Structures of HIV-1 Env V1V2 with broadly neutralizing antibodies reveal commonalities that enable vaccine design

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Antibodies capable of neutralizing a majority of circulating HIV-1 isolates develop in approximately half of all people infected with HIV-1 for over five years1. Intense interest has been focused on these antibodies because they provide clues as to how an effective vaccine might be developed2,3. Specific bNAbs—which arise in multiple donors and share common features of Env recognition and B-cell ontogeny—may have utility as vaccine templates, owing to the potential for similar antibodies to be elicited by a common immunogen (or common set of immunogens) in the general population4,5.

An increasing number of such ‘multidonor’ bNAbs have been identified, such as those of the VRC01 class6–7 (named after the first described antibody of the class), which share ‘class’ features of molecular recognition and B-cell ontogeny—may have utility as vaccine templates, owing to the potential for similar antibodies to be elicited by a common immunogen (or common set of immunogens) in the general population4,5.

Broadly neutralizing antibodies (bNAbs) against HIV-1 Env V1V2 arise in multiple donors. However, atomic-level interactions had previously been determined only with antibodies from a single donor, thus making commonalities in recognition uncertain. Here we report the cocrystal structure of V1V2 with antibody CH03 from a second donor and model Env interactions of antibody CAP256-VRC26 from a third donor. These V1V2-directed bNAbs used strand-strand interactions between a protruding antibody loop and a V1V2 strand but differed in their N-glycan recognition. Ontogeny analysis indicated that protruding loops develop early, and glycan interactions mature over time. Altogether, the multidonor information suggested that V1V2-directed bNAbs form an ‘extended class’, for which we engineered ontogeny-specific antigens: Env trimers with chimeric V1V2s that interacted with inferred ancestor and intermediate antibodies. The ontogeny-based design of vaccine antigens described here may provide a general means for eliciting antibodies of a desired class.

To date, V1V2-directed bNAbs have been identified in four donors: the CHAVI donor 0219 (CH0219), with bNAbs CH01–CH04 (ref. 15); the CAPRISA 256 donor, with bNAbs CAP256–VRC26.01–12 (ref. 16); and the IAVI protocol G donors 24 and 84, with IAVI 24 bNAbs PG9 and PG16 (ref. 17) and IAVI 84 bNAbs PGT141–145 (ref. 18) and PGD1400–1412 (ref. 19). Structures of the ligand-free forms of these antibodies reveal a protruding third heavy-chain complementarity-determining region (CDR H3), which is anionic, often tyrosine sulfated and critical for Env interaction15,16,19–22. The epitope appears to be quaternary in nature and to include a N-linked glycan at residue 160 along with strand C of V1V2 (refs. 15, 16, 18, 22–25). In terms

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of B-cell ontogeny, approximations of the unmutated common ancestor (UCA) have been inferred for V1V2-directed bNAbs lineages from donors CH0219 and CAP256 (refs. 15,16), which have indicated that the long anionic CDR H3 is a product of recombination15,16. Initial recognition of UCA (or of V gene–reverted approximations) appears to be restricted to select strains of HIV-1 (for example, CAP256-SU or ZM233)15,16,20, to use similar D genes and in some cases related V genes, and to contain similar motifs (for example, YYD) in the CDR H3 (refs. 15–18). Despite this extensive commonality, it has been unclear whether these bNAbs recognize HIV-1 Env through a common molecular mechanism.

The only atomic-level information on the interactions of these V1V2-directed bNAbs with HIV-1 Env derives from scaffolded V1V2 structures of antibodies PG9 and PG16 from donor IAVI 24, in which the CDR H3 penetrates and thus interacts in a parallel intermolecular strand association with strand C of V1V2, and N-glycans from residues 160 and 156 (or 173) are recognized by the heavy and light chains of the antibody22,26. The combination of this atomic-level information and negative-stain EM of PG9 in complex with a soluble trimeric Env mimic, BG505 SOSIP.664 (ref. 27), suggest that the quaternary dependency of PG9 arises from its recognition of glycan N160 from a neighboring protomer.^24.

The absence of atomic-level information on the recognition of V1V2-directed bNAbs from other donors raises a number of questions. Do V1V2-directed bNAbs from the other donors use a similar strand-strand association in their recognition? What is the basis of their quaternary specificity: does it arise from glycan recognition, as it does with PG9? And if the molecular features of V1V2-directed bNAbs were indeed similar, how would one devise a common set of immunogens to facilitate their development? Here, we set out not only to provide an atomic-level understanding of V1V2-directed bNAb recognition in another donor but also to use this information in the design of class-specific antigens. To facilitate crystallization, we designed trimeric V1V2 scaffolds capable of interacting with quaternary-specific V1V2-directed bNAbs, and we determined cocrystal structures with the antigen-binding fragments (Fabs) of bNAb CH03 and CH04, both from donor CH0219. We used hydrogen–deuterium exchange (HDX) and arginine scanning to delineate Env–interactive regions of bNAb CAP256-VRC26.03 from donor CAP256 and to model its interaction with V1V2. Finally, we used neutralization screening with UCA and intermediates of V1V2-directed bNAbs to engineer antigens capable of interacting with developmental intermediates. Altogether, the structural similarities in antibody recognition along with ontogeny similarities (and differences) in development indicate that the V1V2-directed bNAbs form an extended class, which we define as antibodies that do not necessarily share genetic commonalities but nonetheless display a characteristic mode of antigen interaction. Extended-class immunogens—such as the soluble chimeric trimers developed here through a neutralization- and ontogeny-based chimera strategy—may provide a general means for eliciting bNAbs against specific sites of Env vulnerability.

RESULTS

Design and crystallization of trimeric scaffolded V1V2s

To obtain a cocrystal structure of HIV-1 Env with a V1V2-directed bNAb from an additional donor, we first screened the V1V2 scaffolds from PDB 1FD6 (ref. 28) and PDB 1JO8 (ref. 29), which are recognized by bNAbs PG9 and PG16 from donor IAVI 24 and have previously been used in the structural analyses of these antibodies22,25. bNAbs CH01–04, CAP256-VRC26.01–12 and PGT141–145 did not bind these monomeric V1V2 scaffolds. One possible explanation for this lack of recognition might be related to the quaternary dependence of bNAbs CH01–04, CAP256-VRC26.01–12 and PGT141–145, which is more stringent than that of antibody PG9. To accommodate their quaternary dependencies, we designed a library of scaffolded V1V2s based on trimeric scaffolds, expressed secreted versions of these scaffolds in a 96-well format30–32 and assessed PGT145 recognition directly from expressed supernatant (Fig. 1a and Supplementary Fig. 1a,b). We chose PGT145 for its high quaternary specificity and its broad and potent neutralization; it recognized V1V2 chimeras from two trimeric scaffolds, PDB 1VH8 (ref. 33) and PDB 4F2K34.

