

## Recurring conformation of the human immunodeficiency virus type 1 gp120 V3 loop

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### Abstract

The crystal structure of the human immunodeficiency virus type 1 (HIV-1) neutralizing, murine Fab 83.1 in complex with an HIV-1 gp120 V3 peptide has been determined to 2.57 Å resolution. The conformation of the V3 loop peptide in complex with Fab 83.1 is very similar to V3 conformations seen previously with two other neutralizing Fabs, 50.1 and 59.1. The repeated identification of this same V3 conformation in complex with three very different, neutralizing antibodies indicates that it is a highly preferred structure for V3 loops on some strains of the HIV-1 virus.

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### Introduction

The human immunodeficiency virus type 1 (HIV-1) virus is a membrane-coated lentivirus whose outer surface is embedded with spikes made up of the envelope glycoproteins gp120 and gp41. These two surface proteins are originally encoded by the single polypeptide chain gp160 and, after proteolytic cleavage (McCune et al., 1988), stay non-covalently associated on the viral surface as oligomeric, probably trimeric, assemblies (Earl et al., 1990; Gelderblom et al., 1987; Ozel et al., 1988; Pinter et al., 1989; Weiss, et al., 1990). The gp41 protein contains a membrane-spanning domain that anchors it to the viral membrane so that gp120 remains noncovalently associated around the membrane-bound trimeric gp41. The gp120 protein is responsible for binding to the major cellular receptor, CD4 (Dalglish et al.,

1984; Klatzmann et al., 1984), as well as to coreceptors, such as CCR5 (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996) or CXCR4 (Feng et al., 1996).

Sequence analysis of gp120 has revealed five variable regions in the protein, termed V1–V5 (Starcich et al., 1986). The third hypervariable region (V3) of gp120 consists of approximately 40 residues (varying in length depending on the viral isolate) with a conserved disulfide bridge (Cys 296–Cys 331; HXB2 numbering) at the base of the loop. The V3 loop region is integral to many aspects of HIV-1 viral infectivity. Variation in the V3 sequence is linked with changes in cell tropism (Cann et al., 1992; Chavda et al., 1994; Chesebro et al., 1992, 1996; Harrowe and Cheng-Mayer, 1995; Hwang et al., 1991; Mammano et al., 1995; Stamatatos and Cheng-Mayer, 1993), the ability to induce syncytia (Bhattacharyya et al., 1996; de Jong et al., 1992b; Fouchier et al., 1992; Hogervorst et al., 1995; Milich et al., 1997), the ability to be neutralized by soluble CD4 (Hwang et al., 1992), and the progression from initial infection to full-blown AIDS (Distler et al., 1995). These features of V3 can all be correlated with its role in the interaction of gp120

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with coreceptors CCR5 or CXCR4 (Cocchi et al., 1996; Speck et al., 1997; Wang et al., 1998; Xiao et al., 1998). The V3 loop has also been termed the “principal neutralizing determinant” (Javaherian et al., 1989) because peptides and protein fragments containing the V3 region were originally described as having a high propensity to elicit neutralizing antibodies (Goudsmit et al., 1988; Palker et al., 1988; Rusche et al., 1988).

Early excitement about the possibility of creating an effective vaccine based only on intact gp120 or smaller fragments containing the V3 loop region has since abated. Although these constructs can induce a strong immune response toward V3, the antibodies generated, for the most part, neutralize only laboratory-adapted isolates (TCLA) (Mascola et al., 1994; Matthews, 1994) and are usually specific for only a small number of different strains (Palker et al., 1988). This puzzling phenomenon is most likely due to differential exposure of V3 on different virus strains, and at different stages of the viral entry process. Primary viral isolates, which utilize the CCR5 coreceptor and are responsible for most initial infections, are extremely resistant to neutralization by the vast majority of antibodies, including most directed against the V3 loop. Thus, the V3 loop may be inaccessible in primary isolates, either due to masking by carbohydrate or because of tertiary or quaternary interactions with the gp120 oligomeric complex. As viruses adapt to live in cell culture, or mutate *in vivo* in the progression toward full-blown AIDS, their V3 regions change in sequence and acquire more positively charged residues (de Jong et al., 1992a; Fouchier et al., 1992). The viruses then become more amenable to neutralization, presumably because their V3 regions become more accessible for interaction with antibodies, due either to local conformational changes within or around V3 or to destabilization of the gp120 oligomeric assembly.

The gp120 glycoprotein appears to adopt several different conformational states, such as after the cleavage of gp160 to gp120 and gp41 (McCune et al., 1988), after CD4 binding (Kang et al., 1993; McKeating et al., 1992; Myszkka et al., 2000; Sattentau and Moore, 1991; Sattentau et al., 1993; Thali et al., 1993; Xiang et al., 2002), and after interaction with coreceptor (Chan and Kim, 1998; Freed and Martin, 1995; Wyatt and Sodroski, 1998). These conformational changes include variation in V3 shape or exposure (Stamatatos and Cheng-Mayer, 1995), as shown by changes in V3 reactivity with conformation-dependent antibodies. Better understanding of the structural changes that can take place in gp120 is essential to fully explain mechanisms of receptor binding and membrane fusion.

To date, the only available crystal structures for gp120 are for deglycosylated core regions of gp120 from HXB2 and YU2 isolates, where 52 N- and 19 C-terminal, 67 V1/V2, and 32 V3 loop residues (298–229) have been deleted (Kwong et al., 1998, 2000). While these groundbreaking structures of monomeric gp120 core regions have provided a plethora of extraordinarily valuable structural

information, many unanswered questions remain about the range of conformational states that gp120 can adopt. By studying the conformations of V3 that are recognized by neutralizing antibodies, we aim to resolve the issue of how the V3 loop is differentially recognized by antibodies and how alterations in its sequence, conformation, or exposure may affect the biological and virological properties of the virus.

To address these questions, our laboratory and others have carried out various structural studies of peptides with sequences corresponding to V3 loops from different HIV-1 isolates. We have previously determined crystal structures for neutralizing Fabs 50.1 (Rini et al., 1993), 59.1 (Ghiara et al., 1994, 1997), and 58.2 (Stanfield et al., 1999) in complex with V3 peptides (encompassing residues P303–P320, KRKRIHIGPGRFYTT) and have shown that two of the antibodies (50.1 and 59.1) recognize one particular V3 conformation, while 58.2 recognizes an alternate V3 conformation that differs mainly at the extreme loop tip encompassing the highly conserved GPGRAF sequence. However, both V3 conformations contain a  $\beta$ -strand (residues KRKIHI) followed by a double  $\beta$ -turn around residues GPGRAF (Fig. 1).

A recent crystal structure has been determined for a V3 fusion protein, where V3 was inserted into the coat protein VP1 from the rhinovirus type 14 virus and the structure of the entire virus was determined (Ding et al., 2002). The insert contained V3 residues IGPGRFYTTKN, with a three-residue linker (ADT) located at the N-terminus. In the virus crystal structure, a type I turn exists around DTIG, a type II turn around GRAF, and another type I turn around TTKN, but, surprisingly, the GPG region, which is always a  $\beta$ -turn in complex with neutralizing Fabs, is in an extended conformation in this viral fusion protein (Fig. 1). The V3 insert is not involved in any crystal contacts; however, it makes many contacts with the chimeric viral protein, possibly influencing its conformation (Ding et al., 2002). This modified virus still binds Fabs 59.1 and 58.2, so presumably the V3 insert can adjust its conformation to conform to these two quite different antibody-combining sites.

