



Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning

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Key Words:	Envelope glycoprotein gp120, HIV neutralization phenotype, molecular dynamics simulation, graph machine learning, sequence-structure-dynamics of protein

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Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning

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Abstract

HIV exploits the sequence variation and structural dynamics of the envelope glycoprotein gp120 to evade the immune attack of neutralization antibodies, contributing to various HIV neutralization phenotypes. Although the HIV neutralization phenotype has been experimentally characterized, roles of rapid sequence variability and significant structural dynamics of gp120 are not well understood. Here, 45 prefusion gp120 from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotype are structurally modeled by homology modeling and investigated by molecular dynamics simulations and graph machine learning. Our results show that the structural deviations, population distribution, and conformational flexibility of gp120 are related to the HIV neutralization phenotype. Per-residue dynamics indicate the relationship between the local regions, especially in the second structural regions with high flexibility may be responsible for HIV neutralization phenotypes. Moreover, a graph machine learning model with the attention mechanism was selected to explore inherent representation related to the classification of the HIV neutralization phenotypes, leading to further distinguish the strong related gp120 sequence variation together with structural dynamics in HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also provides a methodological framework to explore complex relationships between sequence, structure, and dynamics of protein by combining molecular dynamics simulations and machine learning.

KEYWORDS

Envelope glycoprotein gp120, HIV neutralization phenotype, sequence, structure, and dynamics of protein, molecular dynamics simulation, graph machine learning

1. INTRODUCTION

The entry of HIV into target cells is mediated by the molecular interaction between the virus and the cell via the envelope glycoprotein gp120, which is the only virus-encoded protein exposed on the surface of the virion [1]. To avoid the attack of immune neutralization antibodies, gp120 undergoes rapid sequence variation and significant structural dynamics [2]. These features of gp120 pose major obstacles to the development of effective antiviral vaccines [3]. In terms of sequence variation, approximately half of the gp120 surface has more than 10% genetic variability to confuse the host immune system [4]. For structural dynamics, the prefusion gp120, which adopts a conformation of immune defense, has significant structural flexibility to mask the binding sites of the receptor and coreceptor [5]. The sequence variation and structural dynamics of gp120 make HIV present diverse adaptability under different physiological or experimental environments [6]. Therefore, in the environment of interest, the knowledge of sequence patterns that are associated with structural dynamics will inform our understanding of the molecular mechanism of HIV viral entry and immune escape to improve the development of antiviral drugs [7][8].

Based on the experimental assessments in a panel of genetically diverse HIV-positive plasma pools, HIV isolates are usually classified as neutralization 'tier' phenotype [6]. Current tiered categorization of the neutralization phenotype has suggested that HIV isolates exhibit a spectrum of neutralization phenotypes that can be divided into 1 to 3 tiers, corresponding to the sensitive, moderate, and resistant to neutralization antibodies, respectively.[9] Tier 1 is the most sensitive neutralization phenotype and often comes from laboratory-adapted strains without immune selection pressure. Although tier 1 was further classified into two subtypes, e.g. tier 1A and tier 1B, in some studies[9], it is feasible and efficient to simply use tier 1 in terms of neutralization sensitivity. Tier 2 exhibits a moderate neutralization phenotype and represents most circulating strains. Tier 3, isolated from the primary clinical sample, displays a least sensitive or most resistant neutralization phenotype.

Although the HIV neutralization phenotype has been experimentally characterized, the roles of sequence variability and structural dynamics of gp120 are not well understood. Our previous theoretical study of gp120 with the extremely sensitive and resistant neutralization preliminarily revealed the molecular mechanism of HIV neutralization phenotype by employing the molecular dynamics (MD) simulations [10]. It shows that the neutralization-sensitive gp120 from Tier 1 has higher global structural dynamics and richer conformational substates than the neutralization-resistant gp120 from Tier 3, promoting the neutralization-sensitive gp120 into a more flexible conformational state that interacts with the receptor and coreceptor. Although MD can provide an atomic exploration of protein conformational dynamics, it is still time-consuming and computationally expensive to perform a series of long-term simulations on GPU-accelerated clusters for protein with a usual size (approximately 500 amino acids). Moreover, the modern post-simulation trajectory analysis methods, such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), principal component analysis, and free energy landscape (FEL), only extract statistical information during the simulation process, and cannot provide a learnable and trainable model with predictive functions.

In recent years, machine learning (ML), especially deep learning, has gained attention from academia and industry because of its surprisingly great potential in bioinformatics applications such as protein structure modeling [11]. Deep learning algorithms exploit training data to discover potential patterns and build relational mapping models to make predictions. Some well-known deep learning algorithms, such as classic convolutional neural network (CNN), recurrent neural network (RNN), and other neural-network-

based models, have been widely used in biological sequence and structure analysis [12]. It should be noted that using ML to help optimize MD simulations gradually becomes a new trend in molecular science research. Wang et al. combined MD simulations with ML to predict the impacts of mutations-induced variation of protein-ligand binding affinity [13]. Bignon et al. explored the complex combinatorial chemistry of tandem DNA lesions repair and more generally local multiple damaged sites of the utmost significance in radiation chemistry by using MD simulations and ML [14]. Degiacomi mined the protein conformational space by training a neural network based on the MD simulations to supplement the existing conformations of the protein [15]. However, there are few attempts to probe the relationship between the sequence variation and structural dynamics of protein by employing ML and MD simulations. Moreover, for non-Euclidean data such as proteins, the application of graph ML in MD simulations is rarely reported.

In this study, we combine MD simulations and graph ML to probe the roles of rapid sequence variability and significant structural dynamics of gp120 in the HIV neutralization phenotypes. Randomly selected from an experimental panel [9], 45 prefusion gp120 structural models from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotype were built. Subsequently, these models were subjected to MD simulations followed by the comparative analysis of the structural deviations, conformational flexibility, and population distribution. In addition, a graph ML model with the attention mechanism was used to explore inherent representation related to the classification of the HIV neutralization phenotype, leading to further distinguish the strong related gp120 sequence variation together with structural dynamics in HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also provides a methodological framework to explore complex relationships between sequence, structure, and dynamics of protein by combining MD simulations and graph ML.

2. RESULTS

2.1. Structural architecture of the prefusion gp120

As shown in Figure 1A, the structural model of the prefusion gp120 is represented by the atomic coordinate of gp120 in the full-length crystal structure of HIV-1 prefusion envelope glycoprotein (PDB ID: 5FYK) [5]. The structural architecture of gp120 is composed of a relatively conserved functional core, a hydrophobic subdomain centered on the bridging sheet (β_2 , β_3 , β_{20} , and β_{21}), and five variable regions (V1–V5) located in the peripheral areas [16]. The hydrophobic subdomain from the stem of the V1/V2 region to the V3 loop plays a regulatory role in mediating the conformational dynamics of gp120 [17]. There are three layered structural regions above the hydrophobic subdomain [18]. The first layer is located between β_3 and β_0 and mainly contains α_0 ; the second layer is located between β_1 and β_5 and mainly contains α_1 ; the third layer is located between β_7 and β_{25} and mainly contains α_5 .

In the peripheral region, the V1/V2 region presents different structural characteristics. There is a "Greek key" structural folding composed of four β -strands (β_A – β_D) in the V1/V2 region [19]. The variable loop V1 is located between β_A and β_B , V2 is located between β_C and β_D , and the connecting loop L1 is located between β_B and β_C . Extending from the V1/V2 region and the V3 loop, a 4-stranded β sheet, called the bridging sheet, has been considered as a regulatory switch for conformational transitions of gp120 [20].

Based on the experimental assessment of HIV neutralization phenotype with a broad range of genetic and geographic diversity [9], 45 gp120 structural models from different HIV strains with sensitive, moderate, and resistant neutralization phenotype were randomly selected and constructed by homology modeling,

respectively. As shown in Figure 1B, all gp120 models from three neutralization phenotypes exhibit similar structural architecture without some loops have different spatial orientations. These constructed structural models have been validated and used for MD simulations and structure-based ML.

2.2. Structural Deviations of gp120

To quantitatively evaluate the structural deviation of gp120 with different neutralization phenotypes during the simulations, the time evolution of backbone RMSD values (Figure 2) relative to the template structure (PDB ID: 5FYK) were calculated. For the neutralization-sensitive, moderate, and resistant gp120, different relaxation time (about 10, 5, and 3 ns, respectively) is required to reach the equilibrium stage of simulations. A longer relaxation time means that the neutralization-sensitive gp120 has higher structural dynamics, which makes it easier to be exposed and attacked by neutralization immune antibodies [21]. In contrast, the structure of neutralization-resistant gp120 is easier to achieve stability which can be generally considered as a neutralization-resistant conformation after long-term immune selection [22]. For neutralization-moderate gp120, the relaxation time in the middle matches its transition position in the neutralization phenotype of HIV.

After reaching a relatively stable RMSD equilibrium, the neutralization-sensitive, moderate, and resistant gp120 fluctuate in a similar structural region around the average RMSD values of 0.47, 0.44, and 0.42 nm, respectively. Average RMSD is positively correlated with the degree of neutralization phenotype, indicating that gp120 with a more neutralization sensitive phenotype has a higher structural deviation. Except for the subtle differences in the RMSD timing changes, the RMSD values of gp120 with different neutralization phenotypes are distributed in the range of about 0.3–0.7 nm, indicating that gp120 can cover sufficient structural deviations to maintains the basic viral infection function while showing different neutralization phenotypes.

2.3. Dynamics of the Hydrophobic Subdomain

A recent study has pointed out that the hydrophobic subdomain composed of the V1/V2 region, the V3 loop, and the bridging sheet plays a regulatory role in mediating the conformational dynamics of gp120, contributing to the neutralization phenotype of HIV [17]. Therefore, the Rg of this hydrophobic subdomain was calculated to quantitatively analyze the pressure of the surrounding environment on it.

The time evolution of the Rg of the hydrophobic subdomain was plotted in Figure 3. For all phenotypic gp120, the Rg of the hydrophobic subdomain shows a downward trend, which is due to the Brown attack of the surrounding molecules, especially solvent water. However, the hydrophobic subdomains of different phenotypic gp120 shrink to different degrees. For neutralization-sensitive, moderate, and resistant gp120, the minimum value of the Rg is about 2.58, 2.6, and 2.62 Å, respectively. It demonstrates that gp120 with a more neutralization-sensitive phenotype has a looser hydrophobic subdomain and is therefore easy to shrink by the surrounding environment. More neutralization-resistant gp120 has a more stable hydrophobic subdomain to maintain its stable conformation.

2.4. Conformational Population Distribution of gp120

Characterizing by the RMSD relative to the template structure and the Rg of the hydrophobic subdomain, the conformational population distribution of gp120 with different neutralization phenotypes can be evaluated from the perspective of energetics. Three FELs (Figure 4) were constructed by projecting MD

trajectories into subspace spanned by the RMSD and the Rg. All FELs present a funnel-like free energy surface with irregular and divergent edges. The gp120 which has a more neutralization-sensitive phenotype occupies a larger FEL range and has more complex edges, indicating that it exhibits more conformational flexibility. In contrast, neutralization-moderate or resistant gp120 has a relatively concentrated FEL population distribution.

For the neutralization-sensitive gp120, there is a dominant free energy basin and two other metastable conformational distribution regions (in purple, free energy below about -12 kJ/mol). Although the neutralization-moderate gp120 has one main and two attached metastable regions, their areas are smaller than that of the neutralization-sensitive gp120, especially for the metastable regions. The neutralization-resistant gp120 has only one dominant free energy basin. The shrinking of the global minimum free energy region and the reduction of metastable regions indicate that the conformational population distribution of gp120 is positively correlated with the HIV neutralization phenotype.

2.5. Conformational Flexibility of gp120

The RMSF value (Figure 5) of the per-residue C α atom at structurally equivalent residue positions was calculated to characterize the conformational flexibility of each gp120. All RMSF profiles among the three phenotypic gp120 have similar features. Regular secondary structure regions have lower RMSF values, while the N-/C-termini and surface-exposed loops show higher RMSF values. Especially in the V1/V2 region, there is a typical conformational flexibility distribution despite undergoing significant residue mutations, deletions, or insertions. Separated by the β A-D, relatively large RMSF values can be observed in the V1 loop (between β A and β B), the L1 loop (between β B and β C), and the V2 loop (between β C and β D).

However, the conformational flexibility of subregions seems to have different roles among the three phenotypic gp120. As the neutralization phenotype becomes more resistant, both α 1 and α 2 show a trend toward lower RMSF values. This indicates that increasing conformational rigidity of the peripheral regular secondary structure may be one of the strategies to enhance the neutralization resistance. The V3 loop exhibits higher conformational flexibility in some neutralization-sensitive gp120, but it is significantly inhibited in neutralization-moderate and resistant gp120, indicating that the V3 loop perturbs the hydrophobic core to make gp120 more likely to be exposed to immune recognition. As a regulatory switch for conformational transitions of gp120[20], the β 20- β 21 hairpin has a slightly higher RMSF value in the sensitive gp120, implying that forcing gp120 to transfer to various conformations may provide an opportunity to eliminate the virus.

2.6. Building datasets of three phenotypic gp120

According to the characteristics of the protein data, different data preprocessing methods were used to process the sequence, structure, and MD simulation data of the three phenotypic gp120 resulting in five datasets (Table 1). Using natural language processing methods as a reference, the amino acid sequence of three phenotypic gp120 is regarded as a one-hot coding sequence (Table 1 Datasets Seq) for classification training [23]. For the structural datasets (Table 1 Datasets Stru and Snap), a graph-based model is introduced by transferring the structure of gp120 into the graph data. In the Stru dataset, only the gp120 models constructed by homology modeling were used. For MD simulations, 15, 30, and 60 dynamic structural snapshots were extracted from the 30-ns MD simulation trajectory of each gp120 at different time intervals (2, 1, and 0.5 ns), respectively, to construct the dynamic graph (Table 1 Datasets Snap_2, _1,

and _0.5).

Table 1: Datasets of three phenotypic gp120

Datasets	Amounts	Total	Data Model
Seq	3*15*1	45	Sequence
Stru	3*15*1	45	Static graph
Snap_2	3*15*15	675	Dynamic graph
Snap_1	3*15*30	1350	Dynamic graph
Snap_0.5	3*15*60	2700	Dynamic graph

2.7. Selecting machine learning models for the classification of three phenotypic gp120

To choose a suitable model to predict the classification of three HIV neutralization phenotypes, we tested several ML models on different datasets (Figure 6). For the Datasets Seq, the Gated Recurrent Unit (GRU) [24] is trained to learn the contextual representation of the gp120 sequences by maximizing the conditional probability. After transforming the gp120 structures into graphs, two graph ML models, i.e. Graph Convolution Network (GCN) [25] and Graph Isomorphism Network (GIN) [26], were employed to handle the structure-based graph data model. Taking into account the three performance indicators of Accuracy, AUC, and F1 (Table 2), the performance of the GCN model is significantly better than the GRU but slightly worse than the GIN. It reveals that graph ML models (GCN and GIN) can effectively extract complex spatial features from protein structures to obtain better prediction performance in the classification of three HIV neutralization phenotypes. It should be noted that the lower model performance indicators may be because the number of training data (45 sequences or structures) is too small to learn enough classification features for the ML model. However, the comparison of ML models based on sequence or structure provides direction guidance for us to choose the graph ML models as the following training ML model.

Table 2: Performance of the sequence and graph machine learning models training on sequence and structure databases for the classification of three phenotypic gp120.

Models	Datasets	Accuracy	AUC	F1
GRU	Seq	0.480±0.194	0.587±0.124	0.304±0.175
GCN	Stru	0.575±0.117	0.595±0.105	0.304±0.170
GIN	Stru	0.635±0.130	0.577±0.185	0.352±0.271

2.8. Comparison of different gp120 dynamics information in GIN

After testing and comparing different ML models for the classification of three phenotypic gp120, we chose GIN as the basic model. Unlike previous learning on static structures of gp120 (dataset Stru), dynamic structural snapshots (datasets Snap_2, Snap_1, and Snap_0.5) extracted from MD simulations at different time intervals (2.0, 1.0, and 0.5 ns) are used in the GIN model to explore the contribution of dynamic information to the classification of three phenotypic gp120. It can be seen from Table 3 that the performance of the GIN model has been greatly improved after introducing dynamics information through

MD simulations. The increase in training data directly leads to a significant improvement in the performance of the ML model. This shows that using MD simulations to expand the difficult-to-obtain protein structure data is a strategy that can be applied to ML training. As the sampling interval decreases, the accuracy, AUC, and F1 of the GIN model are getting higher and higher, indicating that it is beneficial to expand training data with a smaller sampling interval through MD simulations to exploit more dynamic conformational information of gp120. However, the ML model performance does not continue to increase as the time interval decreases. It is conceivable that a smaller time interval will not further improve the performance of the ML model.

Table 3: Performance of the graph machine learning model (GIN) training on static structures of gp120 and dynamic structural snapshots at different time intervals for the classification of three phenotypic gp120.

