Structural dynamics of V3 loop with different electrostatics: implications on co-receptor recognition: a molecular dynamics study of HIV gp120

Balasubramanian Chandramoulia, Giovanni Chillemib, Emanuela Giombinic, Maria R. Capobianchic, Gabriella Rozera and Alessandro Desideria,b*

aDepartment of Biology, University of Rome Tor Vergata, Via Della Ricerca Scientifica, Rome 00133, Italy; bCASPUR Inter-universities Consortium for Supercomputing Applications, Via dei Tizii 6b, Rome 00185, Italy; cLaboratory of Virology, National Institute for Infectious Diseases ‘L. Spallanzani’, Via Portuense 292, Rome 00149, Italy

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The HIV’s envelope glycoprotein gp120 plays a major role in the entry of the virus into the host cell, through its successive interactions with the cell surface CD4 receptor and a co-receptor (CCR5 or CXCR4). The choice of a specific co-receptor by gp120 has an important consequence on HIV infection and pathogenesis. The third variable region within gp120, the V3 loop, is the principal determinant of the co-receptor usage by gp120. Here, we report the long time molecular dynamics simulations of four gp120 structures, having a V3 loop charge of +3 and +5, from both R5 and X4 specific strains of HIV. The results of the study highlight the properties of the V3 loop that can be critical for dictating the co-receptor recognition and selection in structural context. In detail, we observe that the structural orientation of the V3 loop in the 3D space is modulated by its net charge, whilst its co-receptor choice is likely dictated by a combined effect of both the electrostatics of the loop and its conformational variability at the level of its central crown region.

Keywords: gp120; CD4-binding; Molecular dynamics; V3 loop

Introduction

The envelope spikes of HIV consist of trimeric assemblies of two subunits that include a surface exposed gp120 and a transmembrane gp41 glycoproteins, that are non-covalently attached to each other. Entry of HIV into the host cell is initiated by the interaction of gp120 with cell surface CD4 receptor, followed by a subsequent interaction with co-receptors (either CCR5 or CXCR4). Binding of CD4 to gp120 triggers conformational changes in gp120, leading to the exposure of co-receptor binding sites. Co-receptor binding enables gp41 to insert a fusion peptide into the target cell membrane, resulting in fusion and transfer of HIV’s genomic content into the target cell. Individual viral isolates are presently classified based on the ability to use CCR5, or CXCR4 or both of them and the choice of the co-receptor by HIV is crucial in determining the viral tropism, that according to co-receptor usage, is classified as R5, X4, or R5X4 (Berger et al., 1998). The co-receptor usage by HIV plays an important role in infection and progression of the disease. Over 90% of primary HIV infection occurs with R5 virus irrespective of the route of infection and the evolution of virus from R5 usage into X4 usage occurs at a later stage (~5 years) (Princen & Schols, 2005; Regoes & Bonhoeffer, 2005).

Blocking the interaction of gp120 with co-receptor is an important strategy of antiretroviral therapy. Numerous efforts have been made to understand the structural basis of the mechanism of viral entry. Several X-ray structures of gp120 in complex with a bound CD4 and antibody have been reported (Diskin, Marcovecchio, & Bjorkman, 2010; Huang et al., 2004, 2005, 2007; Kwong et al., 1998, 2000; Zhou et al., 1998, 2000; Zhou et al., 2007). These studies have shown the three-dimensional organization of gp120 to be made up of an inner, an outer domain, and a bridging β sheet, consisting of five conserved (C1–C5) and five variable (V1–V5) regions, that form the surface exposed loops (Figure 1). The structural elements of gp120 that form the co-receptor recognition sites include the bridging β sheet, variable V1/V2 region, and most importantly, the V3 loop. The V3 loop is highly variable and is the principal determinant of co-receptor specificity (De Jong, De

