Folding Molecular Dynamics Simulation of a gp41-Derived Peptide Reconcile Divergent Structure Determinations

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Supporting Information

ABSTRACT: T-20 peptide is the first FDA-approved fusion inhibitor against AIDS/HIV-1 gp41 protein. Part of it, the gp41[659−671] peptide, that contains the complete epitope for the neutralizing 2F5 monoclonal antibody, has been found experimentally in a number of divergent structures. Herein, we attempt to reconcile them by using unbiased large-scale all-atom molecular dynamics folding simulations. We show that our approach can successfully capture the peptide’s heterogeneity and reach each and every experimentally determined conformation in sub-angstrom accuracy, whilst preserving the peptide’s disordered nature. Our analysis also unveils that the minor refinements within the AMBER99SB family of force fields can lead to appreciable differences in the predicted conformational stability arising from subtle differences in the helical- and β-region of the Ramachandran plot. Our work underlines the contribution of molecular dynamics simulation in structurally characterizing pharmacologically important peptides of ambiguous structure.

1. INTRODUCTION

Deletions or mutations in the tryptophan-rich C-terminal region (residues 660−683) of the AIDS/HIV-1 gp41 protein can prevent fusion with the host cell membrane.1,2 This region of gp41 encompasses the synthetic peptide T-20 that corresponds to residues 638−673, which has been approved by the FDA (under the names Fuzeon or Enfuvirtide) as the first fusion inhibitor targeting gp41 with an EC50 of 1 ng/mL.3,4 T-20 is the gp41[659−671] peptide, that contains the complete epitope for the neutralizing 2F5 monoclonal antibody, for the broad-spectrum neutralizing 2F5 monoclonal antibody.5,6

Part of T-20 is the gp41[659−671] peptide which contains the complete epitope (sequence ELDKWA) for the neutralizing 2F5 monoclonal antibody.8−11 Notably, the affinity of the 2F5 antibody is reduced after the binding between gp120 and CD4, suggesting that the gp41[659−671] epitope is solvent exposed in the prefusogenic form but becomes less accessible or restructured after fusion.12−14 It is for these reasons that the solution structure (in prefusogenic form) of the gp41[659−671] has been long recognised as being important for the development of new peptide antigens targeting AIDS/HIV infection.

The structure and dynamics of the gp41[659−671] peptide in solution are a source of controversy in the literature. The NMR/CD study by Anglister’s group15 showed that the peptide takes-up a mostly 310-helical conformation in solution and based on a sufficient number of NOE restraints they deposited their structure (PDB entry 1LCX), lending support to previously published CD data.16 Later UV resonance Raman scattering studies revealed instead a variety of motifs, with half the peptide bonds in helical conformation (mainly 310 and π-helix, less α-helix) and the rest in extended and unfolded conformations (mainly β-turn and polyproline II).17 Follow-up studies by Pessi’s group using a combination of experimental and computational approaches suggested that the initially proposed 310-helical structure by Anglister’s group may be an error: their NMR (PDB entry 1MZI) and CD data suggested that the peptide is mostly disordered, spanning an ensemble of conformers, including helices and turns.18 On top of that, Crain’s group argued as well for a conformational plasticity using a combination of CD and MD data.19,20 However, there was inconsistency even between the experimental findings with their MD data. The group chose for the simulations the CHARMM force field, which has repeatedly been demon-
strated to suffer from an α-helical bias. The result was that only at elevated temperatures their MD data were in qualitative agreement with the experiment, whereas the β-turn motif suggested by Pessi’s group was found unstable. Furthermore in a later study, the peptide was found to exhibit autonomous helical folding in the presence of membrane mimicking sodium dodecyl sulfate micelles, which was tunable by pH variation, a behaviour that was not observed in aqueous solutions. Given the indications for the highly dynamic nature of the gp41[659−671] peptide in solution a direct experimental attack on elucidating the peptide’s structure has been problematic. Herein, we aim to study the folding behaviour of this malleable peptide using unbiased large-scale all-atom molecular dynamics simulation. Using a previously validated force field coupled with an adaptive tempering protocol and a sufficient simulation length to guarantee the convergence of the peptide’s derived atomic structures we attempt to thoroughly characterise its structure and dynamics. We show that simulations can bridge the gap among divergent structure determinations by reaching in sub-angstrom accuracy every experimentally available peptide structure, whilst preserving its disordered nature. Detailed analysis of the simulations interestingly showed that even the best performing force fields still suffer from imbalance between the different secondary structural elements. This diversity seems to impact inadvertently the accurate structural characterisation of highly dynamic peptides such as gp41[659−671]. Our simulations contribute to a better characterisation of the structure and dynamics of this pharmacologically and therapeutically important peptide and to the unceasing refinement of current generation force fields.