In addition, NMR studies have been carried out for the neutralizing antibody 0.5 $\beta$  in complex with a peptide corresponding to the IIIB isolate sequence (Balbach et al., 2000; Tugarinov et al., 1999, 2000; Weliky et al., 1999; Zvi et al., 1995, 1997, 2000), which forms a hairpin turn when bound to the antibody (Fig. 1). Another recent NMR study of an MN V3 peptide bound to antibody 447-52D confirms that the peptide again forms a hairpin turn, but with a  $\gamma$ -turn around residues GPGR (Sharon et al., 2003) (Fig. 1). A large number of other NMR studies have indicated that V3 peptides are mostly disordered in water solution, with some evidence for transient  $\beta$ -turns around the GPGR region (Catasti et al., 1995, 1996; Chandrasekhar et al., 1991; de Lorimier et al., 1994; Dettin et al., 1993, 1997; Ghiara et al., 1997; Gupta et al., 1993; Huang et al., 1996, 1997; Markert et al., 1996; Sarma et al., 1997; Vranken et al., 1996; Vu et

al., 1996, 1999; Zvi et al., 1992). NMR experiments designed to confer more secondary structure on the peptides have included adding 20% TFE (Catasti et al., 1995, 1996; Chandrasekhar et al., 1991; Vranken et al., 1996; Zvi et al., 1992), which usually stabilizes an  $\alpha$ -helical region C-terminal to the GPGR motif (Fig. 1). Other methods that have been used to stabilize V3 loop peptide conformation in solution include peptide cyclization (Cabezas et al., 2000; Chandrasekhar et al., 1991; Gupta et al., 1993; Huisman et al., 2000; Tolman et al., 1993; Vranken et al., 1995, 1996, 2001), addition of the conformationally restricted residue aminoisobutyric acid (Cabezas et al., 2000; Ghiara et al., 1997), glycosylation of the peptide (Huang et al., 1996, 1997; Markert et al., 1996), attachment to resin beads (Jelinek et al., 1997b), attachment to a bacteriophage viral coat protein (Jelinek et al., 1997a), and attachment to other carrier proteins, such as BPTI (Wu et al., 2000), and MUC1 (Fontenot et al., 1995). These stabilized peptides are more ordered, with increased turn propensity in the GPGR region. Interestingly, attachment of V3 peptides to the filamentous bacteriophage fd viral coat protein pVIII (Jelinek et al., 1997a) stabilizes a peptide conformation with a double turn similar to that seen in the the crystal structure of the Fab 59.1–peptide complex.

Antibody 83.1 is one of a group of five antibodies isolated from mice by Repligen Corp. in early HIV-1 neutralization studies (White-Scharf et al., 1993). These antibodies were all raised against a 40-mer disulfide-linked peptide containing the V3 sequence from the MN viral isolate. While over 85,000 different hybridomas were produced in these experiments, only 5 were chosen for further study based on their superior neutralization properties. Some monoclonal antibodies are very isolate-specific, while others neutralize a broader range of viral strains, with two of them (83.1 and 58.2) even capable of neutralizing M-tropic isolates and field isolates passaged only one to two times (White-Scharf et al., 1993).

## Results and discussion

Despite good quality electron density maps (Fig. 2), repeated cycles of refinement and manual rebuilding re-

Table 1  
Average  $l/\sigma$  values as a function of  $l$  index

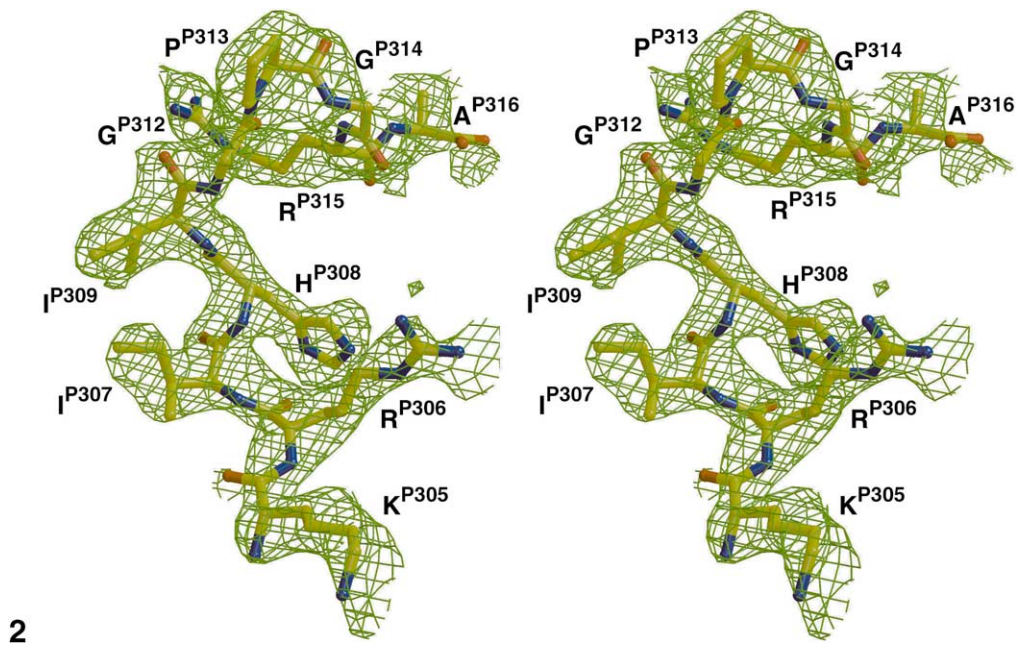
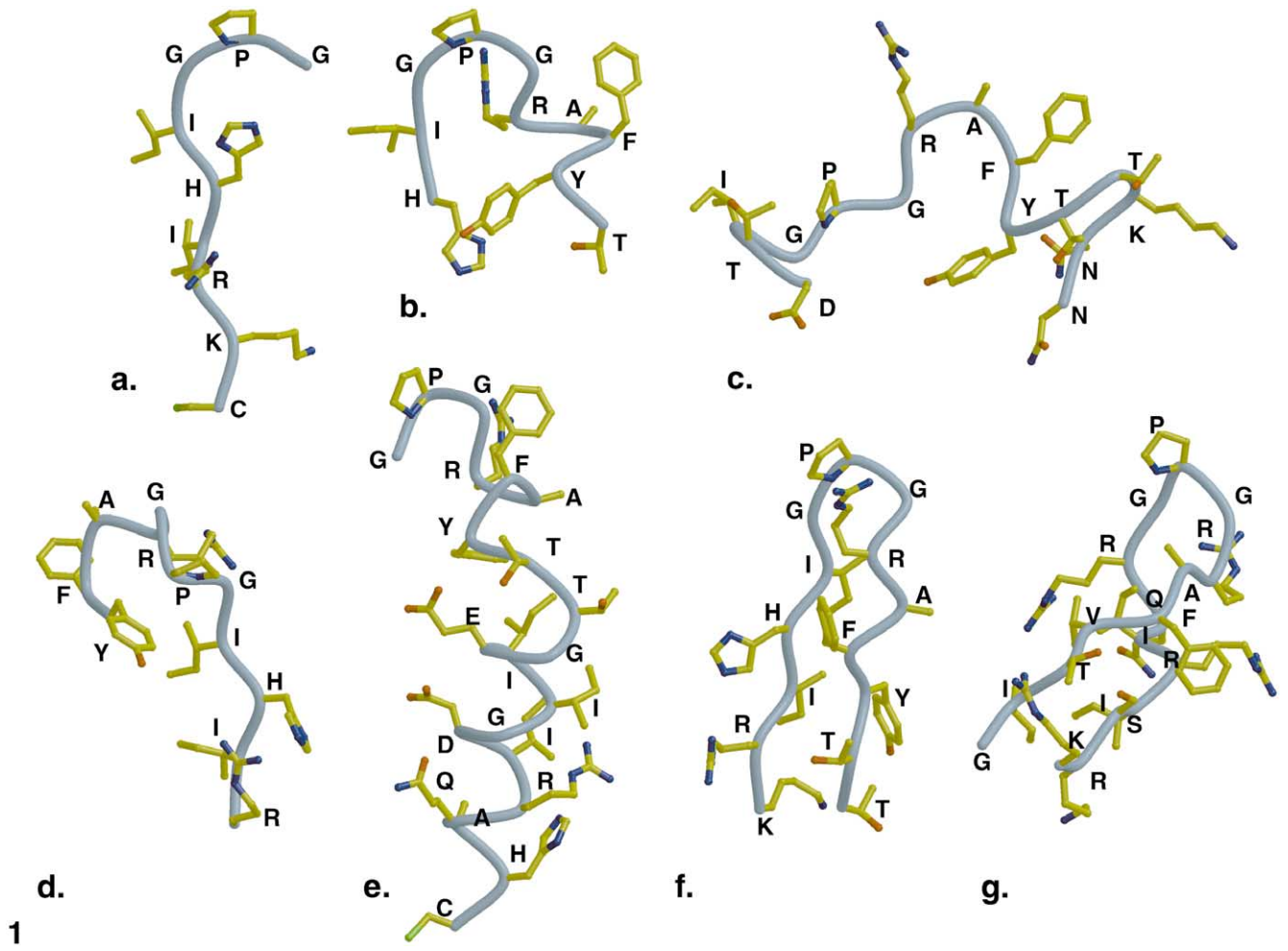
$l$	$l^*0.45$	$\langle l/\sigma \rangle$	No. of reflections
0	0.00	10.3	832
1	0.45	8.6	874
2	0.90	10.0	887
3	1.35	7.8	895
4	1.80	8.1	895
5	2.25	7.7	900
6	2.70	7.8	903
7	3.15	9.7	896
8	3.60	7.3	894
9	4.05	11.4	882
10	4.50	7.3	876
11	4.95	9.9	858
12	5.40	6.0	839
13	5.85	7.3	812
14	6.30	5.4	779
15	6.75	5.9	747
16	7.20	6.2	703
17	7.65	4.7	662
18	8.10	7.4	627
19	8.55	3.8	584
20	9.00	6.3	546
21	9.45	3.4	506
22	9.90	4.8	451
23	10.35	3.2	394
24	10.80	3.2	322
25	11.25	3.1	242
26	11.70	3.0	130

