Supporting information

Array-based Discovery of Aptamer Pairs

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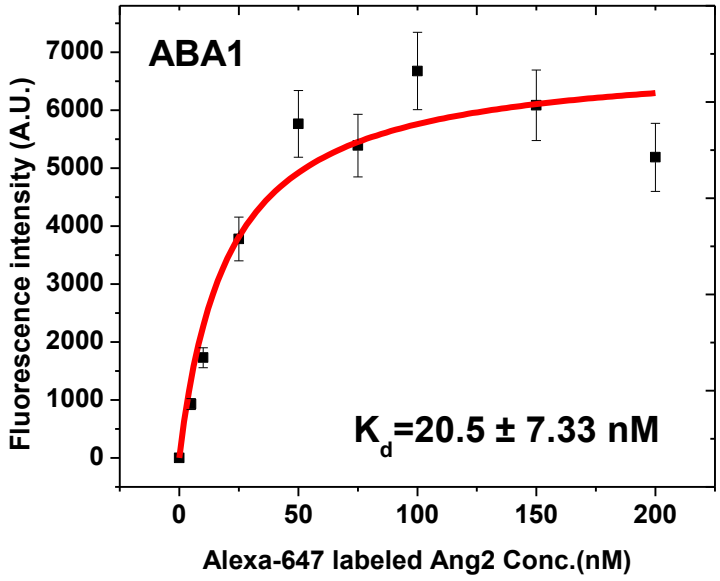

Figure S1. Equilibrium binding curve for ABA1. We obtained fluorescence intensities from eight identical arrays incubated with different concentrations of Alexa Fluor 647-labeled Ang2, and ABA1 exhibited the highest affinity/lowest K_d value (K_d = 20.5 ±7.3 nM) (1).

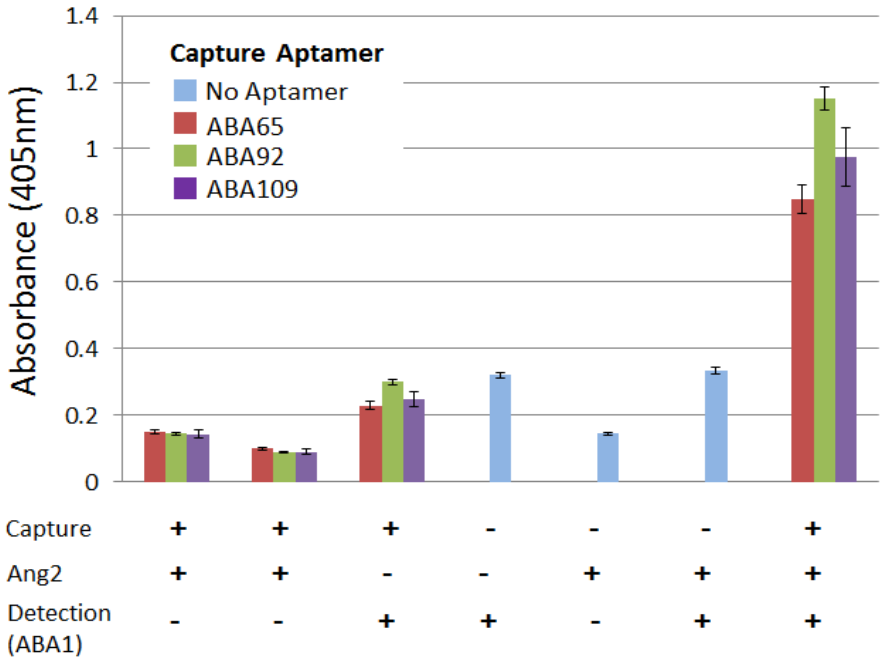

Figure S2. Control experiments for aptamer pair binding. Enzyme-linked oligonucleotide assay (ELONA) experiments demonstrate that labeling both the capture and detection aptamers with biotin achieved sufficient signal-to-noise performance, as shown by the low background signals obtained in control experiments using both biotinylated aptamers. Error bars represent triplicate measurements.

