA
Seq7	TGAGAGGAGCAGATGGACATCCCAGCTACGTTGACTGGATTGCCTCGGTGCATAGGCGGCCAATACAGCATGACACACCCGTAA
Seq8	TGAGAGGAGCAGATGGACATGCGCGGTCAGCCGCGGGTTATCTGTTTGAGCACAGGTAAGTTGTACAGCATGACACACCCGTAA
Seq9	TGAGAGGAGCAGATGGACATACTATGCTACGTGGAGATGTACAGCCTACAAGCTCTGGTGTGATACAGCATGACACACCCGTAA
Seq10	TGAGAGGAGCAGATGGACATTGTGCGACCAGTAGTGACTCTCTCTCTACTGACCTTGGTCGAGGACAGCATGACACACCCGTAA
Seq11	TGAGAGGAGCAGATGGACATGATGAGACGGGCGCGCTGTGTCCTATTCCTGCCTGGGTGTTTGCACAGCATGACACACCCGTAA
Seq12	TGAGAGGAGCAGATGGACATTGCTAGGTGTGAGCTTGCAGGGAAGGCGAATTGTAGGGGTATTCACAGCATGACACACCCGTAA
Seq13	TGAGAGGAGCAGATGGACATCGTCACCAGATGGCTTGAGCTCCTCGAGTTAACCGGGGCGATAAACAGCATGACACACCCGTAA
Seq14	TGAGAGGAGCAGATGGACATGAGCCAGGTGCGGGTCGATGGTGGAGAATGACAGTCGCATGCAGACAGCATGACACACCCGTAA
Seq15	TGAGAGGAGCAGATGGACATGTGAGTCTGCCGTGACATTCGTCAATGTTCTATATACATGGGTCACAGCATGACACACCCGTAA
Seq16	TGAGAGGAGCAGATGGACATGAGTGCGTGCGTGGGAGAGCGCAGAAGTCGTACCTCAGTATCCGACAGCATGACACACCCGTAA
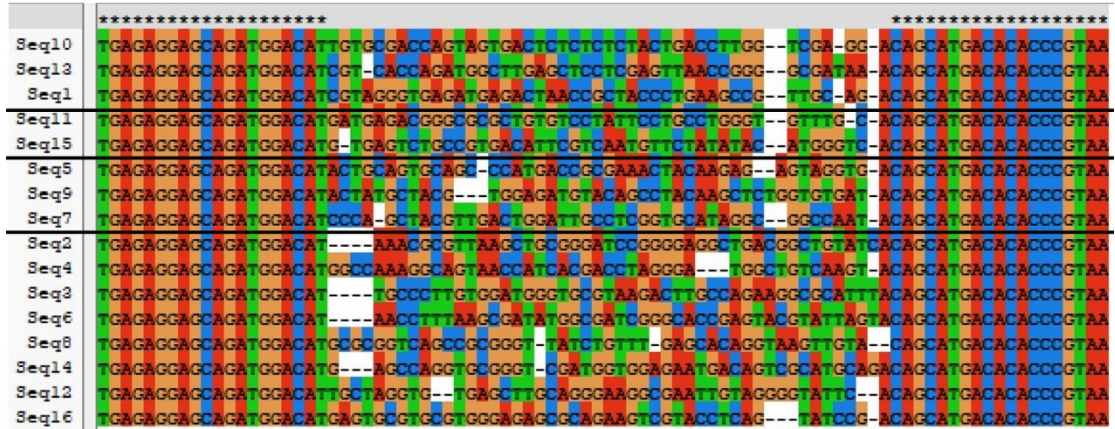
Fig. S2 Multiple sequence alignment of the 16 sequences. The result was obtained by homology matching of 16 sequences obtained from sequencing by ClustalX2 multiple sequence alignment software.
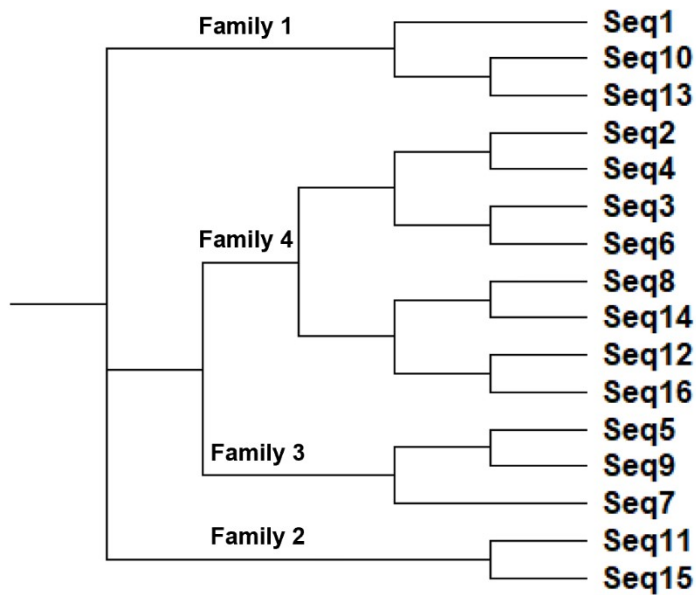

