Somatic mutations within antibody variable and framework regions (FWR) can alter thermostability and structural flexibility, but their impact on functional potency is unclear. Here we study thermostability and use molecular dynamics (MD) simulations to assess the role of FWR mutations during maturation of HIV-1 broadly neutralizing antibodies (bnAbs). The tested bnAbs show lower thermostability than their unmutated ancestor antibodies. FWR mutations in the Fab elbow region are frequently observed in HIV-1 bnAbs and MD simulations show that such FWR mutations alter interdomain flexibility in two HIV-1 bnAbs. In a CD4-binding site lineage, reversion mutations result in a loss of neutralization potency in an early intermediate and affinity-matured bnAb against autologous and heterologous Tier-2 viruses, respectively. Elbow region reversion mutations in a glycan-V3 bnAb modestly reduce potency against an autologous virus isolate. Thus, selection of mutations in the Fab elbow region impacts interdomain conformational flexibility and paratope plasticity during bnAb development.
Affinity maturation of antibodies involves mutations both within the antigen-binding site as well as in distal sites in the antibody framework regions (FWRs)\(^1\)\(^2\). Various roles attributed to FWR residues include being neutral to scaffolding for antibody structural integrity, compensating for destabilizing CDR (complementarity determining region) mutations, and enhancing variable loop flexibility\(^3\)\(^4\). Affinity-enhancing mutations in residues that directly interact with antigen can be detrimental to antibody thermostability, and as such, germline-reverted mutants are thermodynamically less stable when compared to affinity-matured antibodies\(^8\)\(^9\). Since the destabilizing effect of affinity-enhancing mutations is overcome by concurrent selection of stabilizing mutations, affinity maturation has been viewed as a selection process that optimizes both antibody affinity and thermostability\(^5\). As observed in the evolution of enzymes, a function/stability trade-off occurs during antibody affinity maturation and therefore, co-selection of mutations in CDRs and FWRs is required to maintain a balance between antibody function and stability\(^5\)\(^9\)\(^10\).

The relative disposition of the Fv (Fab variable) to the CH1/CL (constant heavy/constant light), and the relative orientation of the heavy and light chain variable domain, VH and VL, can be altered by FWR mutations\(^1\)\(^11\)\(^12\). The former describes a secondary region of flexibility termed the Fab elbow\(^11\)\(^12\), distinct from the well-known antibody hinge region between the Fab and Fc, while the latter determines the geometry of the binding site\(^1\)\(^13\). The Fab elbow adds an additional spatial degree of conformational flexibility which is not necessarily fixed but may display dynamic flexibility, capable of shifting in the presence of ligand\(^1\)\(^4\)\(^14\). Thus, during affinity maturation, the selection of FWR mutations in the Fab elbow residues can be important for optimizing antibody conformational dynamics and adaptation to antigen structure\(^4\)\(^15\)\(^16\).

The role of FWR mutations and the molecular basis for their selection during affinity maturation of HIV-1 broadly neutralizing antibodies (bnAbs) is not clearly understood. Recent studies indicate that distal mutations acquired in exposed loops and the FWRs are not all neutral but rather can contribute to antigen binding or enhance neutralization potency through modification of structural stability and/or loop flexibility\(^8\)\(^17\)\(^20\). FWR mutations can be thermally destabilizing in a fully matured bnAb\(^8\)\(^21\) and improvement in neutralization potency can incur a cost to thermostability\(^22\). However, FWR mutations were reported to provide no functional advantage for weakly neutralizing HIV-1 antibodies, while being essential for bnAb neutralization\(^8\). For a CD4-bs bnAb, germline reversion of a FWR residue that afforded loop flexibility increased the thermostability (melting temperature, T\(_m\)) and decreased the neutralization potency\(^8\), indicating that the bnAb development incurred a stability cost in gaining functional potency. These studies highlight the importance of FWR mutations for bnAb development and the need for understanding the role of specific FWR mutations during bnAb maturation. The relationship between thermostability and gain-of-function for each antibody in a bnAb lineage, including the inferred unmutated common ancestor (UCA) and intermediate antibodies, remains undefined. In the early stages of bnAb development, the selection of mutations that contribute to interdomain flexibility can be advantageous in overcoming the geometric hurdles presented by the HIV-1 Env trimeric arrangement, as well as shifting variable loop lengths and the associated glycan positions\(^23\)\(^24\). Thus, fine-tuning paratope and Fab structural flexibility together likely plays a major role in determining the ability of maturing antibodies to develop heterologous breadth. The above considerations raise the question of whether any key mutations selected early in bnAb maturation impact antibody conformational flexibility and thermostability and pave a path in which concurrent and subsequent affinity-enhancing mutations are selected without further detriment to antibody stability. Thus, we aimed to identify the mutational changes that are destabilizing and/or contribute to interdomain flexibility in HIV-1 bnAb lineages targeting different HIV-1 Env epitopes and to determine if there is an association between the selection of such mutations and recognition of heterologous antigenic sequences and subsequent bnAb development.

Here, we describe the role of specific Ig V\(_H\) mutations in optimizing antibody thermostability and interdomain conformational flexibility during HIV-1 bnAb development. We show that all major classes of bnAbs have lower thermostability than their corresponding inferred UCA antibodies. The thermal destabilization in two bnAb lineages—the CD4-bs CH103\(^3\)\(^4\) and the glycan-V3 DH270\(^25\)\(^26\)—was first observed in an early intermediate and was maintained in the affinity-matured antibodies that developed neutralization potency and breadth. In the CH103 bnAb lineage, we identified heavy chain FWR residues that contribute to thermal destabilization and show that mutations in the Fab elbow region altered interdomain flexibility and were important for bnAb development. We report that Fab elbow region mutations are frequently observed in HIV-1 bnAbs and using long timescale molecular dynamics (MD) simulations, we describe the role of these mutations in enhancing interdomain flexibility in the two bnAb lineages studied. Furthermore, we show that the selection of destabilizing elbow region mutations was important for neutralization in the CH103 lineage, since reversion of destabilizing mutations in an early intermediate and the affinity-matured bnAb resulted in a loss of neutralization potency. In the glycan-V3 DH270 lineage, reversion of elbow mutations resulted in the loss of neutralization potency in a bnAb against more difficult-to-neutralize viruses. Our studies demonstrate that mutations that impact Fab interdomain flexibility are selected early in the bnAb evolutionary path and are important for bnAb development.

