Molecular docking analysis of RN18 and VEC5 in A3G-Vif inhibition

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Abstract:
The HIV-1 protein Vif is essential for *in vivo* viral replication that targets the human DNA-editing enzyme, APOBEC3G (A3G), which inhibits replication of retroviruses. The Vif-A3G interactions are believed to be important targets for antiviral drug development. Since the interactions of A3G and Vif evade the ubiquitination pathways in human host, the viral replication precedes which otherwise spreads infection. In this study, two potent Vif inhibitors RN 18 and VEC5 have been evaluated for their inhibitory potential employing ligand receptor and protein-protein interactions studies. VEC 5 showed better interaction with Vif than RN18. Predicted data show that VEC5 bound Vif and RN18 bound Vif showed diminished interaction to A3G compared to inhibitor unbound Vif. However, this should be further validated using in vitro studies.

Keywords: Vif, Vif inhibitors, RN18, VEC5, Protein – Protein docking, VIF-A3G interactions.

Background:

Vif is an essential protein for *in vivo* viral replication [1- 4] that targets the human DNA-editing enzyme, APOBEC3G (A3G) [5] thereby inhibiting replication of retroviruses [6, 7]. As Vif has no known cellular homologs, it is an attractive target for antiviral intervention. Most currently available antiviral drugs target the *pol*-encoded retroviral enzymes PR, RT, and integrase (IN); in addition, inhibitors that target HIV-1 envelope-receptor interactions have also been recently approved. Recent understanding of the interactions between HIV-1 and host restriction factors has provided fresh avenues for development of novel antiviral drugs. The host restriction factors include apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3) proteins which inhibit viral replication in the absence of Vif [8-10]. Vif suppresses A3G antiviral activity by targeting these proteins for polyubiquitination and proteasomal degradation [11-16].

HIV-1 Vif is a 192 amino acid protein that binds to A3G and forms an E3 ubiquitin ligase complex consisting of Elongin B, Elongin C, Cullin 5, and RING finger protein 1, which results in A3G polyubiquitination and degradation [16]. As a result, A3G's are not packaged into virions, and HIV-1 replication is spared from A3G-mediated inhibition. Pioneering studies of HIV-1Δ*Vif* viruses have shown that their replication is significantly delayed in nonpermissive cells [17], implying that interfering with the Vif- A3G interactions should strongly suppress viral replication. A large proportion of proviruses in infected patients is hypermutated [18-21] and has G-to-A mutations in RT and protease [22]. Interaction should allow the host APOBEC3 protein to carry out their natural activity and inhibit HIV-1 replication.

Methodology:
Molecular Modeling of Vif

Protein sequence of Vif Human immunodeficiency virus 1 retrieved from NCBI using accession num: NP_057851. The protein contains 192 amino acids and has Molecular weight of 22511.79 Daltons. 3D structure of Vif is not available in Protein Data Bank; hence 3D structure prediction of Vif has been

performed using computational homology modeling method. Similarity searching with Vif has been performed against PDB database for finding an appropriate template for homology modeling using BLAST. Top 10 templates were used for the

