Characterizing Broadly Neutralizing Antibody Maturation Pathways Using Reinforcement Learning

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1 Introduction

Humoral immunity, also known as antibody-mediated immunity, plays a crucial role in the adaptive immune response via host protection against a number of viruses. Antibodies generally neutralize viral infections by binding glycoproteins on enveloped viruses or the protein shell of non-enveloped viruses, thereby preventing fusion of the viral and host's cell membranes or penetration into the host's cytosol [1]. However, some human pathogens, such as Human Immunodeficiency Virus (HIV), Influenza, and Coronavirus, can often evade neutralization by rapidly mutating some epitopes and concealing other functionally-important and highly-conserved epitopes, thus preventing antibody binding [2, 3, 4]. While traditional neutralizing antibodies are at the mercy of these viruses—because of their slow generation and high specificity against rapidly mutating regions—broadly neutralizing antibodies (bnAbs) have gained much attention given their ability to neutralize highly variable pathogens by targeting conserved regions [3]. As a result, understanding what differentiates bnAbs from other neutralizing antibodies, and the pathways by which bnAbs are developed, will be crucial in designing drugs and vaccines against the most difficult pathogens encountered today.

1.1 Defining bnAbs

Defining the key characteristics of bnAbs is essential for optimizing the evolution from germline to bnAb. However, bnAbs are defined distinctly depending on the pathogen of interest and the available data. For instance, Kreb et. al. operationalized bnAbs using neutralization breadth calculated on an HIV panel of 34 founder sub-types, classifying those lineages with 50% breadth as the first occurrence of the bnAb [5]. Similarly, Faris et. al. defined bnAbs on a neutralization breadth metric, however, neutralization breadth was calculated as the fraction bnAb-antigen complexes with binding energies above 12 k_BT on a panel of 100 different antigens [6]. Finally, Griffith and McCoy operationalized bnAbs using neutralization breadth on a standard multi-clade panel of 118 Env pseudo-typed viruses, where 21% breadth was still considered broadly neutralizing [7]. They also noted neutralization potency on this panel, or the titers of bnAb required for effective neutralization (less than 4.32 $\mu g/ml$), as well as the level of SHM, similarities in CDRH3 length, and binding affinity to the CD4 receptor site on the Env trimer across the 41 bnAbs they studied [7].

Despite the variety in classifying bnAbs, cross-reactivity among multiple antigen sub-types plus specificity towards the antigen's conserved regions stand out as the most desirable characteristics in bnAbs. We expect that these features are sustained, if not encouraged, through affinity maturation.

1.2 Affinity Maturation

The development of bnAbs follows a similar process to that of traditional neutralizing antibodies, albeit with some unique considerations and challenges. After an antigen is introduced in the host,

B cell receptors (BCRs) weakly bind to the antigen to seed a Germinal Center within lymph nodes, and consequently begin the affinity maturation process [6]. Affinity maturation then replicates B cells, accumulating mutations in the BCRs via somatic hyper-mutation (SHM), in order to increase the binding affinity to the antigen at a specific epitope [6, 7].

1.3 Navigating the Affinity Maturation Pathway

Given a means of characterizing bnAbs and a thorough analysis of a longitudinal repertoire of antibodies produced during affinity maturation, we can gain an understanding of the stages involved in creating effective bnAbs. In this research, we hope to characterize the affinity maturation pathway for a bnAb which targets the membrane-proximal external region (MPER) epitope of the HIV-1 gp41 envelope protein. Using optimal path finding algorithms and reinforcement learning on a repertoire of mutations accumulated through affinity maturation, we can learn the characteristics that drive the production of a bnAb, ultimately generating a pipeline that can navigate an affinity maturation landscape for design tasks.

2 Aims

In this research, I aim to develop a toolkit capable of characterizing the transition from a germline neutralizing antibody to a bnAb for a longitudinal repertoire of antibodies bound to highly conserved (MPER) epitope. This repertoire was produced by participant 40512 from the RV217 cohort (Bio Project: PRJNA486355) taken over 654 days after identification of the Fiebig I infection stage, when patients are first positive for HIV RNA [5]. This participant is particularly interesting because they developed three bnAb lineages (VRC42, VRC43, VRC46) directed towards the MPER epitope [5], which is a known highly conserved region of the HIV-1 gp41 envelope protein [8]. Using an established framework of docking templates from participant 40512's bnAb lineages and the MPER peptide, I will generate data to fill in the temporal affinity maturation pathway from germline to bnAb. Finally, I will use path-finding algorithms and reinforcement learning on the affinity maturation landscape to optimally traverse the pathway from germline to mature bnAb.