Results

**HIV-1 bnAbs are less thermostable than their UCAs.** To determine whether affinity maturation affected the stability of each of the major classes of HIV-1 bnAbs, we measured the melting temperatures (T\(_m\)) of bnAbs and their inferred UCA antibodies by performing thermal denaturation by circular dichroism (CD) and differential scanning calorimetry (DSC) (Fig. 1). Each affinity-matured antibody displayed lower T\(_m\) than their corresponding germline or germline-proximal intermediate mAb, with the largest relative differences (>5 °C) observed for the CD4-bs and glycan-V3 bnAbs (Fig. 1c; Supplementary Table 1). Most notably, the glycan-V3 bnAb DH270 exhibited a ~20 °C reduction in T\(_m\) compared to its UCA. These data suggest that thermal destabilization during affinity maturation is observed in every major class of HIV-1 bnAb specificity.

**FWR mutations impact thermostability in the CH103 lineage.** We previously described the evolution of the CD4-bs CH103 bnAb lineage, which included the inferred UCA, and several inferred intermediates, that preceded the affinity-matured bnAbs with V\(_H\) mutation frequencies of 14.9–16.8% (Fig. 2a)\(^24\)\(^28\). To identify mutations that might have contributed to thermal destabilization of this bnAb, we compared the V\(_H\) sequences of the CH103 lineage antibodies and generated three CH103 UCA mutants (Fig. 2). We produced UCA mutant antibodies with single mutations in the heavy chain FWR1 at position 14 (UCA-P14S) and HCDR1 proximal position 30 (UCA-S30G). An additional mutation was introduced at position 31 of the UCA HCDR1 to generate the double mutant UCA-S30G/S31G. All
three of the above mutations were selected in the germline-proximal intermediate I8 to I4 transition and were retained in each of the later intermediates and the mature antibodies in the upper branch of the CH103 lineage tree (Fig. 2). The positioning of the three substituted residues on a gp120-liganded model of CH103, following superposition of gp120 to the resolved CH103-bound state with gp120 core24, showed the remote location of the FWR1 P14S residue when compared to the closer proximity of HCDR1 proximal residues (S30G/S31G) to the antibody-antigen interface (Fig. 2b). Structural analysis of the UCA versus the CH103 bnAb revealed that the P14S mutation alters a loop between strands A and B while the CDR1 proximal S30G and S31G mutations increase loop and CDR1 flexibility respectively24.

The UCA mutants had Tm values that were lower than the wild-type UCA, with the single mutants (UCA-P14S, UCA-S30G) each reduced by 1 °C and the double mutant (UCA-S30G/S31G) reduced by 1.8 °C (Table 1; Supplementary Table 2). These data suggest that FWR mutations and those that affect loop flexibility can impact thermostability. Furthermore, the binding affinities and rate constants (association/dissociation) of the UCA and each of the UCA mutants to the autologous CH505TF gp120, as well as to CH505TF gp140 SOSIP trimers, were similar, and neither the UCA nor the UCA mutants bound to the heterologous Env B.63521 gp120 (Table 2; Supplementary Table 3). Thus, the selected UCA VH mutations (FWR1/CDR1 proximal) (Fig. 2), when introduced in the UCA, did not have any long-range configurational effect on the antibody’s binding site that provided any detectable affinity improvement beyond impacting thermal stability. Since each of the mutations were retained in the later intermediates and the isolated mature CH103 bnAbs, the destabilizing mutations, once selected, were fixed in the lineage. Therefore, we predicted that the thermal destabilization occurred in one of the earlier intermediate antibodies when the full sequence of mutations (P14S...MGG31) was first acquired, as in intermediate I4 (Fig. 2).
The unmutated common ancestor (UCA) and intermediate antibodies (Is) were inferred and each mAb was produced as described previously. The % VH unmutated common ancestor (UCA) and intermediate antibodies (Is) were inferred and each mAb was produced as described previously. The % VH unmutated common ancestor (UCA) and intermediate antibodies (Is) were inferred and each mAb was produced as described previously. The % VH unmutated common ancestor (UCA) and intermediate antibodies (Is) were inferred and each mAb was produced as described previously. The % VH unmutated common ancestor (UCA) and intermediate antibodies (Is) were inferred and each mAb was produced as described previously.

Thermal destabilization occurs early in the CH103 lineage. To address how affinity maturation affected the thermostability of the intermediates, we measured Tm of the CH103 clonal lineage antibodies (Fig. 3a; Supplementary Figure 1, Table 4). Tm values for the UCA and the germline proximal intermediate I8 were similar, which was expected since I8 retained both the germline FWR1 P14 and HCDR1 proximal S30/31 residues. However, as predicted, I4 with the P14S/S30G/S31G mutations showed a marked decrease in Tm of 11.7 °C. Reversion of these mutations to the UCA and the germline proximal intermediate I8 were similar, which was expected since I8 retained both the germline FWR1 P14 and HCDR1 proximal S30/31 residues.

Table 1 Effect of heavy chain mutations on thermostability in the CH103 lineage

| mAb                  | Tm (°C) | ΔTm (°C) |
|----------------------|---------|----------|
| UCA                  | 75.6 ± 0.3 | --       |
| UCA-P14S             | 74.63 ± 0.06 | -1.0 ± 0.3 |
| UCA-S30G             | 74.56 ± 0.06 | -1.1 ± 0.3 |
| UCA-S30G/S31G        | 73.8 ± 0.4  | -1.8 ± 0.5 |
| I4                   | 64.60 ± 0.01 | --       |
| I4-S14P              | 67.01 ± 0.04 | +2.41 ± 0.04 |
| I4-S14P/G30S         | 68.42 ± 0.02 | +3.82 ± 0.03 |
| CH103                | 65.5 ± 0.1  | --       |
| CH103-S14P           | 66.41 ± 0.03 | +0.9 ± 0.1 |
| CH103-S14P/G30S      | 66.57 ± 0.05 | +1.0 ± 0.1 |

Table 2 Apparent affinity of the CH103 UCA mutant and lineage mAbs for transmitted founder (TF) and heterologous HIV-1 Env

| mAb                  | C.CH505TF gp120 (Kd × 10^-9 M) | C.CH505TF SOSIP.664.v4.1 (Kd × 10^-9 M) | B.635Z1 D11 gp120 (Kd × 10^-9 M) |
|----------------------|---------------------------------|------------------------------------------|---------------------------------|
| UCA                  | 430 ± 20                        | 1,500 ± 300                              | NB                              |
| UCA-P14S             | 370 ± 30                        | 1,700 ± 400                              | NB                              |
| UCA-S30G             | 400 ± 10                        | 1,800 ± 100                              | NB                              |
| UCA-S30G/S31G        | 440 ± 20                        | 800 ± 100                                | NB                              |
| S31G                 |                                 |                                          |                                 |
| I4                   | 53 ± 5                          | 60 ± 20                                  | 14,000 ± 3,000                  |
| I3                   | 9.53 ± 0.06                     | ND                                       | 4200 ± 100                      |
| CH106                | 9.3 ± 0.8                       | 5 ± 1                                    | 14.2 ± 0.9                      |
| CH103                | 40 ± 10                         | 16 ± 3                                   | 11.1 ± 1.0                      |

Antibody thermal denaturation profiles were obtained and analyzed by DSC as described in Methods. Data are shown as the mean and standard deviation from a minimum of two replicate measurements. The ΔTm is calculated for each mutant relative to wild-type with error propagated from the standard deviations.

