Electronic Supplementary Material (ESI) for RSC Advances.

Selection and Application of Aptamers with High-affinity and High-specificity against Dinophysistoxin-1

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Materials and methods

Supplementary Table 1. Summary of sequences involved in the text

Library pool*	GAGGCAGCACTTCACACGAT-N_{27}-CTGCGTAATGACTGTAGTGATG
forward primer	GAGGCAGCACTTCACACGAT
reverse primer	CATCACTACAGTCATTACGCAG
modified reverse primer	poly(dA20)-Spacer18-CATCACTACAGTCATTACGCAG
N11	GAGGCAGCACTTCACACGATCGGACCCAAACTTTACCTTACCCCCATCTGCGTAATGACTGTAGTGATG
N26	GAGGCAGCACTTCACACGATGCCTCCTACCCTGCTGCCTTAGCCGGTCTGCGTAATGACTGTAGTGATG
N16	GAGGCAGCACTTCACACGATATTTGGGGATCAGCCAGGTCAGTGCCACTGCGTAATGACTGTAGTGATG
N50	GAGGCAGCACTTCACACGATCGTCCCTGCCCCTGCCTCCTTTCTATGCTGCGTAATGACTGTAGTGATG
N29	GAGGCAGCACTTCACACGATCGCTGAAGTCAACCTCCCCTACCTGTGCTGCGTAATGACTGTAGTGATG
N59	GAGGCAGCACTTCACACGATCCACCAGGCCAAACACGACCCCAAACACTGCGTAATGACTGTAGTGATG
N63	GAGGCAGCACTTCACACGATTCCCTCCTCCTTTATATCCGGTCCGATCTGCGTAATGACTGTAGTGATG
N72	GAGGCAGCACTTCACACGATCCCCGTTCCTTGTCCCCTTCTCTATATCTGCGTAATGACTGTAGTGATG
N41	GAGGCAGCACTTCACACGATCCCCCCCACACTCTTCCAACCCCCTCTCTGCGTAATGACTGTAGTGATG
N8	GAGGCAGCACTTCACACGATCACGGCAGGAGACCATCACCATTATCGCTGCGTAATGACTGTAGTGATG
N59a	CCACCAGGCCAAACACGACCCCAAACA
N59b	GAGGCAGCACTTCACACGATCCACCAGGCCAAACACGACCCCAAACA
N59c	CCAGGCCAAACACGACCCC
N59a1	CCACCAGGCCAAACA
N59a2	CCACCAGGCCAAACACGACCCC
N59a3	CCACCAGGCCAAACACGACCC
N59a4	CCACCAGGCCAAACACGACC
N59a5	CCACCAGGCCAAACACGA
N59a6	CCACCAGGCCAACC
N59a7	CCAGGCCAAACACGACC

*N：Random base.

Supplementary Table 2. Summary of selection protocol for MB-SELEX

Selection round	Amount of ssDNA pool (pmol)	Incubation time for MB-Counter SELEX (min)	Incubation time for MB-Positive SELEX (min)	Wash times after incubation
1	3000	0	120	3
2-5	200	0	120	3
6-8	120	60	90	3
9-10	120	60	90	4
11-12	120	90	60	5

Supporting Results

Supplementary Figure 1. The relationship between the absorbance and concentration of DTX-1.

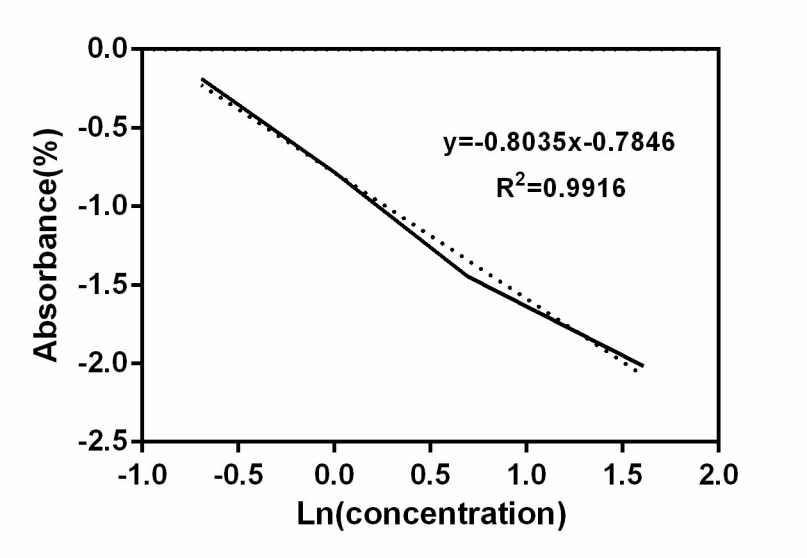


Fig. S1. The relationship between the absorbance and concentration of DTX-1.

