ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Insights from the interfaces of HIV-1 envelope (ENV) trimer viral protein GP160 (GP120-GP41)

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Article History:

Received on: 02 Dec 2020
Revised on: 31 Dec 2020
Accepted on: 02 Jan 2021

Keywords:

HIV-1,
Envelope,
Trimer,
Glycoprotein,
GP160,
GP120,
GP41,
Protein Interface,
Van Der Waals

ABSTRACT

The Human Immunodeficiency Virus (HIV-1) type 1 viral protein is a life threatening virus causing HIV/AIDS in infected humans. The HIV-1 envelope (ENV) trimer glycoprotein GP160 (GP120-GP41) is gaining attention in recent years as a potential vaccine candidate for HIV-1/AIDS. However, the sequence variation and charge polarity at the interacting sites across clades is a shortcoming faced in the development of an effective HIV-1 vaccine. We analyzed the interfaces in terms of its interface area, interface size, and interface energies (van der Waals, hydrogen bonds, and electrostatics). The interfaces were divided as dominant ($\geq$60%) and subdominant (<60%) based on van der Waals contribution to total energies. 88% of GP120 and 74% of GP41 interfaces are highly pronounced with van der Waals energy having large interfaces with interface size (98$\pm$65 (GP120) and 73$\pm$65 (GP41)) and interface area (882$\pm$1166Å^2 (GP120) and 921$\pm$1288Å^2 (GP41)). Nevertheless, 12% of GP120 and 26% of GP41 interfaces have subdominant van der Waals energies having small interfaces with interface size (58$\pm$20 (GP120) and 27$\pm$9 (GP41)) and interface area (581$\pm$1605Å^2 (GP120) and 483$\pm$896Å^2 (GP41)). It was interesting to observe GP41 small interfaces with subdominant van der Waals are stabilized by electrostatics (r^2=0.63) without hydrogen bonds (r^2=0). However, GP120 small interfaces were found to have two fold more hydrogen bonds (r^2=0.59) than electrostatics (r^2=0.20). Therefore, our previous finding stating that small protein-protein interfaces rich in electrostatics holds true in case of GP41 whereas not with GP120 protein interfaces.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i1.4111

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INTRODUCTION

Regardless of the remarkable efforts to develop a vaccine for HIV-1/AIDS has always been a great challenge over the last decade with disappointing results in clinical trials (Shin, 2016). The unsatisfactory clinical trial results from VaxGen's AIDSVAXgp120 vaccine and MRKAd5 HIV-1 Gag/Pol/Nef have been discussed elsewhere (Adis Editorial, 2003; Überla, 2008).

This could be due to the viral human molecular mimicry, protein structural architecture, viral protein mutation and glycosylation. Despite the serious biotechnological challenges there is always an amplified energy to synthesis ENV trimer spike protein.

Table 1: Dataset of GP120 (121) having large (van der Waals Dominant) and small (van der Waals Subdominant) interfaces are listed.

GP120									
Large interfaces van der waals dominant			Small interfaces van der waals subdominant						
1G9M	2NY4	4R4N	1RZ7	3IDX	4J6R	4LSS	4R4H	4YBL	5IES
1G9N	2NY5	4RFN	1RZ8	3LQA	4JB9	4LSU	4RQS	4YC2	5IF0
1GC1	2NY6	4RFO	1RZF	3NGB	4JDT	4LSV	4RWY	4YDI	5IGX
1NAK	3JWD	4XNZ	1RZG	3Q6G	4JO3	4OLU	4RX4	4YDK	5KG9
1RZJ	3JWO	4YDJ	1RZI	3SE8	4JPK	4OLV	4S1Q	4YDL	5T33
1RZK	3MLS	4ZTO	1YYL	3SE9	4JPV	4OLW	4S1R	4YFL	5TE4
2F58	4DVR	5F96	1YYM	3TYG	4JPW	4OLX	4S1S	5CAY	5TE6
2NXY	4JO1	5KJR	2B4C	3U7Y	4JZW	4OLY	4XMK	5F6J	-
2NXZ	4JO2	5KZC	2I5Y	4JAN	4JZZ	4OLZ	4XML	5F9O	-
2NY0	4K0A	5TE7	2I60	4D9L	4LAJ	4OM0	4XMP	5F9W	-
2NY1	4KA2	-	2NY7	4H8W	4LSP	4OM1	4XNY	5FCU	-
2NY2	4LST	-	2QAD	4I3R	4LSQ	4P9H	4XVS	5FEC	-
2NY3	4R4F	-	3F58	4I3S	4LSR	4R2G	4XVT	5I9Q	-

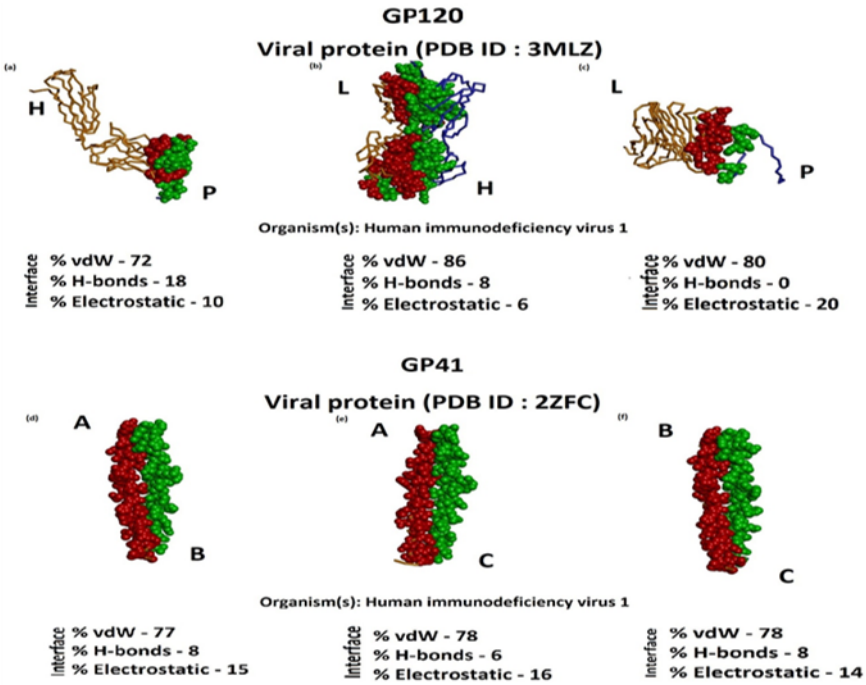

Figure 1: Examples of GP120 (PDB 3MLZ) and GP41 (PDB 2ZFC) viral protein interfaces are illustrated along with the percent contribution of each of interface energies.