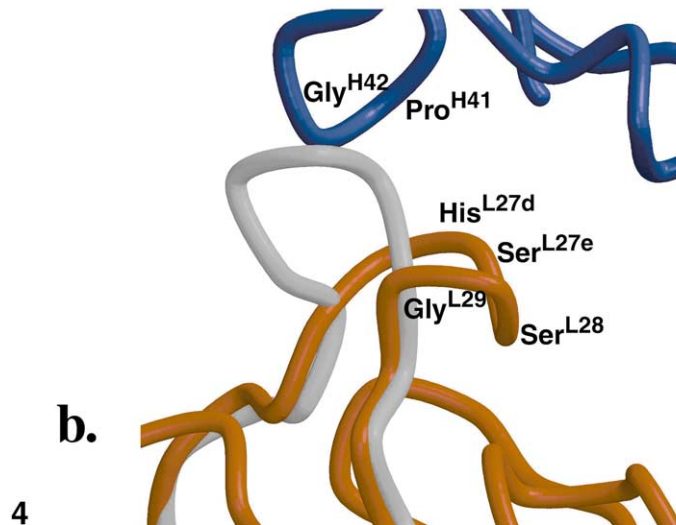
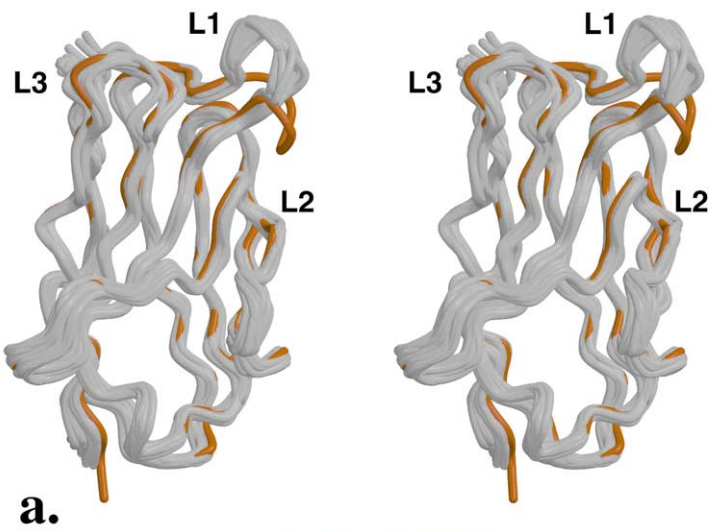
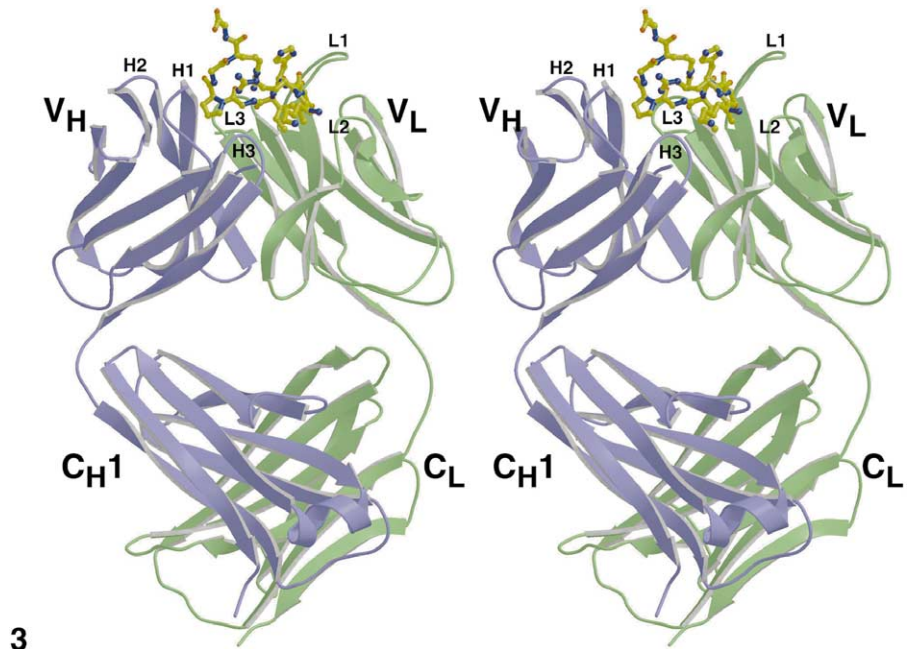
Note. Where  $l^*0.45$  is approximately integer, the average reflection intensity is higher than average. Where  $l^*0.45$  is approximately  $1/2^*$ integer, the average intensities are lower than average.

sulted in slightly higher  $R_{\text{crist}}$  and  $R_{\text{free}}$  values (28.8%, 32.6%) than typically seen for other structures determined at 2.6 Å resolution ( $R_{\text{free}} \cong 25\text{--}29\%$ ) (Kleywegt and Jones, 2002). Tests for twinning (Yeates, 1997) were negative. These slightly higher  $R$ -values appear to reflect a noncrystallographic translation ( $x,y,z = 0,0,0.45$ ) between the two Fab molecules in the asymmetric unit. A noncrystallographic translation of this type will result in reflections where the index ( $0.45^*l$ ) is close to an integer value to be systematically strong, whereas reflections where ( $0.45^*l$ ) is close to one-half integer are systematically weak. For example, the  $h,k,9$  class of reflections should be strong, while