Datasets	Model	Time intervals	Accuracy	AUC	F1
Stru	GIN	-	0.635±0.130	0.577±0.185	0.352±0.271
Snap_2	GIN	2.0 ns	0.732±0.041	0.744±0.085	0.503±0.128
Snap_1	GIN	1.0 ns	0.785±0.032	0.798±0.056	0.587±0.123
Snap_0.5	GIN	0.5 ns	0.822±0.018	0.840±0.097	0.715±0.114

2.9. Comparison of different protein features in GIN

In graph ML, nodes use one-hot encoding and node embedding to introduce their features into the graph model. In the case of the protein graph model, one-hot encoding uses only an N-bit status register to encode 20 amino acids, while node embedding employs a high-dimensional vector to store physical, chemical, and other properties for each amino acid. In this paper, one-hot encoding maps 20 natural amino acid names into integer values and then converts the integer values into binary vectors. For the node embedding, 14 physicochemical properties (e.g. charge, hydrophathy, polarity, volume, etc. in Table S1) of different amino acids and the 3D coordinates of the C_α atom for each residue were transformed into the feature vectors of nodes to enrich the characteristics of nodes in the graph model.

As shown in Table 4, two protein features were used to train the graph ML model on dataset Snap_0.5 by using the GIN model. Compared with the method of one-hot encoding, the method of node embedded achieved better performance. The prediction performance of the GIN model on dataset Snap_0.5 is improved by more than 10%. The results show that appropriate protein features can significantly improve the performance of the ML model.

Table 4: Performance of the graph machine learning model (GIN) training on dynamic structural snapshots at time interval 0.5 ns for the classification of three phenotypic gp120 with different protein features.

Datasets	Model	Features	Accuracy	AUC	F1
Snap_0.5	GIN	One-hot encoding	0.822±0.018	0.840±0.097	0.715±0.114
Snap_0.5	GIN	Node embedded	0.971±0.003	0.986±0.010	0.934±0.036

2.10. Classification-related attention score from GIN with the attention mechanism

To analyze the classification-related information of the HIV neutralization phenotype, the graph ML model with an attention layer (GIN+ATT, Figure 7) was constructed by introducing the attention mechanism

[27] into the basic GIN model. After aggregating node features into graph features in the GIN model, the attention layer was employed to distribute attention scores into different nodes along the gradient descent direction of the classification for the HIV neutralization phenotype. During the training process, the attention score of each node was automatically adjusted, leading to more attention to the important amino acid residues for the classification. Although the classification performance of the GIN+ATT model is weakened after adding the attention mechanism (Table 5), the attention score can indicate the residue or node that plays an important role in the classification. A small difference in classification performance may be a potential signal of the stronger generalization ability of the model. In addition, the attention score of each residue can be used as an indicator to determine the crucial mutations in various sequences of gp120 and to measure the biological contribution of these mutations to the HIV neutralization phenotype.

Table 5: Predictive performance of our graphic-ML model.

Dataset	Model	Accuracy	AUC	F1
Snap_0.5	GIN	0.971±0.003	0.986±0.010	0.934±0.036
Snap_0.5	GIN+ATT	0.941±0.012	0.983±0.007	0.930±0.026

Similar to other attention-based models, attention scores can capture local contributions related to classification in the graph ML model. The attention scores (Figure 8) of the per-residue C_{α} atom at structurally equivalent residue positions were calculated to evaluate the contribution of every residue in gp120 to the classification of the HIV neutralization phenotype. For all attention scores among the three phenotypic gp120, the N-termini, V1/V2 region, and V3 loop have larger scores, suggesting that these local regions play a vital role in identifying the HIV neutralization phenotype. The large attention score of the V1/V2 region and the V3 loop, together with the higher RMSF of these regions, indicate that high sequence variability and conformational flexibility are the molecular basis of the HIV neutralization phenotype. It should be noted that different attention score distributions and amplitude changes can further reveal more subtle residue contributions. For example in the V1/V2 region, larger attention scores locate at the V1 loop, β D, and β 3 for the neutralization-sensitive gp120 (Figure 8, red lines), β B and the L1 loop for the neutralization-moderate gp120 (Figure 8, green lines), and β C for the neutralization-resistant gp120 (Figure 8, blue lines). Compared to the neutralization-sensitive gp120, C-termini seems to contribute more to the classification of the HIV neutralization phenotype in the neutralization-moderate and resistant gp120 and thus shows a larger attention score. Similarly, the β 20- β 21 hairpin also has a more important classification contribution in the neutralization-resistant gp120. These results all indicate that the attention scores can provide a structured explanation of gp120 for the classification of the HIV neutralization phenotype.

3. DISCUSSION

Establishing the relationship between sequence, structure, and function is the core issue in protein research. The sequence variation and structural dynamics of the envelope glycoprotein gp120 determine its function in viral infection, resulting in various HIV neutralization phenotypes [28]. Although MD simulations of gp120 with the extremely sensitive and resistant neutralization phenotypes preliminarily revealed the molecular mechanism of HIV neutralization phenotype [29], broad-spectrum theoretical studies of gp120 with different neutralization phenotypes are still missing. In this paper, 45 pre-fusion

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gp120 structural models from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotype were built and subjected to MD simulations. The comparative analysis of the structural deviations, conformational flexibility, and population distribution together shows that the molecular dynamics properties of gp120 are positively correlated with the HIV neutralization phenotype.

Together with the kinetic of gp120 in the CD4-complex and CD4-free state [29], CD4-binding effects on the conformational dynamics of gp120 [30][31], and conformational transitions of gp120 [32], these results in this paper provide a systematic description of the molecular dynamics of gp120. Although gp120 is a trimer under physiological conditions, it is still reasonable to use the gp120 subunit structure for research because small-angle X-ray scattering data revealed that the single gp120 domain in solution closely resembles that of the gp120 subunit in the context of the oligomeric viral spike/trimer [33]. In addition, the glycosylation effect was ignored in the MD simulations. Considering a single variable parameter (sequence variation) in the same experimental environment (prefusion gp120) can better focus on the purpose of research. Moreover, previous MD simulation studies on gp120 with glycosylated and non-glycosylated variable loops showed no significant differences in molecular fluctuations [34].

Although MD simulations can help explore detailed changes in the molecular structure of viral proteins, it is still impossible and unnecessary to perform long-term MD simulations for large-scale HIV strains. Moreover, modern post-simulation trajectory analysis methods (e.g. RMSD, RMSF, and FEL) only extract statistical information during the MD simulations to summarize the rules of molecular motion. In issues such as the sequence variation and structural dynamics of the envelope glycoprotein gp120 in the HIV neutralization phenotype, MD simulations are difficult to provide global statistical information to help distinguish the potential relationship between gp120 and the HIV neutralization phenotype. Therefore, using ML will be a good attempt to explore complex relationships between the sequence, structure, and dynamics of the protein. However, for the application of ML to protein research, there is a major obstacle that ML models require a lot of data (especially protein structure data that is difficult to obtain) for training. Many attempts solely on protein sequence data for ML training have been reported, but using protein structure data to construct ML models will capture more direct protein function information because the function of a protein is closely related to its structure. In this paper, MD simulations help expand the difficult-to-obtain experimental data into protein datasets for ML training.

After testing different types of data sets (the sequence, structure, and dynamic structure) and ML models (GRU, GCN, and GIN), the dynamic graph representation of protein from the MD simulations were used to train the graph ML model (GIN and GIN+ATT) in this paper. Compared to protein sequences, the use of the structure graph of the protein can improve the performance of ML models. A large number of biological data including the protein is often non-Euclidean form, which has complex topological structures and contains multiple features. GCN and GIN have an excellent performance in processing these data by following the neighborhood aggregation scheme and calculating the representation vector of nodes by recursively aggregating and transforming the representation vector of adjacent nodes. Thanks to the increase of training data and the supplement of dynamic information, the introduction of dynamic graphs significantly improves the performance of ML. In this paper, the advantages of MD simulations and graph ML have been cleverly utilized to decipher the role of envelope glycoprotein gp120 in HIV neutralization phenotype from two aspects of the sequence variation and structural dynamics. It is conceivable that this method that utilizes ML models, especially deep learning, to help analyze calculated data, such as MD simulations holds great research potential.

4. MATERIALS AND METHODS

4.1. Sequence selection and alignment

All HIV sequences were obtained from the UniProtKB database (<http://www.uniprot.org>) with the accession IDs listed in Table 6. By removing the segments corresponding to the signal peptide and gp41, the primary sequence of gp120 was extracted and used as the target to construct structural models of gp120 with different neutralization phenotypes.

4.2. Structural model construction and validation

The atomic coordinate of gp120 in the full-length crystal structure of HIV prefusion envelope glycoprotein (PDB ID: 5FYK) at 3.11 Å resolution [5] in the RCSB PDB database (<https://www.pdbus.org>) was used as the template to construct the structural models. The high sequence identity (higher than 66%, Table S1) between each selected gp120 sequence and the template ensures the reliability of homology modeling, which is implemented in the MODELLER [35] (version 9.17) package. For each structural model of gp120, 20 candidates were generated and the one with the lowest molecular probability density function score was selected. All models were validated using the SAVES server (<https://services.mbi.ucla.edu/SAVES>), in which algorithms of ERRAT [36], PROVE [37], and VERIFY3D [38] suggest that the constructed model and the template have a similar structural quality score (Table S1) which demonstrated that the gp120 structural models have good stereochemical quality. To obtain structurally equivalent positions, all gp120 models were aligned by using the multiple sequence alignment tool MUSCLE [39] within the MEGA [40]. Regular secondary structural elements and variable regions were labeled according to the standard HIV envelope glycoprotein crystal structures (PDB IDs: 1G9M [41] and 3JWD [18]).

4.3. Molecular dynamics simulations

Each structural model of gp120 was individually solvated using TIP3P water molecules [42] in a dodecahedron box with a solute-wall minimum distance of 0.8 nm and a periodic boundary condition. Sodium and chloride ions were added to obtain a 150-mM salt concentration. All simulations were performed by employing GROMACS (version 5.1.4) software [43] with the AMBER99SB-ILDN49 force field [44] at the GPU-accelerated clusters. After energy minimization with steepest descent algorithm and position-restrained simulations with decreasing harmonic positional restraint force constants on the heavy atoms of the protein, 30-ns production MD run for each gp120 was performed with the following protocol: integration time was set as 2 fs due to LINCS [45] algorithm was used to constrain bond lengths involving hydrogen atoms; the partial-mesh Ewald (PME) [46] method and a twin-range cut-off strategy were used to calculate the long-range electrostatic interactions and van der Waals interactions respectively; protein and non-protein components were independently coupled to a 300 K and 1 atm with an external bath; the system coordinates were saved every 2 ps.

The package MDTraj [47] was employed to calculate RMSD, Rg, and RMSF. FELs were constructed by $F(s) = -k_B T \ln(N_i/N_{max})$, where k_B is Boltzmann's constant, T is the simulation temperature, N_i is the population of bin i and N_{max} is the population of the most populated bin.

4.4. Datasets construction

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5 The gp120 sequence data adopted one-hot encoding and filled with the longest sequence length of 500.
6 For the gp120 structure, a graph-based model was introduced by selecting the C_{α} atom of each residue as
7 the node and connecting the edge if the distance of the pairwise C_{α} atom is less than 8 Å. For each residue,
8 14 amino acid properties (Table S2) and the 3D coordinates of the C_{α} atom for each residue were used as
9 the feature vector of each node.
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13 **4.5. Machine learning**

14 The GRU model is implemented by the Pytorch framework. The Deep Graph Library (DGL,
15 <https://www.dgl.ai>) was employed to train and validate graph ML models, i.e. GCN and GIN. When testing
16 the above model, the scikit learn package (<https://scikit-learn.org>) is used to split data into train/test sets
17 and k-fold (k = 10) cross-validation training strategy. All models were trained for 100 epochs, and the
18 learning rate was set to 0.001.
19

20
21 In the GIN+ATT model, the hidden representation H_v ($H_v=[h_1, h_2, \dots, h_n]$, where n is the number of
22 nodes and w is the learnable weight) of all nodes obtained from the last layers of the GIN model were
23 used to construct the attention mechanism by using the following calculation:
24

25
26
$$M = \text{Relu} (H_v), \tag{1.1}$$

27
$$\alpha = \text{softmax}(w^T M), \tag{1.2}$$

28
29
$$H'_v = H_v \alpha^T. \tag{1.3}$$

30
31 The α of formula 1.2 is the attention vector. Through the calculation of formula 1.3, the graph node
32 representation H'_v adjusted by attention weight is obtained. As the first part of the model, the GIN layer
33 is used to extract the potential spatial features of each node. The feature vectors of nodes from the last
34 layers of the GIN model were passed through the attention layer to aggregate as graph representation
35 vectors. The attention layer is implemented by the trainable parameter module of Pytorch.
36
37

38 To measure the performance of the model, three different evaluation indicators, i.e. the accuracy, the
39 area under the ROC curve (AUC), and the F1 score, were used. The calculation method can be obtained
40 from the confusion matrix (Table 7) and formula 2.1.
41
42

43 **Table 7:** The confusion matrix

44
45
46
47
48
49
50

Forecast \ Factual	0	1
0	TN	FN
1	FP	TP

51
52
$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FN + FP} \tag{2.1}$$

53
54

55 ROC curves were plotted with the true positive rate (TPR) and the false positive rate (FPR) as abscissa and
56 ordinate respectively. The definitions are as formula 2.2-2.3.
57
58
59
60

$$TPR = \frac{TP}{TP + FN} \quad (2.2)$$

$$FPR = \frac{FP}{TN + FP} \quad (2.3)$$

The area under the ROC curve (AUC) can help to comprehensively measure the performance of the model. F1 score is the harmonic average of recall (TPR) and precision (P), and its calculation method is shown in formula 2.4-2.5.

$$P = \frac{TP}{TP + FP} \quad (2.4)$$

$$\frac{2}{F} = \frac{1}{P} + \frac{1}{TPR} \quad (2.5)$$

5. CONCLUSION

In this study, 45 prefusion gp120 structural models from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotypes were built and investigated by MD simulations and graph ML to probe the roles of rapid sequence variability and significant structural dynamics of gp120 in the HIV neutralizing phenotype. By integrating homology modeling, MD simulations, and graph ML, we constructed a reliable model for HIV neutralization phenotype. Statistical analysis of gp120 dynamics (e.g. RMSF) and classification-related attention scores together revealed potential structural factors that may affect HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also provides a methodological framework to explore complex relationships between the sequence, structure, and dynamics of the protein by combining MD simulations and ML.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The source data (sequence files, structural models, C_α trajectories, RMSD, and RMSF data) and scripts are available at https://github.com/liyigerry/gp120_role.

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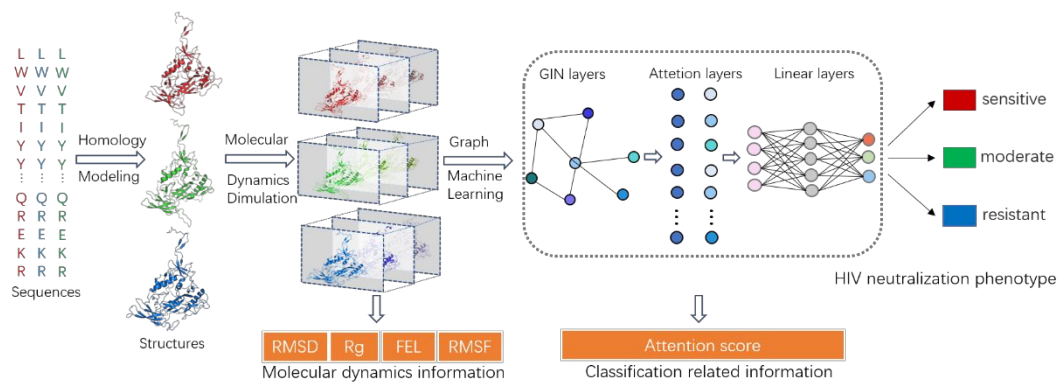
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Graphic abstract

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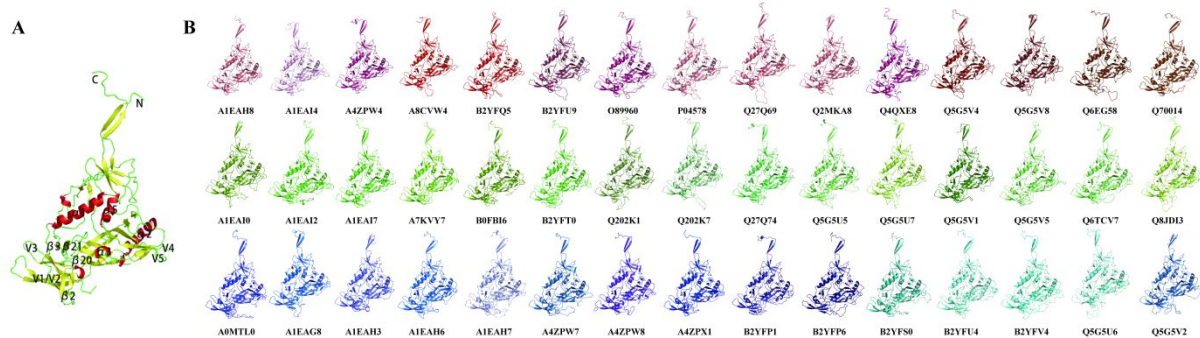


Fig. 1. The structural architecture of gp120. (A) Structure of prefusion gp120 (PDB ID: 5FYK). (B) Structural models of the neutralization-sensitive (reds), moderate (greens), and resistant (blues) gp120 from different HIV isolates with the accession ID of UniProtKB database.