The reasonable efficacy shown in the Thai trail vaccine (RV144 - ENV-GP120, Gag and Pro) is promising (Rerks-Ngarm *et al.*, 2009, 2013). Post Thai trial (RV144), the focus is on envelope (ENV) as a vaccine candidate. In addition, ENV GP160 with least homology is selected by performing a sequence comparison between HIV and human proteome (Kangueane *et al.*, 2008). GP160 ENV trimer spike glycoprotein has gained attention as a potential vaccine candidate in the recent years. Production of native like HIV-1 GP160 envelope trimer glycoprotein is a challenge in designing, developing and validating an effective vaccine from a biochemical, structural and immunological view point (Sanders and Moore, 2017; Doores, 2015). Ringe *et al.* (2015) investigated on the number of factors that are importantly influencing the design, stability and purification of native like HIV-1 envelope trimer glycoprotein. Alsalmi *et al.* (2015) used strep tag method to purify GP160 trimer protein and was resulted

Table 2: Dataset of GP41 (85) having large (van der Waals Dominant) and small (van der Waals Subdominant) interfaces are listed.

GP41								
Large Interfaces van der Waals Dominant					Small Interfaces van der Waals Subdominant			
1CE0	1U8N	2P8L	3DRT	3JWO	1NLD	3MA9	4NGH	5KA6
1TJG	1U8O	2P8M	3EGS	3LEX	1TZG	3MAC	4NHC	5MP6
1TJH	1U8P	2P8P	3F4Z	3LEY	2CMR	3MNW	4U6G	5U3K
1TJI	1U8Q	2PW1	3FN0	4NRX	2FX7	3O40	4XAW	5U3L
1U8H	1U91	2PW2	3IDG	4XC1	2FX9	3O43	4XBE	5U3M
1U8I	1U92	2ZFC	3IDI	4XC3	2Q3I	3OZ9	4XCF	5U3N
1U8J	1U93	3D0L	3IDJ	5F89	2R5B	3P30	5CCK	5U3O
1U8K	1U95	3D0V	3IDM	5U3J	2R5D	3UIA	5CMU	-
1U8L	2F5B	3DRO	3IDN	-	2X7R	4KHT	5IQ7	-
1U8M	2FX8	3DRQ	3JWD	-	3ECB	4KHX	5IQ9	-

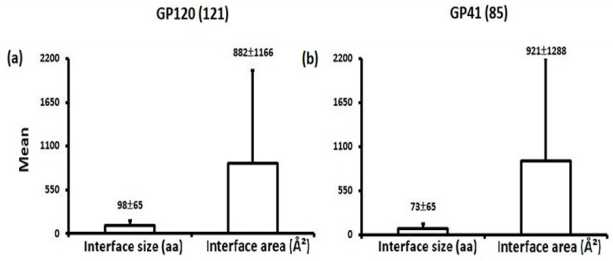

Figure 2: Mean interface size and interface area of GP120 and GP41 protein interfaces.

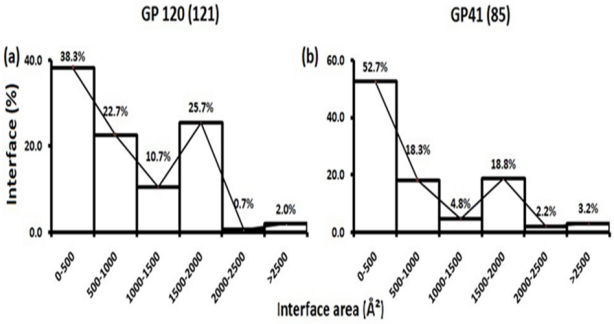

Figure 3: Graph showing interfaces with interface area (Å²) for GP120 and GP41.

with cleaved, uncleaved, fully or partially glycosylated trimers. In addition, they found cleaved gp140 were not required for trimerization, however they played a significant role in triggering conformational changes in channelizing the trimers to generate compact three blade propeller shaped trimers. Verkerke *et al.* (2016) used lectin affinity chromatography to purify native like trimers from diverse HIV-1 isolates. The challenges faced in the production, analysis and synthesis of GP160 ENV trimer glycoprotein are reported Grimm *et al.* (2015); Guenaga (2015).

Surface mutation, charge polarity and glycosylation and sequence variation between known variants in different clades are the significant barriers causing difficulty in imitating a native-like conformation of the glycoprotein. It is evident that assembling individual GP160 into a trimer spike complex structure is a challenge from a protein-protein interaction viewpoint. A large number of GP120 and GP41 structures are available in the PDB deposited using different biophysical techniques to understand the underlying molecular mechanism of the interacting proteins.

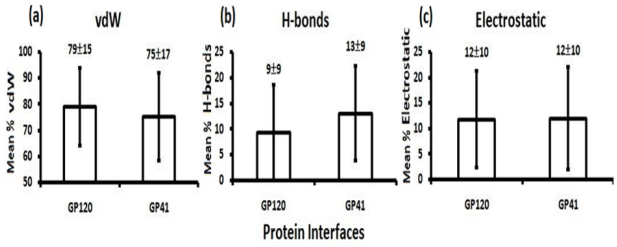

Figure 4: GP120 and GP41 interfaces are shown in terms of mean percent van der Waals, hydrogen bonds and electrostatics.