2.1 Generate protein sequences from a DNA repertoire

Participant RV217.40512's longitudinal repertoire of antibodies against HIV consists of DNA sequences determined using polymerase chain reaction (PCR). In order to perform the analysis in this research, I will need to convert the DNA sequences to protein sequences. To generate protein transcripts, I will use SONAR, a pipeline for inferring antibody ontologies from longitudinal NGS

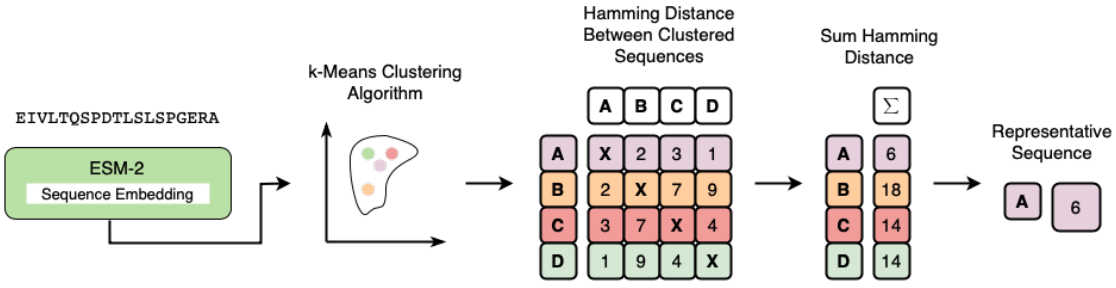

Figure 1: Outline for choosing non-redundant protein sequences. Sequence embeddings are generated using ESM-2 and embeddings are clustered using a k-means clustering algorithm. Hamming distance is then used to choose the most central ie. the most representative sequence.

BCR sequences [9]. Using SONAR we can extract V, J, and CDR3 protein sequences [9], then reconstruct full antibody protein sequences using TITAN which

I will remove redundant sequences by extracting representatives from clustered sequences. First, protein sequences will be encoded using a large language model known as ESM-2, which is a 15 billion parameter model that has been used for structure prediction [10]. Protein language models have shown promise in capturing both functional and structural properties of proteins, meaning that the resulting encoding is well-informed and representative of the entire sequence and potential structure [10, 11].

Once an encoding is generated, I will cluster sequences using a k-means clustering algorithm. To extract a representative sequence from a cluster, the hamming distance between all sequences within one cluster will be computed. For N sequences within the cluster, I generate N 1-dimensional vectors of size $N-1$, where each element in the vector is the hamming distance to all other sequences in the cluster. Summing each vector into a single scalar, I then choose the sequence with the smallest sum to represent the cluster. Choosing the sequence with the smallest total hamming distance from all other sequences, we effectively extract the most central sequence. This process is shown in Figure 1.

2.2 Build Antibody-Antigen Structures using AlphaFold2

Once a smaller set of representative and non-redundant sequences has been developed, structure generation becomes a more feasible and less computationally intensive task. In order to build bound antibody-antigen structures, I will use AlphaFold2(AF2)-Multimer, which is a derivative of the AF2 model tailored for multimeric inputs like an antibody-antigen complex [12]. Structures will be built using an experimentally determined crystal structure of the VRC42 lineage bound to an MPER peptide (6MTP) as template [5]. This lineage most closely resembles bnAb 4E10 (2FX7), which is a well-known bnAb targeting the same MPER region as the VRC42 lineage [8]. By using the mature bnAb bound to the HIV antigen as template, structure predictions similar to the target will be generated, while allowing the structural deviations expected throughout affinity maturation.