2. RESULTS AND DISCUSSION

2.1. Structural Heterogeneity of the T-20 Derived gp41[659−671] Peptide. Even a cursory examination of the collection of structures deposited in the PDB database (Figure 1 and Table S1) reveals that the structures of the T-20 derived peptide, from the 7-mer to the 17-mer, free (PDB entry 1LCX, PDB entry 1MZI) or in complex with the 2F5 antibody (PDB entries 1TJG, 1TJH, 1TJI) can vary from helical to turn to almost fully extended. Turn structures seem to be the dominant population when the peptide 7-mer, 9-mer, 11-mer or 13-mer is in complex with the 2F5 monoclonal antibody (PDB entries 1U8I, 1U8J, 1U8K, 2F5B, 2P8L, 2P8M, 2P8P, 3D0V, 3DRO, 3DRQ, 3DRT, 3MOA, 3MOB, 3MOD, 4G6F, 4N5X). It is worth mentioning however the inconsistency among the studies regarding the resolution of the electron density data for the C-terminal residues. This was attributed to either the end-capping of the peptide through amidation that deprives the extra end-charge or to the extension of the end by just a couple of residues (670WN671). In both cases, the formation of a key hydrogen bond of the peptide’s end with the heavy chain of the antibody stabilises the C-terminal part of the peptide. This conformational stabilisation was found particularly persistent under high ionic strength conditions (PDB entry 3D0L) mostly due to an additional intrachain hydrogen bond between the carbonyl oxygen of 668Trp with the backbone amide of
 Indeed, helical structures seem to be favoured in the larger context like in the 59-mer, that is extended towards the N-terminus this time to include the membrane-proximal external region (MPER), especially in the lipid environment of the viral membrane, with a bend around the epitope to expose the amino acids involved in the antibody binding (PDB entries 2LP7, 2M7W, 4G6F, 4NRX). A significant helical tendency for the gp41[657−669] peptide fragment in water was also found in later studies by the Anglister group in the longer gp41[636−677] peptide, with the preceding N-terminal part unstructured.44

Both findings regarding turn and helical preferences were confirmed by later studies in non-aqueous environments revealing the folding of the 28-mer gp41[656−683] to a continuous kinked helix (PDB entries 2M8M, 2M8O), whilst shorter or longer peptides showed non-continuous partial structuring to 310-α-helix. In particular, the kink comprised residues 664DKW666 preceded by 310-helical conformation for residues 665KW666 that requires a continuous helical structure interrupted by a kink/turn/loop conformation for residues 664DKW666 that redirects the gp41 backbone at the pre-transmembrane region, thus orienting the 666Trp and 669Leu side-chains in parallel and the 664Asp side-chain negative charge projecting from the main chain axis in different direction.39,43 This crucial involvement of side-chain interactions gives the epitope specificity. In total, the conformation of residues 651LELDKWAS668 when bound to neutralizing antibodies is conserved in most observed structures, whereas the conformation of residues 664DKW666 that varies significantly, like in structures 2P8P, 1TJH, and 3DOL.36 One of the most distinct structures is that of a 310-α-helix supported by the seminal study of Anglister’s group. This type of helix was attributed mainly to the strong presence of an i,i+3 hydrogen bond between the carboxyl oxygen of 664Asp and the amide proton of 667Ala together with JH-TT coupling constants and chemical shifts supporting helical conformations and only minor populations of random coil, arguing that the presence of this hydrogen bond could be key to deter antibody interaction. Indeed it seems that the most potent short constrained peptides that inhibit HIV-1 entry are not the ones with high helical propensity.45 Both proper 664DKW666 β-turn conformation and side-chain positioning are also an independent capability to interact with a membrane surface, with different segmental secondary structures being adopted during the fusion process. The bias towards helical folding seems to be pH tuned, with no defined fold type at basic pH and helical near physiological and acidic pH environment.20

