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Supplemental information

Structural dynamics reveal isolate-specific differences at neutralization epitopes on HIV Env

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Supplemental Figures.

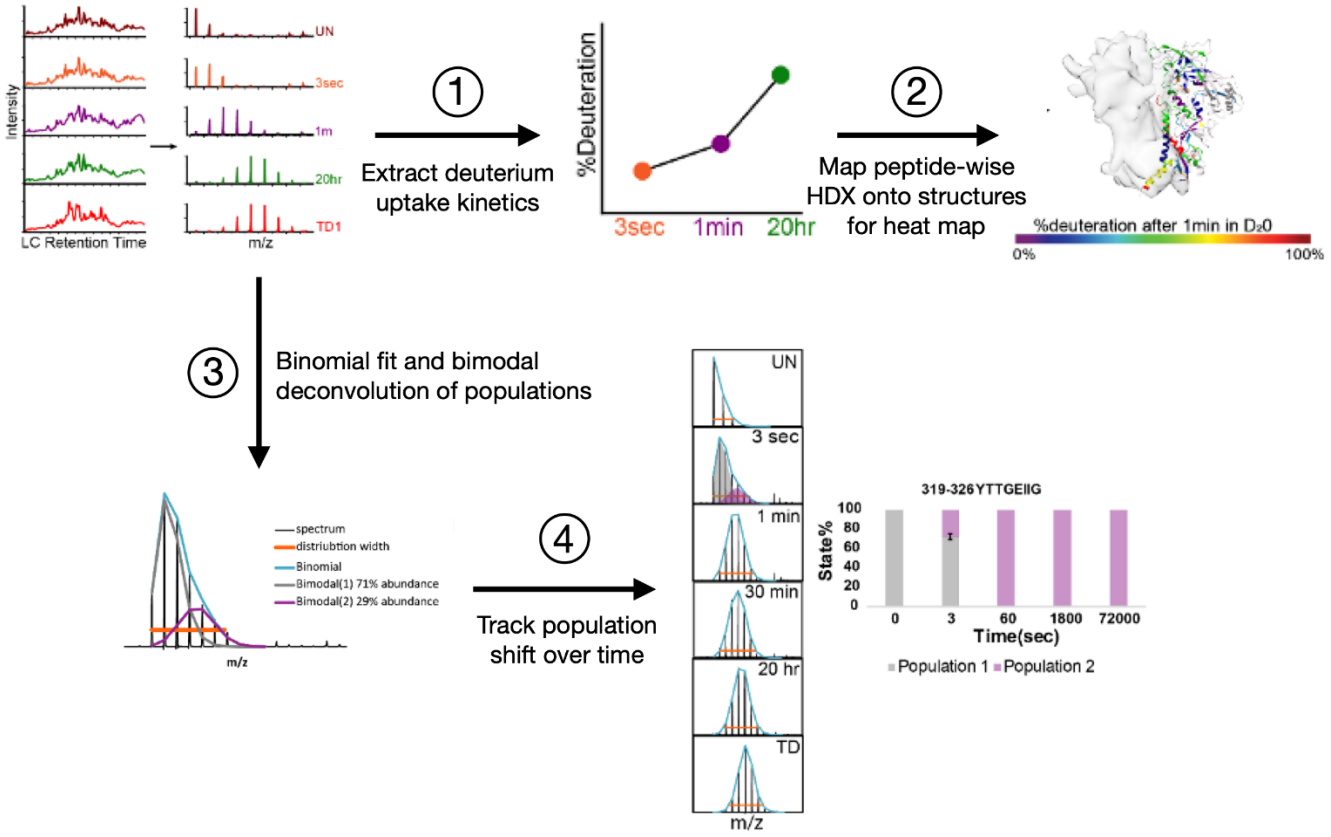


Figure S1. HDX analysis workflow, related to Figures 1-7

Initially after data collection each peptide's m/z, charge state, unique retention time and drift time are uploaded into HDExaminer, which extracts each peptide's mass spectra at each time point (1) and plots the % deuteration with time based on centroid fitting. The percent deuteration of each peptide at the 1minute timepoint is then color coded and mapped onto the structure of a SOSIP (2) to generate an HDX heatmap for each individual isolate. Next the HDExaminer centroid fit data is exported into HX-Expressv2 for binomial fitting (3). The analysis in HX-Express takes into account the mass envelope distribution width. Anything unusually broad is flagged as bimodal, and bimodal deconvolution allows for the identification of EX1 kinetics that are indicative of conformational switching. The percent abundance of the two populations (colored gray and purple respectively) across time are then converted into a bar plot (4) to determine the rate of the conformational transition from the protected to the exposed populations.