The efficiency of coupling between beads and DTX-1 was measured by using an ELISA kit. We got the standard curve according to the standards and related instructions provided in the kit. The linear regression of the curve is: y = -08035x – 0.7846, while R^2 = 0.9916. Then confirmation the immobilization efficiency by measuring the free DTX-1 in the solution before and after incubation.The results showed that before incubation, the free DTX-1 in the solution was 14 μg while after incubation, the free DTX-1 in the solution was 2.5 μg (n=2), that means, 11.5 μg DTX-1 was immobilized onto the magnetic beads(600 μL).

Supplementary Figure 2. Gel electrophoresis results of ssDNA preparation.

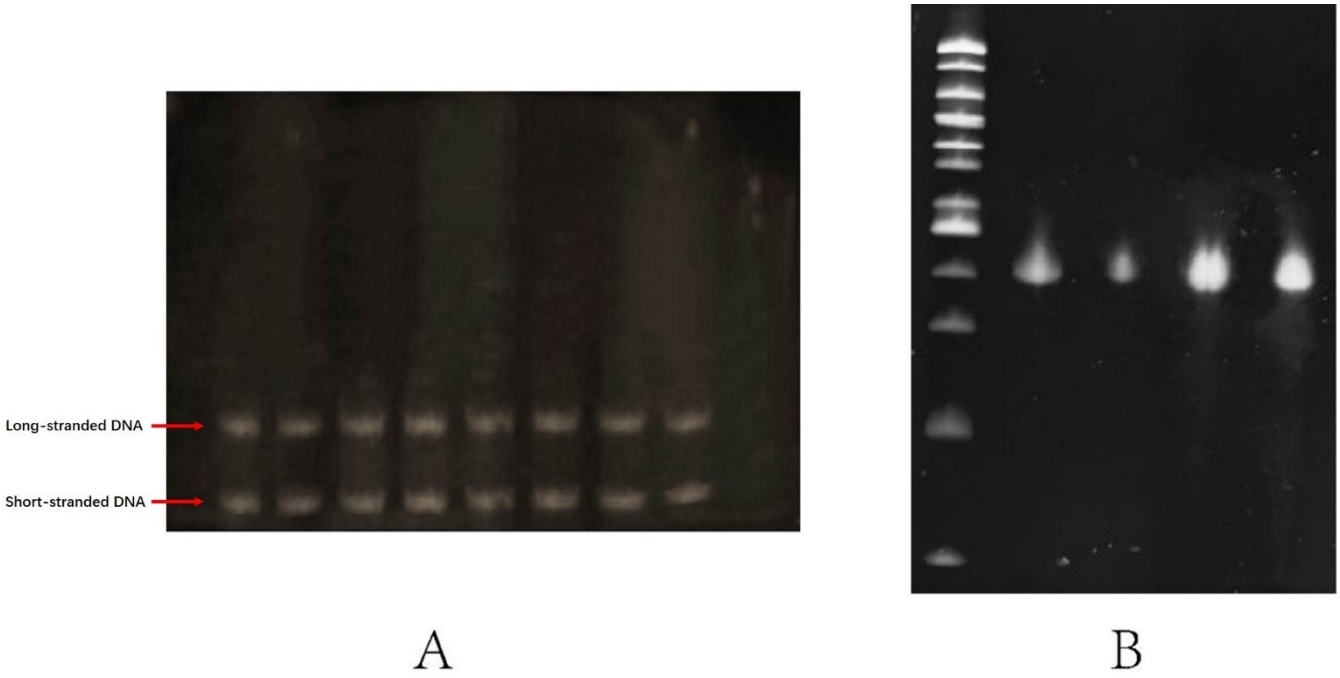


Fig. S2. (A) Gel electrophoresis results of the 12th round of selection. The dsDNA obtained by PCR can be clearly separated by gel electrophoresis due to the difference lengths of forward primer and modified reverse primer. We recovered the following ssDNA for the library preparation. (B) Gel electrophoresis results of the ssDNA preparation from the 3th, 6th, 9th, 12th round.