Figure 2. Similarity to experimental structures. Superposition of the closest simulation-derived structure for the ILDN (left) and ILDN* (right) force fields to each one of the experimentally known structures (see also Figure 1 and Table S1). Secondary structural elements are highlighted using cartoon representation and color coded using STRIDE-derived secondary structure assignments as in Figure 1. The experimental structures are overlayed in transparency for clarity. The occupancy of each ensemble of structures is noted next to each PDB code. The occupancies were calculated using an rmsd cut-off of 2.0 Å as determined from the rmsd histogram distributions in Figure S1, over the stable conformations of the trajectory (T < 340 K).
crucial for designing immunogens capable of eliciting neutralizing antibodies.

2.2. Excellent Agreement between Experimental and Simulation-Derived Structures. The performance of the force fields against folding a peptide with such divergent structures is examined in Figure 2. By using a straightforward root-mean-square deviation (rmsd) calculation, we can identify simulation-derived structures that match each one of the experimental structures to sub-angstrom accuracy. Some of the structures are scarcely visited, as expected due to lesser secondary structural content and consequently increased dynamics. These structures are expected to be further stabilised in the presence of the interacting antibody. On the other hand, helical populations seem to be preferred for this isolated peptide in solution, even though the length of the peptide is smaller compared to the experimental ones’ for which helical conformations were found (see Table S1 for respective peptide lengths per structure). In general, we observe a high agreement on the relative percentage preference for each experimental structure among the two force fields. This means that the delicate balance between helical, beta, turn and coil structures is preserved, as expected for two so closely related force field versions. However, it cannot be overlooked that the ILDN* force field gives constantly higher representation of all the structures. This phenomenically better performance cannot be fully evaluated since its highly disordered nature prevents an accurate description of the peptide’s folding landscape. Nonetheless, when it comes to describe the stable peptide conformations, the ILDN* force field presents itself as a better choice.

To elaborate further, the two earliest solution NMR structures of the 13-mer peptide, 1LCX15 and 1MZI18 although they are the closest to our simulation system, they seem to present only a fraction of the peptide’s available conformations, occupying 10–30% of the stable conformers (see also Figure S1). The 3_10-helix in particular, was proposed after using the 664Asp−667Ala hydrogen bond as a restrain, as mentioned in the previous sections. This hydrogen bond even though is observed, is not persistent (see also Figure S2). In the simulation with the ILDN force field, that hydrogen bond is present in 57% of the low-temperature associated frames (22% of the total simulation time) and in the simulation with the ILDN* force field, in 88% of the low-temperature associated frames (33% of the total simulation time). This implies that the presence of that hydrogen bond is not enough to restrain the conformation to a 3_10-helix, as the persistence of the hydrogen bond is 3–4 times higher than the occupancy of this set of structures. The imposition of this restrain could thus affect the accurate determination of a representative NMR ensemble.

Likewise, turn structures that are observed in the context of the short 7-mer, 9-mer and 11-mer in complex with the 2F5 antibody are restrained by 3 intrapeptide hydrogen bonds (Figure S2). These hydrogen bonds constrain the peptide to a straight conformation with side-chain arrangement that favors antibody recognition.34,35 Apparently one of them, between 664Asp−667Ala, is the same one that contributes to the 3_10-helix formation discussed above. The second hydrogen bond between 666Trp−669Leu, is quite persistent as well, being present in 65% of the low-temperature associated frames (26% of the total simulation time) for the ILDN force field and in 86% of the low-temperature associated frames (32% of the total simulation time) for the ILDN* force field. This hydrogen bond, as discussed in the previous section, was often associated with the initiation of an α-helix extending through the C-terminus of the longer peptides. On the other hand, the third hydrogen bond between 664Asp−666Trp is quite scarce with only 2.2% presence in the low-temperature associated frames (0.9% of the total simulation time) for the ILDN force field and 1.8% presence in the low-temperature associated frames (0.7% of the total simulation time) for the ILDN* force field. This last hydrogen bond seems to “lock” the 666DKW666 β-turn conformation that is crucial for antibody recognition. Thereby, these short turn structures comprising the central peptide epitope residues are visited more often, with 35–45% occupancy, whilst peptide structures with mixed turn/coil conformations along the whole peptide length are still visited but with occupancies of around 5%.

