Supplemental Information

Isolate-Specific Differences in the Conformational Dynamics and Antigenicity of HIV-1 gp120

Thaddeus M. Davenport¹, Miklos Guttman², Wenjin Guo³, Brad Cleveland³, Maria Kahn^{3,4}, Shiu-Lok Hu^{3,5}, Kelly K. Lee^{1,2, #}

¹ Department of Global Health, ² Department of Medicinal Chemistry, ³ Department of Pharmaceutics, University of Washington, Seattle, Washington, 98195, USA

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⁴ Current Address: PATH, Seattle, Washington, 98121, USA

⁵ Washington National Primate Research Center, Seattle, Washington, 98195, USA

[#] Address correspondence to: kklee@uw.edu

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Figure S1. SDS- and Native-PAGE analysis of purified gp120 proteins. (A) SDS-PAGE analysis of purified gp120 proteins. (B) Blue Native-PAGE analysis of purified monomeric gp120.



Figure S2. SAXS reconstruction, Kratky plot, and Porod-Debye analysis of SAXS data. (A) DAMMIN reconstructions of the four full-length gp120 monomers (gray density) overlayed with a space-filling model of the gp120 core (3JWD) including the V3 loop from (2B4C) (blue spheres) (B) Kratky and (C) Porod-Debye analysis of SAXS data. Each isolate is represented by a different color, as shown in the legend. The data are superimposable for all for gp120s, indicating similar degrees of globular structure.

10 TEKLWVTVYY	20 GVPVWKEAKT	30 TLFCASDAKA	40 YEREVHNIWA	50 THACVPTDPN	60 PQELVLENVT
			_		
70 ENFNMWENDM	80 VDQMHEDIIS	90 LWDQSLKP <mark>CV</mark>	100 KLTPLCVTLN	110 CTDVKSANST	120 SEDMRNCSFN
		_			
130	140	150	160	170	180 KVNFDPIPIH
190	200	210	220	230	
ICAPAGIAID	KCWKITWGD	Grennybivg	CINGINEAAS	TQUUUNGUUN	550111K35K
250	260	270	280	290	300
LINNVKTIIV	HLKDIVEIV	TRPNNNTKKS	MRIGPGQAFT	ATGELLONIK	BARCNISGSK
310	320	330	340	350	360
WNNTLQRVKK	KLGEHFPNNT	TIDFKP <mark>SSGG</mark>	DLEITTHSFN	CRGEFFYCNT	SKLFNGTSES
370	380	390	400	410	420
NSTITLPCKI	KQIINMWQGV	GRAM YAPPIA	GNITCKSNIT	GLLLTRDGGN	GNGTEIFRPG
430	440	450	460		\longrightarrow
GGDMRDNWRS	ELYKYKVVKI	EPLGIAPTKA	KRRVVERGKR		

Figure S3. Pepsin digestion map for 1084i gp120. The mature 1084i gp120 amino sequence is indicated (with numbering based on mature 1084i). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

10 TEKLWVTVYY	20 GVPVWKEAKT	30 TLFCASDAKA	40 YEKEVHNIWA	50 THACVPTDPN	60 PQEIVLENVT
70 ENFNMWKDDM	80 VDQMHEDIIS	90 LWDQSLKP <mark>CV</mark>	100 KLTPLCVTLK	110 CSNFTREGNV	120 TYKEEMDKVK
		-			
130	140	150	160	170	180
acsraviigi	RUKKQKVRAL	FIREDITFED	ENNNNSSEIK	LINCASSTIT	QACPRVNEDP
			-		-
190 IPIHYCAPAG	200 YAILKCNNKT	210 FNGTGPCHNV	220 STVOCTHGIK	230 PVVSTOLLLN	240 GSLAEREIII
					<u> </u>
250	260	270	280	290	300
ROBRDIDITI		Dirit and the second	INNOINIOIO	gra intobit	
310 SKENWNKTLQ	320 WVRGKLEEHF	330 PNKTIVFKP <mark>S</mark>	340 SGGDLEITTH	350 SFNCRGEFFY	360 CNTSKLFNGT
370	380	390 KOLINMWOEV	400 GRAMYAPPIE	410 GNITCKSNIT	420 GLLLVRDGG <mark>O</mark>
430 DNSTNNTETF	440 RPGGGDMRNN	450 WRSELYKYKV	460 VEIKPLGMAP	470 TKAKRRVVER	EKR

