Review

gp120 alters its conformation to enhance evasiveness and infectivity

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Abstract: Infection by human immunodeficiency virus type I (HIV-1) requires virus particle binding to host cell-surface receptor CD4 via the viral envelope glycoprotein gp120. HIV-1 therapy and prevention efforts involve development of mimetic or recombinant gp120 vaccines or deployment of antiviral agents that target specific epitopes of gp120. The unliganded conformational state of gp120 is closed, whereas the CD4-bound state is open. However, in between, there exist dynamic conformational states, indicating intrinsically flexible region(s) of structural dynamics, imposing a structural challenge for developing drug or antibody targets. Known conformational states of gp120 were determined by X-ray crystallographic and cryo-electron microscopy, and neither method captures the population of gp120 species arising from conformational plasticity, motions, and transitions. gp120 plasticity brings up several important questions. How will differences in conformation affect receptor binding, antibody recognition, and neutralization? Which regions are crucial for gp120 structural plasticity? How could structural dynamics influence HIV-1 evasiveness against host immunity and drugs or vaccines, and facilitate the viral entry into its host? This review explores the structural constraints presented by conformational states of the glycoprotein to antibodies or drugs and how these conformational states provide structural avenues for the virus to escape neutralizing agents and evade host immunity.

Keywords: Glycoprotein; gp120; HIV-1; conformation; immunity

1. Introduction

Human Immunodeficiency Virus (HIV) attacks the body's immune system. If untreated, infection usually leads to compromised immunity and establishes a condition known as acquired immunodeficiency syndrome (AIDS). AIDS is a public-health crisis that reached pandemic proportions in the early 1990s and still aggressively destroys lives. As early as March 1989, 145 countries had reported 142,000 AIDS cases, and the World Health Organization (WHO) estimated 400,000 cases of HIV infections worldwide **(Burgher, 1989)**. In the middle of the same year, the Centers for Disease Control released the first guidelines to prevent opportunistic infections that were major causes of death among people with AIDS **(CDC, 1989)**. AIDS cases in the USA reached 100,000 (1989) and a decade later (1999), AIDS became the fourth leading cause of death worldwide and number one killer in Africa, killing 14 million and infecting 33 million people **(WHO, 2009)**.

HIV-1 accounts for more than 42 million infections in the world and 1.2 million people in the U.S. are currently living with the disease; as of 2020, nearly 700,000 deaths occurred. However,

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these alarming figures are far less intimidating than a decade ago where annually, over 2.7 million people died globally of this disease. This positive outcome may be attributed to access to HIV prevention, diagnosis, treatment, and care both for the disease and for opportunistic infections [WHO, U.S. Statistics | HIV.gov1]. The regular use of antiretroviral therapy (ART) as promoted by WHO in HIV-1 management has drastically improved the life expectancy of persons living with the virus **(Oguntibeju, 2012)**; nonetheless, there is an urgent need for antiviral drugs that can quickly and safely eliminate HIV-1 and a need for vaccines to protect against infection.

Extensive scientific research has greatly expanded our grasp of HIV-1 transmission and pathogenesis dynamics to reveal the complexities associated with animal viruses. HIV-1 outwits the immune system during infection, then mounts continuous rounds of attacks on the system for many years until the immune system is compromised therefore bringing about the onset of AIDS **(Boasso et al., 2009)**. A critical step of HIV-1 infection involves the virus particle binding to the host cell-surface receptor CD4 through the viral envelope glycoprotein gp120. For this reason, the HIV-1 therapy and prevention efforts typically target the development of mimetic or recombinant gp120 as vaccines, or antiviral agents that target specific epitopes of gp120 **(Nyamweya et al., 2019)**. Furthermore, the host's humoral immunity targets gp120 for neutralization; hence an in-depth understanding of the mechanics involved in the gp120 functional and structural dynamics is valuable **(Zhu et al., 2019; Pancera et al., 2010)**. Antibodies or drugs inhibit the viral particle from infecting cells by either inactivating the virus directly via neutralization or indirectly through Fc-mediated effector functions **(Sherburn et al., 2021; Van Erp et al., 2019)**. Most neutralizing antibodies for HIV-1 bind to the virus and block CD4-gp120 interactions or CCR5-gp120 or CXCR4-gp120 interactions, thereby inhibiting infection **(Gilbert et al., 1993)**. Because this process must occur before virus-cell interaction, most vaccine and drug developers search for peptides, agents, or antibodies that possess a high affinity for one or more epitopes of either gp120, gp41 (another envelope glycoprotein noncovalently attached to gp120), or even to the decorating glycan covering of the surface molecules **(Kowalski et al., 1987)**. Conformational changes are critical processes when the virus binds to target host cell receptors and subsequent fusion **(Richard et al., 1987)**. Therefore, inhibiting this process is an important method of blocking infection.

Another key process in shaping the immune response is Fc-mediated antibody effector deployment, which plays an important role in active immune regulation. IgG antibodies bind to the membrane antigens of HIV-1 infected cells and then the antibody's Fc-part bind to Fcγ receptors, triggering complement-dependent cytotoxicity. Therefore, Fc-mediated effector functions rely on binding to antigens such as exposed epitopes of gp41 or gp120 after fusion **(Richard-Wyatt et al., 1998)**.

1.1. HIV-1 entry

HIV-1 infects target cells first by fusion of its membrane with the host cell, delivering its RNA genome into the cytoplasm of the cell. The RNA is reverse transcribed into to the viral DNA. This is followed by viral DNA integration into the host cell DNA. HIV-1 proteins are transcribed and translated using the host transcription and translational machinery. The virus then matures into new virus by assembling the involving components **(Myszka et al., 2000; Henrik et al., 1995; Sarah Howie et al., 1995; Bashir et al., 2020)**. Viral entry is facilitated by the binding of HIV-1 envelope glycoprotein subunit gp120 to the host CD4 receptor **(Ipinmoroti et al., 2020)**. HIV-1 specifically targets CD4 positive T-helper lymphocytes, macrophages, and dendritic cells **(Cavrois et al., 2019)**. As illustrated in Figure 1, HIV-1 uses a class I fusion protein composed of the glycoproteins gp120-gp41 trimeric complex to initiate entry and infect host cells, instigating a series of events **(Cavrois**

et al., 2019; Venanzi et al., 2019; Williams et al., 2013; Lifeng et al., 2011). The glycoprotein complex interacts with CD4+ recipient cells, and subsequently binds to the coreceptors CXCR4 or CCR5 **(Bashir et al., 2020)**. Alternatively, extracellular vesicles have been demonstrated to be potential routes for viral entry into uninfected cells **(Urbanelli et al., 2019; Rozmyslowicz et al., 2019; Mack et al., 2000)**. Published works concerning microparticles transfer of CXCR4 receptor to CXCR4-null cells have been demonstrated **(Mack et al., 2000)** and this expanded the understanding of the mechanisms and dynamics of HIV infection.

Membrane fusion possesses high kinetic barriers due to repulsive hydration forces **(Rand et al., 1984)**. HIV-1 employs a refolding process to generate free energy to overcome these kinetic barriers via refolding from a high-energy, metastable prefusion conformational state to a low-energy, stable post-fusion state when induced by the CD4 **(Harrison, 2015)**. As shown in Figure 1, the sequential binding of gp120 to CD4 (Figure 1 A–C) and a co-receptor such as CCR5 or CXCR4 (Figure 1 C–D) induces progressive conformational changes in both gp120 and in gp41 **(David et al., 1997)**. The gp41 glycoprotein morphs into a prefusion conformation leading to the cleavage of gp120-gp41 complex. The fusion peptide of gp41 then translocates and inserts into the target cell membrane (Figure 1C) producing a drastic refolding into a hairpin conformation to create a post-fusion conformation, which pulls the two membranes together. The formation of the fusion pore (Figure 1D) leads to release of the HIV-1 capsid into the cytoplasm of the cell. While this process is the canonical pathway of HIV-1 entry, the consequence of this process stimulates both virus-neutralizing and non-neutralizing antibodies that evade cell-aggravated immune response to invasion. Additionally, HIV-1 exploits a receptor-independent pathway involving interaction with cell-derived exosomes released in response to changes in cell physiology during infection. In the process, virus particles form complexes with the released vesicles and are taken up by bystander cells in a receptor-independent process called 'Trojan horse' **(David et al., 1997; Sims et al., 2017; Gu et al., 2017; Sims et al., 2018; Hildreth, 2017)**.

