number of effects that had neither been previously observed in experiments nor predicted by modeling. The setting developed here opens new avenues for the exploration of basic chemistry questions: the nature and number of species involved and their transport mechanisms; the effect of anisotropy; and the effect of dynamic surface reconstructions and of gas-phase coupling. This setting permits the systematic study of pattern formation per se as well as its interactions with domain geometry. The ability to realize elementary geometries will greatly simplify the comparison of simulations and theory with experiments; this should be a serious motivation for further detailed computational and model-building efforts.

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Crystal Structure of the Principal Neutralization Site of HIV-1

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The crystal structure of a complex between a 24–amino acid peptide from the third variable (V3) loop of human immunodeficiency virus–type 1 (HIV-1) gp120 and the Fab fragment of a broadly neutralizing antibody (59.1) was determined to 3 angstrom resolution. The tip of the V3 loop containing the Gly-Pro-Gly-Arg-Ala-Phe sequence adopts a double-turn conformation, which may be the basis of its conservation in many HIV-1 isolates. A complete map of the HIV-1 principal neutralizing determinant was constructed by stitching together structures of V3 loop peptides bound to 59.1 and to an isolate-specific (MN) neutralizing antibody (50.1). Structural conservation of the overlapping epitopes suggests that this biologically relevant conformation could be of use in the design of synthetic vaccines and drugs to inhibit HIV-1 entry and virus-related cellular fusion.

The V3 loop of HIV-1 gp120 plays a critical role in viral infectivity and tropism and contains the principal neutralizing determinant (PND) of HIV-1 viruses. Neutralizing antibodies to the V3 loop can inhibit viral entry even after gp120 has bound CD4, the primary receptor for the virus (*1*). Syncytia formation, a result of the fusion of CD4-positive cells with infected cells expressing gp120, is also inhibited by these antibodies. Furthermore, mutations in the V3 loop give rise to noninfectious viruses that can still bind CD4 (*2*), indicating that the V3 loop is important for subsequent membrane fusion events. It has been proposed that binding or cleavage of the V3 loop by secondary receptors (*3*, *4*) may be required for pH-independent membrane fusion and viral entry (*5*).

The amino acid sequence of the PND is highly variable, except for a short stretch at the crown or tip of the V3 loop (*6*). Several lines of evidence already indicate that this region may adopt a conserved structure in different HIV-1 isolates. The Gly-Pro-Gly-Arg sequence, found in most North American and European isolates, occurs more frequently than any other in known type II β turns (*7*), and proton nuclear magnetic resonance (NMR) techniques have indicated a preference for a β-turn conformation in synthetic V3 peptides (*8*). A secondary structure prediction has further suggested that the V3 loop adopts a β-hairpin structure (β-strand–type II β-turn–β-strand motif), with a short helix at its carboxyl end (*6*).

Despite the sequence (and possible structural) conservation at the crown of the V3 loop, hypervariable flanking sequences cause mainly type-specific neutralizing antibodies to be generated against the various strains of HIV-1. Passive immunization with such a type-specific anti-V3 monoclonal antibody (mAb) has been shown to protect chimpanzees from individual strains of the virus (*9*). Furthermore, antibodies that primarily recognize the conserved crown of the V3 loop have recently been shown to effect a broad neutralization of different HIV-1 isolates (*10*, *11*). A three-dimensional structure for this V3 loop region could then be used in the design of more effective synthetic vaccines and, possibly, of inhibitors of the membrane fusion process.

As a structure for gp120 is not yet available, we investigated the structural features of the HIV-1 PND by determining the conformation of PND peptides in complex with neutralizing mAbs that bind overlapping epitopes within the V3 loop.

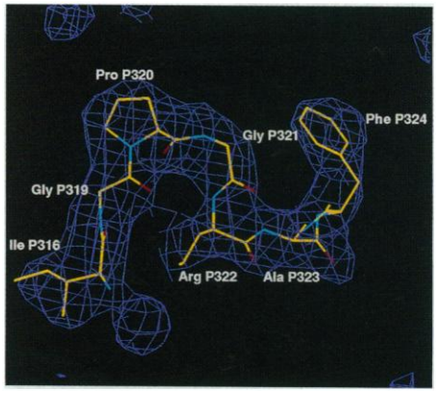

Fig. 1. $F_o - F_c$ omit electron density for the bound peptide. The density at 2.7σ is shown only around the S-shaped double turn formed by residues Gly-Pro-Gly-Arg-Ala-Phe. Peptide residue numbers are according to the BH10 isolate sequence (*12*), preceded by the letter P.

