Supporting Information

Selection Strategy to Generate Aptamer Pairs that Bind to Distinct Sites on Protein Targets

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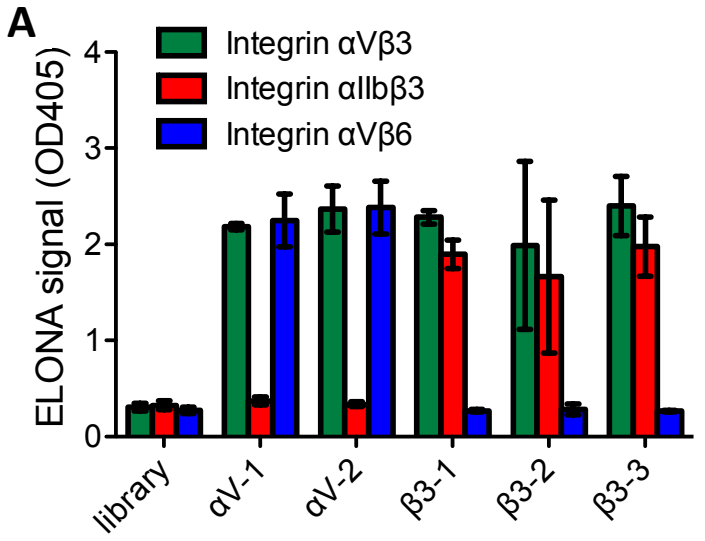
	Copy #	From αV pool
αV-1	4	CACACATTCCCGTCCTCGATACGTCTAGGCTTAGTGCCACTTGCTTAATC
αV-2	2	CACACATTCCCGTCCTCGATAAGTCTAGGCTTAGTGCCACTTACTTAATC
αVβ3-1	15	CACACATTCCCGTCCTCGATAAGTCTAGGCTTAGTGCCACTTGCTTAATC
	1	CACACATCCCCGTCCTCGATAAGTCTAGGCTTAGTGCCACTTACTTAATC

	Copy #	From β3 pool
β3-1	11	CCCAGATTACTGTGGAGTGGTTGTCTGCGAATCCTTCGTCCACCCAATAG
β3-2	1	CCCAGATTACTGTGGAGTGGTTGTCTGCGAATCCTTCGTCCACCCAATAT
β3-3	1	CCCAGATTACTGTGGAGTGGTTGTCTGCGAATCCTTCGTCCACCCTATAG
αVβ3-1	2	CACACATTCCCGTCCTCGATAAGTCTAGGCTTAGTGCCACTTGCTTAATC
	1	GCCAGATTACTGTGGAGTGGTTGTCTGCGAATCCTTGGTCCACCCAATAG
	1	GACGCTTTCACCATATAATAATGAGACCTATTCAGTGCGATTTCGTGCCG

Table S1. Sequencing results for the αV and β3 pools. We selected six representative sequences from the αV and β3 pools for affinity and specificity characterization. The sequences shown above are from 50N random region, which are flanked by the PCR primer sites (see Experimental Section for sequences)


A
Integrin αVβ3
Integrin αIIbβ3
Integrin αVβ6
ELONA signal (OD405)
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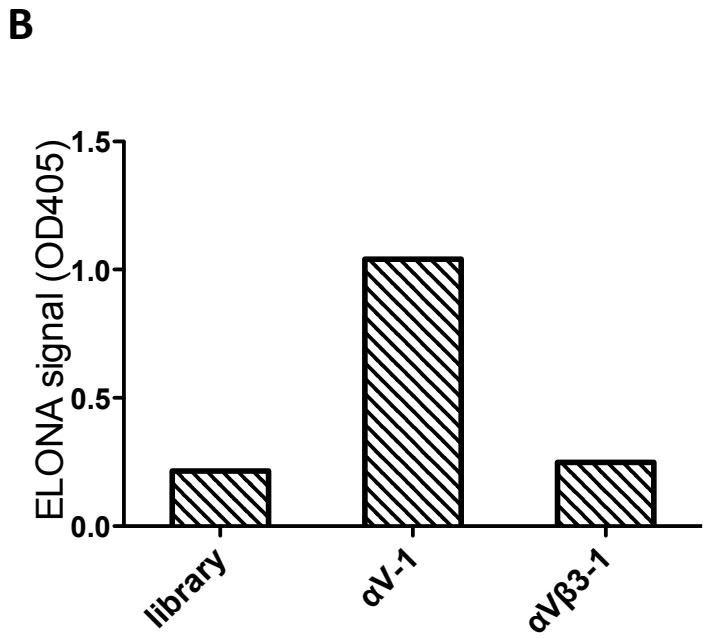
1
2
3
4
library
αV-1
αV-2
β3-1
β3-2
β3-3