A PDB 1VH8–scaffolded V1V2 showed strong binding, and three PDB 4F2K–scaffolded V1V2s with differing linker lengths showed moderate binding to PGT145 (Supplementary Fig. 1b,c). We expressed and purified these trimeric scaffolded V1V2s in GnT1- cells and assessed binding by surface plasmon resonance, which revealed strong to moderate binding for PG9 (Kd 24–500 nM) and weak binding for other V1V2-targeting bNAbs such as CH01

Figure 1 Design of scaffolded V1V2s and cocrystal structure with bNAb CH03 from donor CH0219. (a) Design of trimeric scaffolded V1V2. Yellow, trimeric structure of PDB 1VH8 used as a scaffold; magenta, V1V2 domain of BG505; light blue, residues in PDB 1VH8 replaced with the V1V2 domain. (b) Structure of CH03 (green and blue) bound to a PDB 1VH8–scaffolded (yellow) V1V2 domain (magenta) from the superinfecting strain in CAPRISA donor 256. Three Fabs of CH03 are shown bound to the trimeric PDB 1VH8–V1V2CAP256-SU scaffold. A single Fab and scaffolded V1V2 protomer are shown in ribbon representation, and the two other trimer-related complexes are shown in surface representations. (c) Comparison of PDB 1VH8–scaffolded V1V2 from strain CAP256-SU (magenta) and V1V2 of the prefusion closed trimer from strain BG505 (black, PDB 4TVP).
Structures of scaffolded V1V2 with antibodies CH03 and CH04

We solved the crystal structure of Fab CH03 in complex with the scaffolded V1V2 from HIV-1 strain CAP256-SU by molecular replacement with the previously determined Fab CH04 structure (Table 1). Overall, the structure revealed that CH03 inserts a protruding CDR H3, which is bent to recognize strand C of V1V2 in a parallel strand-strand interaction (Fig. 1b).

The scaffolded V1V2 formed a five-stranded β-barrel similar to that of V1V2 in its prefusion closed Env conformation [35] and to that of V1V2 in complex with antibody 830A [36]. This five-stranded β-barrel differs slightly from the four-stranded Greek key motif observed in the scaffolded V1V2s bound by PG9 (ref. 22) and by PG16 (ref. 25), in which the scaffolded V1V2 dimerized at a lattice contact, thus inhibiting the formation of strand C. Overall, superposition of V1V2s from scaffolded and Env contexts showed high structural similarity, with an r.m.s. deviation of 1.1 Å for 45 β-strand residues (Fig. 1c).

Analysis of the interface between Fab CH03 and scaffolded V1V2 revealed that N-glycan interactions dominated: 1,592 Å² of the CH03 interactive surface was buried by N-linked glycan (82% of the CH03 interface), with protein interactions accounting for only 384 Å² (19% of the CH03 interface) (Fig. 2). We observed electron density for four N-linked glycans emanating from residues N130, N156 and N160 of one V1V2 and from residue N139 of a neighboring V1V2 (Fig. 2a,b). Over a third of the CH03-glycan interactions occurred with the two protein-proximal N-acetylglucosamines at the base of each N-linked glycan; these totaled nine hydrogen-bonding interactions and 741 Å² of buried surface (Fig. 2c). For glycan N130, we observed density for four mannose residues, three of which contacted the CH03 heavy chain. Glycan N156 also interacted with the CH03 heavy chain, but we observed no density beyond the protein-proximal N-acetylglucosamines. For glycan N160, we observed density for five mannose residues, of which four were involved with the CH03 heavy chain, and a fifth interacted with the CH03 heavy chain—light chain interface. Glycan N139 of the neighboring protomer showed clear density for five mannose residues and had the most substantial CH03 interactions, comprising 14 hydrogen bonds (Fig. 2d).

Table 1 Data collection and refinement statistics

|                | CH03–PDB 1VH8–V1V2SU | CH04–PDB 1VH8–V1V2A244 |
|----------------|----------------------|------------------------|
| Data collection|                      |                        |
| Space group    | C2                   | P63                    |
| Cell dimensions| a, b, c (Å)           | 162.69, 98.21, 170.63   |
|                | α, β, γ (°)           | 90.00, 112.82, 90.00    |
| Resolution (Å) | 50.00–3.10 (3.17–3.10) | 50.00–4.20 (4.32–4.20) |
| Rmerge         | 18.4 (70.9)          | 9.7 (76.9)             |
| I / αf         | 7.3 (1.7)            | 19.2 (1.8)             |
| Completeness (%) | 99.7 (98.4)       | 95.8 (77.6)            |
| Redundancy     | 3.4 (2.2)            | 9.6 (5.7)              |
| Refinement     |                      |                        |
| Resolution (Å) | 41.0–3.10            | 50.0–4.2               |
| No. reflections| 44,633 (4,164)       | 13,064 (777)           |
| Rmerge / Rfree | 21.3 / 25.8          | 24.2 / 28.5            |
| No. atoms (total) | 15,133           | 9,388                  |
| Protein        | 14,435               | 9,066                  |
| Carbohydrate   | 779                  | 322                    |
| B factors (Å²) | 67                   | 81                     |
| r.m.s. deviations | 94.5               | 96                     |
| Bond lengths (Å) | 0.004               | 0.006                  |
| Bond angles (°) | 0.8                  | 1.34                   |

Values in parentheses are for highest-resolution shell. One crystal was used to measure the data for each complex.

(Kₐ 20–50 μM) (Supplementary Fig. 1d). We further optimized these scaffolded V1V2s by varying the HIV-1 strain and linker confections. Optimized PDB 1VH8 trimeric scaffolds with V1V2 from HIV-1 strains CAP256-SU or A244 (also known as CM244, which was neutralized well by CH01–04 (ref. 15)) crystallized with the Fabs of bNAbs CH03 or CH04, respectively (Supplementary Fig. 1e).

Figure 2 CH03 V1V2 interactions. (a) CH03 is shown as a molecular surface, with V1V2 shown as magenta ribbons. Mannose and N-acetylglucosamine residues are shown in stick representation, as are the side chains of Asn130, Asn139, Asn156 and Asn160. (b) Ribbon representations of CAP256SU V1V2 (magenta), CH03 heavy chain (green) and light chain (light blue). V1V2 glycans are shown in stick and transparent surface representation. (c) 90° view of b, with CH03 loops shown in ribbon representation and glycans represented as spheres. (d) Schematic representation of glycan moieties recognized by CH03. Blue squares, N-acetylglucosamines; green circles, mannosyl. Hydrogen (H) bonds to CH03 are listed to the right of the symbols, as is the total surface area buried at the interface between CH03 and each sugar. (e) Schematic of the CH03 main chain interaction with V1V2. Disulfide bonds in V1V2 are shown as yellow sticks. (f,g) Ribbon representation of V1V2 (magenta) and CH03 heavy chain (green), showing main and side chain interactions. Hydrogen bonds are represented by dotted lines.
Figure 3  CH03 epitope in PDB 1VH8-scaffold and trimeric Env contexts. (a) V1V2 (magenta) orientation on the trimeric PDB 1VH8 scaffold (yellow). (b) V1V2 (black) in trimeric BG505 SOSIP.664 (gray). (c) Overlay of V1V2 in scaffold (magenta) and Env (black) contexts, in which a single V1V2 has been superimposed. (d) PDB 1VH8-scaffolded V1V2 in ribbon representation with glycans as sticks with a transparent surface shown in color for one protomer and in gray for the other two protomers. The N139 glycan from a neighboring protomer is highlighted in cyan. Inset, binding sites for three CH03 Fabs. (e) HIV-1 Env trimeric V1V2 in ribbon representation with glycans in sticks, shown in color for one protomer and in gray for the other two protomers. The N160 glycan from the right protomer is highlighted in cyan. Inset, binding site for one CH03 Fab (specific glycan numbering differs by strain). (f) Interaction of CH03 with the V1V2 epitope in the PDB 1VH8-scaffold context. The N139 from the neighboring protomer is highlighted. (g) Model of interaction of CH03 with the V1V2 epitope in the Env trimer context. The N160 from the neighboring protomer is highlighted. To obtain the model, the CH03 structure was aligned to the BG505.SOSIP trimer through the V1V2 domain, and the absent mannose moieties were appended from previous V1V2 structures.

