Supplemental Material

STRUCTURAL AND THERMODYNAMIC BASIS OF EPITOPE BINDING BY NEUTRALIZING AND NON-NEUTRALIZING FORMS OF THE ANTI-HIV-1 ANTIBODY 4E10

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Running Title: Neutralizing vs. non-neutralizing anti-HIV antibodies

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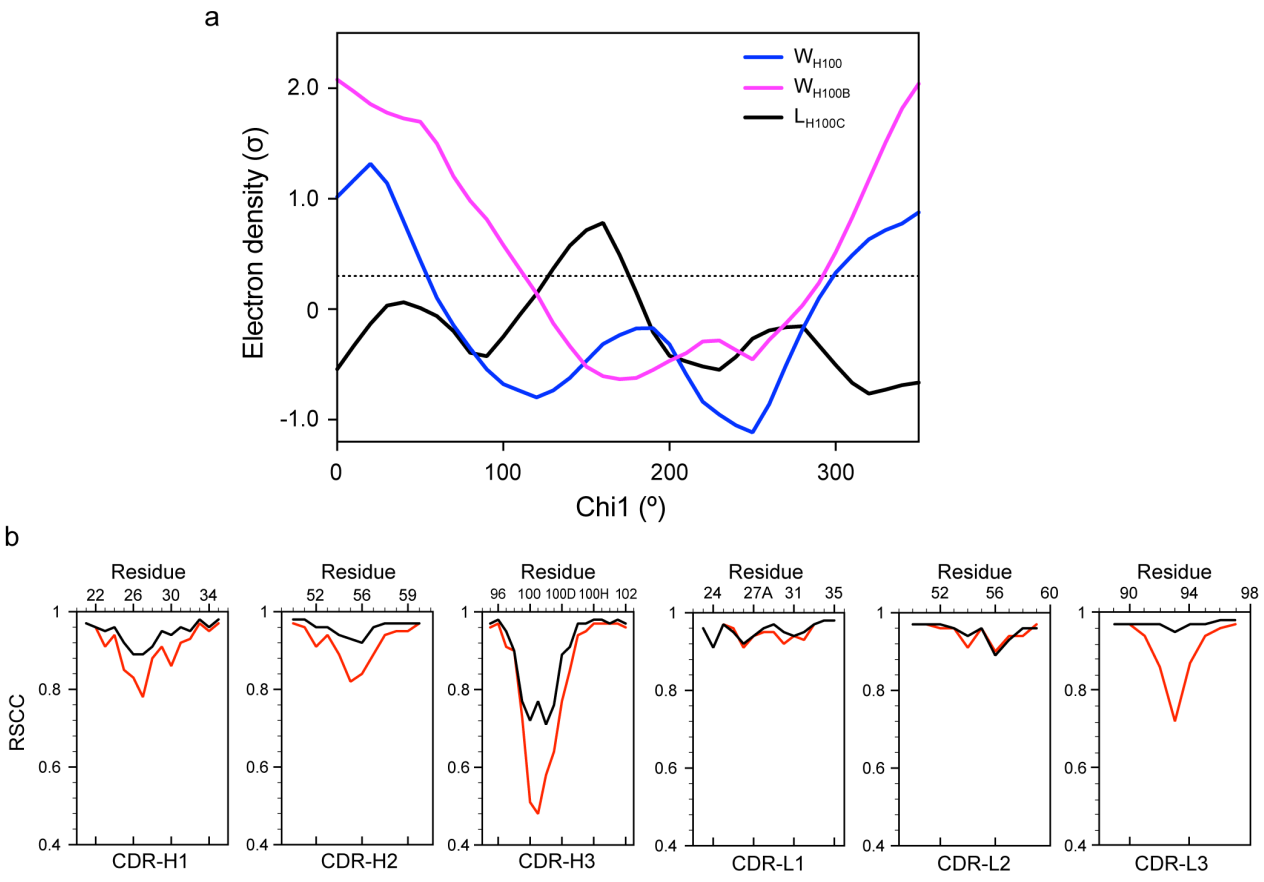

FIG S1. Analysis of residues of the apex region of CDR-H3. (A) Rotamer plot of residues of the CDR-H3 apex of the ligand-free form of 4E10 Fab calculated with RINGER. Values of Chi1 angle for residues W_{H100} (blue), W_{H100B} (magenta) and L_{H100C} (black) of chain H are shown. The dotted line corresponds to the electron density cut-off (σ = 0.3). **(B)** Comparison of RSCC between unbound Fab (red lines, also shown in Figure 5c) and the arithmetic average RSCC of six crystal structures of 4E10 Fab with various types of peptide bound (black lines). The PDB entry codes of the crystal structures employed to prepare these plots were 1TZG (short peptide epitope), 2FX7 (4E10ep), 2FX8 (peptide constrained with a α-amino isobutyric acid), 2FX9 (peptide with a thioether intramolecular bond), 4NHC (stapled peptide), and 4NGH (stapled peptide with a phosphate tether).

