

# Enhanced Dynamics of HIV gp120 Glycoprotein by Small Molecule Binding

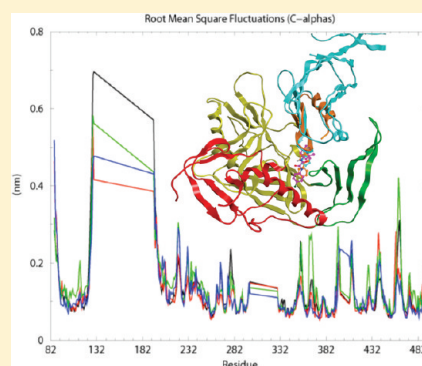
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 Supporting Information

**ABSTRACT:** HIV cell entry and infection are driven by binding events to the CD4 and chemokine receptors with associated conformational change of the viral glycoprotein, gp120. Scyllatoxin miniprotein CD4 mimetics and a small molecule inhibitor of CD4 binding, NBD-556, also effectively induce gp120 conformational change. In this study we examine the fluctuation profile of gp120 in context of CD4, a miniprotein mimetic, and NBD-556 with the aim of understanding the effect of ligand binding on gp120 conformational dynamics. Analysis of molecular dynamics trajectories indicate that NBD-556 binding in the Phe 43 cavity enhances the overall mobility of gp120, especially in the outer domain in comparison to CD4 or miniprotein bound complex. Interactions with the more flexible bridging sheet strengthen upon NBD-556 binding and may contribute to gp120 restructuring. The enhanced mobility of D368, E370, and I371 with NBD-556 bound in the Phe 43 cavity suggests that interactions with  $\alpha$ 3-helix in the outer domain are not optimal, providing further insights into gp120–small molecule interactions that may impact small molecule designs.



HIV infection is mediated by a series of attachment events initiated by the HIV viral coat glycoprotein, gp160, which is cleaved into its two components, gp120 and gp41.<sup>1</sup> The two HIV glycoproteins gp120 and gp41 are assembled as a trimer.<sup>2,3</sup> HIV infection in human T-cell lymphocytes occurs via binding of gp120 to the host T-cell CD4 receptor<sup>4,5</sup> followed by gp120 restructuring.<sup>6,7</sup> This conformational change exposes on gp120 the binding site for the chemokine receptor, either CCR5 or CXCR4, thus permitting, the second obligatory binding event for viral entry.<sup>8–11</sup> Chemokine receptor binding is followed by insertion of the gp41 fusion peptide in the host cell membrane, allowing fusion and viral entry.<sup>12–15</sup> A number of X-ray structures of CD4 bound to gp120 have been described, revealing the inner, outer, and bridging sheet domains that form a large binding cavity.<sup>16–20</sup> Two key CD4 residues, Phe43 and Arg59, bind in the gp120 cavity<sup>21,22</sup> and to Asp 368 on an adjacent  $\alpha$ -helix, respectively. Moreover, when Phe43 and Arg59 are mutated, to alanine, CD4 no longer binds gp120.<sup>23</sup> Crystal structures of several miniprotein CD4 mimetics bound to gp120 have also been solved,<sup>16</sup> revealing a biphenyl group bound deep within the CD4 cavity. The structure of unbound form SIV gp120, which has 35% sequence identity with HIV gp120, indicates an invariant outer domain, with conformational changes occurring in both the bridging sheet and inner domain.<sup>24</sup> Although a crystal structure of the unbound form of HIV gp120 has not been reported, the plasticity of the inner domain and bridging sheet is revealed in the structure of gp120 bound to the CD4-binding site antibody, F105.<sup>18</sup>

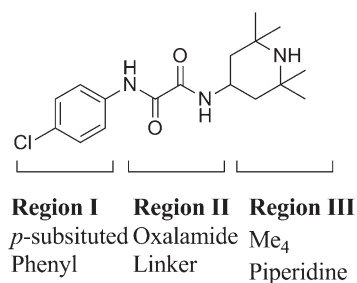
The thermodynamic signature of gp120 conformational change upon CD4 binding has been described<sup>25,26</sup> showing a highly favorable binding enthalpy ( $\Delta H = -63$  kcal/mol) balanced with a highly unfavorable molecular ordering ( $-T\Delta S = 52$  kcal/mol). A similar thermodynamic signature is exhibited by the small molecule inhibitor, NBD-556<sup>27,28</sup> (Figure 1), which induces the CD4 stimulated conformational change in a manner similar to CD4 binding and is capable of enhancing viral infection on CD4 deficient target cells.<sup>27,28</sup> Second generation compounds<sup>29,30</sup> with improved affinity have shown to cause a rapid inactivation of the virus. Furthermore, modification of the region III piperidine modulates the magnitude of viral infection on CD4 deficient target cells and the entropic contribution to binding affinity,<sup>31</sup> establishing this class of compounds as a potential entry inhibitor and antiviral therapeutic agent.

The dynamics of gp120 glycoprotein have also been examined in computational studies.<sup>32–40</sup> Molecular dynamics (MD) simulations of wild-type gp120<sup>35</sup> showed partial unfolding of some of the  $\beta$ -strands while the S375W mutant preferred the bound-like conformation. Nonequilibrium steered MD<sup>36</sup> showed that the bridging sheet strands  $\beta$ -2/3 had more flexibility than the  $\beta$ -20/21 strands which preferred interactions with the inner domain. Da et al. demonstrated that the bridging sheet refolds for the S375A mutant but not for the W112A or S375W/T257S

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**Figure 1.** The three regions of NBD-556.

mutants.<sup>38</sup> Essential dynamics studies by Liu et al.<sup>34</sup> of unbound gp120 homology models indicated that the S375W mutation favors the CD4 bound conformation, while the I423P mutation prefers the unliganded conformation. MD studies by Hsu et al.<sup>32,33</sup> indicate that there are concerted loop motions in the vestibule of the CD4 cavity, stabilization of the bridging sheet, and a coalescing of the bridging sheet and V3 loop to form the coreceptor binding site. Binding entropies extracted from these MD trajectories<sup>33</sup> suggest that the large entropy loss associated with CD4 binding is derived from hydrophobic interactions from CD4 Phe43 insertion into the cavity, the formation of a hydrogen-bond network, and the restructuring of the bridging sheet. Large-scale sampling of gp120 motions with temperature accelerated MD<sup>39</sup> predicted a counter rotation between the inner and outer domains and a disruption of the bridging sheet in the unbound form of gp120. A flexibility index, derived using the floppy inclusion and rigid substructure topography,<sup>37</sup> has also been used to describe the extent and distribution of flexible and rigid regions in the inner, outer, and bridging sheet domains of the 22 gp120 structures bound with various ligands. This analysis indicated that the inner domain and bridging sheet domains are more flexible while the outer domain is more rigid and that gp120 proteins bound with CD4 exhibited less flexibility in the inner domain than when bound by a miniprotein mimetic. We previously applied the coarse-grained Gaussian network model (GNM)<sup>41–44</sup> to gp120. The study of the fluctuation profiles from GNM resulted in the identification of critical residues in the outer domain with minima that line the Phe 43 cavity and may represent a core folding nucleus found in the prebound form of gp120.<sup>40</sup> Furthermore, W427, a highly conserved residue required for CD4 binding exhibited a GNM maximum, which is surrounded by two residues, H105 and M475, of GNM minima, purported to form a key structural element that stabilizes the formation of the CD4 cavity. Determination of residue pairs with efficient communication propensities from GNM and molecular dynamics (MD) simulations delineated an extensive residue network in the outer domain that is topologically suited for signal propagation from the Phe 43 cavity throughout the gp120 outer domain.

