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Structure and Thermodynamics of Surface Recognition

Goutam Gupta*

Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). Interactions of the surface glycoprotein, gp120, with the receptors of host cells define the pathogenesis of HIV-1, the virus that causes AIDS. gp120 is made of several disulfide-bridged loops—the amino acid sequences of some of these loops are fairly conserved whereas the rest are variable. The third variable (V3) loop has been the target of vaccine design for quite some time since this loop is involved in various steps of viral pathogenesis. However, this loop also happens to be the most variable one. We have carried out structural and immunological studies to determine the sequence-structure-antigenicity correlations of the HIV-1 V3 loops. This resulted in the identification of a secondary structure at the tip of the V3 loop that remains invariant in spite of the sequence variation. We designed a multi-valent V3-based antigen that presents multiple copies of the same tip element several times in the same structure. During the course of this project, we realized that the protective epitopes of gp120 should be judged in the context of the native structure. Therefore, we developed a method to obtain a model of gp120 that is consistent with all the immunology and virology data. This model is useful in choosing or designing gp120 subdomains for vaccine development.

Background and Research Objectives

Vaccine development against acquired immunodeficiency syndrome (AIDS) requires a thorough understanding of the pathogenesis of the human immunodeficiency virus type 1 (HIV-1) that causes AIDS. The interaction of the HIV-1 surface glycoprotein, gp120, and the receptors on the host macrophages and T cells define the first step in HIV-1 pathogenesis [70]. The CD4 molecules are the primary receptors on both macrophages and T cells [76,79]. However, co-receptors are different on these cell types, i.e., chemokine receptors, CCR, are present on macrophages whereas chemokine receptors, CXCR, are present on T cells [16,21-23,25,62,96]. Sequence determinants (or epitopes) for CD4 and CCR or CXCR binding reside on gp120 that is made up of several well-defined disulfide-bridged constant (C) and variable (V) subdomains or loops linked as C1-V1-V2-C2-V3-C3-V4-C4-V5-C5 (see Figure 1 and ref. 54). Receptor and antibody binding

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experiments [42,53,71-73,77,85-86] reveal that a discontinuous epitope formed by residues in C2, C3, and C4 define the contact interface of gp120 for CD4 binding. It has been shown that the V3 loop in native gp120 is critical for CCR or CXCR binding [3,21-23]. In fact, the V3 loop has also been implicated in various other aspects of viral pathogenesis: (1) HIV-1 tropism, i.e., sequences inside the loop determines the cell type that is infected by a given virus [9,15,24,44-45,82]. (2) virus-cell fusion, i.e., a set of residues inside the V3 loop are critical determining the process [43,46,61,81,91], and (3) the V3 loop may functionally or spatially interact with the CD4-binding region of gp120 [1,8,28,60,86,90,92-3,95].

Since it is involved in various aspects of HIV-1 pathogenesis, the V3 loop was thought to be an important target for vaccine development [14,47-48,78]. In fact, the use of viral or recombinant gp120 resulted in several monoclonal antibodies (mAb) that map to the V3 loop [33-37]. However, since the V3 loop showed extreme sequence variability across HIV-1 isolates, these antibodies were not cross-reactive, i.e., one V3-specific mAb neutralized only those HIV-1 isolates bearing the same or similar V3 loop and not the others [50-52,57,63]. Therefore, the advantage gained by the enhanced immunogenicity of the V3 loop relative to the CD4-binding regions of gp120 is offset by its extreme sequence variability.

The specific aims of this project were:

- to understand the sequence-structure-antigenicity correlation of the HIV-1 V3 loops, and
- to identify a conserved secondary structural element inside the V3 loop and exploit the same to engineer a set of V3-specific HIV-1 multi-valent antigens that multiply present the same conserved secondary element.

However, during this project, we realized that it was not enough to study a given HIV-1 epitope (such as the V3 loop) in isolation and it should be studied in the context of the native gp120. We also realized that we needed to expand our studies to other gp120 epitopes (i.e., their structures and interactions in the native gp120). Therefore, we added another specific aim:

- to obtain a working model of gp120 that realistically describes the structures and interactions of various constant and variable loops.

We believe that successful completion of this aim would enable us to delineate the structure-antigenicity correlation of continuous and discontinuous surface epitopes in the native gp120 which, in turn, would lead to more realistic choice of gp120 subdomains as vaccine candidates.