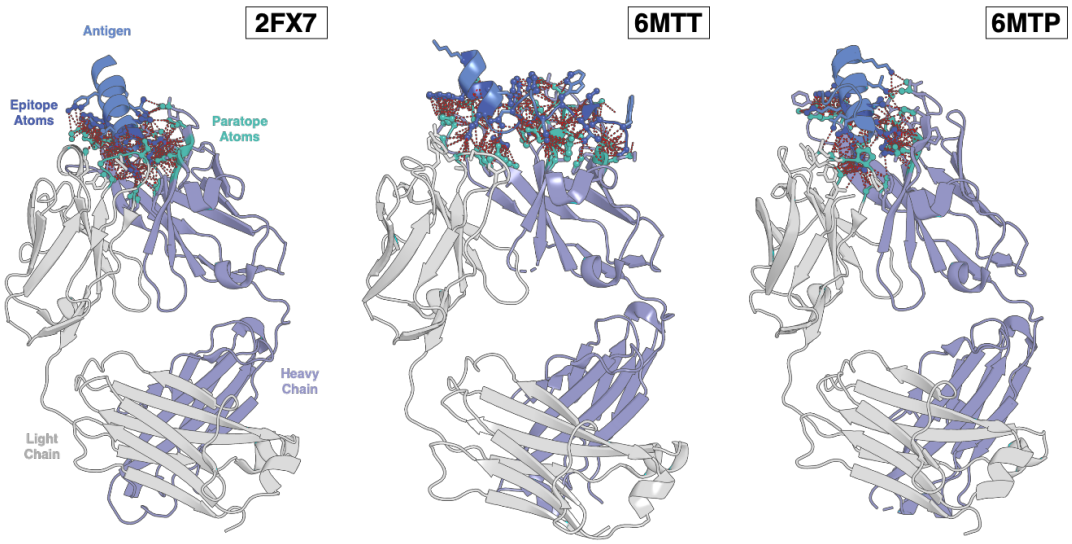

Figure 2: All bnAbs shown target the MPER epitope of the HIV-1 gp41 envelope protein. bnAb 4E10 is shown on the left panel. Two of participant RV217.40512's bnAb lineages are shown in the middle (VRC46.01) and right (VRC42.04) panels.

The purpose of building structures is to both obtain a confidence metric (pLDDT) as well as a means of calculating binding energies. These metrics, along with Levenshtein Distance, will be used to inform our policy in both the shortest path algorithm and reinforcement learning approach.

2.3 Calculate Binding Energies for all Predicted Structures

I will calculate binding energies for all predicted structures using Rosetta's *InterfaceAnalyzerMover* package, which outputs Rosetta Energies (AU). The $InterfaceAnalyzerMover$ determines the change in free energy from bound to unbound state ($\Delta\Delta G$) by calculating the difference in energy of the antigen-antibody complex (ΔG) and the summed energies of the unbound antigen and antibody ($\Delta G_{Antigen} + \Delta G_{Antibody}$) after repacking. Repacking ensures that the monomers find their lowest energy conformation which is important for an accurate imitation of the energy gained from binding. We expect that as we reach the mature bnAb in the affinity maturation pathway, that the binding energy should decrease given a tighter bind between the antibody and antigen, suggesting greater specificity and binding affinity [11].

$$\Delta\Delta G = \Delta G_{Bound} - (\Delta G_{Antigen} + \Delta G_{Antibody}).$$

We are also interested in observing correlations between other composite energies, such as repulsive and attractive forces or hydrogen bonding, and the affinity maturation pathway.

2.4 Optimal Path

2.4.1 Generating an Affinity Maturation Landscape

In order to generate a landscape that represents the affinity maturation pathway, I will build a graph where nodes **V** are the individual sequences recovered from the repertoire and directed edges **E** are weighted according to the Levenshtein Distances between connected nodes. Creating a graph