In this study we focus on MD analysis of the gp120 Phe 43 cavity in the context of binding to the CD4 receptor, a CD4 miniprotein mimetic and a small molecule ligand (NBD-556). A docked conformation of NBD-556 bound to gp120 from strain HXBC2 has been previously described implicating cavity residues important in binding.<sup>30,31</sup> Here we examine CD4, scyllatoxin miniprotein, and NBD-556 interactions with gp120 cavity residues and their influence on residue fluctuations in the inner, outer, and bridging sheet domains. We aim to understand the similarities and differences in the gp120 fluctuation profile in the presence of these three ligands in the context of gp120

structuring and the potential implication on small molecule designs.

## MATERIALS AND METHODS

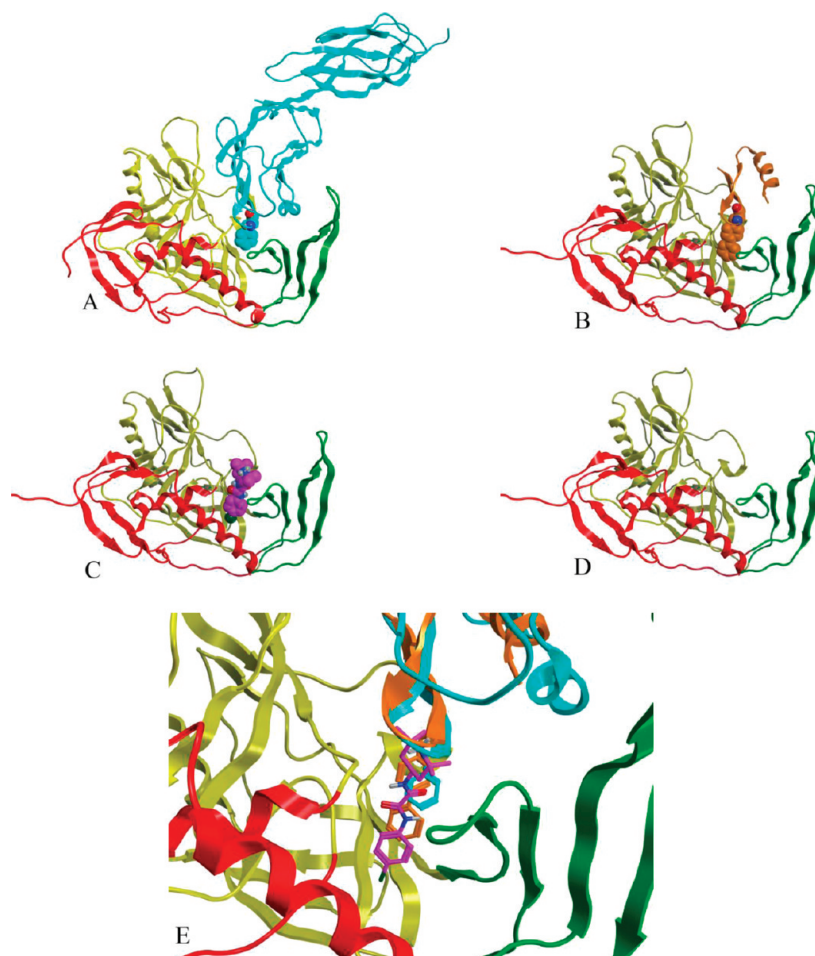
**Small Molecule Modeling.** NBD-556 was constructed in MOE (MOE Molecular Operating Environment Chemical Computing Group, version 2005.06 (Montreal, Canada) (<http://www.chemcomp.com>), ionized using MOE's WashMDB function, and hydrogens were added.<sup>45</sup> The small molecule conformation was minimized to a gradient of 0.01 in the MMFF94x<sup>46,47</sup> force field using a distance-dependent dielectric constant of 1. The minimized NBD-556 was used for docking with GOLD and then in MD simulations.

**Protein Modeling.** Two X-ray crystal structures were prepared for docking and molecular dynamic calculations: CD4-bound HIV-1 gp120 core strain YU2 (PDB code 1G9N)<sup>22</sup> and the scyllatoxin miniprotein (CD4M47A) bound HIV-1 gp120 core strain YU2 (PDB code 2ISY).<sup>16</sup> The *deglycosylated* core gp120 construction used in the two crystal structures has a 19- and 52-amino acid residue deletions from both the N- and C-termini, respectively, is devoid of the V3 variable loop, and contains a tripeptide substitution (Gly-Ala-Gly) for the 67 residues of the V1/V2 loop. Residue numbering in the crystal structures and in this study conforms to the numbering used in the full-length gp120 sequence. A gap in the numbering exists from residues (127 to 194 and 296 to 330) corresponding to the substitution of the V1/V2 loops with the tripeptide and the deletion of the V3 loops. For 2ISY, the missing V4 loop was added from the 1G9N crystal structure and minimized. Four N-terminal residues in 2ISY (S82, E83, V84, K85) which were not present in the 1G9N structure were deleted from the starting coordinates for consistency. Hydrogen atoms were added, and tautomeric states and orientations of Asn, Gln, and His residues were determined with Molprobit (<http://molprobit.biochem.duke.edu/>).<sup>48,49</sup> Hydrogens were added to crystallographic waters using MOE.<sup>45</sup> The OPLS-AA force field<sup>50</sup> in MOE<sup>45</sup> was used and all hydrogens were minimized to a root-mean-square (RMS) gradient of 0.01, holding the heavy atoms fixed. A stepwise minimization followed for all atoms, using a quadratic force constant (100) to tether the atoms to their starting geometries; for each subsequent minimization, the force constant was reduced by a half until 0.25. This was followed by a final cycle of unrestrained minimization. Water molecules, Asn-linked acetyl-D-glucosamine, 2-(acetyl-amino)-2-deoxy- $\alpha$ -D-glucopyranose, and small molecules were removed prior to MD calculations.

