Supporting Information

A Highly Parallel Single Molecule Amplification Approach Based on Agarose Droplet PCR for Efficient and Cost-effective Aptamer Selection

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Optimization of aptamers. To obtain shorter sequence with similar binding affinity to original aptamers, modification mainly based on mutation and truncation of stem-loop regions in sequence of original aptamers was performed. Sense primer and antisense primer were underlined, truncated nucleotides were marked in grey, and mutated nucleotides were in italic capitals and marked in red, shown in Table S1. Modified sequences with truncated or mutated nucleotide were employed for determining their binding ability with Shp2 protein and K_d value using flow cytometry. Selectivity of optimized aptamer against Thrombin, Trypsin, Lysozyme, BSA, GST and Shp2 protein was also investigated by flow cytometry.

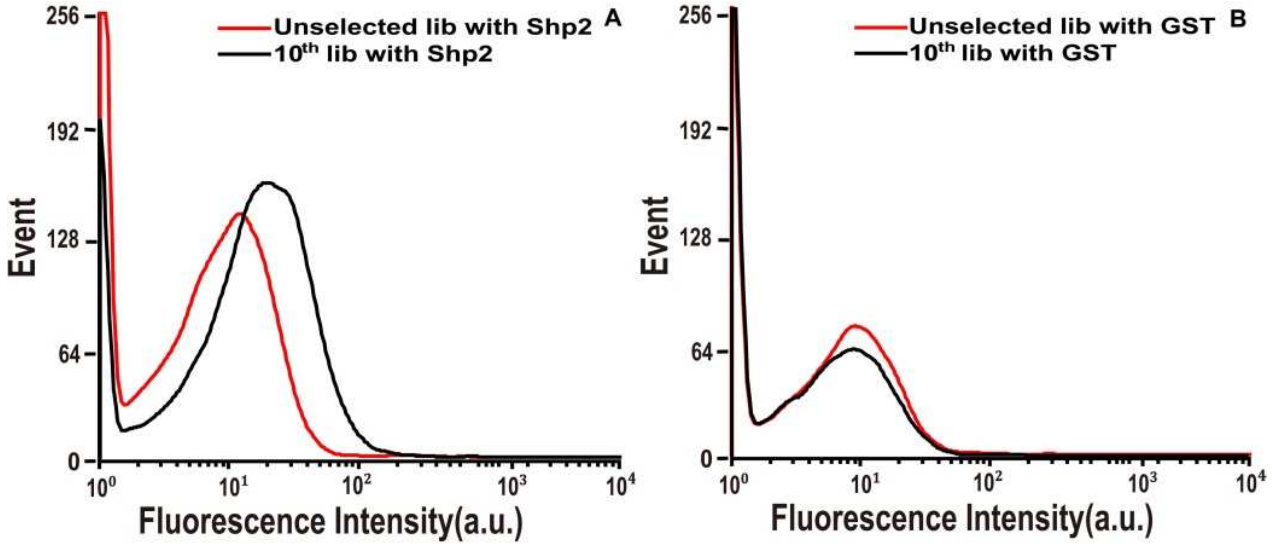

Figure S1. Enrichment of DNA aptamers against Shp2 protein using traditional SELEX technology. After 10 rounds of selection, Shp2-specific aptamer candidates were enriched (A) while showing no affinity towards control protein GST (B).Lib: Library.