Specifically, the current paradigm of HIV-1 infection in the central nervous system (CNS) involves monocyte- and macrophage-mediated entry via the CD4/CCR5 receptor. Exosomes released from replicative-competent or defective HIV-1 cells can activate latent HIV-1 **(Arenaccio et al., 2015)**. More so, CD4 null cells can also be infected with HIV-1 and can act as active HIV-1 reservoirs. One proposed alternative infection pathway involves Trojan exosomes, in which HIV-1 viral particles are packaged into Trojan exosomes; and these been shown to cross the blood–brain barrier **(Urbanelli et al., 2019; Rozmyslowicz et al., 2003; Mack et al., 2000)**.

Beauparlant et al., 2017, analyzed functional envelope phenotypes associated with HIV-1 infection of cells expression low CD4, and found that the virus altered its entry kinetics, for which the gp120 spent longer time at the CD4-induced state and therefore increased sensitivity to neutralizing antibodies targeting the CD4 binding site and the V3 loop. They confirmed the same observation in macrophage-tropic HIV-1 isolates derived from the central nervous system (CNS) of infected individuals **(Beauparlant et al., 2017)**.

Our first set of HIV-1 entry experiments involved mouse neural stem cell (NSC)-derived exosomes and demonstrated that mouse NSC-derived exosomes enhanced HIV/YU-2 viral entry into human target cells. These studies demonstrated that exosomes enhance HIV-1 viral entry into multiple human cell types. In addition, we demonstrated that various human-derived exosomes increase the entry of virus and subsequently that this can be blocked with the addition of CD81 and CD9 antibodies, respectively. Tetraspanins, in particular CD9 and CD81, are found in extracellular vesicles such as exosomes. Thus, it is very plausible that these molecules may be involved in exosome-cellular interactions. Future structural studies, analyzing the interactions between exosomes and HIV-1 binding and interactions are needed to further elucidate these mechanism(s) **(Urbanelli et al., 2019; Rozmyslowicz et al., 2003; Mack et al., 2000)**.

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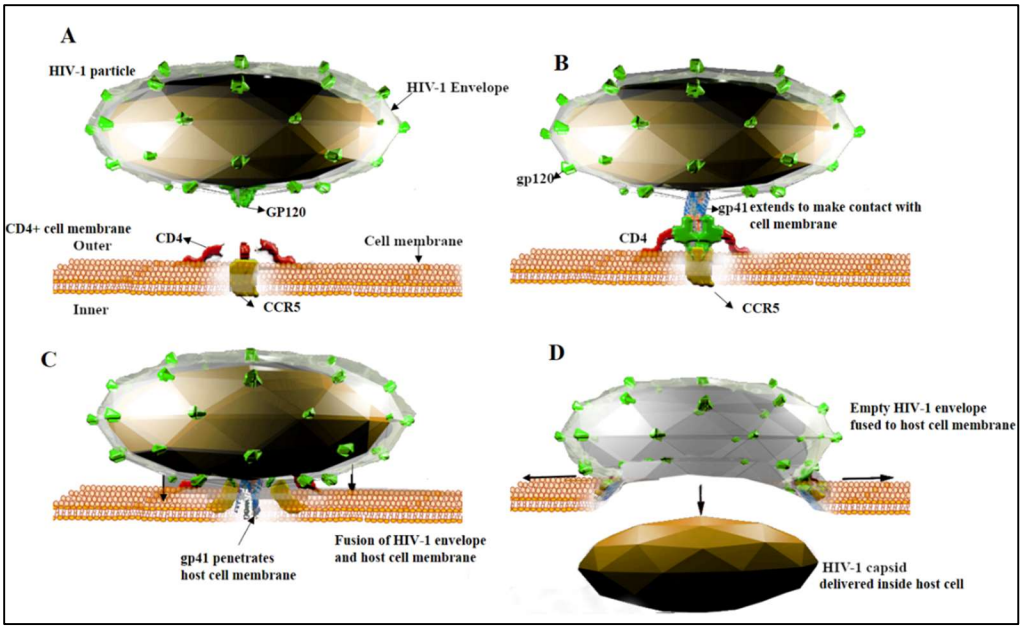

Figure 1. Cartooned model of attachment and entry of HIV-1 into the host cell (not drawn to scale, nor gives exact representation of numbers or sizes of structures displayed). (**A)** Before the interaction of gp120 and the CD4 receptor; (**B**) the interaction of gp120 (green) to its CD4 receptor (red) and the subsequent induction and binding of gp120 to the co-receptor (CCR5 or CXCR4) (light blue). (**C**) Several structural changes in HIV-1 Env leading to the cleavage of gp120-gp41 complex. Following cleavage, the fusion peptide gp41 (blue) inserts into the CD4+ cell membrane, which undergoes refolding to pull the two membranes together. (**D**) Finally, a fusion pore forms that enables the release of the viral capsid containing the genetic material into the host cell cytoplasm.

1.2. Structural composition of gp120-gp41 complex

The crystal structure and cryo-electron tomograms of the HIV-1 gp120-gp41 complex show two major structures: gp120 (the receptor-binding component) and gp41 (the envelope transmembrane subunit which mediates viral fusion to host cells). These two subunits are non-covalently linked heterodimers on the HIV-1 particle surface or protruding from the plasma membrane of HIV-1 infected cells **(Joyce et al., 2013; Kwong et al., 1998)**. gp120 has a globular structure composed of five conserved domains (C1-C5) and five variable loops (V1-V5) and is sectioned into three lower domains: a highly glycosylated outer domain, the inner domain, and the bridging sheet **(Starcich et al., 1986; Willey et al., 1986)**. The outer domain wraps over the inner domain like a sheath and undergoes major structural changes during prefusion; the outer domain also carries the CD4 receptor binding site **(Kwong et al., 1998)**. The conformational change during prefusion triggers a structural alteration in the gp120 outer domain to reveal the inner domain, which sits atop of and shields the gp41. Wang et al., 2020 demonstrated via smFRET that the conformational states of Env trimer affects Nab binding and neutralization **(Wang et al., 2020)**. Their work revealed that Env in State 1 is a pre-triggered metastable state, and upon receptor binding, this state is driven to other conformational states. They showed that the State 2A conformational state of the Env is recognized by antibodies produced against the Env epitoptes. To show the huge significance of Env conformation in vaccine development efforts, they investigated and proved the efficacy of CD4-mimetic compounds in providing protection for gp120-vaccinated monkeys from simian HIV infection **(Wang et al., 2020)**. In the CD4-complexed state, gp120 is known to morph into the three major domains as mentioned earlier **(Starcich et al., 1986; Willey et al., 1986)**. The inner domain is

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composed of two-helix, two-strand bundles, and a five-stranded β-sandwich. A stacked double-barrel consisting of a mixed directional β-sheet with an axis parallel to the axis of the inner domain forms the outer domain. Also, the double-barrel of the outer domain shares one contiguous hydrophobic core that continues from one barrel **(Kwong et al., 1998)**.

The inner domain residues interrelate with gp41 **(Pancera et al., 2010; Finzi et al., 2010)** and are connected via a seven-stranded β-sandwich. The virus' transmembrane subunit (gp41) is thought to be the most susceptible target for antibodies due to its high immunogenicity; however, it is shielded by layers of gp120 structures and an overwhelming glycan shield that covers the envelope protein **(Garg et al., 2011)**.

