Sciencexpress Research Article

Structural Basis for Broad and Potent Neutralization of HIV-1 by Antibody VRC01

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During HIV-1 infection, antibodies are generated against the region of the viral gp120 envelope glycoprotein that binds CD4, the primary receptor for HIV-1. Among these antibodies, VRC01 achieves broad neutralization of diverse viral strains. Here we determine the crystal structure of VRC01 in complex with an HIV-1 gp120 core. VRC01 partially mimics CD4 interaction with gp120. A shift from the CD4-defined orientation, however, focuses VRC01 onto the vulnerable site of initial CD4 attachment, allowing it to overcome the glycan and conformational masking that diminishes the neutralization potency of most CD4-binding-site antibodies. To achieve this recognition, VRC01 contacts gp120 mainly through V-gene-derived regions substantially altered from their genomic precursors. Partial receptor mimicry and extensive affinity maturation thus facilitate neutralization of HIV-1 by natural human antibodies.

Successful vaccine development often takes advantage of clues from humoral responses elicited by natural infection. For HIV-1, neutralizing antibody responses elicited within the first year or two of infection are generally strain-specific (*1*), and thus provide poor leads for vaccine development [reviewed in (*2*)]. A few monoclonal antibodies from HIV-1 infected individuals, however, are broadly neutralizing, and an effort has been made to facilitate vaccine design by defining their structures (*3-4*).

The well-studied broadly neutralizing anti-HIV-1 antibodies, 2G12, 2F5, 4E10, and b12, have unusual characteristics that have posed barriers to eliciting similar antibodies in humans (*5*). Thus, in addition to having broad capacity for neutralization, an appropriate antibody should be present in high enough titers in humans to suggest that such antibodies can be elicited in useful concentrations. We and others have screened cohorts of sera from infected individuals to find broadly neutralizing responses that are detectable in a substantial percentage of subjects (*6-10*). One serum response that satisfies these criteria has been mapped to the site on HIV-1 gp120 envelope (Env) glycoprotein that binds to the CD4 receptor (*8*).

While potentially accessible, the CD4-binding site is protected from humoral recognition by glycan and conformational masking (*11*). The identification of monoclonal antibodies against this site is described in a companion manuscript (*12*). In brief, we created resurfaced, conformationally stabilized probes, with antigenic specificity for the initial site of CD4 attachment on gp120 (*22*). This site, a conformationally invariant subset of the CD4-binding surface, is vulnerable to antibody-mediated neutralization (*22*), and we used probes specific for this site to identify antibodies that neutralize most viruses (*12*). Here, we analyze the crystal structure for one of these antibodies, VRC01, in complex with an HIV-1 gp120 core. We decipher the basis of VRC01 neutralization, identify mechanisms of natural resistance, show how VRC01 minimizes such resistance, examine potential barriers to elicitation, and define the role of affinity maturation in gp120 recognition.

Similarities of Env recognition by CD4 and VRC01 antibody. To gain a structural understanding of VRC01 neutralization, we crystallized the antigen-binding fragment (Fab) of VRC01 in complex with an HIV-1 gp120 from the clade A/E recombinant 93TH057 (*13*). The crystallized gp120 consisted of its inner domain-outer domain core, with truncations in the variable loops V1/V2 and V3 as well as the N- and C-termini, regions known to extend away from the main body of the gp120 envelope glycoprotein (*14*). Diffraction to 2.9 Å resolution was obtained from

orthorhombic crystals, which contained four copies of the VRC01-gp120 complex per asymmetric unit, and the structure was solved by molecular replacement and refined to an R-value of 19.7% (R_{free} of 25.6%) (Fig. 1 and table S1) (*15*).

The interaction surface between VRC01 and gp120 encompasses almost 2500 Å^2, 1244 Å^2 contributed by VRC01 and 1249 Å^2 by gp120 (*16*). On VRC01, both heavy chain (894 Å^2) and light chain (351 Å^2) contribute to the contact surface (table S2), with the central focus of binding on the heavy chain-second complementarity-determining region (CDR H2). Over half of the interaction surface of VRC01 (644 Å^2) involves CDR H2, a mode of binding reminiscent of the interaction between gp120 and the CD4 receptor; CD4 is a member of the V-domain class of the immunoglobulin superfamily (*17*), and the CDR2-like region of CD4 is a central focus of gp120 binding (Figs. 2A and table S3) (*18*). For CD4, the CDR2-like region forms antiparallel, intermolecular hydrogen-bonds with residues 365-368$_{gp120}$ of the CD4-binding loop of gp120 (*18*) (Fig. 2B); with VRC01, one hydrogen-bond is observed between the carbonyl of Gly54$_{VRC01}$ and the backbone nitrogen of Asp368$_{gp120}$. This hydrogen-bond occurs at the loop tip, an extra residue relative to CD4 is inserted in the strand, and the rest of the potential hydrogen bonds are of poor geometry or distance (Fig. 2C and table S4). Other similarities and differences with CD4 are found: of the two dominant CD4 residues (Phe43$_{CD4}$ and Arg59$_{CD4}$) involved in interaction with gp120, VRC01 mimics the arginine interaction, but not the phenylalanine one (Fig. 2B, C). Finally, significant correlation is observed between gp120 residues involved in binding VRC01 and CD4 (fig. S1).

Superposition of the gp120 core in its VRC01-bound form with gp120s in other crystalline lattices and bound by other ligands indicates a CD4-bound conformation (PDB ID 3JWD) (*14*) to be most closely related in structure, with a Cα-root-mean-square deviation of 1.03 Å (table S5). Such superposition of gp120s from CD4-bound and VRC01-bound conformations brings the N-terminal domain of CD4 and the heavy chain-variable domain of VRC01 into close alignment (Fig. 2), with 73% of the CD4 N-terminal domain volume overlapping with VRC01 (*19*). This domain overlap is much higher than observed with the heavy chains of other CD4-binding site antibodies, such as b12, b13 or F105 (table S6). However, when the VRC01 heavy chain is superimposed - based on conserved framework and cysteine residues - on CD4 in the CD4-gp120 complex, clashes are found between gp120 and the entire top third of the VRC01 variable light chain (Fig. 2D) (*20*). In its complex with gp120, VRC01 rotates 43° relative to the CD4-defined orientation, and translates 6-Å away from the bridging sheet, to a clash-free orientation that mimics many of the interactions of CD4 with gp120, though with considerable variation. Analysis of electrostatics shows that the interaction surfaces of VRC01 and CD4 are both quite basic, though the residues types of contacting amino acids are distinct (fig. S2). Thus, while VRC01 mimics CD4 binding to some extent, considerable differences are observed.