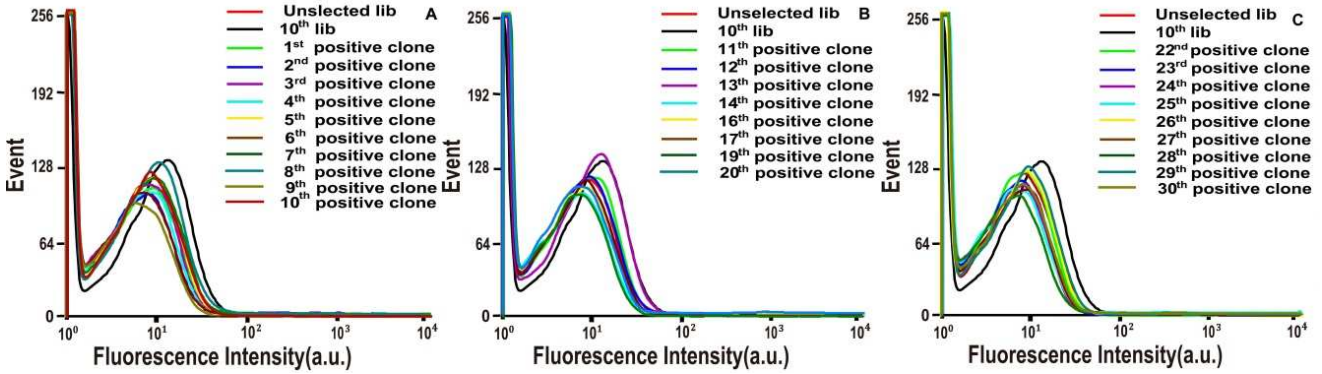

Figure S2. Binding assay of DNA in clonal beads with Shp2 protein monitored by flow cytometry(A-C).

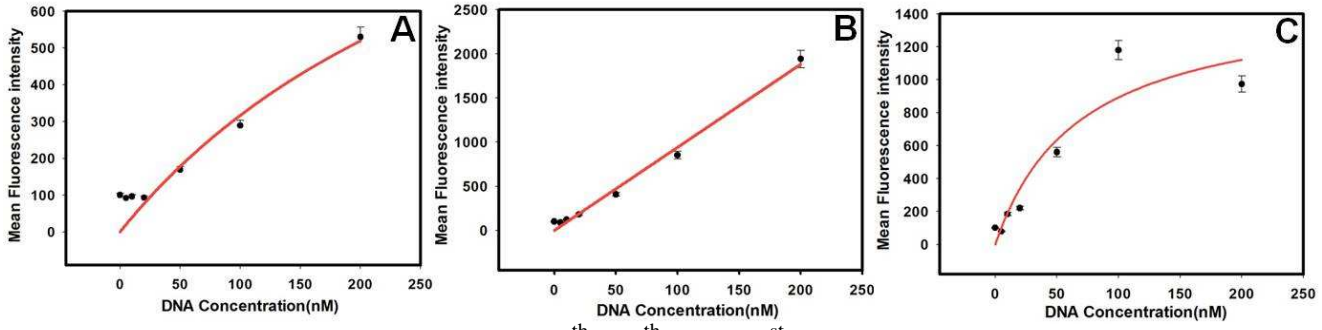

Figure S3. K_d values determination of 15th, 18th and 21st positive clones. The mean fluorescence intensity for each concentration of DNA-Shp2 protein interaction was examined with 3 replicates. K_d values of 15th and 21st positive clones were 354.0 ± 359.9 nM (A) and 68.7 ± 43.9 nM (C), respectively, while K_d value of 18th positive clone was above 1 μM (B).

Table S1. Sequences used for structural optimization.

Name	Sequence
21	5′ -AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA ATG GAG CTC GTG GTC AG-3′
21-1	5′ -AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA AAG GAG CGG TGG TCA G-3′
21-2	5′ -AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA ATG GAG CGG TGG TCA G-3′
21-3	5′ -AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA AAG GAG CTC GTG GTC AG-3′
21-4	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA AAG GAG CGG TGG TCA G-3′
21-5	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA ATG GAG CGG TGG TCA G-3′
21-6	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA ATG GAG CGG TGG TCA G-3′
21-7	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA AAG GAG CTC GTG GTC AG-3′
21-8	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA AAG GAG CTC GTG GTC AG-3′
21-9	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCT TTG TTT AAT TTT GCA CAT CTA ATG GAG CTC GTG GTC AG-3′
21-10	5′ - AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCA TTG TTT AAT TTT GCA CAT CTA ATG GAG CTC GTG GTC AG-3′
21-11	5′ -AGC GTC GAA TAC CAC TAC AGC CCG CAC TCA ACC ACC GTT CCA TTG TTT AAT TTT GCA CAT CTA ATG GAG CTG GTG GTC AG-3′