*Corresponding author. Email: desideri@uniroma2.it

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sequences has shown the loop to be composed of a conserved N- and C-terminal segments (stem), the GPG crest, and a variable region (crown) flanking the crest (Catasti, Fontenot, Bradbury, & Gupta, 1995). A thermodynamic study on the gp120 with chimeric V3 loops has shown the loop to function like two distinct domains, the stem (residues 1–10, 26–35) and the crown (residues 11–25), both the stem and crown are required for co-receptor binding, but the crown alone has been proposed to determine the co-receptor usage (Cormier & Dragic, 2002). Nuclear magnetic resonance (NMR) and X-ray studies on the V3 loop peptides in complex with antibodies have focused in elucidating the antibody recognition by V3 loop (Galanakis, Spyroulias, Rizos, Samolis, & Krambovitis, 2005), but despite these efforts, the V3 loop features that mainly influence the co-receptor preference is still not fully understood.

One useful tool to address this aspect can be the use of molecular dynamics simulation, which provides atomic level insights into the structural dynamics of the molecules (Becker, Roux, & Watanabe, 2001; Chillemi, Fiorani, Benedetti, & Desideri, 2003; Falconi et al., 2001). In a previous study, we carried out a long time simulation on a R5 and X4 specific gp120’s and showed the electrostatics and the plasticity of the V3 loop are important elements of co-receptor recognition (Chandramouli et al., 2012). In our present work, we have focused on the role of V3 loop electrostatics on co-receptor recognition comparing long time simulations on four gp120 structures, having a net charge of +3 and +5, from both R5 (referred as R5P3, R5P5) and X4 (referred as X4P3, X4P5) specific strains. The net charge of the V3 loop is shown to play an important role in determining the orientation of the loop in the three-dimensional space, independently of the co-receptor specificity. The simulations also show that the V3 loop does not reach a co-receptor specific conformation. The conformational variability is larger at the crown region of the loop, letting us to propose that the crown conformation differences coupled with the electrostatics of the loop are the main factors in driving the co-receptor recognition and specificity.

Materials and methods

Model generation

In this work, four simulations constituting two R5 and two X4 specific gp120, having a net charge of +3 and +5 have been compared. The simulation of a R5 gp120 having a V3 loop with charge +3 in the presence of CD4 previously reported (Chandramouli et al., 2012) has been used for the comparison with three other simulations carried out in the present work. To model the starting structure of the R5 gp120, having a +5 V3 loop charge, all the R5 specific gp120 sequences from the
HIV database at the Los Alamos National Laboratory (http://www.hiv.lanl.gov/) have been compared aligning them against the gp120 sequence having the pdb id 2b4c (Huang et al., 2005) using the emboss server (http://www.ebi.ac.uk/Tools/psa/). The sequences having a V3 loop length of 35 residues and a charge of +5 have been filtered using an in-house script. From the filtered set, the gp120 sequence (Los Alamos id: AY426111) with the largest identity (74%) to the reference gp120 (2b4c) has been chosen for 3D modeling. The gp120 protein in the X-ray structure has: (1) deletions in the N- and C-termini, (2) a substitution of GAG tag for the 67 residue long V1-V2 loops and the electron density is observed only for 339 residues. A similar truncation at the N-, C-termini, and substitution of V1/V2 loop by a GAG tag has been made on the chosen R5 gp120, prior to 3D modeling. The truncated sequence consists of 339 residues, sharing an identity of 89.9% with the reference. The X4 gp120 sequences with a V3 loop charge of +3 and +5, have been selected using an identical procedure from the Los Alamos HIV sequence database. The chosen +3 (Los Alamos id: EU743794) and +5 (Los Alamos id: AY173951) X4 gp120 sequences share an identity of 60 and 70% with the reference. After the above mentioned truncation, the sequences consisted of 340 and 339 residues, having an identity of 71.9 and 83.6% with the reference, respectively.

The starting 3D model of all the truncated sequences has been generated using the SWISS-model server (http://swissmodel.expasy.org/) with the gp120 X-ray structure (2b4c) as the template and has been then subjected to energy optimization (Arnold, Bordoli, Kopp, & Schwede, 2006). The backbone root-mean-square deviation (RMSD) of the optimized 3D models from the X-ray structure (Figure 1), after rigid body superposition has been found to be <1 Å. The resulting gp120 models were then superimposed on the X-ray structure to obtain the gp120-CD4 complex with the appropriate orientation of CD4.