Structural basis of VRC01 breadth and potency. When CD4 is placed into an immunoglobulin context by fusing its two N-terminal domains to a dimeric immunoglobulin constant region, it achieves reasonable neutralization. VRC01, however, neutralizes better (Fig. 3A) (*12*). To understand the structural basis for the exceptional breadth and potency of VRC01, we analyzed its interactive surface with gp120. VRC01 focuses its binding onto the conformationally invariant outer domain, which accounts for 87% of the contact-surface area of VRC01 (table S7). The 13% of the contacts made with flexible inner domain and bridging sheet are non-contiguous and are not critical for binding. In contrast, CD4 makes 33% of its contacts with the bridging sheet, and many of these interactions are essential (*18*). The reduction in inner domain and bridging sheet interactions by VRC01 is accomplished primarily by a 6-Å translation relative to CD4, away from these regions; critical contacts such as made by Phe43$_{CD4}$ to the nexus of the bridging sheet-outer domain are not found in VRC01, while those to the outer domain (e.g. Arg59$_{CD4}$) are mimicked by VRC01.

To determine the affinity of VRC01 for gp120 in CD4-bound and non-CD4-bound conformations, we used surface-plasmon resonance spectroscopy to measure the affinity of VRC01 and other gp120-reactive antibodies and ligands to two gp120s: a β4-deletion developed by Harrison and colleagues that is restrained from assuming the CD4-bound conformation (*21*) or a disulfide-stabilized gp120 core, largely fixed in the CD4-bound conformation in the absence of CD4 itself (*18*) (Fig. 3B and fig. S3). VRC01 showed high affinity to both CD4-bound and non-CD4-bound conformations, a property shared by the broadly neutralizing b12 antibody (*21-22*). By contrast, antibodies F105 and 17b as well as soluble CD4 showed strong preference for either one, but not both, of the conformations.

To assess the binding of VRC01 in the context of the functional viral spike, we examined its ability to neutralize variants of HIV-1 with gp120 changes that affect the ability to assume the CD4-bound state. Two of these mutations, His66Ala$_{gp120}$ and Trp69Leu$_{gp120}$, are less sensitive (*23*), while a third, Ser375Trp$_{gp120}$, is more sensitive to neutralization by CD4 (*23-24*). VRC01 neutralized all three of these variant HIV-1 viruses with similar potency (Fig. 3C), suggesting that VRC01 recognizes both CD4-bound and non-CD4-bound conformations of the viral spike. This recognition diversity allows VRC01 to avoid the conformational masking

that hinders most CD4-binding-site ligands (*25*) and to neutralize HIV-1 potently (*26*).

Precise targeting by VRC01. Prior analysis of effective and ineffective CD4-binding-site antibodies suggested that precise targeting to the vulnerable site of initial CD4 attachment is required to block viral entry (*11, 27*). This site represents the outer domain-contact site for CD4 (*22*). Analysis of the VRC01 interaction with gp120 shows that it covers 98% of this site (Figs. 4A, 4B and fig. S4), comprising 1089 $Å^2$ on the gp120 outer domain, about 50% larger than the 730 $Å^2$ surface covered by CD4. The VRC01 contact surface outside the target site is largely limited to the conformationally invariant outer domain and avoids regions of conformational flexibility. This concordance of binding is much greater than for ineffective CD4-binding-site antibodies, as well as for those that are partially effective, such as antibody b12 (*11, 22*) (fig. S4).

The outer domain-contact site for CD4 is shielded by glycan (*22*). Contacts by the VRC01 light chain ($Tyr28_{VRC01}$ and $Ser30_{VRC01}$) are made with the protein-proximal N-acetyl-glucosamine from the N-linked glycan at residue 276_{gp120} (*28*). Thus, instead of being occluded by glycan, VRC01 makes use of a glycan for binding. Other potential glycan interactions may occur with different strains of HIV-1 gp120, as the VRC01 recognition surface on the gp120-outer domain extends further than that of the functionally constrained CD4 interaction surface, especially into the loop D and the often-glycosylated V5 region (fig. S5).

Natural resistance to antibody VRC01. In addition to conformational masking and glycan shielding, HIV-1 resists neutralization by antigenic variation. In a companion manuscript, we show that of the 190 circulating HIV-1 isolates tested for sensitivity to VRC01, 173 were neutralized and 17 were resistant (*12*). To understand the basis of this natural resistance to VRC01, we analyzed all 17 resistant isolates by threading their sequences onto the gp120 structure (fig. S5). Variation was observed in the V5 region in resistant isolates, and this variation – along with alterations in gp120 loop D - appeared to be the source of most natural resistance to VRC01 (Fig. 4C, figs. S5, S6).

Because substantial variation exists in V5, structural differences in this region might be expected to result in greater than 10% resistance. The lower observed frequency of resistance suggests that VRC01 employs a recognition mechanism that allows for binding despite V5 variation. Examination of VRC01 interaction with V5 shows that VRC01 recognition of V5 is considerably different from that of CD4 (fig. S7), with $Arg61_{VRC01}$ in the CDR H2 penetrating into the cavity formed by the V5 and β24-strands of gp120 (fig. S8). Most importantly, the V5 loop fits into the gap between heavy and light chains; thus by contacting only the more conserved residues at the loop base, VRC01 can tolerate variation in the tip of the V5 loop (Fig. 4D).

Unusual VRC01 features and contribution to recognition. We examined the structure of VRC01 for special features that might be required for its function. A number of unusual features were apparent, including a high degree of affinity maturation, an extra disulfide bond, a site for N-linked glycosylation, a 2-amino acid deletion in the light chain, and an extensively matured binding interface between VRC01 and gp120 (Fig. 5 and fig. S9). We assessed the frequency with which these features were found in HIV-1 Env-reactive antibodies (SOM Appendix) or in human antibody-antigen complexes (fig. S10 and tables S8, S9), and measured the effect of genomic reversion of these features on affinity for gp120 and neutralization of virus (Fig. 5A-D, table S10).

Higher levels of affinity maturation have been reported for HIV-1 reactive antibodies in general (*29*), and markedly higher levels for broadly neutralizing ones (*30*). These maturation levels could be a by-product of the persistent nature of HIV-1 infection, and may not represent a functional requirement. Removal of the N-linked glycosylation or the extra disulfide bond, which connects CDR H1 and H3 regions of the heavy chain, had little effect on binding or neutralization (Figs. 5A, 5B and table S10). Insertion of 2-amino acids to revert the light chain deletion had moderate effects, which were larger for an Ala-Ala insertion (50-fold decrease in K_D) versus a Ser-Tyr insertion (5-fold decrease in K_D), which mimics the genomic sequence (Fig. 5C and table S10). Finally reversion of the interface was examined with either single-, 4-, 7- or 12-mutant reversions. For the single-mutant reversions of the interface to the genomic antibody sequence, all 12 mutations had minor effects (most with less than 2-fold effect on K_D, with the largest effect for a Gly54Ser change with a K_D of 20.2 nM) (table S10). Larger effects were observed with multiple (4, 7 or 12) changes, and these reduced the measured K_D by 5-30 fold and EC_{50}s or neutralization percentages by 10-100 fold (Fig. 5D and table S10). Thus, while VRC01 has a number of unusual features, no single alteration to genomic sequence reduced binding or neutralization by more than 10-fold.