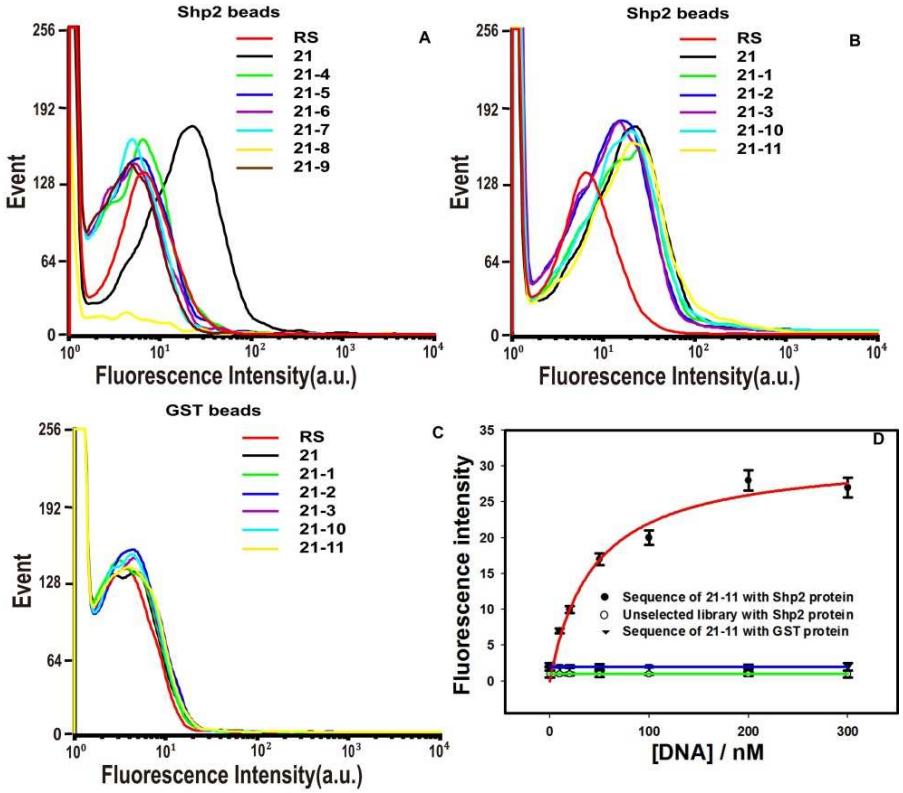

Figure S4. Binding affinity assay of modified sequences based on aptamer 21 with Shp2 protein monitored by flow cytometry. Compared with aptamer 21, sequences of 21-4, 21-5, 21-6, 21-7, 21-8 and 21-9 showed much smaller fluorescence shift (A), while sequences of 21-1, 21-2, 21-3 and 21-10, especially sequence 21-11 maintained similar fluorescence shift (B). RS: random sequence. Binding affinity assay of modified sequences based on aptamer 21 with GST protein monitored by flow cytometry. Similarto RS, sequences of 21-1, 21-2, 21-3, 21-10 and 21-11 as well as aptamer 21 showed negligible binding affinity towards GST protein. (C). K_d determination of sequence 21-11. The mean fluorescence intensity for each concentration of sequence 21-11-Shp2 protein interaction was examined with 3 replicates. K_d of sequence 21-11 was found to be 43.4 ± 9.4 nM (D).