alignment against Vif. All the residues are colored in black; however, those residues in template which are identical to the residue in the query sequence are highlighted in color.
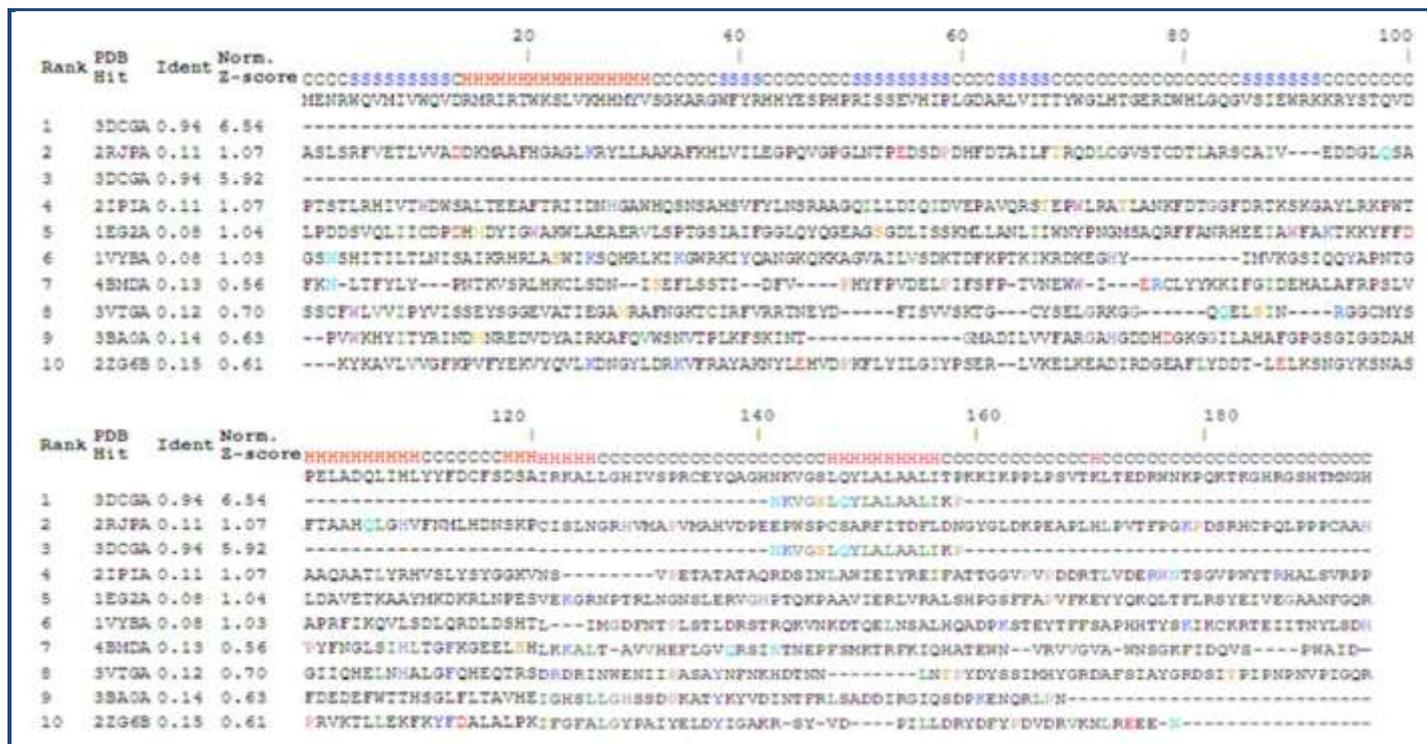
Figure 1: Alignment between top 10 templates and Vif

The entire top 10 template alignment file (.ali) has been used for building loops using modeler **(Figure 1).** MODELLER requires the sequence in PIR format in order to be read. The FASTA was converted to PIR using Readseq, an algorithm developed by EMBL. Structure similarity has been performed by using the profile.build(), an in-built command in MODELLER. The build_profile.py was used for the local dynamic algorithm to identify homologous sequences against target Vif Target sequence. The final model obtained from modeler further been used for structure validation using Procheck **[23, 24].**

Prediction of Inhibitory Site in Vif
The inhibitory site of the modeled structure was identified using Q site finder **[25]**. Q site finder is a method of ligand binding site prediction which works by hydrophobic (CH_3) probes to the protein and finding clusters of probes with most favourable binding energy.

Selection of Inhibitors
To inhibit the Vif-A3G interaction, two potent inhibitors - RN18 **[26]** and VEC 5 **[27]** were selected.

Ligand Receptor Docking
The optimized structures of RN 18 and VEC 5 were docked into the inhibitory site of Vif protein. Docking parameters were set to 0.20 Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood

distance factor of 1. Binding affinity and interactions of inhibitor with protein was evaluated on the basis of the internal ES (Internal electrostatic Interaction), internal hydrogen bond interactions and sp2-sp2 torsions.

Protein-Protein Docking Studies
Apolipo protein 3D structure was retrieved from Protein Databank with PDB accession number: 3V4J. Protein - Protein docking was executed through Patchdock server **[28]**. Patchdock algorithm is inspired by object recognition and image segmentation used in computer vision. Default parameter was set as clustering RMSD at 4.0.

Solvent Accessible Surface Area (SASA) and Interface Property Calculation
Solvent accessible surface area of the complexes was calculated by GETAREA server **[29]**, protein interfaces calculated by Aquaprot **[30]** and interface property was calculated using 2P2I inspector **[31]** online server.