Fig. 1. Comparison of V3 peptide conformations determined by X-ray crystallography and NMR. Peptides bound to 50.1 (a) (Rini et al., 1993; Stanfield et al., 1993) and 59.1 (b) (Ghiara et al., 1994, 1997) are very similar for the five residues in common (HIGPG) with a type II turn around GPGR. (c) The V3 insert from a V3-rhinovirus chimera (Ding et al., 2002). This structure was determined for the unliganded virus with no bound Fab. The V3 conformation has no significant structural homology with any of the others determined to date, yet the chimeric virus will bind anti-V3 neutralizing antibodies. (d) The peptide bound to Fab 58.2 (Stanfield et al., 1999) is similar to (a) and (b) for N-terminal residues RIHI and C-terminal residues AFY; however, the GPGR region has a type I turn conformation. (e) An NMR structure determined for a V3 peptide in solution with 20% TFE (Vranken et al., 1995). The N-terminal region of the peptide is highly disordered, so has not been included in this figure. This structure shows a distorted type I turn around GPGR. (f) The NMR structure of an MN peptide bound to Fab 447-52D (Sharon et al., 2003) is similar to (a) and (b) for the N-terminal extended region KRIHI, but with a  $\gamma$ -turn around GPGR. (g) The NMR structure of a IIIB peptide bound to Fab 0.5 $\beta$  (Balbach et al., 2000; Tugarinov et al., 1999, 2000; Weliky et al., 1999; Zvi et al., 1995a, 1995b, 1997, 2000) has an unclassified turn around residues GPGR, and a QR insert prior to GPGR, characteristic of IIIB-like viruses.

Fig. 2. Stereoview of electron density for the V3 peptide bound to 83.1. The peptide electron density is from a  $\sigma_A$ -weighted,  $2F_o - F_c$  map contoured at  $1.0\sigma$  with the final peptide coordinates superimposed. This figure was calculated with BobScript (Esnouf, 1999) and rendered with Raster3D (Merritt and Bacon, 1997).





**4**

the  $h,k,10$  class of reflections should be weak (Table 1). This atypical intensity distribution is reflected in the value for mean  $|E^{**2} - 1|$ , which is expected to be 0.74 for noncentrosymmetric space groups with a random intensity distribution (Karle et al., 1965), but is higher (0.80) than normal for the Fab 83.1 data. Thus, the larger percentage of systematically weak data results in slightly higher than expected  $R_{\text{cryst}}$  and  $R_{\text{free}}$  values. If reflections with  $F < 2.0\sigma F$  are removed from the calculation;  $R_{\text{cryst}}$  and  $R_{\text{free}}$  for the final model are 24.9 and 28.7%, respectively (4514 or 14.8% of the reflections would be rejected, if these criteria were used).

One residue (Val<sup>L51</sup>) from each Fab molecule is located in the disallowed region of the Ramachandran plot. This residue is the central residue in the highly conserved, three residue  $\gamma$ -turn which is the primary canonical structure seen for L2 complementarity determining region (CDR) loops (Al-Lazikani et al., 1997). Residues Ser<sup>L27e</sup> and Ser<sup>L28</sup>, which are in a mobile region at the tip of L1, are in the generously allowed region, as is Ser<sup>H15</sup>, which is correctly positioned as judged from the strong and clear electron density at this position. Ser<sup>H82b</sup>, from the second molecule in the asymmetric unit, is also in the generously allowed region and has clear electron density. Final statistics for the refined structure are listed in Table 2. The two Fab molecules in the asymmetric unit both have elbow angles of 133° (Fig. 3).

With the exception of L1, the CDR loops all fall into their expected canonical classes (Al-Lazikani et al., 1997; Chothia and Lesk, 1987; Martin and Thornton, 1996), with L2, L3, H1, and H2 belonging to Chothia canonical classes 1, 1, 1, and 1, respectively. The L1 CDR has a five amino acid insertion after residue L27, and, in both Fabs in the asymmetric unit, the tip of this loop bends away from the antigen binding site in an unusual manner. Electron density is weak for the tip of the loop (residues His<sup>L27d</sup>, Ser<sup>L27e</sup>, Ser<sup>L28</sup>, and Gly<sup>L29</sup>), and, as a result, Ser<sup>L27e</sup> and Ser<sup>L28</sup> have torsion angles in the generously allowed region of the Ramachandran plot. A comparison of this loop in 83.1 with several CDR L1 loops of the same size (Fig. 4a) shows the tip of the loop moves about 9 Å away from its corresponding position in these other Fabs, due to crystal packing, as the loop would otherwise clash with residues Pro<sup>H41</sup> and Gly<sup>H42</sup> from a symmetry-related molecule (n.b. this same crystal contact is present for both molecules in the asymmetric unit) (Fig. 4b). Residues H128–H135 for both molecules are disordered, as seen in other Fab structures, and have been assigned occupancies of 0.0 in the coordinate file.

Table 2  
Summary of crystallographic data

Data collection		
Wavelength (Å)	1.033	
Resolution (Å)	48.2–2.57 (2.66–2.57) <sup>a</sup>	
No. of observations	87,725	
No. of unique reflections	29,751	
Completeness (%)	96.5 (80.0)	
$R_{\text{sym}}$ (%) <sup>b</sup>	11.5 (39.4)	
Average $I/\sigma$	9.4 (2.1)	
Refinement statistics		
	all refl. > 0.0 $\sigma F$	All refl. > 2.0 $\sigma F^c$
Resolution (Å)	48.2–2.57 (2.66–2.57)	
Total no. of reflections	29,740	25,226
No. in test set	1469	1238
$R_{\text{cryst}}$ (%) <sup>d</sup>	28.8 (46.4)	24.9 (30.0)
$R_{\text{free}}$ (%) <sup>e</sup>	32.6 (52.5)	28.7 (35.9)
No. of Fab atoms	6615	
No. of peptide atoms	156	
No. of waters	107	
Average B values (Å <sup>2</sup> )		
Fab variable domain 1	26.9	
Fab constant domain 1	30.5	
Fab variable domain 2	28.4	
Fab constant domain 2	32.3	
Peptide 1	41.1	
Peptide 2	37.4	
Waters	19.6	
Ramachandran plot (%)		
Most favored	86.9	
Additionally allowed	11.9	
Generously allowed	0.9	
Disallowed	0.3	
r.m.s. deviations		
Bond lengths (Å)	0.007	
Angles (°)	1.6	
Dihedral (°)	26.9	
Improper (°)	0.98	

<sup>a</sup> Numbers in parentheses are for the highest resolution shell of data.

<sup>b</sup>  $R_{\text{sym}} = \sum_{\text{hkl}} |I^{\sigma} - \langle I \rangle| / \sum_{\text{hkl}} I$

<sup>c</sup> All data, including negative intensities, were used throughout the refinement; these values were calculated for the final model only.

<sup>d</sup>  $R_{\text{cryst}} = \sum_{\text{hkl}} |F_o - F_c| / \sum_{\text{hkl}} |F_o|$

<sup>e</sup>  $R_{\text{free}}$  is the same as  $R_{\text{cryst}}$  but for 5% of the data excluded from the refinement.