Scientific Approach and Accomplishments

On the sequence-structure-antigenicity correlation of the HIV-1 V3 loops

Theoretical studies [40-41] revealed that the variability in sequence and structure of the V3 loop is confined to the N- and C-terminal sides of the conserved GPG-crest. This leaves three regions of the V3 loop conserved both in sequence and secondary structure. Figure 2 shows the V3 loop sequences of various HIV-1 isolates; the three conserved secondary structural elements are underlined.

We have carried out NMR studies [10-11,39] to test the validity of our theoretical predictions. Structural studies were performed for the HIV-MN V3 loop in the linear and cyclic (S-S bridged) forms. While the linear V3 loop in water is devoid of ordered structure except for a loose turn at the GPG-crest, the cyclic form shows a well defined structure in water. Moreover, in (7:3) water: TFE mixed solvent (less polar than water), the cyclic V3 loop shows higher order both in terms of secondary structure content and rigidity (see Figure 3). The three conserved regions of the HIV-MN V3 loop in the mixed solvent adopt the predicted secondary structural elements. TFE-induced helix formation in the C-terminal segment is also documented by us in the Haitian V3 loop. TFE induced, less polar environment for helix stabilization is biologically relevant in the context of the native structure of gp120. As discussed in the following section, our modeling studies show that the proximity of the C3 region provides a hydrophobic environment for the C-terminal segment of the V3 loop (see Figure 7).

The observation of the three conserved secondary structures in the V3 loop leads to a simple rule that the sequence variability of the V3 loop can be tracked by finding the associated variability brought about by different structural elements on either side of the GPG-crest. Finally, the conformational requirement of the ND in the V3 loop-antibody interaction is tested by monitoring the mAb binding to the HIV-MN V3 loop in the linear and cyclic forms by ELISA. The binding data in Figure 4 reveal that the cyclic V3 loop is a better ligand for the mAbs than the linear form although the latter has the same sequence. The cyclization through the (S-S)-bridge between C1 and C35 and changing the solvent environment provide interesting insights into the structure-function correlation of the HIV V3 loop.

Sequence Variability at the Two Ends of the ND: Camouflaging of the Conserved Secondary Structural Element

NMR studies on the V3 loop sequences listed in Figure 2 are summarized as follows (also see Figure 3). (i) A GPG type II turn is present at the crest of the V3 loop in all the sequences. (ii) Stretches of β -strand adjacent to the GPG-turn on the N- and C-terminal side are common to all the sequences. (iii) The residues in the C-terminal segment form a few turns in water and a helix in the less polar mixed solvent. (iv) In spite

of the constraints of secondary structures [(i)-(iii)] and the disulfide bridge, the V3 loop exhibits conformational flexibility as evidenced by the absence of long range NOESY interactions commonly observed in well folded globular proteins. However, a "protruding knob" formed by the central GPG-turn and the β -strands on either side emerges as the secondary structural feature conserved among diverse V3 loop sequences.

The single crystal structure of the HIV-1 neutralizing antibody (mAb 50.1) complexed to 16-residue long linear MN V3 fragment shows the hint of such a "protruding knob" although the segment on the C-terminal side of the GPGR type turn remains disordered [32,94]. The crystallographic observation suggests that the protruding knob of the V3 loop that includes the neutralizing epitope might well be specifically recognized by the antibody. However, we cannot count on the fact that the conserved "protruding knob" of the V3 loop will always be presented in its conformationally pure form because HIV will always find a way to mask this conserved secondary structural element.

In this work we report one such mechanism of masking as revealed by the "close" state in Figure 5. In this form of the Haitian V3 loop, the NMR data indicate an arching of the residues on the C-terminal side of the GPGK-turn. This is a departure from the "protruding knob" motif that contains the central GPG-turn and two β -strands on either side. Such an arched conformation of the neutralizing epitope has also been observed in an antibody (mAb 59.1) complexed with a linear V3 fragment [36]. When combined with the single crystal data on mAb-V3 complex, our NMR data indicate that the "closed" or "arched" conformation of the neutralizing epitope of the V3 loop is possible and can be recognized by the antibody. In addition, our data also indicate that an equilibrium between the "closed" and "open state" (Figure 5) is possible. The arching around A20-F21 tends to mask A20 and F21 as shown by the solvent exposure data of the open and close forms of the Haitian V3 loop.