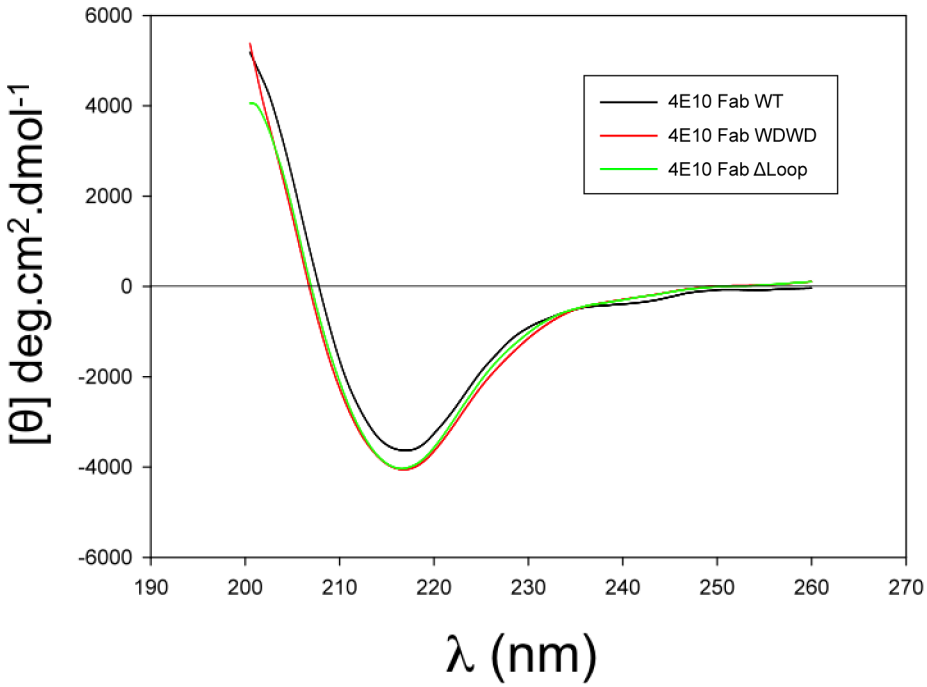

FIG S2. Secondary structure. CD spectra of WT (black), WDWD (red) and ΔLoop (green) in the unbound form. Measurements were done in PBS at 25 ºC. Fab concentration was 30 μM in all samples.