Antibody thermal denaturation pro...
profiles indicated that the drop in $T_m$ was due to Fab thermal instability (Fig. 3a; Supplementary Figure 1).

We conclude that the decrease in $I_4$ thermostability was a result of destabilizing mutations acquired early in the affinity maturation process ($I_8$ to $I_4$) and was associated with the acquisition of $14S_{...}GG^{31}$ sequences in the $V_H$ FWR1. Although the above FWR1 motif accounted for much of the thermal destabilization effect, other residues outside this region may be involved. However, unlike the $14S_{...}GG^{31}$ residues (Figs. 2, 3a), $V_{H}$ mutations of other residues outside the binding site were not fixed in the lineage and variation in the mutational changes was observed as maturation progressed. Thus, the thermally destabilizing $14S^{30}/330G^{331}$ substitutions were necessary mutational changes for development of $CH103$ bnAbs.

**Thermostability and gain-of-function in the CH103 lineage.** A critical feature in a bnAb lineage is the ability to bind to diverse clades of viral antigens, and therefore, a key step in bnAb development is a gain-of-function with respect to binding Env variants. The $CH103$ UCA binds to the transmitted/founder (TF) $CH505$ Env but not to heterologous Env. To address how gain in affinity for heterologous Env ($B.63521$) developed in the lineage, we analyzed the kinetic rates (association rate, $k_a$ and dissociation rate, $k_d$) of each antibody in the $CH103$ lineage. We observed that $CH103$ lineage binding affinity enhancement ($K_d > 100 \mu M$ to $1 nM$) involved distinct kinetic rates improvement at different stages of affinity maturation (Fig. 4a; Supplementary Table 5). In the early stage ($I_8$ to $I_3$), affinity enhancement was primarily due to improved $k_a$ ($>50$-fold faster), while the late stage maturation ($I_3$ to mature mAbs) was dominated by improved $k_d$ (100-fold slower). The earliest gain in affinity to heterologous Env was observed in $I_4$ primarily due to improved $k_a$. The $\sim$16-fold increase in $k_d$ of $I_4$ ($2.7 \times 10^3 M^{-1} s^{-1}$) when compared to that of the germline-proximal intermediate $I_8$ ($0.17 \times 10^3 M^{-1} s^{-1}$) contributed to an order of magnitude difference in $K_d$ between the two intermediates. The observed difference in the kinetic rates of $CH103$ lineage antibodies binding to heterologous Env is in contrast to the kinetic rates of binding to the autologous TF Env. The faster $k_a$ ($>10^3 M^{-1} s^{-1}$) observed for UCA binding to the TF Env was maintained in the lineage and the order of magnitude gain in affinity ($K_d = 430 nM$ (UCA) to $40 nM$ (CH103)) (Table 2; Supplementary Figure 2, Table 5) was due to reduction in dissociation rates ($k_d = 101 \times 10^{-14} s^{-1}$ (UCA) to $2.6 \times 10^{-13} s^{-1}$ (CH103)). These results demonstrated that the $k_a$ increase observed early in the lineage is associated with gain-of-function with respect to binding heterologous Env (Fig. 4a). Since $I_4$, with a substantial increase in mutation frequency (Fig. 2a), showed lower $T_m$ than $I_8$ and UCA, there is an inverse relationship between gain-of-function (heterologous Env binding) and antibody thermostability (Fig. 4b).

In the lower branch of the $CH103$ lineage tree, the germline FWR1 $P_{14}$ residue was mutated to $T$ in each of the matured non-bnAbs ($V_{H}$ mutations 7.7–15.2%) (Fig. 2a, c). Binding to the heterologous Env ($B.63521$) was orders of magnitude weaker ($K_d \sim 1–15 \mu M$) for the mAbs in the lower branch when compared to those in the $CH103$ bnAb branch ($K_d \sim 1–14 nM$). Furthermore, the matured lower branch antibodies did not develop neutralization breadth even when compared to either intermediate $I_7$ or UCA, there was a concomitant decrease in $T_{onset}$ (Supplementary Figure 1), indicating that there was overall structural instability in the mature antibody that was distinct from the $14S/330G^{331}$ associated Fab destabilization observed in the $CH103$ branch of the lineage tree. These results show the importance of the early selection of the mutations in $I_4$ and the subsequent evolvability of higher affinity antibodies and the acquisition of neutralization breadth.

The $14S/330G$ mutations alter Fab orientation preferences. While paratope mutations are known to impact $Ab$ loop flexibility, additional sites affecting structural flexibility include the well-known antibody hinge point (Fab-$F_C$) as well as the Fab elbow joint ($F_C-C_{HL}/C_{L}$) (Fig. 5a–c). A structural alignment of the $C_{HL}/C_{L}$ regions of the available $CH103$ bnAb and UCA crystal structures indicated a marked shift in the orientation of the $F_C$, relative to the $C_{HL}/C_{L}$, via rigid body rotation of the two regions about the elbow hinge point yielding elbow angles of 132° and 245° for the UCA and $CH103$, respectively (Fig. 5d). Since the $CH103$ $P_{14}$ residue is in the Fab elbow region, we hypothesized that the acquisition of the $14S$ mutation, together with the FWR1 $330G$ mutation, increased flexibility at the 14 Fab elbow and gave rise to increased CDR and $V_{HL}/V_{L}$ orientational...
flexibility. We employed long timescale (µs) molecular simulations to elucidate the source of the apparent increase in CH103 lineage antibody flexibility observed in the thermal denaturation experiments. Results from five 1 µs simulations of both the UCA and UCA-P14S/S30G Fabs indicated marked shifts between the UCA and bnAb elbow orientation states (Fig. 5e, f; Supplementary Figure 3, Movie 1). The distribution in elbow angle states for the UCA indicated it strongly favors its initial UCA state, accounting for 82.6% of the simulation time, while the UCA-P14S/S30G mutant displayed a bimodal distribution in elbow angle states, accessing the CH103 bnAb state for 64.7% of its simulation time (Fig. 5e–f; Supplementary Figure 4). Additional changes in the V_{H1}-V_{L} orientation toward that of the bnAb orientation accompanied these mutations (Supplementary Figure 5). Remarkably, these mutation-induced changes in orientation resulted in major increases in flexibility of CDR loops in both chains and limited potential C_{H1}/C_{L} clash with the adjacent protomer in the CH505 Env trimer (Fig. 5g–i). In order to verify the apparent shift in elbow angle preference in these simulations, we used structures of the shifted elbow angle state from the previous simulations of both the CH103 UCA and CH103-P14S/S30G. We extracted Fab coordinates from each individual simulation in which the elbow angle shifted to beyond 180 degrees (Supplementary Figure 6). The differences observed in the simulations of these constructs is consistent with the measured changes in Tm and with the V_{H1}-V_{L} orientation changes in the bnAb crystal structure. The details of these domain changes and their residue influences are discussed further in the supplemental material (Supplementary Discussion, Fig. 3–7). Together, these results suggest that the dramatic shift in the domain interaction network of the CH103 UCA with the destabilizing P14S and S30G mutations was likely critical for shaping downstream affinity maturation of the CH103 bnAb lineage.