Elicitation of VRC01-like antibodies. The probability for elicitation of a particular antibody is a function of each of the three major steps in B cell maturation: 1. Recombination to produce nascent antibody heavy and light chains from genomic V_H-D-J and $V_{\kappa/\lambda}$-J precursors; 2. Deletion of auto-reactive antibodies; and 3. Maturation through hypermutation of the variable domains to enhance antigen affinity. For the recombination step, a lack of substantial CDR L3 and H3 contribution to the VRC01-gp120 interface (table S2) indicates that specific $V_{\kappa/\lambda}$ -J or V_H(D)J- recombination is not required (*31*) (fig. S11). The majority of recognition occurs

with elements encoded in single genomic elements or cassettes, suggesting that specific joints between them are not required. Within the V_H cassette, a number of residues associated with the IGHV1-02*02 precursor of VRC01 interact with gp120; many of these are conserved in related genomic V_Hs, some of which are of similar genetic distance from VRC01 (fig. S12). These results suggest that appropriate genomic precursors for VRC01 are likely to occur at a reasonable frequency in the human antibody repertoire.

Recombination produces nascent B cell-presented antibodies that have reactivities against both self and nonself antigens. Those with auto-reactivity are removed through clonal deletion. With many of the broadly neutralizing anti-HIV-1 antibodies, such as 2G12 (glycan reactive) (*32*-*33*), 2F5 and 4E10 (membrane reactive) (*34*-*35*), this appears to be a major barrier to elicitation. While this remains to be characterized for genomic revertants and intermediates, no auto-reactivity has so far been observed with VRC01 (*12*).

The third step influencing the elicitation of VRC01-like antibodies is affinity maturation, a process involving hypermutation of variable domains combined with affinity-based selection that occurs during B cell maturation in germinal centers (*36*). In the case of VRC01, 41 residue alterations were observed from the genomic V_H-gene and 25 alterations from the V_K-gene (including a deletion of two residues) (fig. S13) (*37*). To investigate the effect of affinity maturation on HIV-1 gp120 recognition, we reverted the V_H- and V_K-regions of VRC01, either individually or together, to the sequences of their genomic precursors. We tested the affinity and neutralization of these reverted antibodies (Fig. 6A), and combined these data with the genomic reversion data obtained while querying the unusual molecular features of VRC01 (previous section) (Fig. 6B).

No antibodies containing genomic V_H and V_κ regions bound gp120 or neutralized virus (*38*). Binding affinity and neutralization showed significant correlations with the number of affinity-matured residues ($p<0.0001$). Interestingly, binding to stabilized gp120 did not correlate well with other types of gp120 or to neutralization (table S11), related in part to greater retention of binding to VRC01 variants with genomically reverted V_κ regions. Extrapolation of the correlation to the putative genomic V-gene sequences predicted binding affinities of 0.6 μM K_D for gp120 stabilized in the CD4-bound conformation and substantially weaker affinites for non-stabilized gp120s (Fig. 6B and fig. S14).

Notably, no single affinity maturation alteration appeared to affect affinity by more than 10-fold, suggesting that affinity maturation occurs in multiple small steps, which collectively enable tight binding to HIV-1 gp120. When the effects of VRC01 affinity maturation reversions are mapped to the structure of the VRC01-gp120 complex, they are broadly distributed throughout the VRC01 variable domains, rather than focused on the VRC01-gp120 interface. Non-contact residues therefore appear to influence the interface with gp120 through indirect protein-folding effects. Thus, for VRC01, the process of affinity maturation entails incremental changes of the nascent genomic precursors to obtain high affinity interaction with HIV-1 Env surface.

Receptor mimicry and affinity maturation. The possibility of antibodies using conserved sites of receptor recognition to neutralize viruses effectively has been pursued for several decades. The recessed canyon on rhinovirus that recognizes the unpaired terminal immunoglobulin domains of ICAM-1 highlights the steric role that a narrow canyon entrance may play in occluding bivalent antibody-combining regions (*39*), although framework recognition can in some instances permit entry (*40*). Partial solutions such as those presented by antibody b12 (neutralization of 40% of circulating isolates) (*22*) or by antibody HJ16 (neutralization of 30% of circulating isolates) (*41*), a recently identified CD4-binding-site antibody, may allow recognition of some HIV-1 isolates.

With VRC01, the potency and breadth of neutralization (over 90%) suggests a more general solution. It remains to be seen how difficult it will be to guide the elicitation of VRC01-like antibodies from genomic rearrangement, through affinity maturation, to broad and potent neutralization of HIV-1. Accumulating evidence suggests that the VRC01-defined mode of recognition is used by other antibodies (*12*). These findings suggest that VRC01 is not an isolated example, and likely provides a template for a general mode of recognition. The structure-function insights of VRC01 described here thus provide a foundation for rational vaccine design based not only on the particular mode of antibody-antigen interaction, but also on defined relationships between genomic antibody precursors, somatic hypermutation, and required recognition elements.

References and Notes

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15. The four independent copies of the VRC01-gp120 complex in the asymmetric unit resembled each other closely for the antibody variable domain-gp120 components, with an Cα-root-mean-square deviation of less than 0.2 Å. Elbow variation, however, between variable and constant domains was apparent, and we found one copy (molecule 1) to be more ordered than the others. In figures, we display molecule 1; see Fig. S15 for a comparison of all of the molecules in the asymmetric unit.
16. Surface areas of interaction reported in this paper were determined with the program PISA, as implemented in CCP4 (*43*). Values were about 20% higher than those reported previously for the gp120-CD4 complex (*18*), which were obtained using the program MS (*44*).
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26. In a companion paper (*12*), we show that VRC01 binding induces 17b and CCR5 binding in the context of monomeric gp120 with an unusual entropy signature characteristic of transiting to the CD4-bound state (*45*); neutralization data, however, shows that VRC01 does not induce 17b or CCR5 binding in the context of the viral spike. This difference likely arises from the more constrained gp120 conformation in the trimeric spike. Thus, although VRC01 induces large conformational changes in monomeric HIV-1 gp120 that resemble those induced by CD4, VRC01 interaction with gp120 does not depend upon these conformational changes.
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31. Four residues are provided by the CDR H3, $Asp99_{VRC01}$-$Trp100B_{VRC01}$, with a combined interaction surface of 123 $Å^2$ (tables S3 and S12). These four residues are likely contributed by the D segment (IGHD3-16*02), and none of them appears critical to VRC01 recognition, as changes are observed in two of these residues in the closely related broadly-neutralizing antibody VRC03, which was one of two antibodies we isolated along with VRC01 (*12*). Meanwhile, three residues are provided by the CDR L3, $Tyr91_{VRC01}$, $Glu96_{VRC01}$ and $Phe97_{VRC01}$, with a combined interaction surface of 190 $Å^2$ (tables S3 and S13). These three residues lie at the junction between V- and J-genes. They make important hydrophobic interactions with loop D of gp120, and two of them are conserved between VRC01 and VRC03. While it is difficult to know how precisely the CDR L3 needs to be aligned, with only three contact residues, variation at the V_K-J gene junction should provide sufficient diversity for it to be represented in the repertoire.
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37. Analysis of the HIV-1 Env-reactive antibody repertoire from infected individuals shows increased levels of affinity maturation (*29*). Analysis of a subset of this data (SOM Appendix) containing 147 heavy and 147 light chains from HIV-1 Env-reactive antibodies reveals an average of 15 alterations (30 maximum) for the heavy chain and an average of 8.6 alterations (22 maximum) for the light chain (fig. S13). In terms of the subset of HIV-1 Env-reactive antibodies that are broadly neutralizing (e.g. 2G12, 2F5, 4E10 and b12), antibodies b12 and 2G12 have 45 and 51 changes, respectively, relative to nearest genomic precursors in their V_H- and J-segments of the heavy chain (*30*).
38. Similar significant reductions in affinity have been observed with reversion of other broadly neutralizing, anti-HIV-1 antibodies to putative genomic sequences (*46-48*); these observations have led to the suggestion that the dramatically reduced germline affinity for gp120 might hinder the initiation of affinity maturation of these antibodies (*49*). That is, if the affinity for gp120 of the genomic precursor of a broadly neutralizing antibody were below the threshold required for the nascent B cell to