bonds and 729 Å² of buried surface. Four of the N139 mannose residues interacted with the light chain; a fifth was extensively buried at the heavy chain–light chain interface, with six hydrogen bonds and 204 Å² of buried surface.

In addition to the glycan interactions, parallel strand–strand interactions occurred between a hairpin in the CH03 CDR H3 and strand C of V1V2 (Fig. 2e). Other than the four strand–strand hydrogen bonds, most of the protein–protein interactions were hydrophobic in nature. Ile100E and Tyr100G (Kabat notation) in the CDR H3 stack over the V1V2-strand C backbone and aliphatic portions of the side chains (Fig. 2f,g). A salt bridge between Glu30 in the CDR H1 of CH03 and Lys171 of V1V2 provided the only notable electrostatic interaction.

We also obtained cocryostals of Fab CH04 in complex with a PDB 1VH8-scaffold V1V2 from HIV-1 strain A244 and solved the structure of the complex (Table 1). Overall, the CH04–V1V2 structure showed a similar glycan-dominated interface as that of CH03–V1V2, and CH04 also inserted a CDR H3 hairpin to recognize strand C of V1V2 in a parallel strand–strand manner (Supplementary Fig. 2).

Quaternary recognition of glycan in scaffold and Env contexts

Because CH03 showed extensive contacts with glycan N139 on a neighboring protomer, yet this glycan had not been previously implicated in CH03 interaction, we investigated quaternary scaffold interactions further. Although the V1V2 domain in the trimeric scaffold and trimeric Env aligned well as individual domains (Fig. 1c), they showed substantially different trimeric arrangements (Fig. 3a,b). Indeed, alignment of a single V1V2 domain from scaffold and Env contexts would position the trimeric counterparts in mostly nonoverlapping locations (Fig. 3c).

We observed that glycan N139 on the neighboring V1V2 in the trimeric scaffold was positioned similarly to glycan N160 on the neighboring V1V2 in trimeric Env (Fig. 3d,e). Notably, the protein-proximal N-acetylglucosamines emanating from N139 (scaffold context) and N160 (Env context) were positioned to within 3.5 Å. This suggests that in the trimeric Env context, CH03 would be able to recognize glycan N160 on a neighboring V1V2 in a manner similar to its recognition of N139 in the scaffold context (Fig. 3f,g). In the scaffold context, these CH03 interactions would be symmetric and would lead to three CH03s, each interacting in a similar way with four glycans. However, in the Env context, the interactions were not compatible with binding of three Fabs, and only a single CH03 Fab would be able to interact with each Env trimer (Fig. 3d,e).

Modeled interaction between antibody CAP256-VRC26 and V1V2

Similarly to the quaternary interaction proposed for CH03 and observed with IAVI 24, V1V2-directed antibodies from both donor CAP256 and donor IAVI 84 also showed a single Fab recognizing V1V2 at the trimer apex. However, despite substantial effort, we were unable to produce cocryostals for any of the broadly neutralizing antibodies from donors CAP256 or IAVI 84 with V1V2. Modeling of the Fab PGDM1400 structure from donor IAVI 84, which displays a straight extended CDR H3, into a similar orientation as that seen in the negative-stained two-dimensional (2D) class-average EM density of the Fab–Env trimer (PDB 4RQQ) indicated a possible strand-strand interaction in the ligand-free Fab crystal structure (stabilized by a strand-strand antiparallel lattice interaction in the ligand-free Fab context) would otherwise clash. Modeling of the CAP256-VRC26 structures from donor CAP256 (ref. 16), which display bent CDR H3s that are more axe-like than hammerhead-like, into the negative-stain EM density, indicated a possible strand-strand interaction.
Figure 4  Modeling of bNAb CAP256-VRC26 bound to Env suggests a common mode of recognition. (a) HDX plots of bound versus unbound CAP256-VRC26 (top), highlighting seven CDR H3 residues (red lines, bottom right) that are protected upon binding to the BG505 SOSIP Env trimer. Specific peptides are colored according to fragment, and asterisks indicate sulfated tyrosines within the peptide. (b) Structure of CAP256-VRC26, displaying paratope mapping results with regions associated with loss of neutralization shown in red. (c) Atomic-level model of CAP256-VRC26 (cyan) bound to V1V2, based on crystal structures of CAP256-VRC26 Fab, HDX, paratope mapping and EM data. CDR H3 residues protected from HDX are shown in red. (d) Schematic of the CAP256-VRC26 main chain topology with V1V2. Disulfide bonds in V1V2 are shown as yellow sticks. (e-g) Common mode of recognition by V1V2-directed bNAbs from three donors. A representative antibody for each donor is shown with the atomic model of CAP256-VRC26.09 (e), the crystal structure of CH03–V1V2 (f) and the crystal structure of PG9–V1V2 (g). Intermolecular hydrogen-bond interactions between CDR H3 and V1V2 strand C are highlighted with main chain interactions in gray and side chain interactions in black.

between antibody CDR H3 and V1V2 strand C (Supplementary Fig. 3).

To provide additional experimental constraints, we analyzed rates of HDX from CAP256-VRC26.03 either free of ligand or bound to the BG505 SOSIP.664 Env trimer (Fig. 4a); we used CAP256-VRC26.03 because this was the only structure with structural definition of the entire CDR H3 (ref. 16). HDX information from four overlapping peptides pinpointed reduced HDX rates to a seven-residue stretch in the CDR H3; on Env, the HDX information pinpointed a short peptide in the strand-C region of V1V2 (Supplementary Fig. 3b). Interestingly, the peptides bearing double tyrosine sulfation appeared to become substantially more protected upon binding, thus suggesting that the doubly sulfated Fab may form a more stable complex with the epitope than does the singly sulfated variant (Fig. 4a). We also used an arginine-scanning approach22 to map the CAP256-VRC26.03

Figure 5 Properties of UCA or the earliest known ancestor of V1V2-directed bNAb lineages. (a) For each of the four donors from which V1V2-directed bNAbs have been identified, a phylogenetic tree is displayed with the heavy-chain sequences of lineage members. The gray bars denote percentage SHM, with UCA (0% mutated) on the far left; white bars denote earliest ancestors. Mature bNAbs are labeled. Properties of the CDR H3 are provided for select bNAbs and UCA or earliest well-defined maximum-likelihood intermediate (I). The overall charge of the CDR H3 appears in parentheses followed by the length (Kabat system). (b) Histograms of CDR H3 lengths, charge and germline divergence from 9,721 unique and productive human sequences. Values for select lineage members are indicated by colored arrows.