B
ELONA signal (OD405)
0.0
0.5
1.0
1.5
library
αV-1
αVβ3-1

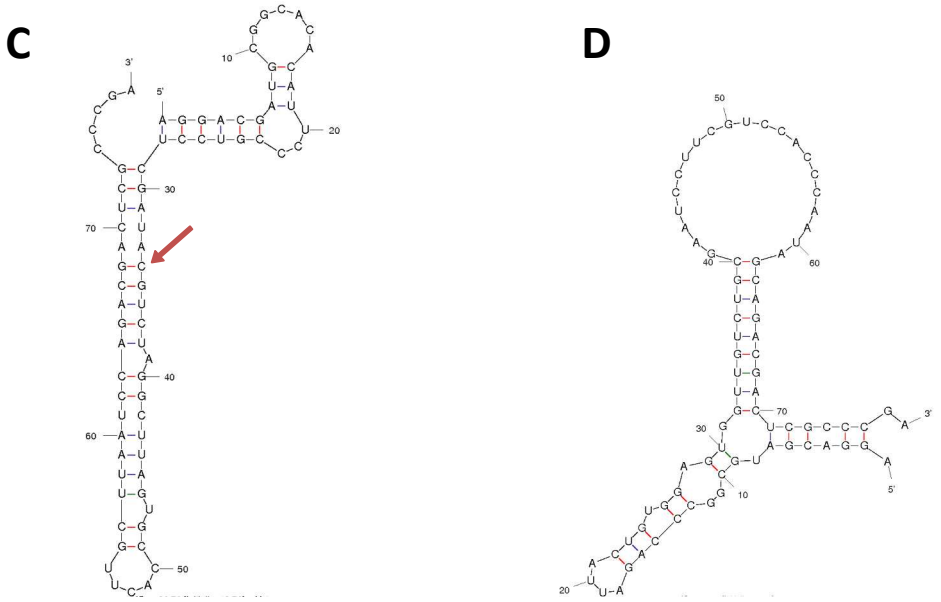


Fig. S1. Characterization of aptamers from αV and β3 pools. (A) We performed ELONA with individual aptamers to characterize their affinity for different integrin proteins. Both αV-1 and αV-2 showed only background levels of binding to integrin αIIbβ3, but significant binding to integrin αVβ3 and αVβ6; On the other hand, all the aptamer sequences from the β3 pool showed significant binding to integrin αVβ3 and αIIbβ6, but not integrin αVβ6. This result indicated that, as expected from MAI-SELEX design, aptamers from the αV pool selectively bind the αV subunit while aptamers from the β3 pool specifically bind the β3 subunit. (B) The αVβ3-1 sequence, which appeared in both αV and β3 pools, exhibits negligible binding to integrin αVβ3. We suspect that the sequence may originate from biases during synthesis or selection. (C) & (D) Model of secondary structure of αV-1 and β3-1 aptamers obtained using the mfold[1] software. The arrow in (C) indicates the single base difference between αV-1 and αVβ3-1, and is responsible for the dramatically different binding properties between the two aptamers. Such large differences binding properties arising from single base differences have been previously reported in literature.[2]