Figure S4. Pepsin digestion map for 1157ip gp120. The mature 1157ip gp120 amino sequence is indicated (with numbering based on mature 1157ip). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

10 TEKLWVTVYY	20 GVPVWKEATT	30 TLFCASDAKA	40 YDTEVHNVWA	50 THACVPTDPN	60 PQEVVLVNVT
			-		
70 ENFNMWKNDM	80 VEQMHEDIIS	90 LWDQSLKP <mark>CV</mark>	100 KLTPLCVSLK	110 CTDLKNDTNT	120 NSSSGRMIME
_					
130 KGEIKNCSFN	140	150 KEYAFFYKLD	160	170	180
190 EPIPIHYCAP	200 AGFAILKCNN	210 KTF <mark>N</mark> GTGPCT	220 NVSTVQCTHG	230 IRPVVSTQLL	240 LNGSLAEEEV
				_	
250 VIRSVNFTDN	260 AKTIIVQLNT	270 SVEIN <mark>CTRPN</mark>	280	290 RGPGRAFVTI	300 GKIGNMRQAH
310 <mark>C</mark> NISRAKWNN	320 TLKQIASKLR	330 EQFGN <mark>N</mark> KTII	340 FKQ <mark>SSGGDPE</mark>	350 IVTHSFNCGG	360 EFFY <mark>CNSTQL</mark>
370	380 STEGSNNTEG	390	400 KOIINMWOKV	410 GKAMYAPPIS	420 GOIRCSSNIT
430 GLLLTRDGG <mark>N</mark>	440 SNNESEIFRP	450 GGGDMRDNWR	460 SELYKYKVVK	470 IEPLGVAPTK	480 AKRRVVQREKR

Figure S5. Pepsin digestion map for HXB2 gp120. The mature HXB2 gp120 amino sequence is indicated (with numbering based on mature HXB2). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

10 TEKLWVTVYY	20 GVPVWKEATT	30 TLFCASDAKA	40 YDTEVHNVWA	50 THACVPTDPN	60 PQEIVLENVT
70	80	90	100	110	120
ENFNMWKNNM	VEOMHEDIIS	LWDOSLKPCV	KLTPLCVTLH	CTNLKNATNT	KSSNWKEMDR
		_			
130	140	150	160	170	180
OD T VIO OD VII	mmorphyuou	PULL PUUL PU	UNTRUDUCCU	MT THOMOUT	BOLODE
GEIKNCSFKV	TTSIKNKMQK	ETALFIKLDV	VPIDNDNTSI	KLINCNTSVI	TOACPRVSFE
100	0.0.0	214	000	0.0.0	2.4.0
190	200	210	220	230	240
PIPIHYCAPA	GFAILKCNDK	KFNGSGPCTN	VSTVOCTHGI	RPVVSTOLLL	NGSLAEEGVV
				THE FEEL REPORT	
250	260	270	280	290	300
TRSENETDNA	KTTTVOLKES	VETNCTRRNN	NTRESTATOR	CRAEVATODT	TODTROAHCN
INDENTIONA	KI I I VYDKDO	VEINGINERI	MIKKOIIIGE	OKAI IAIODI	TOPTINET
310	320	330	340	350	360
TECERLINIT	VATUMPTAN	PONYMTURY	RECODDRTIN	HERNOCORRE	VONCEOT PUC
ISGEKWNNTL	KÖTALKPÖVÖ	FGNKTIVEKQ	SSGOPEIVM	HSPNCGGEFF	ICNSTQLENS
270	200	200	400	410	420
370	380	390	400	410	420
TWNNTIGPNN	TNGTITLPCR	IKQIINRWQE	VGKAMYAPPI	RGQIRCSSNI	TGLLLTRDGG
430	440	450	460	470	
KEISNTTEIF	RPGGGDMRDN	WRSELYKYKV	VKIEPLGVAP	TKAKRRVVQR	EKR
370 TWNNTIGPNN	380	390 IKQIINRWQE	400 VGKAMYAPPI	410 RGQIRCSSNI	420 TGLLLTRDGG
430	440	450	460	4/0	
NEISWITEIF	REGGGDMRDN	WRODDININV	VETERLOVAR	TVAVKKAAŐK	DVV

Figure S6. Pepsin digestion map for SF162 gp120. The mature SF162 gp120 amino sequence is indicated (with numbering based on mature SF162). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis. Yellow lines indicate coverage by subtraction of overlapping peptides.

