Supplementary Information for

"Selection of Aptamers for AMACR Detection from the DNA Libraries with Different Primers"

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Materials

Bovine serum albumin (BSA), human serum albumin (HSA), acid-washed glass bead, and (3glycidyloxypropyl) trimethoxy-silane (GLYMO) were all purchased from Sigma-Aldrich (St. Louis, MO). Homemade recombinant AMACR protein for comparison was from Prof. Hsu, Chun-Hua's lab. Synthesized oligonucleotide sequences were purchased from Integrated DNA Technologies (Coralville, IA). Real-time quantitative PCR kit was purchased from Qiagen (Venlo, Netherlands). PCR kit and Centri-Sep™ Spin Column were purchased from Life Technologies (Grand Island, NY). Streptavidin magnetic bead was purchased from PerkinElmer (Waltham, MA). Alkaline phosphatase-conjugated streptavidin (Strep-AP) was purchased from Abcam (Cambridge, MA). AttoPhos® AP fluorescent substrate system component was purchased from Promega (Madison, WI). The 96 well polystyrene plates were purchased from Nunc™ A/S (Rochester, NY). All the other reagents were analytical grade.

Equipment and software

The ultrapure water (18.2 MΩ·cm) was produced by a Milli-Q System (Merck Millipore, Direct-Q 3, Billerica, MA) and used for buffer preparation in this work. The concentration of DNA and protein were measured by UV/Visible spectrophotometer (Biochrom, WPA Biowave II, Holliston, MA). The amplification of DNA, and evolution of ssDNA ligand pool were monitored by qPCR machine (QIAGEN, Rotor-Gene® Q, Venlo, Netherlands). The conformations of aptamers were characterized by using a circular dichroism spectropolarimeter (Jasco, J-810) (Easton, MD). The signal of ELISA was measured by a microplate reader (Molecular Devices, FlexStation 3) (Sunnyvale, CA). Secondary structures of aptamers were predicted by RNAstructure version 5.6 (Mathews lab) (Rochester, NY). Origin 8.5.1 (OriginLab) (Northampton, MA) was used to analyze and plot the data in this work.

