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Research Article

Membrane-induced organization and dynamics of the N-terminal domain of chemokine receptor CXCR1: insights from atomistic simulations

Shalmali Kharche[a,b], Manali Joshi[c], Durba Sengupta[a,b,*], Amitabha Chattopadhyay[d,*]

[a] CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India
[b] Academy of Scientific and Innovative Research, New Delhi, India
[c] Bioinformatics Center, S.P. Pune University, Ganeshkhind Road, Pune 411 007, India
[d] CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

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ABSTRACT

The CXC chemokine receptor 1 (CXCR1) is an important member of the G protein-coupled receptor (GPCR) family in which the extracellular N-terminal domain has been implicated in ligand binding and selectivity. The structure of this domain has not yet been elucidated due to its inherent dynamics, but experimental evidence points toward membrane-dependent organization and dynamics. To gain molecular insight into the interaction of the N-terminal domain with the membrane bilayer, we performed a series of microsecond time scale atomistic simulations of the N-terminal domain of CXCR1 in the presence and absence of POPC bilayers. Our results show that the peptide displays a high propensity to adopt a β-sheet conformation in the presence of the membrane bilayer. The interaction of the peptide with the membrane bilayer was found to be transient in our simulations. Interestingly, a scrambled peptide, containing the same residues in a randomly varying sequence, did not exhibit membrane-modulated structural dynamics. These results suggest that sequence-dependent electrostatics, modulated by the membrane, could play an important role in folding of the N-terminal domain. We believe that our results reinforce the emerging paradigm that cellular membranes could be important modulators of function of G protein-coupled receptors such as CXCR1.

1. Introduction

G protein-coupled receptors (GPCRs) are important signaling hubs (Granier and Kobilka, 2012; Chattopadhyay, 2014) that serve as important drug targets in all clinical areas (Jacobson, 2015). The receptors typically have seven transmembrane helices interconnected by extracellular and intracellular loops (Pierce et al., 2002; Venkatakrishnan et al., 2013). The structural details of the transmembrane domains of several GPCRs have been addressed by crystallographic studies with increasing resolution (Zhang et al., 2015; Cherezov et al., 2007; Rosenbaum et al., 2007; Wacker et al., 2017). In addition, computational and magnetic resonance approaches have helped uncover conformational changes in the transmembrane domain that are coupled to receptor activation (Nygaard et al., 2013; Perez-Aguilar et al., 2014; Provasi et al., 2011; Lee et al., 2014; Kohlhoff et al., 2014; Schmidt et al., 2014; Manglik et al., 2015). The extracellular and intracellular domains exhibit higher dynamics and remain less characterized. For example, the intracellular loop 3 of GPCRs in many cases are replaced by folded proteins or monoclonal antibodies (more recently, with nanobodies) to help in crystallization (Venkatakrishnan et al., 2013; Cherezov et al., 2007; Rosenbaum et al., 2007). The N-terminal domains are usually truncated in structural studies, and if not, they are difficult to be resolved by NMR, as well as crystallography (Cherezov et al., 2007; Rosenbaum et al., 2007; Park et al., 2012). The importance of these domains is apparent from the fact that they are involved in ligand binding and G-protein coupling (Wheatley et al., 2012; Peeters et al., 2011; Timossi et al., 2002; Chen et al., 2011). The interaction of the extra-membranous regions of GPCRs with the membrane bilayer is an emerging concept, which needs to be addressed for a comprehensive understanding of GPCR function.

Chemokines (chemotactic cytokines) are a large family of small soluble proteins (70-120 residues) that play important regulatory roles in innate immunity, inflammation, host cell defense against infection, embryogenesis and metastasis (Rajagopalan and Rajarathnam, 2006; Allen et al., 2007). Chemokines are classified either as CC, CXC, CX_3C, or C based on the presence of conserved cysteine residues near the N-

Abbreviations: CXCR1, CXC chemokine receptor 1; GPCR, G protein-coupled receptor; IL-8, interleukin-8; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

* Corresponding authors.
E-mail addresses: d.sengupta@ncl.res.in (D. Sengupta), amit@ccmb.res.in (A. Chattopadhyay).