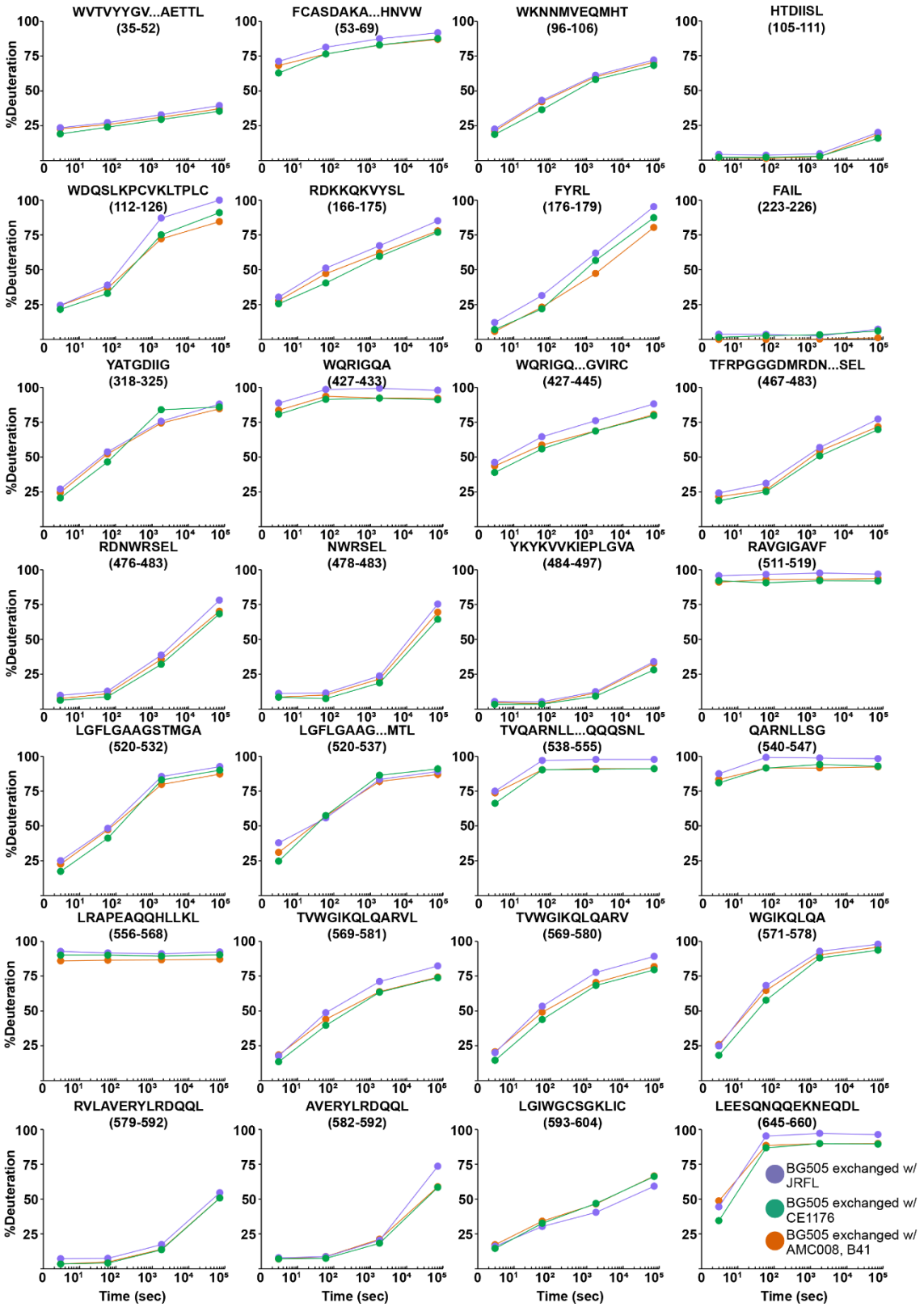
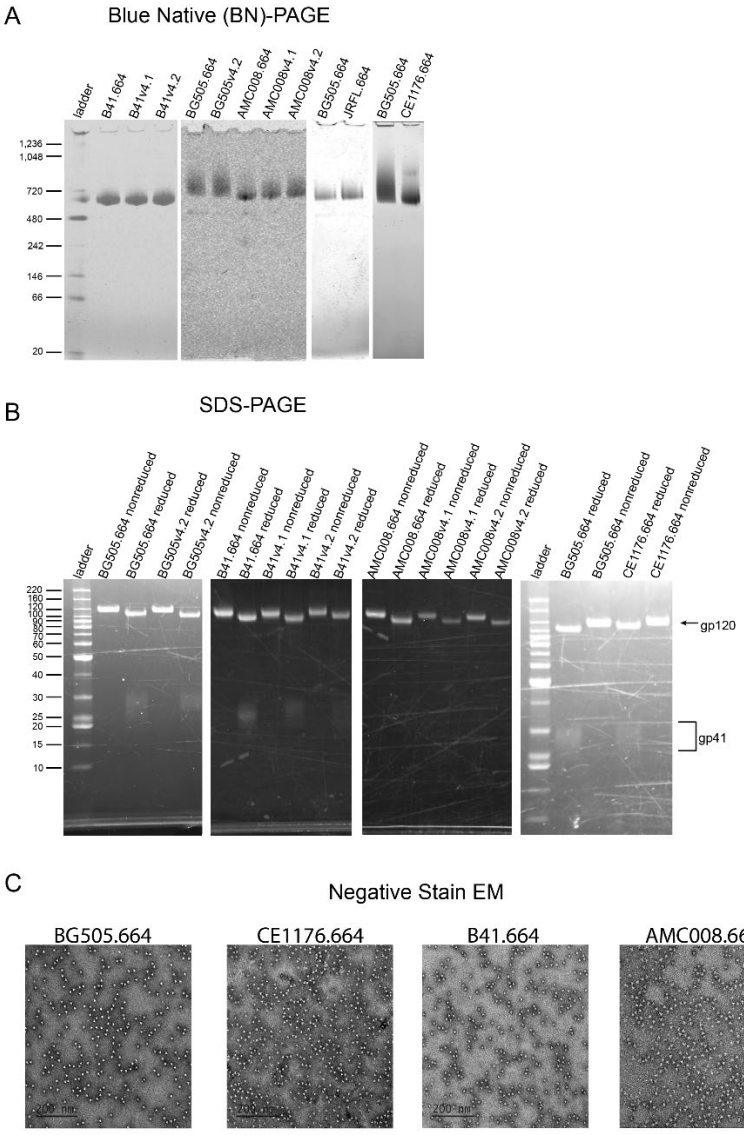

WVTVYYGV...AETTL
(35-52)
FCASDAKA...HNVW
(53-69)
WKNNMVEQMHT
(96-106)
HTDIISL
(105-111)
WDQSLKPCVKLTPLC
(112-126)
RDKKQKVYSL
(166-175)
FYRL
(176-179)
FAIL
(223-226)
YATGDIIG
(318-325)
WQRIGQA
(427-433)
WQRIGQ...GVIRC
(427-445)
TFRPGGGDMRDN...SEL
(467-483)
RDNWRSEL
(476-483)
NWRSEL
(478-483)
YKYKVVKIEPLGVA
(484-497)
RAVGIGAVF
(511-519)
LGFLGAAGSTMGA
(520-532)
LGFLGAAG...MTL
(520-537)
TVQARNLL...QQQSNL
(538-555)
QARNLLSG
(540-547)
LRAPEAQQHLLKL
(556-568)
TVWGIKQLQARVL
(569-581)
TVWGIKQLQARV
(569-580)
WGIKQLQA
(571-578)
RVLAVERYLRDQQL
(579-592)
AVERYLRDQQL
(582-592)
LGIWGCSGKLIC
(593-604)
LEESQNQQEKNEQDL
(645-660)
%Deuteration
Time (sec)
BG505 exchanged w/ JRFL
BG505 exchanged w/ CE1176
BG505 exchanged w/ AMC008, B41

Figure S2. BG505 HDX reference controls, related to Figures 3-4

Percent deuteration of commonly found homologous peptides across different isolates from BG505 biological replicates that were exchanged alongside other isolates as an HDX control standard. Purple uptake curves represent BG505 exchanged alongside JRFL, green was BG505 exchanged in tandem with CE1176, and orange indicates BG505 exchanged in tandem with AMC008 and B41 constructs. Each time point is the average of duplicate measurements with error bars displaying standard deviation.