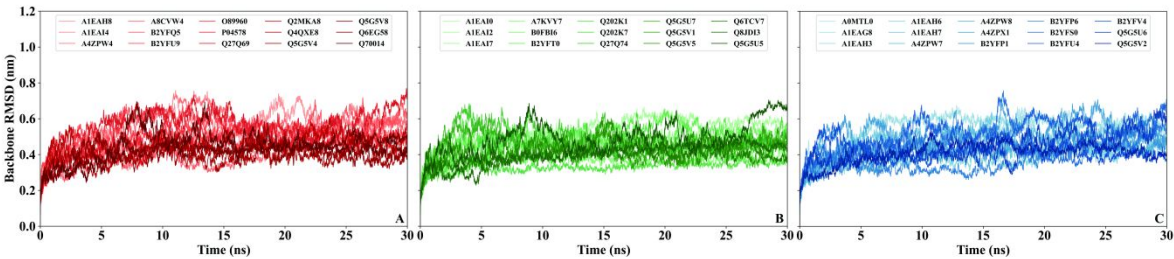


Fig. 2. Time evolution of backbone root-mean-square deviation (RMSD) values of the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 relative to the template structure (PDB ID: 5FYK) during the molecular dynamics simulations.

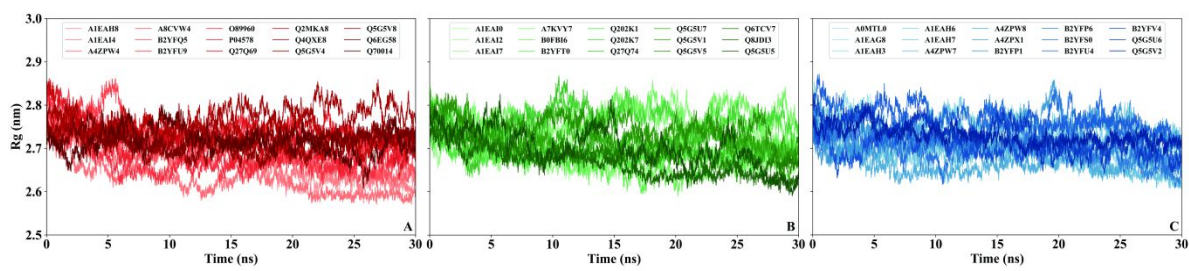


Fig. 3. Time evolution of radius gyration (Rg) of the hydrophobic core in the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 during the molecular dynamics simulations.

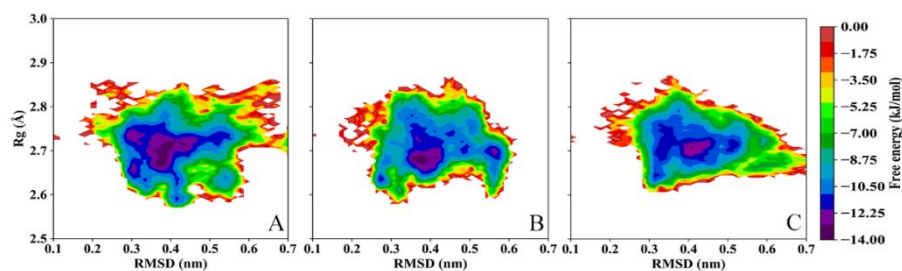


Fig. 4. Free energy landscapes of the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 by projecting trajectories into the subspace of root-mean-square deviation (RMSD) relative to the template structure and the radius gyration (Rg) of the hydrophobic subdomain.

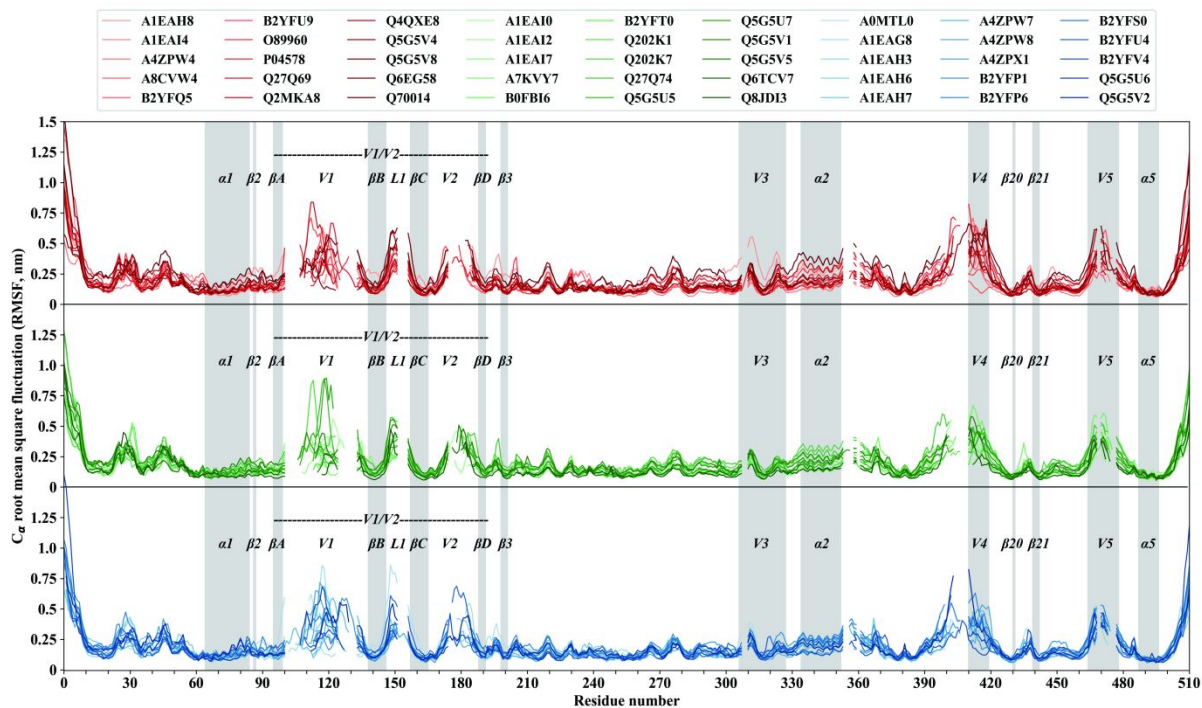


Fig. 5. C α root-mean-square fluctuation (RMSF) values of the neutralization-sensitive (red lines), moderate (green lines), and resistant (blue lines) gp120 during the molecular dynamics simulations.

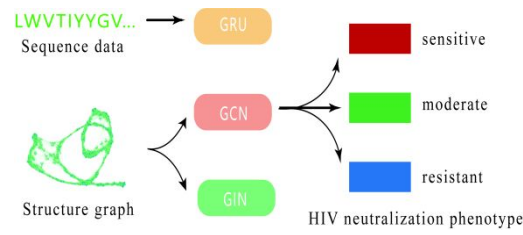


Fig. 6. The Gated Recurrent Unit (GRU) was used to handle the sequence data. The Graph Convolution Network (GCN) and Graph Isomorphism Network (GIN) were trained by the structure graph. All ML models were used for the classification of HIV neutralization phenotypes.

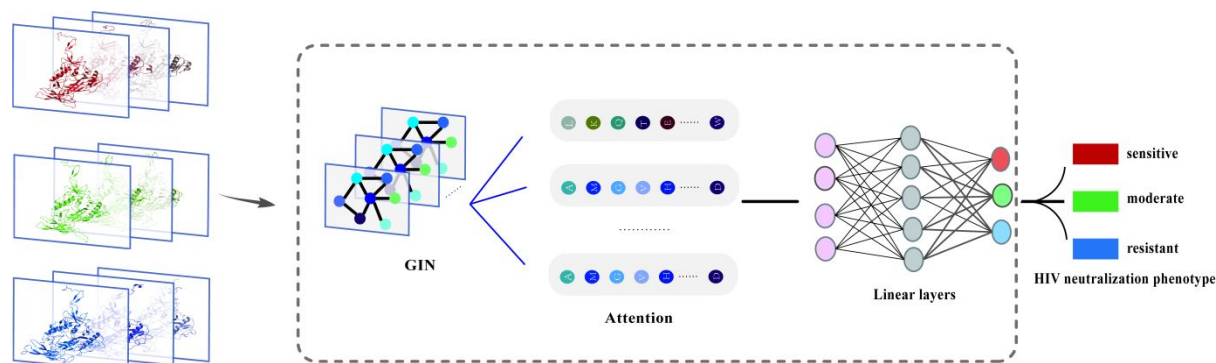


Fig. 7. Diagram of the Graph Isomorphism Network (GIN) with the attention mechanism (GIN+ATT).

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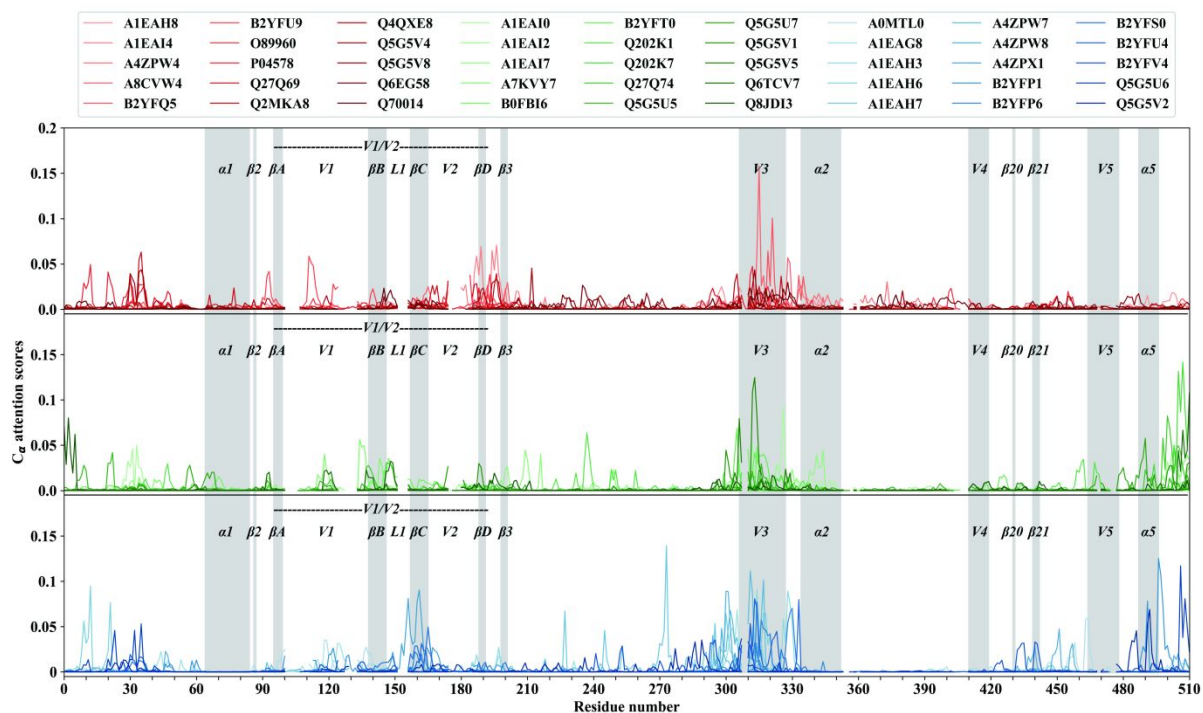


Fig. 8. C_{α} attention scores of the neutralization-sensitive (reds), moderate (greens), and resistant (blues) gp120.

Supporting Information

Table S1: 14 physicochemical properties of different amino acids.

Amino acid	alpha	beta	charge	core	hydropathy	pH	polarity	rim	surface	turn	volume	strength	disorder	high_contact
A	1.29	0.9	0	0.049	1.8	0	0	0.047	0.065	0.78	67	0	0	1
C	1.11	0.74	0	0.02	2.5	-2	0	0.015	0.015	0.8	86	1	-1	0
D	1.04	0.72	-1	0.051	-3.5	-2	1	0.071	0.074	1.41	91	0	1	1
E	1.44	0.75	-1	0.051	-3.5	-2	1	0.094	0.089	1	109	0	1	0
F	1.07	1.32	0	0.051	2.8	0	0	0.021	0.029	0.58	135	1	-1	0
G	0.56	0.92	0	0.06	-0.4	0	0	0.071	0.07	1.64	48	0	1	1
H	1.22	1.08	0	0.034	-3.2	1	1	0.022	0.025	0.69	118	0	-1	0
I	0.97	1.45	0	0.047	4.5	0	0	0.032	0.035	0.51	124	1	-1	0
K	1.23	0.77	1	0.05	-3.9	2	1	0.105	0.08	0.96	135	0	1	0
L	1.3	1.02	0	0.078	3.8	0	0	0.052	0.063	0.59	124	1	-1	1
M	1.47	0.97	0	0.027	1.9	0	0	0.017	0.016	0.39	124	1	1	0
N	0.9	0.76	0	0.058	-3.5	0	1	0.062	0.053	1.28	96	0	1	1
P	0.52	0.64	0	0.051	-1.6	0	0	0.052	0.054	1.91	90	0	1	0
Q	1.27	0.8	0	0.051	-3.5	1	1	0.053	0.051	0.97	114	0	1	0
R	0.96	0.99	1	0.066	-4.5	2	1	0.068	0.059	0.88	148	0	1	1
S	0.82	0.95	0	0.057	-0.8	-1	1	0.072	0.071	1.33	73	0	1	1

Amino acid	alpha	beta	charge	core	hydropathy	pH	polarity	rim	surface	turn	volume	strength	disorder	high_contact
T	0.82	1.21	0	0.064	-0.7	-1	0	0.064	0.065	1.03	93	0	0	1
V	0.91	1.49	0	0.049	4.2	0	0	0.048	0.048	0.47	105	1	-1	0
W	0.99	1.14	0	0.022	-0.9	1	1	0.007	0.012	0.75	163	1	-1	0
Y	0.72	1.25	0	0.07	-1.3	-1	1	0.032	0.033	1.05	141	1	-1	1

This table is taken from https://github.com/mikessh/vdjtools/blob/master/src/main/resources/profile/aa_property_table.txt

Table S2: Structural models of gp120.

	Isolate	Residues ¹	Identity ²	RMSD ³ (nm)	VERIFY3D ⁴	ERRAT ⁵	PROVE ⁶	MD&ML ⁷
Template	5FYK_G	480	-	-	85.26%	84.7727	5.00%	No
Tier 1	A1EAH8	468	71.55%	0.684	83.33%	63.2690	8.20%	Yes
	A1EAI4	485	72.58%	0.67	84.71%	55.7235	7.00%	Yes
	A4ZPW4	470	69.10%	0.72	55.74%	58.658	8.90%	Yes
	A8CVW4	470	71.07%	0.697	77.87%	64.6421	6.30%	Yes
	B2YFQ5	477	72.26%	0.684	84.70%	59.1075	9.30%	Yes
	B2YFU9	481	68.32%	0.667	73.80%	57.3561	7.10%	Yes
	O89960	486	70.61%	0.706	81.89%	63.0252	5.80%	Yes
	P04578	478	67.08%	0.706	82.01%	59.743	6.20%	Yes
	Q27Q69	494	67.94%	0.653	84.41%	56.1728	7.20%	Yes
	Q2MKA8	476	70.00%	0.686	85.50%	68.4665	6.50%	Yes
	Q4QXE8	466	69.87%	0.691	83.48%	68.1223	6.40%	Yes
	Q5G5V4	473	69.83%	0.725	83.51%	67.0978	6.20%	Yes
	Q5G5V8	477	68.46%	0.677	87.42%	61.1940	7.80%	Yes
Tier 2	Q6EG58	488	68.16%	0.742	83.47%	59.1667	6.60%	Yes
	Q70014	469	71.34%	0.76	77.40%	57.7007	6.20%	Yes
	A1EAI0	483	69.53%	0.706	84.68%	63.2911	6.90%	Yes
	A1EAI2	489	71.84%	0.722	79.75%	58.6134	7.10%	Yes
	A1EAI7	463	71.76%	0.696	82.94%	58.6813	5.70%	Yes
	A7KVY7	469	68.27%	0.677	84.43%	59.9129	8.40%	Yes
	B0FBI6	475	69.48%	0.777	85.26%	61.9355	6.60%	Yes
	B2YFT0	486	69.96%	0.748	84.98%	58.9958	8.00%	Yes
	Q202K1	472	69.52%	0.679	77.97%	70.6897	7.20%	Yes
	Q202K7	470	71.07%	0.692	78.72%	61.1354	5.70%	Yes
	Q27Q74	471	71.04%	0.739	84.50%	61.1231	9.00%	Yes

		Q5G5U5	472	70.35%	0.67	76.06%	64.5022	7.00%	Yes
		Q5G5U7	478	68.05%	0.685	86.82%	55.8190	7.00%	Yes
		Q5G5V1	484	70.72%	0.723	85.33%	59.8739	6.10%	Yes
		Q5G5V5	476	69.48%	0.703	85.50%	63.4615	6.40%	Yes
		Q6TCV7	470	69.63%	0.674	86.81%	61.2554	6.40%	Yes
		Q8JDI3	468	72.41%	0.703	83.12%	63.0435	6.50%	Yes
		A0MTL0	475	71.99%	0.699	77.47%	48.8223	8.90%	Yes
		A1EAG8	487	71.84%	0.656	83.37%	58.7866	6.50%	Yes
		A1EAH3	475	72.41%	0.667	81.47%	58.9247	6.40%	Yes
		A1EAH6	486	71.19%	0.772	82.72%	56.9328	7.40%	Yes
		A1EAH7	473	71.43%	0.695	90.27%	60.9914	7.40%	Yes
		A4ZPW7	490	67.61%	0.701	83.47%	59.5833	6.60%	Yes
		A4ZPW8	475	70.19%	0.716	78.32%	60.8137	5.50%	Yes
	Tier 3	A4ZPX1	478	67.63%	0.703	85.36%	64.8936	6.80%	Yes
		B2YFP1	487	68.65%	0.843	81.00%	55.9499	7.20%	Yes
		B2YFP6	472	68.68%	0.687	79.24%	63.6364	6.50%	Yes
		B2YFS0	478	69.55%	0.713	87.87%	66.3793	7.70%	Yes
		B2YFU4	482	67.89%	0.708	80.91%	57.5949	7.90%	Yes
		B2YFV4	493	68.61%	0.706	81.95%	55.3719	6.40%	Yes
		Q5G5U6	477	66.53%	0.721	80.71%	57.7827	5.80%	Yes
		Q5G5V2	487	68.64%	0.695	81.52%	66.9456	5.80%	Yes

1. The number of residues in gp120.
2. The identity between the target sequence and the sequence of the template (PDB ID: 5FYJ, chain G).
3. The root means square deviation (RMSD) to the template (PDB ID: 5FYJ, chain G).
4. The percentage of the residues have averaged 3D-1D score ≥ 0.2 calculated from the algorithm VERIFY3D.
5. Overall quality factor of the algorithm ERRAT.
6. The percentage of buried outlier protein atoms in the algorithm PROVE.
7. Whether the structural model is used for molecular dynamics (MD) simulations and machine learning (ML) training.

