SUPPORTING INFORMATION

A Simple Method for Eliminating Fixed-Region Interference of Aptamer Binding During SELEX

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Supporting materials and methods

Preparation of modified carboxylic acid beads displaying the protein target

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and ethanolamine were obtained from Sigma-Aldrich (Oakville, ON, Canada). Carboxylic acid beads (0.5 mg) were washed 3X in MES buffer (25 mM 2-[N-morpholino]ethane sulfonic acid, pH 6.0)), and the beads were then recovered using a magnetic particle separator (Magna-Sep, Invitrogen Life Technologies) before being activated in a well-mixed 250 μl solution of 400 mM EDC and 100 mM NHS for 30 min at room temperature. After activation, the beads were washed 3X in MES buffer and incubated for 2 hrs in a solution containing 1.35 nmoles of the target protein (*e.g.* human α-thrombin). After incubation, the beads were washed 3X in 2 M NaCl Tris-buffer (20 mM Tris-HCl pH 7.4, 2 M NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$), followed by 3X wash in MES buffer. The modified beads were then blocked in a 250 μl solution of 1 M ethanolamine for 30 min, before being subjected to a final 3X wash in MES buffer containing 0.02% Tween-20. The protein-modified beads were stored at a 10 mg ml^{-1} concentration at 4 °C until further use.

SELEX-based aptamer screening

The 80-mer Library-1 form of the streptavidin binding aptamer (SBA29-L1), where the 29-mer consensus sequence is fully contained within the core region, was introduced into the ssDNA library (1 nmole; 10^{14} sequences) at a 1% molar ratio to permit detection of the aptamer in the bound and unbound pools during the first round of selection. Equimolar amounts of the complementary oligonucleotides 5'- and 3'-Comp-L1 were added to and equilibrated with the library. A 2 µl portion of the MyOne Streptavidin C1 Dynabead stock solution was washed three times with 500 µl of 1X binding buffer (20 mM Tris-HCl pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.02% Tween-20). The washed beads (~10^6 beads) were incubated with the folded aptamer library in 500 µl of 1X binding buffer for 1 hr at room temperature with gentle rotation. Unbound and weakly bound aptamers were removed by washing with binding buffer containing 140 mM NaCl. All remaining aptamers were eluted from the beads with denaturing buffer (40 mM Tris-HCl pH 8.0, 3.5 M urea, 10 mM EDTA, 0.02% Tween-20) at 85 °C. All wash and elution steps were performed three times for 5 min in a total volume of 500 µl. Each collected fraction was desalted using centrifugal filter units (Amicon Ultra-0.5 MWCO 10K, Millipore) and the aptamers recovered in nanopure water (18.2 MΩ-cm) according to the manufacturer's instructions. The same procedure was applied to all SELEX-based screenings in which no complementary oligonucleotides were present.

Real-time amplification and detection of TBA15-L1 in recovered Library L1 fractions

Desalted aptamer fractions were mixed with amplification reagents and cycled in a CFX Real-Time PCR detection system (Bio-Rad, Mississauga, ON, Canada) in the presence of a TaqMan™ probe (5'- FAM-TGC TGA ATT CGG TTG GTG TGG TTG GGA-Iowa Black FQ -3') generated against the TBA15-L1 aptamer. A 5 µl aliquot of each fraction was mixed with iQ Supermix (Bio-Rad) with 300 nM each of the forward and reverse primers, as well as 200 nM of TaqMan probe to form a 25 µl reaction volume. The thermal cycling conditions were as follows: initial denaturation for 3 min at 95 °C, then 50 cycles each comprised of 95 °C for 30 s and 59.5 °C for 30 s. The results were analyzed using CFX Manager Software (Bio-Rad).

Amplification and regeneration of recovered Library-2 aptamers

Desalted aptamer fractions were amplified in 50 μl PCR reactions containing 1X PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 250 nM each of FOR-L2 and phosphorylated REV-L2 primer, as well as 2 units of Platinum Taq DNA polymerase. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by an optimal number of cycles (10 – 18) comprised of denaturation at 94 °C for 15 s, annealing at 64 °C for 20 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 3 min. PCR reactions were pooled, ran on a 1.5% TBA agarose gel, and purified using a QIAEXII Gel Extraction Kit (Qiagen, Toronto, ON) following the manufacturer's protocol. The purified double stranded product was digested with 5 units of lambda exonuclease in the supplied buffer for 60 min at 37 °C. The resulting single stranded product was then purified by phenol extraction and ethanol precipitation for the next round of selection.