SOSIP	Avg radius (nm)	Radius std dev	Avg %polydispersity	Avg %abundance
BG505.664	6.7	0.10	9.0	100
BG505v4.2	6.9	0.11	11.4	100
B41.664	6.8	0.21	12.9	100
B41v4.1	7.0	0.12	14.5	100
B41v4.2	6.8	0.1	15.8	100
AMC008.664	7.0	0.1	10.6	100
AMC008v4.1	7.0	0	13.7	100
AMC008v4.2	7.0	0	16.6	100
JRFL.664	7.0	0.06	12.9	100
CE1176.664	7.0	0.07	13.1	100

Figure S3. SOSIP characterization by blue native (BN)-PAGE, SDS-PAGE, and dynamic light scattering (DLS), related to STAR Methods.

To assess trimer purity each SOSIP was characterized by BN-PAGE (A), SDS-PAGE (B), negative stain EM (C), and DLS (D) after purification and prior to HDX and BLI experiments. On the SDS-PAGE each sample was run non-reduced and reduced via addition of DTT. The single band in the non-reduced lane runs at a slightly higher MW due to the presence of an additional inter-subunit disulfide bond linking gp120 and gp41. The weakly visible gp41 bands in the reduced lane are smeared likely due to glycan heterogeneity.

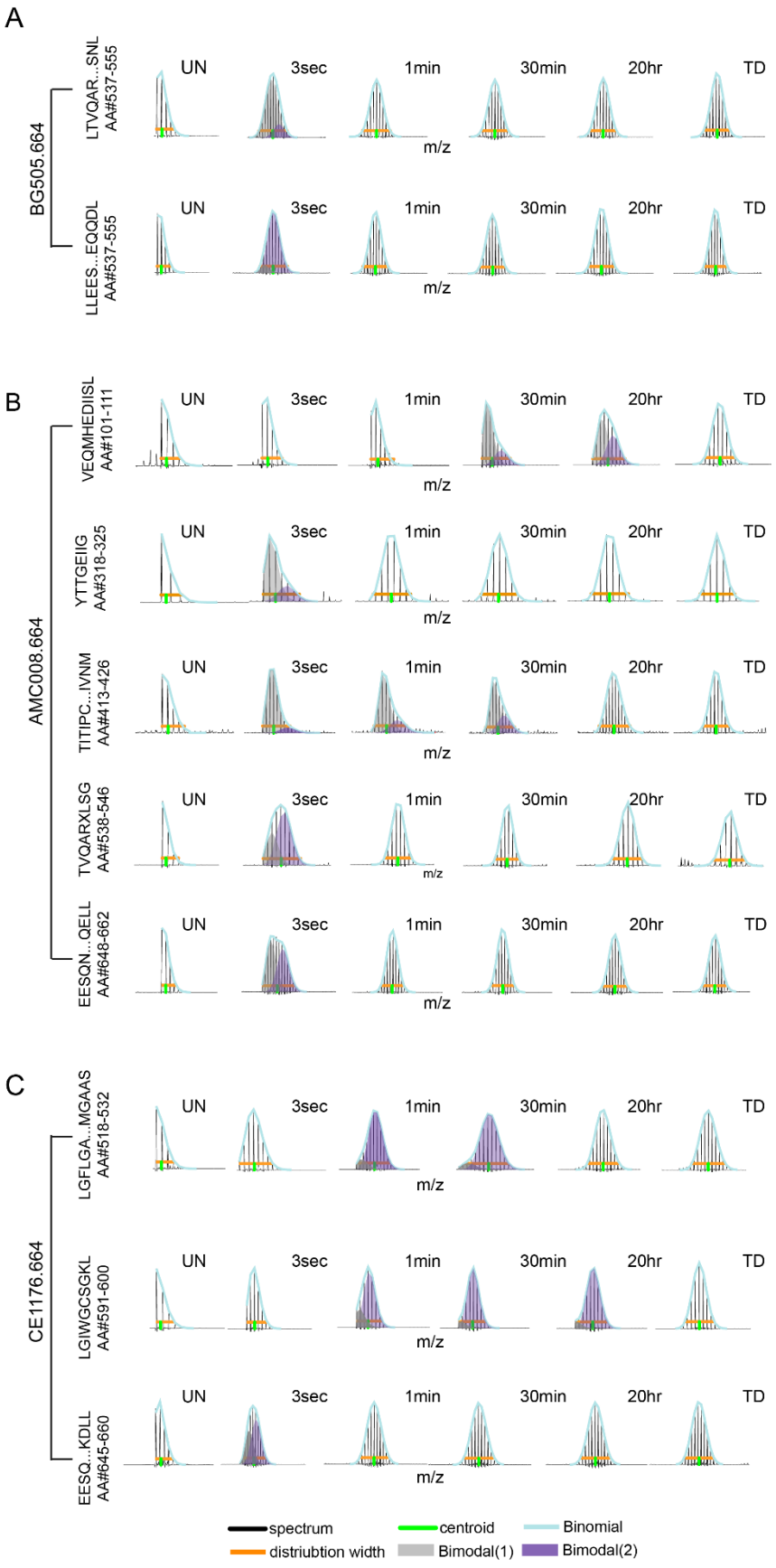

A
BG505.664
LTVQAR...SNL
AA#537-555
LLEES...EQQDL
AA#537-555
UN
3sec
1min
30min
20hr
TD
m/z
B
AMC008.664
VEQMHEDIISL
AA#101-111
YTTGEIIG
AA#318-325
TITIPC...IVNM
AA#413-426
TVQARXLSG
AA#538-546
EESQN...QELL
AA#648-662
C
CE1176.664
LGFLGA...MGAAS
AA#518-532
LGIWGCSGKL
AA#591-600
EESQ...KDLL
AA#645-660
spectrum
centroid
Binomial
distriubtion width
Bimodal(1)
Bimodal(2)

Figure S4. Bimodal spectra suggestive of conformational switching across strains, related to Figure 6.