Helical conformations persist longer, with occupancies of no less than 25% that can reach up to 66% of simulation time (accounting for the low-temperature associated frames). This seems contradictory to the fact that the peptide is found experimentally in helical conformations only in a larger context (21-mer to 59-mer) and either in complex with an antibody or in membrane-mimicking environment. It seems though that there is an intrinsic helical preference for this isolated 13-mer peptide in aqueous solutions as well. If this is the case, then such a computational approach to characterise the full ensemble of available structures for a highly disordered peptide as this, is extremely valuable as it presents all the peptide’s accessible conformations with their relative stabilities and preferences.

Closing this section of successfully reproducing all available experimental structures, it is worth mentioning that no other significant population (with occupancy of more than 1% of the low-temperature associated frames) of stable structures was found during our simulations, that differed by more than 2 Å from at least one of the experimental structures included in the collection of structures in Figure 1.

2.3. Differential Secondary Structure Preferences between ILDN and ILDN* Force Fields. From a general perspective, the ILDN and ILDN* simulations show an overall agreement on the preferred peptide structures, in the sense that they give similar relative representation of the helical structures. This phenomenically better performance cannot be completely attributed to the accurate determination of a representative NMR ensemble. To elaborate further, the two earliest solution NMR structures of the 13-mer peptide, 1LCX15 and 1MZI18 although they are the closest to our simulation system, they seem to present only a fraction of the peptide’s available conformations, occupying 10–30% of the stable conformers (see also Figure S1). The 3_10-helix in particular, was proposed after using the 664Asp−667Ala hydrogen bond as a restrain, as mentioned in the previous sections. This hydrogen bond even though is observed, is not persistent (see also Figure S2). In the simulation with the ILDN force field, that hydrogen bond is present in 57% of the low-temperature associated frames (22% of the total simulation time) and in the simulation with the ILDN* force field, in 88% of the low-temperature associated frames (33% of the total simulation time). This implies that the presence of that hydrogen bond is not enough to restrain the conformation to a 3_10-helix, as the persistence of the hydrogen bond is 3–4 times higher than the occupancy of this set of structures. The imposition of this restrain could thus affect the accurate determination of a representative NMR ensemble.

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structures, over the turn/coil ones, with minimal interstitial \( \beta \)
structures. This is nicely captured in Figure 3, where we
calculate the per residue secondary structural preferences
for the two force fields. A closer look reveals that the first and last
couple of residues are quite flexible, as expected for such a
short peptide. On the other hand, the central part of the
peptide comprising the \( 65^1 \text{LELDKWAS}^{668} \) epitope sequence
shows some discrepancy between the two force fields. The
preference for turn conformation of each residue is preserved
among them but there is difference in the preference for \( \alpha \)
helix and \( 3_{10} \)-helix. The ILDN* force field is prone to helical
conformations overall, as is observed in the per residue
secondary structure analysis over time (Figure S3, bottom
panel). But this helical preference is closer to an \( \alpha \)-helix and
lesser to a \( 3_{10} \)-helix, the last one being more favoured by the
ILDN force field. Likewise, most of the helical experimental
structures (9 out of 30) are found in \( \alpha \)-helical conformations, a
couple of them present with a combination of turn and \( 3_{10} \)-
helix and only one (out of 30) folds to a \( 3_{10} \)-helix, as discussed
previously. From this perspective, ILDN* force field seems to
capture this behaviour more accurately. Nonetheless, the
balance between these conformations is critical for accurate
representation of the peptide’s conformations which can play a
role in antibody recognition and interaction.