mature, then maturation would either not occur or would need to occur in response to a different immunogen. This lack of guided initiation of the maturation process may provide an explanation for the absence of such broadly neutralizing antibodies in the first few years of infection. Conversely, the introduction of modified gp120s with affinity to genomic precursors and affinity maturation intermediates could provide a mechanism by which to elicit antibodies like VRC01.
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Supporting Online Material

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Materials and Methods
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25 May 2010; accepted 1 July 2010
Published online 8 July 2010; 10.1126/science.1192819
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Fig. 1. Structure of antibody VRC01 in complex with HIV-1 gp120. Atomic-level details for broad and potent recognition of HIV-1 by a natural human antibody are depicted with polypeptide chains in ribbon representations. The gp120 inner domain is shown in gray, the bridging sheet in blue, and the outer domain in red, except for the CD4-binding loop (purple), the D loop (brown), and the V5 loop (orange). The light chain of the antigen-binding fragment (Fab) of VRC01 is shown in light blue with complementarity-determining regions (CDRs) highlighted in dark blue (CDR L1) and marine blue (CDR L3). The heavy chain of Fab VRC01 is shown in light green with CDRs highlighted in cyan (CDR H1), green (CDR H2), and pale yellow (CDR H3). Both light and heavy chains of VRC01 interact with gp120: the primary interaction surface is provided by the CDR H2, with the CDR L1 and L3 and the CDR H1 and H3 providing additional contacts.

Fig. 2. Structural mimicry of CD4 interaction by antibody VRC01. VRC01 shows how a double-headed antibody can mimic the interactions with HIV-1 gp120 of a single-headed member of the immunoglobulin superfamily such as CD4. (**A**) Comparison of HIV-1 gp120 binding to CD4 (N-terminal domain) and VRC01 (heavy chain-variable domain). Polypeptide chains are depicted in ribbon representation for the VRC01 complex (right) and the CD4 complex with the lowest gp120 RMSD (left) (table S5). The CD4 complex (3JWD) (*14*) is colored yellow for CD4 and red for gp120, except for the CDR-binding loop (purple). The VRC01 complex is colored as in Fig. 1. Immunoglobulin domains are composed of two β-sheets, and the top sheet of both ligands is labeled with the standard immunoglobulin-strand topology (strands G, F, C, C', C"). (**B,C**) Interface details for CD4 (**B**) and VRC01 (**C**). Close-ups are shown of critical interactions between the CD4-binding loop (purple) and the C" strand as well as between $Asp368_{gp120}$ and either $Arg59_{CD4}$ or $Arg71_{VRC01}$. Hydrogen bonds with good geometry are depicted by blue dotted lines, and those with poor geometry in gray. Atoms from which hydrogen bonds extend are depicted in stick representation and colored blue for nitrogen and red for oxygen. In the left panel of **C**, the β15-strand of gp120 is depicted to aid comparison with **B**, though because of the poor hydrogen-bond geometry, it is only a loop. (**D**) Comparison of VRC01- and CD4-binding orientations.

Polypeptides are shown in ribbon representation, with gp120 colored the same as in **A** and VRC01 depicted with heavy chain in dark yellow and light chain in dark gray. When the heavy chain of VRC01 is superimposed onto CD4 in the CD4-gp120 complex, the position assumed by the light chain evinces numerous clashes with gp120 (left). The VRC01-binding orientation (right) avoids clashes by adopting an orientation rotated by 43° and translated by 6-Å.