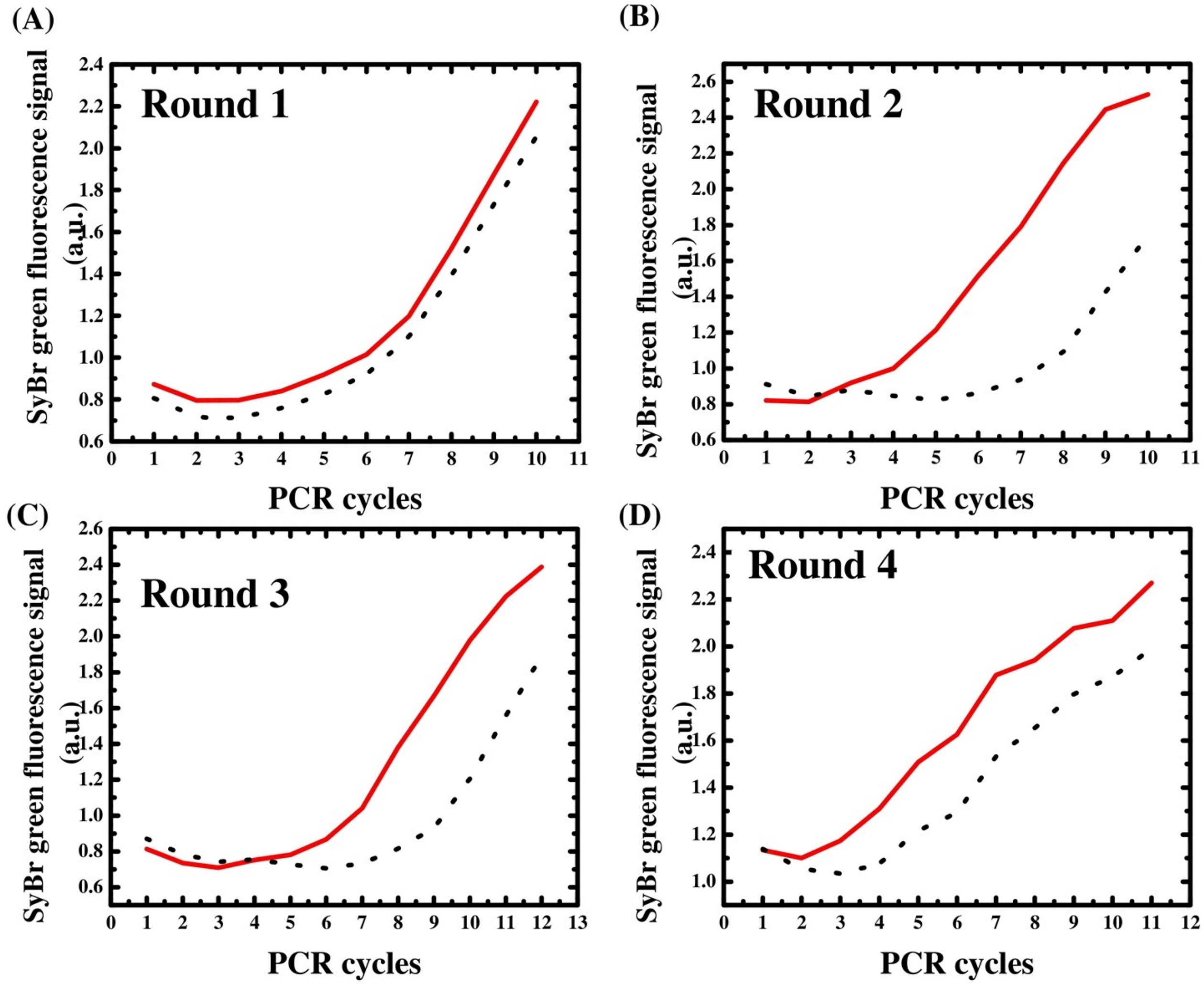


Fig. S1 Differential qPCR analysis for monitoring the ssDNA ligand evolution of Library A from the 1st to 4th SELEX round ((A) to (D)). The specific ssDNA ligand binding to AMACR-coated beads (solid red line) is compared with the non-specific ssDNA ligand binding to HSA-coated beads (dash black line) in each sub-figure.