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terminus. Chemokines induce transmembrane signaling by activation of a subclass of GPCRs, known as chemokine receptors. The chemokine receptor family represents the largest subfamily of peptide-binding GPCRs (Onuffer and Horuk, 2002) of which CXCR1 is a representative member. CXCR1 is predominantly expressed on the surface of neutrophils and is a major mediator of immune and inflammatory responses (Holmes et al., 1991). The natural ligand for this receptor is interleukin-8 (IL-8), also known as CXCL8 (Rossi and Zlotnik, 2000) and CXCR1 binds IL-8 with high affinity (Rajagopalan and Rajarathnam, 2004; Park et al., 2011).

It has been reported in a number of studies that the extracellular N-terminal domain of chemokine receptors plays a crucial role in binding affinity, receptor selectivity, and regulation of signaling (Rajagopalan and Rajarathnam, 2006; Prado et al., 2007; Szpakowska et al., 2012). Specifically, in case of CXCR1, the recognition and selective binding of IL-8 was previously demonstrated to be mediated by the N-terminal domain of the receptor (Rajagopalan and Rajarathnam, 2004). However, the extracellular N-terminal domain of CXCR1 is highly dynamic and therefore could not be resolved in the NMR structure of the receptor (Park et al., 2012). The structure of a short truncated fragment of the CXCR1 N-terminus has been co-crystallized with IL-8 in the absence of membranes and it has been shown that this short peptide does not fold to any defined secondary structure (Skelton et al., 1999). In order to explore the organization and dynamics of the N-terminal region of CXCR1, several studies using fluorescence and NMR have been performed on this domain. A peptide construct, corresponding to the N-terminal residues of CXCR1 was shown to interact with the membrane (Haldar et al., 2010). Upon interaction with the membrane, the N-terminal domain exhibited motional restriction, and tryptophan residues in the N-terminal domain were implicated in interaction with the membrane (Park et al., 2011; Haldar et al., 2010). Interestingly, IL-8 was observed to bind the N-terminal peptide with significantly higher affinity in micelles than in solution (Rajagopalan and Rajarathnam, 2004), suggesting the role of the membrane bilayer in imparting ligand specificity. In a subsequent study, the secondary structure adopted by the peptide was shown to have a β-sheet character in reverse micellar system (Chaudhuri et al., 2013). In line with these observations, IL-8 binding to CXCR1 is proposed to be a multi-step process in which the first step involves the interaction of the N-terminal region of CXCR1 with the membrane (Park et al., 2011). However, the molecular mechanism by which the membrane confers structure and selectivity to the N-terminal region of CXCR1 is still not understood. In this overall context, the dynamics of the N-terminal region with respect to the membrane assumes relevance.

In this work, we carried out all atom molecular dynamics simulations of the N-terminal domain of CXCR1 in the presence and absence of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayers. Our results show large conformational dynamics in the N-terminal region in general, independent of the presence of the membrane bilayer. Interestingly, our simulations show an increased structural propensity for β-sheet formation for the N-terminal domain of CXCR1 in the presence of membranes, in agreement with previous experimental results. An interesting feature of our results is the transient interactions observed between the peptide and the membrane. Our results provide novel insights into the dynamics of the interaction between the N-terminal region of CXCR1 and the membrane, which could help in understanding ligand specificity and overall function of the receptor.

2. Experimental

2.1. System setup

The sequence of the N-terminal region of rabbit CXCR1 was taken from UniProtKB (residues 1-34 from P21109) and corresponds to MEVNVWNMTD LWTWFEDEFA NATGMPPVEK DYSP. The three dimensional structure of the peptide was built in an extended conformation using Discovery Studio version 3.5. The modeled structure was energy minimized and used as an input for the molecular dynamics simulations. The peptide was placed in a simulation box containing a pre-equilibrated, hydrated POPC bilayer at a distance of 2 nm. The bilayer was constructed from the CHARMM lipid builder (Jo et al., 2008) and equilibrated for 100 ns. As a control, the peptide was simulated in a water box containing TIP3P water molecules. Six sodium ions were added to both the systems to neutralize the net