**NBD-556 Docking.** Docking calculations with GOLD (version 3.2)<sup>51,52</sup> were performed without crystallographic water molecules in the cavity. One hundred genetic algorithm (GA) docking runs were performed with the following parameters: initial\_virtual\_pt\_match\_max = 3.5, diverse\_solutions = 1, divsol\_cluster\_size = 1, and divsol\_rmsd = 1.5. All other parameters were set as defaults. The best scoring pose was used as a starting conformation in MD simulations.

**Cavity Analysis.** The MOE (45) site finder utility was used for cavity analysis and calculation of volume for starting and simulated coordinates.

**Molecular Dynamics Simulations.** A preliminary energy minimization, followed by a short molecular dynamics simulation in an explicitly solvated environment, is expected to relax the X-ray structure, driving it closer to its true minimum. We thus performed MD simulations of solvated GPO3, GCD2, GCD3



**Figure 2.** The four constructs used in MD simulation are shown in panels A–D with the three gp120 domains: inner, outer, and bridging sheet colored as red, yellow, and green ribbons, respectively. (A) The gp120-CD4 complex (GCD2) with CD4 shown in blue with Phe 43 depicted as space-filling model. (B) The gp120-CD4M47 complex (GCD3) with the scorpion toxin derived miniprotein shown in orange with the residue 23 biphenyl depicted as space-filling model. (C) NBD-556 docked to gp120 (GPO3\_NBD) with NBD-556 shown as purple space filling model. (D) The unbound gp120 structure (GPO3). (E) Comparison of the NBD-556 (purple), biphenyl (orange), and Phe-43 (cyan) as bound in the cavity gp120 for GPO3\_NBD, GCD3, and GCD2, respectively.

and GPO3\_NBD structures (Table 2) and analyzed the time evolution of their dynamics. 30 ns simulation trajectories were generated, using GROMACS<sup>53,54</sup> in a fully solvated environment, at 310 K and under constant number of particles, pressure, and temperature (NPT) conditions.

Molecular dynamics simulation trajectories were generated using Gromacs software package version 3.3.1,<sup>53,54</sup> using the GROMOS 43a1 force field and the SPC water model<sup>55</sup> for solvation. The solvated protein molecule was *initially* energy minimized with 1000 steps of steepest descent, followed by a short equilibration run of 200 ps, during which the protein backbone atom were position restrained and the side-chain atoms and water molecules were allowed to relax. During the production run of 30 ns, the position restraints were removed. A dielectric permittivity  $\epsilon = 1$  and time step of 2 fs were used. The LINCS<sup>56</sup> algorithm was used to constrain all bond lengths and the particle mesh Ewald (PME) method<sup>57</sup> to compute the electrostatic term. In all simulations, the temperature was kept constant ( $T = 310$  K) by coupling to an external temperature bath with a coupling constant of 0.1 ps, and isotropic pressure coupling was employed to maintain a constant pressure of 1 bar.

## RESULTS AND DISCUSSION

Two crystal structure complexes from the HIV YU2 strain were used to form the basis of this study: the CD4, 17b antibody, gp120 complex (1G9N)<sup>22</sup> and the CD4M47A scyllatoxin, 17b antibody, gp120 complex (2I5Y)<sup>16</sup> (Figure 2 A,B). The gp120 coordinates from the CD4M47 scyllatoxin bound complex were used to dock NBD-556 to the Phe 43 cavity (Figure 2C). Previously published structure activity relationships combined with docking and mutagenesis experiments indicate that the NBD-556 chlorophenyl group binds at the bottom of the Phe 43 cavity while the tetramethylpiperidine is positioned in the Phe 43 cavity vestibule.<sup>30,31</sup> The docked model predicts cavity lining residues that are within interaction distance to NBD-556 (less than 0.5 nm) (Table 1). Also listed in Table 1 are characteristics of cavity residues, such as whether they are conserved, display GNM slow mode minimum or maximum, or have efficient communication propensities with nearby residues. The docked NBD-556-gp120 complex was used as a starting point in MD simulations and is abbreviated as GPO3\_NBD. MD simulations were used to elucidate the fluctuation profiles of these residues in comparison to the three other coordinate sets: the CD4-gp120



**Table 1. gp120 Residues within 5.0 Å of Cavity Binding Ligands<sup>a</sup>**

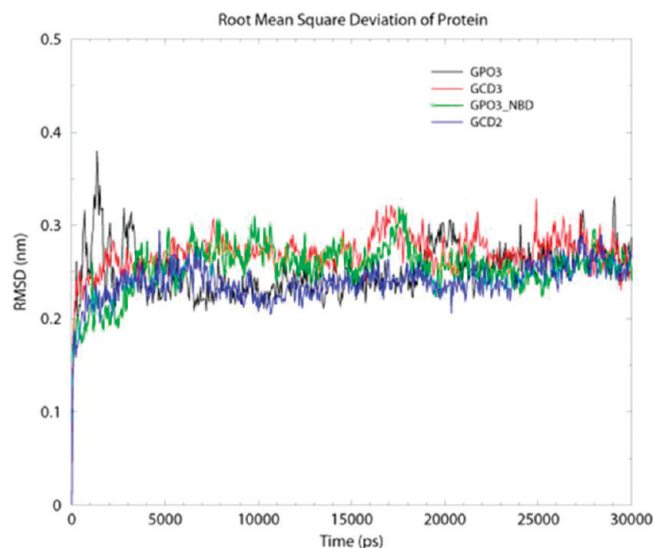
domain	GPO3-NBD-556	GCD3 biphenyl	GCD2 Phe 43	GPO3 GNM profile	GPO3 residue CP < 0.20
inner	<i>H105</i>			min	
inner	W112	W112			
inner	V255	V255		min	
outer	S256	S256		min	L260, S375
outer	T257	T257	T257	min	I371, H374 S375, F376
outer	D368	D368	D368		
outer	<b>E370</b>	<b>E370</b>	<b>E370</b>	min	
outer	I371	I371	<u>I371</u>	min	H374
outer	S375	S375		min	F382,F383,Y384, C385
outer	F376	F376		min	F382, F383, Y384
outer	N377	N377		min	F382, F383
outer	F382	F382			
outer	Y384	Y384			
bridging sheet	I424	I424		max	C418, I420
bridging sheet	N425	N425	N425	max	
bridging sheet	M426	M426	M426	max	
bridging sheet	<b>W427</b>	<b>W427</b>	<b>W427</b>	<b>max</b>	
bridging sheet	<u>N428</u>			max	
bridging sheet	E429			max	
bridging sheet	V430			max	
bridging sheet	G431			max	
outer domain	G472			max	
outer domain	<u>G473</u>	<u>G473</u>	<u>G473</u>	min	
outer domain	D474	D474	D474	min	
inner domain	<u>M475</u>	<u>M475</u>	<u>M475</u>	min	