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The crystal structure of a 24–amino acid V3 loop peptide (RP142) (*12*) in complex with the Fab fragment of R/V3-59.1 (59.1), a broadly neutralizing mAb (*10*), is described here and compared to the x-ray structure (*13*) of another V3 loop peptide (MP1) (*12*) as complexed to Fab 50.1, a neutralizing mAb specific for MN and MN-like HIV-1 isolates (*10*).

The x-ray diffraction data were collected to 3 Å resolution from a single crystal of the Fab 59.1–RP142 complex (*14*), and the structure was determined by molecular replacement (*15, 16*). The entire Fab model was built into 10% $2F_o - F_c$ omit maps with FRODO (*17*) and refined with the positional and simulated annealing protocols in X-PLOR (*16*). Three alternating cycles of model building and refinement were performed before building the HIV-1 peptide into $F_o - F_c$ electron density maps. A total of eight cycles of model building and refinement resulted in the current structure, which has an *R* value of 0.21 (for all data in the 12.0 to 3.0 Å range and an overall temperature factor of 28 Å^2) and root-mean-square (rms) deviations from ideality for bond lengths and angles of 0.018 Å and 3.8°, respectively.

Clear electron density was interpretable for the central 10 residues (His-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Tyr-Thr; P315 through P316 and P319 through P326) (*12*) of the 24–amino acid peptide RP142 in the antigen-binding site of Fab 59.1 (Fig. 1). The highly conserved Gly-Pro-Gly-Arg-Ala-Phe sequence adopts an S-shaped conformation (Fig. 2) consisting of a type II β turn (Gly-Pro-Gly-Arg) followed by a type III (Gly-Arg-Ala-Phe) β turn. The type III turn is also part of a double bend, as the residues Arg-Ala-Phe-Tyr additionally form a type I turn. These turn conformations are stabilized in part by three intrapeptide hydrogen bonds. Gly^{P319} (O) makes a hydrogen bond with the side chain of Arg^{P322} (NHε), replacing the main chain *i* to *i* + 3 bond often seen in reverse turns (Fig. 2), accounting for the preference for Arg at the fourth (*i* + 3) position of type II turns (*7*). A variant of the V3-loop crown sequence, Gly-Leu-Gly-Gln, is frequently found in African isolates of HIV-1 (*4*). Although Leu is not common at the second (*i* + 1) position of a type II turn, Gln is the only other residue (besides Arg) that is preferred at the fourth position (*7*) because its side-chain NHε can form an equivalent interaction with the main-chain carbonyl oxygen at the first residue of the turn. Two hydrogen bonds occur in the III-I double bend, between Gly^{P321} (O) and Phe^{P324} (N) and between Arg^{P322} (O) and Tyr^{P325} (N). The conformation of the III-I double bend is close to that of a short segment of 3_{10} helix, but the torsional angles of Phe^{P324} are closer to the β region of φ, ψ space. Multiple bends of this type are not uncommon; in a study of 23 proteins, each representing a different group of homologous proteins, 235 single bends, 58 double bends, and 11 higher order bends were found (*18*). An HIV-1 variant with Thr substituted for Ala^{P323} has been proposed to escape neutralization through a local conformational change in the V3 loop (*19*). However, this substitution is unlikely to change the backbone conformation of the crown region unless it introduces new interactions with other regions of the V3 loop (or elsewhere in gp120).

The S-shaped peptide binds in a relatively flat binding pocket of the Fab, as compared with the deeper, concave pockets seen in other Fab-peptide complexes (Fig. 3) (*20*). The Fab surface area buried by the peptide is 469 Å^2, whereas the corresponding area on the peptide is 429 Å^2, indicating a good fit between the peptide and Fab combining site (*21*). The peptide makes contacts with all complementarity determining regions (CDRs), or hypervariable loops, of the antibody, except for CDR L2. The amide NHS from Gly^{P319}, Gly^{P321}, and Ala^{P323} each form a hydrogen bond with the Fab (*22*). The side chain of Arg^{P322} is perpendicular to the plane of the S-shaped conformation and inserts into a deep pocket in the antibody combining site, where it makes two hydrogen bonds and one buried salt bridge with residues from CDR L3 (*22*). There are 62 van der Waals contacts between the peptide and Fab residues from CDRs L1 (12 contacts), L3 (16), H1 (5), H2 (10), and H3 (19).