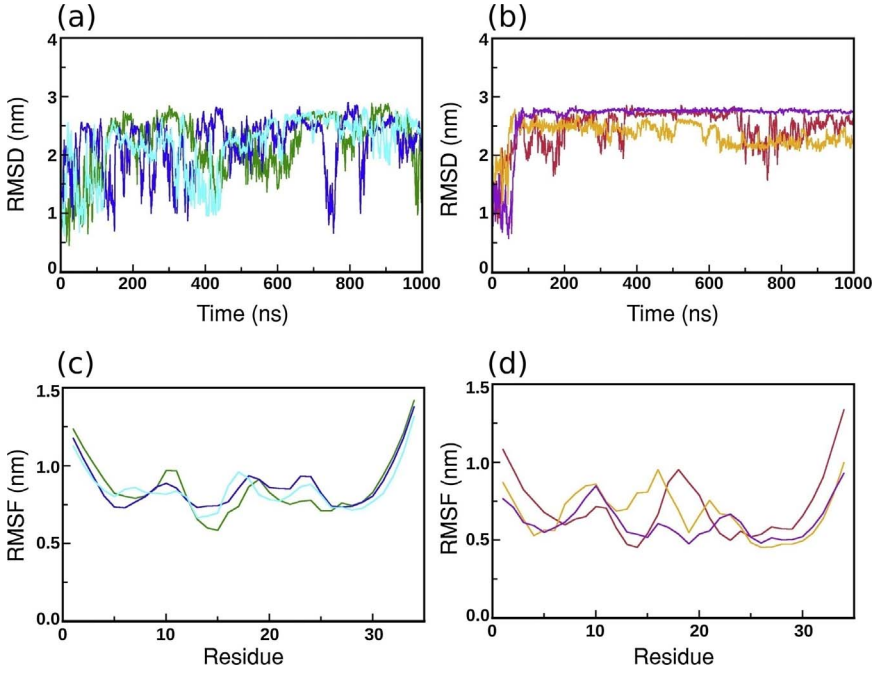

Fig. 1. Structural characterization of the CXCR1 N-terminal peptide. RMSD plots of the backbone of the N-terminal region of CXCR1 in (a) water (simulations 1-3, shown in green, blue and cyan), and (b) in the presence of POPC bilayers (simulations 1-3, shown in red, orange and purple). RMSF plots of the backbone of the N-terminal peptide in (c) water and (d) POPC bilayers. The coloring scheme is the same as in (a) and (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

charge. The scrambled peptide was designed with the same residues in a randomized order. The scrambled sequence was generated using a random sequence generator from the shuffle protein module of the sequence manipulation suite (www.bioinformatics.org/sms2/shuffle_protein.html; Stothard, 2000). The scrambled sequence corresponds to: KMVDTVDPNF DEEMTPEMWN PGFWTWNEYA VASL. The structural models and the simulation system was setup for the scrambled peptide using the same workflow as used for the CXCR1 N-terminal peptide.

2.2. Simulation parameters

All atom simulations were performed using GROMACS 4.5.5 software (Van Der Spoel et al., 2005) with the CHARMM36 force field (Bjelkmar et al., 2010; Klauda et al., 2010). Energy minimization was carried out using the steepest descent algorithm. Systems were equilibrated under NVT conditions for 100 ps using the v-rescale algorithm (Andersen, 1983) with positional restraints on the peptide. This was followed by 100 ps of NPT equilibration. Production runs were performed for 1 μs in triplicate for both water and POPC bilayers. Electrostatic interactions were represented using the Particle-mesh Ewald (PME) method (Darden et al., 1993). Temperature coupling was applied to maintain the temperature at 300 K using the v-rescale algorithm. Semi-isotropic pressure was maintained using Parrinello-Rahman pressure coupling (Parrinello and Rahman, 1981) with a pressure of 1 bar independently in the plane and perpendicular to the bilayer. A time step of 2 fs was used. The lengths of all bonds were constrained using the LINCS algorithm (Hess et al., 1997). Analysis was carried out using GROMACS and in-house tools; images were rendered using VMD (Humphrey et al., 1996).

3. Results

To understand the dynamics of the N-terminal domain of CXCR1 and identify the molecular details, we carried out extensive atomistic molecular dynamics simulations. A peptide corresponding to the truncated N-terminal region of the CXCR1 (see Section 2 for sequence) was built in an extended conformation and was simulated in the presence and absence of POPC bilayers. This was followed by analysis of atomic level differences induced by the bilayer. As a control, we compared our results to a peptide containing the same residues in a randomly scrambled sequence.