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paratope (Supplementary Fig. 3c); arginine scanning disrupts interactions through steric interference, thus allowing for a more robust delineation of contacts than can be achieved through alanine scanning. This paratope mapping implicated the CDR H3 as the primary means of Env contact (Fig. 4b).

We combined constraints from Fab–Env negative stain, from HDX and from arginine scanning to model the CAP256–VRC26 interaction with V1V2 in the context of the BG505 SOSIP.664 trimer (Fig. 4c). We used the CAP256–VRC26.09 antibody for this modeling because this antibody showed high affinity for the SOSIP trimer, and the negative-stain EM map with the SOSIP.664 trimer had been obtained with this antibody. This model suggested a strand-strand interaction, occurring in an antiparallel manner, between the seven residues of the CDR H3 implicated by HDX and strand C of V1V2 (Fig. 4d). Tryptophan side chains could stack against the strand-C backbone, and electrostatic interactions were facilitated by two sulfated tyrosine and two asparagine residues; because of the greater distance between strand-strand interactions and other CDR regions, interactions with N-linked glycans would be expected to occur with the mannose branches to a greater extent than with the N-acetylgalactosamine stalks.

A common mode of V1V2 recognition by antibody

The cocrystal structures of V1V2 with CH03 and CH04 from donor CH0219 and the modeled interaction of V1V2 with CAP256–VRC26 from donor CAP256 provide atomic-level insight into V1V2-directed bNAbs from two donors, and we integrated this information into the previously published characterization of V1V2 with PG9 and PG16 from donor IAVI 24 (Fig. 4e–g). In all three donors, a strand-strand interaction was implicated between an extended CDR H3 on the antibody and the C strand of V1V2. With bNAbs from donors CH0219 and IAVI 24, the interaction involved parallel hydrogen-bonding, whereas in donor CAP256, the interaction involved antiparallel hydrogen-bonding. In terms of CDR H3 side chain interactions, bNAbs from CH0219 used primarily hydrophobic interactions, those from IAVI 24 primarily used electrostatic interactions, and those from CAP256 used a mixture of hydrophobic and electrostatic interactions.

bNAbs from all three donors bound a single Fab per trimer (Supplementary Fig. 4). For bNAbs from CH0219, the crystal-structure data indicated this stoichiometry and the known quaternary specificity to derive from recognition of N-linked glycans that emanate from neighboring V1V2s. For bNAbs from IAVI 84, the published structural data22,24,25 have also indicated quaternary recognition of N160. For bNAbs from CAP256, modeling indicated protein–protein interactions to occur mostly within a single V1V2 protomer; however the model implicated electrostatic interactions with each of the neighboring protomers, thus suggesting that the CAP256–VRC26 bNAb lineage has strong quaternary specificity that derives from quaternary recognition of a bivalent. Minor differences in the precise engagement of the glycans between IAVI 24 and CH0219 may result in varying tolerances to different glycoforms present on the HIV-1 Env, as has been seen with V3 N332-directed lineages.

We observed some CDR H3 features, such as an enrichment of aspartate and tyrosine residues in the CDR H3s of all of the V1V2-directed antibodies. These appeared to have diverse roles. For example, a D gene–encoded YDY motif occurred in the CDR H3s of both PG9 and CAP256–VRC26.09, and in both antibodies both tyrosines were sulfated. However, in PG9, the sulfated YDY tyrosines interact with strand-C side chains; in contrast, in the CAP256–VRC26.09 model, the sulfated YDY tyrosines appeared to interact with a neighboring protomer (Supplementary Fig. 4). The conservation of the YDY motif in spite of its different roles may relate to the preferred anionic character of the paratope16,22, in which the YDY motif would

Figure 6 Inferred ancestor and intermediates of V1V2-directed bNAbs neutralize a common set of HIV-1 isolates. (a) Mature and reverted V1V2-directed bNAb neutralization across –200 HIV-1 isolates. Neutralized, number of HIV-1 strains with half-maximal inhibitory concentration (IC50) less than 50 μg/ml; total, number of HIV-1 strains tested. Antibodies are organized by donor, with mature antibodies to the left of the dashed vertical line and reverted antibodies to the right. IC50 values for select strains are indicated by enlarged dots colored according to the representative strain shown in the inset. Inset, rank order of strains neutralized by revertants according to the probabilities obtained by frequentist analysis. Enhancement in likelihood of interaction with the earliest neutralizers is provided. Nomenclature of the revertants is as follows: UCA, unmutated common ancestor; gHgL, reverted V gene, mature CDR H3; I1, early intermediate from next-generation sequencing; CH0219-UCA, previously inferred common ancestor15; gHL, reverted V-gene heavy chain with mature light chain; HgL, mature heavy chain with reverted V-gene light chain; gHgL, reverted V genes in both heavy and light chains, mature CDR H3. Likelihood analysis is shown in Supplementary Table 1. (b) Design of soluble HIV-1 Env trimers with chimeric V1V2. Residues 126–196 (magenta) of strains found to preferentially interact with germline-reverted V1V2-directed antibodies were modeled into a trimeric scaffold. (c) Gel filtration analysis and negative-stain EM (2D class averages) of BG505 SOSIP.664.DS.368R.CAP256SU, a representative chimera. A280, absorbance at 280 nm.
### Table 2 Neutralization and binding of selected V1V2 strains and Env-trimer antigens

| Strain     | CAP256   | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 | CAP256-2.29 |
|------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| B          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| B          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |
| C          | ++       | +++         | +++         | +++         | +++         | +++         | +++         | +++         | +++         |

**Strain Clade Assay**

**CH0219** CAP256 IAVI24 IAVI84

**WITO** CAP256 IAVI24 IAVI84

**PG9** CAP256 IAVI24 IAVI84

**PGT145** CAP256 IAVI24 IAVI84

**ZM33.6** CAP256 IAVI24 IAVI84

**T-250** AG CAP256 IAVI24 IAVI84

**CH070.1** AG CAP256 IAVI24 IAVI84

**KER2018.11** A CAP256 IAVI24 IAVI84

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**Ontogenies of V1V2-directed antibodies**

The finding of a common mode of V1V2 recognition, shared by bNAbs from multiple donors in the absence of a common genetic origin, suggested an extended class, which we define as antibodies lacking specific genetic commonalities but nonetheless using a characteristic mode of antigen interaction. We further investigated the development of these V1V2-directed bNAbs to determine whether similarities in molecular recognition might extend to similarities in B-cell ontogeny. In particular, we sought to understand how the structural characteristics of the V1V2-directed bNAbs such as long anionic CDR H3s—which are needed to penetrate the glycan shield and interact with the exposed cationic strand C in V1V2—are created by B-cell processes of recombination and somatic hypermutation and whether these processes are similar from donor to donor.