Fig. S3 Phylogenetic tree analysis of sequences. This result was obtained by evolutionary tree analysis of the results of Fig. S3 by TreeView-X software.
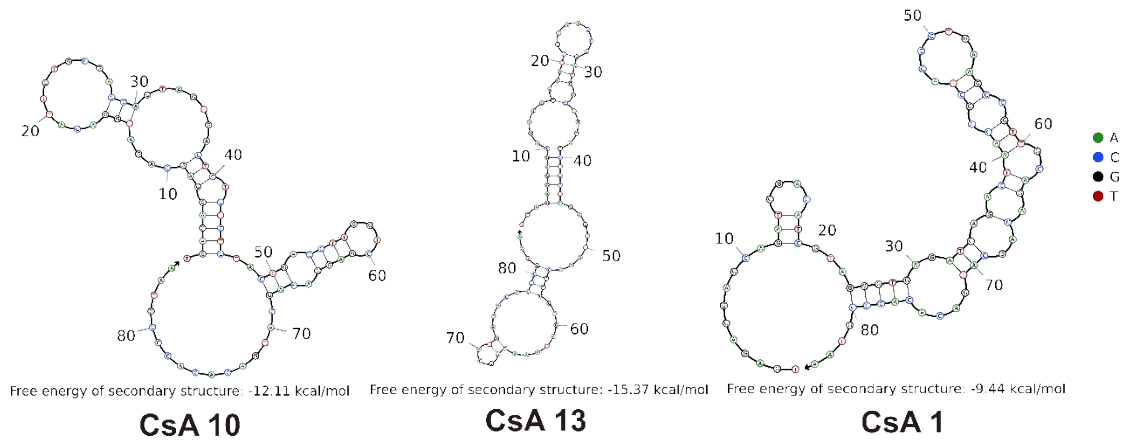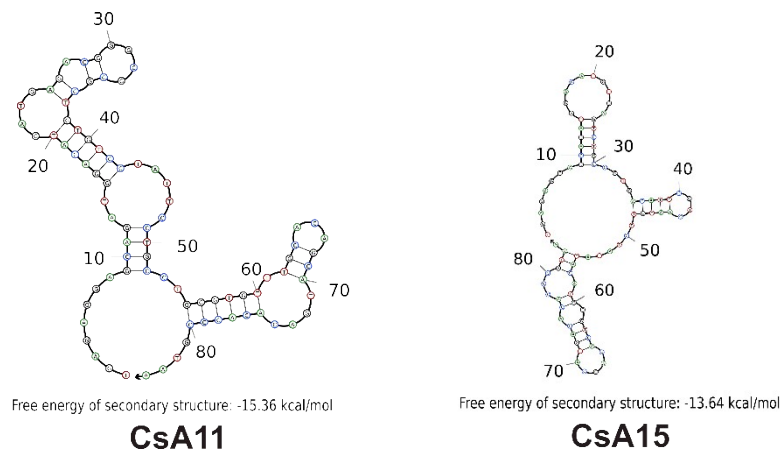
Fig. S4 Secondary structure of Family 1.
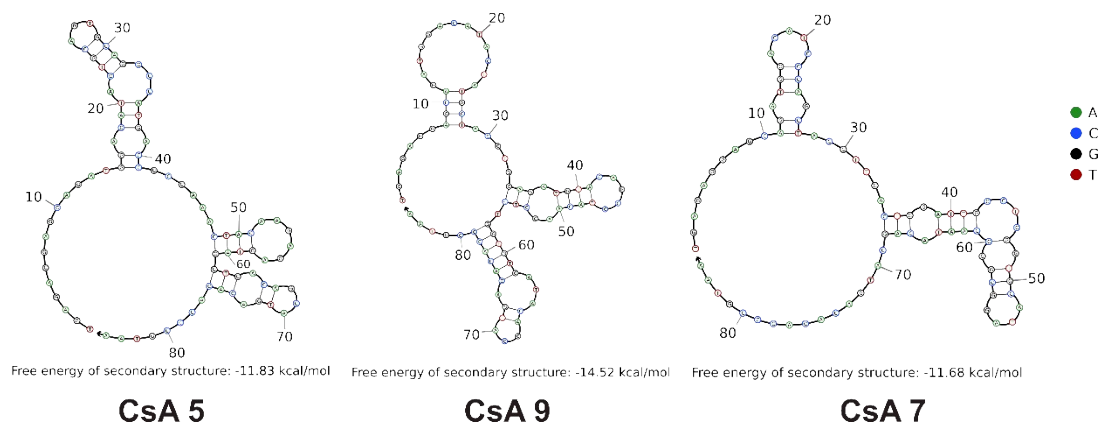