Sowmya *et al.* (2011) demonstrated the correlation between sequence polarity and mean Shannon entropy by calculating sequence polarity for surface residues in GP120 and GP41 and concluded stating the use of protein modification in the enhancement of HIV-1 vaccine across different clades, blood, and brain. Nilofer *et al.* (2017) characterized the interfaces of GP120-GP120, GP120-GP41 and GP41-GP41 and reported that the interfaces of GP120-GP120 are largely polar. The interfaces of GP120-GP41 and GP41-GP41 are characteristics of polar and non-polar residues. We characterize a manually curated dataset of 121 GP120 and 85 GP41 (Figure 1) pro-

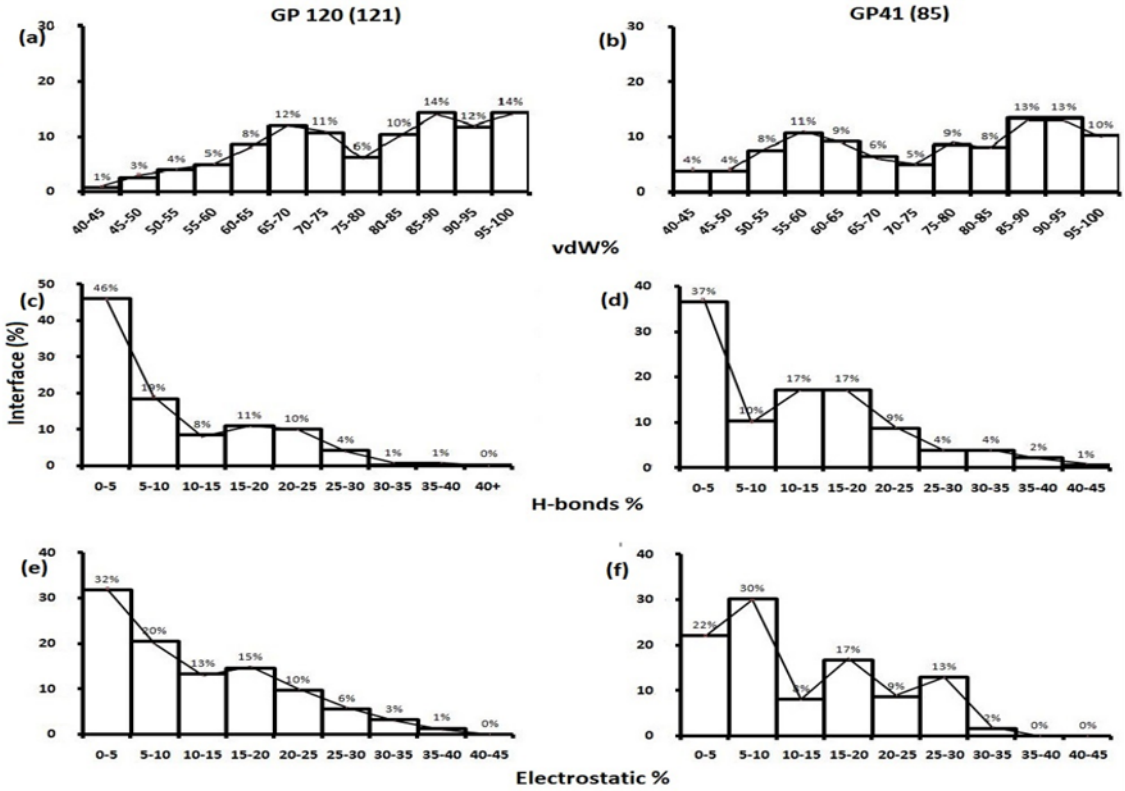

Figure 5: GP120 and GP41 interfaces are shown with varying percent contribution of van der Waals, hydrogen bonds and electrostatics.

tein interfaces reported by Nilofer *et al.* (2017) using interface features including interface area, interface size (number of residues at the interface), van der Waals, hydrogen bonds and electrostatics, to verify our previous finding stating that small protein interfaces are rich in electrostatics are often linked to regulatory proteins (Nilofer *et al.*, 2020). The residues at the interface are displayed using CPK depiction (Discovery Studio® (Systèmes, 2020).

MATERIALS AND METHODS

Dataset

We used a dataset of 206 interfaces manually curated as reported by Nilofer *et al.* (2017). It consists of 121 GP120 (Table 1) and 85 GP41 (Table 2) interfaces. It should be noted that GP120 structures in the PDB are available in ligand-bound state.

Interface area

Interface area was estimated for each of 121 interfaces of GP120 and 85 interfaces of GP41 using Naccess (Hubbard and Thornton, 1993). Naccess uses Lee and Richards method (Lee and Richards, 1971), wherein a probe with radius 1.4Å (Jones and Thornton, 1996) roll over the protein complex in monomer state and dimer state to find the accessible surface area and the interface area using delta ASA. Delta ASA (change in accessible surface area) is calculated using a formula: [ASA (Monomer subunit 1) + ASA (Monomer subunit 2) - AB (Dimer complex)]/2.

Interface size & interface energies

Interface size and interface energies were estimated for each of 121 interfaces of GP120 and GP41 using PPCheck (Sukhwal and Sowdhamini, 2015). PPCheck uses distance criteria to identify the non-covalent interactions between atoms of the two interacting proteins. It should be noted that the role of water is ignored in this analysis.

Large interface area and small interface area

Interfaces with large interface size (98±65 (GP120) and 73±65 (GP41)) and interface area (882±1166Å^2 (GP120) and 921±1288Å^2 (GP41)). Having dominant van der Waals energy (≥60%) at the interface are defined as large interface whereas interfaces with small interface size (58±20 (GP120) and 27±9 (GP41)) and interface area (581±1605Å^2 (GP120) and 483±896Å^2 (GP41)) having subdominant van der Waals energy (<60%) are defined as small interface.

Dominant and subdominant van der Waals interface

Interfaces with van der Waals contribution ≥60% to total energy (sum of van der Waals, hydrogen

bonds and electrostatics) is defined as dominant interfaces, while interfaces with van der Waals contribution <60% to total energy is defined as subdominant interfaces. A cutoff of 60% was used as the larger part of van der Waals contribution was at this cutoff on a scale of 0-100% and hence used.

Statistical analysis

We calculated interface energies of GP120 and GP41 using the statistical (Microsoft® Office Excel (version 2003)) variables including mean, mode, distribution, standard deviation and frequency at definite bin and range. We also carried out multiple linear regressions analysis for each interface with interface size against van der Waals, hydrogen bonds, electrostatic, total energy and interface area using regression tool. Its co-efficient of determination (r^2) was predicted with an evaluation of p-value using ANOVA (statistical test) at 95% confidence limit.