The close form of the V3 loop may camouflage some essential elements of the neutralizing epitope from the immune system. For instance, this masking will interfere with the binding of antibodies that recognize the PGRAF epitope. Most importantly such a local masking of A20 and F21 should affect the proteolysis of the R/Q/K19-A20 peptide bond by thrombin and tryptase [17,66]; the second enzyme lies on the T-cell surface. When gp120 is used as a substrate, unlike other proteases, these two enzymes show exceptional specificity for cleavage of the R/Q/K19-A20 peptide bond inside the V3 loop. The most striking is the observation that the V3 loops of T-cell tropic virus strains are 1,000 times more susceptible to cleavage by these two enzymes than the V3 loops of macrophage tropic strains. The T-cell tropic V3 loops are more positively charged than the macrophage tropic V3 loops. Our studies reveal that the open state of the neutralizing epitope of the V3 loop is exclusively preferred for MN and RF V3 loops with net charges $\geq$

+5 whereas the close state of the neutralizing epitope begins to appear for the Haitian V3 loop with net charge of +3. Therefore, we believe that the proteolysis data [17,66] are consistent with our structural conclusions.

We have carried out two-dimensional (2-D) NMR and molecular modeling studies on three mini V3 loops, which are 17-amino-acids-long derived from the MN, Florida, and Thailand V3 sequences (Figure 2). These mini V3 loops contain the central GPG and the flanking sequences that are required for antibody binding. We show that the presence of the (S-S) bridge between the 1st and the 17th C leads to a β -hairpin conformation for all three mini V3 loops with quite different sequences. Therefore, by this design the conformational camouflaging can be avoided.

Effect of Single Site Mutations at the GPGR-Crest of the V3 Loop of gp120

It has been shown that any mutation in the GPG-sequence of the V3 loop that destabilizes the type II turn also affects the fusion activity of the virus. This suggests that the type II turn in the V3 loop is critical in the life cycle of the virus. The four residues in GPGR/K/Q are numbered as G1, P2, G3, and R/K/Q4, respectively. The positions 1 and 3 in the (ϕ, ψ)-plot show stereochemical preference for G. This is especially true for the position 1, which should strictly prefer G and probably can accommodate A with a distortion in the turn [75]. Interestingly HIV-1 mutant with G1->T1 mutation in the type II turn leads to a non-infectious virus for Sup-T1 T cells [61]. However, after 40 days of coculture a T1->A1 revertant is identified. This revertant becomes infectious to Sup-T1 T cells. Note, that T->G mutation requires 3 base changes in the codon while T->A reversion requires a single change in the second position of the codon.

Experiments discussed above and our NMR data prove the importance of a type II turn at the GPG-crest of the V3 loop for viral pathogenesis. However, it remains unclear whether residues like R/K/Q are also critically needed following the GPG sequence. We (in collaboration with the NCI/NIH) set up the following experiment (unpublished data) in order to examine the importance of a basic (R/K) or a neutral (Q) residue after the conserved GPG sequence. We performed molecular modeling of various V3 loop sequences with a GPGE-crest (instead of GPGR/K/Q) and our results showed that the global structure of the V3 loop remains the same as well as the type II turn at GPGE. We then constructed HIV-1 mutants with GPGR->E mutants inside the V3 loop of gp120. The mutant virus replicated as well as the wild one. The mutant virus also expressed the same number of gp120 molecules on the surface as the wild one. However, the mutant virus was the NSI type while the wild one was the SI type. But after a passage of three weeks in the co-culture, a revertant population of the virus (70%) was discovered. This revertant virus changed from GPGE to GPGK sequence and the virus became SI active. Note that E->K reversion requires only a single base change in the first position of the triplet codon. This

leads us to conclude that R, K, or Q after GPG sequence is important in the life cycle of the virus.