<sup>a</sup>Residues conserved in all primates viruses, all human viruses, and moderately in human viruses are indicated in bold, underlined, and italic fonts, respectively. Residues with Gaussian network model calculated slow mode maximum and minimum as well as residues pairs with efficient communication propensity (CP < 0.20) are also listed

**Table 2. Coordinate Sets and Ligands Used in MD Studies**

coordinate set	PDB code	ligand	total atoms	production run (ns)
GCD2	1G9N	CD4	94 345	30
GCD3	2ISY	CD4M47-biphenyl	95 047	30
GPO3_NBD	2ISY	NBD-556 docked	71 019	30
GPO3	2ISY	none	71 007	30

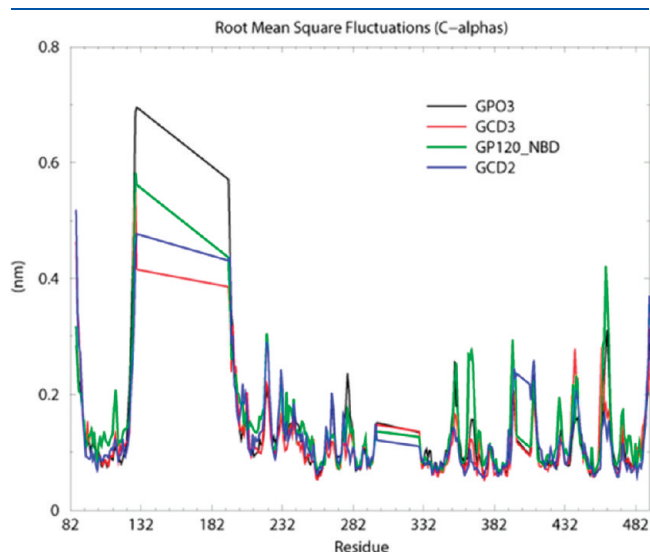
complex, with Phe 43 bound in the cavity (GCD2, pdb code 1G9N), the CD4M47-gp120 complex, with biphenyl bound in the cavity (GCD3, pdb code 2ISY), and gp120 in the CD4 bound form but without ligand bound in cavity during simulation (GPO3, pdb code 2ISY) (Table 2). MD simulation with GROMACS<sup>53,54</sup> produced stable 30 ns trajectories for the four coordinate sets (Figure 3). As we have previously reported, GNM and MD produced similar fluctuation profiles for receptor and miniprotein bound gp120.<sup>40</sup> Here, root mean square fluctuation (RMSF) from the average structure (averaged over 30 ns simulation trajectory for each amino acid residue (C-α)) shows similar profiles between the four coordinate sets for the majority of gp120 residues (Figure 4). However, in general, the fluctuations are enhanced, i.e. peak height, when the Phe 43 cavity is occupied by NBD-556 (Figure 4). Individual RMSF difference plots among the bound gp120 systems compared to the unbound GPO3 also indicate that the complexes with the deepest cavity



**Figure 3.** Calculated RMSDs for backbone C-α atoms from the starting structure for the four constructs, GPO3 (black), GCD2 (red), GCD3 (blue), and GPO3\_NBD (green). The mean RMSDs and standard deviations in nm for the four complexes, GPO3, GCD2, GCD3, and GPO3\_NBD, are 0.25 (0.03), 0.24 (0.02), 27 (0.02), and 0.25 (0.02), respectively.

binding ligands have dynamics that are enhanced compared to GPO3 (Figure S1). When RMSF's are mapped to the gp120 fold,

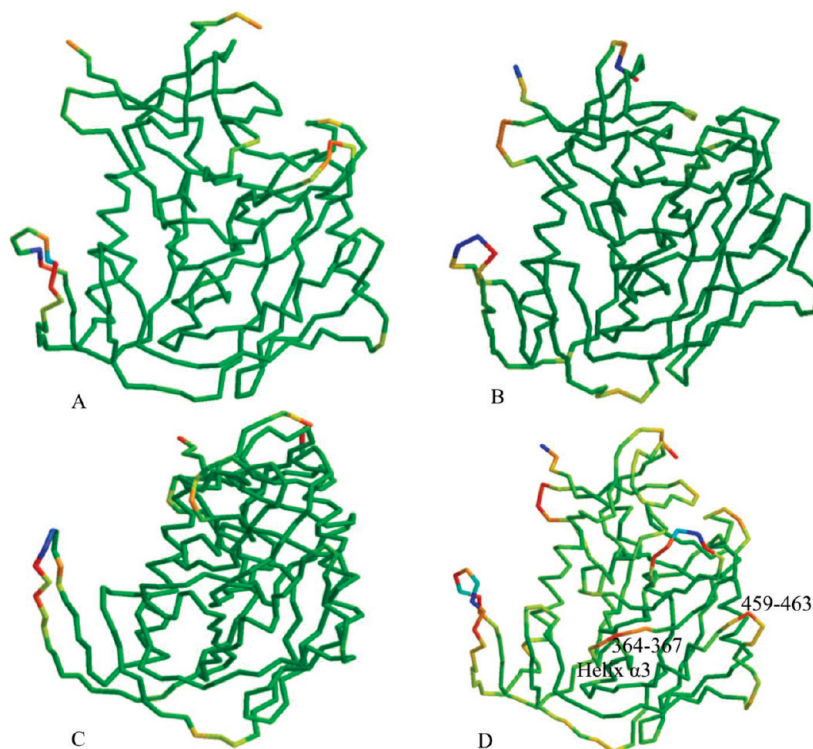
a picture emerges that with NBD-556 bound in the cavity the dynamics of the gp120 are accentuated (Figure 5). The two regions of largest enhancement in backbone fluctuation when