The crystallographic observations on the contribution made by each residue of the peptide to its binding correlate well with results obtained from epitope mapping (*10*). Competition enzyme-linked immunosorbent assays (ELISA), with a series of peptides containing Ala substitutions at each position, showed that Gly^{P319}, Arg^{P322}, or Phe^{P324} cannot be substituted (Table 1) and that replacement of Pro^{P320} or Gly^{P321} is disfavored. Gly^{P319} has tor-

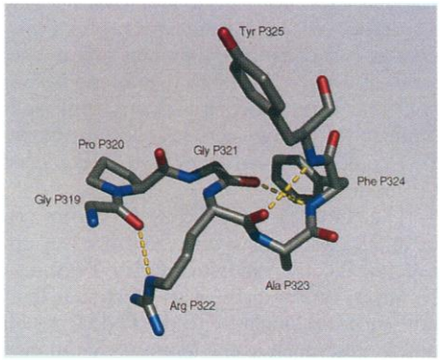

Fig. 2. Conformation of the bound peptide. The three turns in the peptide are formed by residues Gly-Pro-Gly-Arg (type II), Gly-Arg-Ala-Phe (type III), and Arg-Ala-Phe-Tyr (type I). The three intrapeptide hydrogen bonds are indicated by dotted yellow lines.

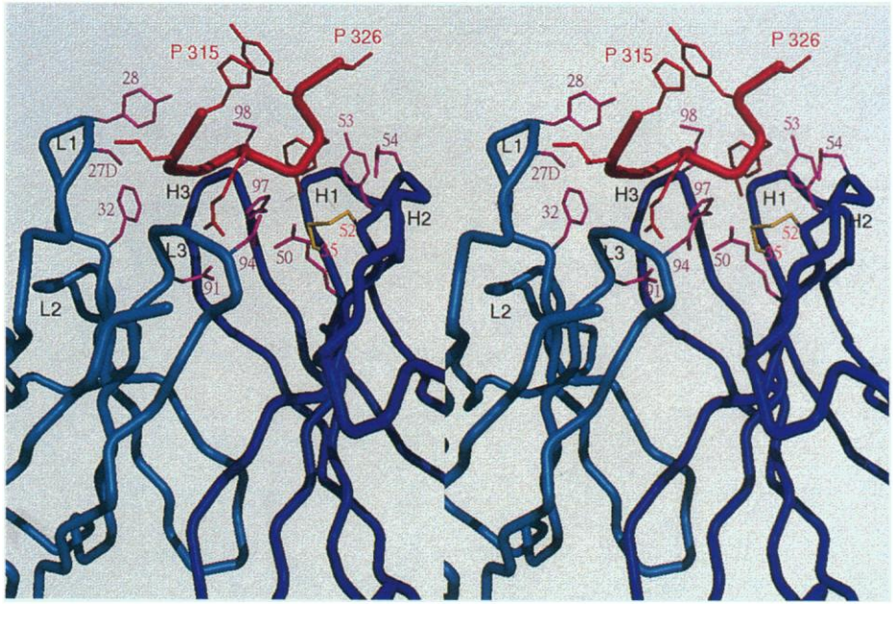

Fig. 3. Stereo view of the Fab 59.1–RP142 complex. The light (L) and heavy (H) chains are shown in light and dark blue, respectively, for only the variable (V_H and V_L) regions of Fab 59.1. The peptide is shown in red. The CDR loops of the light and heavy chain of the Fab are labeled L1 through L3 and H1 through H3, respectively. The unusual disulfide bond between CDRs H1 (Cys^{H35}) and H2 (Cys^{H52}) is shown in yellow and forms part of the binding site. The Fab side chains involved in salt bridge, hydrogen bond, or van der Waals interactions with the peptide are shown in magenta.

sional angles ($\phi = 98°$, $\psi = -173°$) corresponding to the epsilon region of ϕ, ψ space (*23*), a region not normally accessible to non-Gly residues. The most favored residue at position P320 is Pro because its inherent restriction in ϕ to about $-60°$ corresponds well with the $\phi(i + 1)$ requirement for type II turns. Also, 11 of the 14 van der Waals contacts made by Pro^{P320} involve the Cδ and Cγ atoms. Ala cannot be substituted for Gly^{P321} because non-Gly residues are disfavored at the third position of type II turns due to a steric clash between the Cβ atom and the preceding carbonyl oxygen (*7, 24*). The single most important residue for antibody-peptide binding appears to be Arg^{P322}. Its guanidinium group makes two hydrogen bonds with Asn^{L91} and one salt bridge with Asp^{L94} of CDR L3. Phe^{P324} is buried in a hydrophobic pocket that includes Tyr^{H53} (H2), Met^{H98} (H3), and a disulfide bond between Cys^{H35} (H1) and Cys^{H52} (H2). Although this disulfide (*25*) does not appear to affect the general disposition of CDR loops H1 and H2, it is significant because it covalently bridges two CDR loops, a feature not reported for any other antibody structures to date.