	40	50	60		70 80
нхв2	TEKLWVTVYY	GVPVWKEATT	TLFCASDAKA	YDTEVHNVW	A THACVPTDPN
SF162	TEKLWVTVYY	GVPVWKEATT	TLFCASDAKA	YDTEVHNVW	A THACVPTDPN
1084i	TEKLWVTVYY	GVPVWKEAKT	TLFCASDAKA	YEREVHNIW	A THACVPTDPN
1157ip	TEKLWVTVYY	GVPVWKEAKT	TLFCASDAKA	YEKEVHNIW	A THACVPTDPN
-	90	100	110		120 130
	POPUVIVIV	PNPNMMENDM	VPANNEDTTE	LNDOSLKRC	V KITPLOVSIV
HXB2	PORTULENUT	PNPNMWVNNM	VEQMEDIIS	LWDOSLKPC	V KLTPLCVSLK
SF102	POFLVLENVT	ENENMWENDM	VDOMWEDITS	LWDOSLKPC	V KLTPLCVTLN
1157 in	POELVLENVT	ENFNMWKDDM	VDOMHEDIIS	LWDOSLKPC	V KLTPLCVTLK
115/12	140	450	10 2 11 10 11 0	2 go ant o	
	140	150			170 180
HXB2	CTDLK N DTNT	NSSSGRMIME	KGEIKNCSFN	ISTSIRGKV	Q KEYAFFY KL D
SF162	CTNLK N ATNT	K S S N W K – E M D	RGEIKNCSFK	VTTSIRNKM	Q KEYALFYKLD
1084i	CTDVKSANST		SEDMRNCSFN	VTTEIKDRK	K LEQALFYRLD
1157ip	CSNFTREGNV	T Y = K E E	MDKVKNCSFN	VTTGIRDKK	Q KVNALFY RLD
	188	196	206		216
нхв2	IIPIDNDT	TSYKLTSC	NTSVITQACP	KVSFEPIPI	H YCAPAGFAIL
SF162	VVPIDND <mark>N</mark>	TSYKLINC	NTSVITQACP	KVSFEPIPI	H YCAPAGFAIL
1084i	IVPLKNSSSS	NFSEYRLINC	NTSTVSQACP	KVNFDPIPI	H YCAPAGYAIL
1157ip	ITPLDENNNN	- SSEYRLINC	NSSTITQACP	KVNFDPIPI	H YCAPAGYAIL
-					266
	VONNETENOT	C B C T N V S T V O	CTRCTPPVVS	TOTILNOST	A PPPUUTDCUM
HAB2	KCNDKKENGS	GPCINVSIVQ	CTHGIRPVVS	TOLLINGSL	A BEEVVIRSVN
SF162	KCNNKTPNOS	GPCINVSIVQ	CTRCINEVUS	TOTILNOSL	A PERITTECEN
10641 1157in	KONNKTENGT	GPCHNVSTVQ	CTHGIKPVVS	TOLLINGSL	A PRETTINGEN
115719	A CHARTENOI	Greakvorvy	CINGINITIUS	T Y D D D O D D	
	286	296	306		316
HXB2	FTDNAKTIIV	Q L N T S V E I N C	TRPNNTRKR	IRIQRGPGR	R FVTIG-KIGN
SF162	FTDNAKTIIV	Q LKESVEIN C	TRPNNTRKS	ITIGPGR	A FYATGDIIGD
1084i	LTNNVKTIIV	H L K D Y V E I V <mark>C</mark>	TRPNNTRKS	MRIGPGQ	A FXATGEIIGN
1157ip	LTDNVKTIIV	HFNESVEIN C	TRPNNTRKS	IRIGPGQ	A FTATGDIIGD
	335	345	355		365 375
нхв2	MRQAHCNISR	AKWNNTLKQI	ASKLREQFGN	NKTIIFKQS	S GGDPEIVTHS
SF162	IRQAHCNISG	EKWNNTLKQI	VTKLOAQFG-	NKTIVFKQS	S GGDPEIVMHS
1084 i	IREAHCNISG	SKWNNTLORV	KKKLGEHFPN	NTTIDFKPS	SGGDLEITTHS
1157ip	IRQAHCNISK	ENWNKTLOWV	RGKLEEHFPN	K-TIVFKPS	S GGDLEITTHS
	385	305	405		A15 A25
					410
HXB2	FNCGGEFFYC	NSTQLFNSTW	FNSTWSTEGS	NNTEGSDTI	T LPCRIKQIIN
SF162	FNCGGEFFYC	NSTQLFNSTW	NNTIGP	NNTNG TI	T LPC RIKQIIN
10841	FNCRGEFFYC	NTSKLFNGTS	E	SNSTI	TLPCKIKQIIN
11571p	FNCRGEFFI	NTSKLFNGTD	NSTHMD	TGNDTVI	TIPCRIKQIIN
	435	445	455		463
HXB2	M W Q K V G K A M Y	A P P I S G Q I R C	SSNITGLLLT	R	N ESEIFRP GGG
SF162	R W Q E V G K A M Y	A P P I R G Q I R C	SS NITGLL LT	R D G G <mark>K – E I S</mark>	N TTEIFRP GGG
1084i	M W Q G V G R A M Y	A P P I A G N I T C	KSNITGLLLT	R D G G N G	N GTEIFRP GGG
1157ip	MWQEVGRAMY	APPIEGNITC	KSNITGLLLV	RDGGQDNST	N NTETFRP GGG
	483	493	503		Inner Domain
нхв2	DMRDNWRSEL	YKYKVVKIEP	LGVAPTKAKR	RVVQREKR	Outer Domain
SF162	DMRDNWRSEL	YKYKVVKIEP	LGVAPTKAKR	RVVQREKR	V1
1084i	DMRDNWRSEL	YKYKVKIEP	LGIAPTKAKR	RVVERGKR	V2
1157ip	DMRNNWRSEL	YKYKVVEIKP	LGMAPTKAKR	RVVEREKR	V3
ľ	Observable Re	sidues			V4
	Missing Resid	ues			CD4 Binding Loop
	Potential N-1	inked Glycosyl	ation Site		Beta 2 and 3 (Bridging Sheet)
	O-linked glyc	osylation site	e		Beta 20 and 21 (Bridging Sheet)