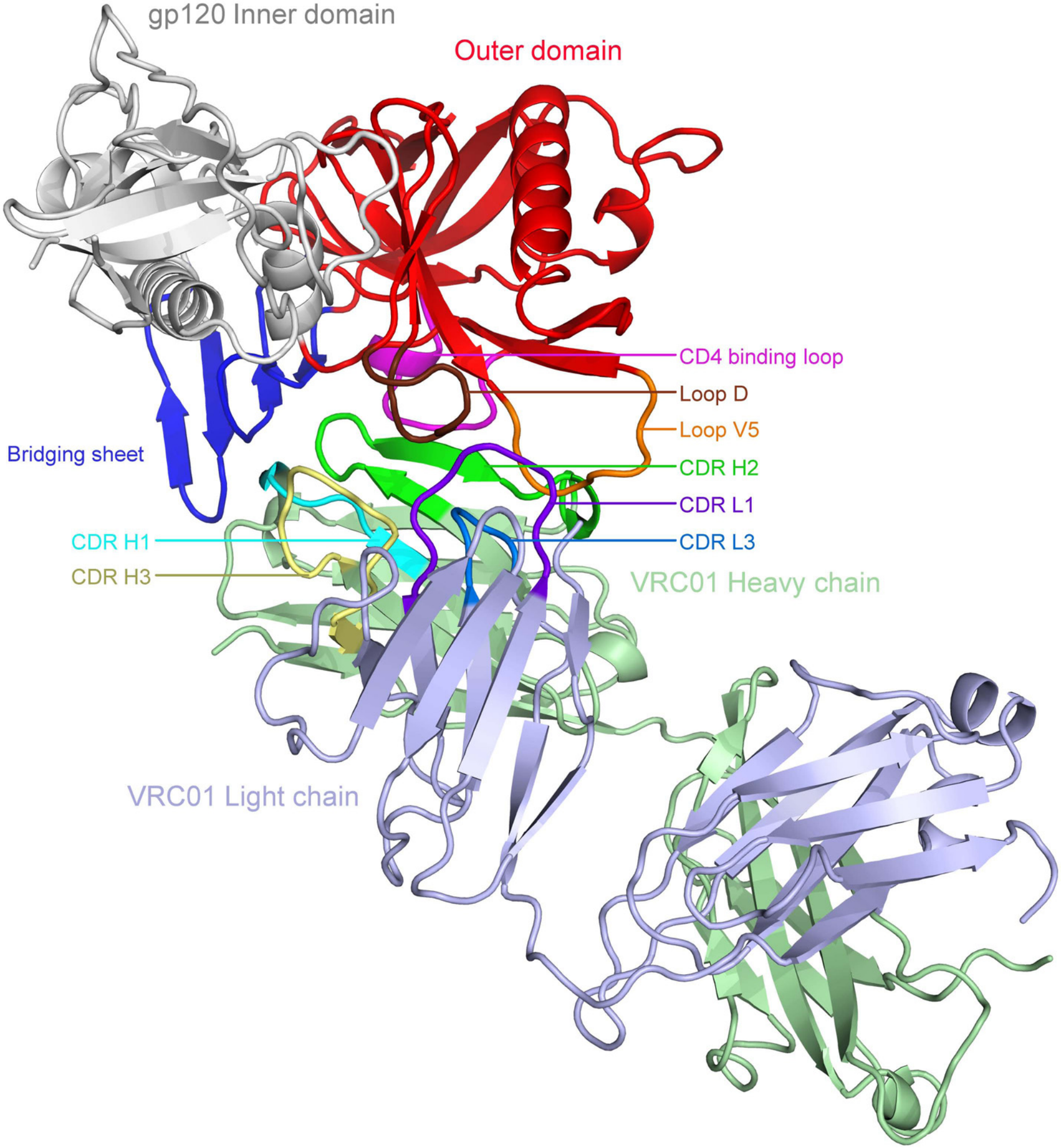
Fig. 3. Structural basis of antibody VRC01 neutralization breadth and potency. VRC01 displays remarkable neutralization breadth and potency, a consequence in part of its ability to bind well to different conformations of HIV-1 gp120. (**A**) Neutralization dendrograms. The genetic diversity of current circulating HIV-1 strains is displayed as a dendrogram, with locations of prominent clades (e.g. A, B and C) and recombinants (e.g. CDR02_AG) labeled. The strains are colored by their neutralization sensitivity to VRC01 (left) or CD4 (right). VRC01 neutralizes 72% of the tested HIV-1 isolates with an IC_{80} of less than 1 ug/ml; by contrast, CD4 neutralizes 30% of the tested HIV-1 isolates with an IC_{80} of less than 1 ug/ml (table S14). (**B**) Comparison of binding affinities. Binding affinities (K_Ds) for VRC01 and various other gp120-reactive ligands as determined by surface-plasmon resonance are shown on a bar graph. White bars represent affinities for gp120 restrained from assuming the CD4-bound state (*21*) and black bars represent affinities for gp120 fixed in the CD4-bound state (*24*). Binding too weak to be measured accurately is shown as with an asterisk and bar at 10^{-5} M K_D. (**C**) Neutralization of viruses with altered sampling of the CD4-bound state. Mutant $S375W_{gp120}$ favors the CD4-bound state, whereas mutants $H66A_{gp120}$ and $W69L_{gp120}$ disfavor this state. Neutralization by VRC01 (left) is similar for wild-type (WT) and all three mutant viruses, whereas neutralization by CD4 (right) correlates with the degree to which gp120 in the mutant viruses favors the CD4-bound state.

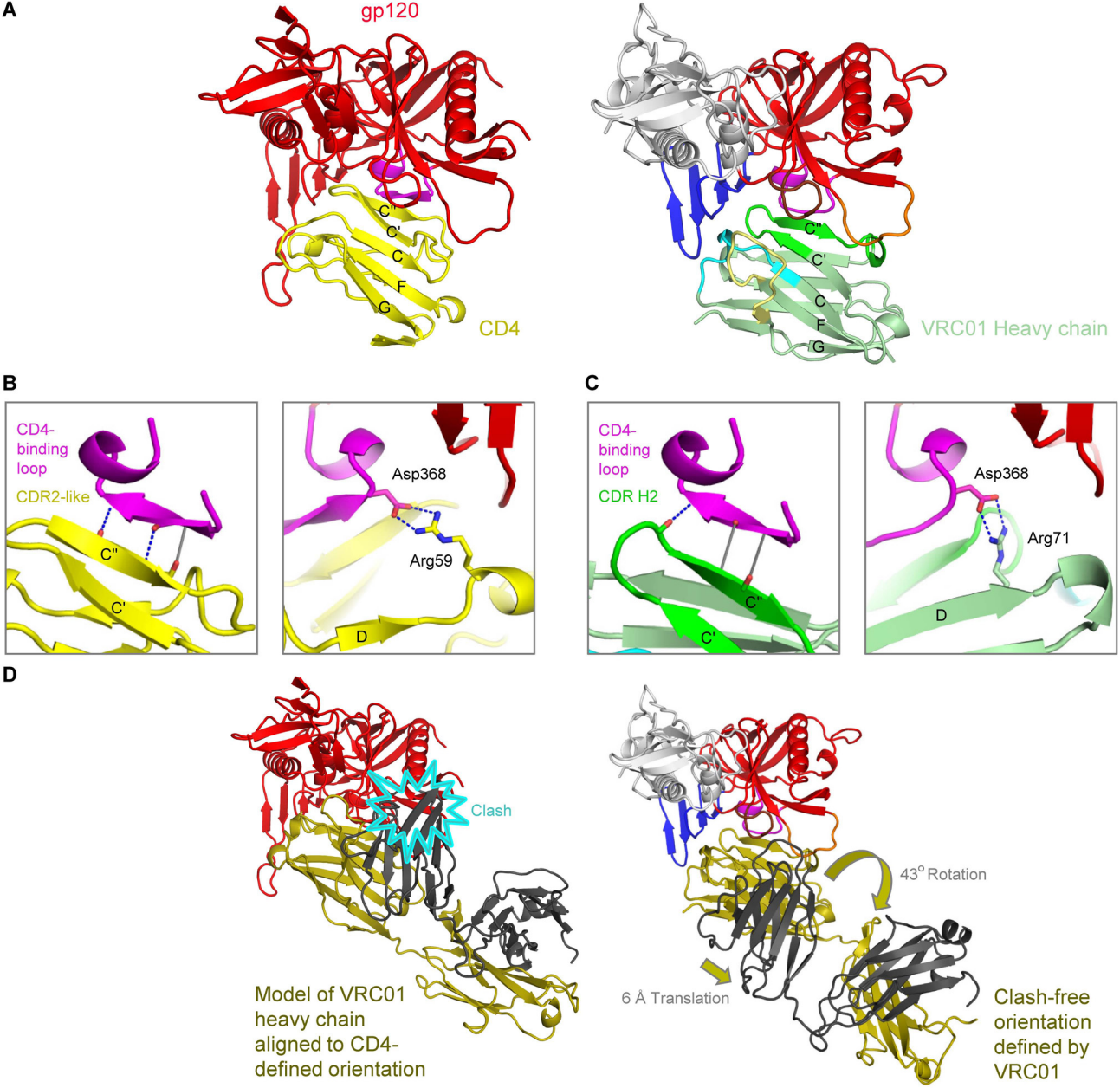
Fig. 4. Natural resistance to antibody VRC01. VRC01 precisely targets the CD4-defined site of vulnerability on HIV-1 gp120. Its binding surface, however, extends outside of the target site, and this allows for natural resistance to VRC01 neutralization. (**A**) Target site of vulnerability. The CD4-defined site of vulnerability is the initial contact surface of the outer domain of gp120 for CD4 and comprises only 2/3 of the contact surface of gp120 for CD4 (*22*). The molecular surface of HIV-1 gp120 has been colored according to its underlying domain substructure: red for the conformationally invariant outer domain, grey for the inner domain and blue for the highly mobile bridging sheet. Regions of the gp120 surface that interact with VRC01 have been colored green, with the CD4-defined site of vulnerability outlined in yellow. The view shown here is rotated 90° about the horizontal from the view in Figs. 1 and 2. (**B**) VRC01 recognition. The molecular surface of gp120 in the VRC01 bound conformation is colored as in **A**. The variable domains of VRC01 are shown in ribbon representation with the heavy and light chains colored as in Fig. 1 and extension to constant regions indicated. (**C**) Antigenic variation. The polypeptide backbone of gp120 is colored according to sequence conservation, blue if conservation is high and red if conservation is low. (**D**) Molecular surface of VRC01 and select interactive loops of gp120. Variation at the tip of the V5 loop is accommodated by a gap between heavy and light chains of VRC01.