Editorial comments:

Experts in the field have carefully reviewed your manuscript entitled "Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning". Although they found merit in your study, they have raised a number of serious concerns that preclude its acceptance in the present form. This paper has been recommended for MAJOR REVISIONS. I invite you to respond to the reviewers' comments and revise your paper accordingly. The revised version will be re-evaluated by the original reviewers. Please be aware that this invitation to revise your manuscript does not guarantee eventual acceptance of your manuscript.

Response

Thanks to the editor for the opportunity to peer review and revise. We thank the reviewer for their careful reading and comments on the manuscript. The reviewers' comments are very professional and constructive. We have carefully revised the manuscript according to the reviewers' comments, and hope that it can be accepted. In addition to main text revisions, we have reorganized the supporting material and online data or code. The name of the online code repository has been changed from the original 'gp120_role' to a more meaningful abbreviation 'gp120_md_gml', i.e. https://github.com/liyigerry/gp120_md_gml.

I hope to get in touch with the reviewers after this review for more academic communication. Since the review is anonymous, please contact my email address (liyigerry@163.com) without affecting the review of this article.

Reviewer 1 comments:

This manuscript presents MD analysis of a set of gp120 variants with diverse sequences, and the utilization of the generated data for applying machine learning approaches toward understanding how sequence features could be associated with neutralization phenotypes (the tiered system of classifying HIV-1 variants on a scale of neutralization sensitive to neutralization resistant). The study presents intriguing results about how HIV-1 Env sequence relates to virus function, but there are several major issues with both study design and data analysis.

Response

We thank the reviewers for the positive comments on our work.

1. There is a lack of precision in the analysis because of the focus on gp120 only. The HIV-1 Env ectodomain is a trimer of heterodimers, and the protein dynamics depend on the interactions within the trimer of gp120/gp41 heterodimers. It will be important to present this as a major limitation of this study. At the least, there should be a detailed discussion of how this limitation could affect the results reported here. Related to this point – the authors refer to gp120 as though it is the only protein on the surface of HIV-1; this language should be corrected throughout the manuscript.

Response

We added the discussion of gp120/gp41 heterodimer and the simulation limitations of this article in the revised manuscript, seeing the second sentence in the Discussion section. The description of the 'only' virus-encoded protein on the surface of HIV has also been modified.

2. The comparisons between the different gp120 groups (e.g., Figures 2-5) currently do not include appropriate statistical tests and p-values. As a result, the claims about differences between groups are not supported throughout the manuscript. In fact, for some of these figures, it is difficult to visually notice differences between groups, and it is difficult/impossible to know if any differences are actually significant. Appropriate statistical tests should be performed and reported, including p-value correction for multiple comparisons.

Response

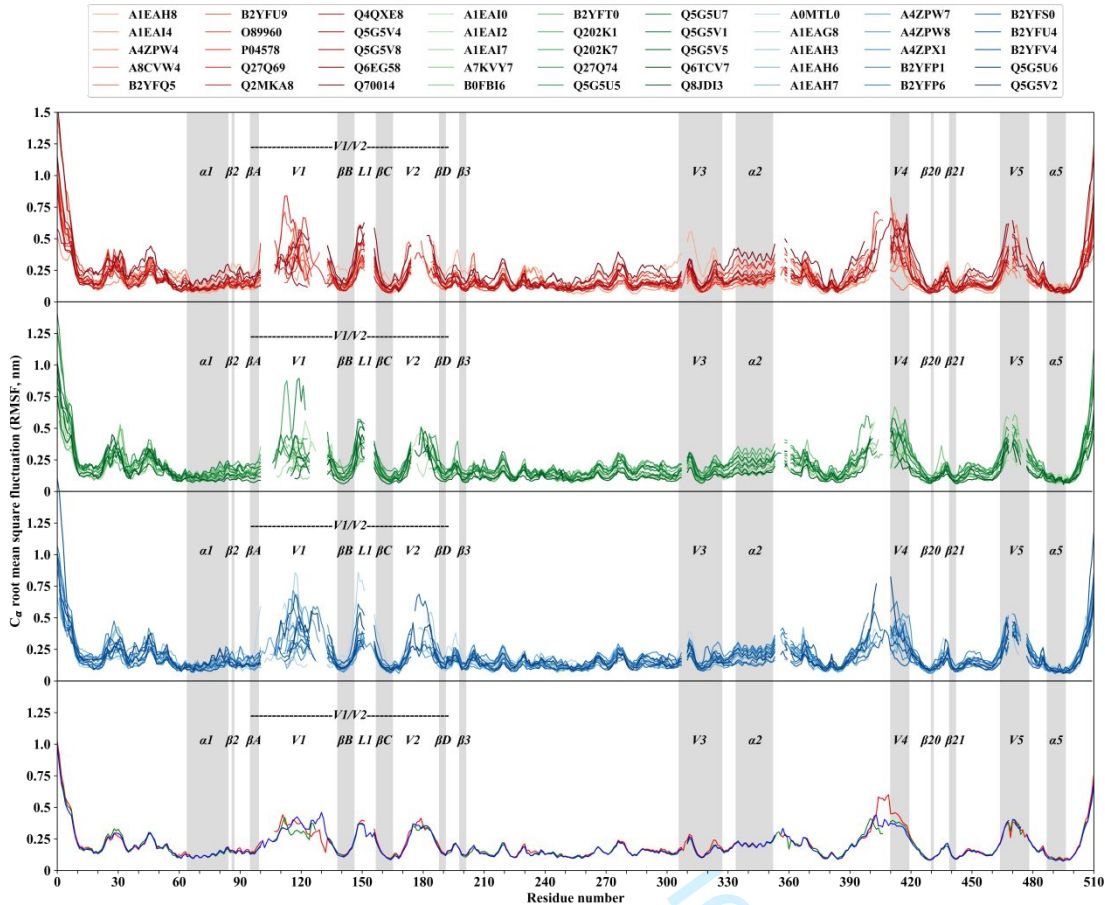
For the RMSD (Fig. 2) and Rg (Fig. 3) values, the Kolmogorov-Smirnov test (K-S test) was used to observe the similarity of the data distributions. The p-values obtained by the pairwise K-S test of the RMSD or Rg values of gp120s with different neutralization phenotypes were all less than 0.05 (see the following table), indicating these values had significantly different distributions. This result has been added to the supporting information.

	RMSD			Rg		
	sensitive	moderate	resistant	sensitive	moderate	resistant
sensitive	1	0	4.13e-237	1	1.46e-107	3.44e-204
moderate	0	1	1.22e-89	1.46e-107	1	6.06e-169
resistant	4.13e-237	1.22e-89	1	3.44e-204	6.06e-169	1

Since the FEL (Fig. 4) was constructed based on two coordinates of the RMSD and Rg values, its K-S test can be omitted.

For the RMSF (Fig. 5) profiles, the protein belonging to the same family (gp120) should have a

similar RMSF distribution along with the residual index. To show the RMSF difference between different gp120 with sensitive, moderate, and resistant neutralization phenotypes, we calculated RMSF mean values (bottom subplot) for each phenotypic gp120 at the equivalent residue position. Then, the mean RMSF of gp120 with three neutralization phenotypes was compared. The RMSF mean comparison plot (see the following figure) was added in the revised manuscript.



3. It appears that there was no separate validation set that could be used to evaluate the cross-validation results from the initial training/test set. This could have led to overfitting toward the gp120 set used in the analysis. To show that this is not the case, models for an additional diverse set of gp120s should be created, and the resulting accuracy, etc. should be reported – this validation set should be diverse enough.

Response

In this study, 45 gp120 were randomly selected from an experimental panel (Seaman et al., 2010) which contains 102 HIV isolates belonging to three tiers of sensitive, moderate, and resistant neutralization phenotype.

Seaman, M.S., Janes, H., Hawkins, N., Grandpre, L.E., Devoy, C., Giri, A., Coffey, R.T., Harris, L., Wood, B., Daniels, M.G., et al. (2010). Tiered Categorization of a Diverse Panel of HIV-1 Env Pseudoviruses for Assessment of Neutralizing Antibodies. *J. Virol.* 84, 1439–1452.

We further selected another untrained 57 gp120s as the separate validation dataset (see the

following table) to examine the predictive performance of our trained ML model. The prediction accuracy was 66%, indicating that our model can better predict the HIV neutralization phenotype through the gp120 sequence and structure. The source file is available online, seeing the [ml_test.ipynb](#).

	sensitive	moderate	resistant
		A1EAI3 B0FBH2 Q8JDL4 B2YFN6 A4ZPW3 Q8JDJ1	
		Q6EFN0 B2YFN1 Q1PHM6 B0FBJ5 B2YFM7 Q5G5V0	
	A4ZPX2 A4ZPX0	B0FBD9 B2YFR5 A1EAH0 A7KVX0 Q8JDN0 B2YFT9	
Isolate	O55774 Q7ZJC0	B0FBD4 Q5G5U4 A4ZPX3 Q202J8 Q202K4 Q27Q59	A4ZPW9
Number	A1EAI5 Q6TCP8	Q1PHM9 Q27Q64 B2YFT4 B0FBG7 A4ZPW5 B2ZSE9	
	A1EAI6 B0FBB9	Q5G5V7 Q5G5U8 Q6TD46 B0FBB4 A1EAI1 Q5G5U9	
		A1EAH2 B2YFR0 A1EAH9 Q202J5 A1EAG7 A4ZPW6	
		A1EAH1 A1EAH4 Q8JDJ9 B2YFQ1 A1EAH5 B2YFV9	

Although machine learning models are commonly used for prediction tasks, in our study graph machine learning models were applied to map protein (gp120) sequence-structure-dynamics to biological function (HIV neutralization phenotype). Given the scarcity of biological data and the inaccessibility of protein dynamics data, our graph machine learning model operates on datasets that lack diversity. We admit that overfitting exists. In machine learning, overfitting usually indicates that the model does not generalize well and should be avoided. However, we emphasize that overfitting pays attention to mining the biological information contained in the training data.

We discuss the limitations of our graph machine learning model in the revised manuscript and reiterate the role of overfitting in this work. We hope that the reviewer will look at our work from the perspective of bioinformatics mining. It is hoped that the revised manuscript will be approved by the reviewers.

4. It is important to report the gp120 sequence identity matrix, clade, and tier information. This should be shown in the context of the global HIV-1 Env diversity, to motivate why these specific gp120 variants were selected, and to show that there is no bias created with highly similar sequences.

Response

Pairwise sequence identity of gp120 sequences with different neutralization phenotypes showed that our randomly selected gp120 sequences have a similar sequence similarity and have no bias among the three neutralization phenotypes. The sequence identity matrix file is available online, seeing the [seq_identity.xlsx](#).

5. Minor comment: the graphical abstract has a typo (“Dimulation”).

Response

Modified.

Reviewer 2 comments:

I have reviewed the manuscript entitled “Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning” by li Yi t al. I am a molecular biophysicist who develops, standardizes, and implement, computational biophysical methods for Genomics (Structural Bioinformatics for Genomics), which is the area of focus for this manuscript. In addition, to my own review, I have also proceeded to compare the findings here reported with those previously existent in the literature. Thus, following, I provide suggestions for the Editor’s consideration.The manuscript present novel information of significant biomedical relevance to the new field of Structural Bioinformatics for Genomics, and should be of interest to the readers of Proteins, if the authors expand significantly the number and types of different molecular mechanic calculations.

Response

We are grateful to the reviewer for affirming that our work is in line with the new field of Structural Bioinformatics for Genomics.

Methodologically, the authors used for standard methods of molecular mechanics and molecular dynamics. In fact, the value of the approach and the conclusions of the manuscript are overstated. This is because, the authors do not develop a comprehensive suite of methods but rather use standard analyses that are available in any computational laboratory.

Response

We modified the description of the approach and the conclusion in the revised manuscript. We still emphasize that the joint application of machine learning and graph molecular dynamics simulation is rare and worth exploring.

In summary, The topic of the current manuscript is extremely timely and represent an opportunity for the journal to project even further than molecular biophysics but enter the era of Structural Bioinformatics for Genomics. Thus, the manuscript is of interest to tis growing and important number of investigations, world-wide. This field, in fact, is only expected to grow exponentially in the following few years.The current manuscript is appropriate and will be of interest to the audience of this journal. However, before accepting the manuscript, it would be important for the authors to:

1. Enhance the analyses of variants using more parameters derived from molecular dynamic simulations

Response

We calculated RMSF values and attention scores for each phenotypic gp120 at the equivalent residue position and identified gp120 variants (mainly in the V1, L1, V2, V3, the head of V4, and V5 loop) associated with the HIV neutralization phenotype. These results were added to the revised manuscript.

2. While in MDs the present data on positive correlations, they should discuss how they arrived at this simple design (the design of the manuscript is very simple).

Response

In our previous studies (Li et al., 2020a, Li et al., 2020b, Li et al., 2020c, and Li et al., 2018),

comparative MD simulations were able to reveal protein dynamics. The dynamic properties of proteins are determined by their intrinsic properties. A simple comparative study using MD can reveal its biological significance. In this work, we also used graph machine learning for classification to complement the results of MD simulations.

Li, Y., Deng, L., Ai, S.-M., Sang, P., Yang, J., Xia, Y.-L., Zhang, Z.-B., Fu, Y.-X., and Liu, S.-Q. (2018).

Insights into the molecular mechanism underlying CD4-dependency and neutralization sensitivity of HIV-1: a comparative molecular dynamics study on gp120s from isolates with different phenotypes. *RSC Adv.* **8**, 14355–14368.

Li, Y., Zhang, X.-L., Yuan, X., Hou, J.-C., Sang, P., and Yang, L.-Q. (2020a). Probing intrinsic dynamics and conformational transition of HIV gp120 by molecular dynamics simulation. *RSC Adv.* **10**, 30499–30507.

Li, Y., Deng, L., Liang, J., Dong, G.-H., Xia, Y.-L., Fu, Y.-X., and Liu, S.-Q. (2020b). Molecular dynamics simulations reveal distinct differences in conformational dynamics and thermodynamics between the unliganded and CD4-bound states of HIV-1 gp120. *Phys. Chem. Chem. Phys.* **22**, 5548–5560.

Li, Y., Guo, Y.-C., Zhang, X.-L., Deng, L., Sang, P., Yang, L.-Q., and Liu, S.-Q. (2020c). CD4-binding obstacles in conformational transitions and allosteric communications of HIV gp120. *Biochim. Biophys. Acta - Biomembr.* **1862**, 183217.

3. Provide a correlation matrix of structural and functional scores (reference provided)

Response

We have carefully read this paper (<https://doi.org/10.1093/bioinformatics/btaa972>) which was provided by the reviewer. Based on the analysis of RMSF and attention scores, we estimated the correlation of gp120 variants with HIV neutralization phenotype by calculating the change in folding free-energy by using FoldX. The positions of the gp120 variants were chosen from large to small according to the deviation of the RMSF or attention score. Top 100 positions with the largest deviation of the RMSF or attention score were extracted, seeing the source file online `top_100_position.xlsx`. We select the top one position with the largest deviation of the RMSF or attention scores and in the V1, L1, V2, V3, the head of V4, and V5 loop, i.e. the 119 position for the RMSF and the 315 position for attention scores to calculate the change in folding free-energy. The relationship of the position and the residual index for each gp120 can be found in the online file (`position_residual.xlsx`). It should be noted that the equivalent positions obtained by the multiple sequence alignment are not the residue numbers of each gp120. The folding free energy was calculated after mapping the respective gp120 residue numbers at the above positions. The source file of FoldX results is available online, seeing the `fold_energy_rmsf.xlsx` and `fold_energy_att.xlsx`.

Given the complexity of the structural score analysis and our paper's focus on gp120 variants indicated by MD and ML, we did not calculate and analyze the full sequence and structural scores. The complete structure and function score correlation matrix has gone beyond the focus of our study. In the revised manuscript, we have added a discussion of gene phenotype related to sequence and structure scores. We think a score-based analysis of variants is very important. We will apply this method in future work. We implore reviewers to focus on the main contributions of our current work.

4. Clarify the data presentation and discussion, as suggested.

Response

Detailed data presentation and discussion in the revised edition have been modified.

5. The authors exaggerate the novelty of the approach used in the study since the measurements used are very straightforward. Therefore, they should reduce or tone down their conclusions. Thus, I underscore that this manuscript would make a good contribution to the journal after the authors have addressed these comments.