Fig. S5 Secondary structure of Family 2.

Fig. S6 Secondary structure of Family 3.

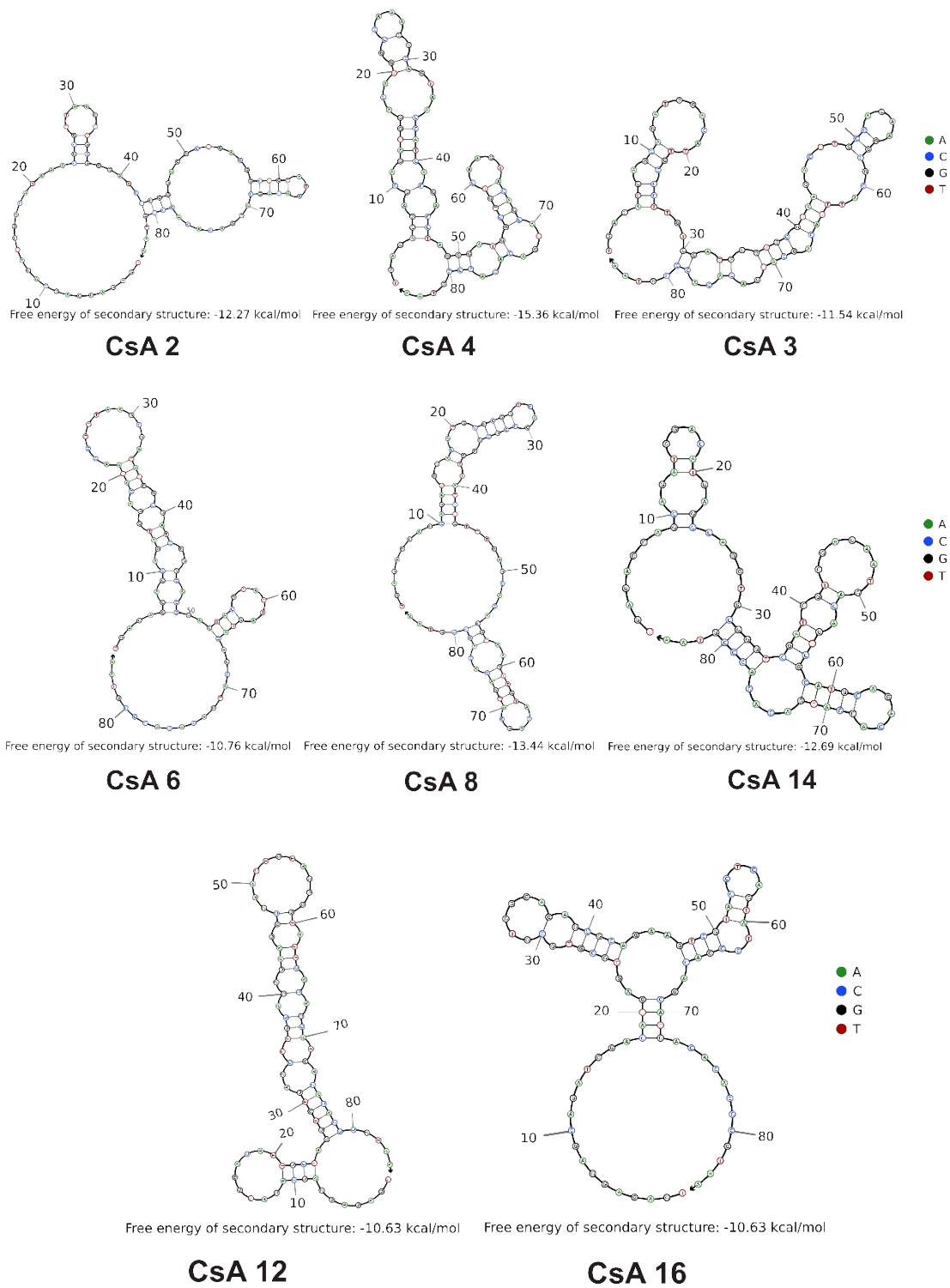Free energy of secondary structure: -12.27 kcal/mol