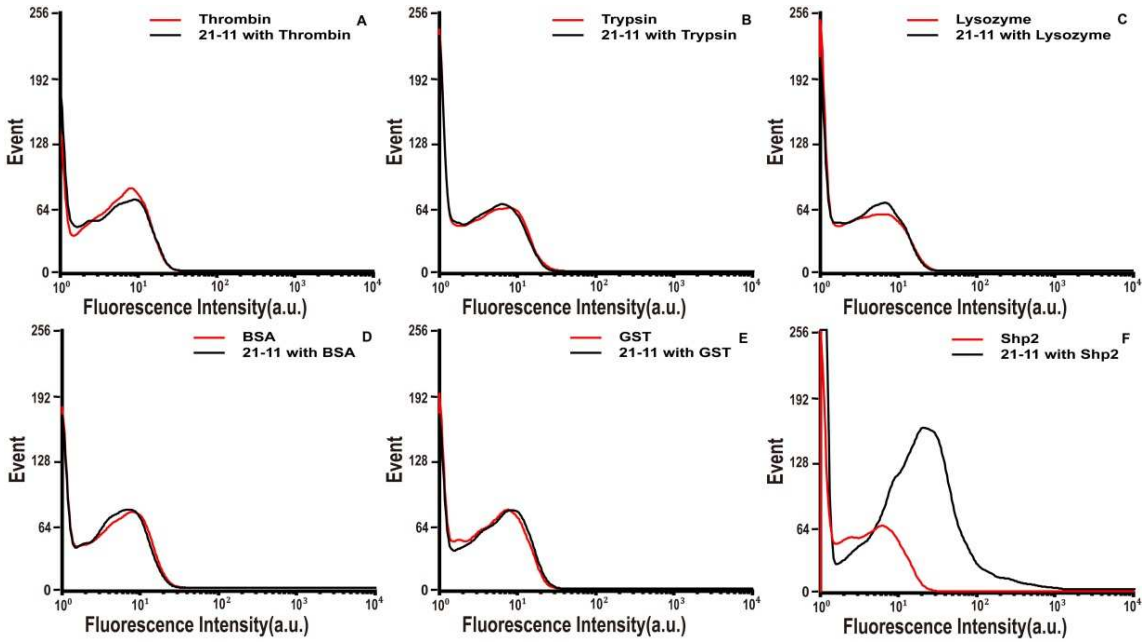

Figure S5. Selectivity of aptamer 21-11 against different proteins. The selectivity of aptamer 21-11 was tested against Thrombin (A), Trypsin (B), Lysozyme (C), BSA (D), GST (E) and Shp2 (F) proteins. Aptamer 21-11 selectively bound with Shp2 protein (F), but no obvious binding was observed for Thrombin, Trypsin, Lysozyme, BSA and GST (A-E).

Table S2. Sequences of the random region in 10th library.

Number	Similarity	Random region sequence	# of Sequences
1, 10	Identical	CTTGTTTTTTTTATTATTATTATTTTTAATTTATTTGTTT	2
2, 15	Identical	CGGCAGGTTGTCATATTTTTTTTCTGTTAGGAACAGTAGG	2
3	Different	TTTTTTTTGTTGGGTTTTGTCTTTATTGTTAGCTTTGGGT	26
4		TTTTTGTTTAGTTCGTTTGTTTTACGGGGGTGTGGATGTT	
5		TGTACCAATCGGTGATTTGTGTAATTTGAATAGATTGTTG	
6		TTTTATATTTTCTGATTTTTGTTTTTGTGAGGGTTTTGTT	
7		GTTTTTTTTCTCATATAGTCACTTTTATTCTGGGCGGGTA	
8		TTGGGGCTATGTTTTGATTGTTTTATATTTGGTTTGGGGG	
9		TTATATCTGTGCTTGTTTTTTTGGTGGTGGGGGGTTGTTT	
11		TCTCTTTTTTGTTCTTGGTTATTTATATTCTTGGTTGTGT	
12		CAGTTAAAGTTTATTGGCCTTGTTATTTTATCTATTAACT	
13		TTTTAACTTTTCTATTTATGTGGCGTAATCTTTTTTAGTT	
14		TTTTGGGTTATTTTTTTGCAGGGGTATTTATGGTTTTAAG	
16		TTTTTTTTCAGTTTTATCTTGGGGTTTAATTTATTTTTTT	
17		ATTGTTTGGGTTATTTTTTTTTGTTCTCTTTGGGGGTGG	
18		GTTGTTGATTTTTTTTGGGGATTGGGTTTGGGGTTTGGT	
19		TTCCGGTACCAATGATAAGTGCAATGTTCAAGTTGAAGTT	
20		TGTTTTTTTTTTGGTTGGAGGTTGGTTGTTGGATTTTTGT	
21		GCTTGCATGGCTTTTTGTTAATCTTTTCTTTTGTGTGGAG	
22		TGAACTAAGAAAGGGGAAGTTGGTAAGTTAGGCACCCTTG	
23		TGTGTGTCTTTTCTTGTGGTTTGTTTTTGTTTTATTTTGT	
24		TTTTGGGTCGTCAGGTTGGTGTAGTTTTTGGGTTTTTTTT	
25		GCTTACATTGTTTTGTTCTTTAGGGAGGTGGATTATGTTC	
26		GGTGGGGTTTGTCTGGGTGGGGGGGGTCGGTGGATTAGTG	
27		TTTGTTTTTTTTGTATTTTGTCGTTGTTTTGTGGTTTAG	
28		TTATTTTTAGAGTATGTATGTCTTTTGGTTTTAATTTTT	
29		TAAAAATATCCATTGGTTGTTTGCGAGTTTTATAGCCTTA	
30		CTTGTTAAAAGAGGGACTTTTCGTAGAAGAAAAAGTCATA	