

Supporting Information for: Side Chain Requirements for Affinity and Specificity in D5, an HIV-1 Antibody Derived from the V_H1-69 Germline Segment

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Sequence comparison of D5 and CR6261 V_L and V_H domains.

Light chain

D5-VL	diqmtqspst	lsasigdrvt	itcrasegiy	hwlawyqqkp	gkapklliyk	asslasgaps
CR6261-VL	qsvltqppsv	saapgqkvti	scsgsssnig	ndyvswyqql	pgtapklliy	dnnkrpsgip

D5-VL	rfsgsgsgtd	ftltisslqp	ddfatyyccq	ysnypltfgg	gtkleikrtv
CR6261-VL	drfsgsksgt	satlgitglq	tgdeanyyca	twdrprtayv	vfgggtkltv

Heavy chaina (CDR regions shown in gray)

				HCDR1		HCDR2
D5-VH	qvqlvqsgae	vrkpgasvk	sckasgdtfs	syaiswvrqa	pgqglewmgg	iipifgtany
CR6261-VH	evqlvesgae	vkkpgssvk	sckasggpfr	syaiswvrqa	pgqgpewm	iipifgttky

HCDR3

D5-VH	aqafqgrvti	taneststay	melsslrse	taiyycardn	ptllgsdywg	agtlvtvssa
CR6261-VH	apkfqgrvti	taddfagtvy	melsslrse	tamyycahkm	gyqvretmdv	wgkgttvtvs

Table S2 – Structures Used for Design of D5-Lib-II

Antibody	PDB ID (ref.)	Target
D5	2CMR (S1)	HIV-1 gp41 (5-Helix)
412D	2QAD (S2)	HIV-1 gp120
M75	2HKF (S3)	Human carbonic anhydrase IX
Ru5	1FE8 (S4)	Von Willebrand factor A3 domain
36-65	2A6D (S5)	Arsonate / peptide mimic
Unnamed	2ZJS (S6)	SecYE
Aqc2	1MHP (S7)	VLA1 I-domain
Unnamed	2B2X (S8)	VLA1 I-domain
23c3	3CXD (S9)	Osteopontin / peptide mimic
80r	2GHW (S10)	SARS S1 receptor binding domain
X5	2B4C (S11)	HIV-1 gp120
GC1008	3EO1 (S12)	Transforming growth factor β
4E10	2FX7 (S13)	HIV-1 gp41
17b	1GC1 (S14)	HIV-1 gp120
Unnamed	3G6J (S15)	C3b
R3Mab	3GRW (S16)	FGF receptor 3
Unnamed	2QQN (S17)	Neuropilin 1/2
E2	3BN9 (S18)	MT-SP1/matriptase

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Design and characterization of 6-Helix-Fd

Amino acid sequence of 6-Helix-Fd

|-----CHR-----|---Link---|-----NHR-----
MWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLGGKGGSSGIVQQQNNLLRAIEAQOHLQLTVWGIKQLQARI