CDR H3 has a “kinked” or “bulged” base (Al-Lazikani et al., 1997; Morea et al., 1998; Shirai et al., 1996) that would not have been predicted from its sequence. Fab 83.1 has an Ala at H93, an Ile at H94, and an Asp at H101 (Table 3). Normally, if Asp<sup>H101</sup> is able to form a salt bridge with a charged residue at H94 (Arg or Lys), then the side chain of Trp<sup>H103</sup> will hydrogen bond to the main-chain carbonyl

Fig. 3. The structure of the Fab 83.1–V3 peptide complex. This stereoview ribbon diagram depicts one of the two Fab molecules in the asymmetric unit, with light and heavy chains in green and blue. All atoms of the bound V3 peptide are shown in a ball and stick representation with carbon atoms yellow, oxygen red, and nitrogen blue. This and subsequent figures were calculated with MolScript (Kraulis, 1991) and rendered as in Fig. 2.

Fig. 4. Noncanonical L1 CDR loop from Fab 83.1. (a) Stereoview of a superposition of the V<sub>L</sub> domain from 83.1 (red) with 27 other V<sub>L</sub> domains (gray) with the same length L1 CDR loop. The L1 CDR loop from 83.1 bends away from the canonical position by as much as 9 Å at the tip of the loop. (b) The extreme movement of the 83.1 L1 loop (red) avoids a potential crystal packing clash with residues Pro<sup>H41</sup> and Gly<sup>H42</sup> from a symmetry-related molecule (blue). The canonical conformation of L1 is shown in gray.



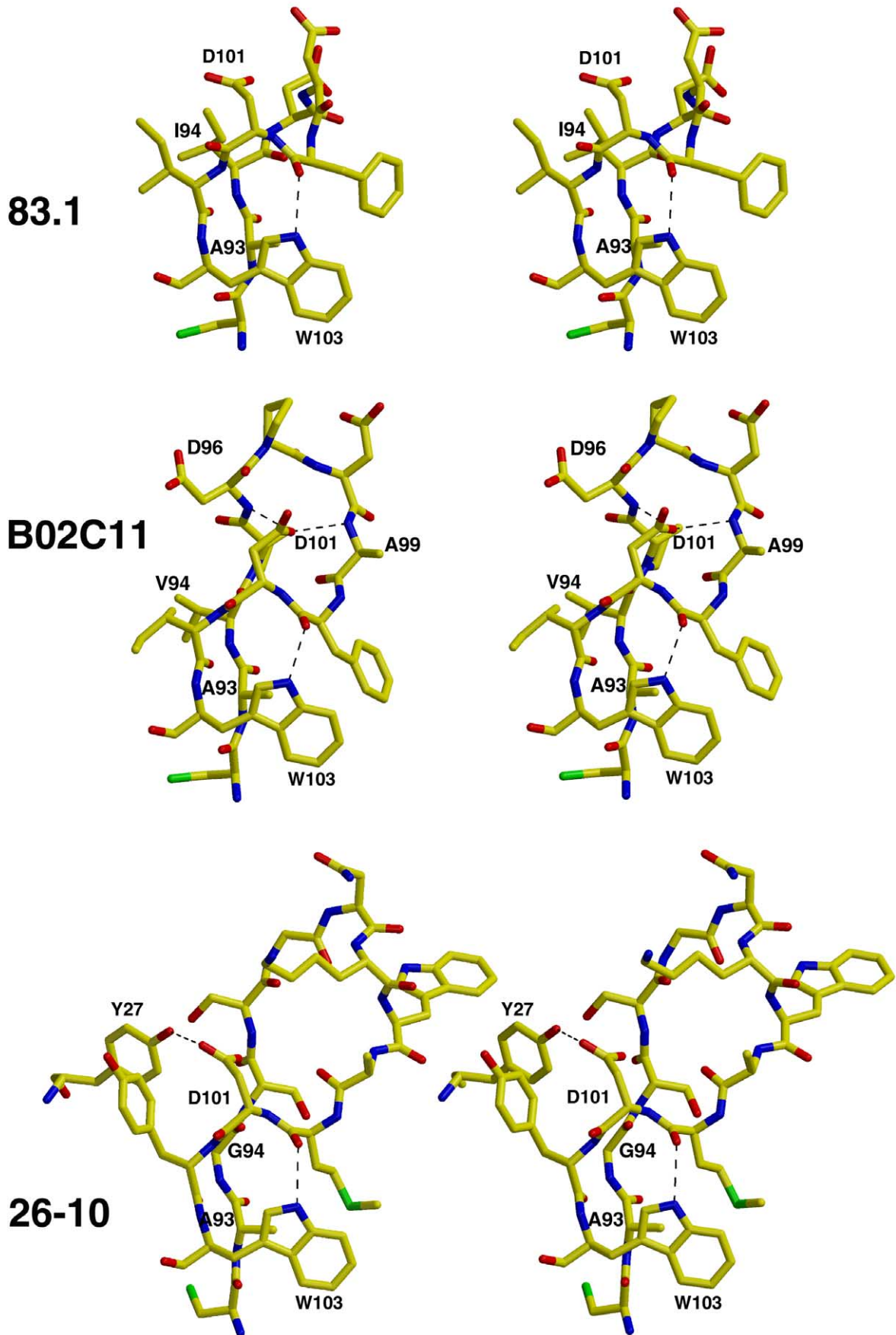


Fig. 5. Stereoview of H3 loop conformations from Fabs 83.1 (top), B02C11 (middle), and 26-10 (bottom). Each of these H3 loops has a “bulged” or “kinked” torso, rather than the predicted extended torso, with AspH101 unexpectedly not interacting with the indole nitrogen from Trp H103.