Two peptides in BG505.664, five peptides in AMC008.664, and three peptides in CE1176.664 exhibited unusually broad isotopic distributions that were binomially fit to two populations (Figures A-C respectively). Each panel displays the spectra from each respective time point. The lighter in mass/more protected population is shaded in gray, and the heavier in mass/faster exchanging population is shaded in purple. The legend describing the centroid, peak envelope, and binomial fit are displayed in the bottom center of the Figure.

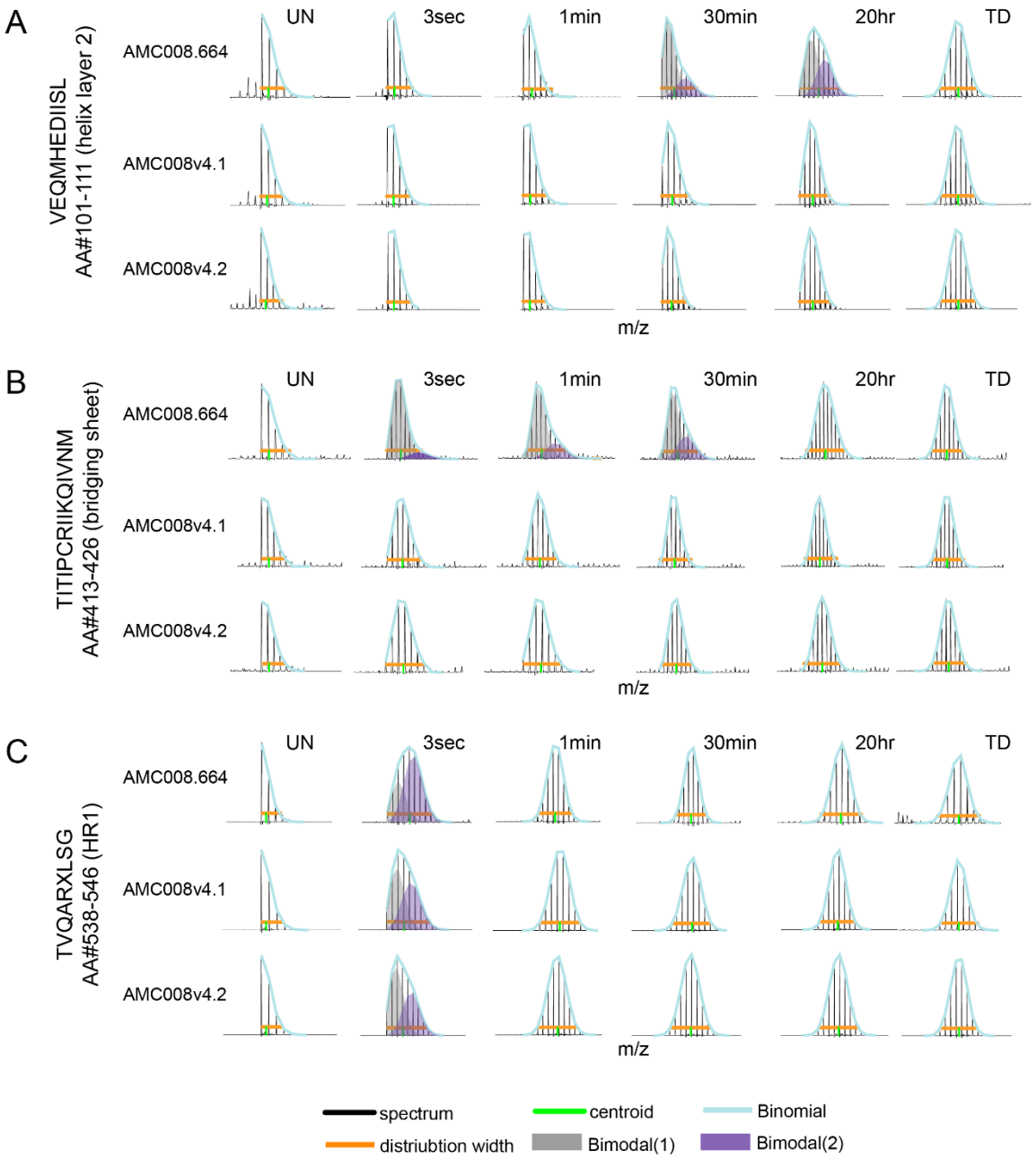


Figure S5. Additional stabilizing mutations quench conformational sampling observed in the dynamic AMC008.664 trimer, related to Figure 7. Mass spectra at each HDX time point of the AMC008 SOSIP constructs (.664, v4.1, and v4.2) are aligned. Spectra with a mass envelope width that was unusually broad were binomially fit to two populations. The lighter in mass/more protected population is shaded in gray, and the heavier in mass/more exposed population is shaded in purple. Peptides are highlighted spanning the gp120 inner domain helix 2 **(A)**, the bridging sheet **(B),** and HR1 in gp41 **(C).** Two of the three peptides displayed bimodal spectra only in the minimally engineered SOSIP.664 trimers, however the peptide 538-546 in gp41, where an X is used for the L543N mutation site in the v4 constructs, displayed bimodal spectra at the earliest time point in all three constructs.