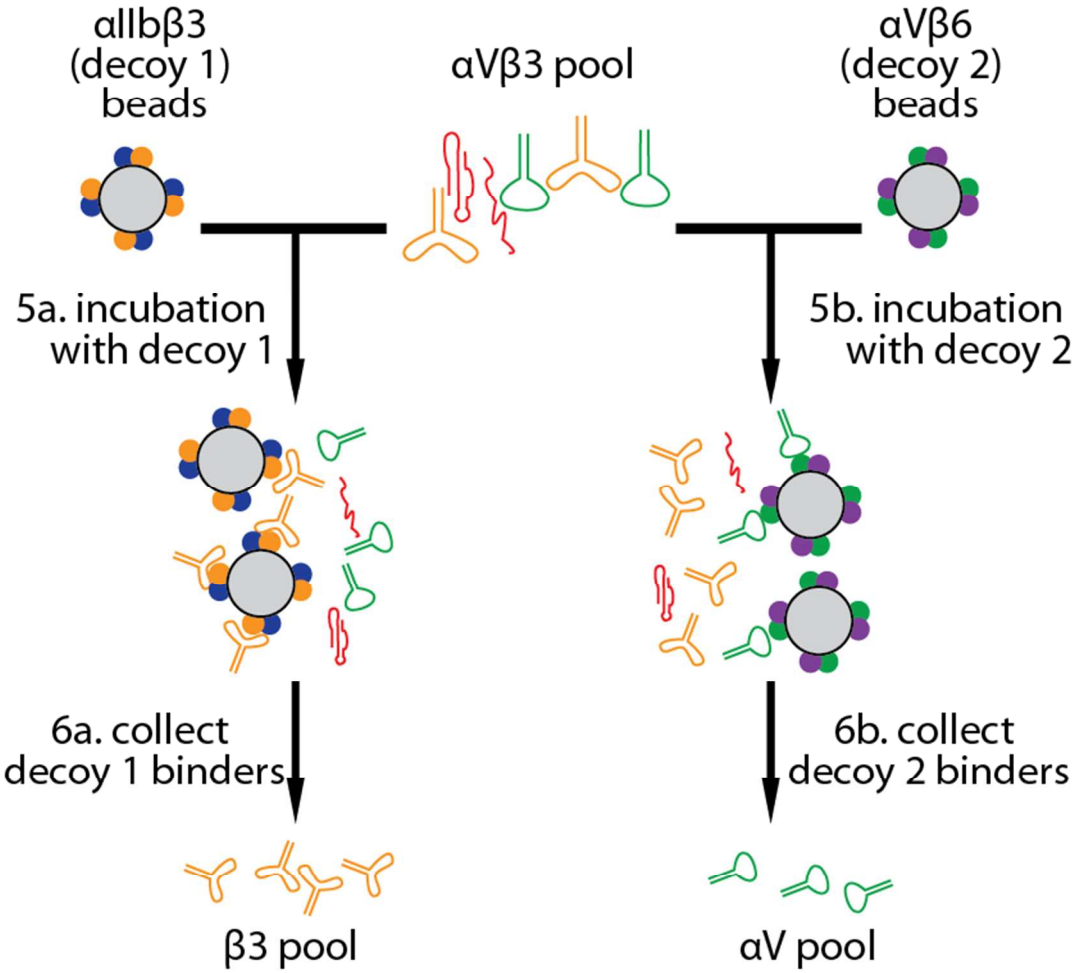


Fig. S2. Alternative MAI-SELEX specificity module scheme. Availability of two decoys would further improve the efficiency of the specificity module. For example, the αVβ3 pool could be incubated with an αV-containing homolog, integrin αVβ6 (step 5b), which will capture αV-binding aptamers but not β3-binding aptamers. Eluting the sequences that bind to integrin αVβ6 will lead to enhanced isolation of the αV pool (step 6b). The β3 pool can be isolated similarly, using integrin αIIbβ3 as the decoy (step 5a, 6a).

Reference:

(1) Zuker, M. *Nucleic Acids Res* **2003**, *31*, 3406.

(2) Katilius, E.; Flores, C.; Woodbury, N. W. *Nucleic Acids Res* **2007**, *35*, 7626.