Figure S7. Sequence coverage of all peptides for every isolate. The four isolates are aligned to HXB2 and numbered based on standard HXB2 numbering. HDX-MS-observable residues are indicated in black; residues that cannot be measured as a result of missing peptide coverage or rapid back-exchange (N-terminal residues of peptic fragments) are indicated in grey. Variable loops, bridging sheet elements, and CD4-binding loop are colored as described in the inset.

	40	50	60		70 80
нхв2	TEKLWVTVYY	GVPVWKEATT	TLFCASDAKA	YDTEVHNVW	A THACVPTDPN
SF162	TEKLWVTVYY	GVPVWKEATT	TLFCASDAKA	YDTEVHNVN	A THACVPTDPN
1084i	TEKLWVTVYY	GVPVWKEAKT	TLFCASDAKA	YEREVHNIN	A THACVPTDPN
1157ip	TEKLWVTVYY	GVPVWKEAKT	TLFCASDAKA	YEKEVHNIN	A THACVPTDPN
	90	100	110		120 130
UVD2			VROVURDITE		
SE162	PORTVLENVT	PNPNMWKNDM	VEQMEEDIIS	LWDQSLKPC	V KLTPLCVSLK
10841	POPLVLENVT	ENENMMENDM	VDOMHEDIIS	LWDOSLERPO	V KLTPLCVILN
1157in	PORTVLENVT	ENENMWKDDM	VDOMHEDIIS	LWDOSLKPC	V KLTPLCVTLK
			100110	2 Seguration	
	140	150			170 180
HXB2	CTDLK N DTNT	NSSSGRMIME	KGEIKNCSFN	ISTSIRGKV	Q KEYAFFY KL D
SF162	CTNLK N ATNT		RGEIKNCSFK	VTTSIRNKM	IQ KEYALFY KL D
10841	CTDVKSANST		SEDMRNCSFN	VTTEIKDRE	KK LEQALFY RL D
115/1p	CSNFTREGNV	T Y = K E E	MDKVKNCSFN	VTTGIRDKE	CQ KVNALFY RL D
	190	196	206		216
нхв2	IIPIDNDT	T S Y K L T S C	NTSVITQACP	KVSFEP IPI	H YCAPAGFAIL
SF162	VVPIDND <mark>N</mark>	TSYKLINC	NTSVITQACP	KVSFEPIPI	H YCAPAGFA IL
1084i	I V P L K N S S S S	NFSEYRLINC	NTSTVSQACP	KVNFDPIPI	H YCAPAGYAIL
1157ip	ITPLDENNNN	- SSEYR LINC	NSSTITQACP	KVNFDPIPI	H YCAPAGYAIL
	236				266
нхв2	KCNNKTFNGT	GPCTNVSTVO	CTHGIRPVVS	TOLLLNGSI	A EEEVVIRSVN
SF162	KCNDKKFNGS	GPCTNVSTVO	CTHGIRPVVS	TOLLLNGSI	A EEGVVIRSEN
1084i	KCNNKTFNGS	GPCNNVSTVQ	CTHGIKPVVS	TQLLLNGSI	A EEDIIIRSEN
1157ip	KCNNKTFNGT	GPCHNVSTVQ	CTHGIKPVVS	TQLLLNGSI	A EREI IIRSEN
	286	296	306		316 325
UVD2		OT NECKETN		TRADADA	
SE162	PTDNAKTIIV	QLNTSVEINC	TRPNNNTRAK	TRIQKGPGP	VIIG-KIGN
10841	LTNNVKTITV	VIKDSVEINC	TRENMATRAS	MRT CRCC	A FYATGDIIGD
1157in	LTDNVKTITV	HENESVEINC	TRENNNTRES	TRTGPGC	A FYATGDIIGD
				202 0103	