Conserved Structure at the Immunogenic Tip of the V3 Loop: Design of a Chimeric Multivalent HIV Antigen that Contains Multiple Copies of this Conserved Structural Element

We carried out molecular modeling and 2-D NMR studies on the V3 loops sequences shown in Figure 5 to identify the structural features of the V3 loop, especially at the neutralizing determinant (ND), that remain conserved irrespective of the sequence variation. The conserved structure of the ND is a solvent accessible protruding motif or a knob (Figure 6). Interestingly, we also showed (Figure 6) by 2-D NMR spectroscopy [13,26] that the HIV ND knobs are structurally isomorphous with the immunodominant knobs in the tandem repeat protein, human mucin Muc-1 (a tumor antigen for breast, pancreatic, and ovarian cancer). Each 20 amino acid repeat of Muc-1 consists of (TSAPDTRAPGSTAPPAHGV). The antigenic knob of Muc-1 is located at APDTR. Therefore, we replaced the mucin antigenic knobs by the HIV ND knobs in a set of chimeric Muc-1/V3 antigens. The repeat sequences of the chimera are: (TSGPGRAFAPGSTAPPAHGV)_n, (IHIGPGRAFAPGSTAPPAHGV)_n, and (HIGPGRAPAPGSTAPPAHGV)_n. The V3 inserts are underlined. This produced multivalent HIV antigens in which NDs are located at regular intervals and separated by extended mucin spacers. We show by 2-D NMR spectroscopy that the multivalent antigens preserve the NDs in their native structure. We have also demonstrated by enzyme-linked-ELISA that the antigens correctly present the NDs to produce binding with monoclonal antibodies (mAbs) and polyclonal antisera from AIDS infected patients [26-27]. The antibody binding of these chimera is equivalent to the cyclic form MN V3 loop.

Muc-1/V3 antigens are unique in the following ways. (i) NMR and antibody binding data [11] verify that they reproduce the native structure of the NDs even when they are presented in the context of a totally unrelated protein like mucin Muc-1. (ii) Immunogens containing identical NDs within the Muc-1 chimeras effectively allows enhanced presentation of a conserved structural feature of the virus in a fashion not possible with non-chimeric HIV antigens. The true advantage of this approach will be to induce either T-dependent or T-independent antibody responses to the ND depending on the precise construction of the antigen. (iii) Multiple NDs, present in these chimeric proteins, may be advantageous in enhancing the immune response by significantly increasing the affinity of antibody binding. The importance of multiple NDs being present in the same antigen becomes clear by analyzing the relative binding of Muc-1/V3 (HIGPGRAPAPGSTAPPAHGV)_{3,6} peptides to different antisera. The data show that the 120 residue peptide is a better ligand than the 60 residue peptide for the majority of the antisera we tested. This is probably due to the fact that the higher number of ND knobs in

the 120 residue peptides are correctly disposed along the long axis of the molecule to facilitate the binding of bivalent antibodies. (iv) Alternatively, the nature of the Muc-1/V3 structure (Figure 6) suggests that if two or more different NDs are grafted alternately along the chain, there is enough flexibility in the spacers such that two or more antibodies specific for two different NDs can both bind bivalently, interdigitating along the molecule. Finally, there is no reason why more than two NDs cannot be introduced in the molecule. This may be critical in designing vaccines for a highly mutating pathogen like HIV.

A Model of gp120

We used molecular modeling to construct a model of gp120 that contains the V1-V2-C2-V3-C3-V4-C4 fragment; the C1 and V5/C5 fragments are ignored. We performed molecular dynamics (MD) simulated annealing to sample different conformations such that the following conditions are met. (i) the local structures of the V1-V2, C2b, V3, V4-C4 (C2b is the two disulfide-bridged loop inside the C2 region in Figure 1) kept close to the model derived from our MC simulated annealing method which was developed for studying the V3 loop. The steric restriction due to glycosylation is crudely imposed on the N's by placing a bulky group with 5 Å van der Waal radius.

We will refine the model later after obtaining the detailed NMR-derived structures of various domains. The presence of an α -helical segment inside V2 is consistent with many secondary structure prediction algorithms and also with our CD data. The presence of an α -helical segment inside C4 is again consistent with various secondary structure prediction algorithms and CD data on linear peptide containing C4 sequences [72-74]. (ii) It is seen that the surface exposure parameters of the model are consistent with the data from the immunochemical map [58]. (iii) It is also seen that the observed long-range interactions between various domains of gp120 are satisfied [1,8,28,60,86,90,92-3,95].