CD4 binds to gp120 via a groove formed by the outer and bridging sheet domains of gp120. It has been demonstrated that the most important CD4 binding region is the Phe43 cavity located at the connection of all three domains of gp120 and covered by Phe43 residue of CD4 **(Dey et al., 2007)**. The Phe43 cavity covers the hydrophobic core of gp120 and has abundant hydrophobic residues **(Olshevsky et al., 1990)**. Residues lining this cavity are conserved. The exact location of the cavity and its direct interaction with CD4 via the CD4 Phe43 residue to form a stable Phe43 cavity is a crucial component of CD4-induced gp120 conformation **(Kwong et al., 1998; Xiang et al., 2002)**. Korkut and Hendrickson demonstrated the structural plasticity and conformational transitions of the gp120 and showed that the CD4-bound state significantly alters its structure **(Korkut and Hendrickson, 2012)**. Additionally, Bohl et al. reported rearrangement in the inner and bridging-sheet domains of gp120 **(Emileh et al., 2011)**. Emileh and Abrams used all-atom simulations to demonstrate the role of the I109C/Q428C disulfide stitch in altering the conformational distribution of engineered HIV-1 gp120 core and how such alterations were relevant for binding of the broadly neutralizing recombinant antibody b12. They demonstrated that the disulfide stitch produced a conformational distribution favoring an unfolded inner domain α1-helix upon binding of b12 antibody. Furthermore, via molecular dynamics, they proved that folded α1 in the b12-bound conformation of gp120 is stable both with and without the stitch; however, when α1 is folded, the stitch required orientation of the β20/β21 sheet that was sterically incompatible with b12 binding. In the b12-bound state, the β2–β3 and β20–β21 strands of the bridging sheet detach from each other, and the α1 helix of the inner domain goes through a helix-to-coil transition **(Emileh et al., 2011)**. As shown in Figure 2, VRC01 is a broadly neutralizing antibody that binds to gp120 at the CD4 binding site through a conformationally less variant CD4-binding site on the outer domain. However, this conformational barrier can be broken and weakens the neutralization potency of CD4 binding site antibodies, such as VRC01 **(Zhou et al., 2010)**. Taken together, such conformational changes and structural rearrangements in gp120 demonstrate both global and local large-scale conformational transitions, which play important roles in both viral entry and resistance to neutralization by antibodies.

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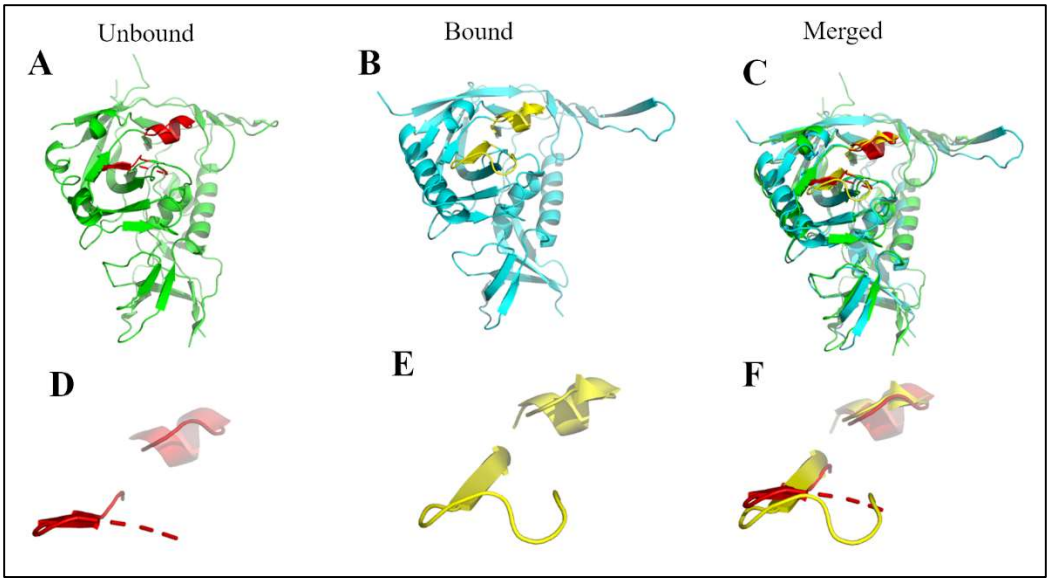

Figure 2. The binding sites of the neutralizing antibody VRC01 in gp120 (**A**) Unbound state (in red cartoon rendering; gp120 PDB ID: 3TIH); (**B**) in the bound state (in yellow cartoon rendering, gp120 PDB ID: 1G9M); (**C**) Representation of the merge between the unbound and bound states; (**D**) The representation of the VRC01 sites only in the unbound state; (**E**) The representation of the VRC01 sites only in the bound state; (**F**) The merged representation of the VRC01 sites only in unbound and bound states.

2. gp120 epitopes as drug or vaccine targets

2.1. Variable loops

Several sequence diversities in the gp120 molecule enhance its ability to hide from the immune system. This variability is partly due to the five variable loops (V1-V5) in the glycoprotein's sequence **(Peachman et al., 2015)**. The V1 domain in HIV-1 sequenced by Silver et al. demonstrated that their strain had 49 amino acids **(Silver et al., 2019)** and included two additional N-glycosylation sites and a pair of cysteines. They also showed that the V1 domain confers resistance to neutralization by monoclonal antibodies of the V3-glycan-dependent class, indicating the role of V1 regions in shielding HIV-1 from recognition by V3-directed broadly neutralizing antibodies **(Silver et al., 2019)**. Cardaci et al. reported a definite interaction between gp120 and Tat at the cell surface, which enhanced virus attachment and entry, and the V1 loop was implicated in this interaction. Using their gp120-mimicking peptide, CT319 in a competitive assay with gp120 for Tat binding, they demonstrated that the V1/V2 loop of gp120 was involved in Tat binding, hence functionally relevant for HIV-1 entry into host cells **(Cardaci et al., 2013)**.

The second variable loop (V2) has extensive sequence variability between HIV-1 strains; nonetheless, its structural role as trimer stabilizer **(Cimbro et al., 2014)**, functioning like a stapler, demands conservation in specific microdomain structural elements critical for functionality. The V2 loop is a conserved evolutionary element in all primate lentiviruses except for HIV-1, which carries variability in the loops sequence **(Bowder et al., 2018)**. Experiments conducted by Bowder et al. demonstrated that two cysteines in the V2 loop of SIVsm/HIV-2 lineage are critical for gp120 stability, and removal of the twin-cysteines decreases envelope trimer stability. They also demonstrated *in silico* that the twin-cysteines form a disulfide bond in the gp120 subunit, which interacts with the V1 loop to stabilize the envelope trimer **(Bohl et al., 2013)**. In HIV-1, the V2 loop enforces trimer stabilization and co-receptor binding **(Bowder et al., 2018)**. Analysis of plasma IgG anti-

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gp70V1V2 antibodies indicated an inverse correlation with risk for HIV-1 acquisition in a RV144 human vaccine efficacy trial **(Powell et al., 2020)**. The immunogenicity of this region also demands that the virus evolve to outwit the immune system; therefore, this loop is highly N-glycosylated **(Silver et al., 2019; O'Connell et al., 2014)**.

The third variable loop, V3, is essential for HIV-1 infectivity **(Albert et al., 1992; López de Victoria et al., 2012)**. Neutralizing antibodies targeting the V3 loop block cell fusion by inhibiting the initial gp120-CXCR4 interaction **(Ivanoff et al., 1992)**, in another study, it was shown that the removal of the V3 loop abolished gp120-CXCR4 interaction **(Mondor et al., 1998)**. The immunodominant V3 loop and its flanking regions possess homologous structural and sequence identity with one of the most studied proteins, the complementary determining region (CDR) of human immunoglobulins. CDR regions permit HIV-1 entry into the immune regulatory network, a mechanism targeted by HIV-1 vaccine development **(Metlas et al., 2004)**. Table 1 highlights some major studies and findings on the variable regions of gp120.

Table 1. Major studies on the variable regions of HIV-1 Env and their specific findings.

Target Variable region of gp120	Key finding	Ref.
V1/V2 loop of gp120	Tat binding; Modulation of virus entry	**(Cardaci et al., 2013)**
V1/V2 loop of gp120	Four heparin-binding domains (1-4) were identified in the V2 and V3 loops, in the C-terminal domain, and within the CD4-induced bridging sheet.	**(Crublet et al., 2008)**
V3 loop of gp120	Polyanionic compounds (Poly (4,4'-stilbenedicarboxylate-alt-maleic acid) (DCSti-alt-MA)) inhibited HIV-1 entry; Electrostatic interactions with the V3 loop of gp120.	**(Hollingsworth et al., 2018)**
gp41	Water and chloride ions interact with the highly conserved arginine bundle, $(R696)_3$, at the center of the membrane and influenced TMD stability; $(R696)_3$ - water - anion network is crucial in viral fusion to host cell by modulating protein conformational changes within the membrane.	**(Hollingsworth et al., 2018)**
V1/V2 to V3	CXCR4 mutations (mutations at aspartic acid in the second extracellular loop (ECL2) (D193A and D193R)) limits HIV-1 infection.	**(Labrosse et al., 2001)**
V1/V2	Substitutions in the V2 loop produced dissociation of the gp120 and gp41 glycoproteins; Alteration in V2 conformation affect subunit assembly.	**(Sullivan et al., 1993)**