To enhance the lineage information of V1V2-directed bNAbs from donor CH0219, we used both paired and unpaired heavy chain–light chain sequencing to obtain sequences of additional lineage members. The new sequences were most similar to antibody CH01 (Supplementary Fig. 5). The earliest well-defined maximum-likelihood intermediate had 0.3% nucleotide-level somatic hypermutation, a CDR H3 of 24 amino acids and a net −4 charge (Fig. 5a). Analysis of glycan-interacting residues indicated that 71% did not require somatic hypermutation (SHM) in intermediate I (Supplementary Fig. 5). Altogether, the lineage information indicated that the long CDR H3 derives from recombination, and residues involved in glycan recognition are partially present in the initial recombinant and evolve upon maturation.

The CAP256-VRC26 bNAb lineage, previously substantially defined by longitudinal sampling16, also indicated that the long CDR H3 is result of recombination (Fig. 5a). For the PG9-bNAb lineage from IAVI 24, which has been defined with only two members, PG9 and PG16, we also carried out maximum-likelihood analysis. The earliest intermediate had 4.4% nucleotide-level SHM, a CDR H3 of 28 amino acids and a net −3 charge (Fig. 5a). Analysis of glycan-interacting residues indicated that 43% were present in the earliest intermediate (Supplementary Fig. 6).

We also analyzed the PGT145-bNAb lineage from donor IAVI 84 (Supplementary Fig. 5). Although the likely conformational change in CDR H3 between free and bound conformations made modeling difficult, many features of PGT145-lineage bNAbs were common with those of V1V2-directed bNAbs from CH0219, CAP256 and IAVI 24, thus suggesting a similar mode of recognition. To the PGT141–145 and PGDM1400–1412 series of bNAbs18,19, we used ‘intradonor’ phylogenetic analysis to add sequences of antibodies from previous published next-generation sequencing of donor IAVI 84 B-cell transcripts39. The earliest well-defined maximum-likelihood intermediate had 6.1% nucleotide-level somatic hypermutation, a CDR H3 of 32 amino acids and a net −5 charge (Fig. 5a).

To determine which class features might provide roadblocks for development, we analyzed the prevalence of antibodies with long anionic CDR H3s and high SHM from normal donors40 and compared these with the four V1V2-directed bNAb lineages (Fig. 5b). In the
CDR H3 length and charge distributions, bNabs from donors CH0219 and IAVI 24 were at the edge of normal, whereas bNabs from donors CAP256 and IAVI 84 showed more extreme values. In the SHM histograms, all antibodies were at the upper range of normal, except for PGDM1400 which was a more substantial outlier. Altogether, the lineage information from all four donors indicated that V1V2-directed bNabs start with a long anionic CDR H3 at the recombination stage, which in a number of donors appeared to have partial recognition of N-linked glycans that evolved during maturation.

HIV-1 strains recognized by developmental intermediates

We have previously found that reversion of V1V2-directed bNabs to UCA (or to V gene–reverted approximations) substantially restrict neutralization potency15,16,20. However, prior neutralization analyses had not been performed with antibodies from all four donors against the same panel of viruses. We therefore assessed the neutralization of mature, intermediate and UCA versions of V1V2-directed bNabs from donors CH0219, CAP256, IAVI 24 and IAVI 84 on a panel of approximately 200 HIV-1 isolates (Fig. 6a).

To define a common set of HIV-1–interactive isolates, we used a frequentist analysis to determine the likelihood that a particular strain might be recognized by a reverted bNAbs. This analysis identified nine strains recognized by many of the reverted bNabs (Fig. 6a). These strains were ~10–20 times more likely than the median of the panel to be neutralized by the earliest neutralizer from each lineage (Supplementary Table 1). The clade B strain WITO was recognized commonly, and the clade C ZM233 strain, which we had earlier shown to be recognized by reverted versions of PG9 and CH01 (refs. 15,20), was also commonly recognized. Direct analysis of strand-C sequences for these strains showed some differences (for example, ZM233 does not contain a glycan at residue N156 or N173, thus differentiating it from others in the group) and many commonalities, such as the presence of glycan N160 and a cationic N-terminal half of strand C (Supplementary Fig. 7). However, we did not observe a specific distinguishing sequence characteristic separating strains neutralized by reverted bNabs from those not neutralized. In general, the V1V2 epitope of these bNabs involves glycan, is quaternary and is known to adopt multiple conformations. For instance, strand C, in a monomeric context, can adopt multiple conformations41, and the V1V2 trimeric interface on virions is conformationally dynamic42. Thus, the mechanism by which specific strains interact more strongly with reverted V1V2-directed bNabs is probably both complex and multifactorial. Nonetheless, we did observe that neutralization by reverted bNabs correlated with the neutralization potency of the mature bNabs. One distinguishing characteristic was the high susceptibility of the select strains to being neutralized not only by the reverted bNabs but also by the mature ones. (This susceptibility was specific to V1V2-directed antibodies because these strains were not generally sensitive to neutralization.) In general, the direct experimental assessment of strains capable of interacting with UCA or reverted bNabs through a neutralization-based screen (such as that shown in Fig. 6) identifies strains from which to engineer antigens capable of binding early members of V1V2-directed bNab lineages.

Figure 7 Ontogeny-based vaccine design. (a) A general scheme for the development of ontogeny-based chimeric immunogens. (b) Different stages of B-cell development for V1V2-directed bNabs and their probabilistic enhancement with ontogeny-based immunogens. The recombination frequency (0.05%) was estimated on the basis of the percentage of long (equal to or greater than 24 amino acids) and anionic (net charge of −3 or lower) CDR H3 present in 9,721 unique and productive human sequences40. Probability enhancements are shown for a single immunogen; substantial additional enhancement could be gained through the use of immunogen cocktails (Supplementary Table 1). (c) Schematic of the B-cell ontogeny for the extended class of V1V2-directed bNabs. Strategies for vaccine enhancement are highlighted in red.
Design and validation of ontogeny-specific chimeric trimers

Although the trimeric V1V2 scaffolds that we designed and optimized for crystallization might have utility as bNAb-specific probes, their altered oligomeric presentation of V1V2 (Fig. 3) along with neoepitopes from both scaffold and exposed regions of V1V2 suggest that they might be less than optimal as vaccine immunogens. This contrasts with the correct oligomeric presentation of V1V2 in SOSIP.664 and DS-SOSIP.664 contexts27,32,35 and the ability of SOSIP.664 to elicit high titers of tier 2 autologous neutralizing titers43. We therefore chose to use soluble DS-SOSIP.664 trimers as a platform to obtain ontogeny-specific antigens.