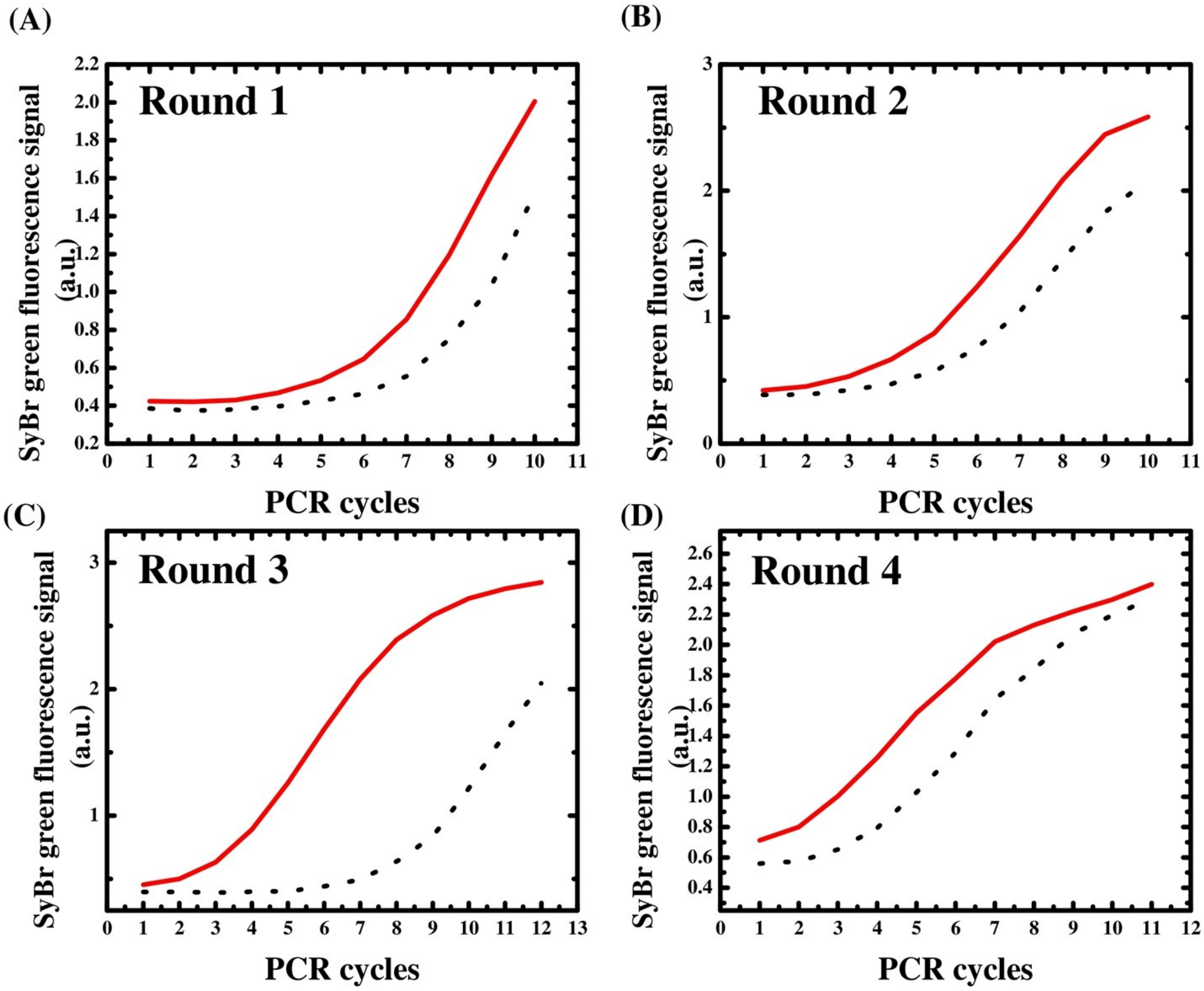


Fig. S2 Differential qPCR analysis for monitoring the ssDNA ligand evolution of Library B from the 1st to 4th SELEX round ((A) to (D)). The specific ssDNA ligand binding to AMACR-coated beads (solid red line) is compared with the non-specific ssDNA ligand binding to HSA-coated beads (dash black line) in each sub-figure.