					365 375
HXB2	MRQAHCNISR	A KWNNTLKQ I	ASKLREQFGN	NKTIIFKQS	SS GGDPEIVTHS
SF162	IRQAHCNISG	EKWNNTLKQI	VTKLQAQFG-	NKTIVFKQS	SS GGDPEIVMHS
10841	IREAHCNISG	SKWNNTLQRV	KKKLGEHFPN	NTTIDFKPS	S GGDLEITTHS
115/1p	I RQAHCNISK	ENWNKTLQWV	RGKLEEHFPN	K – TIVFKPS	S GGDLEITTHS
	385	395	405		415 425
нхв2	FNCGGEFFYC	NSTQLFNSTW	FNSTWSTEGS	NNTEGSDTI	T LPC RIKQIIN
SF162	F N C G G E F F Y <mark>C</mark>	NSTQLFNSTW	${\tt N} \: {\tt N} = + - + {\tt T} \: {\tt I} \: {\tt G} \: {\tt P}$	$N \ N \ T \ N \ G \ \ T \ I$	T LPC RIKQIIN
1084i	FNCRGEFFYC	NTSKLFNGTS		SNSTI	T LPC KIKQIIN
1157ip	FNCRGEFFYC	NTSKLFNGTD	$\mathbf{N} \: \mathbf{S} = \mathbf{T} \: \mathbf{H} \: \mathbf{M} \: \mathbf{D}$	${\bf T} ~ {\bf G} ~ {\bf N} ~ {\bf D} ~ {\bf T} ~ V ~ {\bf I}$	T IPCRIKQIIN
	435	445	455		463 473
нхв2	MWOKVGKAMY	APPISGOIRC	SSNITGLLLT	RDGGN-S-N	IN ESEIFRPGGG
SF162	RWQEVGKAMY	APPIRGQIRC	SSNITGLLLT	RDGGK-EIS	N TTEIFRPGGG
1084i	MWQGVGRAMY	APPIAGNITC	KS NITGLL LT	R D G G N G	N GTEIFRPGGG
1157ip	MWQEVGRAMY	APPIEGNITC	KS NITGLL LV	RDGGQDNST	N NTETFRPGGG
HXP2	DMRDNWPCFT	VEVEVEEP	LOVAPTEAFF	RVVOPPER	Inner Domain
SF162	DMRDNWRSEL	YKYKVVKTEP	LGVAPTKAKP	RVVOREKR	V1
10841	DMRDNWRSEL	YKYKVVKIEP	LGIAPTKAKR	RVVERGER	V2
1157in	DMRNNWRSEL	YKYKVVEIKP	LGMAPTKAKE	RVVEREKR	V3
	Desidues com	anable server	4 2 2 icela	+ 0.5	V4
	Missing Resid	ues	4, 3, Z 1SOLA	tes	V5
	Potential N-1	inked Glycosvl	ation Site		Beta 2 and 3 (Bridging Sheet)
	O-linked glvc	osylation site	•		Beta 20 and 21 (Bridging Sheet)
	2010				

Figure S8. Sequence coverage of peptides that are comparable across isolates. The four isolates are aligned to HXB2 and numbered based on standard HXB2 numbering. Black, blue and green text indicates residues that are comparable across 4, 3 and 2 isolates, respectively; residues that cannot be compared across isolates as a result of missing peptide coverage or rapid back-exchange (N-terminal residues of peptic fragments) are indicated in grey. Variable loops, bridging sheet elements, and CD4-binding loop are colored as described in the inset.