Thermostability and elbow angles in a glycan-V3 bnAb lineage.

In the glycan-V3 bnAb lineage DH270, the UCA and early intermediate (I) antibodies exhibited higher Tm than the mature bnAbs (Fig. 3). As observed in the CH103 lineage, the germline-proximal intermediate (I4) of the DH270 bnAb lineage had similar Tm to that of the DH270 UCA, and the earliest, and largest, decrease in Tm was observed in the next intermediate I2, followed by an additional decrease in I1 and a relatively small increase in the mature DH270.6 bnAb (Fig. 3b; Supplementary Table 6). Similarly, in the lower branch of the DH270 lineage that diverged at I4, the intermediate I3 showed a marked reduction in Tm that was further reduced in the DH270.2 bnAb (Tm = 59.3 °C) (Supplementary Table 6). Thus, in a second bnAb lineage of differing specificity (glycan-V3 DH270 versus CD4-bs CH103) we observed that thermal destabilization first occurred in an earlier intermediate and thereafter the lower Tm was maintained through to the affinity-matured bnAbs (Fig. 3).

We next asked whether the dramatic reduction in Tm (12.7 °C) between the DH270 UCA and I2 (Fig. 3b) could have also involved shifts in the elbow angle distribution. As in the CH103 lineage, an elbow region mutation in V_{H} FWR1 (V11M)
**Fig. 5** Antibody structure and CH103 Fab elbow flexibility. 

- **a** Surface representation of an IgG antibody. The heavy chain (blue) and light chain (green) pair in the Fab region made up of the antigen binding Fv region and the CH103 UCA state.

- **b** Cartoon representation of an IgG antibody. The portion of the structure connecting the CH103 UCA region to the Fc region is a site of flexibility termed the antibody hinge. **c** Top: Additional antibody hinge point between the Fab Fv and CH103 UCA region termed the Fab elbow. Bottom: Fab Fv VH and VL orientation flexibility about two planes.

- **d** Structural alignment of the CH103 and CH103 UCA crystal structures (PDB ID 4JAM chains H and L and 4QHK chains O and P, respectively) CH103 UCA region depicting the elbow angle differences. **e** Elbow angle distribution for the five 1 μs simulations of the CH103 UCA (solid) and the CH103 UCA-P145/S30G mutant (dashed).

- **f** RMSD to the CH103 bnAb (PDB ID 4JAM) distribution for the five 1 μs simulations of the CH103 UCA (solid) and the CH103 UCA-P145/S30G mutant (dashed).

- **g** Structural superposition of the CH103 UCA and CH103 UCA-P145/S30G onto a CH505 TF SOSIP trimer homology model based upon the CH103 bound gp120 configuration.

- **h** Aggregate simulation VH RMSF difference between the CH103 UCA-P145/S30G mutant and the CH103 UCA. Dotted lines indicate one standard deviation from the mean. Red brackets indicate HCDR positions.

- **i** Aggregate simulation VL RMSF difference between the CH103 UCA-P145/S30G mutant and the CH103 UCA. Dotted lines indicate one standard deviation from the mean. Blue brackets indicate LCDR positions.
Elbow mutations are common among HIV-1 bnAbs. We next sought to determine whether mutations in the elbow region, including the V_{H} ball-and-socket residues 11, 110, and 112, are common among the various classes of bnAbs (Table 3). The evaluated residues were selected based on the correlation between the CH103 UCA-P14S/S30G elbow joint destabilization and the CH103 T_{m} data and included ball-and-socket adjacent and neighboring residues between V_{H} positions 8–14 and 107–113 (Supplementary Figure 11). Sequence analysis revealed elbow mutations, with ~91% of the bnAbs containing mutations in the elbow region and ~55% of the bnAbs containing mutations in the ball-and-socket residues (Table 3). The CD4-bs bnAbs displayed the greatest degree of elbow region mutation, consistent with the potential role of such mutations in the development of neutralization breadth, as demonstrated in the CH103 lineage. While elbow mutations tend to increase with increasing mutation rates in the FWR, several bnAbs (e.g., BF520) with relatively limited V_{H} FWR mutation rates (BF520 = 5.7%) had numerous elbow mutations (Table 3), and in two bnAb lineages (i.e., CH103 and DH270 lineages), the destabilizing elbow region mutations were selected in germline-proximal intermediates with relatively lower numbers of V_{H} mutations. Analysis of V_{H} elbow region sequences revealed fewer mutations, suggesting mutation in the V_{H} elbow region is the dominant source of elbow conformational control in bnAb development (Supplementary Table 7).