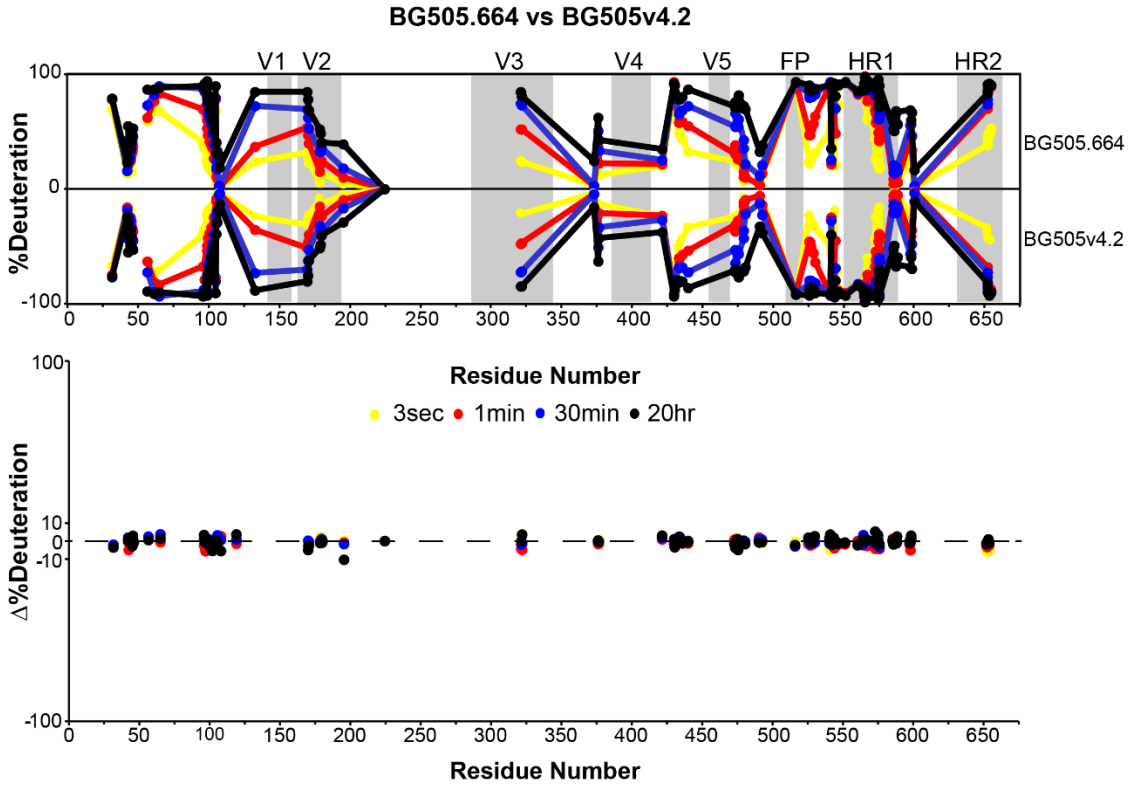


Figure S6 BG505.664 and BG505v4.2 butterfly and differential plots, related to Figure 7 and Data S1

The top Figure is a butterfly plot. The mid-point of each homologous peptide found in BG505.664 (top half) and BG505v4.2 (bottom half) is plotted to show deuterium uptake with time (3 second time point in yellow, 1 minute time point in red, 30 minute time point in blue, and 20 hour time point in black). The bottom Figure is a differential plot where the %difference in exchange between BG505.664 and v4.2 is plotted. Peptides falling below the 0 dashed line are regions more protected from exchange in the v4.2 construct, and peptides falling above the dashed line are regions that became less protected from exchange in the v4.2 construct. Deuterium uptake plots comparing homologous BG505.664 (blue) and BG505v4.2 (red) peptides are in an attached excel sheet (Data S1). Each data point is the average % deuteration from duplicate measurements at each time point (3sec, 1min, 30min, and 20hours) with standard deviation bars. Peptides spanning the H66R or A316W mutation site are displayed as X.