3.1. β-sheet conformation is preferentially adopted in the presence of bilayer

The initial orientation and structure of the N-terminal peptide in water and POPC bilayers are shown in Fig. S1. Each system was simulated in triplicate for 1 μs. The time evolution of the peptide was monitored by plotting the RMSD and RMSF (see Fig. 1). High structural dynamics was observed with large fluctuations persisting in water at longer time scales (Fig. 1a). The corresponding simulations in POPC bilayers deviate significantly from the initial extended structure, but appear to stabilize toward the end of the simulation (see Fig. 1b). The N-terminal peptide is flexible in both the systems with the termini exhibiting higher dynamics (Fig. 1c,d). To visualize the difference in the conformation of the peptides, representative structures from the most populated clusters (of the last 400 ns) are shown in Fig. 2. The peptide appears to be more compact in the presence of the membrane bilayer and adopts a folded state (Fig. 2d-f). A distinct β-sheet fold was observed in the presence of POPC bilayers while several β turns were observed in water.

To quantify the folding of the peptide, the secondary structure per residue was plotted over time as per the DSSP nomenclature (see Fig. 3; Kabsch and Sander, 1983). In general, the peptide exhibited β-sheet conformation in water and in POPC bilayers. However, the extent and persistence of β-sheet character was higher in the presence of POPC bilayers. The peptide forms a short β-hairpin (two antiparallel β-sheets with an intervening turn) between residues 15 to 20 across the simulations in water that subsequently unfolds. In contrast, in the presence of the POPC bilayer, a β-sheet is formed and retained in all three replicate simulations, although the β-sheet is formed by different residues in the replicate simulations. In the first set (Fig. 3d), a β-hairpin is observed between residues 14 to 23 which remains folded till the end of the simulation. In addition, transient β-sheets are formed between residues 4-6, and 31-33. In the second set (Fig. 3e), the initial β-sheet formation is observed between residues 4-7 and 19-25, that unfolds to residues 4,5 and 19-21. In the third set (Fig. 3f), a stable β-hairpin conformation is adopted between residues 2-21. β-sheet probabilities were calculated from the time average of the secondary structure evolution and it shows that the peptide prefers to form a β-sheet in the presence of POPC bilayers (shown in Fig. 4). The β-sheet character of the peptide observed in the simulations is in agreement with previously reported CD spectra of the CXCR1 N-domain peptide in reverse micellar environment (Chaudhuri et al., 2013).

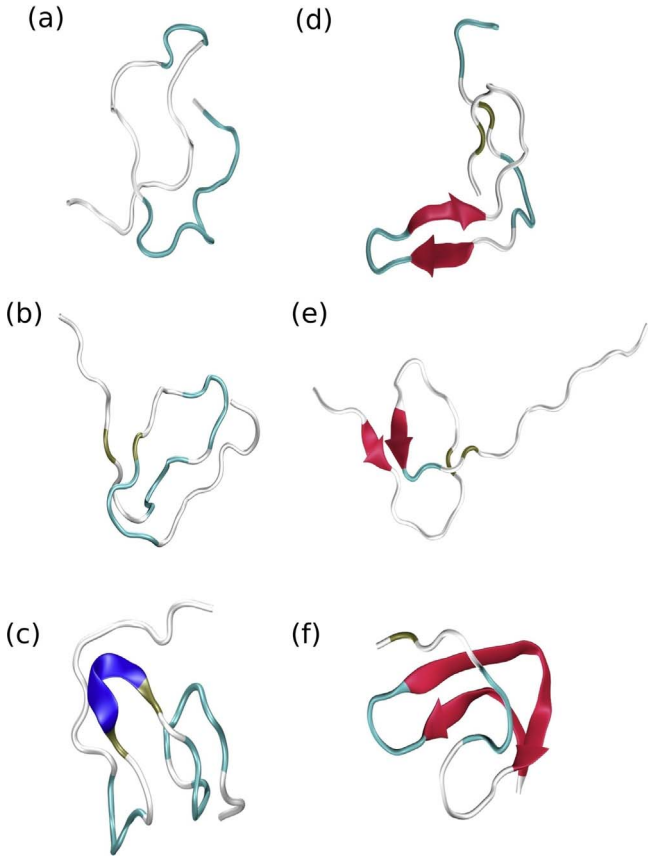

Fig. 2. Representative snapshots of secondary structures of the CXCR1 N-terminal peptide. The conformers representing the most populated clusters for three independent simulations in water (a-c) and in the presence of POPC bilayers (d-f) are shown. The N-terminal peptide is rendered in the cartoon representation and colored according to secondary structures, in which β-sheet, turn and α-helix are represented by red, cyan and blue, respectively. The unstructured coil regions are shown in white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