Results:
Protein Model Evaluation and Details of Predicted Inhibitory Site
The procheck results revealed the modeled Vif protein to be bonafide **(Figure 2A).** In final model of the protein out of 167 amino acids 133 were in core region, 21 were in additional and 9 were in generous allowed region. Overall 97.6 percentages of amino acids were in allowed region. Hence the same has been used for the Protein - Protein studies with Apolipo protein. Three cavities with different volume were detected by Qsite

finder in Vif protein. As a convention, the volume with highest volume was considered to be an active (inhibitory) site. The inhibitory site had a volume of 841 Cubic Angstroms with minimum coordinates of (-21, -15, -10) and maximum coordinates of (-1, 14, 14) **(Figure 2B).**
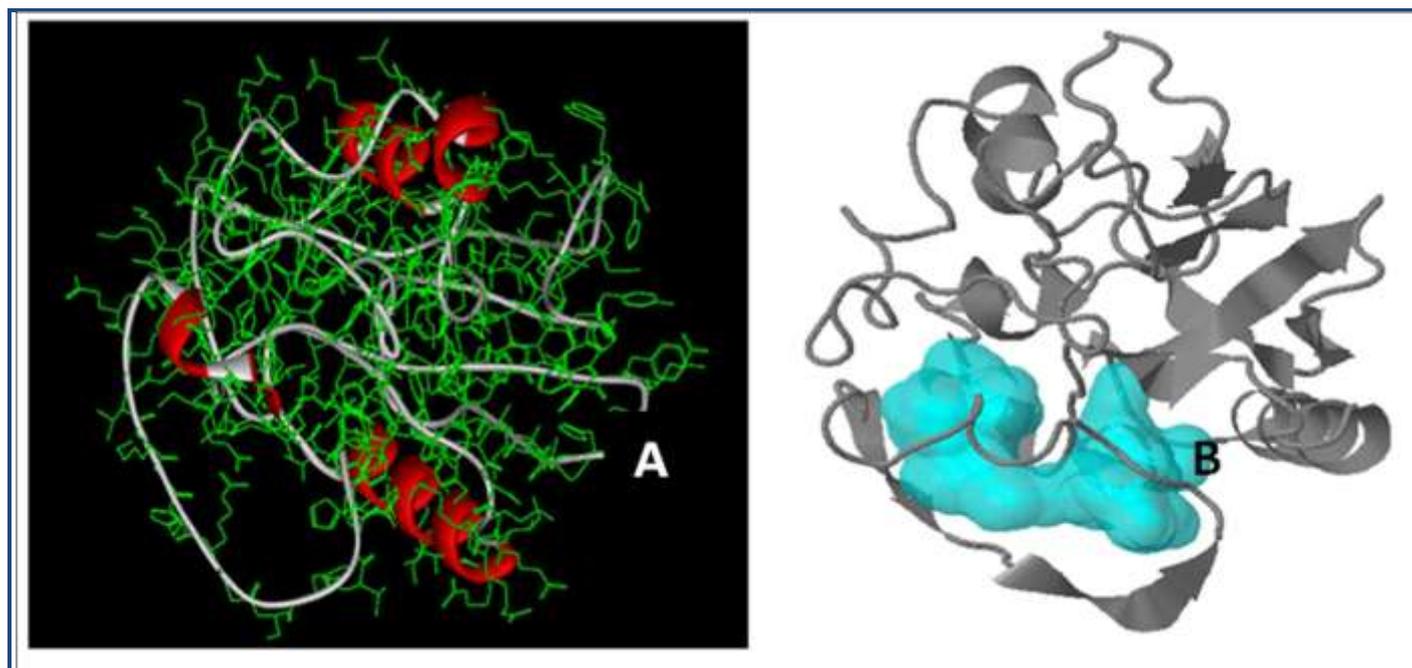
Figure 2: **A)** Modeled structure of Vif; **B)** Predicted inhibitory site in Vif protein.

Binding Affinity of RN18 and VEC5 against Vif

As evident from rerank score derived from Moldock and PLP aided algorithm, VEC5 showed better receptor ligand affinity to Vif compared to RN 18. **Table 1 (see supplementary material)** represents the docking scores of RN 18 and VEC 5 against Vif.