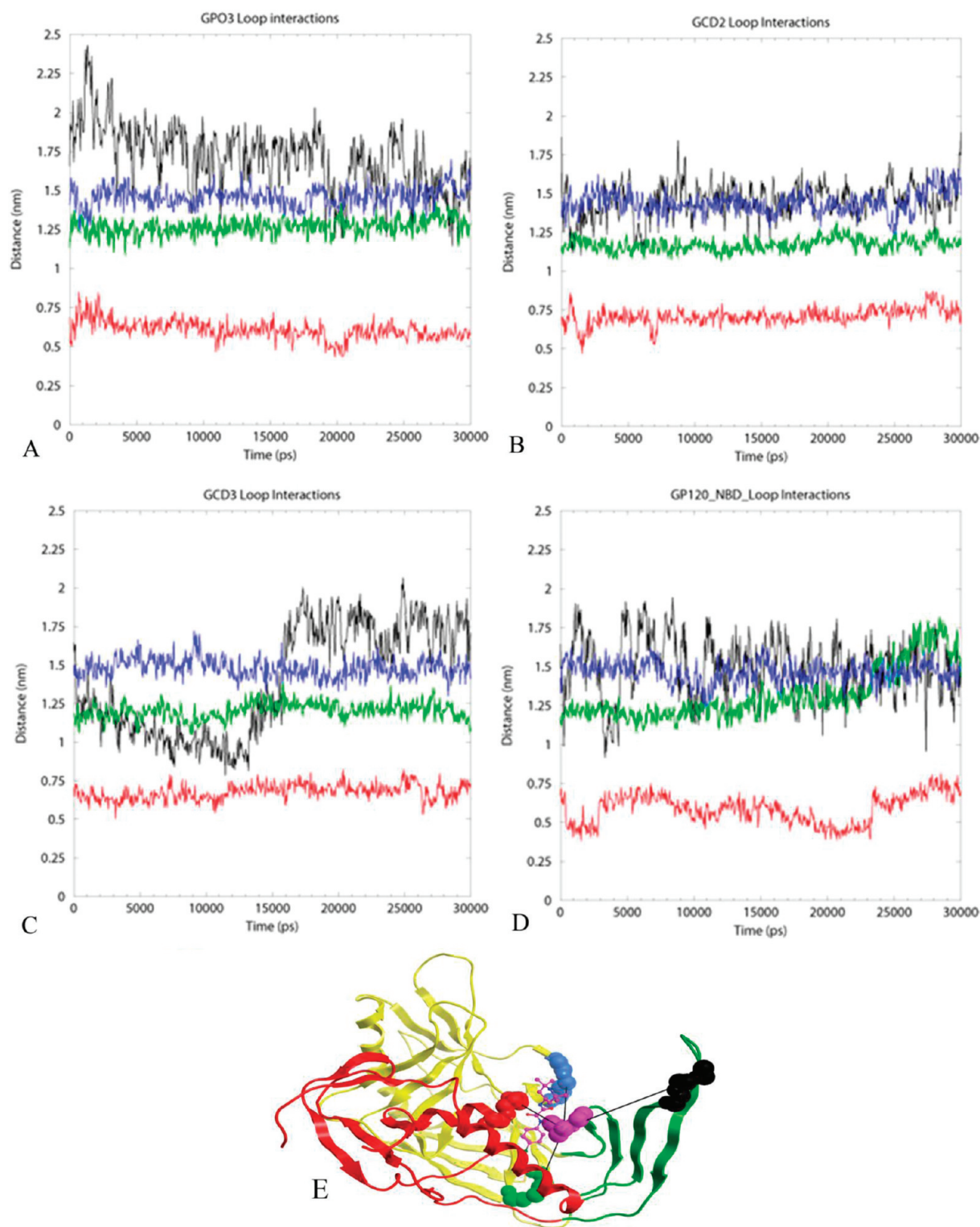
**Figure 4.** Calculated root-mean-square fluctuations (RMSFs) from the average, for gp120 residue backbone C- $\alpha$  atoms for the four constructs, GPO3 (black), GCD2 (red), GCD3 (blue), and GPO3\_NBD (green). Residue numbering for the core gp120 crystal structures maintains the numbering from the full-length gp120 sequence (residues 82–492). A gap in the numbering exists from residues (127 to 194 and 296 to 330) corresponding to the substitution of the V1/V2 loops with the tripeptide and the deletion the V3 loops. The peaks at residue 127 and 296 correspond to these gaps.

NBD-556 is bound are residues 364–367 (located adjacent to helix  $\alpha$ -3 in the outer domain) and 459–463 (V5 loop in the outer domain) (Figure 5D) compared to bound CD4 (Figure 5B and Figure S1B) or the miniprotein (Figure 5C). Residues 364–367, adjacent to the cavity, are within van der Waals contact of the  $\beta$ -strand from CD4M47 miniprotein or CD4 residues and a hydrogen bond is formed between the backbone of D368 and the mini-protein or CD4. Residues 459–463 are quite distant from the cavity and do not form interprotein contacts with CD4M47 and only sparingly with CD4. Enhanced mobility in loop V5 (residues 459–463) in GPO\_NBD (Figure 5D) can be attributed to the inherent flexibility in this loop as has also been observed in various crystal structures,<sup>16,22,58</sup> whereas enhanced RMSF for residues 364–367 appears ligand dependent.

To better understand overall fluctuation patterns in the Phe 43 cavity, we choose to first analyze the behavior of several residues of moderate to high sequence conservation in Phe 43 cavity (I109, P124, D368, W427, V430) and their interacting residue partners for the four simulation trajectories. The inter-residue distances over the course of the simulation (Supporting Information, Figure S2) indicate that there is a tight interaction (the distance between the center of mass of the interacting pairs of residues is less than  $\sim 0.3$  nm and exhibits low fluctuation) between F210-W112, W427-I109, D113-K117, and K432-L122 residue pairs while V430-T123, V430-P124, and K432-D368 are loosely associated (the distance greater than 0.3 nm and exhibits high fluctuation). We observe that for the gp120-CD4 complex (GCD2) V430-T123 (Figure S2C, black curve) is a rather loose association (distance increases to greater than 0.5 nm from 5 to 15 ns), while in the presence of NBD-556 (GPO3\_NBD) this association (Figure S2D, black curve) strengthens (distance decreases to less than 0.25 nm from 15 ns onward) over the course of



**Figure 5.** Calculated root-mean-square fluctuations (RMSFs) for gp120 residue backbone C- $\alpha$  atoms mapped to ribbon diagram for the four constructs: (A) GPO3, (B) GCD2, (C) GCD3, and (D) GPO3\_NBD. High to low RMSFs are colored from blue, red, orange, yellow, and green, respectively.



**Figure 6.** The internode distance to the residue pair W427-E429 (purple) on  $\beta$ -20/21 was measured (nm) for the four constructs: (A) GPO3, (B) GCD2, (C) GCD3, and (D) GPO3\_NBD for the residues (E) G473-M475 (red), G367-P369 (blue), G379-E381 (green), and V127-A129 (black), corresponding to turns or loops between  $\beta$ -strands  $\beta$ -24/ $\beta$ -25,  $\beta$ -14/ $\beta$ -15,  $\beta$ -16/ $\beta$ -17 and the V1/V2 stem, respectively.

the simulation. Conversely, W427-I109 is stable (distance less than 0.3 nm) in the CD4, CD4M47 and unliganded constructs GCD2 (Figure S2C, green curve), GCD3 (Figure S2B, green curve), and GPO3 (Figure S2A, green curve), respectively, while in GPO3\_NBD (Figure S2D, green curve) this interaction

weakens (distance greater than 0.3 nm from 20 ns onward) during the course of the simulation. This weakening of the interactions is characterized by movement of W427 away from the Phe 43 cavity with an accompanying shift in neighboring inner domain residues H105, M475, and W112 (Figure S3).