Table 1. Contribution of individual peptide residues to Fab binding.

Residue	Competition* (%)	Van der Waals contacts (no.)	Hydrogen bonds (HB) and salt bridges (SB)	Peptide surface area buried (*21*) (Å²)
His^{P315}	84	0	0	6
Ile^{P316}	82	3	0	52
Gly^{P319}	0	1	1 HB	28
Pro^{P320}	35	14	0	77
Gly^{P321}	32	4	1 HB	23
Arg^{P322}	0	14	2 HB +1 SB	86
Ala^{P323}	95†	9	1 HB	53
Phe^{P324}	0	17	0	93
Tyr^{P325}	85	0	0	2
Thr^{P326}	ND	0	0	9
Total		62	5 HB +1 SB	429

*Binding of mAb 59.1 to RP70 was analyzed by competition with Ala-substituted peptides in an ELISA (*10*). The antibody was preincubated with competitor peptide before being added to an ELISA plate coated with RP70. Antibody molecules not bound by the competitor were reacted with RP70 and detected with a horseradish peroxidase–labeled antibody to mouse immunoglobulin G. Results are expressed as a percentage of the antibody bound by RP70 in the absence of competitor peptides. An unsubstituted control peptide gave 75% competition for RP70 binding by 59.1. ND = not determined. †The Ala residue was replaced by a Gly in this case.

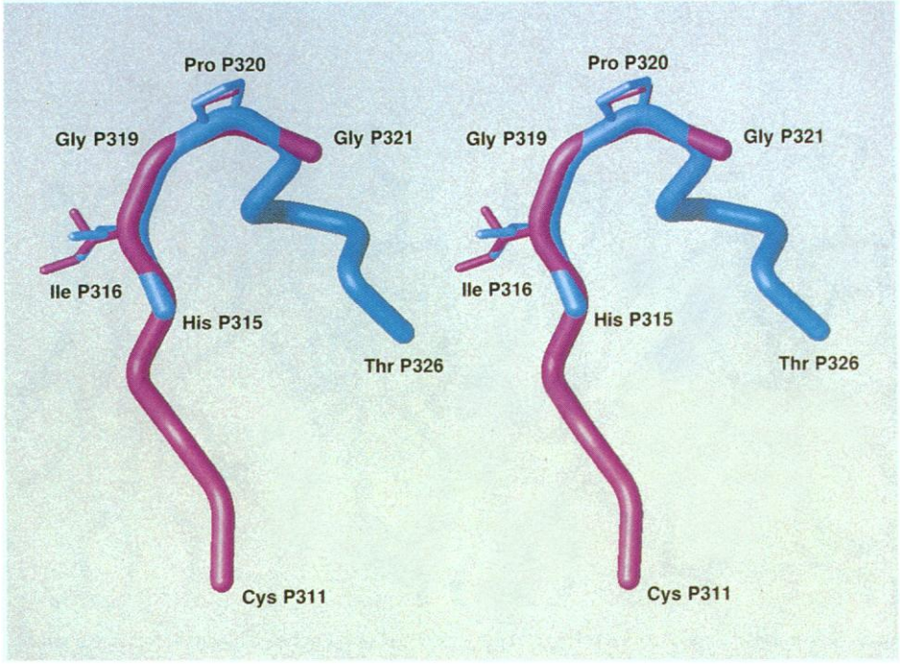

Fig. 4. Structural conservation of the V3 loop in two independent Fab-peptide complexes. Stereo pairs of the Cα traces of peptides from the 50.1 (magenta) and 59.1 (cyan) complexes are shown with their partially overlapping epitopes superimposed. Side chains for Ile^{P316} and Pro^{P320} residues of the sequence His^{P315}-Ile^{P316}-Gly^{P319}-Pro^{P320}-Gly^{P321}, observed in both peptide structures, are also shown.