**Simulation protocol**

The starting structures have been immersed in a periodic box of TIP3P water model (Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983), which extended from 15 to 17 Å from the solute and neutralized with chloride ions. All simulations were performed using AMBER11 package with ff99SB force field (Hornak et al., 2006) and periodic boundary conditions. The PME method was used to treat the long-range electrostatics (Darden, York, & Pedersen, 1993). Bond lengths involving bonds to hydrogen were constrained using SHAKE algorithm (Ryckaert, Ciccotti, & Berendsen, 1977). A time step of 2 fs was used. The conformational sampling was done at a constant pressure of 1 atm and a temperature of 303 K. Langevin coupling with a collision frequency of 1.0 was used for temperature regulation (Izaguirre, Catarello, Wozniak, & Skeel, 2001). The equilibration procedure involves (1) two rounds of minimizations (1500 iteration each) and dynamics (25 ps each) of the solvent and chloride ions in the bulk solvent, keeping the solute constrained to its initial position with decreasing force constants of 500, 100, 300, and 50 kcal/(mol Å²); (2) four rounds of 2000 steps of minimization of whole system where the solute restraint was kept as 100, 50, 25, and 5 kcal/(mol Å²); and (3) an unrestrained minimization of the whole system. Finally the system was heated to 303 K at constant volume and equilibrated for 300 ps at constant pressure. The production phase was started at this stage. Recently there is a debate on carrying out multiple short time simulations with different starting velocities instead of a single long time simulation, to well sample the phase space (Caves, Evanseck, & Karplus, 1998; Huang, Bowman, Bacallado, & Pande, 2009; Smith, Daura, & van Gunsteren, 2002). In this work, we have opted to carry out a long simulation, up to 50 ns for each system, as we recently reported that the conformational changes in gp120 occur in long time scales (Chandramouli et al., 2012). The conformations were collected in the trajectories at intervals of 2 ps.

**Analysis of trajectories**

The RMSD, root-mean-square fluctuation (RMSF), dynamics cross correlation matrix, and hydrogen bond analysis were carried out with PTRAJ program in AMBER package and salt-bridge calculation was done with visual molecular dynamics (VMD) (Humphrey, Dalke, & Schulten, 1996). The hydrogen bond analysis was done with acceptor–donor distance cutoff of 3 Å and acceptor–hydrogen–donor angle cutoff of 180 ± 60°. A salt-bridge was considered to exist if the Oxygen–Nitrogen distance is less than 4.5 Å (Kumar & Nussinov, 2002). Clustering analysis was carried out using the K-clust program of MMPBSA toolset (Feig, Karanicolas, & Brooks, 2004), after multiple trials with different RMSD values in the range of 2.2–2.7 Å, to select just a limited number of clusters to be compared in the different simulations. The electrostatic potential was calculated with APBS software using 2 and 80 as the solute and solvent dielectrics at zero salt concentration (Baker, Sept, Joseph, Holst, & Mc Cammon, 2001). The figures were generated with UCSF chimera (Pettersen et al., 2004). The plots were generated using matplotlib library (Hunter, 2007).

**Results**

**RMSD and RMSF analyses**

The plot of RMSD as a function of simulation time, reported in Figure 2, shows that the RMSD of the whole gp120 (black line) is larger than after excluding the V3 loop from the calculation (red line). This is confirmed by
the low average RMSD values, calculated over the last 30 ns of the simulation, obtained upon omitting the V3 loop. These results indicate the large contribution of the V3 loop to the overall structural deviation of gp120, and confirm the previously observed tendency of the loop to undergo conformational changes in the presence of CD4 receptor (Chandramouli et al., 2012; Hsu & Bonvin, 2004). The RMSD of the bound CD4 (green line) is well stabilized throughout the whole simulation with an average RMSD value ranging from 1.5 to 2 Å, as previously found in the simulation of other CD4-gp120 binary complexes (Chandramouli et al., 2012).