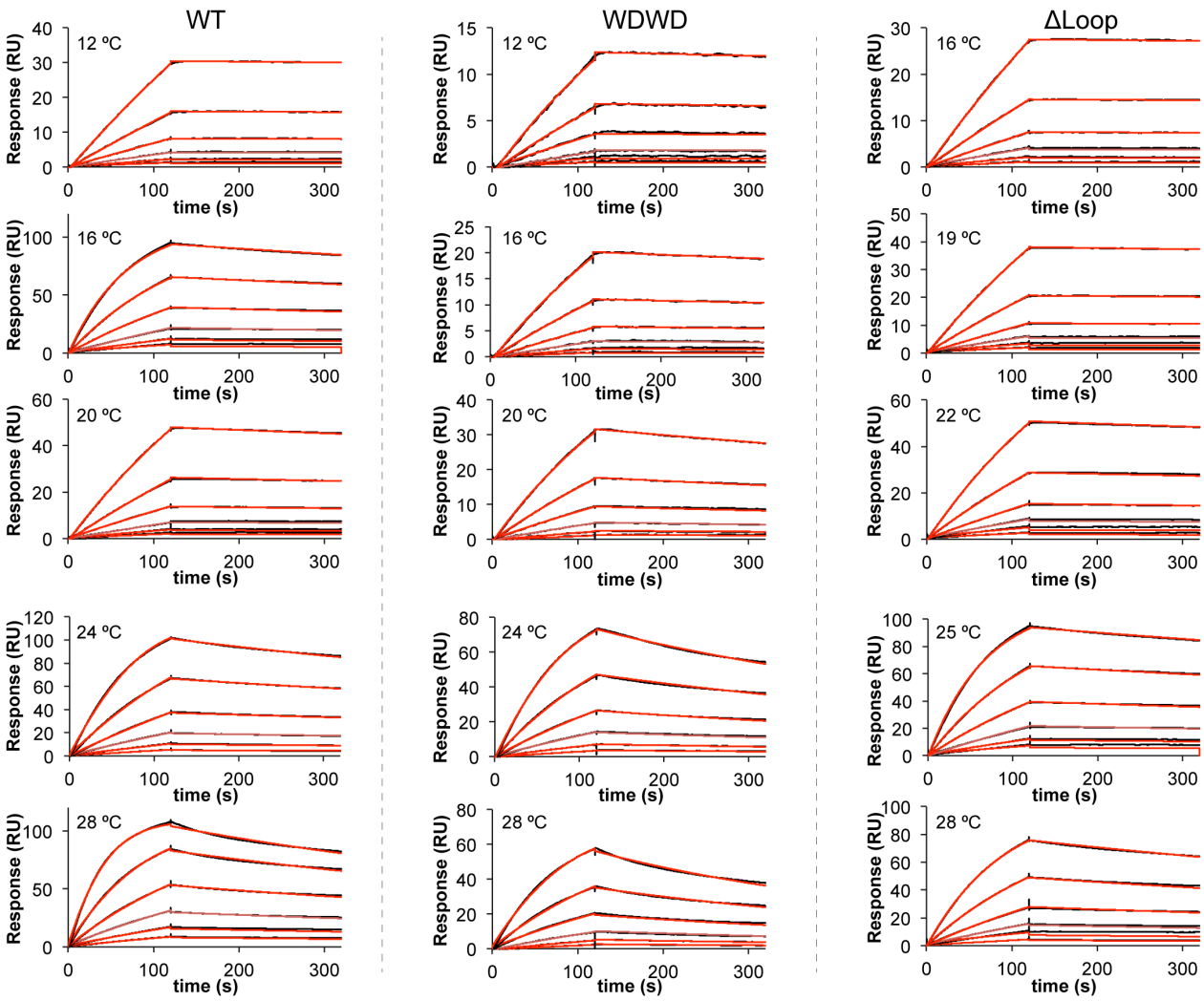

FIG S3. Temperature dependence of binding of peptide to Fabs determined by SPR. Data correspond to WT Fab (left), WDWD (center) and ΔLoop (right). Temperatures are indicated in each panel. Experimental data (black) and best fit (red) are shown. In these experiments the peptide is flowed into a chip decorated with Fab. Prior to the injection of peptide, the Fab was captured by an anti-Fab antibody immobilized on a CM5 chip.