Table S-I. DNA aptamer sequences used in this study

Name	Sequence (5′ – 3′) [a]	Comments
TBA15 (15 nt)	GGTTGGTGTGGTTGG	Thrombin aptamer [Bock et al. 1992]
TBA15-L1 (80 nt)	**AGCAGCACAGAGGTCAGATG–** GCTGCTGAATTCGGTTGGTGTGGTTGGGAATTCTACGG AT**–CCTATGCGTGCTACCGTGAA**	Library 1 80-mer form of TBA15
TBA29 (29 nt)	AGTCCGTGGTAGGGCAGGTTGGGGTGACT	Thrombin aptamer [Tasset et al. 1997]
TBA29-L1 (80 nt)	**AGCAGCACAGAGGTCAGATG–** TTCAGTCCGTGGTAGGGCAGGTTGGGGTGACTGTTCA CGT**–CCTATGCGTGCTACCGTGAA**	Library 1 80-mer form of TBA29
TBA29-L2 (80 nt)	**TCGCACATTCCGCTTCTACC–** TTCAGTCCGTGGTAGGGCAGGTTGGGGTGACTGTTCA CGT**–CGTAAGTCCGTGTGTGCGAA**	Library 2 80-mer form of TBA29
SBA29 (29 nt)	ATTGACCGCTGTGTGACGCAACACTCAAT	Streptavidin aptamer [Bing et al. 2010]
SBA29-L1 (80 nt)	**AGCAGCACAGAGGTCAGATG–** ACTACTATTGACCGCTGTGTGACGCAACACTCAATTTC AC**–CCTATGCGTGCTACCGTGAA**	Library 1 80-mer form of SBA29
VBA25 (25 nt)	CCGTCTTCCAGACAAGAGTGCAGGG	Vascular endothelial growth factor aptamer [Potty et al. 2009]
VBA25-L1 (80 nt)	**AGCAGCACAGAGGTCAGATG–** GGCTGATCCGTCTTCCAGACAAGAGTGCAGGGATCGA GCC**–CCTATGCGTGCTACCGTGAA**	Library 1 80-mer form of VBA25

a Primer sites in bold; aptamer binding sequence underlined.

Table S-II. Thermodynamic data for binding of aptamers to their respective targets[a] measured by isothermal titration calorimetry.

Aptamer	K_D (nM)[b]	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})
TBA15	31.25 ± 7.37	-27.23 ± 0.30	-16.99	-10.24
TBA15-L1	n.d.	n.d.	n.d.	n.d.
TBA15-L1 + Comp-L1's	20.00 ± 2.74	-23.51 ± 0.14	-13.00	-10.51
SBA29	20.79 ± 2.18	-38.10 ± 0.14	-27.61	-10.49
SBA29-L1	n.d.	n.d.	n.d.	n.d.
SBA29-L1 + Comp-L1's	20.79 ± 2.39	-34.12 ± 0.13	-23.58	-10.54
VBA25	36.76±4.91	-24.77±0.21	-14.61	-10.16
VBA25-L1	34.48±3.17	-10.88±0.05	-0.70	-10.18
VBA25-L1 + Comp-L1's	35.46±6.96	-11.75±0.11	-1.59	-10.16
TBA29	3.94 ± 0.78	-18.70 ± 0.08	-7.22	-11.48
TBA29-L1	5.49 ± 1.46	-12.62 ± 0.08	-1.35	-11.27
TBA29-L1 + Comp-L1's	5.71 ± 2.31	-9.91 ± 0.09	1.34	-11.25
TBA29	3.94 ± 0.78	-18.70 ± 0.08	-7.22	-11.48
TBA29-L2	454.54 ± 30.72	-28.44 ± 0.74	-19.79	-8.64
TBA29-L2 + Comp-L2's	4.02 ± 0.31	-10.79 ± 0.07	0.68	-11.46

a Experiments were performed at 25 °C in 20 mM Tris-HCl (pH 7.4), 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$.

b n.d.: no detectable binding; the iTC200 instrument does not detect binding weaker than 10^2 M^{-1}

Supporting references:

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