To explore further the source of this discrepancy, we
calculated the fraction of each secondary structure assignment
for each residue as a function of temperature (Figure S4). This
updated view reveals that \( \alpha \)-helical conformations are
favourably assigned to the central part of the peptide by
both force fields, but they are more stable in the case of the
ILDN* force field, as supported by the higher occupancy in the
low temperature regime (see blue-coloured region in the
difference-plot in the middle column). These same residues
have a higher preference for \( 3_{10} \)-helix in the ILDN force
field simulation, whilst ILDN* force field assigns a small \( 3_{10} \)-helical
population only to residues in the right end part of the epitope
sequence (notice the two small red and blue regions in the
difference-plot in the middle column). On the other hand, \( \beta \)
structures are not observed in any of the experimentally
determined peptide structures (Figure 1). The ILDN* force

Figure 4. Ramachandran plots. Density distributions of the dihedral angles of the peptide calculated over the whole trajectory for the ILDN (left) and ILDN* (right) force fields, with yellow-red colours representing high densities. The middle graph is the difference plot between the two force fields where blue colors correspond to higher preferences for ILDN* (right) and yellow-red colors correspond to higher preference for ILDN (left) force field.

Figure 5. Extent of sampling through application of Good–Turing statistics. Probability of unobserved structures as a function of rmsd from the observed ones, for ILDN (black curve) and ILDN* (orange curve) force fields as produced by the GoodTuring MD program (https://github.com/pkoukos/GoodTuringMD). The main curves are the estimates obtained using structures of the whole trajectories whereas the curves in the inset are estimates for only structures that are associated with temperatures of \( T < 340 \) K (corresponding to structurally more stable peptide conformations).
field again seems to represent this feature better as they are only slightly visited at elevated temperatures (note also the almost complete absence of $\beta$-structures in the per residue secondary structure assignment over simulation time in Figure S3, bottom panel). On the contrary, ILDN force field allows the peptide to accommodate these structures at low temperatures, and particularly for residues in both the ends of the central epitope sequence, $^{66^\text{b}}$LL$^{66^\text{c}}$ and $^{66^\text{b}}$SL$^{66^\text{c}}$ (Figures 3, S3, and S4). On the other hand, turn and coil preferences among the two force fields seem very comparable with no outstanding differences, apart from some slightly higher preference for turn conformation on the N-terminal part and for coil conformation on the C-terminal part of the peptide by the ILDN* force field.

ILDN’s bias over $3_{10}$-helix is illustrated in the dihedral angle calculation presented in Figure 4 (see also the per residue Ramachandran plots and corresponding difference-Ramachandran plots in Figure S5). In these Ramachandran plots, the prevalence of helical populations is clear, but with subtle differences in the exact area that is occupied: ILDN’s helical population tends more to $3_{10}$-helix (note the slightly raised values in the helical region, coloured red in the middle difference Ramachandran plot), ILDN*’s helical population tends more to $\alpha$-helix (note the characteristic blue-coloured region in the difference Ramachandran plot in the lower part of the helical region) and there is an oversampling of the $\beta$-region by ILDN force field. This behaviour is replicated if we repeat the calculation only for the stable structures of the trajectory associated with low temperatures (data not shown). Furthermore, it is transferred on a per residue level calculation (see Figure S5) and is particularly evident for the central epitope residues $^{66^\text{b}}$KWAS$^{66^\text{c}}$ and $^{66^\text{b}}$DKW$^{66^\text{c}}$, which are critical for antibody recognition. This implies that the calculated secondary structure population. This trend has been observed in other experimental structures (Figures 2 and S1). However, the local conformations that they proposed for certain residues are indeed supported by both our simulations and from other experimental structures (Figures 2 and 3). This highlights the difficulty in interpreting complex NMR data of disordered peptides to build accurate models that can cover the entire peptide’s landscape.

In a recent MD study, the peptide was found to have significant amount of helical population, though less in explicit solvent compared to implicit solvent and visited the two experimental peptide structures that were examined (PDB entries $^{1}$LCX and $^{1}$TJH) only upon end-to-end distance restraints. We believe that our unbiased molecular dynamics simulation with the properly selected force field successfully managed to capture the peptide’s heterogeneity and offered the plethora of accessible peptide conformations with relative probabilities. Thus, our folding simulations offer an invaluable method to tackle such a disordered peptide that can accommodate different structures depending on the sequence context, the interaction partner and the surrounding environment.