All further analyses have been carried out on the last 30 ns portion of the trajectory, i.e. in a fully stabilized time window. Per-residue averaged backbone RMSF of the gp120 residues, reported in Figure 3, shows that the V3 loop in the R5 systems experiences fluctuation larger than other regions of the protein (Figure 3(A, B)). In the case of the X4 systems, both V3 and V4 loops show large fluctuations in the X4P3 system (Figure 3(C)), but not in the X4P5 system (Figure 3(D)), where the V3 loop reaches a stable not fluctuating conformation, as can be also appreciated from the RMSD plot (Figure 2(D)).

**CD4-gp120 interaction analysis**

The interaction of CD4 with gp120, examined analyzing the hydrophobic and polar contacts between the two proteins in each system, are summarized in Table I–III (supporting information). The Phe43 residue of CD4 is highly solvent exposed and its insertion into the gp120 binding cavity, located between the inner and outer domains, mediates the initial interaction between the two
proteins. In line, the Phe43-gp120 contacts, analyzed inspecting the gp120 atoms located within 4 Å from the phenyl ring (Table I), are conserved and maintained fairly well in all the simulations independently of the co-receptor specificity, indicating that these interactions are a common CD4-gp120 recognition feature. These contacts facilitate a tight docking of Phe43 into the gp120 cavity providing a strong CD4-gp120 association. In agreement, mutations of E370 and W427 residues of gp120, that are forming conserved contacts over the entire trajectory of the simulations (Table I), have been shown to play an important role in the CD4 binding to gp120 (Kwong et al., 1998).

The binding of CD4 to gp120 has been experimentally shown to be associated with a large change in enthalpy and entropy (Myszka et al., 2000). This results in a significant structural reordering within gp120 and enables the exposure of the regions important for co-receptor binding. A strong bonding energy in the CD4-gp120 interaction is necessary to compensate the entropy loss due to the structural reordering within gp120. In correlation, the presence of a strong network of polar contacts between CD4 and gp120, some of which are conserved with high percentage of occurrence, has been observed in all simulations. The list of the salt-bridge contacts between CD4 and gp120, present for more than 50% of the simulation time are reported in Table II (supporting information). The interaction of D368 of gp120 with R59 of CD4 is highly maintained and conserved in all the systems. In line, the importance of D368 to CD4 binding has been demonstrated by a previous experimental study (Saphire et al., 2001). The D279-K29 interaction is seen only in the R5P5 and X4P5 system (Table II, (B, D)), due to the absence of residue D279 in the gp120 sequence of the R5P3 and X4P3 systems. The other two residues E102 and D457, although present in all four gp120 sequence, are involved in salt-bridge formation only in the R5P5 and X4P5 systems (Table II). The statistics of hydrogen bond contacts between CD4 and gp120, reported in Table III (supporting information), not only confirm the presence of a strong network of hydrogen bond interaction but also the conservation of many of these contacts in all the simulations. In detail, the gp120 residues D368, G366, N280, and D268 form persistent hydrogen bonds via both main chain and side chain interactions with CD4 in all the simulations. The residues G366, D368, and N280 are well conserved across many HIV-1 isolates and their deletion has been shown to diminish the affinity of the antibody specific for CD4 binding site (Olshevsky et al., 1990; Saphire et al., 2001). The residues involved in interactions at the gp120-CD4 interface are in agreement with the ones previously reported in gp120-CD4 simulations having different sequences (Chandramouli et al., 2012; Hsu & Bonvin, 2004), indicating that these interactions are conserved and that the modeling does not perturb the gp120-CD4 interface.