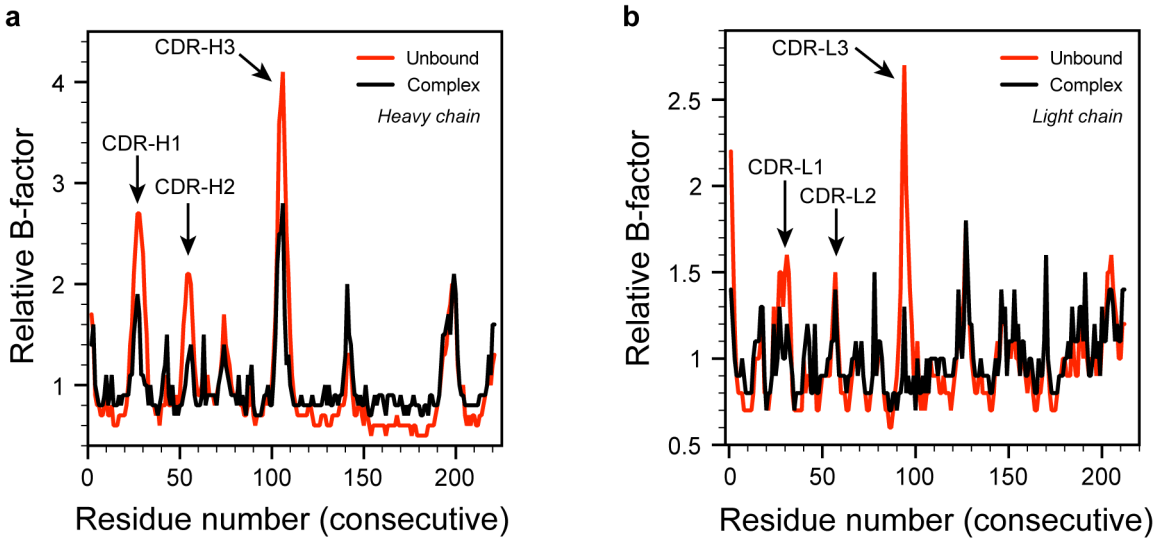

Fig. S4. Crystallographic B-factors of Fab in the bound and unbound state. (A) Heavy chain. **(B)** Light-chain. Relative B-factors are plotted to facilitate the comparison between crystal structures at different resolutions and quality. The relative B-factors were obtained by normalization with respect to the average B-factor of each structure.

Table S1. Hydrogen bonds between 4E10 and 4E10ep.

		WT	WDWD	ΔLoop
Residue1	**Residue 2**	**Distance (Å)**		
H-Ile56	Pep-Trp672	3.21	3.23	3.32
H-Glu95	Pep-Thr676	2.50	2.64	2.61
H-Leu100C[a]	Pep-Trp680	3.30	3.31	2.73
L-Tyr91	Pep-Asn671	2.93	2.93	2.97
L-Ser94	Pep-Trp672	2.91	2.91	2.96
L-Ser94	Pep-Asn671	3.30	3.33	3.39
L-Lys32	Pep-Asp674	2.80	2.81	2.77

[a]In ΔLoop this residue corresponds to Gly100A.

Table S2. Interaction surface between the light chain of 4E10 Fabs and 4E10ep.

	WT	WDWD	ΔLoop
Residue	**Buried surface area (Å^2)**		
L-Asn30	9.2	11.9	11.4
L-Lys32	28.6	29.8	27.7
L-Gln89	3.8	3.7	4.0
L-Tyr91	38.2	38.6	38.9
L-Gly92	13.5	13.2	14.1
L-Gln93	12.5	14.8	14.7
L-Ser94	58.5	57.4	56.7
L-Ser96	12.5	12.4	12.8
Subtotal	177	182	180
Pep-Asn671	62.9	64.4	60.9
Pep-Trp672	22.3	22.3	23.4
Pep-Phe673	80.1	79.5	79.5
Pep-Asp674	32.9	34.1	34.1
Subtotal	198	200	201
TOTAL	375	382	381

Table S3. Interaction surface between the heavy chain of 4E10 Fabs and 4E10ep.

	WT	WDWD	ΔLoop
Residue	**Buried surface area (Å^2)**		
H-Ser28	0	8.0	0
H-Thr31	46.9	51.3	44.6
H-Tyr32	6.2	7.1	2.9
H-Ala33	25.5	24.2	25.7
H-Leu34	0	0.2	0.2
H-Ser35	7.5	7.8	7.6
H-Trp47	17.9	18.6	17.3
H-Gly50	6.7	7.2	7.3
H-Val51	1.8	2.2	2.0
H-Ile52	56.9	59.1	56.9
H-Leu53	35.4	9.0	34.9
H-Leu54	30.0	34.5	32.2
H-Ile56	26.4	27.9	27.2
H-Thr57	0.4	0.7	0.5
H-Asn58	53.7	53.7	52.7
H-Glu95	13.6	13.2	13.1
H-Leu100C[a]	12.5	12.2	23.6
H-Gly100D	7.4	7.4	6.7
H-Lys100E	59.4	61.0	56.9
H-Pro100F	55.4	55.8	57.8
H-Gly100H	27.7	27.8	28.0
H-Phe100J	13.1	12.8	13.7
Subtotal	504	501	512
Pep-Trp672	160.1	160.9	161.1
Pep-Phe673	78.3	79.9	79.3
Pep-Ile675	38.7	35.5	41.8
Pep-Thr676	97.5	94.7	94.9
Pep-Asn677	44.8	48.9	45.3
Pep-Trp678	9.4	0	11.9
Pep-Leu679	54.7	66.3	56.7
Pep-Trp680	89.0	85.8	95.9
Pep-Lys683	35.8	31.0	31.5
Subtotal	608	603	618
Total	1,113	1,104	1,130

[a]In ΔLoop this residue corresponds to Gly100A.