The Fab-peptide structure demonstrates why mAb 59.1 is broadly neutralizing. The highly variable residues that flank the conserved crown of the V3 loop do not contribute to the antibody-peptide interactions. Indeed, all of the hydrogen bond and salt bridge contacts, and 59 of the 62 van der Waals contacts, involve only the Gly-Pro-Gly-Arg-Ala-Phe sequence. The three other van der Waals contacts are with the Cα and Cβ atoms of Ile^{P316} and hence do not dictate specificity for any particular residue at this position. Thus, the Fab can make all of the interactions seen in this structure with a wide variety of HIV-1 isolates. In addition, the Gln-Arg dipeptide insert found prior to the Gly-Pro-Gly-Arg sequence in the IIIB isolate should not prevent binding of this sequence to 59.1, which is consistent with its HIV-1 IIIB neutralizing activity (*10*).

Mutagenesis experiments (*26*) have established that alteration of Gly^{P319}, Gly^{P321}, or Arg^{P322} of gp120 abolishes the infectivity of HIV-1, although the processing, transport, and CD4-binding properties of the mutant proteins are comparable to those of wild-type gp120. Our crystallographic data demonstrate a strong structural preference for Gly residues at positions P319 and P321 of the PND. A putative protease cleavage site has been identified at Arg^{P322} (Gly-Pro-Gly-Arg*-Ala-Phe), and the cellular protease inhibitor trypstatin, which contains a homologous Gly-Pro-Cys-Arg-Ala-Phe sequence, does indeed inhibit syncytium formation (*3, 4*). The positioning and conformation of Arg^{P322}, as observed in the Fab complex, would permit a similar interaction to be made with any secondary receptor. If V3-loop cleavage, or association with a secondary receptor (*27*), does in fact constitute a crucial step in the series of events leading to membrane fusion and viral entry, the virus-neutralizing and syncytia-inhibiting activities of antibody 59.1 could be explained by restricted access to this site.

The broadly neutralizing properties of mAb 59.1 also suggest that this crown-region structure is conserved in many HIV-1 isolates. However, on the basis of its functional (but not sequence) similarity to the PND loops of other viruses such as the foot-and-mouth disease virus (*28*), the intact V3 loop structure in gp120 may have some segmental flexibility in the absence of a receptor.

The 59.1 complex can now be compared with the crystal structure of another V3-loop Fab-peptide complex (50.1-MP1), in which

eight residues of the V3 loop (Lys-Arg-Ile-His-Ile-Gly-Pro-Gly) are ordered in the antibody-binding pocket (*12*). These two antibodies thus recognize partially overlapping epitopes on the V3 loop. When their corresponding V3-loop peptide structures are superimposed (Fig. 4), the conformation of the five common residues (His-Ile-Gly-Pro-Gly) is remarkably similar (*29*). Residues Gly-Pro-Gly-Arg form a type II β turn in the 59.1-peptide structure, and although Arg^{P322} is not observed in the 50.1 structure, Pro^{P320}-Gly^{P321} have ϕ_2, ψ_2, and ϕ_3 angles in the range found for type II β turns. In both structures, Gly^{P319} has main-chain torsional angles corresponding to the epsilon region (*23*) of conformational space, further confirming a structural requirement for Gly at position P319. The 50.1-peptide complex also suggests that a hydrogen-bonded β hairpin structure is not a feature of V3-loop recognition (*13*).

The similarity in the conformation of the five overlapping peptide residues in two different Fab complexes suggests that the V3 loop structure we have assembled is biologically relevant. The Fab binding sites of 59.1 and 50.1 are completely different in shape, consistent with a low-sequence identity (46%) between their CDR loop sequences. Hence, the peptides do not adopt similar conformations merely as a result of a fit to similar binding sites. As these antibodies neutralize HIV-1 and prevent syncytia formation (*10*), it would appear that the peptide structures are representative of their cognate structures in gp120, when the virus is bound to the neutralizing antibody (*30*). Further structural studies will confirm the extent to which the V3 loop structure proposed here represents its conformation as bound to any secondary receptor.

The V3-loop conformation observed in the antibody-peptide complexes is not exactly as predicted by neural network analysis of the amino acid sequences from different HIV-1 isolates (*6*). In the prediction, greater confidence was assigned to the Gly-Pro-Gly-Arg turn and the first β strand than for the helix and the second β strand. The Fab-peptide structures provide experimental evidence for the presence of the first β strand and the β turn. However, this turn does not appear to be followed by a β strand as predicted. Instead a type III-I double bend is formed by residues Gly-Arg-Ala-Phe-Tyr, giving rise to an S-shaped conformation for the crown of the V3 loop. Cyclized peptides, and other analogs that restrict the peptide conformations to those that enhance the formation of the appropriate turn structures in the V3 loop, may generate a better immune response toward HIV-1. In addition, compounds that closely mimic the conserved crown structure of the V3 loop should be capable of generating a more broadly neutralizing response. Such structure-based analogs may also prove to be useful as inhibitors for the proposed secondary receptors of HIV-1.