Response

We modified the description of the conclusion.

Additional comments:

Brief Paper Description: HIV-1 entry is mediated firstly by the molecular recognition between the viral glycoprotein gp120 and its receptor CD4 on host T-cells. As a key antigen that can be targeted by neutralizing antibodies, gp120 has been a focus for extensive studies with efforts to understand its structural properties and conformational dynamics upon receptor binding (Phys Chem Chem Phys. 2019 Dec 4;21(47):26003-26016). The marked mutability of the HIV-1 virus presents a significant challenge to immune responses as epitope contacts shift during infection, rendering most neutralizing antibody responses specific to a particular Env sequence (PLoS Biol. 2015;13:e1002251). Thus, the HIV-1 envelope's structure and dynamics have been the focus of intense investigation owing to its critical role in the virus lifecycle and its importance as an immunogenic target for vaccines. Notably, HIV-1 Envelope (Env) variants are grouped into tiers by their neutralization-sensitivity phenotype. Tier 1 neutralization responses can be elicited readily, but do not protect against new infections. Tier 3 viruses are the least sensitive to neutralization. Because most circulating viruses are tier 2, vaccines that elicit neutralization responses against them are needed.

The current study contributes to the field by evaluating 45 prefusion gp120 from different HIV strains belonging to these three tiers by homology modeling and molecular dynamics simulations. The authors report that 1. structural deviations, population distribution, and conformational flexibility of gp120 are related to the HIV neutralization phenotype, 2. the second structural regions with high flexibility may be responsible for HIV neutralization phenotypes, 3, a graph machine learning model indicates that gp120 sequence variation contribute together with structural dynamics in HIV neutralization phenotype.

Strengths:

1. The study of mutational profiles using computational biophysics lags behind and is in dear need of being expanded. Therefore, the goal of the current study is laudatory.
2. This is an original and informative study on a topic of significant biomedical relevance.
3. For the most part, the study design, execution, and analyses are appropriate.

General Evaluation: The manuscript is very appropriate for publication in Proteins, due to its originality in the area of "Structural Genomics". In other words, the manuscript report how sequence variation influences the molecular biophysical properties of gp120. However, the writing, presentation, and fair representation of what is novel in the manuscript is important.

1. There is a significant large amount of informative measurements and correlations to be done by molecular mechanic calculations that can help to expand the study (example: Bioinformatics,

Volume 37, Issue 10, 15 May 2021, Pages 1367–1375)

Response

We carefully read this paper (<https://doi.org/10.1093/bioinformatics/btaa972>) which was provided by the reviewer. We calculated some 3D structure-based scores, e.g. folding free-energy, $\Delta\Delta G_{\text{fold}}$ using FoldX. However, we think a score-based analysis of variants is very important but has gone beyond the focus of our study. We will apply this method in future work.

2. While the study is appropriate, some of the conclusions must be tone down. For instance, the approach used by the authors for the most part, is not new, but rather standard measurements from the output of molecular dynamics. However, the authors emphasize that the present a new approach that can be followed. Similarly, the fact that sequence variation, influences structure and dynamics of a proteins to reflect a particular phenotype, is practically a well-known dogma, yet the authors make statements that seems as this was a novel finding of the study. In fact, the learning model used by the authors draw correlations that any other method would, without advancing significantly what other methods could provide. While it is important that this data is left in the manuscript, the authors should also tone down their conclusion in this area.

Response

We modified the description of the approach and the conclusion.

3. The authors have chosen a few standard measurements like RSMD, rg, and RMSF to evaluate the impact of sequence variation. However, in a molecular dynamics simulation, many additional measurements can be derived. It would have been important to know what parameters, that can be measured are not only positively but also negatively correlated. In other words, while the authors did not used other measurements? Nothing else in the MD was useful besides these values? There is a paucity of molecular mechanic calculations that could have served to further characterized the mutations (eg: frustration index, time-dependent bonding patterns, etc). In summary, readers would like to know more as why these simple measurements and not others were chosen.

Response

We calculated RMSF values (Fig. 5 in the revised manuscript) and attention scores (Fig. 8 in the revised manuscript) for each phenotypic gp120 at the equivalent residue position and identified gp120 variants (mainly in the V1, L1, V2, V3, the head of V4, and V5 loop) associated with the HIV neutralization phenotype in the revised manuscript.

4. The manuscript can be significantly shorten by removing unnecessary explanations in the results section by focusing exclusively on the description of the data and leaving speculations and conjectures for the discussion.

Illustrations: The illustrations are substandard. The graphs are almost unreadable. The authors are encouraging not to use the graphs as they are generated by their pipelines, because they are not publication ready. The authors should replot the values in a manner that is clear to read with large fonts and clear scales. Differences must be clearly outline in the graph.

Response

We have re-plotted RMSD (Fig. 2), Rg (Fig. 3), FEL (Fig. 4), and RMSF (Fig. 5) in the revised manuscript to show the difference between different HIV neutralization phenotypes.

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Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning

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Abstract

HIV exploits the sequence variation and structural dynamics of the envelope glycoprotein gp120 to evade the immune attack of neutralization antibodies, contributing to various HIV neutralization phenotypes. Although the HIV neutralization phenotype has been experimentally characterized, roles of rapid sequence variability and significant structural dynamics of gp120 are not well understood. Here, 45 prefusion gp120 from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotype are structurally modeled by homology modeling and investigated by molecular dynamics simulations and graph machine learning. Our results show that the structural deviations, population distribution, and conformational flexibility of gp120 are related to the HIV neutralization phenotype. Per-residue dynamics indicate the relationship between the local regions, especially in the second structural regions with high flexibility may be responsible for HIV neutralization phenotypes. Moreover, a graph machine learning model with the attention mechanism was selected to explore inherent representation related to the classification of the HIV neutralization phenotypes, leading to further distinguish the strong related gp120 sequence variation together with structural dynamics in HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also ~~provides a methodological framework to~~ explores complex relationships between sequence, structure, and dynamics of protein by combining molecular dynamics simulations and machine learning.

KEYWORDS

Envelope glycoprotein gp120, HIV neutralization phenotype, sequence-structure-dynamics of protein, molecular dynamics simulation, graph machine learning

1. INTRODUCTION

The entry of HIV into target cells is mediated by the molecular interaction between the virus and the cell via the envelope glycoprotein gp120, which is the **only** virus-encoded protein exposed on the surface of the virion [1]. To avoid the attack of immune neutralization antibodies, gp120 undergoes rapid sequence variation and significant structural dynamics [2]. These features of gp120 pose major obstacles to the development of effective antiviral vaccines [3]. In terms of sequence variation, approximately half of the gp120 surface has more than 10% genetic variability to confuse the host immune system [4]. For structural dynamics, the prefusion gp120, which adopts a conformation of immune defense, has significant structural flexibility to mask the binding sites of the receptor and coreceptor [5]. The sequence variation and structural dynamics of gp120 make HIV present diverse adaptability under different physiological or experimental environments [6]. Therefore, in the environment of interest, the knowledge of sequence patterns that are associated with structural dynamics will inform our understanding of the molecular mechanism of HIV viral entry and immune escape to improve the development of antiviral drugs [7][8].

Based on the experimental assessments in a panel of genetically diverse HIV-positive plasma pools, HIV isolates are usually classified as neutralization 'tier' phenotype [6]. Current tiered categorization of the neutralization phenotype has suggested that HIV isolates exhibit a spectrum of neutralization phenotypes that can be divided into 1 to 3 tiers, corresponding to the sensitive, moderate, and resistant to neutralization antibodies, respectively.[9] Tier 1 is the most sensitive neutralization phenotype and often comes from laboratory-adapted strains without immune selection pressure. Although tier 1 was further classified into two subtypes, e.g. tier 1A and tier 1B, in some studies[9], it is feasible and efficient to simply use tier 1 in terms of neutralization sensitivity. Tier 2 exhibits a moderate neutralization phenotype and represents most circulating strains. Tier 3, isolated from the primary clinical sample, displays a least sensitive or most resistant neutralization phenotype.

Although the HIV neutralization phenotype has been experimentally characterized, the roles of sequence variability and structural dynamics of gp120 are not well understood. Our previous theoretical study of gp120 with the extremely sensitive and resistant neutralization preliminarily revealed the molecular mechanism of HIV neutralization phenotype by employing the molecular dynamics (MD) simulations [10]. It shows that the neutralization-sensitive gp120 from Tier 1 has higher global structural dynamics and richer conformational substates than the neutralization-resistant gp120 from Tier 3, promoting the neutralization-sensitive gp120 into a more flexible conformational state that interacts with the receptor and coreceptor. Although MD can provide an atomic exploration of protein conformational dynamics, it is still time-consuming and computationally expensive to perform a series of long-term simulations on GPU-accelerated clusters for protein with a usual size (approximately 500 amino acids). Moreover, the modern post-simulation trajectory analysis methods, such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), principal component analysis, and free energy landscape (FEL), only extract statistical information during the simulation process, and cannot provide a learnable and trainable model with predictive functions.

In recent years, machine learning (ML), especially deep learning, has gained attention from academia and industry because of its surprisingly great potential in bioinformatics applications such as protein structure modeling [11]. Deep learning algorithms exploit training data to discover potential patterns and build relational mapping models to make predictions. Some well-known deep learning algorithms, such as classic convolutional neural network (CNN), recurrent neural network (RNN), and other neural-network-

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based models, have been widely used in biological sequence and structure analysis [12]. It should be noted that using ML to help optimize MD simulations gradually becomes a new trend in molecular science research. Wang et al. combined MD simulations with ML to predict the impacts of mutations-induced variation of protein-ligand binding affinity [13]. Bignon et al. explored the complex combinatorial chemistry of tandem DNA lesions repair and more generally local multiple damaged sites of the utmost significance in radiation chemistry by using MD simulations and ML [14]. Degiacomi mined the protein conformational space by training a neural network based on the MD simulations to supplement the existing conformations of the protein [15]. However, there are few attempts to probe the relationship between the sequence variation and structural dynamics of protein by employing ML and MD simulations. Moreover, for non-Euclidean data such as proteins, the application of graph ML in MD simulations is rarely reported.

In this study, we combine MD simulations and graph ML to probe the roles of rapid sequence variability and significant structural dynamics of gp120 in the HIV neutralization phenotypes. Randomly selected from an experimental panel [9], 45 prefusion gp120 structural models from different HIV strains belonging to three tiers of sensitive, moderate, and resistant neutralization phenotype were built. Subsequently, these models were subjected to MD simulations followed by the comparative analysis of the structural deviations, conformational flexibility, and population distribution. In addition, a graph ML model with the attention mechanism was used to explore inherent representation related to the classification of the HIV neutralization phenotype, leading to further distinguish the strong related gp120 sequence variation together with structural dynamics in HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also provides a methodological framework to explore complex relationships between sequence, structure, and dynamics of protein by combining MD simulations and graph ML.

2. RESULTS

2.1. Structural architecture of the prefusion gp120

As shown in Figure 1A, the structural model of the prefusion gp120 is represented by the atomic coordinate of gp120 in the full-length crystal structure of HIV-1 prefusion envelope glycoprotein (PDB ID: 5FYK) [5]. The structural architecture of gp120 is composed of a relatively conserved functional core, a hydrophobic subdomain centered on the bridging sheet ($\beta 2$, $\beta 3$, $\beta 20$, and $\beta 21$), and five variable regions (V1–V5) located in the peripheral areas [16]. The hydrophobic subdomain from the stem of the V1/V2 region to the V3 loop plays a regulatory role in mediating the conformational dynamics of gp120 [17]. There are three layered structural regions above the hydrophobic subdomain [18]. The first layer is located between $\beta \bar{3}$ and $\beta 0$ and mainly contains $\alpha 0$; the second layer is located between $\beta 1$ and $\beta 5$ and mainly contains $\alpha 1$; the third layer is located between $\beta 7$ and $\beta 25$ and mainly contains $\alpha 5$.

In the peripheral region, the V1/V2 region presents different structural characteristics. There is a "Greek key" structural folding composed of four β -strands (βA – βD) in the V1/V2 region [19]. The variable loop V1 is located between βA and βB , V2 is located between βC and βD , and the connecting loop L1 is located between βB and βC . Extending from the V1/V2 region and the V3 loop, a 4-stranded β sheet, called the bridging sheet, has been considered as a regulatory switch for conformational transitions of gp120 [20].

Based on the experimental assessment of HIV neutralization phenotype with a broad range of genetic and geographic diversity [9], 45 gp120 structural models from different HIV strains with sensitive, moderate, and resistant neutralization phenotype were randomly selected and constructed by homology modeling,

respectively. As shown in Figure 1B, all gp120 models from three neutralization phenotypes exhibit similar structural architecture without some loops have different spatial orientations. These constructed structural models have been validated and used for MD simulations and structure-based ML.

2.2. Structural Deviations of gp120

To quantitatively evaluate the structural deviation of gp120 with different neutralization phenotypes during the simulations, the time evolution of backbone RMSD values (Figure 2) relative to the template structure (PDB ID: 5FYK) were calculated. For the neutralization-sensitive, moderate, and resistant gp120, different relaxation time (about 10, 5, and 3 ns, respectively) is required to reach the equilibrium stage of simulations. A longer relaxation time means that the neutralization-sensitive gp120 has higher structural dynamics, which makes it easier to be exposed and attacked by neutralization immune antibodies [21]. In contrast, the structure of neutralization-resistant gp120 is easier to achieve stability which can be generally considered as a neutralization-resistant conformation after long-term immune selection [22]. For neutralization-moderate gp120, the relaxation time in the middle matches its transition position in the neutralization phenotype of HIV.

After reaching a relatively stable RMSD equilibrium, the neutralization-sensitive, moderate, and resistant gp120 fluctuate in a similar structural region around the average RMSD values of 0.47, 0.44, and 0.42 nm, respectively. Average RMSD is positively correlated with the degree of neutralization phenotype, indicating that gp120 with a more neutralization sensitive phenotype has a higher structural deviation. Except for the subtle differences in the RMSD timing changes, the RMSD values of gp120 with different neutralization phenotypes are distributed in the range of about 0.3–0.7 nm, indicating that gp120 can cover sufficient structural deviations to maintains the basic viral infection function while showing different neutralization phenotypes.

2.3. Dynamics of the Hydrophobic Subdomain

A recent study has pointed out that the hydrophobic subdomain composed of the V1/V2 region, the V3 loop, and the bridging sheet plays a regulatory role in mediating the conformational dynamics of gp120, contributing to the neutralization phenotype of HIV [17]. Therefore, the Rg of this hydrophobic subdomain was calculated to quantitatively analyze the pressure of the surrounding environment on it.

The time evolution of the Rg of the hydrophobic subdomain was plotted in Figure 3. For all phenotypic gp120, the Rg of the hydrophobic subdomain shows a downward trend, which is due to the Brown attack of the surrounding molecules, especially solvent water. However, the hydrophobic subdomains of different phenotypic gp120 shrink to different degrees. For neutralization-sensitive, moderate, and resistant gp120, the minimum value of the Rg is about 2.58, 2.6, and 2.62 Å, respectively. It demonstrates that gp120 with a more neutralization-sensitive phenotype has a looser hydrophobic subdomain and is therefore easy to shrink by the surrounding environment. More neutralization-resistant gp120 has a more stable hydrophobic subdomain to maintain its stable conformation.

2.4. Conformational Population Distribution of gp120

Characterizing by the RMSD relative to the template structure and the Rg of the hydrophobic subdomain, the conformational population distribution of gp120 with different neutralization phenotypes can be evaluated from the perspective of energetics. Three FELs (Figure 4) were constructed by projecting MD

trajectories into subspace spanned by the RMSD and the Rg. All FELs present a funnel-like free energy surface with irregular and divergent edges. The gp120 which has a more neutralization-sensitive phenotype occupies a larger FEL range and has more complex edges, indicating that it exhibits more conformational flexibility. In contrast, neutralization-moderate or resistant gp120 has a relatively concentrated FEL population distribution.

For the neutralization-sensitive gp120, there is a dominant free energy basin and two other metastable conformational distribution regions (in purple, free energy below about -12 kJ/mol). Although the neutralization-moderate gp120 has one main and two attached metastable regions, their areas are smaller than that of the neutralization-sensitive gp120, especially for the metastable regions. The neutralization-resistant gp120 has only one dominant free energy basin. The shrinking of the global minimum free energy region and the reduction of metastable regions indicate that the conformational population distribution of gp120 is positively correlated with the HIV neutralization phenotype.

2.5. Conformational Flexibility of gp120

The RMSF value (Figure 5) of the per-residue C α atom at structurally equivalent residue positions was calculated to characterize the conformational flexibility of each gp120. All RMSF profiles among the three phenotypic gp120 have similar features. Regular secondary structure regions have lower RMSF values, while the N-/C-termini and surface-exposed loops show higher RMSF values. Especially in the V1/V2 region, there is a typical conformational flexibility distribution despite undergoing significant residue mutations, deletions, or insertions. Separated by the β A-D, relatively large RMSF values can be observed in the V1 loop (between β A and β B), the L1 loop (between β B and β C), and the V2 loop (between β C and β D).