Protein –Protein Docking Studies

Three modes of protein-protein interactions were investigated in the present study. One, interaction between inhibitor unbound Vif with A3G, Two, interaction between RN18 bound Vif with A3G and three, VEC5 bound Vif and A3G interaction. **Table 2 (see supplementary material)** represents the comparative binding scores of RN18 and VEC5. Vif bound to RN18 and Vif bound to VEC5 showed almost similar binding affinity against A3G. Evident from the scores, Vif bound to RN18 and VEC5 showed declined interaction with A3G. **Figure 3A, B & C** respectively shows, inhibitor unbound Vif A3G interactions, RN18 bound Vif –A3G interactions and VEC5 bound Vif-A3G interactions

Interface Analysis

Table 3 (see supplementary material) shows complete profile of solvent accessible surface areas of the protein complexes. Complex of A3G and Vif bound to RN18 or VEC5 shows higher solvent accessible surface area than complex of unbound Vif-A3G (ΔSASA =1145.86), indicating declined interaction of A3G and Vif due to presence of inhibitors. Further the poor interaction of A3G and Vif is complemented by the calculated interface properties **Table 4 (see supplementary material).** There was almost twice (1.90) the decline in the interface area between A3G-Vif when bound to RN 18 or VEC 5. Further the gap index in the complex was increased by two folds double (2.05) in presence of RN 18 or

VEC 5 compared to the complex unbound by either inhibitor. A closer perusal in the interface in Vif unbound A3G interaction revealed that the interface areas are mostly comprised of coils. Since coils are flexible in nature they may assist the better protein-protein interactions. In case of RN 18 or VEC5 bound Vif, the interfaces prominently are helices which are less flexible in nature thereby discouraging the decline interactions between Vif and A3G. **Figure 4** shows the interacting residues and electrostatic interfaces.

Discussion:

In the absence of an effective vaccine or antiviral treatments, AIDS is likely to expand and continue to claim the lives of millions for decades. Despite heroic efforts over the last 25 years, a protective vaccine is not currently on hand, and the recent suspension of the Merck vaccine trial suggests that an effective vaccine is not likely to be available in the near future [32, 33]. Since the approval of AZT in 1987 [34] approximately 30 anti-HIV drugs or drug combinations have been approved for clinical use. An understanding of the interactions between Vif, A3G, and the proteasomal degradation pathway is essential for developing novel drugs for therapeutic intervention. In a view of above, we made a possible attempt at the molecular planes to probe into the A3G-Vif interactions in presence and absence of inhibitors. The study revealed the potential inhibitory actions of drugs RN18 and VEC5. Both inhibitors successfully hampered the A3G-Vif interactions as evident from interface analysis where in, there was almost twice decline in the interface area between A3G and Vif in presence of these inhibitors. In addition, flexible coils were replaced by rigid helices in the interface area confirming the declined protein-protein interactions. Further, the interface area between inhibitor (RN18 or VEC5) bound Vif and A3G has twice the number of charged residues in comparison to