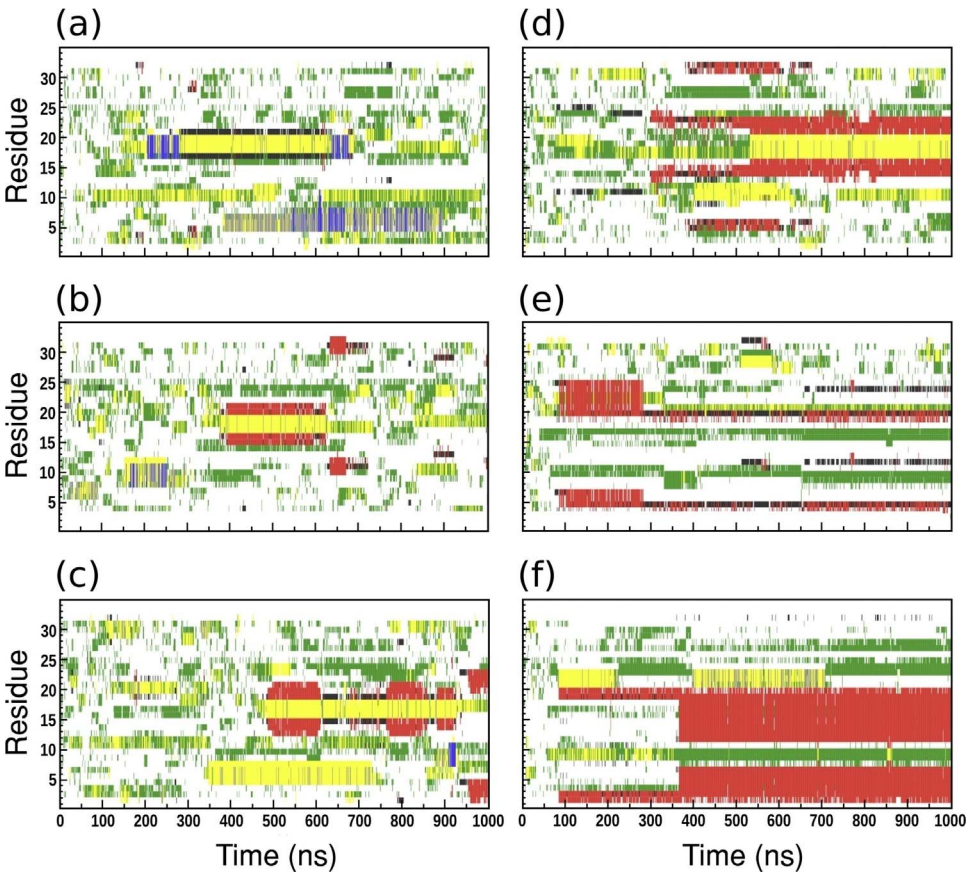

Fig. 3. Plots of secondary structural elements per residue of the CXCR1 N-terminal peptide along the simulation trajectory in water (a-c) and in the presence of POPC bilayers (d-f). Secondary structures were calculated as per the DSSP nomenclature, where red, yellow, green, blue and black represent β-sheet, turn, bend, α-helix and β-bridge, respectively. The white stretches represent unstructured coil regions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

3.2. Transient interactions are observed between the N-terminal peptide and the membrane

To examine whether interactions with the membrane led to the preferential β-sheet content, the minimum distance between the peptide and membrane was plotted over the simulation time (see Figs. 5 and S2a). The peptide was observed to interact transiently with the membrane. Most of the adsorbed states lasted for less than 50 ns. Multiple interactions between the peptide and membrane were observed in the first 100 ns. Interestingly, the third set (Fig. 5c) exhibiting the highest β-sheet content (Fig. 3f) had least interactions with the membrane.

Experimental data using tryptophan fluorescence have indicated a role of tryptophan residues in anchoring the peptide to the membrane (Park et al., 2011; Haldar et al., 2010). Consequently, we monitored the minimum distance between the tryptophan residues (positions 6, 12 and 14) and the membrane (Figs. 5 and S2b-d). In presence of the membrane bilayer, a few contacts between tryptophan residues and the membrane were observed. Maximum contacts were observed between the C-terminal residues (positions 32 to 34). Interestingly, two of the tryptophan residues (positions 12 and 14) are involved in high relative contacts with the membrane.