CsA 2

Free energy of secondary structure: -15.36 kcal/mol

CsA 4

Free energy of secondary structure: -11.54 kcal/mol

CsA 3

Free energy of secondary structure: -10.76 kcal/mol

CsA 6

Free energy of secondary structure: -13.44 kcal/mol

CsA 8

Free energy of secondary structure: -12.69 kcal/mol

CsA 14

Free energy of secondary structure: -10.63 kcal/mol

CsA 12

Free energy of secondary structure: -10.63 kcal/mol

CsA 16

Fig. S7 Secondary structure of Family 4.

5. Chemical structure of CsA and its interfering substances
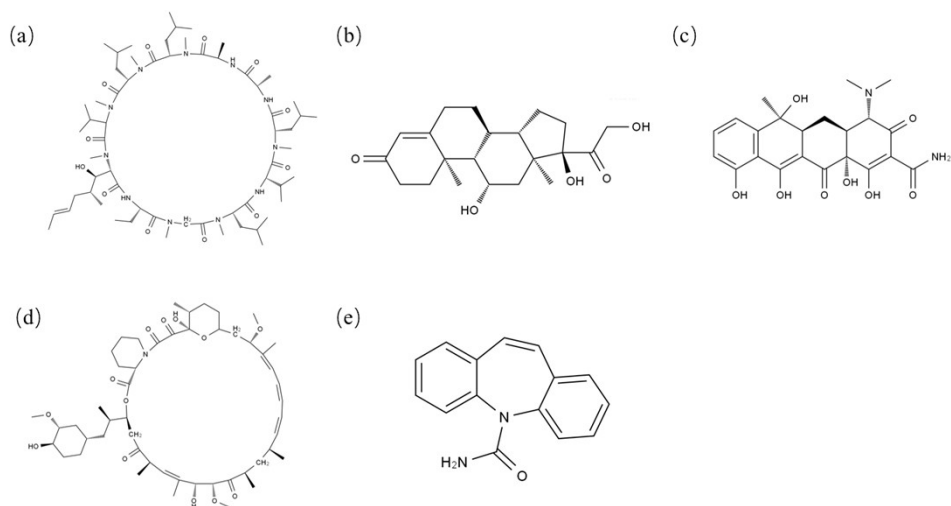
Fig. S8 Chemical structure of CsA and its interfering substances.(a) cyclosporine A;(b)

Hydrocortisone;(c) Tetracycline;(d) Rapamycin;(e) Carbamazepine

6. MOE molecular simulation docking analysis of the interaction between the CsA1, CsA8, CsA11 aptamer and CsA
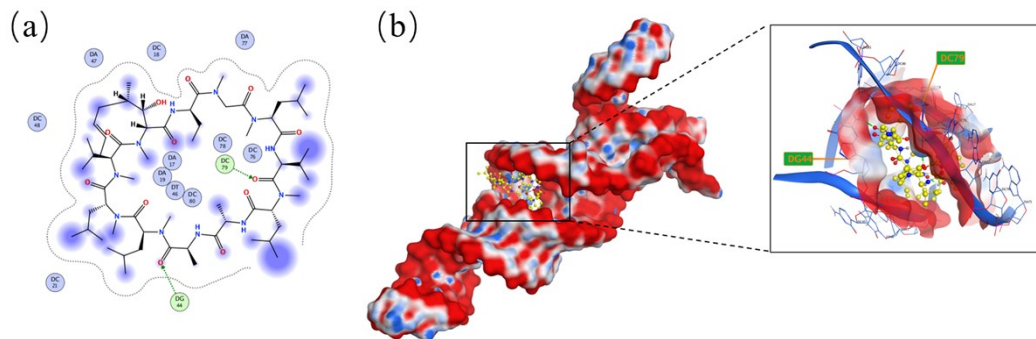