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V1/V2	Elimination of a single glycosylation site at asparagine 197 in the V1/V2 stem is sufficient for CD4-independent gp120 binding to CCR5 and HIV-1 entry into CD4-negative cells expressing CCR5; Deletion of the V1/V2 loops also allowed CD4-independent viral entry and gp120 binding to CCR5.	**(Kolchinsky et al., 2001)**
V1/V2/V3 mutations	Of the glycosylation sites evaluated, those proximal to the V1/V2 loops (N135, N141, N156, N160) and the V3 loops (N301) of gp120 were functionally critical; The glycosylation site mutations near the V1/V2 loop used CCR5 and CXCR4 equally. In contrast, a mutation within the V3 loop preferentially inhibited the usage of CCR5; Revertants altered tropism of the parental virus from dual tropic to T-tropic; Carbohydrate moieties near the V1/V2 and the V3 loops play critical roles in maintaining the proper conformation of the variable loops for optimal interaction with receptors.	**(Ogert et al., 2001)**
V2i and V3	Manα1-2Man-binding lectins enhance the exposure of V2i (V2 induced) and V3 epitopes and consequentially increase the neutralization strength of antibodies against these epitopes.	**(Jan et al., 2017)**
gp120 ligand-free, gp120 bound	HIV-1 gp120 is intrinsically dynamic to sample and transfer various conformational states, including from the open state to the closed state; The binding of CD4 hinders conformational transitions; Ligand-free gp120 exhibits an intrinsically conformational transition from the open state to the closed state, whereas CD4-bound gp120 is mainly restricted in the open state.	**(Li et al., 2020)**
Bridging sheet & V2 loop	The unliganded extended monomeric core of gp120 assumes an intermediate CD4 inducible conformation in solution that further undergoes detectable rearrangements upon association with CD4.	**(Kaplan et al., 2016)**
Beta16/beta17 Beta-hairpin	Beta-hairpin structure governs interactions between the surface of gp120 with native CCR5 Beta16/beta17 beta-hairpin, modulate gp120 binding to CD4 mCD4 protein cannot bind gp120 due to steric clashes.	**(Mechulam et al., 2005; Kassler et al., 2012)**

The conformational flexibility of mCD4 peptides allows interaction; mCD4-peptide stably interacts with gp120 via an intermolecular β-sheet.

2.2. Implication for HIV-1 evasiveness

The gp120 glycoprotein is known to exhibit two major conformational states—the closed and open states; in between, there are many dynamic conformational states **(Wang et al., 2020)**, indicating an intrinsically flexible region(s) that allows for structural dynamics. Several gp120 crystal structures are solved including gp120 and its outer domain in complex with soluble CD4 (sCD4) and gp120 and co-receptor mimics **(Huang et al., 2005; Zhou et al., 2007; Roark et al., 2020; Jones et al., 2018)**. Additionally, the crystal structure of gp120 bound to different antibodies has been well documented **(Roark et al., 2020; Jones et al., 2018; Shen et al., 2017; Georgiev et al., 2013; Klein et al., 2013)** and these structures reveal a high degree of structural plasticity. The transient structural morphing of the gp120 molecule presents a challenge to drugs and antibody binding because in one state, the HIV-1 particle can present binding pockets that have a high affinity to drugs or antibodies, then quickly shift to another state with lower or no affinity. The shapeshifting effectively eludes immunity and therapeutic drugs and is one of the major challenges for drug and vaccine development targeting the HIV-1 envelope glycoprotein **(Garbelli et al., 2017)**. X-ray crystallographic and cryo-electron microscopy determined the open and closed conformational states of gp120 **(Gristick et al., 2016; Gristick et al., 2017)**; however, the population of gp120 species arising from the glycoprotein's conformational plasticity, motions, and transitions cannot be fully captured in such studies. The obvious question is how these differences in conformation will affect receptor binding, antibody recognition and neutralization, and which specific regions of the glycoprotein are most crucial to such structural plasticity, and finally, how such structural dynamics influence HIV-1 evasiveness against host immunity, drugs, and vaccines as well as facilitate the viral entry into its host. In an innovative work carried out using smFRET and Nab binding assay, Wang et al., 2020 were successful in demonstrating that CD4-mimetic compounds were potent in protecting gp120-vaccinated from simian monkeys. Their work highlighted the potential of modulating Env conformation for HIV-1 infection prevention efforts **(Wang et al., 2020)**.

In this review, we explore the structural constraints presented by the several conformational states of the glycoprotein to antibodies and drugs. We examine how such differences are helpful for the viral elusiveness against the host's immunity, thus, providing structural avenues for the virus to escape neutralizing agents.

For instance, the transmembrane subunit gp41 is a good target for antibodies and is known to be highly immunogenic. Drugs targeting the gp41 region first must overcome the several different protective layers arising from gp120 and the glycan shield. Hence most drugs and antibodies directed against the gp41 perform poorly. Fuzeon (also termed T-20), an HIV-1 drug available on the market, is a fusion inhibitor targeting the gp41 subunit with a short half-life and low bioavailability **(Nelson et al., 2007; Ashkenazi et al., 2011)**. While former reports stated that the gp41 is shielded from direct contact by the layers of gp120 structures and the overwhelming glycan shield **(Garg et al., 2011; Robinson et al., 1991)** hence making gp41 only accessible when the HIV-1 particle is docked to its receptor and has initiated its host cell internalization process, current works by Kong et al., 2016 demonstrated that the N-terminal terminal eight residues of a fusion peptide of gp41 could be made solvent accessible **(Kong et al., 2016)** and hence cause vulnerability to antibody neutralization. VRC34, an antibody isolated from a chronically infected HIV patient has been demonstrated to be potent against circulating strain of HIV-1. VRC34.01 is the best performing

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member of the VRC34 lineage, with a neutralization breadth of 50.5% on our panel of 208-diverse HIV-1 strains **(Kong et al., 2016)**. Additionally, Kong et al., 2016 used BG505. SOSIP.664, which is the BG505 strain Env, truncated at residue 664 to present only the ectodomain, and stabilized with an interprotomer disulfide (SOS) and a gp41 Ile to Pro single point mutation **(Kong et al., 2016)**. Studies by Cheng et al., 2019 showed that VRC34.01 branch required a $Y33P_{HC}$ to achieved a broad neutralization. The structure of VRC34.01 in complex to HIV-1 Env reveals that VRC34.01 targets a linear peptide that corresponds to the N-terminal 8 residues of the gp41 transmembrane subunit **(Shen et al., 2020)**. SOSIP.664 has been demonstrated to present all known bNAb epitopes **(Ringe et al., 2019)**. The use of stabilized (via genetic modification) viral proteins as immunogens in vaccines is well-established **(Sanders et al., 2021; Zhou et al., 2020; Duan et al., 2020)**. Furthermore, epitope-based design has been used to generate vaccine immunogens that elicit fusion peptide-directed antibodies with cross-strain neutralization breadth **(Xu et al., 2018)**. The fusion peptide is highly conserved, and this makes it an excellent drug/antibody target if it is accessible, thus the insightful research by Kong et al., 2016 demonstrated that the fusion peptide is accessible in the prefusion, unliganded state is an important finding for drug discovery **(Kong et al., 2016)**. They also showed that CD4-binding to Env reduced the binding of VRC34 to Env, which could be mitigated by adding an additional stabilizing disulfide (DS-SOSIP), demonstrating that the fusion peptide is indeed solvent accessible in the receptor-unbound state and can neutralize CD4-induced conformational transitions **(Kong et al., 2016)**.

The HIV-1 particle cleverly uses different methods to escape the immune system and drug attacks. Since its envelope protein is highly immunogenic, it employs several strategies to circumvent and hide its identity, including constant amino acid mutations, random and fewer matured prefusion state gp120 displayed on the surface, dense glycan shield covering, and conformational masking **(Pancera et al., 2010; Gristick et al., 2017; Lyumkis et al., 2013; Julien et al., 2013; Stewart-Jones et al., 2016; Galimidi et al., 2015)**. Most antibodies that can effectively neutralize HIV-1 usually require at least one long CDR to permit effective envelope glycoprotein gp120 recognition **(Loos et al., 2015)**. Antibodies with long CDR H3s are far less available and require several rounds of selection and somatic hypermutation to function effectively against HIV-1 **(Sadanand et al., 2016; Sundling et al., 2012)**; such circumstances are usually present only in individuals afflicted with the disease for many years **(Hraber et al., 2014)**.