RESULTS AND DISCUSSION

The HIV-1 envelope trimer glycoprotein GP160 is a potential vaccine candidate for HIV-1/AIDS (Burton *et al.*, 2004). Structural data of GP160 available in the PDB are always found to be coupled with ligand shows the degree of stability of GP160 without supportive ligand (Moore *et al.*, 1992).

The trimer interfaces are unstable when produced *invitro* and this may be due to its sequence composition and structural conformation (Moore *et al.*, 1990; Chen *et al.*, 2005). Nilofer *et al.* (2017) reported that the interfaces of GP120 are largely polar whereas the interfaces of GP120-GP41 and GP41 are characteristics of equal contribution of polar and non-polar residues. Interfaces with high polarity have an immense impact on protein's surface, immunological and stability properties. High polarity at the interface is bottleneck in the *invitro* synthesis and production of GP160 in a stable form. The instability of GP160 could also be due to the complicatedness in mimicking the *invivo* environment in *invitro* for protein folding and assembly of the complex (Abagyan and Batalov, 1997; Kinjo *et al.*, 2001).

Therefore, we characterized the interfaces of GP120 and GP41 using interface area, interface size and interface energies using PPCheck (identifies non-covalent interactions using distance criteria). To verify our pervious findings, we used the manually curated dataset of 121 and 85 interfaces of GP120 and GP41 proteins. The statistical analysis show that the mean interface size (98±65 (GP120) and 73±65 (GP41)) and interface area (882±1166Å^2 (GP120) and 921±1288Å^2 (GP41)) to be in close proximity for GP120 and GP41 interfaces (Figure 2). In contrast to our previous study we observed, most of the interfaces to have an interface area <1000Å^2 in both GP120 (60%) and GP41 (71%) and about 25% of GP120 and 19% of GP41 to have interface area between 1500Å^2 to 2000Å^2 (Figure 3).

Subsequently, we described each interface of GP120 and GP41 using van der Waals, H-bond, electrostatics and total energies along with their varying proportion of contribution at the interface. Thus, we calculated each individual contribution in percentage towards total energy. We observed interfaces to have high percentage of van der Waals (77%) and a low percentage of hydrogen bonds (12%) and electrostatics (11%) on average for GP120 and GP41 complexes (Figure 4). In addition, we noticed the interfaces of GP120 and GP41 to be normally distributed with increasing percentage of van der Waals (Figure 5). While a proportion of the interface decrease with increasing percentage of hydrogen bonds and electrostatics unlike van der Waals energy. It should be noted that interfaces of GP120 and GP41 are similar with the percentage contribution of van der Waals, hydrogen bonds, electrostatics, interface area and interface size. We further grouped the interfaces of GP120 and GP41 as dominant (≥60%) and subdominant (<60%) van der Waals based on its contribution towards total energy. As a result dominant interfaces have ≥60% of van der Waals with less magnitude of hydrogen bonds and electrostatics.

We observed majority of interfaces of GP120 (88%) and GP41 (74%) to be van der Waals dominant with less that 10% contribution of hydrogen bonds and electrostatics while the remaining 12% of GP120 and 26% of GP41 have subdominant van der Waals with more than 15% of hydrogen bonds and electrostatic (Figure 6). We performed statistical analysis on dominant van der Waals and subdominant van der Waals interfaces to highlight the contribution of hydrogen bonds and electrostatics in the small interfaces. We observed subdominant van der Waals interfaces of GP120 and GP41 to have three fold more of both hydrogen bonds and electrostatics when compared to dominant van der Waals interfaces (Figure 7). Furthermore, we noticed subdominant van der Waals interfaces to be more pronounced with more than 20% of hydrogen bonds and electrostatics distinct compared to dominant van der Waals interfaces (Figure 8). It is evident from (Figure 9) that the interface size and interface area of small interfaces are only half when compared to the large interfaces. Most of the interfaces with subdominant van der Waals have interface area less than 500Å^2 (Figure 10).

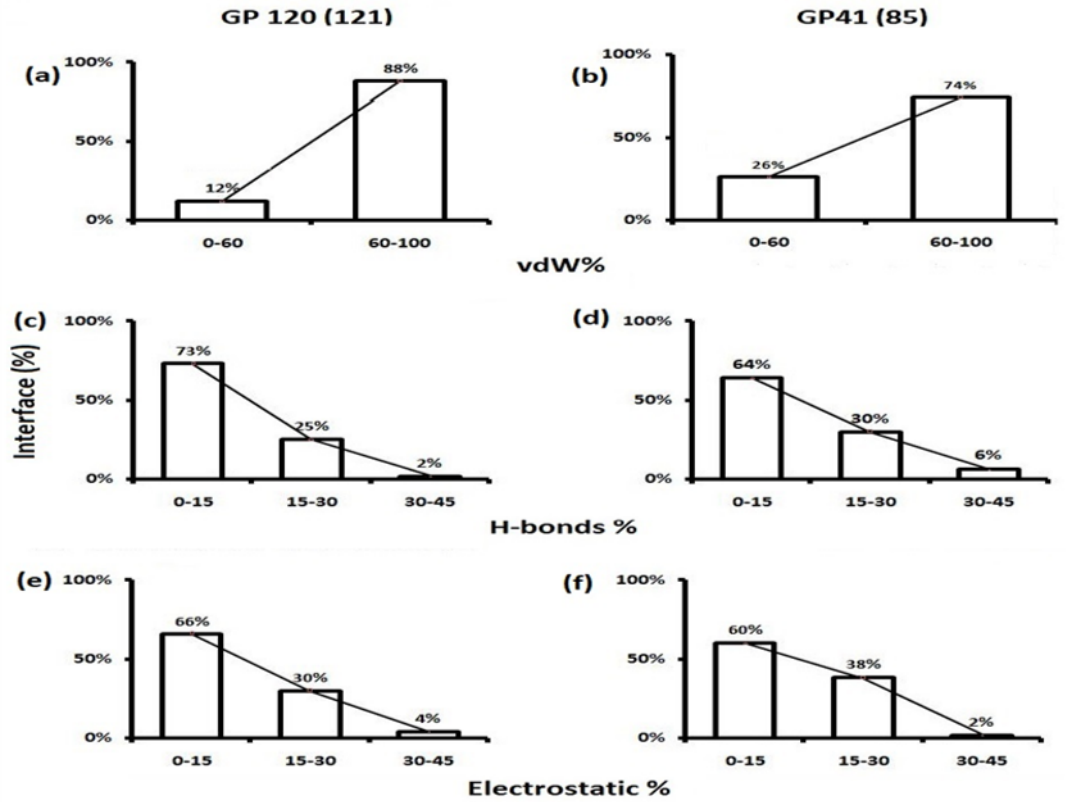

Figure 6: GP120 and GP41 interfaces are shown with increasing percentages of van der Waals, hydrogen bonds and electrostatics.