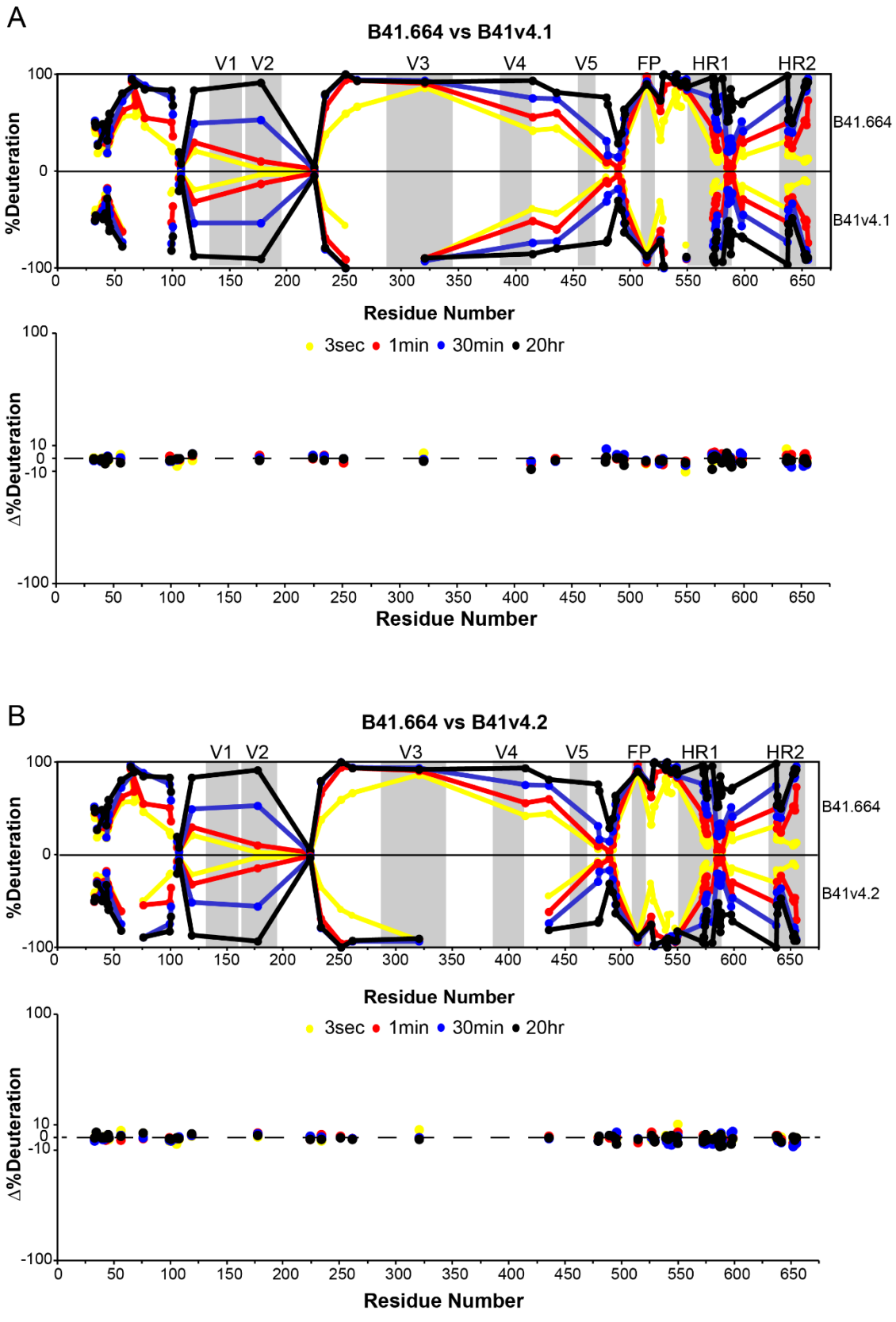

A
B41.664 vs B41v4.1
V1 V2
V3
V4
V5
FP
HR1
HR2
%Deuteration
B41.664
B41v4.1
Residue Number
3sec 1min 30min 20hr
Δ%Deuteration
Residue Number
B
B41.664 vs B41v4.2
V1 V2
V3
V4
V5
FP
HR1
HR2
%Deuteration
B41.664
B41v4.2
Residue Number
3sec 1min 30min 20hr
Δ%Deuteration
Residue Number

Figure S7 B41.664, B41v4.1, and B41v4.2 butterfly plots, and differential plots, related to Figure 7 and Data S1

(A) The top Figure is a butterfly plot. The mid-point of each homologous peptide found in B41.664 (top half) and B41v4.1 (bottom half) is plotted to show deuterium uptake with time (3 second time point in yellow, 1 minute time point in red, 30 minute time point in blue, and 20 hour time point in black). The bottom Figure is a differential plot where the %difference in exchange between B41.664 and v4.1 is plotted. Peptides falling below the 0 dashed line are regions more protected from exchange in the v4.1 construct, and peptides falling above the dashed line are regions that became less protected from exchange in the v4.1 construct. Figure (B) is similar to Figure (A), but compares B41.664 to B41v4.2. An attached excel sheet (Data S1) displays the deuterium uptake plots comparing homologous B41.664 (blue), B41v4.1 (green), and B41v4.2 (red) peptides. Each data point is the average % deuteration from duplicate measurements at each time point (3 sec, 1 min, 30 min, and 20 hours) with standard deviation bars. Peptides spanning the E64K, H66R, A316W, I535M, and L543N mutation sites are displayed as X.

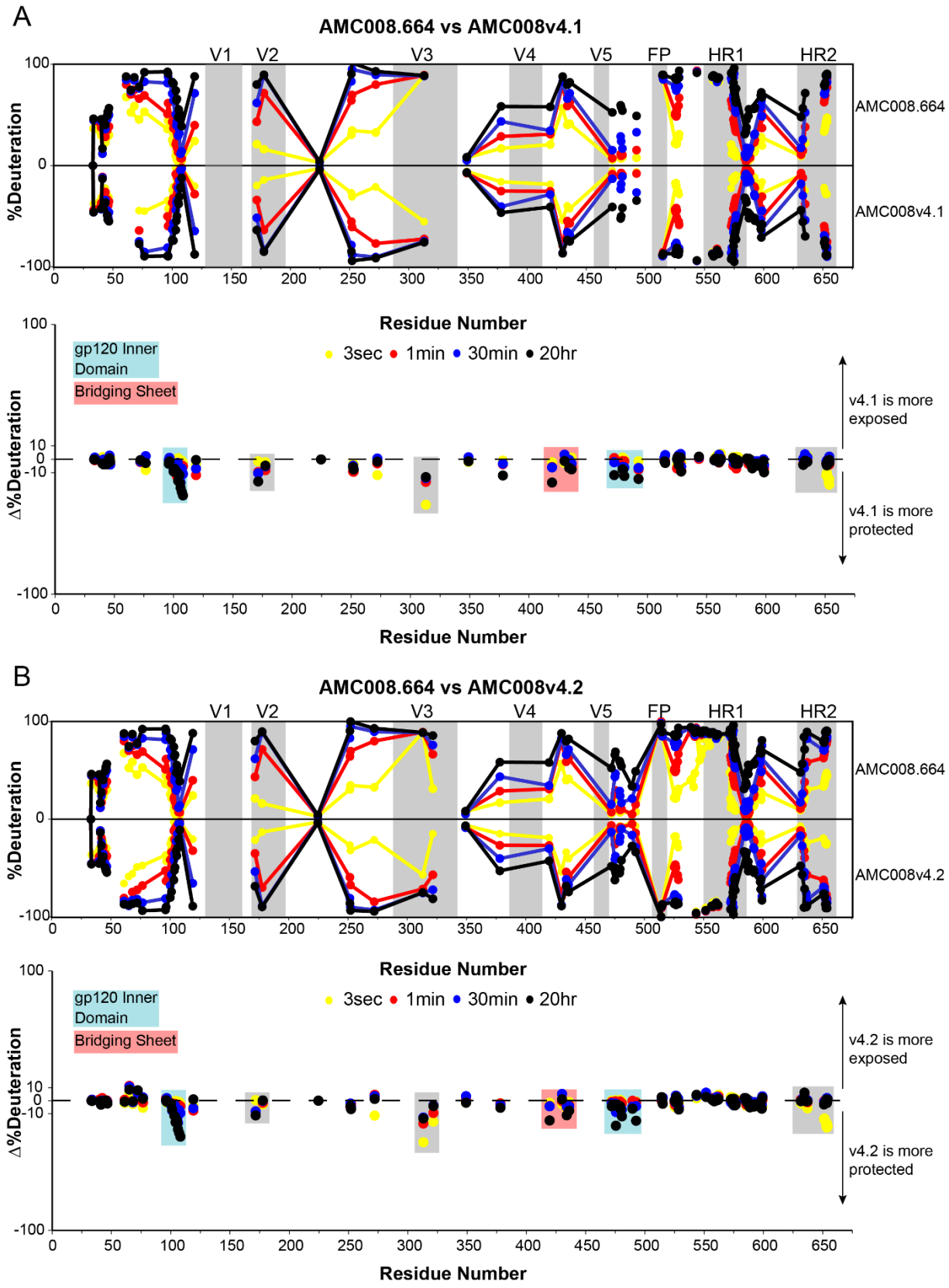

A
AMC008.664 vs AMC008v4.1
V1
V2
V3
V4
V5
FP
HR1
HR2
%Deuteration
AMC008.664
AMC008v4.1
Residue Number
gp120 Inner Domain
Bridging Sheet
3sec
1min
30min
20hr
Δ%Deuteration
v4.1 is more exposed
v4.1 is more protected
Residue Number
B
AMC008.664 vs AMC008v4.2
V1
V2
V3
V4
V5
FP
HR1
HR2
%Deuteration
AMC008.664
AMC008v4.2
Residue Number
gp120 Inner Domain
Bridging Sheet
3sec
1min
30min
20hr
Δ%Deuteration
v4.2 is more exposed
v4.2 is more protected
Residue Number