**Dynamic cross correlation analysis**

The dynamic cross correlation (DCC) matrix of the Cα atoms of the gp120 protein residues has been calculated to understand the motional correlation within the protein. Positive and negative $C_{ij}$ values indicate a correlated and anti-correlated movement between a pair of residues $i$ and $j$, respectively. Positively correlated residues tend to move toward the same direction during the dynamics, while negatively correlated residues move in the opposite direction. The 2D representation of DCC map, reported in Figure S1 (supporting information) indicates that gp120 in the R5 systems (Figure S1(A, B)) experiences a larger correlation at the level of the outer domain (residues 348–453) when compared to the X4 systems (Figure S1(C, D)). The correlation within the inner domain is highly similar in all simulations. The V3 loop, in all cases, does not have strong correlated motions with other parts of the protein, but it behaves as an independent unit of the gp120 protein (Figure S1).

The 3D representation of the DCC map of the gp120 is shown in Figure 4, where the Cα pairs with correlation values larger than 0.7 have been connected by black lines on the average structure obtained from the respective simulations. The figure, in all cases, indi-

![Figure 4. 3D mapping of the cross correlation values on the average structure of gp120 for (A) R5P3, (B) R5P5, (C) X4P3, and (D) X4P5 systems. Each Cα pair having a correlation value of $C_{ij} > 0.70$ is connected by black lines. The secondary structures are colored as α-helix in orange, β-sheet in cyan, and coils in gray.](image-url)
icates the presence of three distinct regions corresponding to (1) the inner domain (2) the outer domain and iii) the region forming the bridging β sheet, that act as three rigid clusters having strong intra, but not inter-domain correlated motions (Figure 4). The conservation of the correlation within the bridging β sheet supports the CD4 induced stabilization of this region observed in previous simulation studies on different gp120 structures (Hsu & Bonvin, 2004; Pan, Ma, Keskin, & Nussinov, 2004). The figure also confirms that the V3 loop does not have any strong concerted motion with other parts of the protein, but only within the residues of the loop. As suggested by a reviewer, we have examined the sensitivity of the correlations to the length of the simulation, calculating the DCC matrix over the first and last 15 ns of the trajectory. The corresponding 2D and 3D representations of the DCC matrix reported in Figures S2–S5 (supporting information) show a relatively similar correlation features as detailed above. In addition, principal component analysis has been carried out on the gp120 Cα atoms and the motion along the first eigenvector depicted in Figure S6 (supporting information), indicates that it is dominated by the motion of the V3 loop that does not appear to be correlated with the motion of other parts of the protein.

Cluster analysis

The conformations in the trajectory have been clustered using the RMSD as a measure of similarity and the optimal number of clusters has been chosen, after multiple trials, using a RMSD threshold in the 2.2–2.7 Å range. The time evolution of the conformational clusters reported in Figure S7 (supporting information) indicates that each system is characterized by 2 or 3 dominant clusters, occurring for large percentage of time. To understand the average conformational feature of the protein in each cluster, the snapshot having the least RMSD to the centroid of the cluster has been selected as the cluster representative. The superimposition of the cluster representatives (Figure 5) indicates that the core region of gp120 has a conserved conformational rigidity, since they fully overlap in this part of the protein.

An interesting feature at the level of the V3 loop is its preference to a spatially separated orientation, depending on its net charge. In detail, in the R5P3 and X4P3 systems, the V3 loop, having a net charge of +3,
localizes close to the basal region of the bridging β sheet (Figure 5(A, C)). In contrast, in the case of the R5P5 and X4P5 systems (Figure 5(B, D)), the V3 loop, having a net charge of +5, localizes away from the bridging β sheet. The shift of the V3 loop movement in the direction of the bridging sheet can be appreciated by a visual inspection of the R5P3 and X4P3 representatives (Figure 5(A, C)) as a function of simulation time, where the time flows from black to red in R5P3 and red to black in X4P3 simulation (Figure 5(A, C)). The opposite i.e. preferential movement of the loop away from the bridging β sheet can be appreciated from the R5P5 and X4P5 representatives (Figure 5(B, D)). In a previous simulation, we have reported that in a gp120, having a V3 loop with a net charge of +8, the loop was moving away from the bridging β sheet similar to the V3 loop with +5 charge studied here (Chandramouli et al., 2012). These results suggest that the orientation of the V3 loop is modulated by its charge, orientating itself away from the bridging β sheet for value larger than +5 and it is not related to its preference towards a specific co-receptor.