interface between A3G and inhibitor unbound Vif. The charged residues may likely confer repulsive columbic forces at the interfaces which perhaps may result in increase in gap volumes in turn decreasing the interface areas.
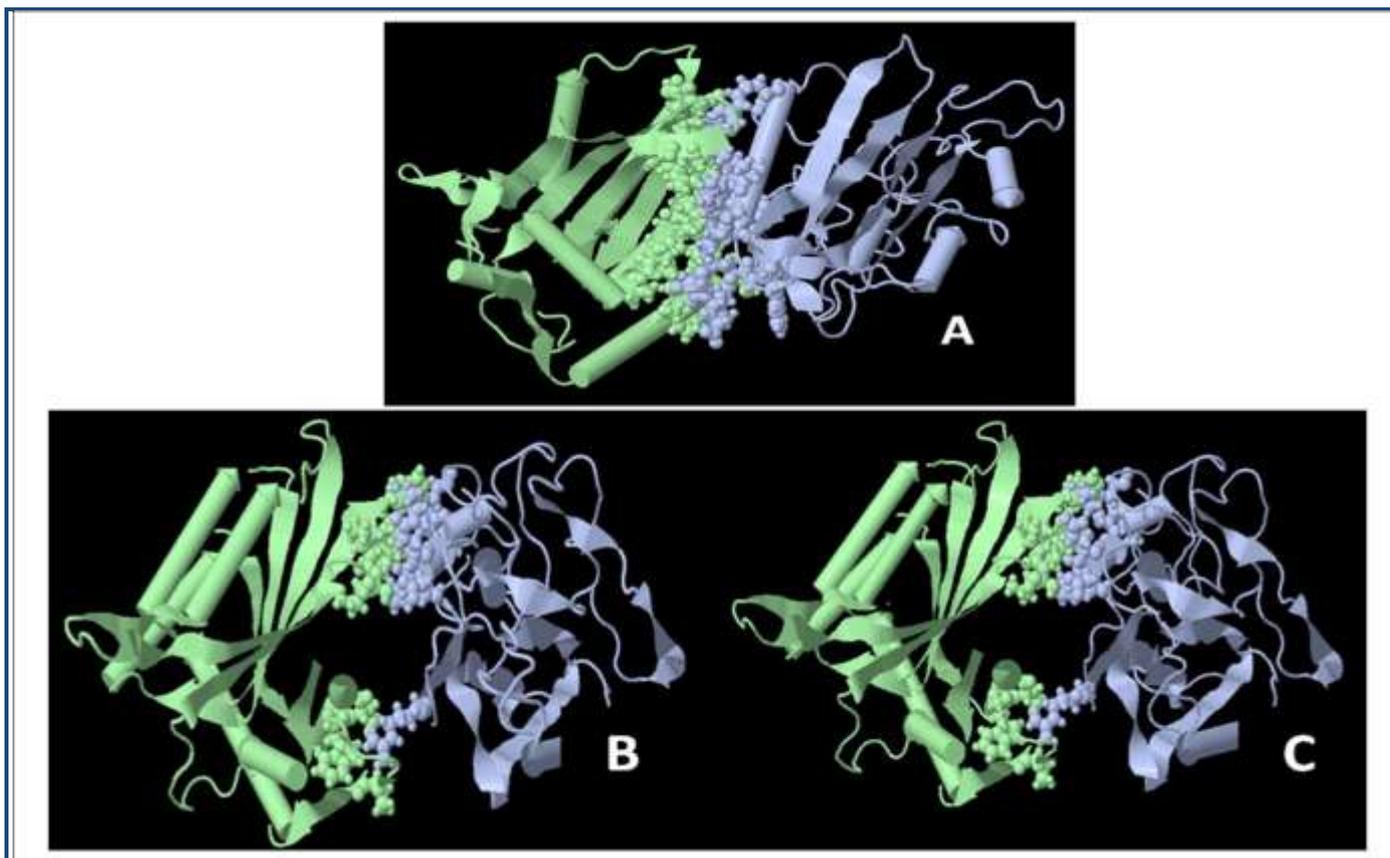
Figure 3: Interface residues (in ball and stick representation) between A3G (violet) and Vif (green) in **A)** A3G-Vif (unbound); **B)** A3G-Vif (bound to RN18); **C)** A3G-Vif (bound to VEC5)
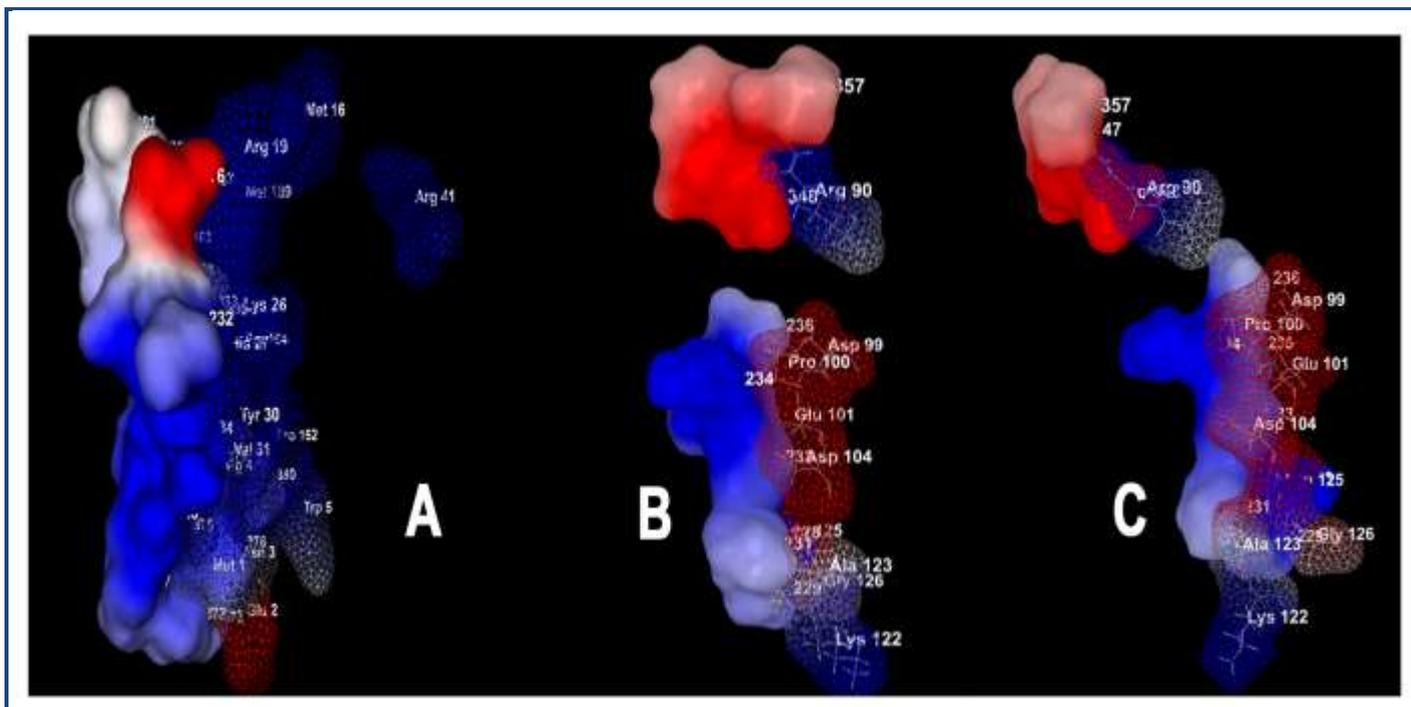
Figure 4: Electrostatic surfaces of Vif (Solid) and A3G (mesh) in **A)** A3G-Vif (unbound); **B)** A3G-Vif (bound to RN18); **C)** A3G-Vif (bound to VEC5) interactions.