Elbow mutations in weakly neutralizing Abs. We next asked whether weakly neutralizing HIV-1 antibodies also frequently accumulate V_{H} elbow mutations. Comparison of nine previously investigated mature non-bnAbs to their germline

Table 3 BnAb elbow region mutation frequencies

| bnAb     | Epitope | % FWR | % Elbow | V_{H} Elbow Mutations | V_{H}*** | V_{H}* | V_{H} |
|----------|---------|-------|---------|-----------------------|----------|--------|-------|
| 8ANC131  | CD4 BS  | 31    | 42.9    | A9G,E10Q*,V11L***,T107S,T110I***,S112T*** | 3        | 1      | 6     |
| CH103    | CD4 BS  | 20.7  | 42.9    | L11V***,P14S,T107S,T110S***,S112T***,S113A* | 3        | 1      | 6     |
| VRC13.01 | CD4 BS  | 44.8  | 57.1    | A9T,E10A*,V11M***,K135P144L108P, T110R***,S113P* | 2        | 2      | 8     |
| N6       | CD4 BS  | 31    | 42.9    | A9T,E10A*,V11M***,M108T,T110V***,S113A* | 2        | 2      | 6     |
| VRC01    | CD4 BS  | 34.5  | 35.7    | A9G,E10Q*,V11M***,L108P,T110I*** | 2        | 1      | 5     |
| VRC16.01 | CD4 BS  | 19.5  | 28.6    | L11F***,Q13K,T110G***,S113A* | 1        | 2      | 4     |
| CH235.12 | CD4 BS  | 36.8  | 50      | A9G,E10Q*,K13R,P14L,T107S,T110P,T110I*** | 1        | 1      | 7     |
| VRC27.01 | CD4 BS  | 34.5  | 35.7    | A9P,E10Q*,K13R*,L108R,T110V*** | 1        | 2      | 5     |
| VRC-CH31 | CD4 BS  | 29.9  | 28.6    | E10A,K12R*,L108P,T110V*** | 1        | 2      | 4     |
| VRC18.02 | CD4 BS  | 29.9  | 28.6    | E10A,K12R*,T110P | 0        | 2      | 4     |
| NIH45-46 | CD4 BS  | 35.6  | 36.7    | A9G,E10Q*,V11M***,T107A,L108P | 1        | 1      | 5     |
| 1B2530   | CD4 BS  | 32.2  | 28.6    | A9T,E10A*,K12R*,T107S | 0        | 2      | 4     |
| 38NC17   | CD4 BS  | 28.7  | 21.4    | E10A,K12T*,L108Q | 0        | 2      | 3     |
| 38NC60   | CD4 BS  | 32.2  | 21.4    | E10A,K12T*,L108Q | 0        | 2      | 3     |
| 8ANC195  | gp120-gp41 | 35.6 | 28.6    | A9T,V109I*,T110S***,S113A* | 1        | 2      | 4     |
| 35O22    | gp120-gp41 | 26.4 | 14.3    | V11L***,V109L* | 1        | 1      | 2     |
| PG151    | gp120-gp41 | 16.1 | 0       | VT9* | 0        | 0      | 0     |
| VRC34    | gp41    | 19.5  | 7.1     | T108S | 0        | 0      | 1     |
| DH511.2  | MPER    | 19.5  | 7.1     | T108I*** | 1       | 0      | 1     |
| 2F5      | MPER    | 11.5  | 21.4    | T10P*,T107V,V111* | 0        | 2      | 3     |
| 4E10     | MPER    | 10.3  | 7.1     | K13R | 0        | 0      | 1     |
| 10E8     | MPER    | 20.7  | 0       | -  | 0        | 0      | 0     |
| PGDM4100 | Glycan V1/V2 | 32.2 | 21.4    | A9P,K12R*,T108A | 0        | 1      | 3     |
| PGT145   | Glycan V1/V2 | 20.7 | 7.1     | T108A | 0        | 0      | 1     |
| PG9      | Glycan V1/V2 | 13.8 | 0       | -  | 0        | 0      | 0     |
| CH01     | Glycan V1/V2 | 26.4 | 21.4    | G9A,G10N*,T110S*** | 1        | 1      | 3     |
| BF520    | Glycan V3 | 5.7   | 21.4    | V11M***,K13M,T110S*** | 2        | 0      | 3     |
| DH270.6  | Glycan V3 | 17.2  | 28.6    | E10Q*,V11M***,K13V,V109L* | 1        | 2      | 4     |
| PGT128   | Glycan V3 | 24.1  | 21.4    | G10T*,K13E,P14A | 0        | 1      | 3     |
| PGT135   | Glycan V3 | 21.8  | 14.3    | T107V,L108Q | 0        | 0      | 2     |
| PGT121   | Glycan V3 | 20.7  | 7.1     | T108Q | 0        | 0      | 1     |
| 2G12     | Glycan V3 | 29.9  | 21.4    | P14A,M108V,S113* | 0        | 1      | 3     |

V_{H} framework and elbow region mutation frequencies (%) are relative to the unmutated common ancestor. Asterisks indicate whether residues occur in the ball-and-socket (**) or are ball-and-socket adjacent (*). The V_{H} list includes all residues in the elbow region.
encoded sequences revealed that, unlike bnAbs, $V_{H}$ elbow mutations are relatively uncommon (Supplementary Table 8). As mutation frequency in these antibodies is typically lower than their bnAb counterparts, we compared the $V_{H}$ framework and elbow mutation frequencies of these non-bnAbs to the frequencies in the CH103, DH270, CH235, and N6 bnAb lineages. In spite of similar framework mutation frequencies between several bnAb lineage members and non-bnAbs, elbow mutation frequencies were generally higher in bnAbs than non-bnAbs at similar framework mutation frequencies (Supplementary Figure 12). That is, the bnAbs display a tendency of disproportionately accumulating elbow mutations at overall comparable framework mutation frequencies.

In comparison, the gp120 V2 non-bnAb CH58, isolated from an RV144 ALVAC/AIDSVAX vaccinee, that binds to the same region as glycan-V2 bnAbs,$^{27}$ had no elbow region mutations, and therefore, we hypothesized that major shifts in the elbow angle distribution would not occur. MD simulations showed that unlike the UCAs of CH103 and DH270 bnAbs, the CH58 UCA remains largely trapped in its initial elbow angle state (Supplementary Figure 13a, Movie 3). The mature CH58 displays a wider elbow angle distribution than its UCA but also fails to access wide elbow angle states (Supplementary Figure 13). Nevertheless, the $T_m$ for CH58 (63.0 °C) was lower than its respective UCA (69.4 °C), and MD simulations revealed shifts in the $V_{H}$-$V_{L}$ orientations with an increase in HCDR3 flexibility (Supplementary Figures 14–15). While CH58 demonstrates a reduced thermostability relative to its UCA, it lacks FWR elbow mutations and does not exhibit the characteristic elbow angle flexibility observed in the bnAbs. Interestingly, a related V2 non-bnAb CH59, isolated from the same vaccinee as CH58$^{27}$, also showed no elbow region mutations but demonstrated increased $T_m$ (73.7 °C) relative to its UCA (67.4 °C) (Supplementary Figure 15). It is likely that the observed $T_m$ differences in CH58 and CH59 are a consequence of different antibody structural dynamic characteristics and/or a means of thermal stabilization/destabilization that does not involve elbow flexibility.