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12. Amino acid sequences of the V3-loop peptides with residues numbered according to the BH10 isolate sequence [L. Ratner *et al.*, *Nature* **313**, 277 (1985)], preceded by the letter P, are RP142 (YNKRKRI**HIGPGRAFYT**TKNIIGC*, P308 through P316 and P319 through P333), MP1 (***CKRIHIGPG**RAFYTTC*, P311 through P316 and P319 through P328), and RP70 (INCTRP-NYNKRKRIHIGPGRAFYTTKNIIGTIRQAHCNIS, P301 through P316 and P319 through P341). Peptide residues P317 and P318 form a dipeptide insert (QR) in the BH10 isolate but are absent in the MN strain. The mAb 59.1 (immunoglobulin G1; κ) was elicited with RP70, which corresponds to the complete disulfide-linked (at the underlined Cys residues) loop of the HIV-1 MN isolate. The epitope residues RP142 and MP1 identified in the crystal structures of their Fab complexes are shown in bold. The asterisk indicates that the Cys residues at those amino and carboxy terminal positions were acetamidomethylated. The mAb 59.1 binds the conserved sequence GPGRAF, which occurs in about 60% of known PND sequences. (A, Ala; C, Cys; F, Phe; G, Gly; H, His; I, Ile; K, Lys; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and Y, Tyr).
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14. Fab 59.1 was prepared by enzymatic digestion of the mAb with pepsin followed by controlled reduction in the presence of cysteine. Crystals of Fab 59.1 in complex with peptide RP142 were grown by vapor diffusion in sitting drops (5 μl), containing 1.65 M mixed phosphate [NaH_2PO_4 and K_2HPO_4 (pH 6.75)], with 10 mg of Fab per milliliter at 22°C. The space group was determined by precession photography to be $C222_1$, with unit cell dimensions a = 89.7 Å, b = 154.0 Å, and c = 121.9 Å. The Matthews coefficient (V_M) of the crystals [B. W. Matthews, *J. Mol. Biol.* **33**, 491 (1968)] is 4.2 $Å^3$ per dalton, and the solvent content is 71% for one Fab molecule per asymmetric unit. Data were collected from a single, large crystal (1.25 mm by 0.4 mm by 0.16 mm) on a Siemens multiwire area detector mounted on a GX-20 rotating anode, equipped with Franks focusing mirrors with a 200-μm focal spot and a helium cone, at a maximum power of 40 mA and 50 kV. Detector data were processed with the XENGEN program package [A. J. Howard *et al.*, *J. Appl. Crystallogr.* **20**, 383 (1987)]. The data consist of 79,118 total observations of 16,265 unique reflections and are 94% complete to 3 Å with an $R_{sym}(I)$ of 11.6%.
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16. We computed cross-rotation functions for various Fab models with MERLOT [P. M. D. Fitzgerald, *J. Appl. Crystallogr.* **21**, 273 (1988)] using data in the 8.0 to 4.0 Å resolution range. On the basis of these results, we constructed a model by independently superimposing the variable and constant regions of Fab 50.1 onto the corresponding regions of McPC603 [Y. Satow, G. H. Cohen, E. A. Padlan, D. R. Davies, *J. Mol. Biol.* **190**, 593 (1986)] to incorporate the elbow angle of McPC603 (133.2°). Appropriate residues were replaced with the BIOPOLYMER module of Insight II [version 1.0, Biosym Technologies (1990)] to build the 59.1 sequence into this model. The rotational solution was optimized by Patterson correlation refinement, and the translation function was solved with X-PLOR [A. T. Brünger, *Acta Crystallogr. Sect. A* **46**, 46 (1990); X-PLOR, version 2.1].
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30. Both apomyohemerythrin and a 19–amino acid synthetic peptide adopt similar conformations in the binding site of Fab B13I2 [R. L. Stanfield and I. A. Wilson, unpublished results; R. L. Stanfield, T. M. Fieser, R. A. Lerner, I. A. Wilson, *Science* **248**, 712 (1990)].

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