We also monitored the distances between the loops surrounding the Phe 43 cavity to ascertain cavity dynamics over the course of the simulation. Nodes were defined by the center of mass of residues G473-M475, G367-P369, G379-E381, and V127-A129, corresponding to turns or loops between  $\beta$ -strands  $\beta$ -24/ $\beta$ -25,  $\beta$ -14/ $\beta$ -15,  $\beta$ -16/ $\beta$ -17 and the V1/V2 stem, respectively (Figure 6). The internode distances to the residue pair W427-E429 on  $\beta$ -20/21 was chosen since W427 is a highly conserved residue and is predicted to interact with the NBD-566 *p*-chlorophenyl group. These distances for the four gp120 coordinate sets are plotted in Figure 6. Significant fluctuation of the V1/V2 stem in GPO3, GCD3, and GPO3\_NBD is observed (Figure 6A,C,D) while more overall cavity instability is seen in GCD3 (Figure 6C) and GPO3\_NBD (Figure 6D) compared to GPO3 and GCD2 (Figure 6A,B). Of note, GCD3-biphenyl and GPO3\_NBD ligands are more deeply bound in the cavity compared to GCD2 (Figure 2E). This cavity instability is consistent with the conclusion drawn from root-mean-square fluctuations in Figure 4 that NBD-556 bound in the gp120 cavity enhances over all gp120 mobility. Although dynamics were enhanced overall, significant changes in Phe 43 cavity shape and volume did not occur when comparing GPO3 (790 Å<sup>3</sup>) and GPO3\_NBD (809 Å<sup>3</sup>) coordinates after 30 ns of MD simulation.

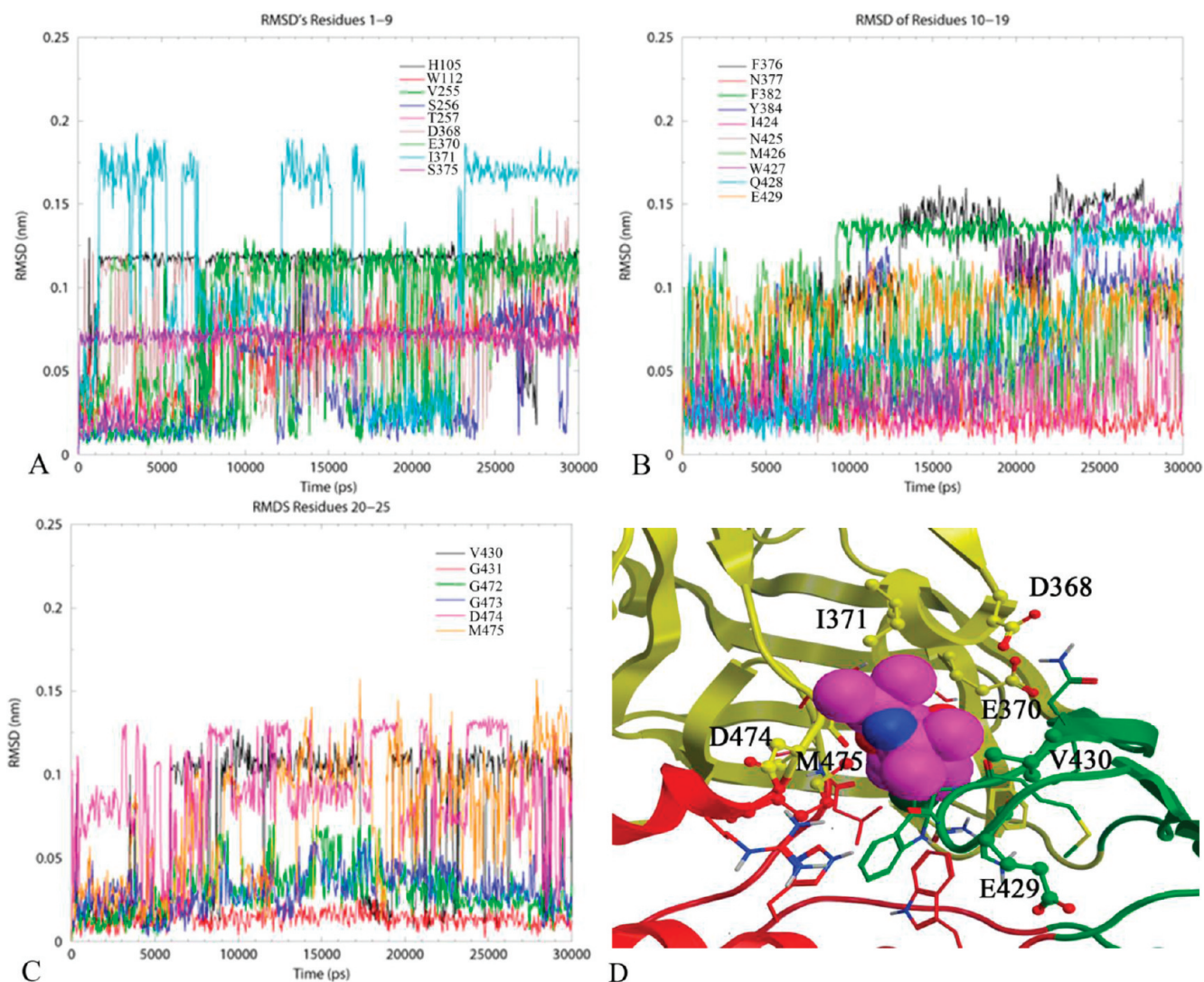
To follow up the observation of enhanced cavity mobility, we examined the extent of NBD-556 fluctuation as predicted to bind in the cavity of GPO3\_NBD during the simulation. The RMSDs of the three functional regions of NBD-556, as defined in Figure 1, do not fluctuate significantly over the 30 ns time frame (Supporting Information Figure S4). However, it is apparent that the region II oxalamide stem displays larger RMSDs during the simulation. (We note that only one tautomer for the region II oxalamide in Figure 1 was considered here as there is no experimental evidence suggesting another active tautomer.) The residue RMSDs are the deviation from the starting conformation of all residue atoms as a function of time. Such an analysis at the residue level allows examination of the stability (or flexibility) of residues in a localized region. We thus examined residue RMSDs to delineate the dynamics of those residues which are predicted to be within interacting distance (less than 0.5 nm) (Table 1) of the bound NBD-556 as indicated in GPO3\_NBD (Figure 7) and in GPO3, GCD2, and GCD3 (Figures S5, S6, and S7, respectively). Inspection of Figure 7 indicates that cavity residues S256, N377, I424, N425, G431, G472, and G473 have low mean RMSDs (less than 0.05 nm). Of these, the RMSD's of residues N377, G431, G472, and G473 also have low standard deviations (less than 0.02 nm) (Table S1). We can attribute these residues with low RMSDs to maintaining the structural integrity of the Phe 43 cavity in the presence of NBD-556. Two other residues T257 and S375 transitioned to a different conformation from the starting structure, as evidenced from the jump in the RMSD (Figure 7A) and remained stable at the new conformation for the duration of the simulation with a low standard deviation of 0.019 and 0.004, respectively (Table S1). An inspection of the starting and final structure revealed that the change in the residue conformation, facilitated interaction of these two residues with the NBD-556 aromatic group, simultaneously. This phenomenon is not observed in GPO3 and GCD2 for residues T257 and S375 which display stable trajectories, while in GCD3, T257, and S375 exhibit highly fluctuating positions. Several other residues I371 (Figure 7A), W427, and Q428 (Figure 7B) exhibit distinct substates throughout the GPO3\_NBD simulation. Residues W427 and Q428 reside on the turn of  $\beta$ -20/ $\beta$ -21 on the bridging