2.3. Determinants of neutralization resistance of gp120

Currently, researchers are engaged in finding strategies to expose immunogenic epitopes. Using CD4 mimetics, Richard et al., 2015 demonstrated that these CD4 mimetics could engage Env in a CD4-like nature **(Jonathan et al., 2015)**. Determinants of neutralization resistance and CD4 binding affinity do not reside on the core but in the oligomeric interactions of the major variable loops **(York et al., 2001)**, which serve to control or modulate the action of the core. Results from Li et al. in an *in silico* molecular dynamics experiment revealed distinct differences in conformational dynamics and thermodynamics between the unliganded and the CD4-bound states of HIV-1 gp120. They reported that CD4-bound gp120 produced global structural alterations, unraveled tightly packed domains, and increased conformational flexibility. They also demonstrated that CD4-induced gp120 was characterized by complex molecular motions and increased conformational entropy, with lower thermostability **(Li et al., 2020)**.

Their conformational dynamics data demonstrated that the CD4-free equilibrium portions of the protein's backbone root mean square deviation (RMSD) curves ranged between 0.3 and 0.6 nm, and CD4-complexed gp120s stood between 0.4 and 1.1 nm **(Li et al., 2020)**. Also, they demonstrated

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that the CD4-complexed gp120 sampled a larger range in the essential subspace during MD simulations, hence increasing gp120 conformational entropy upon CD4 binding, more so, their CD4-complexed free-energy levels (FEL) depicted rugged surface compared to the unliganded FEL, to answer this observation, they inferred that it was due to higher presence of free-energy minima in the CD4-complexed gp120 than the unliganded gp120 **(Li et al., 2020)**. They concluded that the CD4-complexed gp120 is more structurally unstable and conformationally flexible than the unliganded gp120. Munro et al., demonstrated using single-molecule fluorescence resonance energy transfer (smFRET) study that the unliganded, closed state was the most abundant among the three states of the ligand-free Env/gp120 **(Munro et al., 2014)**. An outstanding study by Stadtmueller et al., 2018 evaluated the conformational landscapes of ligand-free, CD4-bound, inhibitor-bound, and antibody-bound SOSIPs using double electron-electron resonance (DEER) spectroscopy and compared results to soluble and virion-bound Env structures, and smFRET-derived dynamics of virion-bound Envs **(Stadtmueller et al., 2018)**. They demonstrated that the unliganded SOSIP results showed similarity to the native or the neutralizing antibody-bound structures. Their study revealed an increased flexibility near Env base, without intra-subunit flexibility near Env apex. Hence, they inferred that CD4 binding increased inter-subunit distances and heterogeneity **(Trkola et al., 1996)**.

The structural fluctuation in the unbound state discourages antibody binding and neutralization; and also in the bound state, it may be too late for HIV-1 neutralization since the capsid is already delivered into the cytoplasm of the cell. Neutralizing the "scaffold" bound to the surface of the CD4 positive cells will not affect the replicating HIV-1 particle inside; however, it is important to understand that neutralizing the native Env, via antibodies or therapeutics, is crucial to prevent the infection of new hosts, and thus to mitigate the transmission of the HIV-1 virus. For this reason, the neutralization of the Env has significant clinical utility as a preventative (i.e. a prophylactic) rather than a treatment.

The gp120 molecule directly interacts with CD4 during receptor-ligand interactions that lead to receptor binding (109). Some studies have reported that the antibody interacting surface undergoes significant dynamic motion, as "if fastening to gp120 and wrenching it" **(Néstor et al., 2010)**. The receptor might have employed these conformation adjustments to tightly bind to its ligand. Conformational distortions have been observed for substrate specificities analogous to the mechanism observed in enzyme-substrate interactions **(Néstor et al., 2010)**.

More so, the large number of structural ensembles **(Pancera et al., 2010)** existing between the unliganded state of gp120 and CD4-bound state further exacerbate the difficulty of antibody binding and subsequent neutralization of the glycoprotein. For this reason, any interaction that occurs between a rigid agent and the glycoprotein may lead to nonproductive binding since there is the absence of a complementary structural alteration, which possibly enhanced a tighter bond. Figure 3 shows that the binding site for HIV-1 neutralizing antibody Y498 has fewer structural and topological changes than the binding site of VRC01 (Figure 2A-D), which demonstrated larger structural and topological changes between the unbound and the CD-complexed states of gp120. There are four key amino acid sites, G367, D368, E370, and V372, located on the CD4 binding loop on gp120 that determine the neutralization of antibody Y498 **(Cao et al., 2020)**. The limited number of residues with little conformational effect on the entire molecule seems to be the major reason for its limited neutralization spectrum. However, the VRC01 binding site is noted to have a conformationally invariant outer domain of gp120 (48, 72). It is a good indicator of great neutralizing potential against several HIV-1 strains (48), comprising a large topological area of 1089 $Å^2$ on the gp120 outer domain, about 50% larger than the 730 $Å^2$ surfaces utilized by CD4 (112). Table 2 highlights most HIV-1 antibodies and their sites of action in the HIV-1 Env macromolecule.

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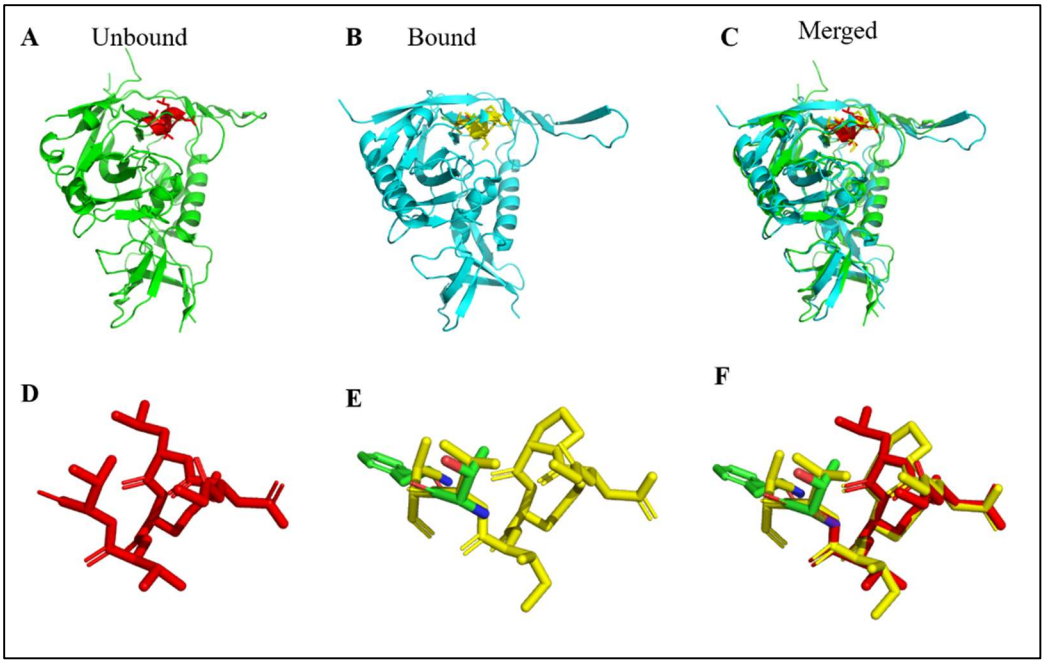

Figure 3. Depiction of 3D Structural conformational changes associated with the Y498 antibody binding site of gp120. (**A**) Y498 binding site (red) in gp120 unbound state; (**B**) Y498 binding site (yellow) in gp120 bound state; (**C**) Merged depiction of the unbound (gp120 PDB ID: 3TIH) and the bound states of gp120 (gp120 PDB ID: 1G9M); The Y498 binding site residues depicted in red (unbound state) (**D**), and yellow (bound state) (**E**), the merge of the two states (**F**).

Table 2. HIV-1 Antibodies/Inhibitors and their sites of action in the HIV-1 Env macromolecule.