Supplementary Figure 3. Multiple sequence alignment of the selected sequences

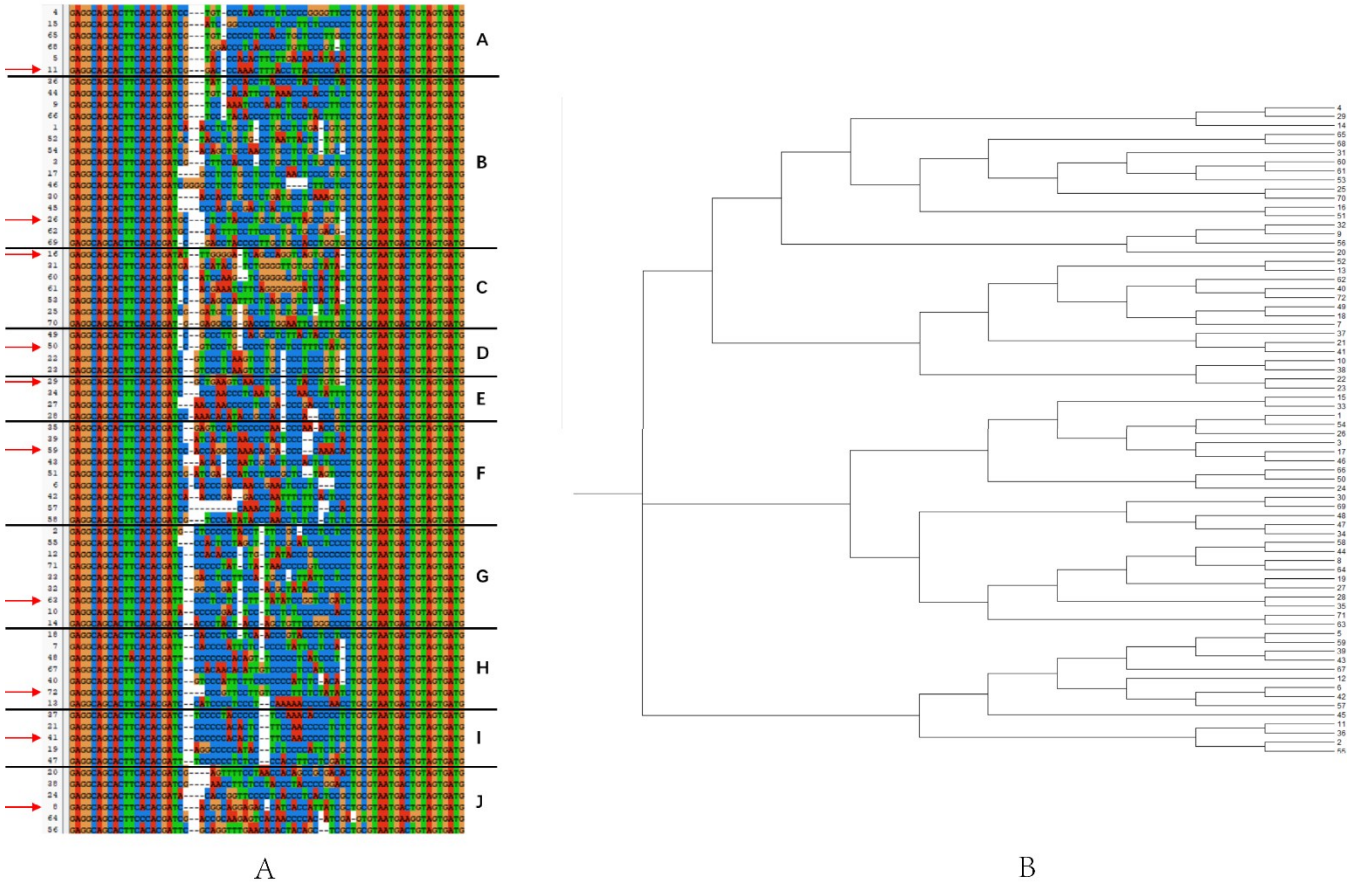


Fig. S3. (A)Multiple sequence alignment of cloned sequencing results by Clustal X software. These sequences were grouped based on conservation into 10 families (A-J), and a representative sequence was chosen from each group for further analysis (N11, 26, 16, 50, 29, 59, 63, 72, 41 and 8). (B) The guide tree of cloned sequencing results.