sheet. Furthermore, W427 is predicted to form an aromatic–aromatic stacking interaction with the *p*-chlorophenyl group of region I. Inspection of the first frame and last frame indicates that at later stages of simulation the NBD-556 *p*-chlorophenyl group rotates and that W427 may adjust its position to maintain the stacking interaction. Inspection of I371, W427, and Q428 RMSDs calculated from the simulations trajectories (Figures S5, S6, and S7) for GPO3, GCD2, and GCD3 does not show distinct states for these residues and only in the case of GCD2 is an overall increase in fluctuation observed for W427 and Q428.

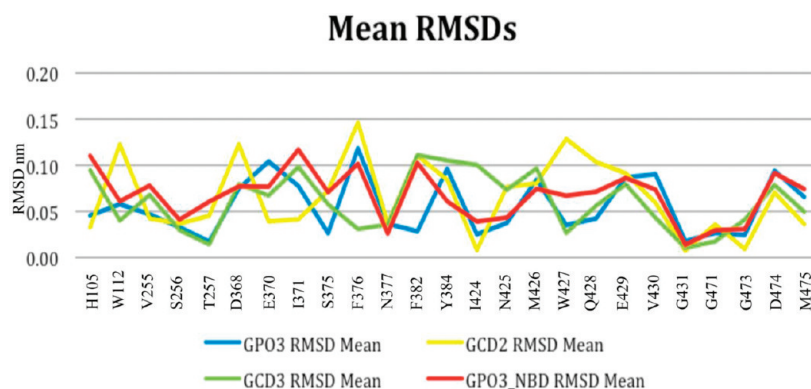
The remaining residues, D368, E370, M426, E429, V430, D474 and M475, exhibit the highest degree of fluctuation (RMSD standard deviation >0.02 (Table S1)). These residues are located at the rim of cavity vestibule (Figure 7D) within 4.5 Å of region III tetramethylpiperidine. Three of these, D368, E370, and D474, are from the outer domain and demonstrated slow mode minima in GNM analysis of GCD2, GCD3, and GPO3.<sup>40</sup> (The GNM studies did not include a gp120-NBD-556 complex because the small molecule is inadequately represented in the Gaussian network model.) Thus, three dynamics states—stable, transitional, and highly mobile—are exhibited by residues that form the Phe 43 cavity when bound by the NBD-556 ligand.

We also analyzed the extent for which these 25 residues have coordinated fluctuations with NBD-556 by measuring the pairwise residue distances with NBD region I, II, and III as shown in Figures S8, S9, and S10. Interaction distances of less than 0.40 nm (minimum distance between the center of mass of the respective NBD region and the residue) and with low standard deviations reflect tight interactions. Residues with tight interaction for region I are V255, S256, T257, S375, F376, F382, I424, N425, and W427; for region II are N425, M426, W427, G473, and D474; and for region III are M426, W427, E429, V430, G473, D474, and M475. Those residues with the tightest NBD-556 interactions and lowest residue RMSDs are S256, N377, I424, N425, and G473, all from the outer domain suggesting that these residue-NBD-556 interactions provide the maximum stabilization to the protein–ligand complex. Those residues that have higher RMSDs but tight NBD-556 interactions are F376, F382, M426, W427, Q428, E429, V430, D474, and M475, suggesting these residues have relaxed away from the corresponding crystal structure positions, allowing a more favorable interaction with the bound NBD-556 molecule. With the exception of F376 and F382, all reside in the bridging sheet domain. Given that the bridging sheet region is highly flexible and displays alternate conformations in several gp120-antibody crystal structures,<sup>59</sup> tight NBD-556 interactions with these same residues can be postulated to be important in driving NBD-556 structuring of gp120.

While some residues which were initially within interacting distance of the bound NBD-556 (distance <0.45 nm) maintained their interactions (V255, S256, T257, S375, F376, F382, I424, N425, W427, M426, G473, D474, E429, and V430 (Table S1)), other residues moved away from NBD-556, during the course of the simulation. Of note are residues with large pairwise distances (>0.6 nm) to NBD-556 during MD simulation when docking predicted interactions less than 0.45 nm. Residue D368 exhibits an average pairwise distance of 0.7 nm with region III with significant fluctuation during the simulation. Nearby residues I371 and E370 also display large mean pairwise distance to the NBD-556 region III, 0.78 and 1.07 nm, respectively. Thus, region III contact with outer domain residues in the vicinity of D368, E370, and I371 is not stabilized by NBD-556 binding. Most of the



**Figure 7.** RMSDs for the 25 residues that form the Phe 43 cavity of GPO3\_NBD and are within 4.5 Å of NBD-556 as predicted by docking. (A) Residues are H105, W112, V255, S256, T257, D368, E370, I371, and S375. (B) Residues are F376, N377, F382, Y384, I424, N425, M426, W427, Q428, and E429. (C) Residues are V430, G431, G472, G473, D474, and M475. (D) Residues surrounding NBD-556 in the Phe 43 cavity vestibule that exhibit larger fluctuations during simulation.



**Figure 8.** Mean residue RMSDs for the 20 residues in the Phe 43 cavity within 5.0 Å of the docking binding mode of NBD-556 for each simulation complex: GPO3 (blue), GCD2 (yellow), GCD3 (green), and GPO3\_NBD (red).

residues interacting with NBD-556 regions I and II also have a low standard deviation ( $\leq 0.05$  nm), while those interaction with

region III have a higher standard deviation ( $\geq 0.05$  nm). This suggests that the residues interacting with the NBD-556



molecule are not rigid but have local flexibility, allowing the bound NBD-556 molecule some flexibility as well.