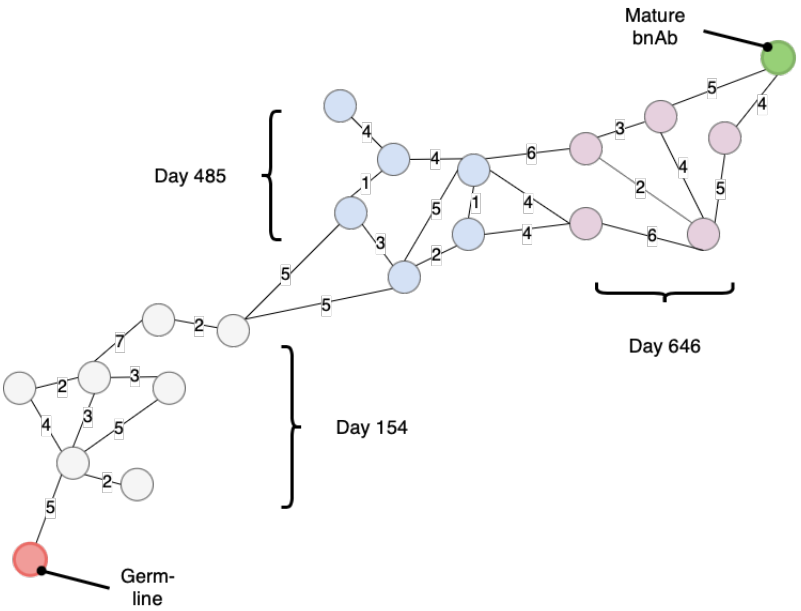

Figure 3: Graph representation of affinity maturation landscape weighted by Levenshtein Distances and time at which repertoires were created.

landscape is ideal since I will be able to use reinforcement learning to mimic affinity maturation in design tasks, by choosing the best sequence from a set of adjacent ones.

To build a graph, I will use an algorithm that starts at the germline and computes pairwise Levenshtein Distances from one sequence to all other available sequences. Sequences within a threshold distance of 5, which is the expected mutation per V region of the VDJ gene at the Germinal Center [13], will be considered adjacent. Note that we can cross-validate other thresholds to find the best performance. Since participant RV217.40512's repertoire is longitudinal, connections in the graph can be informed using collection time. In other words, we know that sequences in repertoires collected at earlier times must connect to the sequences in repertoires collected at later dates.

2.4.2 Shortest Path Algorithms

Assuming a graph landscape of nodes and edges that captures the mutations that occur throughout affinity maturation, shortest path algorithms can be used to determine the optimal path from germline to mature bnAb.

Using A* search algorithm, which is an informed search algorithm [14], finding the shortest path from germline to mature bnAb boils down to minimizing the cost function $f(n)$ from one node or sequence n to the next. The cost function $f(n)$ is composed of a sum of an exact cost $g(n)$ from moving from one node to the next, and a heuristic cost $h(n)$ calculating the "distance" between current node and end node.

$$f(n) = g(n) + h(n)$$

In terms of this investigation's affinity landscape, the exact cost $g(n)$ is defined using the Levenshtein Distance, ie. the weights, between adjacent sequences,

$$g(n) = LD$$

while the heuristic cost function $h(n)$ is defined either on global Levenshtein Distance between the current sequence and final bnAb, changes in binding energies ($\Delta\Delta G$), AF2 pLDDT, or a combination of all these metrics.

The following are proposed heuristic functions to account for these metrics, where n is the current node, S is the starting node (germline), T is the target node (mature bnAb):

- Levenshtein distance (LD): $h_{LD}(n) = |LD(T) - LD(n)|$
- Binding energies: $h_{\Delta\Delta G}(n) = |\Delta\Delta G_T - \Delta\Delta G_n| + \Delta\Delta G_n - \Delta\Delta G_{n-1}$
- pLDDT: $h_{pLDDT}(n) = |pLDDT_T - pLDDT_n| + pLDDT_n - pLDDT_{n-1}$
- Aggregate: $h_{Agg}(n) = h_{LD}(n) + h_{\Delta\Delta G}(n) + h_{pLDDT}(n)$

2.4.3 Deep Reinforcement Learning

2.4.4 Reward Predictor

3 Broader Impacts

Taking advantage of participant RV217.40512's longitudinal repertoire, this investigation will inform whether there exists some deterministic component of the affinity maturation pathway that triggers the development of bnAbs. Combined with a reinforcement learning approach to traversing

the pathway from germline to mature bnAb, this research will contribute to design tasks for bnAbs. Specifically, the agent trained using participant RV217.40512's repertoire should be able to choose the next optimal mutation from a set of antibody sequences and predicted structures. Ultimately, my investigation will provide insight for drug and vaccine targeting rapidly mutating viruses like HIV, Influence, and Coronavirus. I plan to present preliminary results to the computational biochemistry community in a poster at RosettaCON 2023 and a research paper later this year/early next year, in the hopes that other researchers may use it to inform their findings.

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