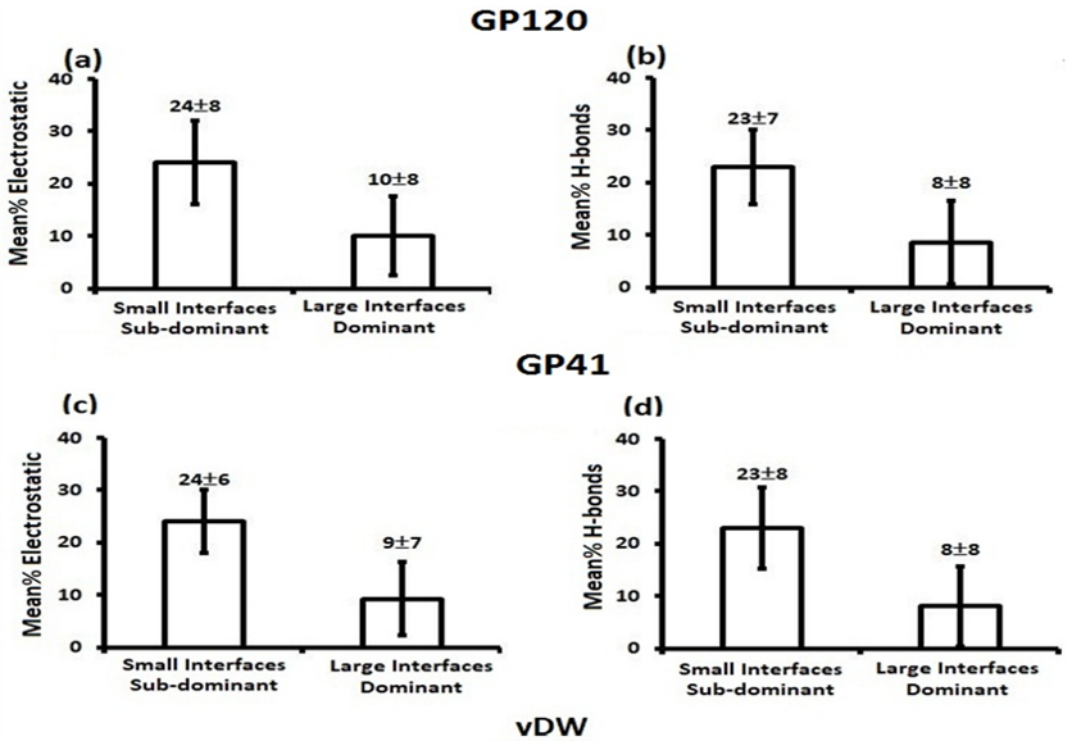

Figure 7: Mean percentage of hydrogen bonds and electrostatics in large and small interfaces of GP120 and GP41 are shown.

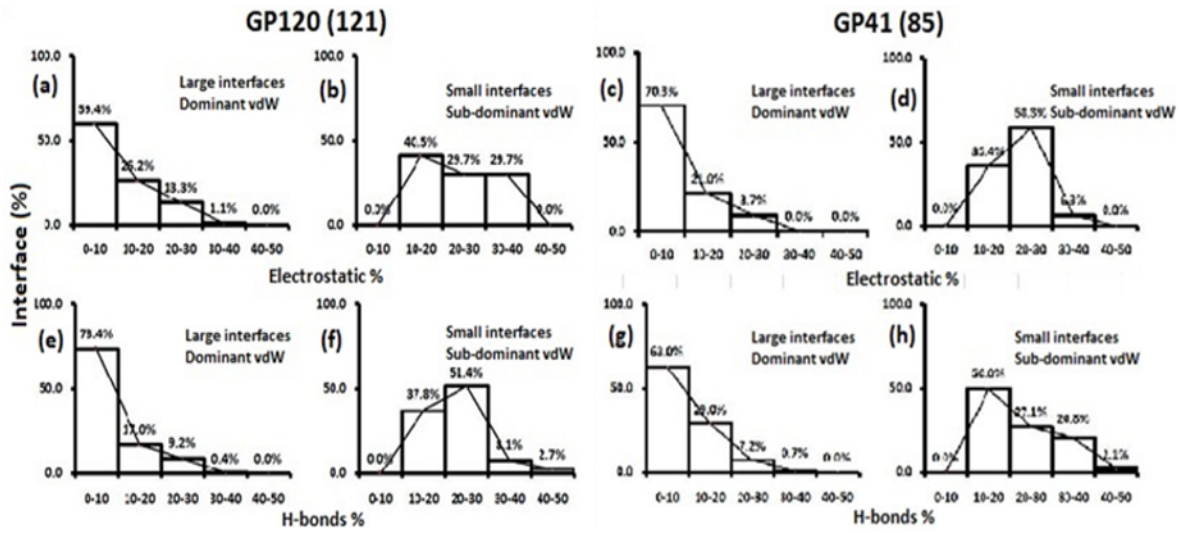

Figure 8: Percentage of electrostatic and hydrogen bonds in large and small interfaces of GP120 and GP41 are shown.