Antibody/ inhibitor	Target	Ref
10E8	HIV-1 gp41 membrane-proximal external region (MPER)-specific antibody, named 10E8, which neutralizes ~98% of tested viruses MPER is highly conserved; The target for potent, non-self-reactive neutralizing antibodies 10E8 is a broad and potent neutralizing antibody 10E8 bind highly conserved residues within MPER; 10E8 makes crucial contacts with highly conserved residues Trp672, Phe673, Trp676 and Lys/Arg683; 10E8 binding site NWFDISNWLWYIK **(**for WT JR2 isolates, for amino acid positions 672 to 683**).**	**(Huang et al., 2012)**

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N123-VRC34.01	N123-VRC34.01 targets the fusion peptide and blocks viral entry by inhibiting conformational changes in gp120 and gp41 subunits of Env required for entry; Molecular dynamics showed that the N-terminal portion of the fusion peptide can be solvent-exposed; N123-VRC34.01 binding site A512-F519 of the gp41 N123-VR34.01 binds to cell-free virus before cell surface attachment.	**(Kong et al., 2016)**
2F5	The MPER adopts an altered conformation, from the usual helical structure to an extended loop when bound to 2F5 mAb; 2F5 binding site is ELDKWA (amino acid positions 662-667) of the gp41.	**(Guenaga et al., 2012)**
4E10	4E10 binding site to the MPER of gp41 is WFDIT (amino acid positions 671-676, respectively); 4E10 fails to elicit immunity; 4E10 displays limited and focused, but unexceptional, poly-specificity Hence poor candidate for vaccine development.	**(Nelson et al., 2007)**
Fostemsavir (FTR)	Inhibitor of HIV Env-CD4 binding	**(Cahn et al., 2018)**
10-1074	Glycan-dependent bNAbs	**(Laura et al., 2009)** **(Sievers et al., 2015)**
PGT121	Binds to glycan	**(Sievers et al., 2015)**
PGT128	Binds to glycan	**(Sievers et al., 2015)**
PGT135	Binds to glycan	**(Sievers et al., 2015)**
2G12	Binds to glycan	**(Sievers et al., 2015)**
PG9	Asn160 glycan-V1/V2	**(Sievers et al., 2015)**
PG16	Asn160 glycan-V1/V2	**(Sievers et al., 2015)**
CH01–04	Asn160 glycan-V1/V2	**(Sievers et al., 2015)**
PGT141–145	Asn160 glycan-V1/V2	**(Sievers et al., 2015)**

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b12	CD4-binding site	**(Sievers et al., 2015)**
VRC07	CD4-binding site	**(Sievers et al., 2015)**
NIH45–46	CD4-binding site	**(Sievers et al., 2015)**
12A12	CD4-binding site	**(Sievers et al., 2015)**
3BNC117	CD4-binding site	**(Sievers et al., 2015)**
VRC-PGV04	CD4-binding site	**(Sievers et al., 2015)**
VRC-CH31	CD4-binding site	**(Sievers et al., 2015)**
8ANC131	CD4-binding site	**(Sievers et al., 2015)**
1B2530	CD4-binding site	**(Sievers et al., 2015)**
CH103	CD4-binding site	**(Sievers et al., 2015)**
HJ16	CD4-binding site	**(Sievers et al., 2015)**
8ANC195	gp120/gp41-interface	**(Sievers et al., 2015)**
35O22	gp120/gp41-interface	**(Sievers et al., 2015)**
PGT151	gp120/gp41-interface	**(Sievers et al., 2015)**
BMS-378806	Interferes with the interaction of the HIV surface protein gp120 with the host cell receptor CD4.	**(Wang et al., 2003)**
Y498	The key amino acid sites, G367, D368, E370, and V372, located on the CD4 binding loop of gp120, provided for neutralization by antibody Y498; hmAb Y498 acts on different subtypes of HIV-1 strains; G367 and T372 are critical to hmAb Y498 recognition on the CNE40-WT variant of HIV-1.	**(Cao et al., 2020)**
VRC01	VRC01 achieves broad neutralization of diverse viral strains; VRC01 partially mimics CD4 interaction with gp120 VRC01 binds to gp120 site for initial CD4 attachment (i.e., SSGGDPEIVT, position 364 to 373);	**(Zhou et al., 2010)**

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	VRC01 overcomes the glycan and conformational masking that diminishes the neutralization potency of most CD4-binding-site antibodies; VRC01 has a high degree of affinity maturation, an extra disulfide bond, a site for N-linked glycosylation, and a matured binding interface between VRC01 and gp120; VRC01 engages extensive interactions with V5 (GGNSNNE at positions 458-464, respectively) and β24 (SEIFR at positions 465-469, respectively) via hydrogen bonds and a salt bridge.	
NBD-556 and NBD-557	N-phenyl-NV-(2,2,6,6-tetramethyl-piperidin-4-yl)-oxalamide analogs blocks the gp120–CD4 interaction hence inhibit HIV-1 entry; NBD-556 and NBD-557 bind to unliganded HIV-1 gp120 but not to the cellular receptor CD4; NBD-556 and NBD-557 are active against HIV-1 laboratory-adapted strains.	**(Zhao et al., 2005)**
NSC 13778	NSC 13778 exerts potent anti-HIV-1 activity, blocks the entry of X4-, R5-, and X4/R5-tropic HIV-1 strains into CD4(+) cells but shows little or no activity in CD4-negative cells or against vesicular stomatitis Virus-G pseudotyped virions; The compounds compete with gp120 for binding to CD4.	**(Yang et al., 2005)**

2.4. Movement for association/avoidance

Li et al. postulated that the ligand-free gp120 conformations were the stable ''ground-state'' whereas the CD4-bound gp120 was the unstable ''excited state'' of gp120 **(Li et al., 2020)**. Therefore, the larger structural alterations in the excited state inevitably lead to the exposure of the gp120 hidden core epitopes as targets for antibody neutralization. The gp120–CD4 interactions comprise a huge conformational reorganization **(Myszka et al., 2000)**. The entropies of gp120 binding to CD4 or antibodies have been measured and demonstrate a unique mechanism where the recognition of gp120 by receptor-binding-site antibodies prompts conformational changes in gp120 **(Prabakaran et al., 2007; Forsell, 2008)**. Studies of the neutralization potency and analysis of receptor–antibody thermodynamic cycles showed a phenomenon of HIV-1 escaping neutralization via the receptor binding site conformational masking mechanism **(Kwong et al., 2002)**. Kwong et al. demonstrated this escape mechanism by testing soluble dodecameric receptor molecules against the virus. The receptor molecules effectively neutralized primary HIV-1 isolates by simultaneously binding gp120 to multiple receptors to create sufficient avidity that compensated for the masking. Thus, conformational masking enables HIV-1 to maintain receptor binding and simultaneously evading antibody neutralization **(Kwong et al., 2002; Thomas et al., 2017)**.

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Liu et al. used CONCOORD computer simulation to generate ensembles of gp120, and using essential dynamics analysis, identified the molecule's preferred concerted motions. Their work showed that collective fluctuations of the gp120 are controlled by complex rotation/twisting, flexing/closing, and shortness/elongation motional modes occurring between or within the inner, outer, and bridging-sheet domains. They inferred that such modes were related to gp120 association with CD4/CCR5/CXCR4 and neutralization avoidance **(Liu et al., 2008)**. Similar observation has been reported on the structural transformations of gp120 **(Thomas et al., 2017)**.

2.5. The anti-HIV-1 compound NSC 13778 acts through competitive binding to CD4

HIV-1 gp120 and the CD4 receptor are highly specific and involve relatively small contact surfaces, presenting an opportunity for antiviral targeting. Yang et al. reported using a compound with ID NSC 13778 as a potent anti-HIV-1 inhibitor **(Yang et al., 2005)**. They showed that the compound blocked the entry of X4-, R5-, and X4/R5-tropic HIV-1 strains into CD4(+) cells. Through computational analyses, we demonstrated such a mechanism in Figure 4A-F. The inhibitor interacted with the following amino acids of CD4 molecule, ILE24, HIS27, HIS27, HIS27, HIS27, VAL86, GLU85 and ASP88 to form alkyl, Pi-Pi T-shaped, Pi-Pi stacked, Pi-cation, Pi-cation, halogen, halogen, and conventional hydrogen bonds establishing bonds with bond distances of 4.83Å, 4.59Å, 3.91Å, 4.75Å, 4.47Å, 3.5Å, 3.29Å, and 2.47Å respectively.