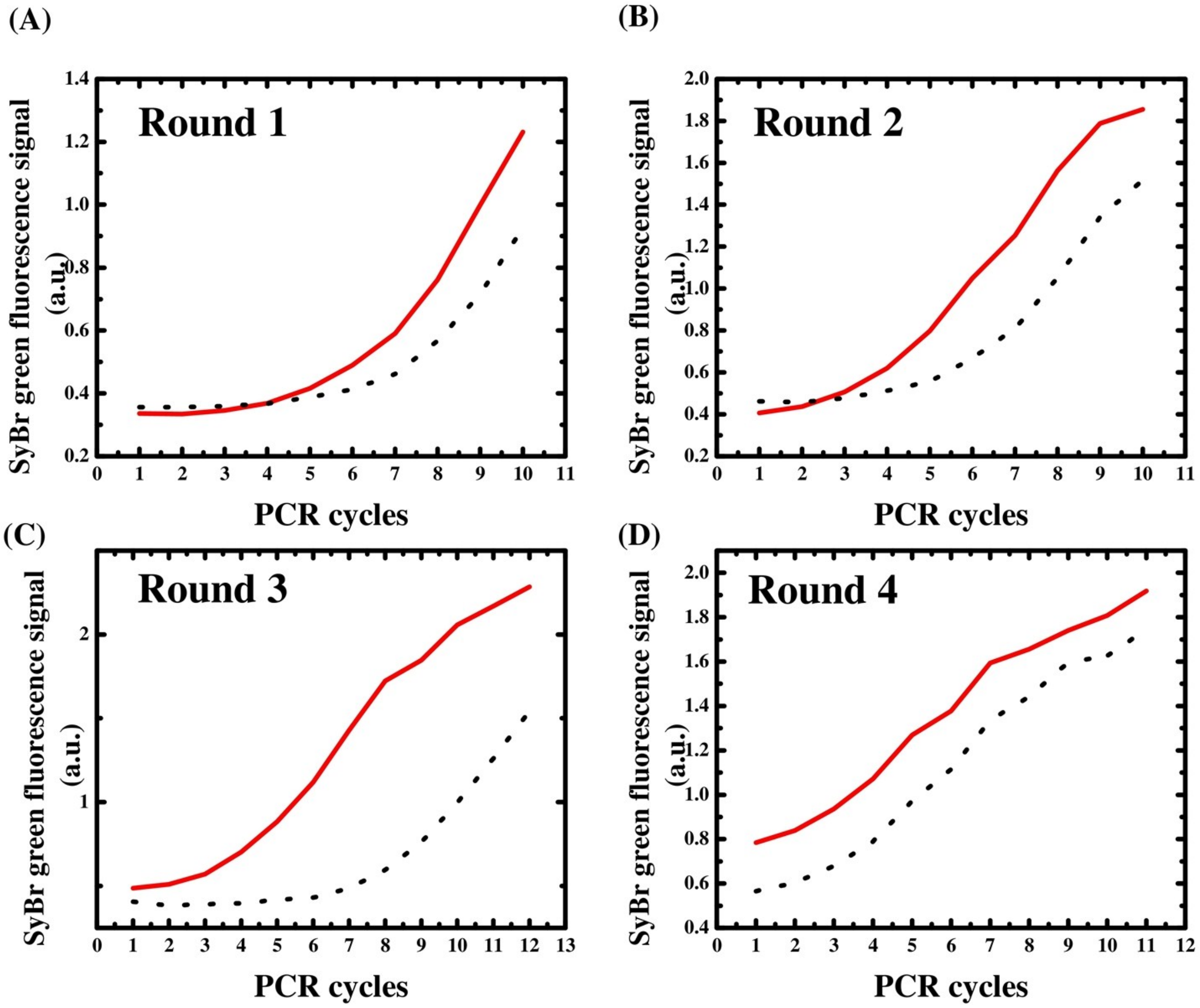
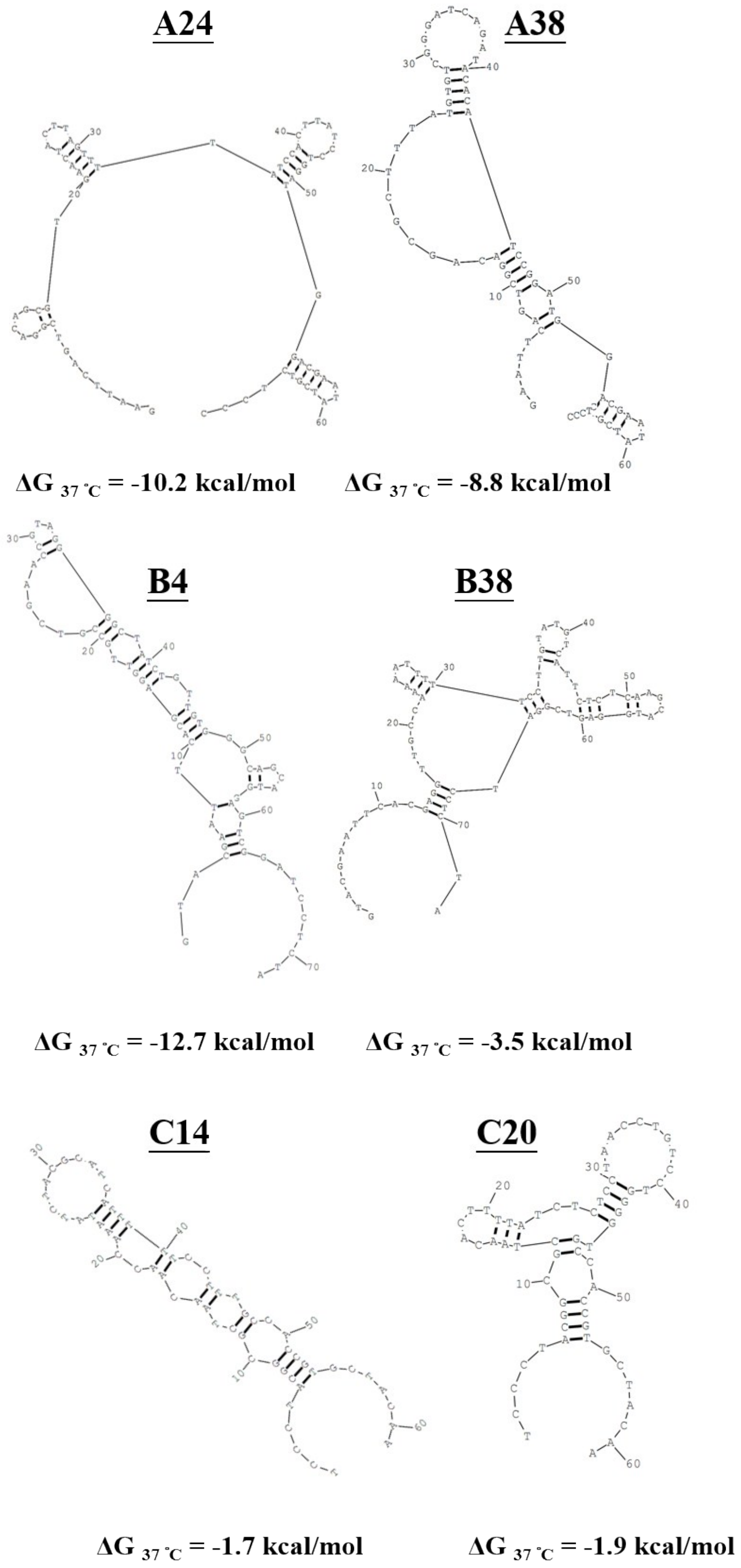