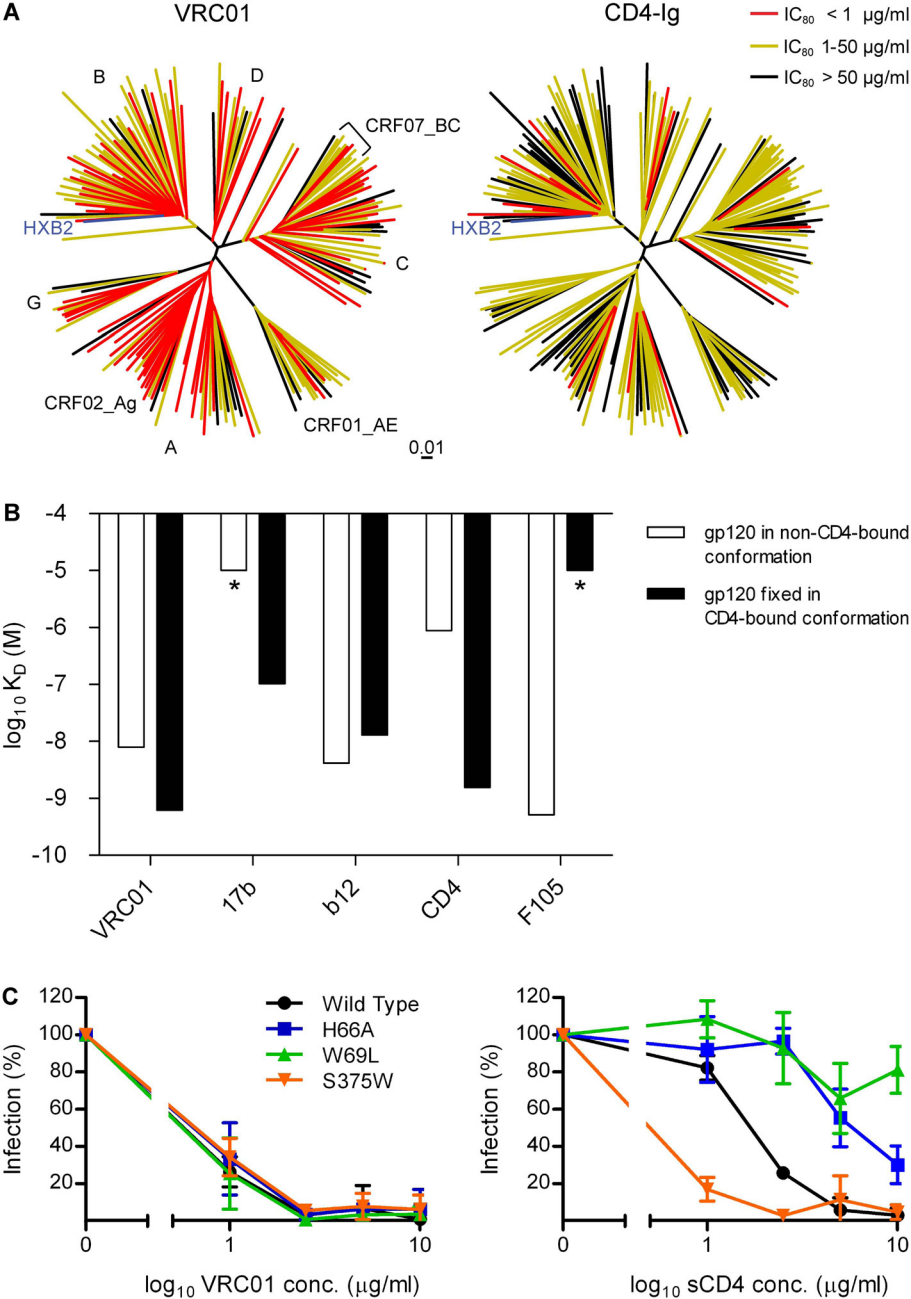
Fig. 5. Unusual VRC01 features. The structure of VRC01 displays a number of unusual features, which if essential for recognition might inhibit the elicitation of VRC01-like antibodies. In **A-D**, unusual features of VRC01 are shown structurally (far left panel), in terms of frequency as a histogram with other antibodies (second panel from left), and in the context of affinity and neutralization measurements after mutational alteration (right two panels). Affinity measurements were made by ELISA to the gp120 construct used in crystallization (93TH057), and neutralization measurements were made with a clade A HIV-1 strain Q842.d12. Additional binding and neutralization experiments are reported in Table S10. (**A**) N-linked glycosylation. The conserved tri-mannose core is shown with observed electron density, along with frequency and effect of removal on affinity. (**B**) Extra disulfide. Variable heavy domains naturally have two Cys, linked by a disulfide; VRC01 has an extra disulfide linking CDR H1 and H3 regions. This occurs rarely in antibodies, but its removal by mutation to Ser/Ala has little effect on affinity. (**C**) CDR L1 deletion. A two amino acid deletion in the CDR L1, prevents potential clashes with loop D of gp120. Such deletions are rarely observed; reversion to the longer loop may have a 10-100-fold effect on gp120 affinity. (**D**) Somatically altered contact surface. The far left panel shows the VRC01 light chain in violet and heavy chain in green. Residues altered by affinity maturation are depicted with "balls" and contacts with HIV-1 gp120 are colored red. About half the contacts are altered during the maturation process. Analysis of human antibody-protein complexes in the protein-data bank shows this degree of contact surface alteration is rare; reversion of each of the contact site to genome has little effect (table S10), though in aggregate the effect on affinity is larger.