What’s more, the peptide’s structural variance allowed us to compare the performance of two very closely related force field versions, ILDN and ILDN*, and look for signs of discrepancy. Both these refined force field versions reproduced all available experimental structures in sub-angstrom accuracy while preserving the disordered nature of the peptide. Nonetheless, the ILDN* force field offered a better representation of all the structures by accommodating them for a longer simulation time (although the actual simulation time was 3 times less compared to the ILDN force field). Last but not least, the backbone corrections in this modified ILDN* force field version provided a better balance between the different secondary structural elements with discrete differences in the helical region in the Ramachandran plot: ILDN force field shows an overestimation of $3_{10}$-helical conformations wherein ILDN* force field favours $\alpha$-helices instead.

Closing this section and based on this study, ILDN* force field emerges out of the AMBER99SB-ILDN family, as the choice of preference. The gp41$^{659–671}$ peptide through the availability of many divergent structure determinations served as an excellent candidate to examine the performance of molecular dynamics and current generation force fields not only to reproduce all of them but also to indicate structural preferences and nevertheless reveal sensitivities of the force fields. We believe that current generation force fields have matured enough in predicting peptide structure and dynamics that could complement current experimental schemes that suffer from the weakness of mixed folded/unfolded populations and the dependency on each experimental condition and aim to a better interpretation of experiments.

3. CONCLUSIONS

In this communication we tried to present the divergent structure determinations for this debatable small peptide and unravel its structural complexity by gathering the collection of representative available structures. From our perspective, the view of Anglister’s group of a $3_{10}$-helix (PDB entry 1LCX$^{35}$) holds only partially true for this peptide, due to the imposition of a hydrogen bond, which seems that is a necessary but not a sufficient condition for the folding to a $3_{10}$ type of helix. On the other hand, Pessi’s group argued that this $3_{10}$-helix might be false and proposed instead that the peptide exists in solution as a complex mixture of conformers (PDB entry 1MZI$^{18}$), with some local stable conformation for residues $^{66^\text{b}}$Leu, $^{66^\text{c}}$LD$^{66^\text{d}}$, $^{66^\text{e}}$Trp and $^{67^\text{e}}$Trp. In particular, they argued for a helical tendency for $^{65^\text{c}}$ELLE$^{66^\text{c}}$ residues followed by $\beta$-turn for $^{66^\text{b}}$DKW$^{66^\text{c}}$ and coil for $^{67^\text{c}}$ASLWN$^{67^\text{d}}$ residues. According to our simulations, their ensemble of conformations (PDB entry 1MZI$^{18}$) is not that often visited (10–20% of the low-temperature associated frames and 3–6% of the total simulation time, Figures 2 and S1). However, the local conformations that they proposed for certain residues are indeed supported by both our simulations and from other experimental structures (Figures 2 and 3). This highlights the difficulty in interpreting complex NMR data of disordered peptides to build accurate models that can cover the entire peptide’s landscape.

4. METHODS

4.1. Selection of Experimental Structures. Figure 1 illustrates the collection of experimental structures deposited in the PDB to date, that comprise the complete 13-mer peptide sequence $^{65^\text{c}}$ELLELDKWASLWN$^{67^\text{d}}$ as well as smaller 7-mer, 9-mer and 11-mer peptide sequences that contain the complete central epitope sequence $^{66^\text{b}}$ELDKWA$^{66^\text{c}}$. PDB entries that
contain partially the sequence of gp41 [659–671] (like PDB entry 2X7R), were not included for structural comparison with the full 13-mer peptide sequence.

4.2. System Preparation and Simulation Protocol. The starting peptide structure, 659ELLEDWKASLWMN671, was in the fully extended form, as obtained from the program Ribosome with both terminal ends unprotected. Addition of missing hydrogen atoms and solvation–ionisation were performed with the program LEAP from the AMBER tools distribution. The simulation was performed using periodic boundary conditions and a cubic unit cell sufficiently large to guarantee a minimum separation between the PBC-related images of the peptide of at least 16 Å, filled with pre-equilibrated TIP3P water molecules.49 We followed the dynamics of the peptide’s folding simulation using the program NAMD for a grant total of 13 μs [10 μs with the AMBER99SB-ILDN,51 hereafter referred to as “ILDN” and 3 μs with the AMBER99SB-STAR-ILDN52 force field, hereafter referred to as “ILDN*FF”] and adaptive tempering with an inclusive temperature range of 280–480 K53 as implemented in the program NAMD (adaptive tempering can be considered as a single-copy replica exchange method with a continuous temperature range).