Fig. S3 Differential qPCR analysis for monitoring the ssDNA ligand evolution of Library C from the 1st to 4th SELEX round ((A) to (D)). The specific ssDNA ligand binding to AMACR-coated beads (solid red line) is compared with the non-specific ssDNA ligand binding to HSA-coated beads (dash black line) in each sub-figure.



A24

ΔG $_{37\,^\circ C}$ = -10.2 kcal/mol

A38

ΔG $_{37\,^\circ C}$ = -8.8 kcal/mol

B4

ΔG $_{37\,^\circ C}$ = -12.7 kcal/mol

B38

ΔG $_{37\,^\circ C}$ = -3.5 kcal/mol

C14

ΔG $_{37\,^\circ C}$ = -1.7 kcal/mol

C20

ΔG $_{37\,^\circ C}$ = -1.9 kcal/mol

Fig. S4 The secondary structure predictions for A24, A38, B4, B38, C14 and C20.

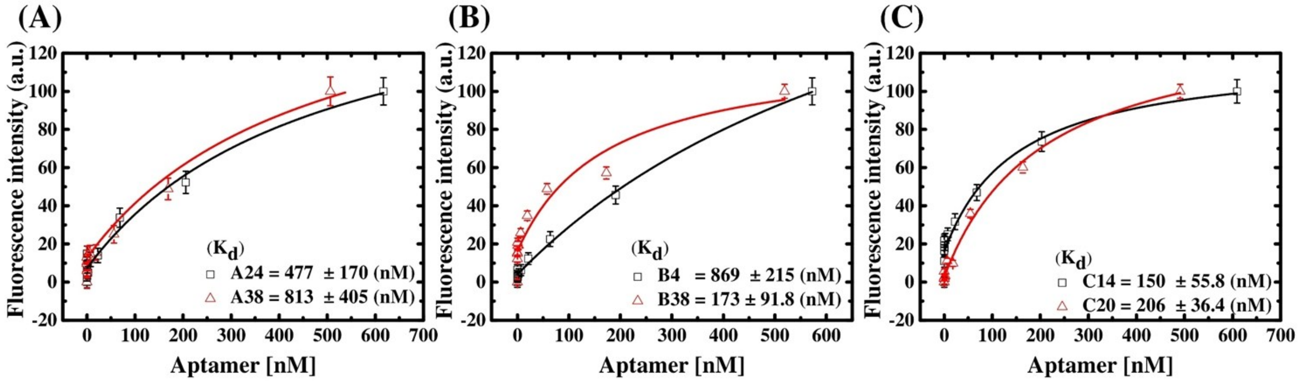


Fig. S5 ELAA-based total-binding assays for the binding affinity estimation of the truncated aptamers ((A) A24 and A38; (B) B4 and B38; (C) C14 and C20). The AMACR concentration for microtiter plate's well coating was 0.2 μM.