Figure S8 AMC008.664, AMC008v4.1, and AMC008v4.2 butterfly plots, differential plots, and deuterium uptake plots, related to Figure 7 and Data S1

(A) The top Figure is a butterfly plot. The mid-point of each homologous peptide found in AMC008.664 (top half) and AMC008v4.1 (bottom half) is plotted to show deuterium uptake with time (3 second time point in yellow, 1 minute time point in red, 30 minute time point in blue, and 20 hour time point in black). The bottom Figure is a differential plot where the %difference in exchange between AMC008.664 and v4.1 is plotted. Peptides falling below the 0 dashed line are regions more protected from exchange in the v4.1 construct, and peptides falling above the dashed line are regions that became less protected from exchange in the v4.1 construct. Figure (B) is similar to Figure (A), but compares ACM008.664 to AMC008v4.2. An attached spreadsheet (Data S1) displays the deuterium uptake plots comparing homologous AMC008.664 (blue), AMC008v4.1 (green), and AMC008v4.2 (red) peptides. Each data point is the average % deuteration from duplicate measurements at each time point (3 sec, 1 min, 30 min, and 20 hours) with standard deviation bars. Peptides spanning the E64K, H66R, A316W, I535M, and L543N mutation sites are displayed as X.

Supplementary Tables.

Dataset	BG505.664	BG505v4.2	
HDX reaction details	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	
HDX time course	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs	
HDX controls	PPPI, PPPF	PPPI, PPPF	
Back-exchange	10.2 +/- 7.4%	11.5 +/- 11.4%	
Number of peptides	154	154	
Sequence coverage	58.70%	58.70%	
Average peptide length/redundancy	12.1 residue length/ 2.8 redundancy	12.1 residue length/2.8 redundancy	
Replicates (biological or technical)	two technical replicates, 3 biological replicates	two technical replicates	
Repeatability	stddev of 1.1% across technical replicates	stddev of 1.5% across technical replicates	
Dataset	**AMC008.664**	**AMC008v4.1**	**AMC008v4.2**
HDX reaction details	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA
HDX time course	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs
HDX controls	BG505.664, PPPI, PPPF	BG505.664, PPPI, PPPF	BG505.664, PPPI, PPPF
Back-exchange	12.8 +/- 8.0%	12.1 +/- 7.9%	12.3 +/- 7.3%
Number of peptides	135	135	135
Sequence coverage	61.10%	61.10%	61.10%
Average peptide length/redundancy	13.0 residues/2.6 redundancy	13.0 residues/2.6 redundancy	13.0 residues/2.6 redundancy
Replicates (biological or technical)	two technical replicates	two technical replicates	two technical replicates
Repeatability	stddev of 0.90% across technical replicates	stddev of 1.1% across technical replicates	stddev of 0.96% across technical replicates
Dataset	**B41.664**	**B41v4.1**	**B41v4.2**
HDX reaction details	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.457, labeled at RT (23.4°C), Quenched at pH 2.51 in 200mM TCEP, 8M urea, 0.2%FA
HDX time course	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs
HDX controls	BG505.664, PPPI, PPPF	BG505.664, PPPI, PPPF	BG505.664, PPPI, PPPF

Back-exchange	11.5 +/- 13.1%	13.2 +/- 13.4%	10.5 +/- 6.8%
Number of peptides	89	89	89
Sequence coverage	60.60%	60.60%	60.60%
Average peptide length/redundancy	12.0 residues/1.7 redundancy	12.0 residues/1.7 redundancy	12.0 residues/1.7 redundancy
Replicates (biological or technical)	two technical replicates	two technical replicates	two technical replicates
Repeatability	stddev of 1.8% across technical replicates	stddev of 1.7% across technical replicates	stddev of 0.93% across two technical replicates
Dataset	**JRFL.664**	**CE1176.664**	
HDX reaction details	85% D2O buffer, pH* 7.514, labeled at RT (22.1°C), Quenched at pH 2.509 in 200mM TCEP, 8M urea, 0.2%FA	85% D2O buffer, pH* 7.514, labeled at RT (23.3°C), Quenched at pH 2.511 in 200mM TCEP, 8M urea, 0.2%FA	
HDX time course	3 sec, 1 min, 30 min, 20 hrs	3 sec, 1 min, 30 min, 20 hrs	
HDX controls	BG505.664, PPPI, PPPF	BG505.664, PPPI, PPPF	
Back-exchange	8.4 +/- 7.9%	8.2 +/- 7.3%	
Number of peptides	98	80	
Sequence coverage	60.40%	61.00%	
Average peptide length/redundancy	13.0 residues/2.0 redundancy	13.7 residues/1.3 redundancy	
Replicates (biological or technical)	two technical replicates	three technical replicates	
Repeatability	stddev of 1.6%	stddev of 0.87%	

Table S1, related to Figure 2 and Data S1.

HDX reaction details and coverage information across each SOSIP construct.