Figure S9. Deuteration plots of all unique 1084i peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration vs time are shown for each unique peptide observed for 1084i gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering as in Figure S3) are indicated above each plot.



Figure S10. Deuteration plots of all unique 1157ip peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration vs. time are shown for each unique peptide observed for 1157ip gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering as in Figure S4) are indicated above each plot.



Time (sec)

Figure S11. Deuteration plots of all unique HXB2 peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration over time are shown for each unique peptide observed for HXB2 gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering scheme as in Figure S5) are indicated above each plot.





Figure S12. Deuteration plots of all unique SF162 peptides. Deuterium uptake plots with percent deuteration over time are shown for each unique peptide observed for SF162 gp120. Data for unliganded gp120 is shown in blue, data was not collected for sCD4-bound SF162, as it has been previously characterized. Amino acid sequence and position of each peptide (using the same numbering scheme as in Figure S6) are indicated above each plot.







Homologous Inner Domain Peptides (Unliganded)



Figure S14. Deuteration plots for all comparable inner domain peptides (unliganded gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 inner domain, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Half-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.



Figure S15. Deuteration plots for all comparable outer domain/variable loop/bridging sheet peptides (unliganded gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 outer domain, variable loops, or bridging sheet, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2, unless a peptide for HXB2 was not observed) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Partial-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.



Figure S16. Heat map summary of sCD4-bound gp120 deuteration and difference maps. Data for each gp120 isolate (1084i, 1157ip, and HXB2) is presented in a separate column, and each row reflects a different time point (12 sec, 1 min, 5 min, 30 min, and 4 hr). Within each column, the colors mapped onto gp120 core (PDB ID: 3JWD) indicate percent deuteration (left) or difference in percent deuteration relative to unliganded gp120 (right). Deuteration levels are indicated in the legend on the left, with red colors corresponding to high levels of deuterium uptake (dynamic regions) and blue colors corresponding to low levels of deuteration (ordered or "protected" regions). Regions where peptide information is missing are indicated in gray. Deuteration difference values are indicated in the legend on the right, where white colors reflect regions that are similarly deuterated in unliganded and sCD4-bound gp120 and blue colors reflect regions that are stabilized as a result of sCD4-binding.



Homologous Inner Domain Peptides (sCD4-bound)



Figure S17. Deuteration plots for all comparable inner domain peptides (sCD4-bound gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 inner domain in sCD4-bound gp120, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2, unless a peptide for HXB2 was not observed) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Half-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.



Homologous Outer Domain Peptides (sCD4-bound)

Time (sec)

Figure S18. Deuteration plots for all comparable outer domain/variable loop/bridging sheet peptides (sCD4-bound gp120) Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 outer domain, variable loops or bridging sheet, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is described by the colored boxes surrounding the graphs, as defined in the figure legend. Partialboxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.







Figure S20. Linear epitope-specific antibody SPR binding curves. Double-reference subtracted SPR binding curves are shown for gp120 binding to captured linear-epitope specific antibodies CA13, C4, or B18 over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit 1:1 binding model curves are in red. Each column shows data for a different gp120 isolate, and each row shows data for a different antibody. Y-axis is SPR response (in RU) and x-axis is time. Note changes in gp120 concentration range and scale for the Y-axis for each antibody.



Figure S21. Conformation-dependent antibody SPR binding curves. Double-reference subtracted SPR binding curves are shown for gp120 binding to immobilized (sCD4) or captured conformation-dependent ligands CD4-IgG2, IgG1-b12, and M90 over a series of gp120 concentrations (indicated above each graph, note changes in concentration range among the isolates). Raw data is shown in blue, and best-fit 1:1 binding model curves are in red. Each column shows data for a different gp120 ligand, and each row shows data for a different isolate. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis for each ligand. Because IgG2-CD4 dissociation was relatively slow, a longer dissociation phase was used. The early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).



gp120(+/- sCD4) Binding to N5i5

Figure S22. N5i5 SPR binding curves +/- sCD4. Double-reference subtracted SPR binding curves are shown for gp120 (in the presence or absence of 625 nM sCD4) binding to immobilized N5i5 over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit curves for a 1:1 binding model with the dissociation rate fixed at 0 s⁻¹ are in red. Each row shows data for a different gp120 isolate, and each column shows data for that isolate in the absence (left) or presence (right) of sCD4. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis, and differences in gp120 concentration series used in the experiment. Because N5i5 dissociation was slow, a longer dissociation phase was used. Within each column, the early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).