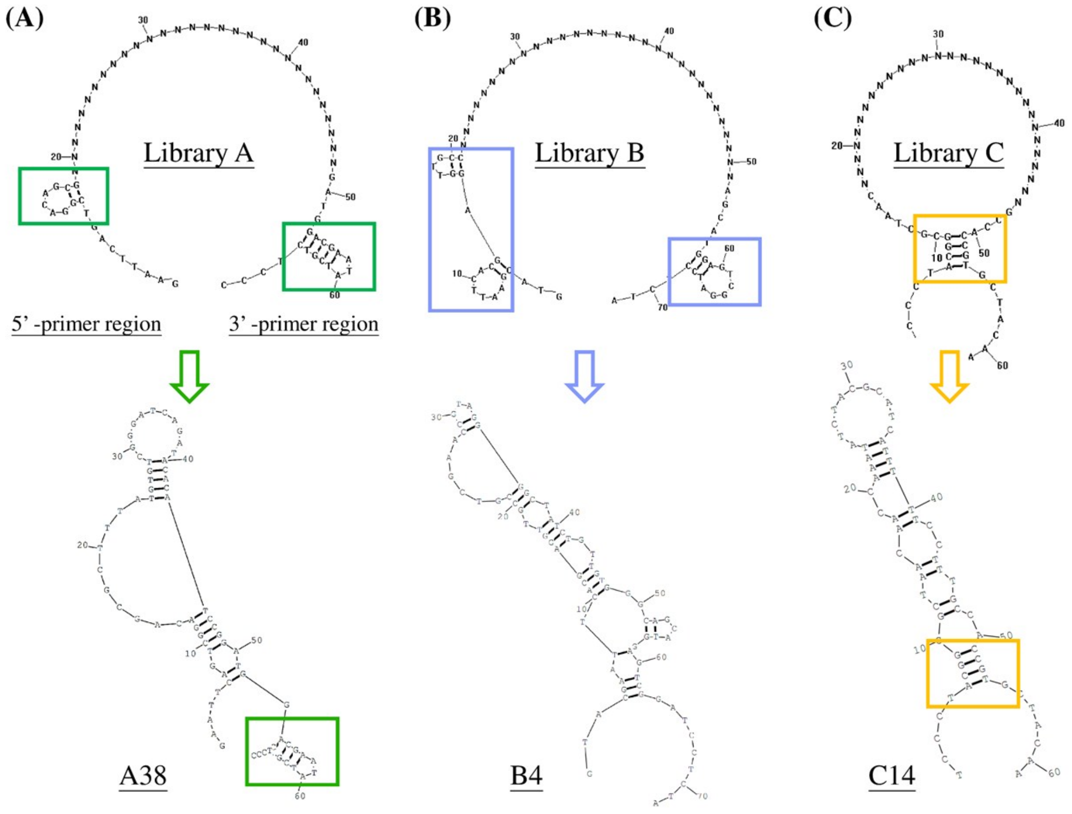


Fig. S6 The 2nd structure prediction for (A) Library A and A38; (B) Library B and B4; (C)Library C and C14.

Table S1 The sequences of the three kinds of ssDNA libraries and related primers in this study.

ID	Sequence (5'--->3')	Length(mer)
Forward primer A	GAATTCAGTCGGACAGCG	18
Reverse primer A	GGGAGACGATATTCGTCCATC	21
Reverse primer-biotin A	Biotin-GGGAGACGATATTCGTCCATC	21
Library A	GAATTCAGTCGGACAGCG-(N30)-GATGGACGAATATCGTCTCCC	69
Forward primer B	GTACGAATTCACGAGGTTGCC	21
Reverse primer B	TAGAGGATCCGACTCCATGCT	21
Reverse primer-biotin B	Biotin-TAGAGGATCCGACTCCATGCT	21
Library B	GTACGAATTCACGAGGTTGCC-(N30)-AGCATGGAGTCGGATCCTCTA	72
Forward primer C	TCCCTACGGCGCTAAC	16
Reverse primer C	TTGTAGCACGGTGGC	15
Reverse primer-biotin C	Biotin-TTGTAGCACGGTGGC	15
Library C	TCCCTACGGCGCTAAC-(N30)-GCCACCGTGCTACAA	61