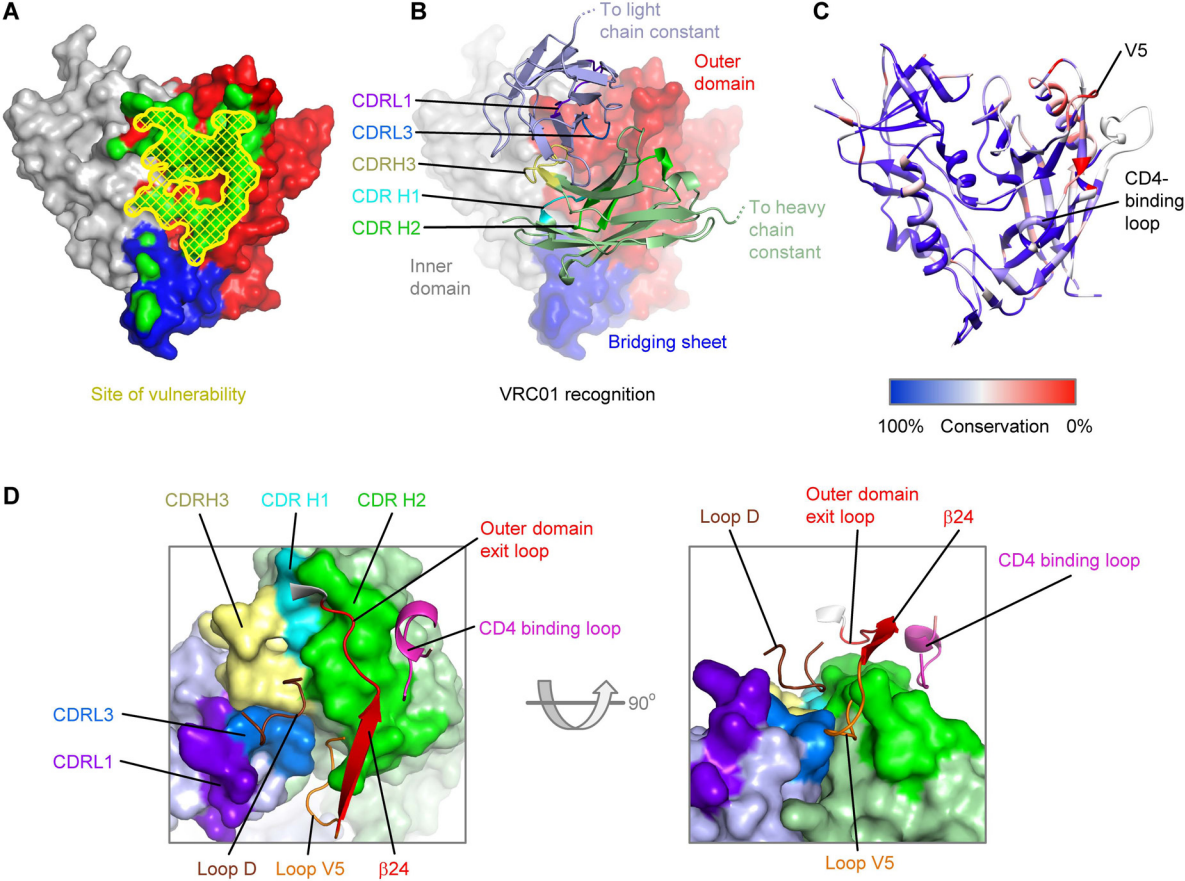
Fig. 6. Somatic maturation and VRC01 affinity. Hypermutation of the variable domain during B cell maturation allows for the evolution of high affinity antibodies. With VRC01 this enhancement to affinity occurs principally through the alteration of non-contact residues, which appear to reform the genomic contact surface from

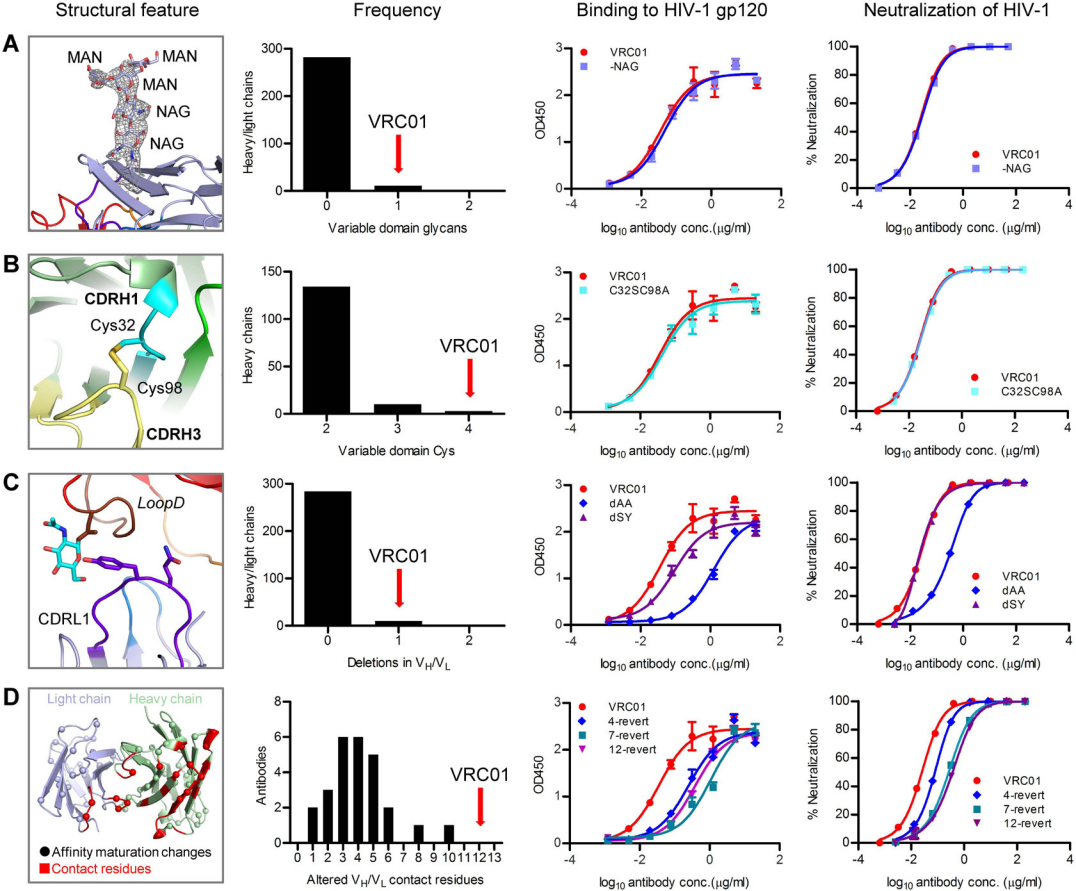
affinity too low to measure to a tight (nM) interaction. (**A**) Effect of genomic reversions. The V_H- and V_K-derived regions of VRC01 were reverted to the sequences of their closest genomic precursors, expressed as immunoglobulins and tested for binding as V_H- and V_K-revertants (gHgL), as a V_H-only revertant (gH), or as a V_K-only revertant (gL) to the gp120 construct used in crystallization (93TH057) or to a stabilized HXBc2 core (*22*). These constructs were also tested for neutralization of a clade A HIV-1 strain Q842.d12. Additional neutralization experiments with clade B and C viruses are reported in the supplemental materials. (**B**) Maturation of VRC01 and correlation with binding and neutralization. Affinity and neutralization measurements for the 19 VRC01 mutants created during the structure-function analysis of VRC were analyzed in the context of their degree of affinity maturation. Significant correlations were observed, with extrapolation to V_H- and V_K-genomic revertants suggesting greatly reduced affinity for gp120.