**Electrostatics analysis**

The similar orientation of the V3 loops having an identical charge number irrespective of the gp120’s co-receptor specificity poses the question on what is the V3 loop’s feature that permits the protein to discriminate between the CCR5 or CXCR4 co-receptor. A possibility could be the protein electrostatic potential distribution that has been calculated for the representative conformers of the most populated cluster (Figure 6). The spatial distribution of the positive and negative iso-surfaces shows a clear difference upon comparison of the R5P3 and X4P3 gp120 representatives, especially at the level of the V3 loop (Figure 6(A, C)). However, there is not a pronounced difference between the R5P5 and X4P5 gp120 representatives (Figure 6(B, D)), suggesting that the protein electrostatic distribution cannot be the only determining factor in dictating the co-receptor selection. Following a reviewer suggestion, the electrostatic analysis has been calculated also in the absence of the V3 loop Figure S8 (supporting information) to identify residues outside the V3 loop responsible for the orientation of the loop in the 3D space. However no significant differences have been detected.

**2D-RMSD and secondary structure analyses**

The V3 loop conformations have been compared extracting snapshots at an interval of 0.5 ns from each simulation for a total of 60 snapshots. The comparison has been done calculating the pair-wise 2D RMSD matrix between the snapshots of (1) R5P3 vs. X4P3 and (2) R5P5 vs. X4P5 systems, having the same net positive charge but different co-receptor specificity. The 2D RMSD matrix calculated as a function of time over the Cα atoms of the whole V3 loop (Figure 7(A, B)) shows a structural deviation in the range of 6–7.0 Å in the initial part of the simulation (20 ns) that is reduced to 3.5–4.5 Å towards the end of the simulations (~46 to 49 ns), indicating that the overall structure of the V3 loop converges towards a similar conformation independently of the co-receptor specificity. This result seems to indicate that the co-receptor specificity is not dictated by conformational variability of the V3 loop.

Studies on sequence comparison of V3 loops, from various HIV isolates, have shown that the N- (residue 1–10) and C-terminal (residue 26–35) segments of the V3 loop are conserved, whilst the main variability occurs in the crown (residues 11–25), that has been indicated as an important factor in determining the co-receptor usage (Catasti et al., 1995; Cormier & Dragic, 2002). A more detailed comparison of the V3 loop conformations, taking in consideration the N-, C-terminal segments and the crown, has been then carried out. The 2D-RMSD matrix indicates that the terminal segments converge towards a similar conformation, while this does not happen for the crown of the loop (Figure 8(A, B)). The structural dissimilarity observed at the level of the crown region provides a further indication that these residues may be an important factor in dictating the co-receptor preference. In line, the evolution of the secondary structure of the V3 loop residues, reported in Figure S9 (supporting information),
confirms significant differences within the +3 charged V3 loops (Figure S9(A, C)) and the 5 charged V3 loops (Figure S9(B, D)), which is more pronounced at the level of the crown region (shaded portion in Figure S9). However, the comparison of the 2D-RMSD matrix of the crown region of V3 loop conformations having the same co-receptor specificity but different net charge and orientation (i.e. X5P3 vs. X5P5 and X4P3 vs. X4P5) indicates the absence of any close structural similarity (Figure S10, supporting information), indicating that the crown region

Figure 7. 2D RMSD in Å (of Cα atoms) between V3 loop conformations extracted from (A) R5P3, X4P3 and (B) R5P5, X4P5 systems. Each color dot in the plot represents the RMSD between the two conformations obtained from the corresponding simulation. X and Y axes correspond to R5 and X4 systems, respectively.