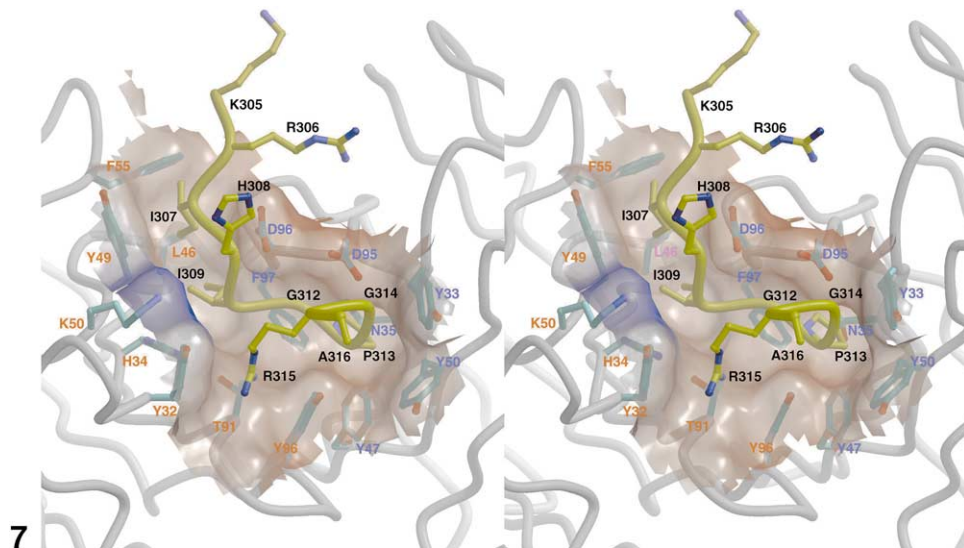
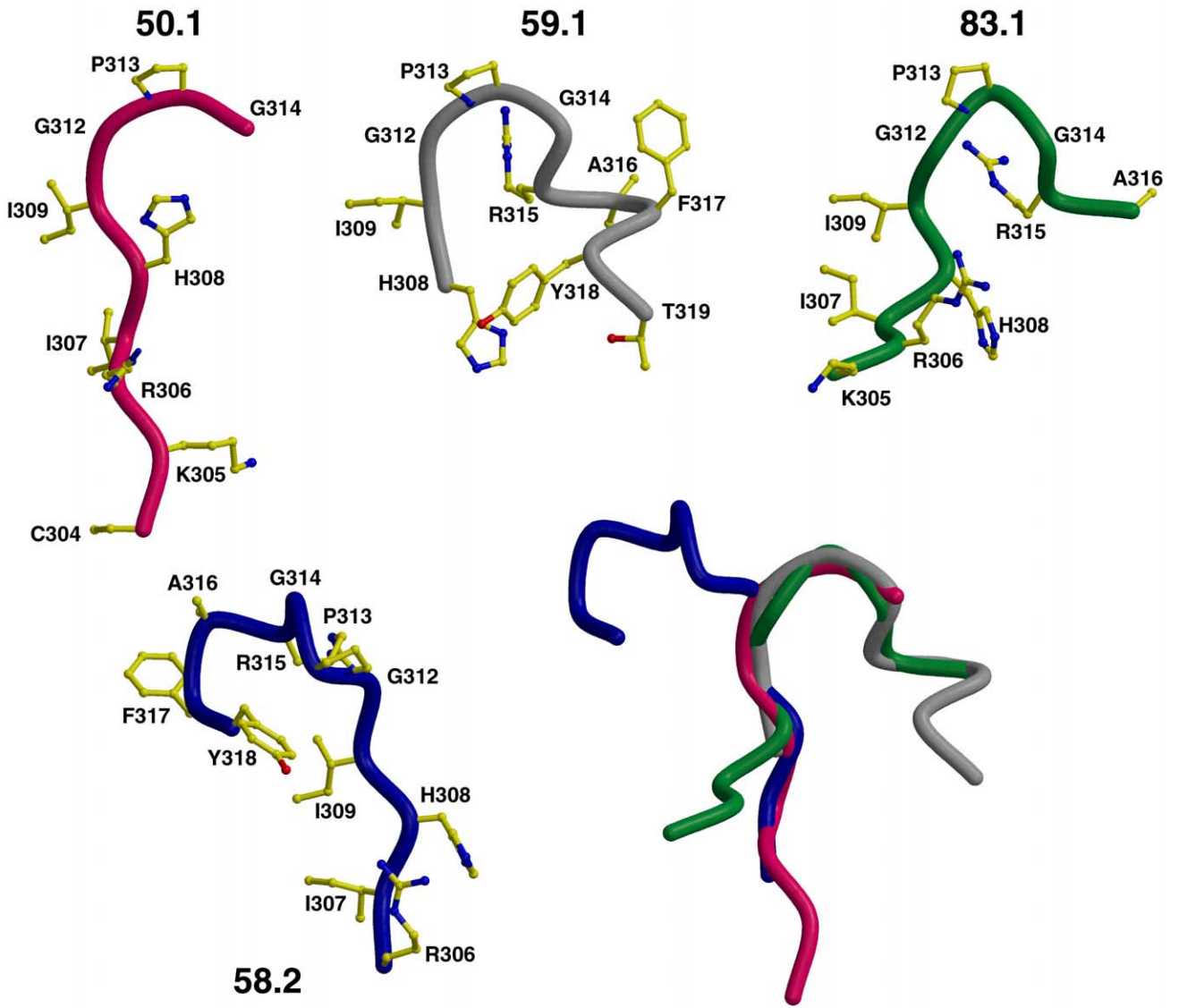




Table 3

Sequences of the anti-V3 Fabs 83.1, 59.1 (Ghiara et al., 1994, 1997), 50.1 (Rini et al., 1993), and 58.2 (Stanfield et al., 1999)

(a) Light chains	
	10 20 abcde 30 40 50
83.1	DVVMTQSPVSLPVSLGDAQASISCRSSQSLGHSSGNTYLHWFLQKPGQSPKLLIYKVSNRFS
59.1	DIVMTQSPASLVSLGQRATISCRASESVDS-YGKSFMHWYQQKPGQPPKVLIIYASNLIS
50.1	DIVLTQSPGLAVSLGQRATISCRASESVDD-DGNSFLHWYQQKPGQPPKLLIYRSSNLIS
58.2	DIVLTQSPASLAVSLGQRATISCKASQGVDF-DGASFMNWYQQKPGQPPKLLIFAASTLKS
	CDR 1 CDR 2
	60 70 80 90 100
83.1	GVPDRFSGSGSSTDFTFKISRVEAEDLGVYFCFQTHDPTFFGGGKLEI
59.1	GVPARFSGSGSRTDFTLTIDPVEADDAATYYCQONNEDPPTFGAGTKLEI
50.1	GIPDRFSGSGSRTDFTLTINPVEADDAATYYCQSNEDPLTFGAGTKLEI
58.2	GIPARFSGRSGSSTDFTLNIHPVEEEDAATYYCQOSHEDPLTFGAGTKLEI
	CDR 3
(b) Heavy chains	
	10 20 30 ab 40 50 60
83.1	EVQLQESGSPVSKPSQTLSTLTCSTVTVGVSITSGYWN--WIRKFPGNKFEYMGYISKSGSAYYN
59.1	QVKLQESGPAVSKPSQSLSLTLCIVSGFSITRTNYCWHWIRQAPGKGLEWWMGRICYEGSIYS
50.1	QVQLQESGPGILQPSQTLSTLTCSTVTVGVSITSGYWN--WIRKFPGNKFEYMGYISKSGSAYYN
58.2	DVQLQESGPDLPVSKPSQSLSLTCTVTGVSITSGYSWH--WIRQFPGNKLEWWMGYIHSAGTNYN
	CDR 1 CDR 2
	70 80 abc 90 100 abcdefghi 110
83.1	PSLKSRIISFTRDTSKNQFYKLNLSVTTEDTATYYCAIDDF-----DIWGAGTTVTVSS
59.1	PSIKSRSTISRDTSLNKFFIQLISVTNEDTAMYYCSRKNHMYETTF-----DVGWQGTVTVSS
50.1	PSLKSRLKISKDTSNNQVFLKITSVDTDATATYYCVQEGY-----IYWQGTSTVTVSS
58.2	PSLKSRIISITRDTSKNQFFLQLNLSVTTEDTATYYCAREEAMPYGNQAYYAMD CWGQGTVTVSS
	CDR 3

Note. CDR residues are shown in bold. CDR definitions and numbering according to standard conventions (Kabat et al., 1991).

oxygen of the residue three positions upstream, resulting in a kinked base. 83.1 has no charged side chain at position H94 to interact with Asp<sup>H101</sup>, and, surprisingly, the only interaction made by the Asp<sup>H101</sup> side chain is a single hydrogen bond to a water molecule. Asp<sup>H101</sup> is close (~6 Å) to the positively charged N-terminus of the peptide ligand (Lys<sup>P305</sup>, Arg<sup>P306</sup>), but makes no direct interactions with the peptide in the crystal structure. At least two other Fabs have kinked H3 bases not predicted by their sequence, Fab 26-10 (1IGJ) (Jeffrey et al., 1993) with Ala<sup>H93</sup>, Gly<sup>H94</sup>, and Asp<sup>H101</sup>, and Fab B02C11 (1IQD) (Spiegel et al., 2001) with Ala<sup>H93</sup>, Val<sup>H94</sup>, and Asp<sup>H101</sup> (Fig. 5). In the 26-10 structure, Asp<sup>H101</sup> is stabilized by a hydrogen bond to Tyr<sup>H27</sup> OH, and in the B02C11 structure, Asp<sup>H101</sup> makes two hydrogen bonds to the main-chain amide nitrogens of Asp<sup>H96</sup> and Ala<sup>H99</sup>.