However, the conformational flexibility of subregions seems to have different roles among the three phenotypic gp120. As the neutralization phenotype becomes more resistant, both α 1 and α 2 show a trend toward lower RMSF values. This indicates that increasing conformational rigidity of the peripheral regular secondary structure may be one of the strategies to enhance the neutralization resistance. The V3 loop exhibits higher conformational flexibility in some neutralization-sensitive gp120, but it is significantly inhibited in neutralization-moderate and resistant gp120, indicating that the V3 loop perturbs the hydrophobic core to make gp120 more likely to be exposed to immune recognition. As a regulatory switch for conformational transitions of gp120[20], the β 20- β 21 hairpin has a slightly higher RMSF value in the sensitive gp120, implying that forcing gp120 to transfer to various conformations may provide an opportunity to eliminate the virus.

To show the RMSF difference between different gp120 with sensitive, moderate, and resistant neutralization phenotypes, we calculated RMSF values for each phenotypic gp120 at the equivalent residue position. Then, the mean RMSF of gp120 with three neutralization phenotypes was compared (Figure 5, bottom subplot). The regions with significant differences in the RMSF mean values are mainly concentrated in the V1, L1, V2, V3, the head of V4, and V5 loop, indicating that the sequence variation of gp120 in these loops has a direct effect on its structural dynamics.

2.6. Building datasets of three phenotypic gp120

According to the characteristics of the protein data, different data preprocessing methods were used to process the sequence, structure, and MD simulation data of the three phenotypic gp120 resulting in five datasets (Table 1). Using natural language processing methods as a reference, the amino acid sequence of

three phenotypic gp120 is regarded as a one-hot coding sequence (Table 1 Datasets Seq) for classification training [23]. For the structural datasets (Table 1 Datasets Stru and Snap), a graph-based model is introduced by transferring the structure of gp120 into the graph data. In the Stru dataset, only the gp120 models constructed by homology modeling were used. For MD simulations, 15, 30, and 60 dynamic structural snapshots were extracted from the 30-ns MD simulation trajectory of each gp120 at different time intervals (2, 1, and 0.5 ns), respectively, to construct the dynamic graph (Table 1 Datasets Snap_2, _1, and _0.5).

Table 1: Datasets of three phenotypic gp120

Datasets	Amounts	Total	Data Model
Seq	3*15*1	45	Sequence
Stru	3*15*1	45	Static graph
Snap_2	3*15*15	675	Dynamic graph
Snap_1	3*15*30	1350	Dynamic graph
Snap_0.5	3*15*60	2700	Dynamic graph

2.7. Selecting machine learning models for the classification of three phenotypic gp120

To choose a suitable model to predict the classification of three HIV neutralization phenotypes, we tested several ML models on different datasets (Figure 6). For the Datasets Seq, the Gated Recurrent Unit (GRU) [24] is trained to learn the contextual representation of the gp120 sequences by maximizing the conditional probability. After transforming the gp120 structures into graphs, two graph ML models, i.e. Graph Convolution Network (GCN) [25] and Graph Isomorphism Network (GIN) [26], were employed to handle the structure-based graph data model. Taking into account the three performance indicators of Accuracy, AUC, and F1 (Table 2), the performance of the GCN model is significantly better than the GRU but slightly worse than the GIN. It reveals that graph ML models (GCN and GIN) can effectively extract complex spatial features from protein structures to obtain better prediction performance in the classification of three HIV neutralization phenotypes. It should be noted that the lower model performance indicators may be because the number of training data (45 sequences or structures) is too small to learn enough classification features for the ML model. However, the comparison of ML models based on sequence or structure provides direction guidance for us to choose the graph ML models as the following training ML model.

Table 2: Performance of the sequence and graph machine learning models training on sequence and structure databases for the classification of three phenotypic gp120.

Models	Datasets	Accuracy	AUC	F1
GRU	Seq	0.480±0.194	0.587±0.124	0.304±0.175
GCN	Stru	0.575±0.117	0.595±0.105	0.304±0.170
GIN	Stru	0.635±0.130	0.577±0.185	0.352±0.271

2.8. Comparison of different gp120 dynamics information in GIN

After testing and comparing different ML models for the classification of three phenotypic gp120, we chose GIN as the basic model. Unlike previous learning on static structures of gp120 (dataset Stru), dynamic structural snapshots (datasets Snap_2, Snap_1, and Snap_0.5) extracted from MD simulations at different time intervals (2.0, 1.0, and 0.5 ns) are used in the GIN model to explore the contribution of dynamic information to the classification of three phenotypic gp120. It can be seen from Table 3 that the performance of the GIN model has been greatly improved after introducing dynamics information through MD simulations. The increase in training data directly leads to a significant improvement in the performance of the ML model. This shows that using MD simulations to expand the difficult-to-obtain protein structure data is a strategy that can be applied to ML training. As the sampling interval decreases, the accuracy, AUC, and F1 of the GIN model are getting higher and higher, indicating that it is beneficial to expand training data with a smaller sampling interval through MD simulations to exploit more dynamic conformational information of gp120. However, the ML model performance does not continue to increase as the time interval decreases. It is conceivable that a smaller time interval will not further improve the performance of the ML model.

Table 3: Performance of the graph machine learning model (GIN) training on static structures of gp120 and dynamic structural snapshots at different time intervals for the classification of three phenotypic gp120.

Datasets	Model	Time intervals	Accuracy	AUC	F1
Stru	GIN	-	0.635±0.130	0.577±0.185	0.352±0.271
Snap_2	GIN	2.0 ns	0.732±0.041	0.744±0.085	0.503±0.128
Snap_1	GIN	1.0 ns	0.785±0.032	0.798±0.056	0.587±0.123
Snap_0.5	GIN	0.5 ns	0.822±0.018	0.840±0.097	0.715±0.114

2.9. Comparison of different protein features in GIN

In graph ML, nodes use one-hot encoding and node embedding to introduce their features into the graph model. In the case of the protein graph model, one-hot encoding uses only an N-bit status register to encode 20 amino acids, while node embedding employs a high-dimensional vector to store physical, chemical, and other properties for each amino acid. In this paper, one-hot encoding maps 20 natural amino acid names into integer values and then converts the integer values into binary vectors. For the node embedding, 14 physicochemical properties (e.g. charge, hydropathy, polarity, volume, etc. in Table S1) of different amino acids and the 3D coordinates of the C_α atom for each residue were transformed into the feature vectors of nodes to enrich the characteristics of nodes in the graph model.

As shown in Table 4, two protein features were used to train the graph ML model on dataset Snap_0.5 by using the GIN model. Compared with the method of one-hot encoding, the method of node embedded achieved better performance. The prediction performance of the GIN model on dataset Snap_0.5 is improved by more than 10%. The results show that appropriate protein features can significantly improve the performance of the ML model.

Table 4: Performance of the graph machine learning model (GIN) training on dynamic structural snapshots at time interval 0.5 ns for the classification of three phenotypic gp120 with different protein features.

Datasets	Model	Features	Accuracy	AUC	F1
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Snap_0.5	GIN	One-hot encoding	0.822±0.018	0.840±0.097	0.715±0.114
Snap_0.5	GIN	Node embedded	0.971±0.003	0.986±0.010	0.934±0.036

2.10. Classification-related attention score from GIN with the attention mechanism

To analyze the classification-related information of the HIV neutralization phenotype, the graph ML model with an attention layer (GIN+ATT, Figure 7) was constructed by introducing the attention mechanism [27] into the basic GIN model. After aggregating node features into graph features in the GIN model, the attention layer was employed to distribute attention scores into different nodes along the gradient descent direction of the classification for the HIV neutralization phenotype. During the training process, the attention score of each node was automatically adjusted, leading to more attention to the important amino acid residues for the classification. Although the classification performance of the GIN+ATT model is weakened after adding the attention mechanism (Table 5), the attention score can indicate the residue or node that plays an important role in the classification. A small difference in classification performance may be a potential signal of the stronger generalization ability of the model. In addition, the attention score of each residue can be used as an indicator to determine the crucial mutations in various sequences of gp120 and to measure the biological contribution of these mutations to the HIV neutralization phenotype.

Table 5: Predictive performance of our graphic-ML model.

Dataset	Model	Accuracy	AUC	F1
Snap_0.5	GIN	0.971±0.003	0.986±0.010	0.934±0.036
Snap_0.5	GIN+ATT	0.941±0.012	0.983±0.007	0.930±0.026

Similar to other attention-based models, attention scores can capture local contributions related to classification in the graph ML model. The attention scores (Figure 8) of the per-residue C_{α} atom at structurally equivalent residue positions were calculated to evaluate the contribution of every residue in gp120 to the classification of the HIV neutralization phenotype. For all attention scores among the three phenotypic gp120, the N-termini, V1/V2 region, and V3 loop have larger scores, suggesting that these local regions play a vital role in identifying the HIV neutralization phenotype. The large attention score of the V1/V2 region and the V3 loop, together with the higher RMSF of these regions, indicate that high sequence variability and conformational flexibility are the molecular basis of the HIV neutralization phenotype. It should be noted that different attention score distributions and amplitude changes can further reveal more subtle residue contributions. For example in the V1/V2 region, larger attention scores locate at the V1 loop, β D, and β 3 for the neutralization-sensitive gp120 (Figure 8, red lines), β B and the L1 loop for the neutralization-moderate gp120 (Figure 8, green lines), and β C for the neutralization-resistant gp120 (Figure 8, blue lines). Compared to the neutralization-sensitive gp120, C-termini seems to contribute more to the classification of the HIV neutralization phenotype in the neutralization-moderate and resistant gp120 and thus shows a larger attention score. Similarly, the β 20- β 21 hairpin also has a more important classification contribution in the neutralization-resistant gp120. These results all indicate that the attention scores can provide a structured explanation of gp120 for the classification of the HIV neutralization phenotype.

3. DISCUSSION

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Establishing the relationship between sequence, structure, and function is the core issue in protein research. The sequence variation and structural dynamics of the envelope glycoprotein gp120 determine its function in viral infection, resulting in various HIV neutralization phenotypes [28]. Although MD simulations of gp120 with the extremely sensitive and resistant neutralization phenotypes preliminarily revealed the molecular mechanism of HIV neutralization phenotype [29], broad-spectrum theoretical studies of gp120 with different neutralization phenotypes are still missing. In this paper, 45 pre-fusion gp120 structural models from different HIV strains belonging to three tiers of sensitive, moderate, and resistant neutralization phenotype were built and subjected to MD simulations. The comparative analysis of the structural deviations, conformational flexibility, and population distribution together shows that the molecular dynamics properties of gp120 are positively correlated with the HIV neutralization phenotype.

The HIV envelope glycoprotein (Env) is expressed as a gp160 precursor that is subsequently cleaved into gp120 and gp41 heterodimers. Although the MD simulations in this paper focus on the gp120, the impact of this design on the results reported here is limited. Studies of sequence analysis and structural elucidation indicated that the gp120 has a highly variable surface, whereas the gp41 is more conserved. It should be noted that gp120 contains most of the sequence variation and structural dynamics of the Env. Considering gp41 is deeply buried in the phospholipid bilayer, gp120 contains the vast majority of sites associated with the HIV neutralization phenotype. Small-angle X-ray scattering data revealed that the gp120 free in solution closely resembles that of gp120 in the context of the Env [30]. It is reasonable to use gp120 extracted from the Env for deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype.

Together with the kinetic of gp120 in the CD4-complex and CD4-free state [29], CD4-binding effects on the conformational dynamics of gp120 [31][32], and conformational transitions of gp120 [33], these results in this paper provide a systematic description of the molecular dynamics of gp120. Although gp120 is a trimer under physiological conditions, it is still reasonable to use the gp120 subunit structure for research because small-angle X-ray scattering data revealed that the single gp120 domain in solution closely resembles that of the gp120 subunit in the context of the oligomeric viral spike/trimer [34]. In addition, the glycosylation effect was ignored in the MD simulations. Considering a single variable parameter (sequence variation) in the same experimental environment (prefusion gp120) can better focus on the purpose of research. Moreover, previous MD simulation studies on gp120 with glycosylated and non-glycosylated variable loops showed no significant differences in molecular fluctuations [35].

Although MD simulations can help explore detailed changes in the molecular structure of viral proteins, it is still impossible and unnecessary to perform long-term MD simulations for large-scale HIV strains. Moreover, modern post-simulation trajectory analysis methods (e.g. RMSD, RMSF, and FEL) only extract statistical information during the MD simulations to summarize the rules of molecular motion. In issues such as the sequence variation and structural dynamics of the envelope glycoprotein gp120 in the HIV neutralization phenotype, MD simulations are difficult to provide global statistical information to help distinguish the potential relationship between gp120 and the HIV neutralization phenotype. Therefore, using ML will be a good attempt to explore complex relationships between the sequence, structure, and dynamics of the protein. However, for the application of ML to protein research, there is a major obstacle that ML models require a lot of data (especially protein structure data that is difficult to obtain) for training. Many attempts solely on protein sequence data for ML training have been reported, but using protein structure data to construct ML models will capture more direct protein function information because the function of a protein is closely related to its structure. In this paper, MD simulations help expand the

difficult-to-obtain experimental data into protein datasets for ML training.

After testing different types of data sets (the sequence, structure, and dynamic structure) and ML models (GRU, GCN, and GIN), the dynamic graph representation of protein from the MD simulations were used to train the graph ML model (GIN and GIN+ATT) in this paper. Compared to protein sequences, the use of the structure graph of the protein can improve the performance of ML models. A large number of biological data including the protein is often non-Euclidean form, which has complex topological structures and contains multiple features. GCN and GIN have an excellent performance in processing these data by following the neighborhood aggregation scheme and calculating the representation vector of nodes by recursively aggregating and transforming the representation vector of adjacent nodes. Thanks to the increase of training data and the supplement of dynamic information, the introduction of dynamic graphs significantly improves the performance of ML. In this paper, the advantages of MD simulations and graph ML have been cleverly utilized to decipher the role of envelope glycoprotein gp120 in HIV neutralization phenotype from two aspects of the sequence variation and structural dynamics. It is conceivable that this method that utilizes ML models, especially deep learning, to help analyze calculated data, such as MD simulations holds great research potential.

Although ML models are commonly used for prediction tasks, the trained graph ML model in this paper was applied to map the sequence, structure, and dynamics of gp120 to the HIV neutralization phenotypes. Given the scarcity of biological data and the difficult accessibility of protein dynamics data, this graph ML model operates on datasets that lack diversity, leading to the potential for overfitting. In machine learning, overfitting usually indicates that the model does not generalize well and should be avoided. However, this study emphasizes that overfitting pays attention to mining the biological information contained in the training data.

4. MATERIALS AND METHODS

4.1. Sequence selection and alignment

All HIV sequences were obtained from the UniProtKB database (<http://www.uniprot.org>) with the accession IDs listed in Table 6. By removing the segments corresponding to the signal peptide and gp41, the primary sequence of gp120 was extracted and used as the target to construct structural models of gp120 with different neutralization phenotypes.

Table 6 45 gp120 from different HIV isolates belong to sensitive, moderate, and resistant neutralization phenotype

	HIV neutralization phenotype		
	sensitive	moderate	resistant
Isolate Number	A1EAH8 A1EAI4 A4ZPW4	A1EAI0 A1EAI2 A1EAI7	A0MTL0 A1EAG8 A1EAH3
	A8CVW4 B2YFQ5 B2YFU9	A7KVV7 B0FBI6 B2YFT0	A1EAH6 A1EAH7 A4ZPW7
	O89960 P04578 Q27Q69	Q202K1 Q202K7 Q27Q74	A4ZPW8 A4ZPX1 B2YFP1
	Q2MKA8 Q4QXE8 Q5G5V4	Q5G5U5 Q5G5U7 Q5G5V1	B2YFP6 B2YFS0 B2YFU4
	Q5G5V8 Q6EG58 Q70014	Q5G5V5 Q6TCV7 Q8JDI3	B2YFV4 Q5G5U6 Q5G5V2

4.2. Structural model construction and validation

The atomic coordinate of gp120 in the full-length crystal structure of HIV prefusion envelope

glycoprotein (PDB ID: 5FYK) at 3.11 Å resolution [5] in the RCSB PDB database (<https://www.pdbus.org>) was used as the template to construct the structural models. The high sequence identity (higher than 66%, Table S1) between each selected gp120 sequence and the template ensures the reliability of homology modeling, which is implemented in the MODELLER [36] (version 9.17) package. For each structural model of gp120, 20 candidates were generated and the one with the lowest molecular probability density function score was selected. All models were validated using the SAVES server (<https://services.mbi.ucla.edu/SAVES>), in which algorithms of ERRAT [37], PROVE [38], and VERIFY3D [39] suggest that the constructed model and the template have a similar structural quality score (Table S1) which demonstrated that the gp120 structural models have good stereochemical quality. To obtain structurally equivalent positions, all gp120 models were aligned by using the multiple sequence alignment tool MUSCLE [40] within the MEGA [41]. Regular secondary structural elements and variable regions were labeled according to the standard HIV envelope glycoprotein crystal structures (PDB IDs: 1G9M [42] and 3JWD [18]).

4.3. Molecular dynamics simulations

Each structural model of gp120 was individually solvated using TIP3P water molecules [43] in a dodecahedron box with a solute-wall minimum distance of 0.8 nm and a periodic boundary condition. Sodium and chloride ions were added to obtain a 150-mM salt concentration. All simulations were performed by employing GROMACS (version 5.1.4) software [44] with the AMBER99SB-ILDN49 force field [45] at the GPU-accelerated clusters. After energy minimization with steepest descent algorithm and position-restrained simulations with decreasing harmonic positional restraint force constants on the heavy atoms of the protein, 30-ns production MD run for each gp120 was performed with the following protocol: integration time was set as 2 fs due to LINCS [46] algorithm was used to constrain bond lengths involving hydrogen atoms; the partial-mesh Ewald (PME) [47] method and a twin-range cut-off strategy were used to calculate the long-range electrostatic interactions and van der Waals interactions respectively; protein and non-protein components were independently coupled to a 300 K and 1 atm with an external bath; the system coordinates were saved every 2 ps.