One issue with DS-SOSIP.664 is a difficulty in expressing strains other than BG505. To address this, we used our understanding of the V1V2 structure in the context of the prefusion closed HIV-1 Env trimer to construct V1V2 chimeras generated by swapping desired strains of HIV-1 Env into the V1V2 region of BG505 DS-SOSIP.664 trimers (Fig. 6b). We succeeded in expressing chimeric DS-SOSIP trimers with V1V2s from all nine of the special strains that we identified by lineage reversion and HIV-1 neutralization (Fig. 6a and Supplementary Fig. 7). We assessed recognition by mature and reverted bNAbs of all nine V1V2 chimeras (Table 2 and Supplementary Fig. 7). Binding of the reverted bNAbs to each of the nine V1V2 chimeras correlated with their neutralization of the same nine HIV-1 strains (Spearman’s $\rho = 0.67$; $P < 0.0001$, one sided; $n = 9$ strains, 19 antibodies).

**DISCUSSION**

The path to creating a set of antigens capable of interacting with germ-line-reverted versions of V1V2-directed bNAbs involved an information flow from a supersite of vulnerability, through the definition of an extended class and the identification of specific strains capable of being neutralized by germ-line-reverted class members, to the engineering and antigenic validation of V1V2-chimeric trimers (Fig. 7a). We note that for V1V2-directed bNAbs of the PG9-extended class (named after the first described antibody of the extended class), ontogeny analysis indicated three likely roadblocks to development. The first roadblock, recombination to produce a long anionic CDR H3 capable of piercing the glycan shield and backbone hydrogen-bonding to strand C of V1V2 (Fig. 7b), is likely a substantial impediment in the development of V1V2-directed bNAbs, partly because of the rarity of long CDR H3s as a result of tolerance deletion from the naive B-cell repertoire44. Next-generation sequencing and CDR modeling, however, have suggested that suitable CDR H3s are present in the naive human repertoire37. Our ontogeny-based immunogens are not expected to influence recombination frequencies and thus probably have little impact on this B cell stage.

A second roadblock that appears to be a substantial impediment is that B-cell priming requires a sufficiently strong interaction between the initial recombinant on a naive B cell and HIV-1 Env to initiate the maturation of the B-cell lineage (Fig. 7b). For all four bNAb V1V2-directed lineages analyzed here, the UCA or gHgl could neutralize less than 5% of HIV-1 isolates (Fig. 6a), thus suggesting that fewer than 1 in 20 HIV-1 strains can prime the naive B cells of a potential V1V2-directed bNAb lineage. In the case of the CAP256-VRC26 lineage, sequencing of co-variant antibody and virus indicates that successful priming of this lineage has occurred only because the sequence of a CAP256-SU-derived virus fortuitously happened to interact with the lineage ancestor18. Immunization with soluble trimers with V1V2 chimeras of the special strains capable of interacting with initial recombinant, however, should substantially increase the likelihood of successful priming of the lineage. Individually, the enhancement in likelihood of interaction is ~10–20 times higher for each of the nine special strains identified here (Fig. 6a).

Cocktails of these strains are expected to increase the likelihood even more, and a nine-strain cocktail of all of the special strains enhances the probability of interaction by ~40-fold (Supplementary Table 1b).

A third roadblock, somatic development, through which primed B cells mature from having weak interactions with few HIV-1 strains to having strong interactions with most HIV-1 strains (Fig. 7b), is likely to be a substantial impediment as well. Each of the bNAb lineages showed substantial maturation before the emergence of mature antibodies capable of neutralizing most HIV-1 strains (Fig. 5a), and longitudinal data from the CAP256-VRC26 lineage have indicated years of maturation16. It should be possible to quantify the maturation induced by our V1V2 chimeric trimers in mouse systems comprising knockins of germline or early intermediates of the V1V2-directed antibodies, as has been done with knockins of germline or early intermediates for other bNAbs, such as those of the VRC01 class12,13.

The ability of ontogeny-specific vaccine immunogens to affect specific stages of B cell development, such as that shown for the V1V2-extended class (Fig. 7c), may be generally applicable to extended bNAb classes for which steps of UCA priming or of somatic maturation represent roadblocks to antibody development3,5,7,12,13. Currently, the only classes of broadly HIV-1-neutralizing antibodies that have been observed are the VH1–2–derived VRC01 class and the VH1–46–derived 8ANC131 class, both of which target the CD4 supersite8,10,11. For the VRC01 class, a severe roadblock to development involves recognition by the UCA of HIV-1 Env5,7,10,46. Nevertheless, neutralization by VRC01-class reverted intermediates has identified strains with characteristics known to enhance UCA recognition, such as the deletion of glycan 276 in gp120 loop D5,10,47 (Supplementary Fig. 8). It will be exciting to see whether other classes or extended classes of bNAbs targeting HIV-1 will be identified—and whether the reversion-based neutralization strategy for ontogeny-specific vaccine design described here will be useful in their ‘re’-elicitation.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** Coordinates and structure factors have been deposited in the Protein Data Bank under accession codes PDB 5ESV (Fab CH03 with PDB 1VH8–scaffolded V1V2 (strain CAP256-SU)) and 5ESZ (Fab CH04 with PDB 1VH8–scaffolded V1V2 (strain A244)). The new next-generation sequencing data for donor CH0219 (454 pyrosequencing and Illumina) have been deposited in the NCBI Sequence Read Archive under accession number SRP065493.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.G. headed the determination of the V1V2-bound CH03 and CH04 crystal structures, revertant neutralization strategy and chimeric SOSIP design and assembled and wrote the paper, on which all principal investigators commented. R.T.B. and M.K.L. contributed CH0219 materials; M.B. and B.F.H. contributed CH0219 experiments; M.B. and B.F.H. contributed CH0219 materials; J.R. Mascola and NISC contributed next-generation sequencing data; J.G., C.S., N.G. and B.J.D. contributed to reverted VRC01 experiments; M.B. and B.F.H. contributed to V1V2 scaffold design and assessment; C.T., T.M.L. and J.G. contributed to MDFF analysis; M.P. assisted with chimeric SOSIP design; J.M. assisted with figure conception and design; U.B. performed EM; T.Z. and M.G.J. contributed to reverted VRC01 experiments; M.B. and B.F.H. contributed CH0219 materials; P.L.M. and L.M. contributed CAP256 materials; J.G., C.S., L.S. and P.D.K. assembled and wrote the paper, on which all principal investigators commented.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Protein expression and purification. V1V2 scaffolds, codon optimized for mammalian expression, were designed with an artificial N-terminal secretion signal and a C-terminal HRV3C recognition site followed by a His6 tag. The genes were cloned into the XbaI/BamH1 sites of the mammalian expression vector pVRC8400 and transiently transfected into HEK 293S-GnTI- cells (ATCC CRL-3022), which were used because of a requirement for a Man9GlcNAc2. Scaffolds were purified from the medium with Ni-NTA resin (Qiagen), and the eluted proteins were digested with HRV3C (Novagen) before passage over a 16/60 S200 size-exclusion column.

