Figures and figure supplements

Role of framework mutations and antibody flexibility in the evolution of broadly neutralizing antibodies

Victor Ovchinnikov et al
Figure 1. RMSF of the CG residue model of the heavy chains (A–C) and the light chains (D–F). (A,D) 3BNC60 lineage; (B,E) CH103 lineage; (C,F) PGT121 lineage. In order to compare the three lineages, all sequences are multiply aligned. This procedure creates gaps in the traces corresponding to antibodies with shorter loops in the region; i.e., there are no actual missing residue coordinates. The dashed lines bound the region of one standard deviation above and below the average trace. Bullets indicate mutations acquired during AM. The definitions of FWR and CDR regions are taken from (Scheid et al., 2011).

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Figure 1—figure supplement 1. Root mean square distance between simulation and initial structures for the 3BNC60 lineage. The RMSD is computed using the backbone atoms of (A) 3BNC60 heavy chain (HC); (B) 3BNC60 light chain (LC); (C) 3BNC60 HC/LC complex; (D) CH103 heavy chain; (E) CH103 light chain; (F) CH103 HC/LC complex; (G) PGT121 heavy chain; (H) PGT121 light chain; (I) PGT121 HC/LC complex. Five independent trajectories for each structure are concatenated together, and separated by vertical dashed lines.

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Figure 2. MD simulation structures of antibodies. (A) Simulation structure of the mature 3BNC60 antibody. Antibody framework regions are shown in orange, and the CDR regions are in purple. The conformational flexibility of the CDR regions of the heavy chain is illustrated by overlaying 24 conformations of the CDRs in 4ns intervals from each of the five trajectories. The P61 residue is shown in black; (B) simulation structure of the intermediate PGT121 antibody. The structure is colored as in (A), except that the conformational flexibility is illustrated for the FWR region of the light chain using 24 overlaid orange curves; (C) simulation structure of the mature CH103 antibody. Twenty-four gray curves are overlaid to illustrate the relatively lower overall flexibility of this antibody.

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**Figure 2—figure supplement 1.** Interactions between the CDR1 and FWR3 regions of the PGT121 light chain. Stabilizing hydrogen bonds between S27 (CDR1) and G68 (FWR3) are indicated in black lines. Letters a-c in parentheses represent insertion codes.

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Figure 2—figure supplement 2. Structure of antibody 3BNC60 (Scheid et al., 2011). The entire structure was simulated for 400ns (see Materials and methods). The initial structure before simulation is shaded in gray, and the final structure is in color. The variable and constant LC regions are in light and dark blue, respectively. The variable and constant HC regions are in yellow and orange, respectively. For the main MD simulations analyzed in this work only the variable chains were included, as indicated by the rectangle.

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Figure 3. Evolution of flexibility differs for our three bnAb lineages. Absolute classical quasi-harmonic entropy for the CG antibody model of the heavy (A) and light (B) chains, shown as the contribution to the absolute free energy (−TS). The absolute entropy magnitude tends to be larger for the heavy chains than for the light chains because they have more amino acids (e.g. 123 aa. for 3BNC60 HC vs. 98 aa. for 3BNC60 LC).

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Figure 4. Antibody maturation proceeds along alternate routes that depend on the initial binding strength with the conserved region of the antigen. Here we show statistics of the maturation pathways of the largest clones in our affinity maturation simulations. These figures therefore summarize typical features of antibody maturation across many parallel and independent germinal center reactions. (A) Antibody lineages that initially bind strongly with the conserved region of the antigen are likely to accumulate mutations that increase their binding strength and reduce flexibility. Starting parameters are indicated with an arrow. In our simulations, mutations in the CDR affect binding energies directly, while mutations in the FWR affect...
flexibility. Typical binding free energies with panel antigens, a proxy for breadth, strengthen steadily over the course of maturation (represented for generations 25, 150 and 400). (B) Antibodies that initially bind weakly with the conserved region typically become more flexible while increasing their binding strength. Such antibodies may subsequently begin to rigidify as they mature. The typical binding free energies with panel antigens increase slightly faster than in the strong conserved binding case above, but final binding free energies are not as strong. (C) The most potent antibodies at the end of the maturation process (generation 400), measured by median binding energy with panel antigens, are those that are the least flexible.