The package MDTraj [48] was employed to calculate RMSD, Rg, and RMSF. For the RMSD and Rg values, the Kolmogorov-Smirnov test (K-S test) was applied (Table S2), observing that these values have significantly different distributions among three phenotypic gp120. FELs were constructed by $F(s) = -k_B T \ln(N_i/N_{max})$, where k_B is Boltzmann's constant, T is the simulation temperature, N_i is the population of bin i and N_{max} is the population of the most populated bin.

4.4. Datasets construction

The gp120 sequence data adopted one-hot encoding and filled with the longest sequence length of 500. For the gp120 structure, a graph-based model was introduced by selecting the C $_{\alpha}$ atom of each residue as the node and connecting the edge if the distance of the pairwise C $_{\alpha}$ atom is less than 8 Å. For each residue, 14 amino acid properties (Table S32) and the 3D coordinates of the C $_{\alpha}$ atom for each residue were used as the feature vector of each node.

4.5. Machine learning

The GRU model is implemented by the Pytorch framework. The Deep Graph Library (DGL,

<https://www.dgl.ai>) was employed to train and validate graph ML models, i.e. GCN and GIN. When testing the above model, the scikit learn package (<https://scikit-learn.org>) is used to split data into train/test sets and k-fold ($k = 10$) cross-validation training strategy. All models were trained for 100 epochs, and the learning rate was set to 0.001.

In the GIN+ATT model, the hidden representation H_v ($H_v=[h_1, h_2, \dots, h_n]$, where n is the number of nodes and w is the learnable weight) of all nodes obtained from the last layers of the GIN model were used to construct the attention mechanism by using the following calculation:

$$M = \text{Relu}(H_v), \quad (1.1)$$

$$\alpha = \text{softmax}(w^T M), \quad (1.2)$$

$$H'_v = H_v \alpha^T. \quad (1.3)$$

The α of formula 1.2 is the attention vector. Through the calculation of formula 1.3, the graph node representation H'_v adjusted by attention weight is obtained. As the first part of the model, the GIN layer is used to extract the potential spatial features of each node. The feature vectors of nodes from the last layers of the GIN model were passed through the attention layer to aggregate as graph representation vectors. The attention layer is implemented by the trainable parameter module of Pytorch.

To measure the performance of the model, three different evaluation indicators, i.e. the accuracy, the area under the ROC curve (AUC), and the F1 score, were used. The calculation method can be obtained from the confusion matrix (Table 7) and formula 2.1.

Table 7: The confusion matrix

Forecast \ Factual	0	1
0	TN	FN
1	FP	TP

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FN + FP} \quad (2.1)$$

ROC curves were plotted with the true positive rate (TPR) and the false positive rate (FPR) as abscissa and ordinate respectively. The definitions are as formula 2.2-2.3.

$$\text{TPR} = \frac{TP}{TP + FN} \quad (2.2)$$

$$\text{FPR} = \frac{FP}{TN + FP} \quad (2.3)$$

The area under the ROC curve (AUC) can help to comprehensively measure the performance of the model. F1 score is the harmonic average of recall (TPR) and precision (P), and its calculation method is shown in formula 2.4-2.5.

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$$P = \frac{TP}{TP + FP} \tag{2.4}$$

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$$\frac{2}{F} = \frac{1}{P} + \frac{1}{TPR} \tag{2.5}$$

5. CONCLUSION

In this study, 45 prefusion gp120 structural models from different HIV strains belong to three tiers of sensitive, moderate, and resistant neutralization phenotypes were built and investigated by MD simulations and graph ML to probe the roles of rapid sequence variability and significant structural dynamics of gp120 in the HIV neutralizing phenotype. By integrating homology modeling, MD simulations, and graph ML, we constructed a reliable model for HIV neutralization phenotype. Statistical analysis of gp120 dynamics (e.g. RMSF) and classification-related attention scores together revealed potential structural factors that may affect HIV neutralization phenotype. Our study not only deciphers gp120 sequence variation and structural dynamics in the HIV neutralization phenotype but also ~~provides a methodological framework to~~ explores complex relationships between the sequence, structure, and dynamics of the protein by combining MD simulations and ML.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The source data (sequence files, structural models, C_α trajectories, RMSD, and RMSF data) and scripts are available at https://github.com/liyigerry/gp120_md_gml.

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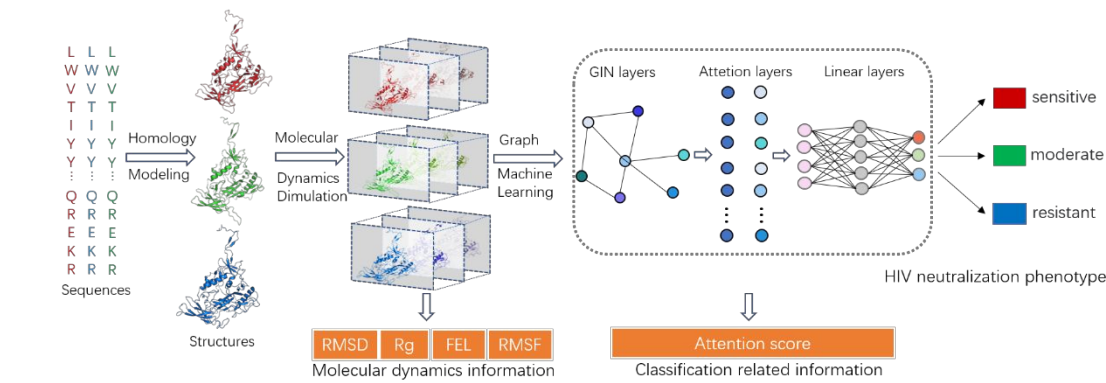
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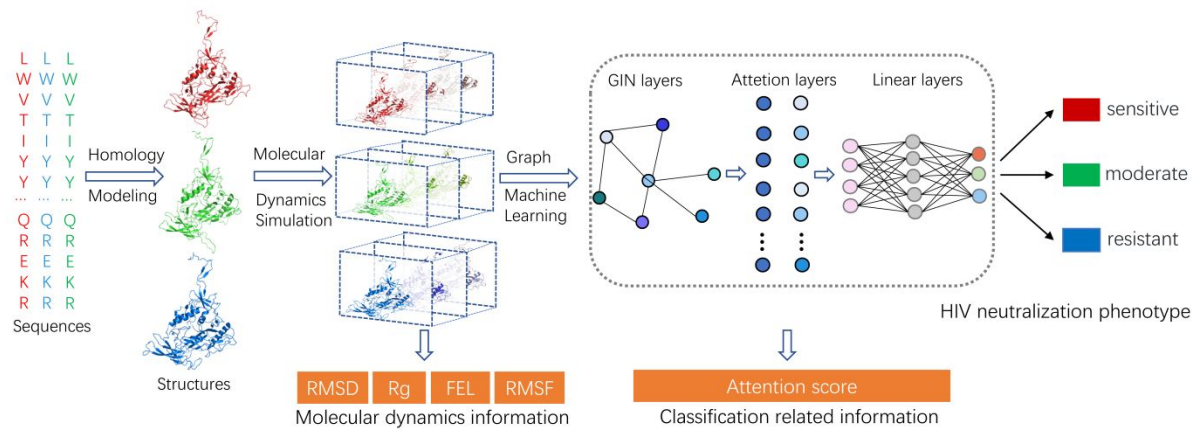
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Revised



Graphic abstract

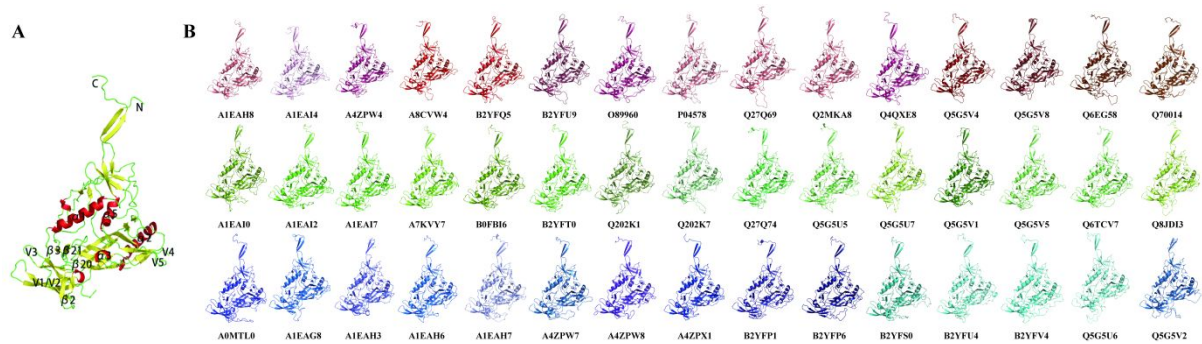
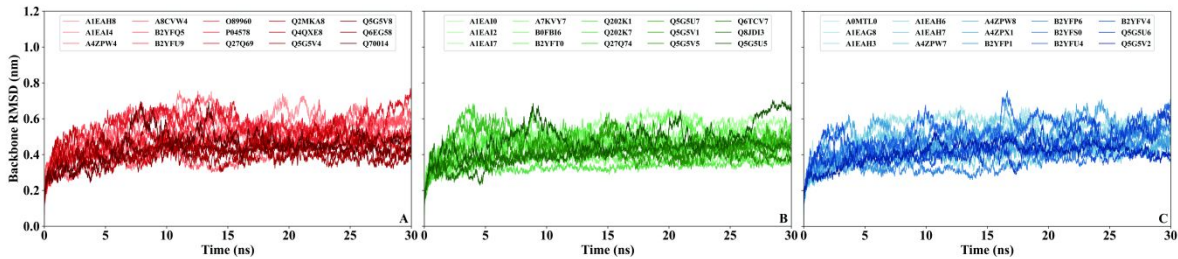


Fig. 1. The structural architecture of gp120. (A) Structure of prefusion gp120 (PDB ID: 5FYK). (B) Structural models of the neutralization-sensitive (reds), moderate (greens), and resistant (blues) gp120 from different HIV isolates with the accession ID of UniProtKB database.



Revised

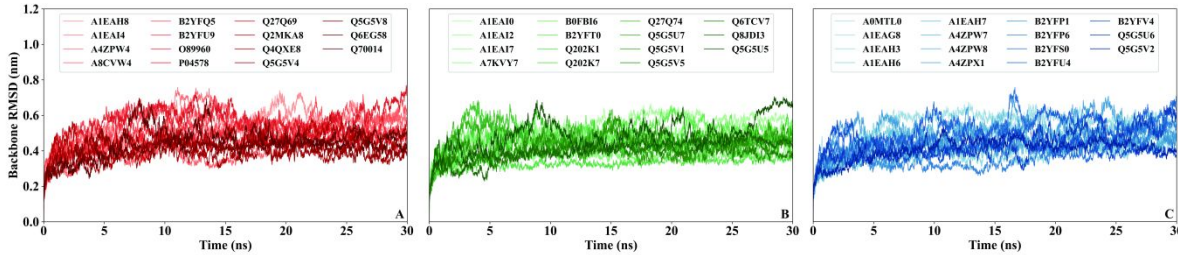
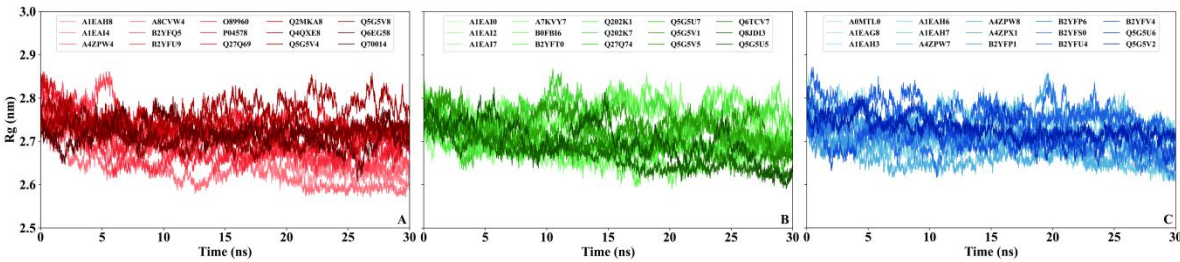


Fig. 2. Time evolution of backbone root-mean-square deviation (RMSD) values of the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 relative to the template structure (PDB ID: 5FYK) during the molecular dynamics simulations.



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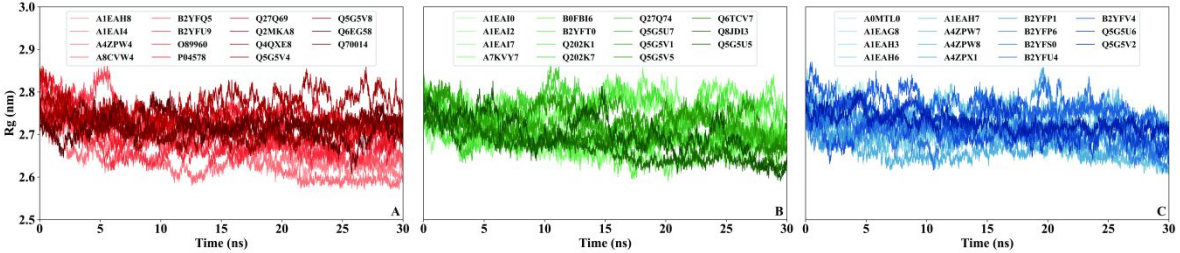


Fig. 3. Time evolution of radius gyration (Rg) of the hydrophobic core in the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 during the molecular dynamics simulations.

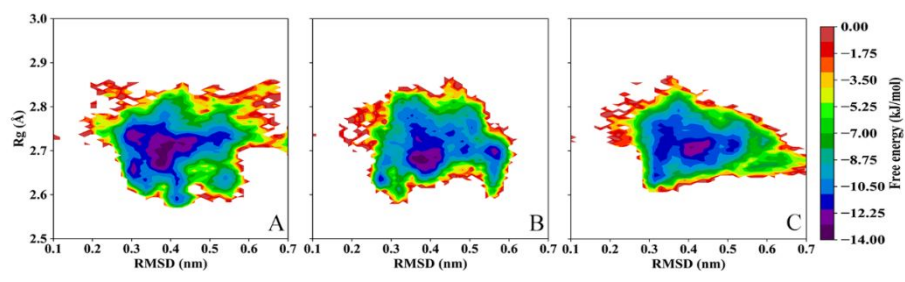
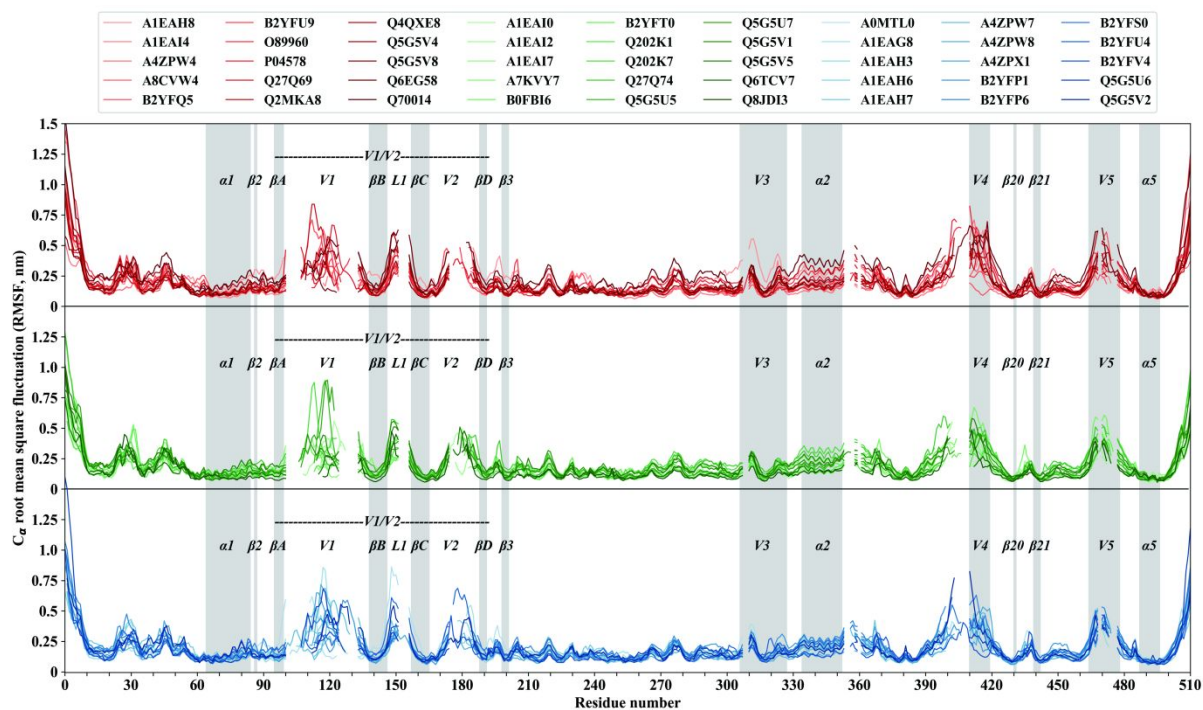


Fig. 4. Free energy landscapes of the neutralization-sensitive (A), moderate (B), and resistant (C) gp120 by projecting trajectories into the subspace of root-mean-square deviation (RMSD) relative to the template structure and the radius gyration (Rg) of the hydrophobic subdomain.