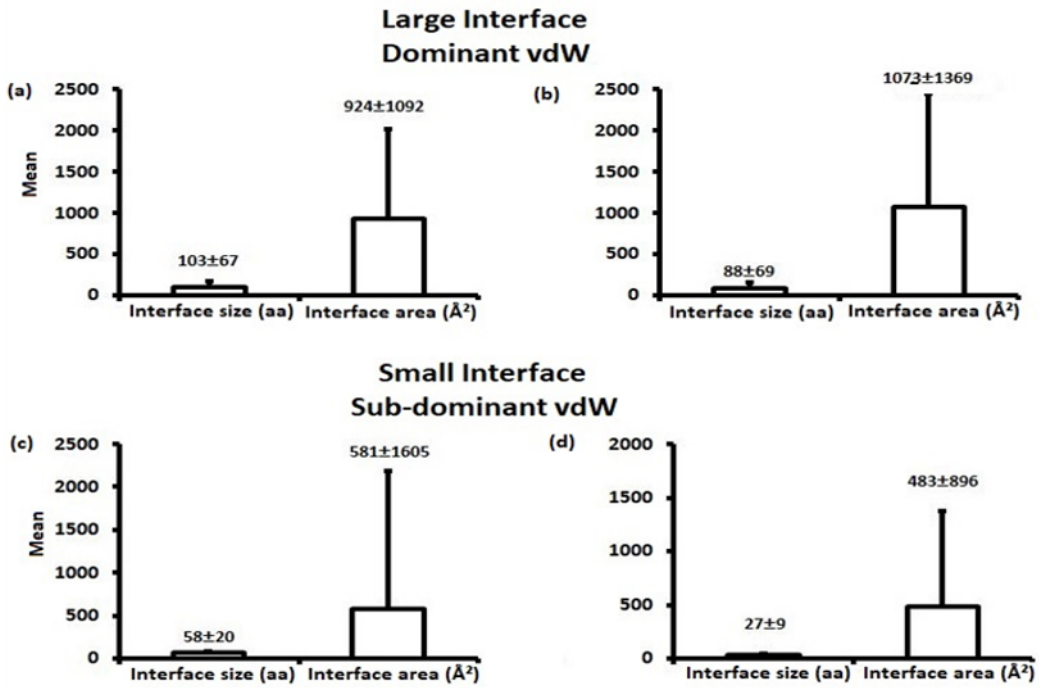

Figure 9: Interface size and interface area in large and small interfaces of GP120 and GP41 interfaces are shown.

Therefore, it was stated that the small interfaces with subdominant van der Waals energy and small interface area are rich in electrostatics.

However, in context to the small interfaces (sub-dominant van der Waals) of GP120 and GP41, small interfaces of GP120 are rich in hydrogen bonds and GP41 is rich in electrostatics.

Interface size increases with interface area is a known fact and hence we correlated interface size and interface energies.

It was reported that total energy, van der Waals and hydrogen bonds increase with interface size but electrostatics decrease with increasing interface size (Nilofer *et al.*, 2020).

While the results of our current study shows that van der Waals and total energies of GP120 and GP41 interfaces increase with interface size but hydrogen bonds and electrostatics decrease with increasing interface size (Figure 11).

Hence we divided our interfaces as large (dominant van der Waals) and small (subdominant van der Waals) interface based on their percent contribution towards total energy to check for correlation.

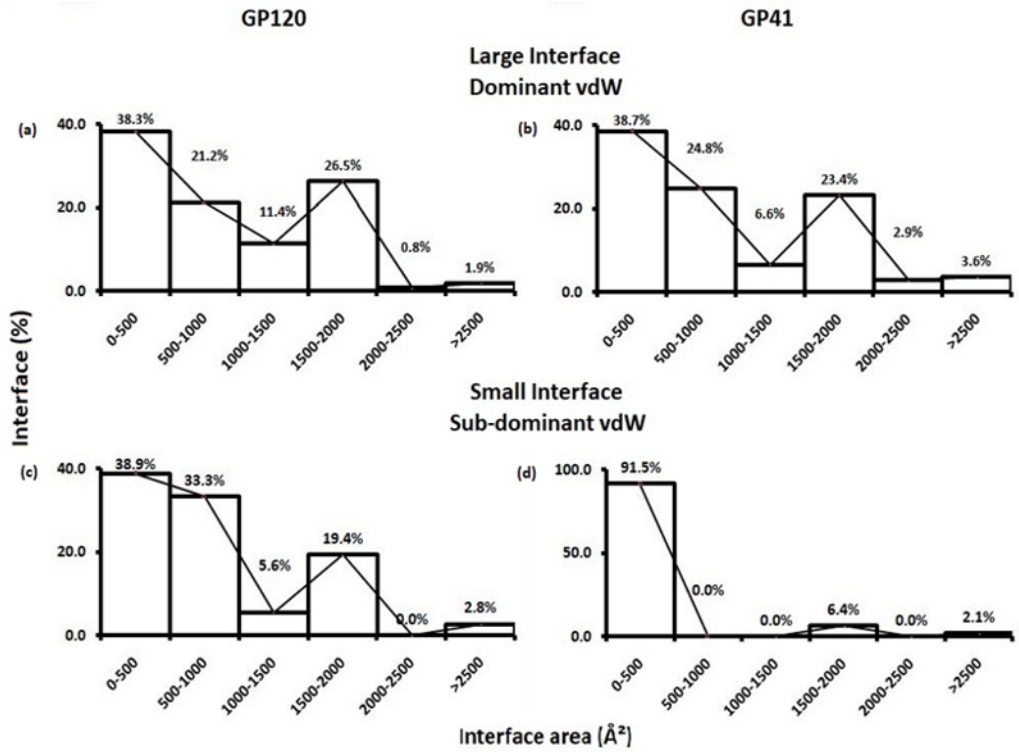

Figure 10: Interface area in large and small interfaces among GP120 and GP41 is shown.