**Elbow mutations and neutralization breadth in HIV-1 bnAbs.** In order to determine whether elbow mutations accompany increases in heterologous breadth and potency, we examined the timing of the selection of elbow region mutations during bnAb maturation. In the CH103 lineage, the first thermally destabilizing elbow region mutation (P14S) was selected in I4, which neutralizes the Tier 2 autologous virus (Supplementary Figure 16) and binds weakly to heterologous Env protein but does not neutralize heterologous viruses (Fig. 4). Nevertheless, the initial selection of the destabilizing elbow mutation P14S in I4 was required for interdomain flexibility and the subsequent development of neutralization breadth in the lineage. As such, we observed that a progressive increase in neutralization breadth and potency is associated with selection of additional elbow mutations in the later intermediates which are retained in each of the bnAbs, both in the ball-and-socket and in adjacent residues (Supplementary Figure 16). In contrast, antibodies in the lower branch of the CH103 lineage with no destabilizing FWR elbow region mutations do not neutralize heterologous viruses, and even with ~15% $V_{H}$ mutations, the most somatically mutated mAb isolated in this branch only weakly neutralizes the autologous virus (Supplementary Figure 16).

The association of FWR elbow region mutations and neutralization breadth was also observed in the glycan-V3 bnAb DH270. Specifically, the first elbow mutation (V11M) is in I2, the intermediate at which a reduction in $T_m$ was first observed, which coincides with the acquisition of neutralization breadth in the lineage (Supplementary Figure 17). Further increases in breadth and potency among DH270 bnAbs were associated with the acquisition of additional mutations in residues adjacent to the ball-and-socket region (K13N, V109L). Considering that our simulation and experimental results indicate the potential for elbow mutations to have profound impacts on antibody physical properties, elbow mutations likely had an impact on the development of heterologous breadth in both the CH103 and DH270 bnAb lineages. Consistent with these observations, elbow mutations in the CD4-bs N6 bnAb lineage between intermediates I2 and I3 were associated with dramatic increases in neutralization breadth and potency.$^{28}$

Interestingly, in another CD4-bs bnAb lineage (CH235)$^{24,26}$, the CH235 bnAb, which accumulated less mutations and displayed narrower neutralization than more mutated clone members$^{26}$, contained a single elbow mutation at position 113 (Supplementary Figure 18), adjacent to the ball-and-socket region.$^{26}$ This particular position, 113, is distal to the elbow region and solvent exposed, limiting its ability to influence overall Fab architecture. However, the more affinity matured members of the lineage, CH235.9 and CH235.12, which reached broader neutralization, contained five and seven mutations in the elbow region, respectively, which included both a P14L and a ball-and-socket residue mutation (T110D) (Table 3). Therefore, we predicted that, while CH235 was unlikely to be thermally destabilized relative to the UCA, CH235.9 and CH235.12 would likely display a markedly reduced $T_m$. Indeed, the $T_m$ for CH235 (76.7 °C) was higher than that of the UCA (74.0 °C), and as predicted, the $T_m$ values for CH235.9 and CH235.12 were greatly reduced to 66.9 and 67.7 °C, respectively (Supplementary Figure 19). The reduced $T_m$ in the later antibodies is in accord with our observations in the CH103 and DH270 lineages and is similarly associated with an increase in neutralization breadth from 18% in CH235 to 77% in CH235.9 and 90% in CH235.12.$^{26}$

**Effect of reversion mutations on neutralization.** To directly determine the functional impact of destabilizing as well as Fab elbow region mutations on neutralization potency and breadth, we produced revertant mutants of key early intermediates and affinity-matured bnAbs of both the CD4-bs CH103 and the glycan-V3 DH270 bnAb lineages. We measured the effect of reversion mutations on thermostability and neutralization potency for each of the intermediates and bnAbs.

For the CH103 lineage, we produced two CH103 germline-revertant mutants, a single-residue mutant with the SI4P mutation (CH103-S14P) and a double mutant (CH103-S14P/G30S) with both SI4P and G30S mutations. Similarly, for the intermediate I4, a single mutant I4-S14P and a double mutant I4-S14P/G30S were produced. CH103 I4 revertant mutants showed improved thermostability compared to WT ($\Delta T_m = +2.4 ^\circ C$; $\Delta T_m,I4-S14P/G30S = +3.8 ^\circ C$) and an overall 2.5-fold reduction in affinity for the autologous CH505TF SOSIP trimer ($K_{d,I4} = 160 \text{ nM}$, $K_{d,I4-S14P/G30S} = 160 \text{ nM}$) (Table 1; Supplementary Tables 2-3). CH103 revertant mutants had marginally improved thermostability compared to WT ($\Delta T_m,\text{CH103-S14P} = +0.9 ^\circ C$; $\Delta T_m,\text{CH103-S14P/G30S} = +1.1 ^\circ C$) but a 10-fold reduction in affinity for the autologous CH505TF SOSIP trimer ($K_{d,\text{CH103-S14P}} = 16 \text{ nM}$, $K_{d,\text{CH103-S14P/G30S}} = 150 \text{ nM}$) (Table 1; Supplementary Tables 2-3). Reversion of the two destabilizing residues that increased elbow flexibility in the CH103 lineage had nominal effect on the autologous CH505TF neutralization potency of the CH103 bnAb (Fig. 6a, $IC_{50} = 6.4$, 12.8, and 15.5 $\mu$/mL for CH103, CH103-S14P, and CH103-S14P/G30S respectively). However, the neutralization potency of the SI4P mutant of CH103 was reduced against two of the Tier 2 heterologous pseudoviruses
We previously reported that the development of the V3 and N332-glycan-dependent DH270 bnAb lineage involves overcom-

We have studied the thermostability of bnAbs with different thermal destabilization. Using CD4-bs and glycan-V3 bnAb