The simulation protocol was the following: the system was first energy minimised for 1000 conjugate gradient steps. Then the temperature was increased with a ΔT step of 20 K until a final temperature of 300 K over a period of 32 ps. Subsequently the systems were equilibrated for 10 ps under NpT conditions without any restraints until the volume equilibrated. This was followed by the production NpT runs with the temperature and the pressure controlled using the Nose–Hoover Langevin dynamics and Langevin piston barostat control methods as implemented by the NAMD program, with adaptive tempering applied through the Langevin thermostat, while the pressure was maintained at 1 atm. The Langevin damping coefficient was set to 1 ps⁻¹ and the piston oscillation period to 200 fs, with a decay time of 100 fs. The production run was performed with the impulse Verlet-1 multiple timestep integration algorithm48 as implemented by NAMD. The inner time step was 2 fs, with non-bonded interactions calculated every 1 time steps and full electrostatics every 2 time steps using the particle mesh Ewald method55 with a grid spacing of approximately 1 Å and a tolerance of 10⁻⁶. A cutoff for the van der Waals interactions was applied through a switching function, acting between 7 and 9 Å. The SHAKE algorithm55 with a tolerance of 10⁻⁸ was used to restrain all bonds involving hydrogen atoms. Trajectories were obtained by saving the atomic coordinates of the whole system every 0.8 ps.

4.3. Extend of Sampling and Convergence. Given the highly dynamic nature of the gp41 [659–671] peptide in solution, even 13 μs of cumulative folding simulation time might be insufficient to characterise its folding landscape, especially the vast configurational space of the disordered/unfolded conformations. This can be nicely illustrated in Figure 5, where we demonstrate the application of a recently proposed probabilistic method that applies Good–Turing statistics to molecular dynamics trajectories.57 According to that, there is a small probability (1 out of 10) to find structures that differ by more than 4 Å rmsd from the ones already observed, if you account for the whole trajectory and all-heavy atoms. This value drops down to 2.6–2.8 Å if you consider the central peptide epitope sequence 660ELDKWA667 or the backbone atoms of the 13mer peptide (data not shown).

However for the stable peptide conformations (T < 340 K), it seems improbable to find structures with statistically significant difference as there is a probability 1 out of 1000 to observe a structure with rmsd > 0.5 Å from the ones already observed (inset in Figure 5, 13mer, all-heavy atoms). This demonstrates the fine sampling of the stable peptide conformations, offering statistical significance to our observations regarding the experimentally observed structures (Figure 1 and Table S1). As for the unfolded fraction for which convergence is difficult to achieve, parameters that are affected by this shortcoming are explicitly discussed in the corresponding sections.

4.4. Trajectory Analysis. The programs CARMA and GRCARMA59 together with custom scripts were used for most of the analyses, including removal of overall rotations/translations, calculation of the evolution of the Cα distances, calculation of rmsd’s from a chosen reference structure, calculation of frame-to-frame rmsd matrices, principal component analysis in both Cartesian60,61 and dihedral space62,63 and corresponding cluster analysis, calculation of phi/psi dihedral angles, calculation of average structures and of the atomic root mean square fluctuations and production of PDB files from the trajectories. Secondary structure assignments were calculated with the program STRIDE.64 All molecular graphics work and figure preparation was performed with the programs VMD and CARMA.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01579.

Summarised table of experimental structures found in the PDB; histogram rmsd distributions; hydrogen bond persistence over the trajectories; comparative molecular dynamics analysis over simulation time; per residue secondary structure preferences versus temperature; per residue Ramachandran and difference-Ramachandran plots (PDF).

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Notes

The authors declare no competing financial interest.

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HIV-1 gp41 contains exposed C-peptide regions.

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