Clear electron density is present for 10 residues of the 16-mer bound V3 peptide, allowing the structure of residues P305–P316 (KRIHIGPGRA) to be interpreted from the maps (Fig. 2). Additional weak electron density is seen at

either end of this 10-mer peptide, but has not been modeled due to difficulty in its interpretation. Residues at the N-terminus (KRIHIG) are in an extended conformation, followed by a type I turn around residues GPGR and the start of a type II turn around GRA. The peptide forms a double turn, very similar in shape to that seen in both the Fab 59.1-peptide (Ghiara et al., 1994, 1997) and the Fab 50.1-peptide structures (Fig. 6). Differences in main-chain torsion angles arise compared to the 59.1 peptide, due to different locations of the carbonyl oxygens of P309, P313, and P315 in the 83.1 or 59.1 structures; nevertheless, the overall shape of the peptide remains the same. Although the medium resolution data of these structures (2.8 and 3.0 Å for 59.1, and 2.57 Å for 83.1) can sometimes make the exact positioning of carbonyl oxygens problematic, repeated inspections of the 83.1 and 59.1 electron density maps indicate that the carbonyl oxygens are correctly positioned. The 83.1 peptide also differs slightly from the 50.1 and 59.1 peptides in the relative disposition of the extended region

Fig. 6. Comparison of the V3 peptide conformations from complexes with Fabs 50.1 (pink), 59.1 (gray), 83.1 (green), and 58.2 (blue). The peptides have all been superimposed onto the V3 loop peptide from 50.1, and their overlapped C $\alpha$  traces are shown at the bottom right. The 83.1 peptide has a very similar shape to the 50.1 and 59.1 peptides for both its extended region (KRIHIG) and its crown (GPGR); however, a slight difference is seen in the disposition of these two segments. The superposition for 83.1 was carried out using the crown residues.

Fig. 7. The antibody combining site of Fab 83.1. Stereoview of the Fab binding site and its interactions with the V3 peptide. Contacting residues were identified with Contacsym (Sheriff et al., 1987a). The peptide residues are labeled in black; the light chain residues are labeled in red, and the heavy chain residues are labeled in blue. The molecular surface for the Fab was calculated with GRASP (Nicholls et al., 1991) and is colored according to the electrostatic potential (blue = positive, red = negative, surface potential from -15 to 15 kT).

Table 4  
Hydrogen bonding distances between Fab and peptide

Fab atom	Peptide atom	distance (Å)	
		mol1	mol2
Asp <sup>H96</sup> Oδ2	Ile <sup>P307</sup> N	3.68	3.31
Lys <sup>L50</sup> Nζ	His <sup>P308</sup> O	3.12	2.96
Asp <sup>H96</sup> Oδ2	His <sup>P308</sup> N	3.23	3.20
Thr <sup>L91</sup> Oγ1	Ile <sup>P309</sup> O	3.30	2.93
Asp <sup>H96</sup> Oδ1	Ile <sup>P309</sup> N	2.71	2.71
Asp <sup>H96</sup> Oδ1	Gly <sup>P312</sup> N	2.83	2.82
Asp <sup>H95</sup> Oδ1	Gly <sup>P314</sup> N	3.01	3.22
Thr <sup>L91</sup> O	Arg <sup>P315</sup> NH2	2.66	2.69

*Note.* Hydrogen bonds calculated with HBPLUS (McDonald and Thornton, 1994).

(P305–P312) versus the double turn region (P312–P316), by an angle of about 60° (Fig. 6).

The peptide makes contact with both the light and the heavy chains from the Fab, with 110 (107) total contacts for molecule 1 (molecule 2) (Fig. 7). Of these contacts, 7 (8) are hydrogen bonds, with no charge-charge interactions (Table 4). Six (7) hydrogen bonds are to peptide main-chain atoms, with 1 (1) bond to the Arg<sup>P315</sup> side chain. The H3 CDR makes the most contacts (39%, 37%), followed by H1 (20%, 15%), L1 (10%, 12%), L2 (4%, 4%), L3 (9.1%, 14.0%), H2

(7%, 6%), light-chain framework residues (10%), and heavy-chain framework residues (1%). The peptide buries 478 (479) Å<sup>2</sup>, while the Fab buries 490 (549) Å<sup>2</sup> of molecular surface, with about 50 (59)% of the total from the heavy chain.

The 83.1-peptide structure is the fourth crystal structure determined for a neutralizing antibody–V3 peptide complex. Analysis of the conformations of the four peptides reveals that three of the peptides share very similar conformations (Fig. 6), while that of the fourth (58.2) differs around the residues at the tip of V3 (GPGR). The four anti-peptide antibodies (83.1, 50.1, 59.1, 58.2) were all generated during the same set of experiments from related mice, and the peptides used for immunization and for cocrystallization all correspond to the MN viral isolate sequence. However, the antibodies themselves do not have strong sequence (Table 3) or structural homology (Fig. 8), and in fact, have CDR loops of various size, shape, and sequence composition. Thus, the similarity among conformation of the three peptides bound to 50.1, 59.1, and 83.1 is not because the Fabs themselves are highly similar. In fact, the peptides, although adopting the same shapes, bind in different orientations and locations in the antibody-combining site (Fig. 8). Because the antibodies were chosen for their ability to neutralize, and, hence, to bind to intact virus, these

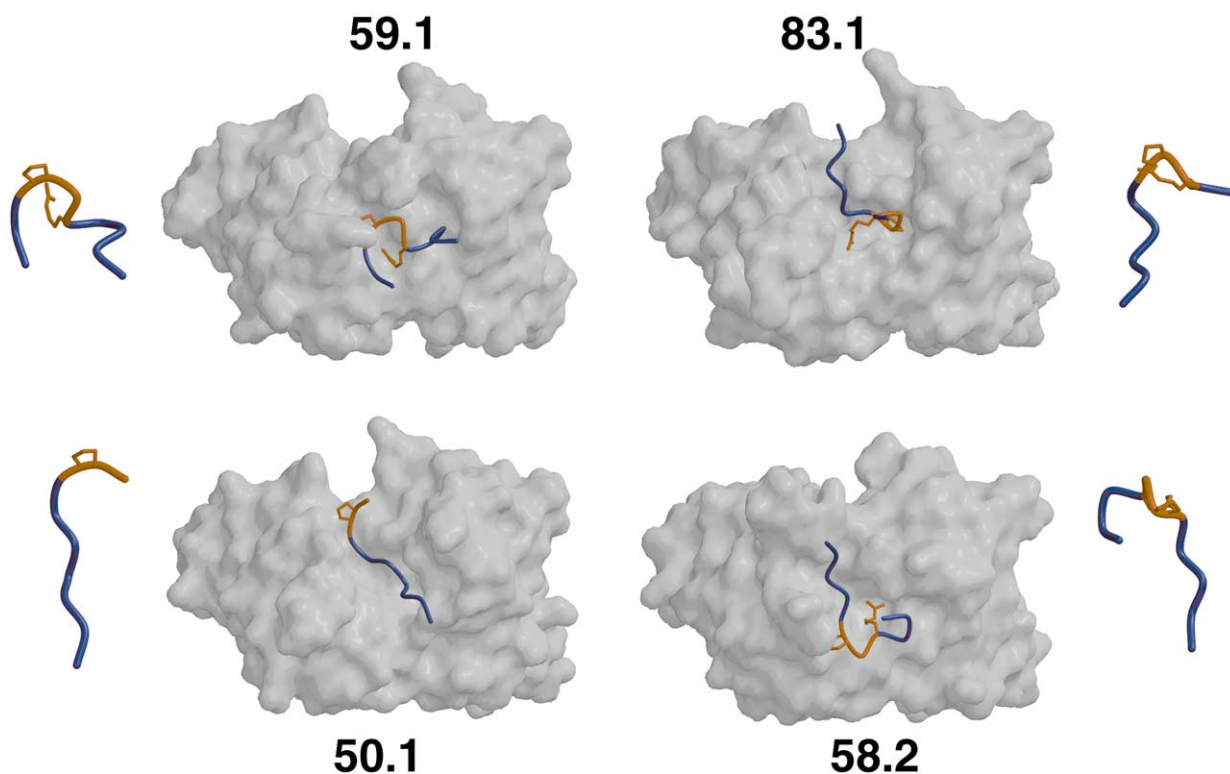


Fig. 8. Antigen binding sites of Fabs 83.1, 59.1, 58.2, and 50.1. These four Fabs have only limited sequence homology in their variable domains, reflected in their binding sites for the V3 loop, which are shaped and oriented differently in each Fab; nevertheless, the striking similarity in peptide conformation persists despite very different antigen binding sites. Residues GPGR are shown in red, with their side chains also represented, while the backbone of the remainder of the peptide is shown in blue. Molecular surfaces calculated with GRASP (Nicholls et al., 1991).