Discussion

We have studied the thermostability of bnAbs with different specificities for HIV-1 Env gp120 and gp41 epitopes and report that all major classes of bnAb development are associated with thermal destabilization. Using CD4-bs and glycan-V3 bnAb lineages with inferred UCAs and intermediates, we show that thermostability is selected early in affinity maturation, at an intermediate state when both affinity gain for diversity in antigen
recognition and thermal destabilization are concurrently achieved. Once selected, the destabilizing mutations are fixed in the lineage and the lower thermostability is maintained in the affinity-matured bnAbs, indicating that selection of key destabilizing mutations are important for bnAb development. In the CD4-bs CH103 bnAb lineage, we identified FWR mutations that contributed to thermal destabilization, and we show that a key mutated residue resides in the Fab elbow region and facilitates the development of paratope plasticity for heterologous Env recognition and development of neutralization breadth. Using long timescale (µs) molecular dynamics simulations, we demonstrated that the V_{H} FWR elbow mutations impact inter-domain dynamics due to altered Fab elbow and V_{H}-V_{L} orientational flexibility and similar mutational changes are required in several key bnAb lineages. We report that, unlike weakly or strain-specific neutralizing antibodies, elbow region mutations are frequently observed in HIV-1 bnAbs, and are selected consistently in bnAbs that target the CD4-bs and glyc-an-V3. In four bnAb lineages, we charted the progressive selection of Fab elbow region mutations and showed that selection of elbow mutations coincided with increases in heterologous neutralization breadth and potency. In contrast, antibodies that matured in the CH103 bnAb indicated that these mutations were important for autologous neutralization prior to development of neutralization breadth. The impact of the revertant mutations in the affinity-matured CH103 bnAb was observed only against heterologous and not autologous viruses. Thus, the destabilizing elbow region mutations are important both at an early maturation stage when the intermediate has not yet achieved sufficient affinity for potent neutralization and later in the affinity-matured bnAb for difficult-to-neutralize heterologous viruses. In the DH270 lineage, the impact of reversion mutations was limited to DH270.2 that branches out from I3, an earlier intermediate that unlike I2 has limited breadth and potency and no elbow region mutations. The intermediate I2, unlike the I4 in the CH103 lineage, shows breadth in neutralization and potently neutralizes autologous viruses with relatively shorter V1 lengths. The DH270 I2, therefore, represents an intermediate at a more advanced maturation stage, and as observed with the most potent DH270.6 bnAb, reversion of elbow region mutations had no impact on neutralization potency against the tested panel of viruses. Thus, the impact of reversion mutations on neutralization was limited to the bnAb in the lower branch of the DH270 lineage and against viruses that were relatively more difficult to neutralize. The observed differences in the two evolutionary branches of the DH270 lineage is not unique and has been described for the glyc-an-V3 PGT121 lineage that showed a selective preference for glycans on different residues for antibodies on the two branches. Our current studies addressed only the impact of V1 length on neutralization potency of selected DH270 mutants and therefore, further studies with glycans may provide a more complete understanding of the role of the elbow region mutations for antibodies in the upper branch of the DH270 lineage.

The decrease in thermostability and altered elbow region flexibility could be selected against under normal antibody development, therefore suggesting a barrier for bnAb elicitation that needs to be accounted for in vaccine design strategies. A common trait associated with all major classes of matured HIV bnAbs is polyreactivity and/or autoreactivity. While CH103 bnAbs were polyreactive, the UCA and the intermediates with destabilizing elbow mutations were not self or polyreactive, suggesting that selection of interdomain flexibility itself would not be disfavored. However, the impact of antibody elbow flexibility on B cell signaling and survival is unclear. While murine V_{H} chain usage with a more flexible elbow is positively selected in mature B cells, elbow conformational flexibility can impact initiation of cell signaling if antigen binding induces conformational changes and BCR reorganization is required for full activation. It is likely that vaccine design strategies aimed at recapitulating the first step of increased global Fab flexibility...
could increase the probability of selection of the more favored mbAb precursor pools and allow subsequent selection of key affinity-enhancing mutations that lead to the acquisition of neutralization breadth. As the MD simulations and previous work suggest elbow region mutations alter VH-VL orientations and therefore paratope distribution, immunogen design efforts that include steric challenges to the VH-VL orientation are likely to select for elbow region mutations via reduced stress at the paratope-epitope interface.

Methods

Proteins. Procedures for purification of each of the studied mbAbs are described in the previous publications. Computational inferences and production of the UCA and intermediate antibodies were performed by methods previously described. For the CH103 lineage, the individual heavy chains of UCA, 18, 14, 13, and 17 mbAbs were expressed with the UCA-light chain. The heavy chains of later intermediates (12, 11) and lower branch Abs (1A1O2R6, 1AZCTE5) were expressed with the 12-light chain. The mature CH103 bnAbs were coupled with somatically mutated light chains unique to that antibody. All antibody samples were buffer exchanged to the final PBS buffer 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4. The final concentrations (per IgG) of the antibody samples were determined from absorption measurements using a molar extinction coefficients (M−1 s−1 per IgG) at 280 nm. Although no aggregates were observed by size exclusion chromatography analysis, as a standard procedure all antibody and protein samples were spun down to remove any potential small scale aggregates prior to performing any measurements.

HIV-1 Env gp120 and gp140 proteins were produced in Freestyle 293 cells (Invitrogen) as previously described. Briefly, HIV-1 gp120 and SOSIP gp140 were purified with lectin affinity and PGT145 affinity respectively. The envelope was size fractionated by gel filtration chromatography to purify monomeric gp120 and trimeric gp140.

Circular dichroism (CD). CD melting measurements were carried out on antibody samples in PBS buffer, 0.3–1.5 µM (per IgG molecule) in a 1 mm pathlength cuvette using the Aviv (Lakewood, NJ) Model 202 spectropolarimeter. CD signals at 208 nm were monitored every 1 °C as the temperature of the antibody samples increased at a ramp rate of 0.5 °C/min. The samples were allowed to equilibrate for two minutes at each temperature before CD signals were recorded at an averaging time of 2 min. The fraction folded after each incremental increase in temperature was calculated using the formula provided by Greenfield. A Savitzky-Golay 13-point quadratic first derivative function was applied to the fraction folded curves to approximate the melting temperature of each antibody.

Differential scanning calorimetry (DSC). Antibody thermal denaturation profiles were obtained in HERPES Buffered Saline (HBS; 10 mM HEPES, 150 mM NaCl pH 7.4) at concentrations ranging from 0.2–0.4 mg/mL using the NanoDSC platform (TA instruments; New Castle, DE). All antibody samples were extensively dialyzed into HBS, diluted in filtered dialysate, and degassed for 15 min at room temperature. Perturbation buffer cells were conditioned at filtered, degassed dialysate prior to sample loading. Antibody samples were heated from 10–20 °C to 100 °C at 1 °C/min under 3 atm pressure using the corresponding dialysate as the reference buffer. The observed, irreversible denaturation profiles were buffer subtracted, converted to molar heat capacity, baseline corrected with a 6th-order polynomial, and fit with three Gaussian transition models using the NanoAnalyze software (TA Instruments). The primary transition temperature (Tonset) was calculated as the temperature at which the sum deviates from the baseline by 2% of its maximum amplitude.