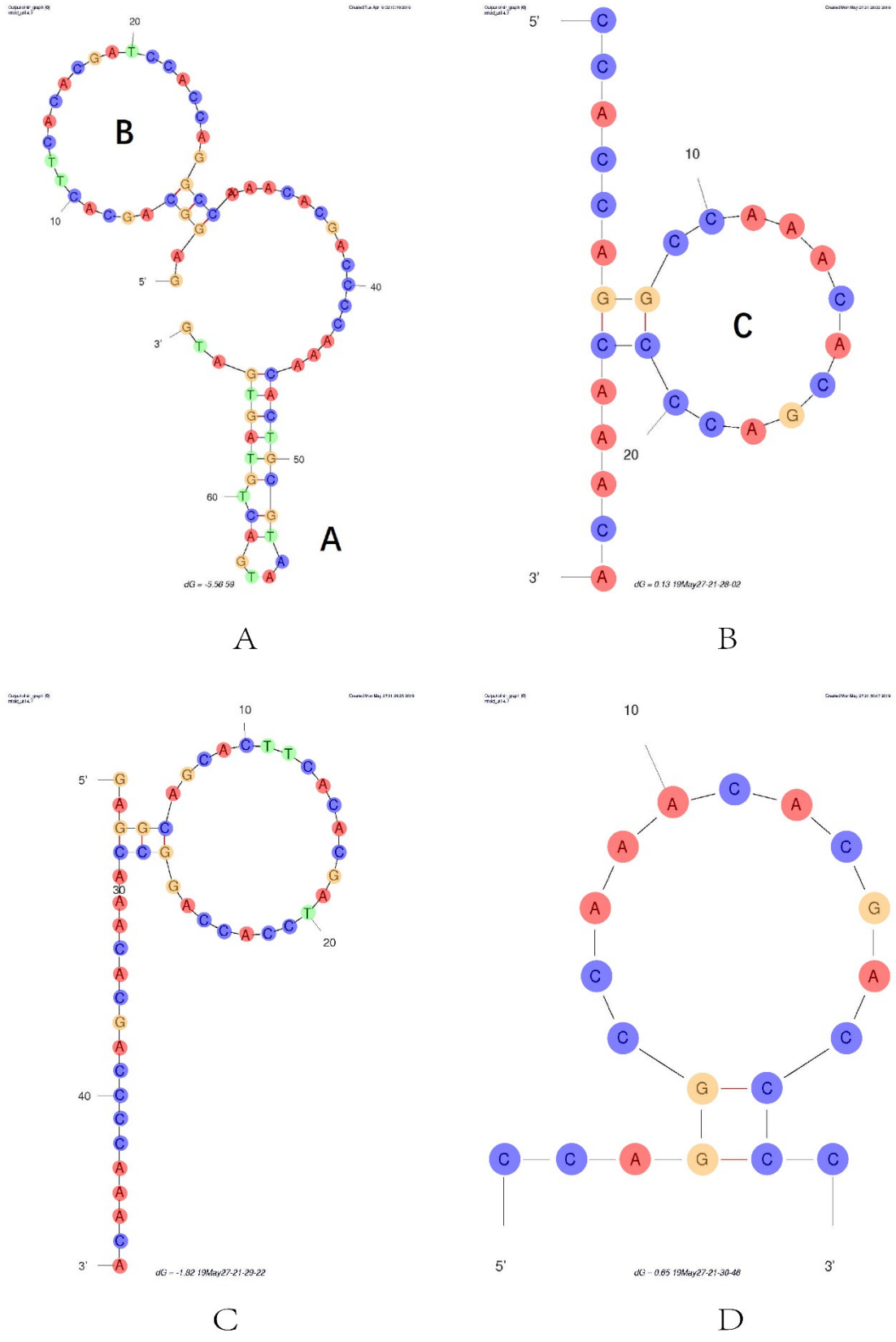
Supplementary Table 3. Affinity constant (*Kd*) between DTX-1 and truncated aptamers

Name	Sequence	*Kd*(nM) *
N59	GAGGCAGCACTTCACACGATCCACCAGGCCAAACACGACCCCAAACA CTGCGTAATGACTGTAGTGATG	170
N59a	CCACCAGGCCAAACACGACCCCAAACA	64
N59b	GAGGCAGCACTTCACACGATCCACCAGGCCAAACACGACCCCAAACA	370
N59c	CCAGGCCAAACACGACCCC	340
N59a1	CCACCAGGCCAAACA	NB
N59a2	CCACCAGGCCAAACACGACCCC	278
N59a3	CCACCAGGCCAAACACGACCC	178
N59a4	CCACCAGGCCAAACACGACC	142
N59a5	CCACCAGGCCAAACACGA	NB
N59a6	CCACCAGGCCAACC	NB
N59a7	CCAGGCCAAACACGACC	NB

*NB: No Binding.

Table S3. Sequences and affinity constants between DTX-1 and optimized aptamers.

Supplementary Figure 4. Secondary structure of truncated sequences for aptamers.



Supplementary Figure 4. Secondary structure of truncated sequences for aptamers.
(A) N59. (B) N59a. (C) N59b. (D) N59c.