To verify NBD-556 enhancement of cavity dynamics when docked in the Phe 43 cavity, residue RMSDs are compared to GPO3, GCD2, and GCD3 (Figure 8 and Figures S5–S7). Overall, residues with the largest RMSDs in GPO3\_NBD also exhibit larger RMSDs in GPO3, GCD2, and GCD3 throughout the simulations. However, for residues D368, E370, and I371 the magnitude and type of fluctuation differ among the four simulation sets. GCD3 and GPO3\_NBD have similar RMSDs for D368 and E370 and I371, while only GPO3\_NBD exhibits distinct dynamic states for I371. Moreover, in GCD2, D368 dynamics are accentuated (mean RMSD 0.12 nm) while E370 and I371 fluctuations are substantially reduced (mean RMSDs 0.04). A second area of differing dynamics is W427 and Q428 (mean RMSD of 0.13 and 0.10 nm, respectively) on the bridging sheet. This contrasts with moderate dynamics with transitional states for W427 and Q428 in GPO3\_NBD and very stable dynamics for these residues in GPO3 and GCD2. Greater fluctuations in the bridging sheet for M426, E429, V430, and D474 as exhibited in the GPO3\_NBD simulation are similarly observed for GPO3, GCD2, and GCD3. Thus, MD simulations of GPO3\_NBD in comparison with GPO3, GCD2, and GCD3 show differential dynamics for both outer domain (D368, E370, and I371) and bridging sheet residues (W427 and Q428) in the Phe 43 cavity.

## CONCLUSIONS

In this study we compare the fluctuation dynamics of the gp120 core (residues 86–492) when bound to its cognate ligand (CD4 receptor), a CD4 miniprotein mimetic derived from the scyllatoxin scaffold (CD4M47), a small molecule ligand (NBD-556) and without ligand bound, but in the CD4 bound structured form. The docked binding mode of NBD-556 with *p*-chlorophenyl group bound most deeply in the Phe 43 cavity is consistent with mutagenesis experiments<sup>30</sup> and SAR studies,<sup>31</sup> thus providing the rationale for further computational studies using this model.

As a docked model of NBD-556 bound to gp120 is used rather than a crystal structure, trends in weakening and strengthening of specific protein–ligand interactions can be interpreted as an adaptation of the MD relaxed complex to a form closer to the native state. Nonetheless, the overall dynamics are revealing and consistent with previous studies. Molecular dynamics studies have shown that the bridging sheet is highly mobile and that two strands  $\beta$ -2/3 and  $\beta$ -20/21 have distinct dynamics.<sup>35</sup> In the context of NBD-556, interactions with W427 are tight and intra-bridging sheet contacts as measured between V123 and W427 strengthen, while interactions between the bridging sheet and inner domain weaken consistent with the counter rotation between the inner and outer domain and disruption of the bridging sheet observed in computations by Abrams<sup>39</sup> and Tan.<sup>37</sup>

While NBD-556 has a stable fluctuation profile the simulations indicate that its presence in the Phe 43 cavity enhances the overall mobility of the gp120 envelop protein in comparison to the CD4, miniprotein bound and uncomplexed forms. Evaluation of gp120 intraprotein interactions and cavity stability indicated that in response to NBD-556 binding the bridging sheet  $\beta$ -2/ $\beta$ -3 interaction strengthens and the interface between inner domain and bridging sheet weakens and is less stable. Overall, individual RMSDs reveal three dynamic states for cavity residues: stable, transitional, and highly mobile depending on the cavity filling capacity of the ligand. At the cavity bottom, residues

T257 and S375 have notably increased dynamics with biphenyl (GCD3) and NBD-556 (GPO3\_NBD) bound, compared to GCD2 with Phe43 in the cavity. At the cavity vestibule the largest enhancement is observed for in residues 364–368 and 370 and 371. Enhancement of residues 364–368 dynamics in this region is not surprising as there is no compensating D368-CD4-R59 interaction predicted to form in the NBD-556-gp120 complex to stabilize the  $\alpha$ 3-helix. Indeed, the lack of gp120 binding for CD4-R59A<sup>23</sup> mutant indicates that this is an important region of protein–ligand interaction. The concurrent enhancement of E370 and I371 dynamics, however, was also unexpected, as both residues are well conserved and were thought to form a stable surface on the outer domain as assessed by GNM studies.<sup>40</sup> Moreover, in the absence of the D368-CD4 R59 stabilization several dynamics states are observed for these two residues, in the case of GPO3\_NBD. In summary, residues with low RMSD and tight interactions with regions I, II, and III in the outer domain (S256, I424, N425, and G473) may provide the most stabilization to the protein–ligand complex. Conversely, those residues with larger RMSDs but tight NBD-556 interactions (S375, F382, M426, W427, Q428, E429, V430, D474, and M474) may represent residues and regions that contribute to NBD-556-induced gp120 structuring.

The delineation of cavity dynamics in the context of NBD-556 binding suggests that tight interactions with the bridging sheet are important and drive structuring. Interactions in the outer domain with residues 472–475 between  $\beta$ -24 and the  $\alpha$ 5-helix in the inner domain may dominate binding rather than interactions with  $\alpha$ 3-helix and D368. Overall enhancement of gp120 dynamics may result from the small molecule ligand binding more deeply in the Phe 43 cavity and/or a lack of stabilizing protein–protein interactions in the absence of protein ligand (cf. CD4 or CD4 miniprotein mimetic). The thermodynamic signature of NBD-556 while similar to CD4 has diminished enthalpic and entropic terms ( $\Delta H = -24.5$  kcal/mol and  $T\Delta S = -57.4$  kcal/mol) when compared to CD4 ( $\Delta H = -34.5$  kcal/mol and  $T\Delta S = -79$  kcal/mol).<sup>27,28</sup> The enhanced dynamics observed in this study provides an indication for which gp120 residues may be contributing to less efficient binding of NBD-556 in comparison to the CD4 receptor. A previous report has noted that several NBD analogues have differential effects on gp120 structuring and viral enhancement when the NBD region III is modified<sup>31,60</sup> and that efficient inhibition of CD4-gp120 binding may be obtained when unwanted triggering of gp120 conformational change is eliminated. Results from this MD study, in the absence of protein–ligand crystal structure, provide insights into which residues and interactions may be responsible for inducing gp120 structuring and suggests which interactions may have the most impact on future small molecule designs.

## ASSOCIATED CONTENT

**S Supporting Information.** Supplementary Figures S1–S9 and Table 1S. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ABBREVIATIONS

MD, molecular dynamics; HIV, human immunodeficiency virus; GNM, Gaussian network model; RMSF, root-mean-square fluctuations; RMSD, root-mean-square deviations; SAR, structure–activity relationships.

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