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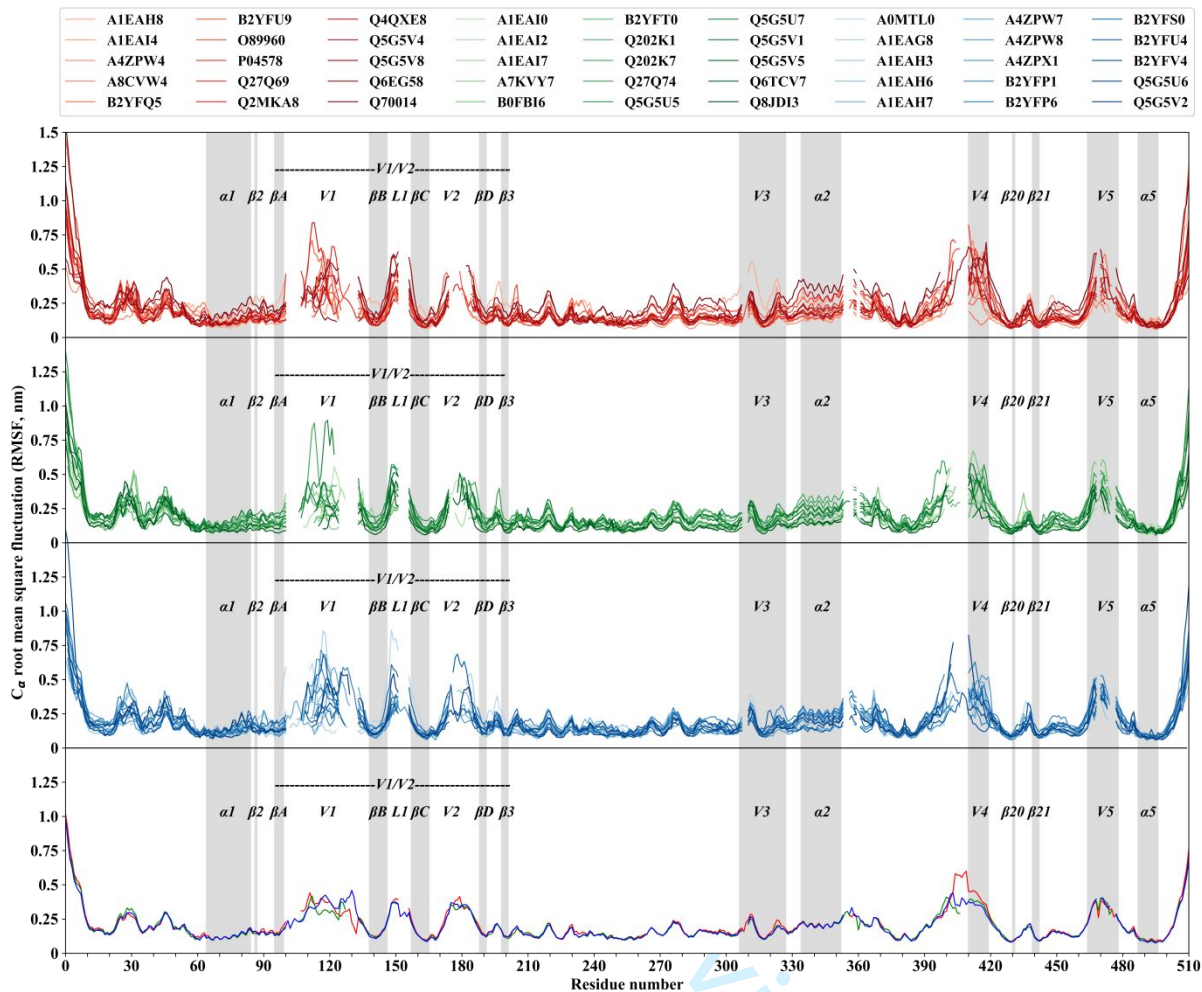


Fig. 5. C_{α} root-mean-square fluctuation (RMSF) values of the neutralization-sensitive (red lines), moderate (green lines), and resistant (blue lines) gp120 during the molecular dynamics simulations. The RMSF mean values for each phenotypic gp120 at the equivalent residue position were calculated and plotted in the same color in the bottom subplot.

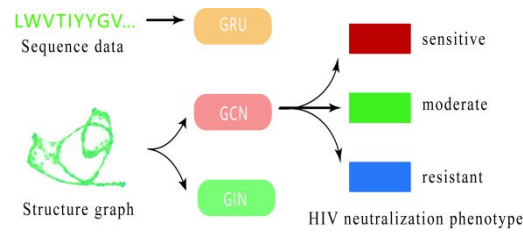


Fig. 6. The Gated Recurrent Unit (GRU) was used to handle the sequence data. The Graph Convolution Network (GCN) and Graph Isomorphism Network (GIN) were trained by the structure graph. All ML models were used for the classification of HIV neutralization phenotypes.

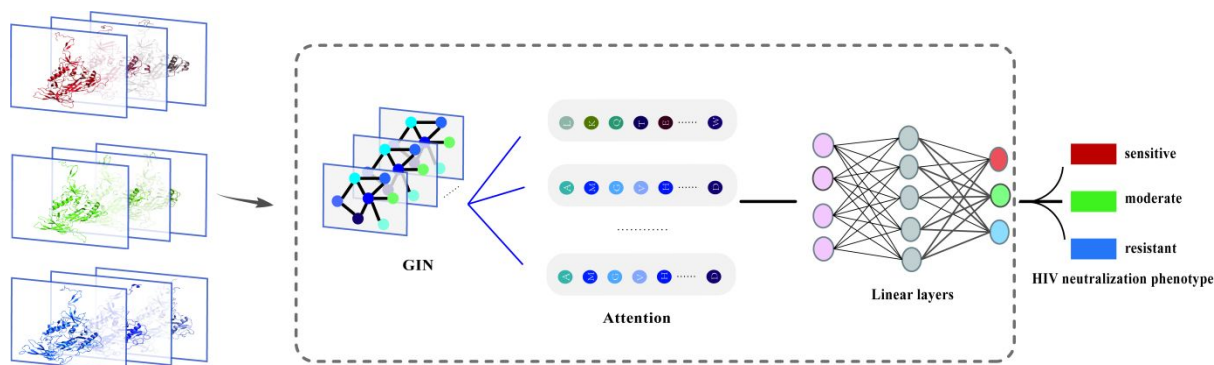


Fig. 7. Diagram of the Graph Isomorphism Network (GIN) with the attention mechanism (GIN+ATT).

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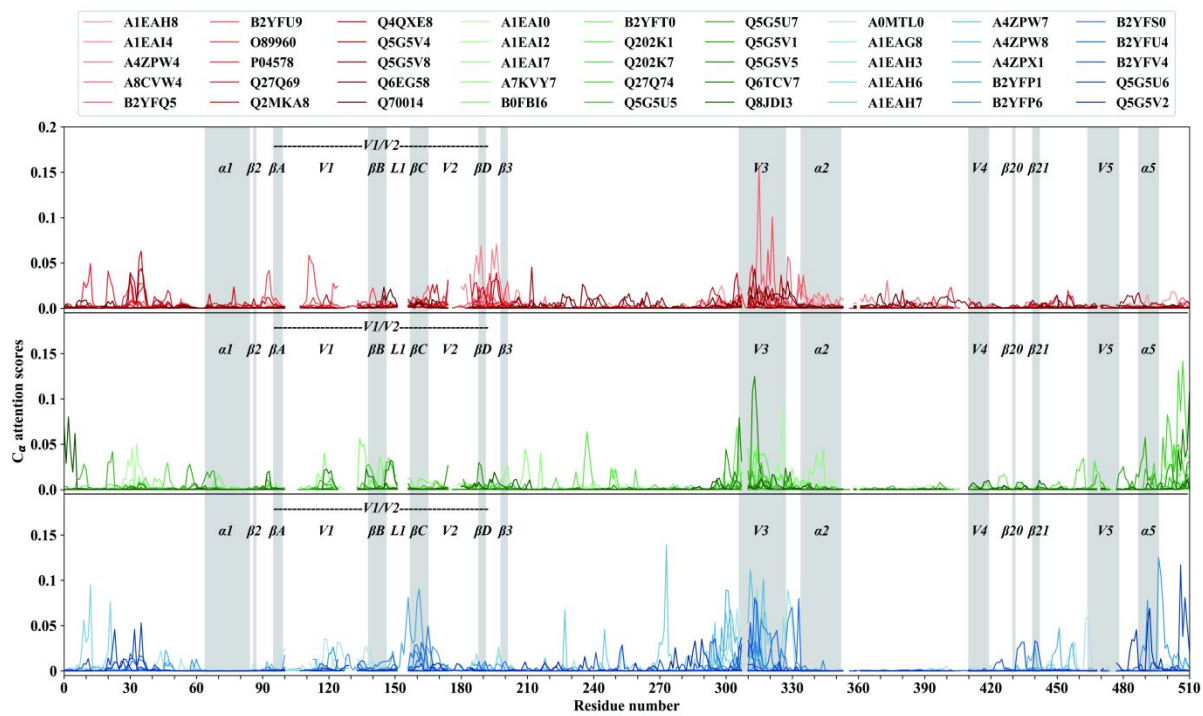


Fig. 8. C_α attention scores of the neutralization-sensitive (reds), moderate (greens), and resistant (blues) gp120.

Supporting Information

Table S1: 14 physicochemical properties of different amino acids.

Amino acid	alpha	beta	charge	core	hydropathy	pH	polarity	rim	surface	turn	volume	strength	disorder	high_contact
A	1.29	0.9	0	0.049	1.8	0	0	0.047	0.065	0.78	67	0	0	1
C	1.11	0.74	0	0.02	2.5	-2	0	0.015	0.015	0.8	86	1	-1	0
D	1.04	0.72	-1	0.051	-3.5	-2	1	0.071	0.074	1.41	91	0	1	1
E	1.44	0.75	-1	0.051	-3.5	-2	1	0.094	0.089	1	109	0	1	0
F	1.07	1.32	0	0.051	2.8	0	0	0.021	0.029	0.58	135	1	-1	0
G	0.56	0.92	0	0.06	-0.4	0	0	0.071	0.07	1.64	48	0	1	1
H	1.22	1.08	0	0.034	-3.2	1	1	0.022	0.025	0.69	118	0	-1	0
I	0.97	1.45	0	0.047	4.5	0	0	0.032	0.035	0.51	124	1	-1	0
K	1.23	0.77	1	0.05	-3.9	2	1	0.105	0.08	0.96	135	0	1	0
L	1.3	1.02	0	0.078	3.8	0	0	0.052	0.063	0.59	124	1	-1	1
M	1.47	0.97	0	0.027	1.9	0	0	0.017	0.016	0.39	124	1	1	0
N	0.9	0.76	0	0.058	-3.5	0	1	0.062	0.053	1.28	96	0	1	1
P	0.52	0.64	0	0.051	-1.6	0	0	0.052	0.054	1.91	90	0	1	0
Q	1.27	0.8	0	0.051	-3.5	1	1	0.053	0.051	0.97	114	0	1	0
R	0.96	0.99	1	0.066	-4.5	2	1	0.068	0.059	0.88	148	0	1	1
S	0.82	0.95	0	0.057	-0.8	-1	1	0.072	0.071	1.33	73	0	1	1

Amino acid	alpha	beta	charge	core	hydropathy	pH	polarity	rim	surface	turn	volume	strength	disorder	high_contact
T	0.82	1.21	0	0.064	-0.7	-1	0	0.064	0.065	1.03	93	0	0	1
V	0.91	1.49	0	0.049	4.2	0	0	0.048	0.048	0.47	105	1	-1	0
W	0.99	1.14	0	0.022	-0.9	1	1	0.007	0.012	0.75	163	1	-1	0
Y	0.72	1.25	0	0.07	-1.3	-1	1	0.032	0.033	1.05	141	1	-1	1

This table is taken from https://github.com/mikessh/vdjtools/blob/master/src/main/resources/profile/aa_property_table.txt

Table S2 Kolmogorov-Smirnov test of RMSD and Rg of three phenotypic gp120.

	RMSD			Rg		
	sensitive	moderate	resistant	sensitive	moderate	resistant
sensitive	1	0	4.13e-237	1	1.46e-107	3.44e-204
moderate	0	1	1.22e-89	1.46e-107	1	6.06e-169
resistant	4.13e-237	1.22e-89	1	3.44e-204	6.06e-169	1

Table S3: Structural models of gp120.

	Isolate	Residues	Identity	RMSD ³	VERIFY3D	ERRAT ⁵	PROVE	MD&ML
	Number	1	2	(nm)	4		6	7
Templat e	5FYK_G	480	-	-	85.26%	84.7727	5.00%	No
Tier 1	A1EAH8	468	71.55%	0.684	83.33%	63.2690	8.20%	Yes
	A1EAI4	485	72.58%	0.67	84.71%	55.7235	7.00%	Yes
	A4ZPW4	470	69.10%	0.72	55.74%	58.658	8.90%	Yes
	A8CVW4	470	71.07%	0.697	77.87%	64.6421	6.30%	Yes
	B2YFQ5	477	72.26%	0.684	84.70%	59.1075	9.30%	Yes
	B2YFU9	481	68.32%	0.667	73.80%	57.3561	7.10%	Yes
	O89960	486	70.61%	0.706	81.89%	63.0252	5.80%	Yes
	P04578	478	67.08%	0.706	82.01%	59.743	6.20%	Yes
	Q27Q69	494	67.94%	0.653	84.41%	56.1728	7.20%	Yes
	Q2MKA8	476	70.00%	0.686	85.50%	68.4665	6.50%	Yes
	Q4QXE8	466	69.87%	0.691	83.48%	68.1223	6.40%	Yes
	Q5G5V4	473	69.83%	0.725	83.51%	67.0978	6.20%	Yes
	Q5G5V8	477	68.46%	0.677	87.42%	61.1940	7.80%	Yes
Tier 2	Q6EG58	488	68.16%	0.742	83.47%	59.1667	6.60%	Yes
	Q70014	469	71.34%	0.76	77.40%	57.7007	6.20%	Yes
	A1EAI0	483	69.53%	0.706	84.68%	63.2911	6.90%	Yes
	A1EAI2	489	71.84%	0.722	79.75%	58.6134	7.10%	Yes
	A1EAI7	463	71.76%	0.696	82.94%	58.6813	5.70%	Yes
	A7KVY7	469	68.27%	0.677	84.43%	59.9129	8.40%	Yes
	B0FBI6	475	69.48%	0.777	85.26%	61.9355	6.60%	Yes
	B2YFT0	486	69.96%	0.748	84.98%	58.9958	8.00%	Yes
	Q202K1	472	69.52%	0.679	77.97%	70.6897	7.20%	Yes
	Q202K7	470	71.07%	0.692	78.72%	61.1354	5.70%	Yes

	Q27Q74	471	71.04%	0.739	84.50%	61.1231	9.00%	Yes
	Q5G5U5	472	70.35%	0.67	76.06%	64.5022	7.00%	Yes
	Q5G5U7	478	68.05%	0.685	86.82%	55.8190	7.00%	Yes
	Q5G5V1	484	70.72%	0.723	85.33%	59.8739	6.10%	Yes
	Q5G5V5	476	69.48%	0.703	85.50%	63.4615	6.40%	Yes
	Q6TCV7	470	69.63%	0.674	86.81%	61.2554	6.40%	Yes
	Q8JDI3	468	72.41%	0.703	83.12%	63.0435	6.50%	Yes
Tier 3	A0MTL0	475	71.99%	0.699	77.47%	48.8223	8.90%	Yes
	A1EAG8	487	71.84%	0.656	83.37%	58.7866	6.50%	Yes
	A1EAH3	475	72.41%	0.667	81.47%	58.9247	6.40%	Yes
	A1EAH6	486	71.19%	0.772	82.72%	56.9328	7.40%	Yes
	A1EAH7	473	71.43%	0.695	90.27%	60.9914	7.40%	Yes
	A4ZPW7	490	67.61%	0.701	83.47%	59.5833	6.60%	Yes
	A4ZPW8	475	70.19%	0.716	78.32%	60.8137	5.50%	Yes
	A4ZPX1	478	67.63%	0.703	85.36%	64.8936	6.80%	Yes
	B2YFP1	487	68.65%	0.843	81.00%	55.9499	7.20%	Yes
	B2YFP6	472	68.68%	0.687	79.24%	63.6364	6.50%	Yes
	B2YFS0	478	69.55%	0.713	87.87%	66.3793	7.70%	Yes
	B2YFU4	482	67.89%	0.708	80.91%	57.5949	7.90%	Yes
	B2YFV4	493	68.61%	0.706	81.95%	55.3719	6.40%	Yes
	Q5G5U6	477	66.53%	0.721	80.71%	57.7827	5.80%	Yes
	Q5G5V2	487	68.64%	0.695	81.52%	66.9456	5.80%	Yes

1. The number of residues in gp120.
2. The identity between the target sequence and the sequence of the template (PDB ID: 5FYJ, chain G).
3. The root means square deviation (RMSD) to the template (PDB ID: 5FYJ, chain G).
4. The percentage of the residues have averaged 3D-1D score ≥ 0.2 calculated from the algorithm VERIFY3D.
5. Overall quality factor of the algorithm ERRAT.
6. The percentage of buried outlier protein atoms in the algorithm PROVE.

7. Whether the structural model is used for molecular dynamics (MD) simulations and machine learning (ML) training.

For Peer Review