CH03 and CH04 heavy and light chains were codon optimized for mammalian expression with an HRV3C recognition site (GLEVLFQGP) inserted after Lys235 of the heavy chain. The sequences were cloned into pVRC8400 and transiently cotransfected into Expi293 cells (Invitrogen) as previously described. 2G12 IgG was purified from the supernatant after 5 d with Protein A agarose (Pierce) and diaлизed into PBS.

To form homogenous complexes, 3 mg of purified CH03 or CH04 HRV3C IgG was bound to 750 µl Protein A Plus agarose (Pierce) in a disposable 10-ml column. To this resin, 10 mg of purified V1V2 scaffold was added. Unbound scaffold was washed through the column with five column volumes of PBS. The column was capped, and 20 µl of HRV3C protease at 2 U µl−1 was added to the resin along with 1 ml of PBS. After 2 h at room temperature, the resin was drained, and the eluate was collected and passed over a 16/60 S200 column in buffer containing 5 mM HEPES, pH 7.5, 150 mM NaCl, and 0.02% NaN3. Fractions corresponding to the CH03–V1V2 complex were pooled and concentrated to 5 mg ml−1.

Crystallization screening. CH03–PDB 1V18–V1V2SU and CH04–PDB 1V18–V1V2AS4A were screened for crystallization with 572 conditions from Hampton, Wizard and Precipitant Synergy screens with a Cartesian Honeybee crystalization robot as described previously and a mosquito robot with 0.1 µl of reservoir solution and 0.1 µl of protein solution. Crystals for CH03–PDB 1V18–V1V2CAP256SU suitable for structural determination were obtained in 10% PEG 8000, 0.1 M CaCl2, 20% MPD, and 0.1 M Na acetate, pH 5.5. Crystals were cryoprotected in a solution supplemented with 25% (2R,3R)-butane-2,3-diol and 140 mM HCl, and then the absorbance was measured at 450 nm. Data were collected at a wavelength of 1.00 Å at the SER-CAT beamline ID-22 (Advanced Photon Source, Argonne National Laboratory).

X-ray data collection, structure solution and model building. Diffraction data were processed with the HKL2000 suite. A molecular replacement solution for the data set consisting of three CH03 Fab molecules per asymmetric unit with the PDB 1V18 trimer was obtained with Phenix. The CH03 complex contained two Fab s and two monomers of PDB 1V18; the trimer conformation was maintained through crystal symmetry. Model building was carried out with COOT, and refinement was performed with Phenix. Final data collection and refinement statistics are presented in Table 1. The Ramachandran plot as determined by MolProbity shows 91% of all residues in favored regions and 97.2% of all residues in allowed regions for the CH04 structure and 92% of all residues in favored regions and 98.4% of all residues in allowed regions for the CH04 structure.

Antigenic analysis of BG505 SOSIP.664.DS.V1V2 by ELISA. 96-well nickel-plated plates (Thermo Fisher Scientific) were coated for 1 h at 100 µl/well with histidine-tagged BG505 SOSIP.664.DS.V1V2 constructs at 1 µg/ml, diluted in 1× PBS. The plates were then blocked at room temperature for 1 h with 200 µl/well of 5% skim milk in 1× PBS. After the plates were washed (wash buffer, 0.05% Tween 20 plus 1× PBS), they were incubated for 1 h at room temperature with five-fold serially diluted V1V2-directed antibodies at concentrations ranging from 20 to pg/ml to 1 ng/ml in 1× PBS plus 0.05% Tween 20. The plates were washed and then incubated for another hour with horseradish peroxidase (HRP)-conjugated anti-human IgG (1:5,000, Santa Cruz Biotech, sc-2543) diluted in 0.2% Tween 20 plus 1× PBS. After a final wash, the plates were then developed with TMB peroxidase substrate for 20 min. The reaction was stopped with 140 mM HCl, and then the absorbance was measured at 450 nm. All incubations were at 100 µl/well at room temperature, except where noted otherwise. Experiments were run in triplicate.

Neutralization panels. Single-round-replication Env pseudoviruses were prepared, titered, and used to infect TZM-bi target cells as described previously. The data were calculated as a reduction in luminescence units compared with that in control wells, and neutralization curves were fit by nonlinear regression with a five-parameter Hill slope equation. Data are reported as 50% inhibitory concentration (IC50) in micrograms per microlitre.

Negative-stain electron microscopy. Negative-stain EM samples were diluted to −0.03 mg/ml, adsorbed to a freshly glow-discharged carbon-film grid for 15 s, and stained with 0.7% uranyl formate. Images were collected semiautomatically with SerialEM® on an FEI Tecnai T20 with a 2K × 2K Eagle CCD camera at a pixel size of 0.22 nm/pixel. Particles were picked automatically, and reference-free 2D classification was performed in EMAN2 (ref. 62).

Hydrogen-deuterium exchange (HDX). To identify Env-interactive residues, within CAP256-VRC26.03, rates of hydrogen-deuterium exchange for CAP256-VRC26.03 Fab alone and in the presence of BG505 SOSIP.664 (2:1 molar ratio, Fab/SOSIP (a ratio for which 50% of CAP256-VRC26.03 Fab and 100% of SOSIP should be bound) were measured. Deuteration reactions were initiated by diluting 10 µl of 0.56 mg/ml CAP256-VRC26.03 Fab (± SOSIP) into 90 µl of phosphate buffered saline containing 85% D2O. Deuterations were carried out for 10 s, 15 s, and 20 s for the addition of 90 µl of quenching solution (500 mM Tris-2-carboxyethyl phosphine (TCEP), 3 M urea, and 0.2% formic acid, with a final pH of 2.5), 10 µl of pepsi (2 mg/ml in 100 mM phosphate, pH 4.0) and digestion on ice for 5 min. Pepsin-digested HDX samples were plunge frozen in liquid nitrogen and stored at −80 °C. Ligand-free and SOSIP-bound VIRTEX203.03 HDX samples were prepared side by side in matched buffers. For LC-MS analysis, samples were thawed on ice for 5 min and manually injected onto a Waters BEH 1.7-µm C18 column (Thermo Scientific) with a gradient of 15% to 40% B in 8 min (A, 0.05% TFA and 5% ACN; B, 0.05% TFA and 80% ACN). Eluted peptides were analyzed in duplicate with a Waters Synapt Q-TOF mass spectrometer. Peptide identification and exchange analysis were as described previously, with the exception that peptide deuteration was calculated with the number of exchangeable backbone amides to estimate theoretical 100% deuteration for each peptide.
In silico arginine scanning. Step one of our in silico arginine-scanning method involved mutating each residue in the CDR region to arginine. Any endogenous arginine or lysine residues were left unmutated but were still used in all subsequent analyses. To carry out all of our in silico mutations, we used the side chain modeling program Scap by default settings. Step two of our method scored and determined the solvent accessibility of each predicted arginine residue. To assess the feasibility of adopting an arginine at a particular position in a CDR region, each mutated residue was scored independently with a modified RAPDF distance-dependent statistical potential. The scores for each residue were computed as follows: $\Delta E = \text{RAPDF}(\text{ARG}) - \text{RAPDF}(\text{NAT})$, where the subscript i denotes the residue position, and ARG and NAT denote the scores of the mutated and native residue, respectively. We used the Surface65 program to determine the solvent-accessible surface area (SASA) of each residue. The SASA was normalized to a reference SASA for the same residue, as determined from a peptide composed of three residues in extended conformation with glycine as the first and last residue. The normalized SASA was computed as follows: $\text{Acc}_i = \text{SASA}_i(S)/\text{SASA}_i$, where S denotes the side chain in the protein environment, and L denotes the side chain in the linear peptide environment. The normalization factor allowed for an unbiased comparison of solvent accessibility for residues of different amino acid types (and sizes). A weighting factor (set to 1.0) was used to convert the normalized SASA value into a score. The final step of our method involved a stochastic search for the optimal set of arginine residues, on the basis of the RAPDF and solvent-accessibility scores determined in step two. Each cycle of the search began by random selection of a seed residue from the set of CDR residues. All residues within a 5.0-Å radius of the seed residue were removed (‘flagged’) from the set of selectable residues in the current cycle. We next determined the closest residue greater than 5.0 Å (measured from the Cα atom) away from the current seed residue. This residue was then set as the seed residue, and all residues within a 5.0-Å radius were removed from the set of selectable residues. The process was repeated until all residues had been selected. At the end of each cycle, the total score for the final set of seed residues was determined from the look-up tables generated in step two. The total score was computed as follows: $F = \sum_{i} \text{Acc}_i$, where $\Delta E_i$ is the RAPDF-based score, and $\text{Acc}_i$ is the weighted normalized SASA score. The stochastic search continued until the lowest F score no longer changed, or the user-specified maximum number of cycles (set to 1,000) had been reached. The set of residues with the lowest F score was selected as the optimal set of residues to mutate to arginine.