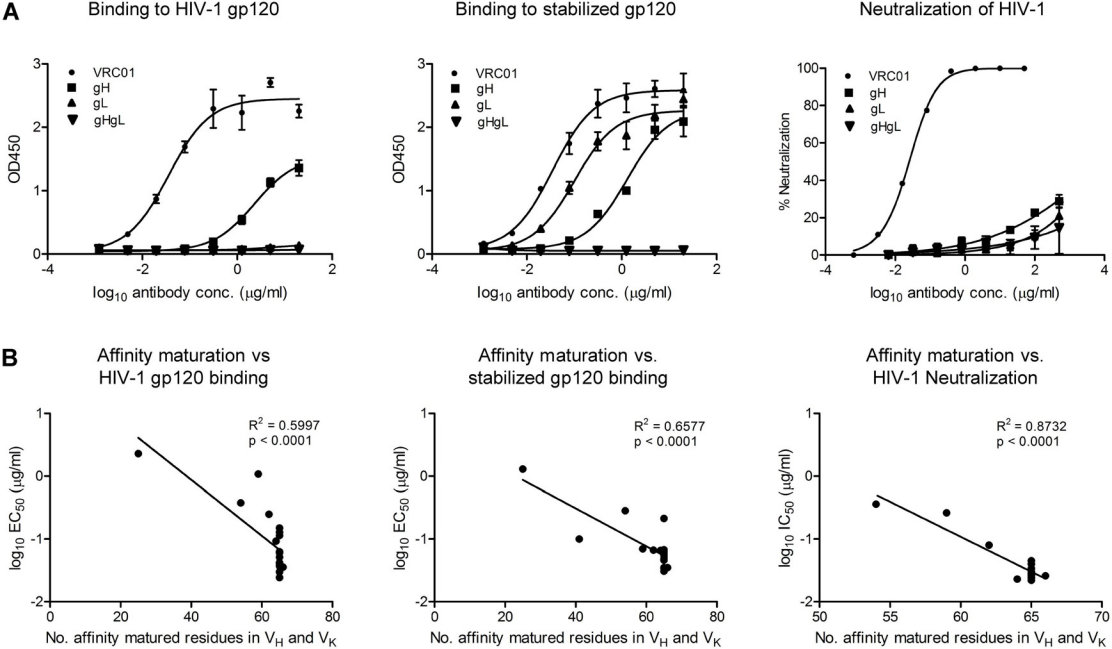

gp120 Inner domain
Outer domain
CD4 binding loop
Loop D
Loop V5
Bridging sheet
CDR H2
CDR L1
CDR H1
CDR L3
CDR H3
VRC01 Heavy chain
VRC01 Light chain


A
gp120
C''
C'
C
F
G
CD4
C''
C'
C
F
G
VRC01 Heavy chain
B
CD4-
binding
loop
CDR2-like
C''
C'
Asp368
Arg59
D
C
CD4-
binding
loop
CDR H2
C''
C'
Asp368
Arg71
D
D
Clash
Model of VRC01
heavy chain
aligned to CD4-
defined orientation
43° Rotation
6 Å Translation
Clash-free
orientation
defined by
VRC01


A
VRC01
CD4-Ig
IC80 < 1 µg/ml
IC80 1-50 µg/ml
IC80 > 50 µg/ml
B
D
CRF07_BC
C
HXB2
G
CRF02_Ag
CRF01_AE
A
0.01
HXB2
B
log10 KD (M)
-4
-5
-6
-7
-8
-9
-10
*
*
gp120 in non-CD4-bound conformation
gp120 fixed in CD4-bound conformation
VRC01
17b
b12
CD4
F105
C
Infection (%)
120
100
80
60
40
20
0
1
10
Wild Type
H66A
W69L
S375W
log10 VRC01 conc. (µg/ml)
log10 sCD4 conc. (µg/ml)


A
Site of vulnerability
B
To light chain constant
Outer domain
CDRL1
CDRL3
CDRH3
CDR H1
CDR H2
To heavy chain constant
Inner domain
Bridging sheet
VRC01 recognition
C
V5
CD4-binding loop
100% Conservation 0%
D
CDRH3
CDR H1
CDR H2
Outer domain exit loop
CD4 binding loop
CDRL3
CDRL1
Loop D
Loop V5
β24
90°
Loop D
Outer domain exit loop
β24
CD4 binding loop
Loop V5


Structural feature
Frequency
Binding to HIV-1 gp120
Neutralization of HIV-1
A
MAN
MAN
MAN
NAG
NAG
Heavy/light chains
VRC01
Variable domain glycans
OD450
VRC01
-NAG
log10 antibody conc.(μg/ml)
% Neutralization
VRC01
-NAG
log10 antibody conc. (μg/ml)
B
CDRH1
Cys32
Cys98
CDRH3
Heavy chains
VRC01
Variable domain Cys
VRC01
C32SC98A
VRC01
C32SC98A
C
LoopD
CDRL1
Heavy/light chains
VRC01
Deletions in VH/VL
VRC01
dAA
dSY
VRC01
dAA
dSY
D
Light chain
Heavy chain
Affinity maturation changes
Contact residues
Antibodies
VRC01
Altered VH/VL contact residues
VRC01
4-revert
7-revert
12-revert
VRC01
4-revert
7-revert
12-revert


A
Binding to HIV-1 gp120
Binding to stabilized gp120
Neutralization of HIV-1
VRC01
gH
gL
gHgL
OD450
% Neutralization
log10 antibody conc. (μg/ml)
B
Affinity maturation vs HIV-1 gp120 binding
Affinity maturation vs. stabilized gp120 binding
Affinity maturation vs. HIV-1 Neutralization
R2 = 0.5997
p < 0.0001
R2 = 0.6577
p < 0.0001
R2 = 0.8732
p < 0.0001
log10 EC50 (μg/ml)
log10 IC50 (μg/ml)
No. affinity matured residues in VH and VK