			Open/Closed Conformation specific				CD4 bs	V3 N332	V3 tip specific			V2i		gp120-41
Tier	Clade	SOSIP	PGT145 K_D	PG16 K_D	b12 K_D	17b K_D	VRC01 K_D	PGT121 K_D	447-52D K_D	3074 K_D	3869 K_D	830A K_D	2158 K_D	35022 K_D
1B	B	AMC008.664	735.4+/- 246.9	ND	20.2 +/- 2.1	135.2 +/- 4.0	24.5 +/- 7.9	8.7 +/- 3.9	27.8 +/- 3.3	48.9 +/- 3.7	13.5 +/- 0.6	ND	ND	72.6 +/- 19.3
1B	B	AMC008v4.2	265.5 +/- 152.0	ND	ND	ND	6.5 +/- 3.9	2.4 +/- 1.4	ND	ND	ND	ND	ND	41.5 +/- 8.7
2	B	B41.664	23.4 +/- 0.9	419 +/- 110.3	102.5 +/- 19.0	ND	90.3 +/- 10.3	115.5 +/- 2.1	125.5 +/- 21.9	662.5 +/- 95.5	97.1 +/- 22.6	145.0 +/- 31.1	ND	160.5 +/- 16.3
2	B	B41v4.2	15.4 +/- 7.9	346.5 +/- 10.6	151.0 +/- 36.8	ND	108.9 +/- 38.4	91.5 +/- 38.9	184.2 +/- 139.8	ND	220.5 +/- 105.4	276.5 +/- 232.6	ND	119.0 +/- 19.7
2	A	BG505.664	15.6 +/- 4.6	86.8 +/- 8.5	1114.4 +/- 315.7	ND	8.1 +/- 1.7	15.8 +/- 5.4	33.0 +/- 12.4	62.8 +/- 1.6	257.5 +/- 191.7	ND	ND	78.1 +/- 28.1
2	A	BG505v4.2	13.2 +/- 5.6	67.4 +/- 21.0	120.8 +/- 20.2	ND	11.2 +/- 6.5	15.3 +/- 4.7	ND	6542 +/- 5412	1036.0 +/- 444.2	ND	ND	28.0 +/- 3.5

	K_D nM
	0-10
	11-50
	50-250
	250-500
	500-1000

Table S2: Isolate specific differences in binding affinities of Abs specific for different regions on Env, related to Figure 8. The BLI determined average binding affinities from at least two independent experiments of each of the five isolates for a panel of Abs specific different conformations or localized regions of interest. Affinities and binding rates are colored as indicated above to highlight differences in binding affinity (K_D).

			Open/Closed Conformation specific				CD4 bs	V3 N332	V3 tip specific			V2i		gp120-41
Tier	Clade	SOSIP	PGT145 k_{on} (1/Ms)	PGT16 k_{on} (1/Ms)	b12 k_{on} (1/Ms)	17b k_{on} (1/Ms)	VRC01 k_{on} (1/Ms)	PGT121 k_{on} (1/Ms)	447-52D k_{on} (1/Ms)	3074 k_{on} (1/Ms)	3869 k_{on} (1/Ms)	830A k_{on} (1/Ms)	2158 k_{on} (1/Ms)	35022 k_{on} (1/Ms)
1B	B	AMC008.664	2.29E+03	ND	2.70E+04	4.02E+03	1.18E+04	3.92E+04	4.19E+04	3.77E+04	3.63E+04	2.58E+04	ND	6.52E+03
1B	B	AMC008v4.2	6.30E+03	ND	ND	ND	1.20E+04	4.96E+04	ND	ND	ND	ND	ND	7.61E+03
2	B	B41.664	2.46E+04	8.78E+03	1.08E+04	ND	3.98E+03	4.82E+03	6.83E+03	4.35E+03	5.59E+03	5.71E+03	ND	6.91E+03
2	B	B41v4.2	3.11E+04	1.67E+04	4.70E+03	ND	3.81E+03	5.53E+03	6.15E+03	ND	3.74E+03	3.64E+03	ND	6.69E+03
2	A	BG505.664	1.29E+05	3.17E+04	1.97E+03	ND	1.42E+04	2.09E+04	2.11E+03	3.36E+03	2.02E+03	ND	ND	6.02E+03
2	A	BG505v4.2	1.38E+05	5.15E+04	4.70E+03	ND	1.72E+04	2.15E+04	ND	2.25E+02	4.43E+02	ND	ND	8.33E+03

	k_{on} 1/Ms
	>1.0E+05
	1.0E+04 - 1.0E+05
	1.0E+03 - 1.0E+04
	1.0E+02 - 1.0E+03
	<1.0E+02

Table S3: Isolate specific differences in association rates of Abs specific for different regions on Env, related to Figure 8. The BLI determined average association rates from at least two independent experiments of each of the five isolates for a panel of Abs specific different conformations or localized

regions of interest. Binding rates are colored as indicated above to highlight differences in binding association rates (k_{on}) across strains.

			Open/Closed Conformation specific				CD4 bs	V3 N332	V3 tip specific			V2i		gp120-41
Tier	Clade	SOSIP	PGT145 k_{off} (1/s)	PGT16 k_{off} (1/s)	b12 k_{off} (1/s)	17b k_{off} (1/s)	VRC01 k_{off} (1/s)	PGT121 k_{off} (1/s)	447-52D k_{off} (1/s)	3074 k_{off} (1/s)	3869 k_{off} (1/s)	830A k_{off} (1/s)	2158 k_{off} (1/s)	35022 k_{off} (1/s)
1B	B	AMC008.664	1.63E-03	ND	5.47E-04	5.43E-04	2.89E-04	3.43E-04	1.18E-03	1.85E-03	4.86E-04	no off rate	ND	4.80E-04
1B	B	AMC008 v4.2	1.44E-03	ND	ND	ND	7.36E-05	1.21E-04	ND	ND	ND	ND	ND	3.18E-04
2	B	B41.664	5.60E-04	3.62E-03	1.11E-03	ND	3.54E-04	5.57E-04	8.57E-04	2.89E-03	5.35E-04	8.27E-04	ND	1.10E-03
2	B	B41v4.2	5.57E-04	2.71E-03	1.08E-03	ND	5.92E-04	8.09E-05	1.17E-03	ND	9.05E-04	ND	ND	8.01E-04
2	A	BG505.664	1.85E-03	2.72E-03	2.17E-03	ND	1.14E-04	3.53E-04	6.35E-05	2.10E-04	2.98E-04	ND	ND	4.52E-04
2	A	BG505v4.2	1.68E-03	3.37E-03	5.67E-04	ND	1.95E-04	3.14E-04	ND	6.10E-04	3.81E-04	ND	ND	2.30E-04

	k_{off} (1/s)
	>1.0E-03
	1.0E-04 - 1.0E-03
	<1.0E-04

Table S4: Isolate specific differences in dissociation rates of Abs specific for different regions on Env, related to Figure 8. The BLI determined average dissociation rates from at least two independent experiments of each of the five isolates for a panel of Abs specific different conformations or localized regions of interest. Dissociation rates are colored as indicated above to highlight differences dissociation rates (k_{off}) across strains.