--|---Link---|-----Fd-----|
LGTGGSGGYIPEAPRDGQAYVRKDGWVLLSTFLGENLYFQSHHHHHH

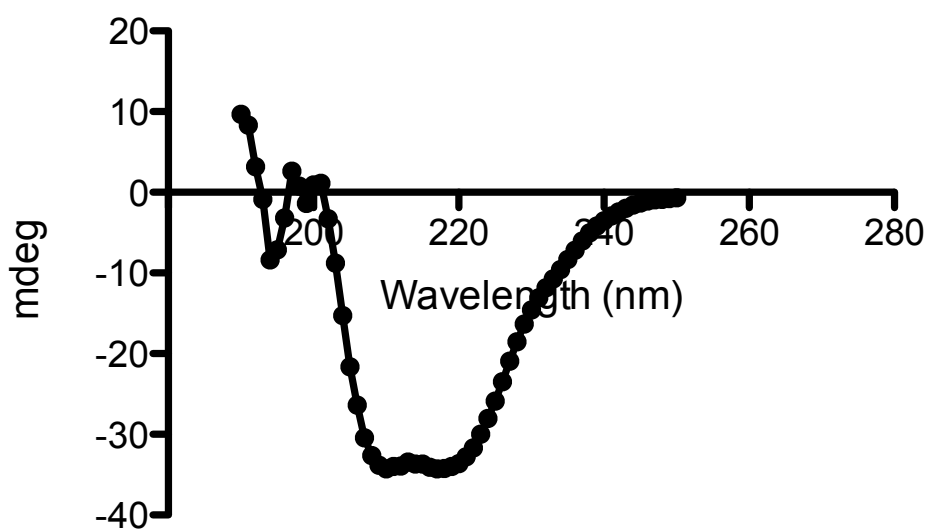


Figure S1 – Circular dichroism spectrum of 6-Helix-Fd.

Competitive ELISAs with 5-Helix and 6-Helix-Fd.

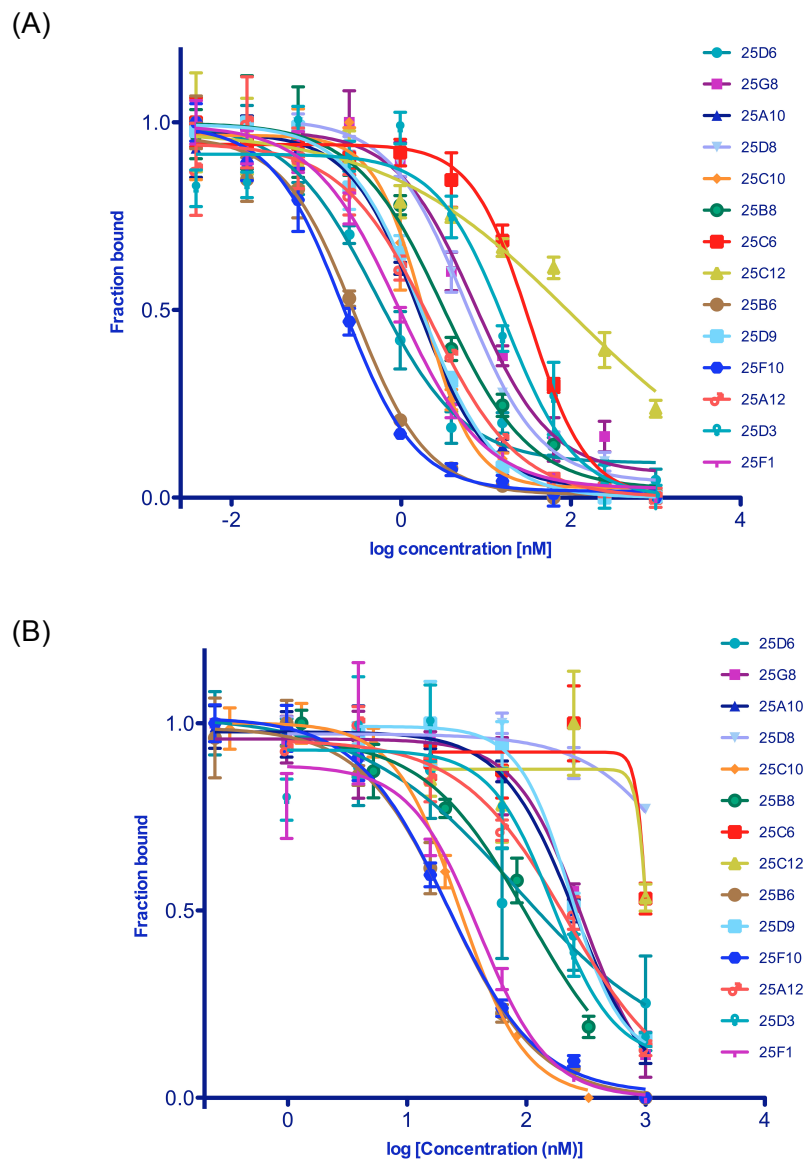


Figure S2 – Competitive ELISA for bivalent phage-displayed scFv fragments. Binding to immobilized 5-Helix was competed with free 5-Helix (A) or 6-Helix-Fd (B).