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Figure 4—figure supplement 1. Simulations of affinity maturation against a single antigen agree with experimental results. (A) Example trajectories of the total B cell population of germinal centers focused on a single antigen. Two cycles correspond to roughly one day. (B) Typical germinal center reactions end after around 50 days. The number of mutations accumulated (C) and final binding energy (D) are congruent with typical numbers recorded for the maturation of antibodies against a single antigen.

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Figure 4—figure supplement 2. Similar values of the overlap parameter lead to qualitatively similar results. Here we consider trajectories of antibody development for antibodies that initially bind (A) strongly or (B,C) weakly with the conserved region. In this case the affinity maturation process occurs against a panel of antigens with overlap $\lambda = 0.8$ instead of 0.9, as in the main text. The results are similar to those shown in Figure 4, but with greater selection for flexibility due to maturation against a more diverse panel of antigens.

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Figure 4—figure supplement 3. Shifting $E_0$ affects the preferred antibody maturation pathway. (A) Stronger, or more negative, $E_0$ makes flexibility-increasing framework mutations more beneficial, and thus drives antibodies along 3BNC60-like trajectories (compare with Figure 4B). Here antibodies only begin to rigidify late in the maturation cycle, after $E_c$ has already become quite strong. (B) In contrast, weaker $E_0$ promotes more CH103-like maturation trajectories (compare with Figure 4A). This is because CDR mutations that improve binding with the conserved region ($E_c$) become relatively more beneficial early in the maturation process. For each set of simulations we have used a weak initial $E_c$, as in Figure 4B.

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The three bnAb lineages we studied evolve through different paths that depend on the binding strength of their germline to conserved epitopes. CH103 (in orange) has a strong starting binding energy for the conserved residues, $E_c$. It follows the traditional evolution pathway and quickly rigidifies while enhancing its $E_c$.

PGT121 (in purple) and 3BNC60 (in green) have germlines with a weaker $E_c$. To survive selection during affinity maturation with multiple different antigens, they follow the same pathway as some enzymes (Raman et al., 2016) in changing environments: they first become more flexible which allows them to bind all antigens with limited potency; later they acquire mutations that enhance $E_c$ and increases their binding potency.

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Figure 5. The three bnAb lineages we studied evolve through different paths that depend on the binding strength of their germline to conserved epitopes. CH103 (in orange) has a strong starting binding energy for the conserved residues, $E_c$. It follows the traditional evolution pathway and quickly rigidifies while enhancing its $E_c$.

PGT121 (in purple) and 3BNC60 (in green) have germlines with a weaker $E_c$. To survive selection during affinity maturation with multiple different antigens, they follow the same pathway as some enzymes (Raman et al., 2016) in changing environments: they first become more flexible which allows them to bind all antigens with limited potency; later they acquire mutations that enhance $E_c$ and increases their binding potency.

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Figure 6. Accumulation of mutations in the CDR and framework regions varies according to the maturation pathway. (A) Antibodies that have a strong initial binding energy for the conserved residues, $E_c$, tend to first accumulate CDR mutations. Later, these antibodies rigidify through framework mutations. (B) Antibodies that have germlines with a weaker starting $E_c$ are more likely to acquire early framework mutations to increase flexibility, improving the odds of surviving selection during affinity maturation with multiple antigens. Note that nearly all lineages possess early framework mutations, in contrast to the maturation trajectories in (A).

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Figure 6—figure supplement 1. Shifting $E_0$ affects the accumulation of mutations in the CDR and framework regions along the maturation pathway. (A) When $E_0$ is made stronger, i.e. more negative, flexibility-increasing framework mutations are more often preferred, especially early in maturation. This shifts maturation pathways towards more 3BNC60-like trajectories (compare with Figure 6B). (B) For weaker values of $E_0$, flexibility-increasing mutations are less beneficial, and therefore antibodies are more likely to acquire early CDR mutations and proceed along a CH103-like trajectory (compare with Figure 6A). For each set of simulations we have used a weak initial $E_0$, as in Figure 6B.

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