In laboratory experimentation using MAGI assay, Yang and his colleagues demonstrated that the inhibitor NSC 13778 exhibited excellent inhibition of HIV-1 with IC_{50} ranging from 0.64 ± 0.40 to 2.47 ± 1.65 μM. They also reported that there was no antiviral activity in chronically HIV-1-infected cells **(Yang et al., 2005)**. While they showed a possible working mechanism via the inhibition of HIV-1 replication and viral integration, our molecular docking analysis seems to supports a mechanism in which the compound competitively binds to CD4, blocking the gp120-CD4 complex from forming.

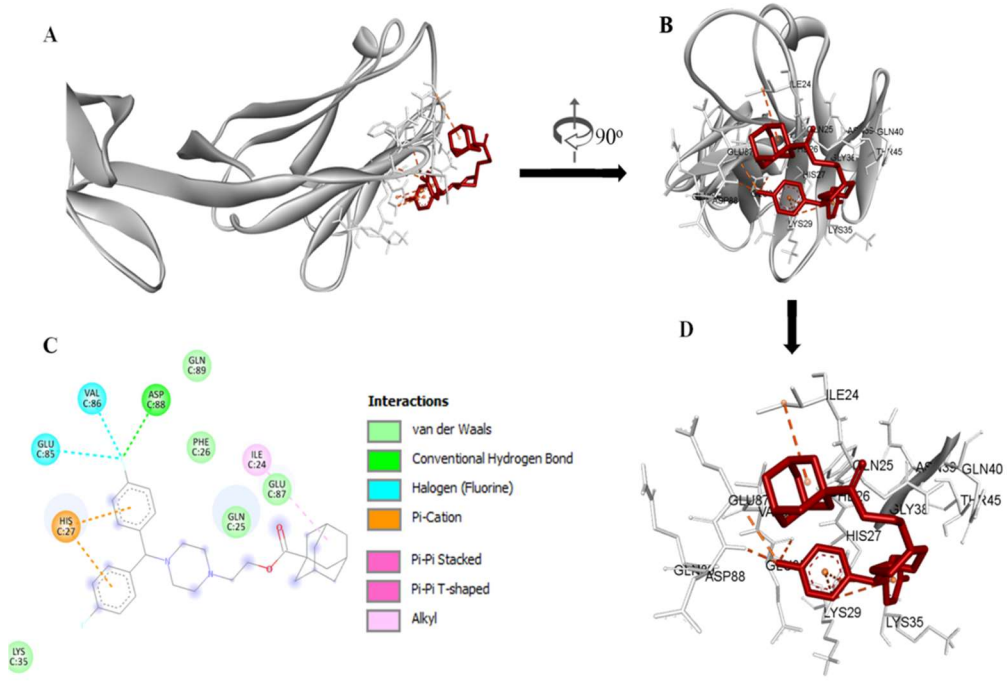

Figure 4. The interaction of gp120 inhibitor NSC 13778 with CD4i molecule (extracted from PDB ID 1G9M). (**A**) NSC 13778 inhibitor (red) model 1 interacts with CD4i (gray) (side view); (**B**) NSC 13778 inhibitor model

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1 interaction with CD4i (centered view); (**C**) A 2D illustration of the interaction between the inhibitor and the interacting atoms in the bindings pocket of CD4i; (**D**)NSC 13778 inhibitor model 1 interacts with CD4i (close up view). The inhibitor interacted with the following amino acids of CD4i molecule: ILE24, HIS27, HIS27, HIS27, HIS27, VAL86, GLU85, and ASP88 to form alkyl, Pi-Pi T-shaped, Pi-Pi stacked, Pi-cation, Pi-cation, Halogen, Halogen and conventional hydrogen bonds establishing bonds with bond distances of 4.830814Å, 4.594680Å, 3.907075Å, 4.745579Å, 4.469074Å, 3.515117Å, 3.295245Å, and 2.468635Å, respectively.

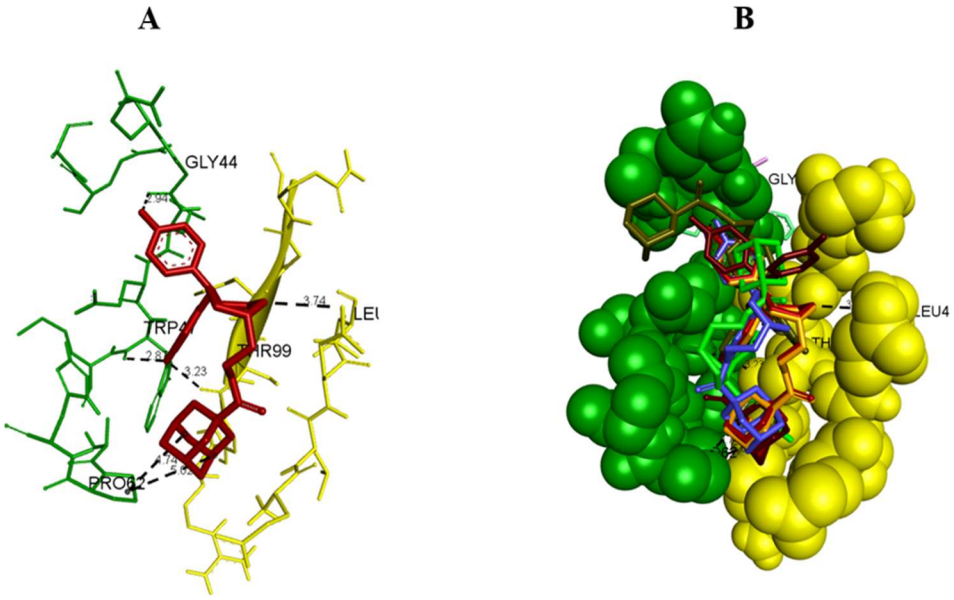

Figure 5. The interaction of gp120 inhibitor NSC 13778 with specific amino acids in the binding pocket of 17b neutralizing antibody (crystal structure of 17b neutralizing antibody extracted from PDB ID: 1G9M). Close up view of the NSC 13778 inhibitor interaction with specific amino acids in the binding pocket of 17b neutralizing antibody and the binding affinities of the inhibitor to the antibody. (**A**) 3D illustration of the interaction of NSC 13778 with atoms in the binding pocket of 17b neutralizing antibody; (**B**) Depiction of the different conformational poses of NSC 13778 in the binding pocket of 17b neutralizing antibody.

2.6. Implication for infectivity

HIV-1 gp120 interacts with its primary receptor CD4 and the co-receptor CXCR4/CCR5 to mediate the virus particles and plasma membrane fusion and entry into target cells **(Berger et al., 1999)**. The gp120 bound state possesses larger structural changes in the rest of the gp120 structure than the unliganded state, and such structural changes prove crucial for the virus' ability to interact with other receptors post CD4 induction. For instance, large dynamic alterations in the V3 loop are important for gp120–gp41 communication requisite for triggering the gp41- entry machinery. Furthermore, the gp120-co-receptor association requires the V3 loop to extend out of gp120 to its receptor CXCR4/CCR5. The active site of gp120 responsible for the interactions between gp120 and CCR5/CXCR4 comprises residues in the V3 loop and the C4 domain **(Liu et al., 2003)**. Cormier and Dragic, using mutagenesis of the gp120's V3 loops, demonstrated that the binding of V3 loops to CCR5 mediates viral entry and that the functionally distinct domain called V3 stem alone mediates soluble gp120 binding to the CCR5 N-terminus **(Cormier and Dragic, 2002)**. In another study, Liu et al. showed via protein structure modeling, docking, and molecular dynamics simulation that the CCR5 is complexed with gp120 by forces such as hydrogen bonds, salt bridges, and van der Waals and that the initial interactions of CCR5 with gp120 involves the negatively charged N-terminus

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region of CCR5 and positively charged bridging sheet region of gp120 leading to subsequent interactions occurring between the extracellular loop2 (ECL2) of CCR5 and the base of V3 loop regions of gp120 **(Liu et al., 2003)**. They also proposed that these interactions induce conformational changes in gp120, a requirement for triggering the gp41. The orientation of gp41 for target cell fusion includes prefusion and fusion stages. To tether this molecule and prevent a misstep during the infection process, the orientation of this domain is in sequential order through both structural states. The mobility of gp120 termini and neighboring β-sandwich with gp41 is tightly associated with the positioning of gp41 in a metastable prefusion conformation. Although wide-ranging gp120 structural changes are occurring, such changes only relate to layer refolding and outer domain movement, without adversely affecting gp41 since this domain is insulated by a fixed β-sandwich **(Liu et al., 2008)**, providing an avenue for segmental flexibility.