Table S2 The list of 28 aptamer sequences obtained from DNA Library A

ID	**Sequence (*randomized* N30 region) _5' to 3'**	**CG%**
A10	TCGATGATTCACTCTCGCATTTATCTTTTC	36.7
A11	TACTCACAGTCTTGGCCCATACACCAGTCA	50.0
A12	TAATCTGACGACGGCTCGGTAAAAGGTCCA	50.0
A20	GACTCGTGGGCGTTACTTCCCAATTTCGCT	53.3
A22	ACATGATCAACCGTCGAACTCTAACCCAAC	46.7
A24	TGAACTACTTAGTTTTATCCACTTATCCTG	33.3
A26	ATGAACTCACCAATTTATCCCTATATCTTT	30.0
A38	CTTTATGTGTCGGGATCAGATACACATCCG	46.7
A46	GCATGTGATCGTACGGTCTGTTTTTTCTGC	46.7
A52	CACAATGCATTCTTGCCTGTAAAGCATCTC	43.3
A53	CCGTTGTTATCTGTTTCAACTCCACTTCTT	40.0
A54	ACAATAGTTACAACTCTTCTGTTCCTCGTA	36.7
A56	TAACAGGTAAATGCAGCCTAGTTCATTCAT	36.7
A6	CAGATTCCAATTTTCCAAATTTTTACATGT	26.7
A60	TTGGAATCCGTTAATCCCTTTAAGCGCATT	40.0
A61	CGATCGCAGACGCCCAGTGAAGCGCAAGCA	63.3
A70	TATCTTTCGTTTCTAATCATTTACATATTG	23.3
A71	TACTTGACTGGTAATGTAGTTTGCGCAACC	43.3
A72	TGTTGGGTTAATTTCGTTATCGTCTGACAT	36.7
A74	TCTATAATAAATGCCCATAAATCCGGCGAG	40.0
A77	GTGATTTAGCGAGTTTCATGCATGCACACA	43.3
A82	CTATAATCCGTTATCACAACCTACTTCCCA	40.0
A84	GAGTACTTTATAGGGATGCAGTGTGTAAGA	40.0
A86	CATAATCAGTCTACATACATCCGCCCCGCT	50.0
A89	ACTTAGGTTATCTTGCAACATCCATTTATT	30.0
A92	ATCTCTTCACAGTCATGGGATAGTTACACA	40.0
A94	TCTATATGCCATGATTCATATCAGATCCGG	40.0
A96	ATGGGCCAATTTTATACTCCATATTATATA	26.7
	Avg.	40.5
	Stdv.	8.66

Table S3 The list of 33 aptamer sequences obtained from DNA Library B

ID	**Sequence (*randomized* N30 region) _5' to 3'**	**CG%**
B1	GTGTGCCTACTCCGGCTGCACCTTCTATAG	56.7
B10	ACTTAGTACTTTTTCTTTTTTTGTTTCCCG	30.0
B11	GTCGGGGTGAGGCTCAGTTTGCCATAATCC	56.7
B12	TCCCTTATCCACACTGATATTGTTTGTCAG	40.0
B13	TGAAGCAATTCCATGCCTTGACAGGATAAC	43.3
B17	TTTAATTCACACATTTATTGTATGTTTAGT	20.0
B2	TCGGTAGCTGGTTTACGAATGTTGGTTGGG	50.0
B20	ATCTCGGCACCTTATGTGTTTTCCCCTTAA	43.3
B24	TGGTTTTCCTTCAGTATGTTTCTAGATATG	33.3
B25	TATTTTTTCTCTTGGTATCTCATCTCGCTT	33.3
B3	CACTGGTCCTGGCCTTCTTCTACTTCTTCG	53.3
B30	TCCTTATAAATCCAATGTTTTCTTATGGTT	26.7
B34	CCAGCTTCTCCCTTATGTTTTCCCCTACGG	53.3
B38	AAAAATTTTTCCTTGTATGTCATTCTCTCA	26.7
B4	GTCGAACCGTAGGGGCTATCTGTTGTGGGC	60.0
B46	TGTTATTACACTGCATTTTTCCTATCCTAC	33.3
B5	ATCTATGCCTGTTCTCTGCTCAGTCGTAGA	46.7
B50	AATATGATGAAATTCGGTGTATTGTGTCTG	33.3
B51	ATTTTTTATCTTTACGTTTGTTATAGTGCA	23.3
B59	CCACTACCTACCCAAACTGCTAACCCACCG	56.7
B6	CCCACGGTCTTCTGACTTAGTCGCGTTGCC	60.0
B60	TGTAGCTTCTATTATCTCGCCTTTCCGCTT	43.3
B61	ACCTACTCATTTTTATCTTCTTATGATACC	30.0
B62	TCTCCTTATTCCGAACGGGTTTTTAATCTA	36.7
B65	GCCCTTTCATCCTCTGTGTAGGAATTCTCC	50.0
B68	CCTGAGGGGTTGTCGGGAAGTGAGGTTGGG	63.3
B7	CGCGGCTCACATTACCCTTTTTCACTCATG	50.0
B74	GATTGTTCTTCGGACCCTATCCTCATACAC	46.7
B8	TTTGCGAGTAGGGTCAGTCTCGGAGTGGGC	60.0
B84	CGGCACCAATTTATAATTCTTCCCCTAATA	36.7
B86	GATCAAGCTTTTTTTATGATCTTACCATTT	26.7
B9	AGCTCTTTTTTTTCCATTAAACTTATCATT	23.3
B92	AGTAGTCGTTTCCTTTGGGCTCTTTTTATC	40.0
	Avg.	42.0
	Stdv.	12.4