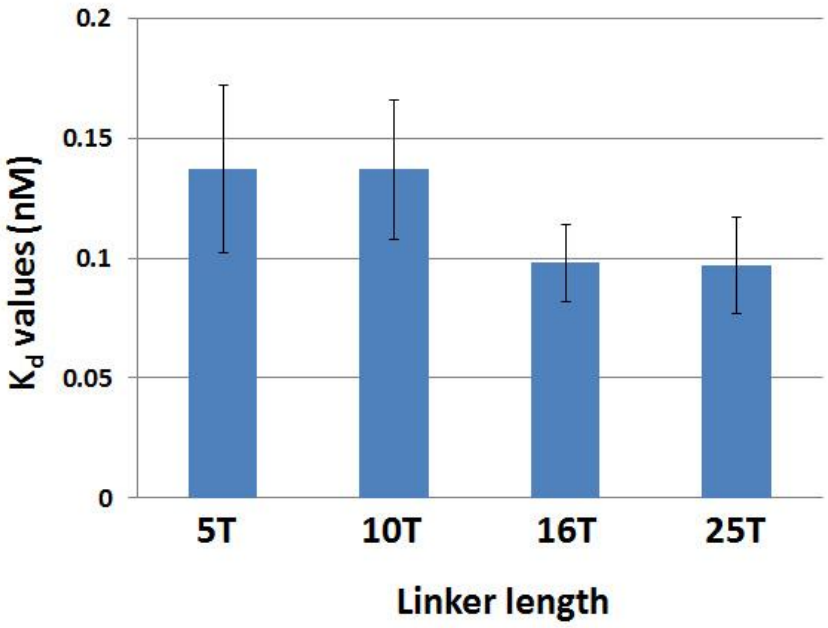

Figure S3. Contribution of linker length to binding affinity for bidentate aptamer reagents. We connected ABA1 to ABA65 with flexible poly-T linkers of different lengths, and measured their binding affinities using a bead-based fluorescence assay. The ABA1-ABA65 construct with a 25T linker showed the highest binding affinity (K_d = 97±20 pM). Error bars represent triplicate measurements.

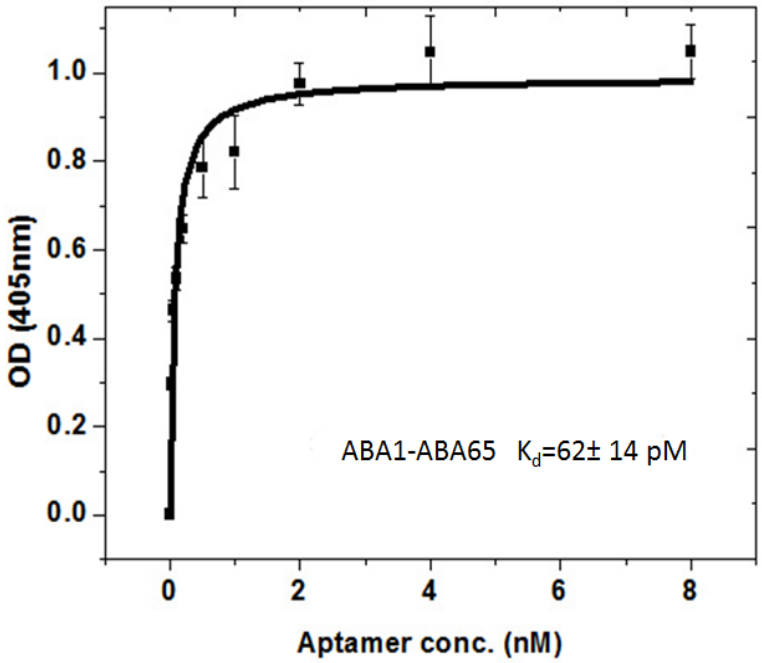

Figure S4. Binding affinity measurement for ABA1-ABA65 using ELONA. ABA1-ABA65 showed similar affinity as measured with ELONA in comparison to bead-based fluorescence binding assay measurements.