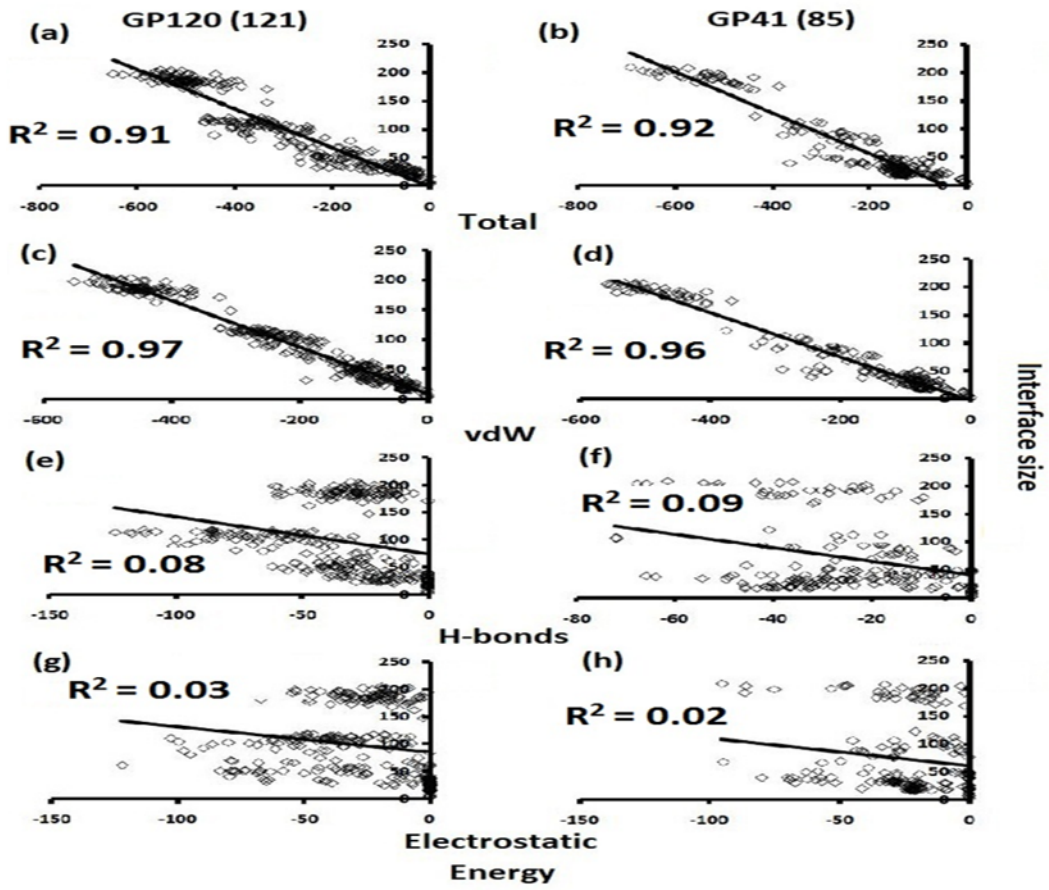

Figure 11: Correlation between interface energy and interface size of GP120 and GP41 interfaces is shown.

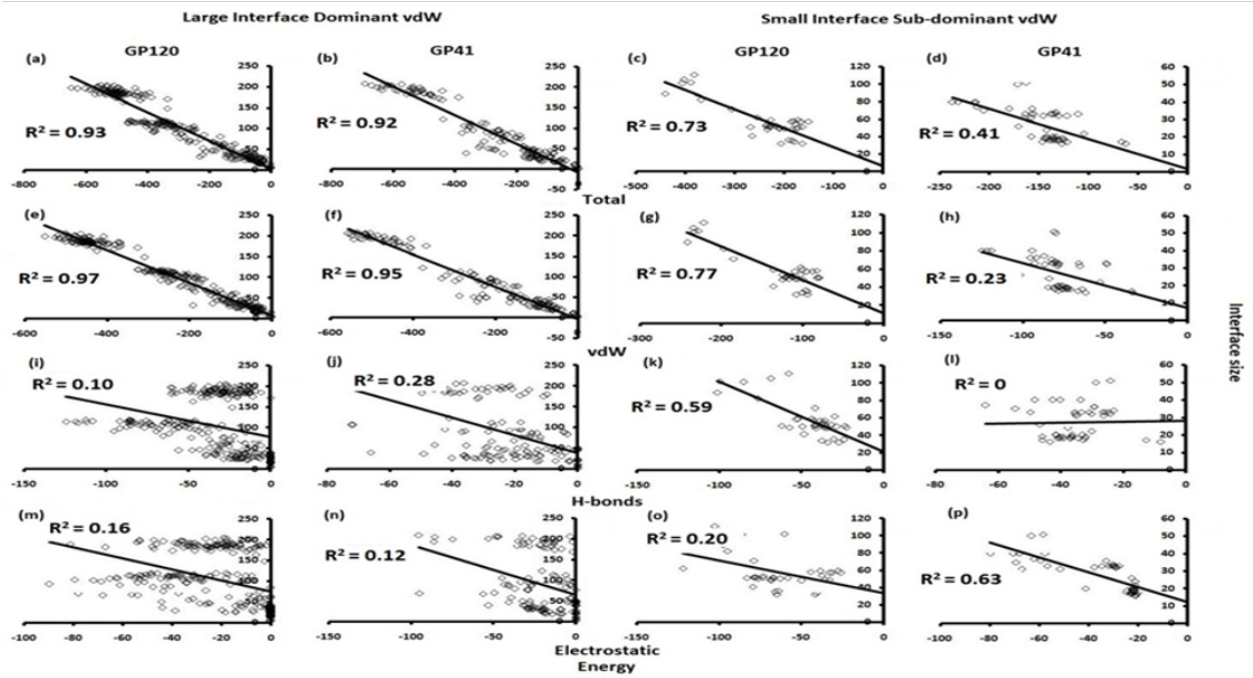

Figure 12: Correlation between interface energy and interface size for GP120 and GP41 interfaces in terms of large and small interfaces is shown.

It has also been reported that in small interfaces, total energy, van der Waals and hydrogen bonds decreases considerably with the increasing interface size whereas electrostatics moderately increases with interface size (Nilofer *et al.*, 2020). But, this is not the case with the small interfaces of GP120 and GP41. Surprisingly, we found electrostatics (r^2=0.63) (Figure 12p) to be highly pronounced in GP41 interfaces with subdominant van der Waals having van der Waals (r^2=0.23) (Figure 12h) and without hydrogen bonds (r^2=0) (Figure 12p) contribution. Contrastingly, we observed the small interfaces of GP120 to be highly stabilized by hydrogen bonds (r^2=0.59) (Figure 12k) followed by electrostatics (r^2=0.20) (Figure 12o). Hence, we report that hydrogen bonds (r^2=0.59) (Figure 12k) increases with the interface size in the small interfaces of GP120 and electrostatics (r^2=0.63) (Figure 12p) increases with the interface size in the small interfaces of GP41.

