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Supplemental information

Focal accumulation of aromaticity at the CDRH3 loop mitigates 4E10 polyreactivity without altering its HIV neutralization profile

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Supplemental Information

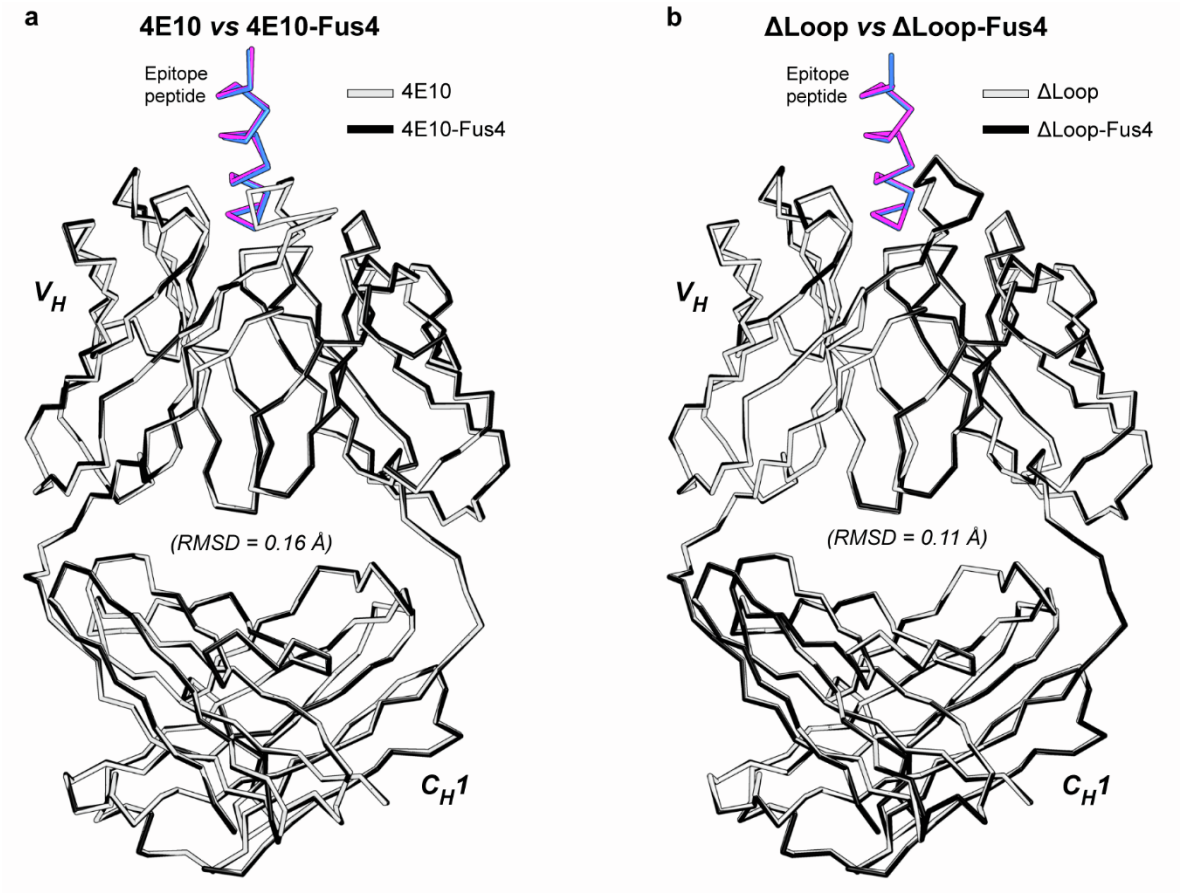

Figure S1: Crystal structures of complexes of variants modified with Fus4. (a) Superposition of the coordinates of the crystal structure of 4E10 (light gray) and 4E10 modified with Fus4 (black). The epitope peptide bound to the unmodified (blue) and to the modified antibody (magenta) is also shown. A root-mean-square-deviation (RMSD) value of 0.16 Å was achieved using the entire structure. **(b)** Superposition of the coordinates of the crystal structure of ΔLoop (light gray) and ΔLoop modified with Fus4 (black). The epitope peptide bound to the unmodified (blue) and modified (magenta) antibody is also depicted. A RMSD value of 0.11 Å was achieved.