Figure 8. 2D RMSD in Å (of Cα atoms) calculated for the crown region (residues 11 to 25) of V3 loop conformations extracted from (A) R5P3, X4P3 and (B) R5P5, X4P5 systems. X and Y axes correspond to R5 and X4 systems, respectively.
does not converge toward a co-receptor specific conformation and so it cannot be the unique element for the co-receptor recognition.

Discussion
Gp120 plays an important role in the entry of HIV into the host cells through its interaction with the CD4 receptor and an obligatory co-receptor. The V3 loop within gp120 is known to be the principal determinant of the selection and recognition of appropriate co-receptor during the viral entry. The present work aims at exploring the role of V3 loop structure/electrostatic properties on the co-receptor recognition. To this end, the structural-dynamics of the gp120 protein with a net V3 loop charge of +3 and +5 from both R5 and X4 specific strains have been compared. An interesting observation obtained from the simulations concerns the difference in the spatial orientation of the V3 loop depending on its net charge (Figure 5). The +3 V3 loop prefers to relocate itself close to the bridging β sheet, whilst the +5 V3 loop prefers to shift away from it, irrespective of the gp120’s co-receptor specificity (Figure 5). This result is in line with our previous work where we reported the movement of a +8 charged V3 loop away from the bridging sheet (Chandramouli et al., 2012), suggesting that the net charge of the loop is an important factor for modulating the structural orientation of the loop. However, the loops orientation is not co-receptor dependent. Also the analysis of the electrostatic potential distribution does not indicate any specific distribution that can be attributed as diagnostic for the recognition of a specific co-receptor (Figure 6). The comparison of the electrostatic potential distribution shows some differences in the spatial arrangement of the positive and negative iso-surfaces for the R5P3 and X4P3 systems having a +3 charged V3 loop, but it does not show any distinguishable difference for the R5P5 and X4P5 systems having a +5 charged V3 loop (Figure 6).

An interesting difference between the equally charged V3 loop, having a different co-receptor specificity, concerns the structural dissimilarity that has been identified comparing the 2D RMSD matrix (Figures 7 and 8). The dissimilarity is large at the level of the crown region (Figure 8), a difference that is confirmed by the inspection of the time evolution of the secondary structure of the V3 loop residues (Figure S9). These results suggest that the crown region can be an important element for the co-receptor selection. However, this region does not reach an identical configuration for the V3 loop having same co-receptor specificity, as indicated by the structural comparison of the V3 loop with same co-receptor specificity but different charge (Figure S10). Debatable results have been reported by different groups concerning the correlation between V3 loop conformation and co-receptor specificity. An NMR study on the V3 peptide-antibody complex by Sharon et al. (2003) has suggested that alternate conformations of the V3 loop play a key role in determining the co-receptor specificity of HIV-1 (Rosen, Sharon, Quadt-Akabayov, & Anglister, 2006; Sharon et al., 2003), a study by Sharon et al. (2003) argues that there is a predominant single conformation for both the R5 and X4 variants and that the variability of the sequence features is responsible for specificity towards the respective co-receptor (Scheib, Sperisen, & Hartley, 2006). These reports have the limit to be based on the structure of isolated V3 peptides in complex with neutralizing antibodies. Our results indicate that the crown region has a large conformational variability that together with the V3 loops electrostatic may represent the important indicators for the co-receptor recognition.

Conclusions
In this work, we have employed the molecular modeling and dynamics techniques to investigate the role of the V3 loop features in dictating the co-receptor selection and recognition. The study indicates (1) a conserved CD4-gp120 recognition mechanism independently of co-receptor usage, (2) the orientation of the V3 loop in the 3D space determined by the net loop charge not related to co-receptor specificity, and (3) a crown region characterized by a large structural variability. It is interesting to notice that the starting 3D structure of the V3 loop is identical in all systems, but it evolves toward different configurations. These findings let us to propose that the combined effect of the crown structural variability and V3 loop electrostatics dictates the co-receptor selection capability of the loop.

Supplementary material
The supplementary material for this paper is available online at http://dx.doi.[10.1080/07391102.2012.703068]

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