Figure 7 shows a representative low energy structure of the V1-V2-C2-V3-C3-V4-C4 fragment of gp120. It is clear that the tip of the V3 loop, the helix inside the V2, and a part of the C4 loop are all solvent exposed. Theoretical solvent accessibility data are computed for all the residues in our model of the V1-V2-C2-V3-C3-V4-C4 fragment; the computed values agreed well with the observed data [58]. Three types of H-bonds are present in the model and they originate from three different types of interactions, namely, backbone-backbone, backbone-sidechain, and sidechain-sidechain (the last two interactions are sequence specific). For example, in our model (Figure 7) OH-Y435 (of C4) is H-bonded to OD1-D22 (of V1) and NZ-K341 (of C3) is H-bonded to OE1/OE2-E440 (of C4). Key hydrophobic interactions are also present that involve residue-pairs from different domains. Most of these interactions involve residue-pairs inside a tight cavity showing either van der Waal contacts [e.g., I186 (of V2)---I337 (of V3)] or stacking overlaps [e.g., Y306 (of V3) and W427 (of C4)]. Figure 8 shows the inter-domain interactions involving

(A) (V3 and V1/V2) and (B) (V3 and C4), i.e., N*135-T136-**T137-N138**-N139-N140 (of V1), **N*300**-C301-T302-**R303**-P304-N305-Y306-N307 (of V3), and Q422-I423-I424-N425-M426-**W427**-Q428-E429-V430-G431-K432-A433-M434-**Y435**-A436 (of C4) -- key residues in these segments are marked in bold (N*=glycosylatedN).

As shown in Figure 8a, the linear V2 epitope adopts a helix whereas the conformational V2 epitope adopts a β -hairpin in the folded gp120. Also note that the β -hairpin is involved in several inter-domain long-range interactions with the residues in C2 and V4. The turn at residues 135-140 of V1 makes contact with W427 in C4; W427 is critical for the binding of the CD4-blocking antibodies. Experimental data also support the presence of V1-C4 interactions in the native gp120. K183/L184/D185 of the V2 loop are all involved in inter-domain H-bonds. The C=O of L184 is H-bonded to the basic sidechain of R278 whereas the acidic sidechain of D185 is H-bonded to the basic sidechain of K212; also the sidechains of E274 and L184 are within 5 Å. Therefore, if such a β -hairpin is critical for antibody binding a double site mutation, L184/D185-D184/L185, would be catastrophic because this would bring D184 close to E274 and L185 in steric clash with K212 (both of which would destabilize the β -hairpin).

Indeed, a L184/D185-D184/L185 double mutation reduces the binding affinity of antibodies specific for conformational epitopes inside the V2 loop. R303 and N*300 form sidechain-sidechain H-bonding, which stabilizes a turn at residues 300-303. This turn (as shown in Figure 8b) brings the Y306 ring in close proximity with the W437 ring. The turn at residues 300-303 also locks R303 in H-bonding interactions with (G441 and Q442) and N*300 in H-bonding interactions with (A436 and P437). The key residues, W427 and Y435, also show sidechain-backbone H-bonds with N138 and N*140. Therefore, we predict that the residues N*300 and R303 of V3 and the residue W427 of C4 are critical in bringing V1, V3, and C4 in spatial proximity. Interestingly from antibody binding studies, it has been demonstrated that R303G substitution exposes the N-terminal V3 fragment: from our model we predict that such a substitution would abolish several key interactions involving the (V1 and V3) and (V3 and C4) loops. Similarly, antibody binding studies reveal that W427S substitution exposes the residues 420-435 in the C4 fragment: from our model we also predict that such a substitution would disable several key interactions involving the (V1 and V3) and (V3 and C4) loops.

Conclusion

Results reported in this report show that appropriate use of gp120 surface epitopes for developing vaccine against AIDS require a thorough study that provides the information about these epitopes in the native protein because not only the V3 loop but also

other loops on gp120 can be functionally important and immunogenic [29,50,56,59]. The modeling of gp120 developed by us [10] is significantly more reliable than those previously described [30-31]. The structure of the gp120 model shown in Figures 7 and 8 enables us (a) to identify continuous and discontinuous surface epitopes in the native gp120 and (b) to describe their structures and dynamics. This information is useful in choosing or designing gp120 subdomains for vaccine development.