Fig. S9 MOE software simulates the binding mechanism of the CsA1 aptamer to CsA

molecules. (a) shows a plan view of the key action site of CsA1 aptamer binding to CsA

molecules; (b) shows a 3D image of the key action site of CsA1 aptamer binding to
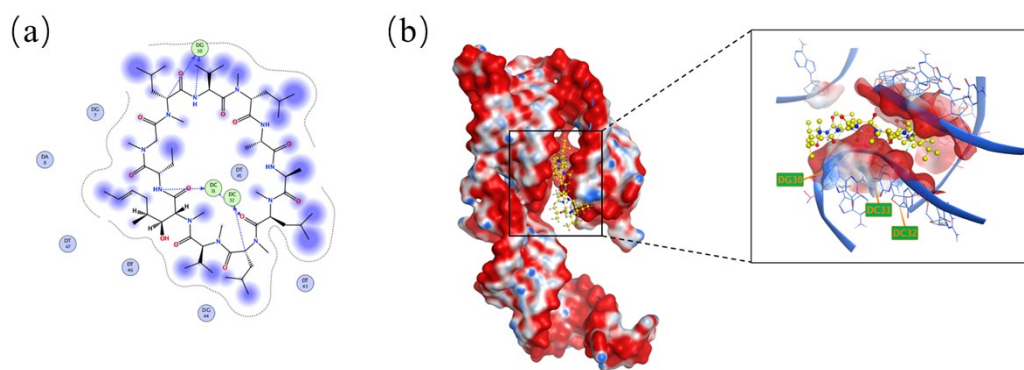
CsA molecules.

Fig. S10 MOE software simulates the binding mechanism of the CsA8 aptamer to CsA molecules. (a) shows a plan view of the key action site of CsA8 aptamer binding to CsA molecules; (b) shows a 3D image of the key action site of CsA8 aptamer binding to CsA molecules.
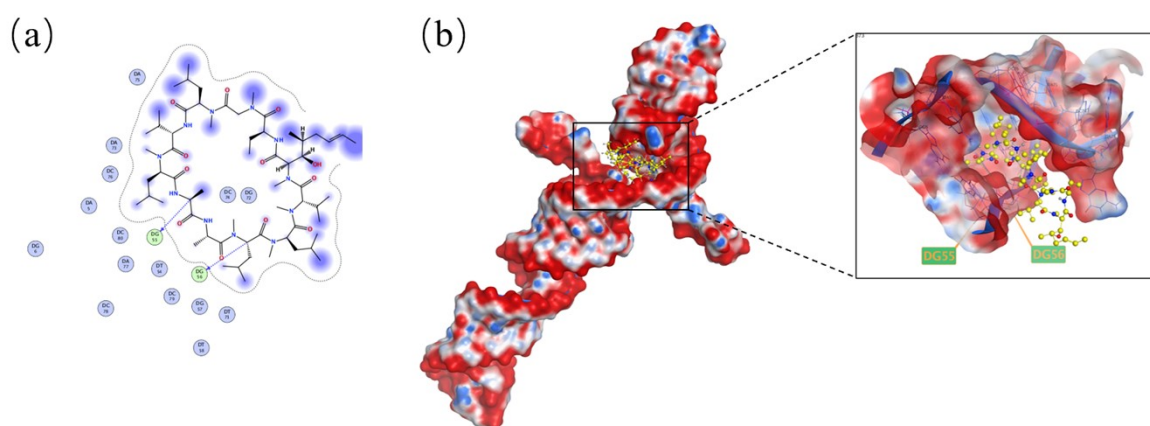

Fig. S11 MOE software simulates the binding mechanism of the CsA11 aptamer to CsA molecules. (a) shows a plan view of the key action site of CsA11 aptamer binding to CsA molecules; (b) shows a 3D image of the key action site of CsA11 aptamer binding to CsA molecules.