Conclusion:
Our interest was to analyze the inhibitory potentials of RN18 and VEC5 in inhibiting Vif and A3G interactions. In the structural planes, we were able to comprehend the possible rationale underlying the decline A3G-Vif interactions in presence of inhibitors like RN18 and VEC5. Though our interpretations were approached *in silico*, our study can be complemented by *in vitro* studies for real time understanding underlying the basis of A3G-Vif interactions.

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Supplementary material:

Table 1: Energy overview of RN18-Vif and VEC-Vif interaction

Energy overview: Descriptors	RN18	VEC5
Total Energy	-112.38	-101.87
External Ligand interactions	-144.54	-128.28
Protein - Ligand interactions	-144.54	-128.28
Steric (by PLP)	-114.01	-103.13
Steric (by LJ12-6)	-27.10	-23.18
Hydrogen bonds	-3.43	-1.97
Internal Ligand interactions	32.16	26.41
Torsional strain	7.08	1.44
Steric (by PLP)	5.18	5.85

Table 2: Binding scores between A3G and inhibitor bound and unbound Vif

Interactions	Score	Area	Transformation
Rn 18 bound Vif and A3G	14046	1851	-1.41 0.30 -0.83 -26.86 11.06 0.51
VEC5 bound Vif and A3G	14054	1852.9	-1.41 0.30 -0.83 -26.86 11.06 0.51
Vif unbound to inhibitors and A3G	15492	2552.8	-3.04 0.36 0.58 11.17 -11.88 -18.65

Table 3: Change in solvent accessible surface area In A3G-Vif complex

ACCESIBLE AREA/ENERGY ($\mathring{A}^2$ Kcal/mol)	A3G-Vif (unbound)	A3G-Vif (RN18)	A3G-Vif (VEC5)	ΔSASA = [SASA $_{A3G\ VIF(RN18)\ COMPLEX}$-SASA $_{A3G\ VIF(UNBOUND)}$ COMPLEX]	ΔSASA = [SASA $_{A3G\ VIF(VEC5)\ COMPLEX}$-SASA $_{A3G\ VIF(UNBOUND)}$ COMPLEX]
POLAR area/energy	6044.71	6399.66	6399.66	354.95	354.95
APOLAR area/energy	11320.41	12111.33	12111.3	790.92	790.92
Total area/energy	17365.13	18510.99	18510.99	1145.86	1145.86

Table 4: Interface properties in complexes of A3G-VIF (unbound), A3G-VIF (RN18) and A3G-VIF (VEC5)

INTERFACE PROPERTIES	A3G-VIF(unbound)	A3G-VIF(RN18)	A3G-VIF(VEC5)
Total interface area ($\mathring{a}^2$)	2297.500	1166.200	1166.200
Gap volume ($\mathring{a}^3$)	5211.000	5447.250	5447.250
Gap index	2.268	4.671	4.671
% Charged residues	26.300	50.000	50.000
Non-bonded contacts	313.000	147.000	147.000
Hydrogen bonds	2.000	2.000	2.000
Salt bridges	1.000	1.000	1.000
disulfide bonds	0.000	0.000	0.000
Secondary structure at interface	Coil	Alpha	Alpha