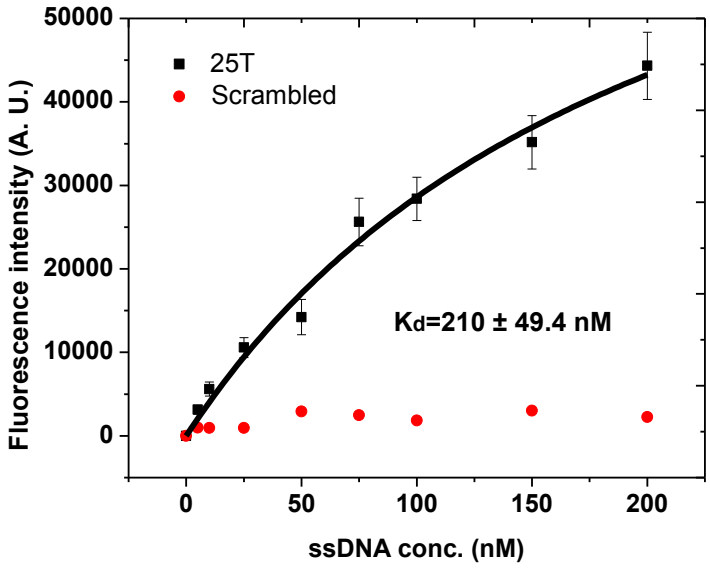

Figure S5. Binding affinity measurements for control sequences. The 25T linker alone showed minimal affinity to Ang2 (K_d = 210 nM) and a scrambled aptamer sequence showed negligible Ang2 binding.

Table S1. Aptamer array features

Aptamer sequences (5'-->3')	
Top 235 sequences	[40nt-aptamer sequence]
Top 235 sequences + T linker	[40nt-aptamer sequence]-TTTTTTTTTTTTTTTTTTT
Top 235 sequences + reverse primer	[40nt-aptamer sequence]-CCTATGCGTGCTACCGTGAA
forward + Top 235 sequences + reverse	AGGTCAGATG-[40nt-aptamer sequence]-CCTATGCGTG
Control sequences (5'-->3')	
20-nt poly T linker	TTTTTTTTTTTTTTTTTTT
40-nt poly-T linker	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
60-nt poly-T linker	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
Reverse primer	CCTATGCGTGCTACCGTGAA
Reverse primer +T linker	CCTATGCGTGCTACCGTGAATTTTTTTTTTTTTTTTTTT
Forward + reverse primer	AGCAGCACAGAGGTCAGATGCCTATGCGTGCTACCGTGAA
Forward+T linker+Reverse primer	AGCAGCACAGAGGTCAGATGTTTTTTTTTTTTTTTTTTTCCTATGCGTGCTACCGTGAA
Scrambled sequence	GGGTTTGCGAAAGGGAAAGGGTTTGTGTTTTTTGGGTTTT
Thrombin aptamer (15 nt) + T linker	GGTTGGTGTGGTTGGTTTTTTTTTTTTTTTTTTTTTTTT
Thrombin aptamer (29 nt) + T linker	AGTCCGTGGTAGGGCAGGTTGGGGTGACTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
PDGF-BB aptamer	CACAGGCTACGGCACGTAGAGCATCACCATGATCCTGTGTTTTTTTTTTTTTTTTTTTT

Table S2. Screened aptamers, bidentate constructs and control sequences

ID	Sequence (5' →3')
Aptamer sequences	
ABA1	ACTTGTAGGGTTCGGCTGGTCAGGGTGTCCAAGTCTGTGG
ABA65	TTTGGGGTGGTTGGGGGTTGTTGTTTTGGGGGTGGAGGGT
ABA92	GGTGCCATTGGGGACTGCTCGGGATTGCGGACTTGGCATC
ABA109	GGGTGCCATTGGGGACTGCTCGGGATTGCGGACTTGGCAT
ABA1-ABA65	[ABA1]-TTTTTTTTTTTTTTTTTTTTTTTTT-[ABA65]
ABA1-ABA92	[ABA1]-TTTTTTTTTTTTTTTTTTTTTTTTT-[ABA92]
ABA1-ABA109	[ABA1]-TTTTTTTTTTTTTTTTTTTTTTTTT-[ABA109]
Control sequences	
SC	GGGTTTGCGAAAGGGAAAGGGTTTGTGTTTTTTGGGTTTT
ABA1-SC	[ABA1]-TTTTTTTTTTTTTTTTTTTTTTTTT-[SC]
SC-ABA65	[SC]-TTTTTTTTTTTTTTTTTTTTTTTTT-[ABA65]
ABA1-25T	[ABA1]-TTTTTTTTTTTTTTTTTTTTTTTTT
25T-ABA65	TTTTTTTTTTTTTTTTTTTTTTTTT-[ABA65]

References

1. M. Cho, S. S. Oh, J. Nie, R. Stewart, M. S. Eisenstein, J. Chambers, J. D. Marth, F. Walker, J. A. Thomson, H. T. Soh, *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 18460-18465.