Figure S23. 17b SPR binding curves +/- sCD4. Double-reference subtracted SPR binding curves are shown for gp120 (in the presence or absence of 625 nM sCD4) binding to immobilized 17b over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit curves for a 1:1 binding model with the dissociation rate fixed at 0 s⁻¹ are in red. In the case of 1084i, there was sufficient dissociation to fit the dissociation rate as well using a standard 1:1 binding model. Each row shows data for a different gp120 isolate, and each column shows data for that isolate in the absence (left) or presence (right) of sCD4. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis and gp120 concentration series used in the experiment. Because 17b dissociation was slow, a longer dissociation phase was used. Within each column, the early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).



Figure S24. sCD4-induced stabilization of peptides within 17b and N5i5 epitopes. Percent deuteration plots for peptides containing CD4-induced antibody contact residues are shown for peptides in the 17b (A) and N5i5 (B) epitopes in the presence and absence of sCD4. Dashed lines with circles indicate data for unliganded gp120, solid lines with squares indicate data for sCD4-bound gp120. Each isolate is represented by a different color as indicated in the figure legend. It can be seen that CD4i antibody epitopes are differentially stabilized in the different gp120 isolates as a result of sCD4 binding.

Table S1. Summary of SPR Experimental Details

Ligand	Immobilization	Density (RU)	Analyte	Flow rate (ul/min)	Concentration Series (nM)	On (sec)	Off 1 (sec)	Off 2 (sec)	Off 2 Concs (nM)	Fitted Concs.
sCD4	EDC/NHS	85	HXB2	40	1000 -> 7.8	240	600	n/a	n/a	500 -> 31.2
		85	SF162	40	1000 -> 15.6	240	780	n/a	n/a	500 -> 31.2
		185	1084i	40	1000 -> 15.6	240	660	n/a	n/a	1000 -> 62
		185	1157ip	40	1000 -> 15.6	240	780	n/a	n/a	1000 -> 62
lgG2-CD4	$G \alpha H Fc cap^*$	80	HXB2	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		80	SF162	40	250 -> 3.9	240	720	3600	125, 62.5	250 -> 15.6
		80	10841	40	2000 -> 31.2	240	720	1800	1000, 500	1000 -> 62
		80	1157ip	40	2000 -> 31.2	240	720	1800	1000, 500	1000 -> 62
lgG1-b12	G α H Fc cap	50	HXB2	50	500 -> 3.9	240	480	n/a	n/a	62 -> 3.9
		75	SF162	40	250 -> 3.9	240	720	3600	125, 62.5	62 -> 3.9
		75	1084i	40	2000 -> 31.2	240	720	1800	1000, 500	not 1:1
		75	1157ip	40	2000 -> 31.2	240	720	1800	1000, 500	2000 -> 125
17b	G α H Fc cap	60	HXB2	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	SF162	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	1084i	40	2000 -> 31.2	240	600	3600	1000, 500	1000 -> 31.2
		60	1157ip	40	2000 -> 31.2	240	600	3600	1000, 500	2000 -> 125*
17b	G α H Fc cap	50	HXB2 + sCD4	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		50	SF162 + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	31.2 -> 1.9*
		50	1084i + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8
		50	1157ip + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8*
N5i5	G α H Fc cap	60	HXB2	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	SF162	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	1084i	40	2000 -> 31.2	240	600	3600	1000, 500	500 -> 31.2*
		60	1157ip	40	2000 -> 31.2	240	600	3600	1000, 500	1000 -> 62.5*
N5i5	G α H Fc cap	50	HXB2 +sCD4	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		50	SF162 + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	31.2 -> 1.9*
		50	1084i + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	62.5 -> 3.9*
		50	1157ip + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8*
M90	G α M Fc cap**	50	HXB2	40	2000 -> 31.2	240	660	n/a	n/a	500 -> 31.2
		50	SF162	40	2000 -> 31.2	240	660	n/a	n/a	500 -> 31.2
		50	1084i	40	2000 -> 31.2	240	660	n/a	n/a	1000 -> 62.5
		50	1157ip	40	2000 -> 31.2	240	660	n/a	n/a	1000 -> 62.5
B18	G α M Fc cap	75	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
		75	SF162	40	1000 -> 31.2	240	720	n/a	n/a	not 1:1
		75	1084i	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
		75	1157ip	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
C4	G α M Fc cap	75	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5
		75	SF162	40	1000 -> 31.2	240	720	n/a	n/a	1000 -> 62.5
		75	1084i	40	2000 -> 31.2	240	900	n/a	n/a	500 -> 31.2
		75	1157ip	40	2000 -> 31.2	240	900	n/a	n/a	500 -> 31.2
CA13	$G \alpha M Fc cap$	70	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	2000 -> 125
		70	SF162	40	2000 -> 31.2	240	720	n/a	n/a	1000 -> 62.5
		70	1084i	40	4000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5
		70	1157ip	40	4000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5