Phylogenetic analysis involving donors CH0219, CAP256, IAV124 and IAV184 and CAP256. Donor CH0219. Pan-B cells were isolated from Donor 0219 PBMC (Miltenyi Biotec) and processed as reported previously for paired heavy- and light-chain sequencing. All 454 and Illumina NGS heavy-chain sequences were processed with an in-house version of IgBlast and then filtered on the basis of their heavy-chain V and J germline gene assignments. All sequences with the IgVH1–8 germline gene assignment were retained for further processing with intradonor phylogenetic analysis. The major objective of intradonor phylogenetic analysis is to bracket all phylogenetically similar sequences on a neighbor-joining (NJ) tree with known neutralizing-antibody sequences derived from the same donor. For the analysis here, we used all possible pairs of neutralizing-antibody sequences derived from this donor PGDM1400–1412 (GenBank KP006370–KP006382) and PGT141–145 (GenBank KJ201906.1–KJ201910.1).

Intradonor phylogenetic analysis works in similar fashion to the cross-donor phylogenetic analysis described previously. Briefly, the method begins by randomly shuffling all the sequences in a data set to remove any potential bias in the order of the sequences and to improve the convergence of the method. After sequence shuffling, the data set was split into FASTA files, each containing up to 5,000 sequences. A pair of neutralizing-antibody sequences along with the germline VH gene sequence was added to each FASTA file. The germline gene sequence was used as the outgroup in the NJ tree. A multiple sequence alignment for each FASTA file was generated with CLUSTAL0 (ref. 67), and each alignment was then converted into a distance matrix with dendist (with default settings). From this matrix, an NJ tree was constructed with the ‘neighbor’ program (with default settings). Both dendist and neighbor are part of the PHYLIP package.

Donor sequences were extracted from each NJ tree with a pair of neutralizing-antibody sequences derived from the same donor. All donor sequences contained in the minimal-spanning tree containing the pair of neutralizing sequences were extracted from the NJ tree and split into FASTA files containing no more than 5,000 sequences, and the above process was repeated five times. The remaining sequences were filtered by their germline J gene retaining all sequences with the IgH6 assignment. We filtered out any transcripts with CDR H3 lengths (Kabat) less than 31 amino acids and greater than 34 amino acids. Duplicate sequences were removed, and problematic sequences were edited to bring the transcript into the correct translational frame. The remaining sequences were aligned and then used to generate an ML tree with dnaml, with the same procedure as for donor CH0219. The inferred intermediates were derived from the ML tree.

Modeling the CAP256-VRC26.9 and the HIV-1 Env trimer complex. A combination of molecular dynamics (MD) and molecular dynamics flexible-fitting (MDFD) simulations were used to model the complex consisting of the prefusion HIV-1 Env trimer and CAP256-VRC26.09 antibody. First, any loop regions on the HIV-1 Env prefusion trimer were modeled in Loopy. We then added mannos 5 to each sequon position. A model for the CAP256-VRC26.09 antibody was obtained by threading the amino acid sequence of CAP256-VRC26.09 onto the CAP256.VRC26.03 antibody structure (PDB 4OD1) with Nest65. Models for the trimer and antibody were docked as rigid bodies into the cryo-EM map (EMD 5856) with Colores in the SITUS package. The complex was then solvated in a 15-Å water box and neutralized by the addition of NaCl at a concentration of 150 mM.

After rigid-body docking into the cryo-EM map, we applied a combination of MDFD and MD to the complex, then MD alone to relax the complex. The simulation was performed with NAMD, using the CHARMM36 force field, including CMAP corrections for the protein. TIP3P water parameterization was used to model the solvent. Periodic electrostatic interactions were computed with particle-mesh Ewald (PME) summation with a grid spacing smaller than 1 Å. Constant temperature was imposed with Langevin dynamics with a damping coefficient of 1.0 ps. A constant pressure of 1 atmosphere was maintained with Langevin piston dynamics, a 200-fs decay period and a 50-fs time constant. During equilibration, the trimer’s secondary structure was restrained with harmonic restraints applied to the backbone’s torsional angles and interacting strands (strand C on the trimer and the strand on CDR H3). The system was minimized using 5,000 steps of conjugate gradient minimization and then equilibrated with a linear temperature gradient, which heated up the system from 0 to 310 K in 5 ns. An additional 5 ns of simulation was carried out, and this was followed by 5 ns MDFD. The protein secondary structure, the chirality and the interacting strains were restrained. The system was relaxed during 20 ns and then subjected to a second 5-ns MDFD run in which the interacting strains were not restrained. Finally, unrestrained molecular dynamics was performed up to 50 ns. The lengths of all bonds involving hydrogen atoms was constrained with the RATTLE algorithm, thus allowing a time step of 2 fs.
Figures. Structure figures were prepared with PyMOL (http://www.pymol.org/). Figure 6 a was generated with GraphPad Prism (http://www.graphpad.com/scientific-software/prism/). EM-structure images were generated with Chimera (http://www.cgl.ucsf.edu/chimera/). Phylogenetic trees were generated with Dendroscope (http://ab.inf.uni-tuebingen.de/software/dendroscope/).

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