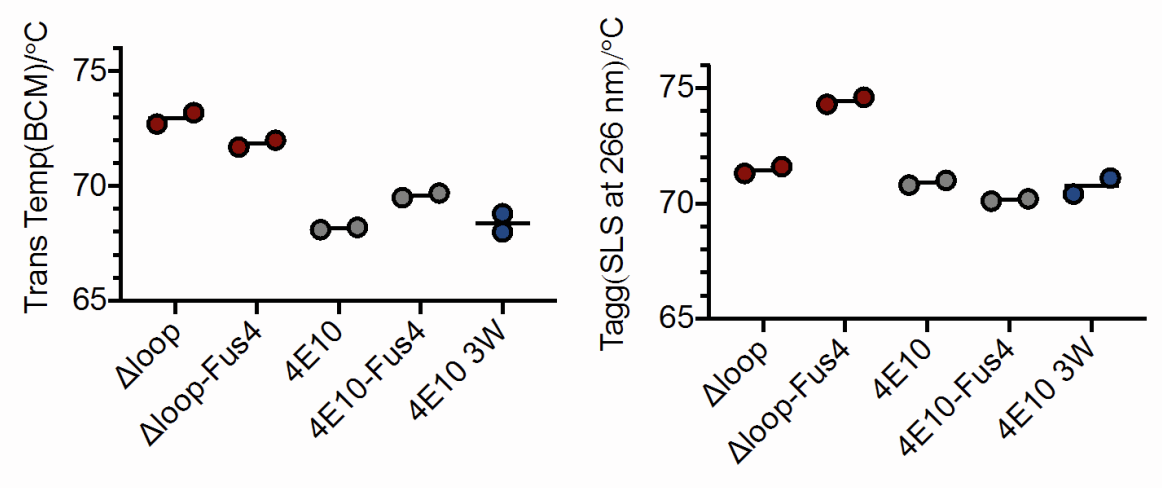

Figure S2: Thermal stability comparison of the modified Fabs. Melting (T_m, left panel) and aggregation temperature (T_{agg}, right panel) of 4E10 3W Fab (blue), the modified and unmodified ΔLoop Fabs (dark red) and the modified and unmodified 4E10 Fabs (grey).

Table S1: Data collection and refinement statistics.

Statistical values given in parenthesis refer to the highest resolution bin.

Data Collection	**4E10-Fus4**	**ΔLoop-Fus4**
Beamline	XALOC (ALMA)	XALOC (ALMA)
Space Group	C 2	C 2
Unit cell		
a, b, c (Å)	158.11, 44.89, 86.04	157.98, 44.80, 85.95
α, β, γ (°)	90, 113.7, 90.0	90, 113.6, 90.0
Resolution (Å)	34.4 – 1.45 (1.53 – 1.45)	34.4 – 1.70 (1.79 – 1.70)
Wavelength	0.97953	0.97953
Observations	537,252 (48,151)	140,231 (12,250)
Unique reflections	96,390 (12,753)	57,219 (6,629)
$R_{merge.}$	0.089 (0.775)	0.095 (0.345)
$CC_{1/2}$	0.996 (0.454)	0.988 (0.770)
$I / \sigma (I)$	11.2 (3.0)	6.8 (2.3)
Multiplicity	5.6 (3.8)	2.5 (1.8)
Completeness (%)	98.1 (89.9)	94.2 (75.1)
Refinement Statistics		
Resolution (Å)	34.4 - 1.45	34.4 - 1.70
R_{work} / R_{free} (%)	12.0 / 15.1	15.8 / 19.1
No. complexes	1	1
No. atoms		
Protein	3,301	3,320
Peptide	147	143
Other	40	31
Water	480	504
B-factor (Å^2)		
Protein	20.3	17.2
Peptide	42.3	23.1
Others	26.0	26.8
Water	32.1	28.4
Ramachandran Plot		
Preferred (%)	92.5	90.9
Allowed (%)	7.3	8.9
Outliers (%)	0.3	0.3
RMSD Bond (Å)	0.013	0.012
RMSD Angle (°)	1.64	1.62
PDB entry code	7EKB	7EKK