* Goat anti-human, Fc-specific antibody capture ** Goat anti-mouse, Fc-specific antibody capture n.d. experiment not done

Table S2. Chi^2 error of 1:1 binding vs. fixed dissociation rate (kd = 0 s⁻¹) fits to CD4i antibody SPR data

		Float	kd	Fixed kd = 0 s ⁻¹	
	Isolate	ka (1/M-s)	Chi2	ka (1/M-s)	Chi2
17b	HXB2	1.52(4) ×10 ⁴ *	0.405(7)	1.51(3) ×10 ⁴	0.36(6)
	SF162	5.06(8) ×10 ⁴	4.4(8)	5.06(7) ×10 ⁴	4.4(8)
	1084i	9.5(1) ×10 ³	0.32(9)	-	-
	1157ip	4.43(3) ×10 ³	0.114(1)	4.38(3) ×10 ³	0.121(2)
17b + sCD4	HXB2	_	-	-	_
	SF162	4.24(5) ×10 ⁵	0.332(2)	4.19(6) ×10 ⁵	0.323(1)
	1084i	$5.8(5) \times 10^4$	0.17(9)	-	-
	1157ip	5.59(9) ×10 ⁴	0.07(2)	5.5(1) ×10 ⁴	0.08(2)
N5i5	HXB2	2.2(1) ×10 ⁴	3.0(3)	2.12(6) ×10 ⁴	3.28(4)
	SF162	3.94(3) ×10 ⁴	4.01(9)	3.94(2) ×10 ⁴	3.98(8)
	1084i	3.11(2) ×10 ⁴	1.5(4)	3.12(4) ×10 ⁴	1.50(3)
	1157ip	9.6(2) ×10 ³	0.51(5)	9.7(2) ×10 ³	0.50(7)
N5i5 + sCD4	HXB2	-	-	-	-
	SF162	2.8(1) ×10 ⁵	1.2(2)	2.9(2) ×10 ⁵	1.2(2)
	1084i	1.6(1) ×10 ⁵	0.3(3)	1.51(3) ×10 ⁵	0.1(4)
	1157ip	6.1(2) ×10 ⁴	0.21(1)	6.1(2) ×10 ⁴	0.214(9)

(-) experiment was not performed or the fixed kd model was not applied

* error on the last significant figure is presented in parentheses as in Table 2 from the main text.

Supporting Methods for Peptide Competition ELISA

Peptides for the peptide competition ELISA were obtained from the NIH AIDS Reagent Program (Consensus Subtype B Env 15-mer peptide set, #9480). The peptide competition ELISA was carried out exactly as described for the standard ELISA in the main text, with a few modifications. SF162 gp120 was plated at 50 ng/well in PBS at 4°C overnight. All peptides were resuspended in dimethyl sulfoxide (DMSO) just prior to use. During the blocking step, antibodies C4 and B18 at 5 μ g/mL (or a 1:40 dilution for the CA13 supernatant) were incubated for 1 hour at room temperature with a 40-fold molar excess of peptide. After washing the plate, the antibody/peptide mixture was diluted in a 4-fold concentration series into antibody dilution buffer containing a constant concentration of peptide (~1.2 μ M). Matched volumes of DMSO were added to the "no peptide" control samples. Following incubation of the primary antibody for 1 hour at room temperature, washing, secondary antibody, and development were carried out exactly as described for the standard ELISA.