peptide conformations should reflect highly populated or energetically preferred conformations of the V3 loop on the intact virus. If V3 were totally disordered and had no stable structure, it is unlikely that three different neutralizing antibodies would recognize such similar peptide conformations. Thus, the V3 loop structures that we have identified would appear to represent a recurring and highly populated conformer on the intact virus, suggesting that the different peptide conformation observed when bound to Fab 58.2 may also be conformationally accessible on the intact virus.

These X-ray crystallographic “snapshots” of V3 peptides in complex with neutralizing antibodies help define the range of conformations that V3 can adopt. As most studies suggest that V3 interacts with coreceptors CCR5 and CXCR4 during viral cell entry, this structural information may be useful in the design of V3-based fusion inhibitors. Ultimately, a better understanding of the quaternary structure of intact gp120/gp41 oligomers as they exist on the viral surface, and of the role of V3 in this complex protein assembly, is essential for truly understanding how HIV-1 carries out its receptor binding and viral fusion activities.

## Materials and methods

### *Fab purification and crystallization*

MAb 83.1 (IgG1, $\kappa$ ) was generated by immunization of ASW mice with cyclic peptide RP70 (INC\*TRPN-YNKRKRIHIGPGRAFYTTKNIIGTIRQAHC\*NIS with a disulfide bond between the two C\* residues) corresponding to the MN sequence (White-Scharf et al., 1993). The antibody was produced in ascites fluid of BALB/c mice and purified with an immobilized protein A column. Fab fragments were produced from immunoglobulin by cleavage with 3% pepsin for 2 h at 37°C, reduction with 15 mM cysteine for 3 h at 37°C, followed by purification on a protein A and then a protein G column (Smith, 1993). Fab was concentrated to 15.0 mg/ml for crystallization studies.

Fab was mixed with linear 16-mer peptide MP1 (CKRI-HIGPGRAFYTTC) in a 6:1 (peptide:Fab) molar ratio. Crystals were grown using the sitting-drop vapor diffusion method with a reservoir solution (1 ml) of 1.6 M Na/K phosphate, 5% isopropanol, pH 6.0. One microliter of Fab/peptide complex was mixed with an equal volume of the reservoir solution. The crystals grow as clusters of thin plates. Crystals were usually observed after 3 days; however, the crystal used for data collection grew over a 2-week period, was slightly thicker than typically seen, and grew singly with no satellite crystals attached.

### *Data collection*

As the 83.1-peptide crystals suffer from significant radiation-induced decay, even when cryocooled to liquid nitro-

gen temperatures, efforts were taken to collect the data as rapidly as possible. Data were collected from a single crystal at the Advanced Photon Source, beamline 19-ID, at 100K, using a wavelength of 1.033 Å and a step size of 0.5° with a CCD detector. The crystals were cryoprotected by a quick plunge into a solution containing 25% glycerol, 1.6 M Na/K phosphate, 5% isopropanol, pH 6.0. The data were reduced in monoclinic space group P2<sub>1</sub> with unit cell dimensions  $a = 60.5$  Å,  $b = 122.6$  Å,  $c = 69.5$  Å, and  $\beta = 108.5^\circ$  and a refined mosaicity of 0.8°. Data were indexed, integrated, and scaled using HKL2000 (Otwinowski and Minor, 1997) using all observations greater than  $-3.0\sigma$ . Data collection statistics are outlined in Table 2.

### *Structure determination*

The Matthews coefficient ( $V_m$ ) (Matthews, 1985) was estimated as  $2.6 \text{ \AA}^3/\text{D}$  based on two Fab molecules in the asymmetric unit (53% solvent). The native Patterson map showed an unexpected peak at  $u, v, w = 0.0, 0.0, 0.45$  with a peak height of  $37\sigma$  (the origin peak height was  $150\sigma$ ) indicative of a noncrystallographic translation between the two Fab molecules in the asymmetric unit of approximately 0, 0, 1/2. Rotation functions were carried out using the Crowther fast rotation function (Crowther and Blow, 1967), as implemented in MERLOT (Fitzgerald, 1988), against our database of 125 intact Fab models. Strong rotation function solutions were found for models with elbow angles of approximately 135°, with Fab 59.1 (1ACY) (Ghiara et al., 1994) providing the best solution. A model was then constructed from the constant region (C<sub>H1</sub>, C<sub>L</sub>) of Fab 58.2 (from unpublished coordinates refined to 1.6 Å resolution, and with the appropriate sequence for an IgG1,  $\kappa$ ) (Stanfield et al., 1999), the V<sub>L</sub> domain of an anti-Dansyl antibody (1DLF) (Nakasako et al., 1999) (88% sequence identity), and the V<sub>H</sub> domain of HyHEL-63 (1DQQ) (Li et al., 2000) (84% sequence identity) assembled with an elbow angle of 135° by superposition over the corresponding domains of Fab 59.1 (1ACY). The EPMP (Kissinger et al., 1999) program was then used to correctly position this model in the cell, followed by positional refinement using four individual domains (the variable and constant regions from each molecule in the asymmetric unit). EPMP located the first Fab molecule in the asymmetric unit with a correlation coefficient of 28.7% and an  $R$ -value of 55.8%, and, subsequently, the second Fab molecule (holding the first molecule fixed) with a correlation coefficient of 50.8% and an  $R$ -value of 46.8%.

### *Model building and refinement*

The hybrid model was mutated to the correct sequence for 83.1, rebuilt using TOM/FRODO (Jones, 1982), and refined with CNS version 1.1 (Brünger et al., 1998) with an overall, anisotropic  $B$ -value correction and bulk solvent correction. All refinement and model building were carried

out using all measured data ( $F > 0.0\sigma F$ ), with tight NCS restraints early on in the refinement, which were released gradually toward the end of refinement. Electron density maps for model building included  $\sigma_A$ -weighted (Read, 1986)  $2F_0 - F_c$ ,  $F_0 - F_c$  and composite annealed omit  $2F_0 - F_c$  maps, as well as solvent flipped/NCS-averaged  $2F_0 - F_c$  maps, all calculated with CNS (Brünger et al., 1998). An  $R_{\text{free}}$  test set consisting of 5% of the reflections was maintained throughout the refinement.

### Structure analysis

The Fab molecules are numbered according to the Kabat convention (Kabat et al., 1991) with light and heavy chains labeled “L” and “H.” The peptide is labeled “P” and is numbered according to the HXB2 isolate sequence (KRI-HIGPGR; 305–309, 312–316). Buried molecular surface areas were calculated with the program MS (Connolly, 1993) with a 1.7 Å probe radius and standard van der Waals radii (Gelin and Karplus, 1979). Hydrogen bonds were evaluated using the program HBPLUS (McDonald and Thornton, 1994) and van der Waals contacts were assigned with the program Contacsym (Sheriff et al., 1987a, 1987b).

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