Table S4 The list of 31 aptamer sequences obtained from DNA Library C

ID	**Sequence (*randomized* N30 region) _5' to 3'**	**CG%**
C10	AGACATAACAGGGGGGTGAGGCAAGTGTGG	56.7
C12	TCCTTACATAGTGGCTTGTCCTCTCTCTCT	46.7
C13	GGTCAGTAGGAACTTGCCTACGCATTAAGT	46.7
C14	AACCAAATATCTACGCATCATTTTTCCTTT	30.0
C15	CCACAAGGAAGAGTGGGCTGAAACATGACC	53.3
C16	CTCTACAGTGATTGGTAACAACGTTACAGT	40.0
C17	TGGTGACACACAGGAACCCTCCTATGCATT	50.0
C19	CAGTATTCTCTTCGTATCATACCTCAGACT	40.0
C2	TTCCATCTCGGGTACACATCCTCCATTTAG	46.7
C20	ACTTTTATCTCTCCTAACCTGTCCTGGGGT	46.7
C21	CACGTTGCACTGATTGACAACACCGATCTC	50.0
C22	TTGCCCTTATTCTGTCCTATTGCCCGGCCG	56.7
C23	GTCATTATCCACCCCCACTTTGGTACTTTG	46.7
C24	GTAACACCGTCCTATTAGATTCCCCCGCTC	53.3
C25	CATAATTGTTAATTGTGTCTGGGCGTGCGG	46.7
C26	ACGGGTTATAGTGCTGACTAACGGTTTGAG	46.7
C27	CAACGTATTCTGGAAGATGAATATTGACAA	33.3
C28	GTGTTTGAAACTGGAAGGTCTTGTTAGGGC	46.7
C29	CCGATTGTTCATTTTTTTATATACATGCTG	30.0
C3	ACAATTAGTTCTTATGAACGTGGTTCCAGG	40.0
C30	ACAATCCCCCGTCTTGCTGTGTGTCTAATA	46.7
C32	GACAGGCGATGGCAGGGTCATCTTCAATGT	53.3
C33	CCCATTATCTAGACAGTCGCCCCCTCCTTC	56.7
C34	GAACAACAAATACACATCTCGGTTTACGAG	40.0
C35	GGAGTTGAATAAGTTTGTTGAGCTTTTTAG	33.3
C4	GTCCTGCTCACATACTCACATCTATTATTT	36.7
C5	GACTTGCTACCAGCCTATCATGAACCCTAA	46.7
C6	AATTCAGGGGAACTGGGTCAAGAGTCCTTC	50.0
C7	GATTCCTTCAGCATCGCGACCTAAGGACGG	56.7
C8	CGACTCCTTCCCGATCTAACCAGTTGTTTA	46.7
C9	CAGATTTAATCTATTTACTTTCCTGAGAT	26.7
	Avg.	45.2
	Stdv.	8.21

Electronic Supplementary material (ESI) for RSC Advances

Table S5 The six pairs of aptamer candidates from Library A, Library B and Library C.

Name	**Sequence (*randomized* N30 region) _5' to 3'**	**Alignment score (%)**
A24	TGAACTACTTAGTTTTATCCACTTATCCTG	77
A26	ATGAACTCACCAATTTATCCCTATATCTTT	
A38	CTTTATGTGTCGGGATCAGATACACATCCG	70
A94	TCTATATGCCATGATTCATATCAGATCCGG	
B25	TATTTTTTCTCTTGGTATCTCATCTCGCTT	73
B38	AAAAATTTTTCCTTGTATGTCATTCTCTCA	
B2	TCGGTAGCTGGTTTACGAATGTTGGTTGGG	70
B4	GTCGAACCGTAGGGGCTATCTGTTGTGGGC	
C20	ACTTTTATCTCTCCTAACCTGTCCTGGGGT	66
C9	CAGATTTAATCTATTTTACTTTCCTGAGAT	
C14	AACCAAATATCTACGCATCATTTTTCCTTT	60
C23	GTCATTATCCACCCCCACTTTGGTACTTTG	