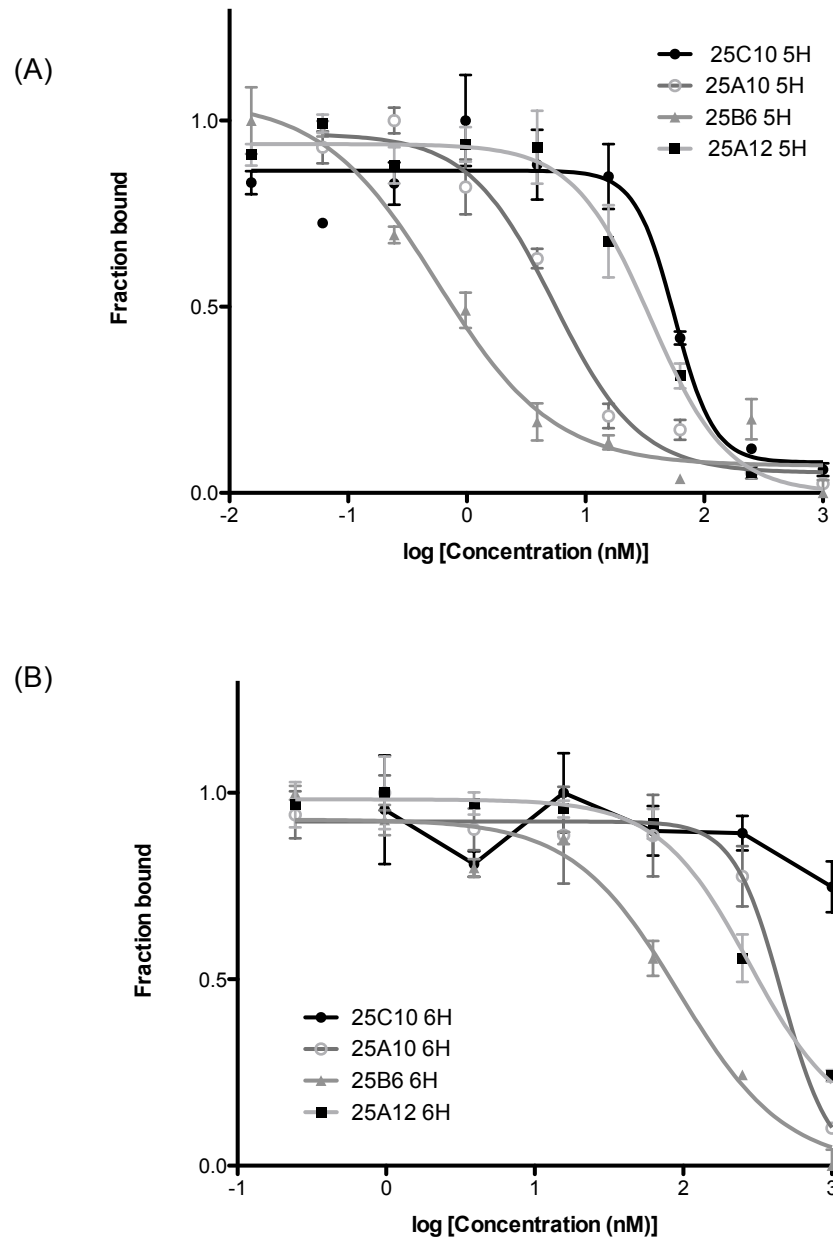


Figure S3 – Competitive ELISA for purified scFv fragments. Binding to immobilized 5-Helix was competed with free 5-Helix (A) or 6-Helix-Fd (B).