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domain	well exposed	poorly exposed	buried or shielded	no information
C1	31-54	85-95	110-120	55-61
	62-71	100-110		71-85
V1	140-150			120-140
				150-158
V2	169-182	158-171		182-195
C2			210-231	195-210
				231-250
		275-280	250-280	280-294
V3	304-322	294-301	320-330	
C3			360-393	330-360
V4		391-405		405-423
C4	423-440		438-451	450-461
V5		461-470		
C5	499-511		465-498	

A schematic representation of HIV-1 gp120

Figure 1. A schematic representation of gp120 with various constant (C) and variable loops. Also shown as an inset on top, the surface accessibility of different epitopes inside constant and variable loops.

turns turn helix

V3-MN:CTRPNYNKRKRIHIGPGRAFYTTKNIIGTIROAHC

Mini V3 : CRIHIGPGRAFYTTKC

V3-RF :-----N-T--S-TK----VI-A-GQ---D--K---

V3-Florida:-----YT--G-R-----V-AAEK---D--R---

Mini V3 : CG-R-----V-AAEC

V3-Haiti :-----D-T--S-PM---K---A-GD---N-----

V3-Thailand :-----SN-T-TS-T----QV--R-GD---D--K-Y-

Mini V3 : CS-T----QV--R-GC

Figure 2. Cyclic V3 and Mini-V3 Loops.

Figure 3. Structures of the MN, Haiti, and RF V3 loops in (top) water and in (bottom) 30% TFE/water mixture. All the V3 loops have a disulfide bridge between C1 and C35. A helical conformation is induced at the C-terminus of the cyclic V3 loops in non-polar mixed solvent.

Figure 4. An ELISA showing the preference of monoclonal antibodies (all derived from patients infected with AIDS) for the cyclic over the linear form of the HIV-MN V3 loop. Human mAbs 1510 (Top) and 1511 (Center) and murine antibody 1289 (Bottom) all bind to a greater extent to the cyclic V3 loop peptide. The recognized epitopes 1510 (Top), 1511 (Center), and 1289 (bottom) are shown on the right. In the schematic representations of the HIV-MN V3 loop shown on the right, solid circles depict hydrophobic residues, open circles charged residues, and outlined circles polar uncharged residues. BIAcore measurements [87] re-confirm the ELISA data.

Structural Camouflaging of the Neutralizing Domain inside the V3 Loop of the gp120 Haiti isolate

Figure 7D

Figure 5. Cryptic nature of the amino acids on the C-terminus of the GPG-turn. The crest of the V3 loop consists of 10-12 residues containing the GPG-turn and the N- and C-terminal flanking sequences. The GPG-crest of the Haitian V3 loop is in an "open" structure in water whereas the same crest adopts a "closed" structure in the non-polar mixed solvent. A key NOE involving G18 and F21 is marked for the "closed" state.

Figure 6. (A) Superimposition of the protruding motifs of two NMR structures: the V3 loop from the HIV-MN isolate (designated as MN) and that from the Thailand TN243 isolate (named TN). The sequences of two motifs are: MN, RIHIGPGRAFYT and TN, SITIGPGQVFYR. Note that the GPGR or GPGQ crests are oriented in the same way. (B) The principle of design. The V3 sequences above the Muc-1 sequences actually replace the Muc-1 residues in the chimeras.

The HIV-1 GP120

Figure 7. A ribbon diagram of the (V1-V2-C2-V3-C3-V4-C4) fragment of MN gp120. This is a representative model of gp120 that is consistent with the data from immunochemical maps and other functional assays. Color coding: green for V1, blue for V2, red for V3, magenta for C4, gray for C2 & C3, and yellow for the (S-S) bridges.

Figure 8A. Inter-domain interactions of the residues in the (V1-V2) sub-domain. Color coding: purple for V1, yellow for the (S-S) bridges, cyan for the linear epitope in V2, magenta for the conformational epitope in V2, gray for the rest of the V2, green for the interacting polar residues, and red for the interacting non-polar residues. Note that the linear V2 epitope adopts a helix whereas the conformational V2 epitope adopts a β -hairpin in the folded gp120. Also note that the β -hairpin is involved in several inter-domain long-range interactions with the residues in C2 and V4. The turn at residues 135-140 of V1 makes contact with W427 in C4; W427 is critical for the binding of the CD4-blocking antibodies.

Figure 8B. Long-range inter-domain interactions involving residues from the V1, V3, and C4 loops. Color coding: magenta for V4, blue for C4, yellow for the (S-S) bridges, green for interacting charged residues from the V1, V3, and C3 regions, red for the interacting non-polar residues from the V1, V3, and C3 regions. A turn at V1, the N-terminal V3 segment, and a helical stretch in C4 form an intricate contact interface. P437 is critical in inducing a sharp turn in C4.