CONCLUSIONS

GP120 viral proteins interact with GP41 to form GP160 the HIV-1 trimer glycoprotein. Statistical analysis on the interfaces of GP120 and GP41 using interface area, interface size and interface energies including van der Waals, hydrogen bonds and electrostatics demonstrate that they are similar. 88% of GP120 and 74% of GP41 interfaces have large interface area and interface size with dominant van der Waals energy; while 12% of GP120 and 26% of GP41 interfaces have small interface area and interface size with subdominant van der Waals energy. In addition, small interfaces were observed to have three fold more of hydrogen bonds and electrostatics than large interfaces. It is shown hydrogen bonds to increase with interface size in the small interfaces of GP120; while electrostatics to increase with interface size in small interfaces of GP41 in absence of hydrogen bonds. These insights from the interfaces of GP120 and GP41 shows that our previous finding stating that small interfaces with small interface area are rich in electrostatics holds true in case of GP41 but not in the case of GP120.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Abagyan, R. A., Batalov, S. 1997. Do aligned sequences share the same fold. *Journal of Molecular Biology*, 273(1):355–368.

Adis Editorial 2003. HIV gp120 Vaccine-VaxGen: AIDSVAX™, AIDSVAX™ B/B, AIDSVAX™ B/E, HIV gp120 Vaccine-Genentech, HIV gp120 Vaccine AIDSVAX-VaxGen, HIV Vaccine AIDSVAX-VaxGen. *Drugs in R & D*, 4:249–253.

Alsalmi, W., *et al.* 2015. A New Approach to Produce HIV-1 Envelope Trimers. *Journal of Biological Chemistry*, 290(32):19780–19795.

Burton, D. R., *et al.* 2004. HIV vaccine design and the neutralizing antibody problem. *Nature Immunology*, 5(3):233–236.

Chen, B., *et al.* 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. *Nature*, 433(7028):834–841.

Doores, K. J. 2015. The HIV glycan shield as a target for broadly neutralizing antibodies. *FEBS Journal*, 282(24):4679–4691.

Grimm, S. K., *et al.* 2015. Directed Evolution of a Yeast-Displayed HIV-1 SOSIP gp140 Spike Protein toward Improved Expression and Affinity for Conformational Antibodies. *PLOS ONE*, 10(2).

Guenaga, J. 2015. Well-ordered trimeric hiv-1 subtype b and c soluble spike mimetics generated by negative selection display native-like properties. *PLoS Pathogens*, 11(1).

Hubbard, S. J., Thornton, J. M. 1993. NACCESS Computer Program. *Journal of Biophysical Chemistry*, 1(3).

Jones, S., Thornton, J. M. 1996. Principles of protein-protein interactions. *Proceedings of the National Academy of Sciences*, 93(1):13–20.

Kangueane, P., *et al.* 2008. Designing HIV gp120 peptide vaccines: Rhetoric or reality for Neuro-AIDS. *Spectrum of Neuro-AIDS Disorders*, pages 105–119.

Kinjo, A. R., *et al.* 2001. Physicochemical evaluation of protein folds predicted by threading. *European Biophysics Journal*, 30(1):1–10.

Lee, B., Richards, F. M. 1971. The interpretation of protein structures: Estimation of static accessibility. *Journal of Molecular Biology*, 55(3):379–383.

Moore, J. P., *et al.* 1990. Enhancement of soluble CD4-mediated HIV neutralization and gp120 binding by CD4 autoantibodies and monoclonal antibodies. *AIDS Research and Human Retroviruses*, 6(11):1273–1279.

Moore, J. P., *et al.* 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. *Journal of Virology*, 66(1):235–243.

Nilofer, C., *et al.* 2017. HIV-1 envelope (ENV) GP160 trimer protein complex SPIKE as a recombinant macromolecular assembly vaccine component candidate: Current opinion. *Global Virology II - HIV and NeuroAIDS*, pages 939–951.

Nilofer, C., *et al.* 2020. Small protein-protein interfaces rich in electrostatic are often linked to regulatory function. *Journal of Biomolecular Structure and Dynamics*, 38(11):3260–3279.

Rerks-Ngarm, S., *et al.* 2009. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *New England Journal of Medicine*, 361(23):2209–2220.

Rerks-Ngarm, S., *et al.* 2013. Extended Evaluation of the Virologic, Immunologic, and Clinical Course of Volunteers Who Acquired HIV-1 Infection in a Phase III Vaccine Trial of ALVAC-HIV and AIDSVAX B/E. *Journal of Infectious Diseases*, 207(8):1195–1205.

Ringe, R. P., *et al.* 2015. Influences on the design and purification of soluble, recombinant native-like HIV-1 envelope glycoprotein trimers. *Journal of Virology*, 89(23):12189–12210.

Sanders, R. W., Moore, J. P. 2017. Native-like Env trimers as a platform for HIV-1 vaccine design. *Immunological Reviews*, 275(1):161–182.

Shin, S. Y. 2016. Recent update in HIV vaccine development. *Clinical and Experimental Vaccine Research*, 5(1):6.

Sowmya, G., *et al.* 2011. HIV-1 envelope accessible surface and polarity: clade, blood, and brain. *Bioinformation*, 6(2):48–56.

Sukhwal, A., Sowdhamini, R. 2015. PPCheck: A webserver for the quantitative analysis of protein interfaces and prediction of residue hotspots. *Bioinformatics and Biology Insights*, 9:141–151.

Systèmes, D. 2020. BIOVIA, discovery studio visualizer, release 2019. *San Diego: Dassault Systèmes*.

Überla, K. 2008. HIV vaccine development in the aftermath of the STEP study: Re-focus on occult hiv infection? *PLoS Pathogens*, 4(8):e1000114.

Verkerke, H. P., *et al.* 2016. Epitope independent purification of native like envelope trimers from diverse HIV-1 isolates. *Journal of Virology*, 90(20):9471–9482.