Additionally, gp120's variable loops are known to possess conserved, key microdomains **(Yuan et al., 2013)** that have served as important domains for broadly neutralizing antibodies **(Powell et al., 2020)**. HIV-1 has solved such a survival challenge via a glycan shield, the V1/V2 loops, and the highly N-glycosylated V3 **(Silver et al., 2019; Ogert et al., 2001)**.

Pancera et al. compared various CD4-bound states of HIV-1 clade B gp120 and proposed that the structurally plastic layers had fewer interlayer interactions than the constant β-sandwich. Furthermore, they demonstrated that the structurally dynamic layer 2 possessed substantial hydration, further enhancing its structural plasticity **(Pancera et al., 2010; Maolin et al., 2019)**. The structural design of gp120 in which there is segmented flexibility, e.g., the structurally plastic elements (layers 1–3) and the invariant 7-stranded β-sandwich, which can refold into alternative conformations **(Pancera et al., 2009)**, help precisely maintain the gp120–gp41 interaction and modulates gp41 orientations to transition into prefusion and fusion states to cause virus infection.

2.7. Ligand binding limits conformational plasticity at the binding site

Functional motions of proteins are crucial aspects of enzyme-substrate recognition and allosteric regulation **(Ivet et al., 2007)**. Novel methods have rapidly provided an understanding of structure–dynamics relations on a global and local scale. The concepts of receptor pre-disposition and their intrinsic ability to go through conformational changes necessary for a specific function and the concomitant evolutionary pressures for choosing such molecular structures have been an area of scientific scrutiny **(Bahar and Rader, 2005; Tama and Brooks, 2006; Changeux and Edelstein, 2005)**. An *in silico* study, in which the residues at the binding site of gp120 were subjected to cross-correlation analysis using full atomic Normal Mode Analysis found no correlating peaks for unbound gp120 in the Phe43 cavity. However, the CD4-bound gp120 residues lining the Phe43 cavity of the CD4 were highly correlated with fluctuations and amplitudes indicative of cooperativity **(Korkut and Hendrickson, 2012)**. Elastic Network Analysis (ENM) **(Bahar et al., 1997)** calculations in the unbound state of gp120 by Korkut and Hendrickson showed higher mean square fluctuations around the bridging sheet than the bound state. Therefore, a higher degree of atomic movement exists in the unbound state than the bound state of the molecule **(Korkut and Hendrickson, 2012)**. Upon receptor binding, they observed a drastic decrease in mobility, especially in the bridging sheet, due to the stabilization effect induced by the CD4 receptor. In their study of the structural plasticity of the Phe43 cavity, they demonstrated that the cavity residues fluctuated with higher amplitudes in the absence of any ligand.

2.8. Conformational selection or induced-fit

In recent times there has been much attention paid to the mechanism of ligand binding and conformational changes in macromolecules **(Gordon et al., 2009; Changeux and Edelstein, 2019;**

Guengerich et al., 2019). The mechanism of gp120 binding to the CD4 receptor and the conformational changes in both macromolecules have a crucial role in their subsequent role in delivering the HIV-1 capsid into the cytoplasm of the host cell; interestingly, access to the open conformations is a requirement for gp120 binding to CD4 and coreceptor **(James and Mothes, 2015; Munro et al., 2014; Guttman et al., 2015; Stadmueller et al., 2018; Trkola et al., 1996)**, and the change from closed to open state is drastic and energy demanding. Both the drastic conformational changes and the thermodynamic fluctuations occurring during the transition of the gp120 unbound state to the gp120-CD4 complex state **(Li-Quan et al., 2013; Xiaochu et al., 2018)** has been demonstrated to take place through several molecular and enzymatic processes. For instance, proteolytic processing of the Env has been demonstrated to affect its conformational dynamics, and via smFRET analysis Alsahafi et al., 2019, showed that the normal and unliganded Env displayed higher intrinsic sampling of states (41% combined occupancy) as compared to the processed counterpart (13% for Env) **(Nirmin et al., 2019)**.

The question remains whether the limiting mechanisms involved in the binding of gp120 to CD4 are governed only by the induced-fit mechanism or by conformational selection. Or do both mechanisms exist simultaneously but switch **(Reed and Kinzel, 1991)** from one pathway to the other depending on gp120 concentration? Dual mechanisms have been reported in some studies in substrate enzyme reactions **(Peter et al., 2008)**. Another interesting question is, does the time over which the gp120 molecule resided on the CD4 receptor during the receptor-ligand interaction affect binding? To a large extent, a highly flexible substrate-binding site usually supports the induced-fit, whereas a rigid substrate-binding site acts more toward a conformational selection mechanism. Korkut and Hendrickson **(Korkut and Hendrickson, 2012)** reported that HIV-1 gp120-CD4 binding affects the concerted motions of gp120, especially in the Phe43 cavity. A simple and elegant method was employed to investigate if the binding site of the unbound and the CD4 complexed states of gp120 showed significant differences in motion.

Studies on the HIV-1 gp120/CD4 interaction have reported unprecedented entropy/enthalpy compensation, fine-tuning recognition capacity, and a broad range of affinity especially at the Phe43 cavity of gp120 **(Shang-Te et al., 2005)**.

3. Conclusion

In conclusion, we reviewed many published works on the conformational plasticity of gp120 and the effect of HIV-1 binding to host cell surface receptors. We demonstrated the significance of the plasticity of gp120 in HIV-1 infection process. Secondly, we reviewed research works that demonstrated that HIV-1 evades neutralization by altering its structural conformation of its gp120. We elucidated the mechanism by which the anti-HIV-1 compound NSC 13778 might act by competitive binding to its CD4 receptor to block the gp120-CD4 complex from forming. Also, we highlighted various *in silico* investigation centered on the computational analysis of gp120 conformations that exams hint at an induced-fit mechanism of the gp120 during binding to its receptors.

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1.0 Methodology

1.1 Preparation of the CD4i Molecule for molecular docking

The CD4i crystal structure was extracted from the RCSB Protein Data Bank (PDB ID:1G9M (**1**) and imported into AutoDock Vina (**2**) (AutoDock Vina, an open-source program, available at http://vina.scripps.edu/) and the molecule prepared for docking. First, the HIV-1 glycoprotein (gp120) bound to the CD4i molecule was removed; the neutralizing antibody 17b bound to the HIV-1 gp120, and water molecules were all removed. Next, missing atoms were calculated and replaced then followed by adding polar hydrogen atoms. Finally, the total charge of the molecule was calculated, and the molecule was saved as a PDB file for downstream studies. Similarly, all other proteins used in this study followed a similar preparation process.

1.2 Molecular docking of NSC 13778 to CD4i

To study the binding pattern of the NSC inhibitor to the specific amino acid residues which are crucial for the various interactions such as pi-pi orbital bonding, electrostatic interactions, hydrophobic, hydrogen bonding, and alkyl and pi-alkyl bonding, the crystal of CD4i and molecular docking analysis carried out using PyRx molecular docking software (**3, 4, 5, 6**) (freely available at https://pyrx.sourceforge.io). This crystal structure which is complexed to HIV-1 HXBC2 was extracted using Biovia Discovery Studio (available at https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio), then water molecules were removed, missing atoms replaced, and charges evaluated. The ligand (the NSC 13778 inhibitor) was retrieved from the PubChem Data Base, its structure was minimized using OpenBabel (http://openbabel.org/wiki/Main_Page 1) and converted to PDBQT format using the same free software. The refined CD4i structure was then loaded into the PyRx engine using the built-in AutoDock Vina wizard. Docking was carried out using the AutoDock Vina wizard for which the Vina search space center was; X:42.3929, Y: -15.520, Z: 66.5869, and the grid box dimensions in angstrom were; X:38.2299, Y: 24.7420, Z: 37.612 with exhaustiveness of 9. The mode of interactions of NSC 13778 with CD4i was analyzed using Biovia Discovery Studio Visualizer. The model consisting of the minimal interaction energy was selected, and its binding pattern with the CD4i molecule was analyzed.

1.3 Molecular docking of NSC 13778 to 17b neutralizing antibody

To investigate the possible interaction between the NSC inhibitor and 17 neutralizing antibody, the crystal structure of the antibody was extracted from its complexed molecule (PDB ID: 1G9M). Similar procedure was followed as in section 1.2 above in order to dock the 17b neutralizing antibody with the inhibitor.

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