Surface plasmon resonance (SPR). SPR measurements of Env protein binding to mbAbs were performed on a BioaCore T200 instrument (GE Healthcare) as described earlier. Approximately 200–500 RU of each antibody was captured on an anti-human IgG immobilized sensor surface (CM5, GE Healthcare). Monomeric gp120 proteins were diluted from 0.5 to 200 µg/mL (20–489 nM for CH505TF gp120 and 9–374 nM for B6.63521 gp120) and injected for 5 min at 50 µL/min using the high-performance injection setting. The 5 min injection was followed by a 10 min dissociation period and then a 20 h injection pulse of glycine pH 2.0 for sensor surface regeneration. Non-specific binding was accounted for by using in-line reference subtraction of signal on a flow cell captured with control mbAb (S三亚, anti-RSV, ASV). Binding rate constants (k on and k off), dissociation (k off,∞) constants, and total association (k on,∞) were determined using two or at least two measurements for each MbAb. The goodness of fit was assessed by examination of randomness in residual plots and chi² values < 1 was observed for all affinity measurements.

Biolayer interferometry (BLI). BLI measurements of Env protein binding to mbAbs were performed on an OctetRed96 instrument (ForteBio). Antibodies were loaded onto anti-human IgG Fc capture sensor chips (AHC, ForteBio) by submerging tips in 20 µg/mL mAb in PBS for 300 sec and then washed in PBS for 60 sec at 1000 rpm. Association measurements were performed by submersion in 4.75–475 nM CH505TF SOSIP.664.v4.1 for 400 sec at 1000 rpm, and dissociation measurements were collected by submersion in PBS for 60 sec. Sensor tips were regenerated using a 20 sec submersion in glycine pH 2.0. Non-specific binding was accounted for by using in-line reference subtraction of signal on a sensor tip captured with control mbAb (anti-Hu Flamaglutinin AB82). Curve fitting analysis was performed with the Octet Data Analysis 10.0 software (ForteBio) using a 1:1 model to derive rate (k on, k off) and dissociation (k on,∞) constants.

Molecular simulation. The unbound crystal structure for the CH103 UCA (PDB ID 4R05)21,22,23,24 and P (PDB ID 4R06)21,22,23,24 were used for the initial construction of the CH103 and mutated CH103 UCA-P14S/S30G, Fab-I2 simulations40,47, The CH103 heavy chain P14S and S30G mutations and CH1702 mutations added using the prepared UCA structures in PyMol46. The proteins were solvated in a truncated octahedral box of TIP3P water molecules with a minimum of 12 Å between the protein and solvent box edge using the AmberTools17 Leap program with the system charge neutralized via addition of chloride atoms. All simulations were run using Amber 16 with the Amber ff14SB forcefield. The systems were prepared for production runs over several steps. First, an initial minimization of the solvent-ion system was performed with the protein atoms fixed for 10,000 steps using a non-bonded interaction cutoff of 10 Å. This was followed by minimization of the entire system without restraints on the protein atoms for 10,000 steps. The system was then heated from 0 K to 300 K in the NVT ensemble over a period of 200 ps. A 2 ps time-step was used for the production runs. Production runs were completed in the NPT ensemble using an 8 Å cutoff for a period of 1 µs for both the UCA and UCA-P14S/S30G with all other parameters identical to the previous 100 ps simulation. Four additional simulations using randomized seed values beginning from the heating run were performed for each construct for a total of 1 µs each. Simulations were visualized and analyzed in VMD and PyMol using tools therein. Alignments and buried surface area calculations were performed in PyMol. Calculation of the root mean square fluctuation of residue alpha carbons was performed in VMD. Elbow angles were determined using the SHAKE algorithm. Production runs were used as a template with superpositions of the CH103 UCA and CH103 bnAb Fab (PDB IDs 4QHK and 4I4N, respectively) performed in PyMol.

Sequence analysis. Sequences for bnAb Fab V1 regions were obtained from GenBank with bnAb UCA sequences determined using Clonanalyse. The sequences were numbered using the Kabat scheme using ANARCI. Framework mutations were determined based upon Kabat assignment of residues in the UCA sequence (PDB ID 4R05). The CDR residues 31–35, 50–53, and 93–96 were defined as residues 8–14 and 107–113. Elbow region residues for the V1 were defined as residues 8–15, 82–83, and 105–107. Mutations and mutation frequencies for the framework and elbow region sequences were determined using custom Perl scripts. Sequence analysis was performed for the following V1 gene sequences (GenBank ID): 10E5 (IN645769)42,1R230 (HE845435)42,1R232 (KM001873)48, BN3C60 (HE845353)47, BNC117 (HE845357)47, BNC131 (HE845459)47, BNC195 (HE845352)47, BFC50 (WX168063)49, CH101 (Q1076523)50, CH103 (KC575845)42, CH105.12 (KU700402)42, DH726 (KY354948)50, DH512 (KY272631)50, N6 (KX931108; 27851912), PG9 (GU272243)50, PGD1400 (KY968070)50, PGT121 (HG901096)63, PGT118 (HG901095)63, PGT119 (HG901094)63, PGT120 (HG901093)63, PGT151 (KJ070028)42, VRC-CH31 (IN519453)50, VIRC10 (GU980702)42, VIRC34 (UI71186)46, HIVAG-1 (HE845453)47, VRC151 (KJ070028)42, VRC131.01 (KJ096914)42, VRC161.01 (KJ096917)42, VRC180.01 (KJ096916)42, VRC27 (KJ096915)42, 2G12 (KJ027166)42, 2G12d 42, CS19A UCA (KC417301)72, CH58 (KC417393)72, CH59 UCA (KC417401)72, CH59 (KC417405)72, CH59 (KC417404)72, CH103 UC and CH103 bnAb intermediates, DH270 UCA-Intermediates 32, CH225 38, and CH235.96. Sequences for non-bnAbs were collected from the table in Klein et al.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.
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Author contributions

S.M.A., H.N.E., R.C.H. and B.E.W. wrote and edited the paper. B.F.H., M.B. and K.W. edited the paper. S.M.A., R.C.H. and H.N.E. designed research study. H.N.E. performed CD and analyzed data. B.E.W. performed DSC and analyzed data. R.C.H. performed molecular modeling, simulation and simulation analysis as well as sequence analysis. K.A. performed SPR and BLI and analyzed data. S.M.X. and M.B. performed neutralization assays and analyzed data. M.B., K.S., H.-X.L., R.P. and A.T. produced and purified antibodies and Env proteins. K.W. provided model structure of CH103-gp120 complex.

Additional information

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Competing interests: The authors declare no competing interests.

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