3.3. Long-range membrane electrostatics could help in driving peptide folding

We explored whether the bilayer could affect the intra-peptide interaction energetics by modulating the electrostatics of the peptide through long-range effects. The total residue–residue interaction energy (*i.e.,* sum of the electrostatic and Lennard Jones terms) was calculated

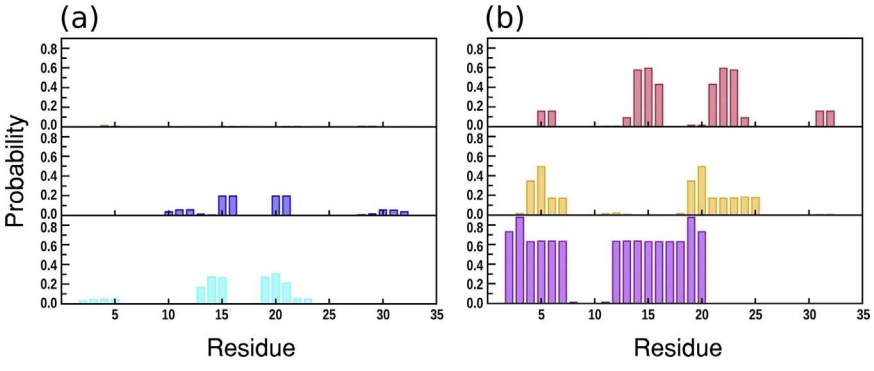

Fig. 4. A quantitative analysis of β-sheet content of the CXCR1 N-terminal peptide. The β-sheet propensity per residue of the N-terminal peptide in (a) water (simulations 1-3 are shown in green, blue and cyan, respectively) and (b) in presence of POPC bilayers (simulations 1-3 are indicated by red, yellow and purple, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

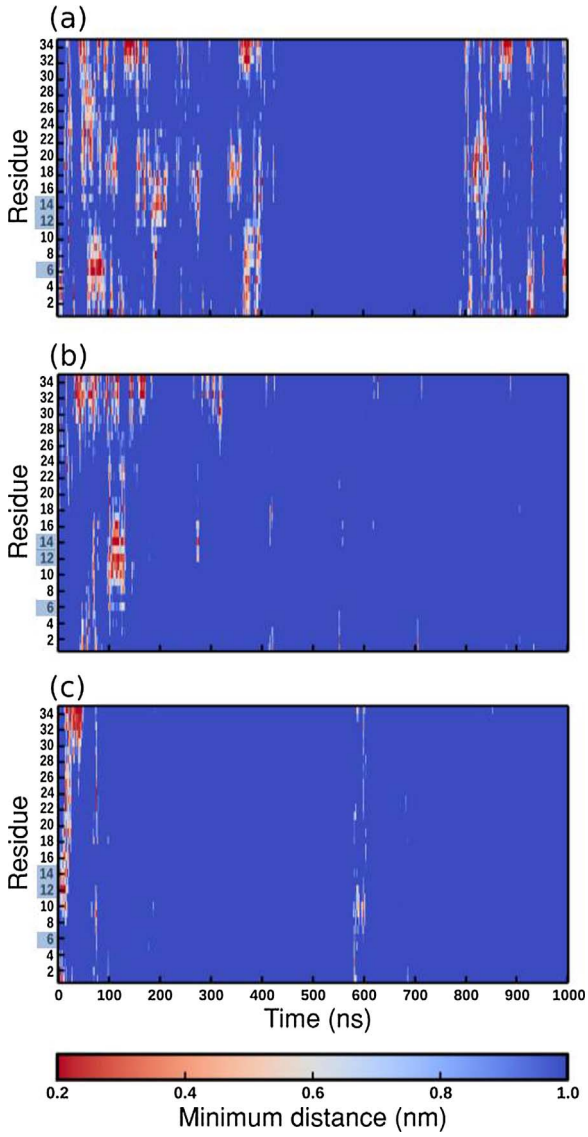

Fig. 5. Contact maps of the distance between the N-terminal peptide and POPC bilayers. The minimum distance between each residue of the peptide and lipid bilayers is shown for three independent simulations (a-c). Red indicates a close contact between a specific residue of the peptide and the bilayer, whereas blue stretches indicate a larger distance between them. A color scale bar corresponding to the contact distances is shown below the figure. The tryptophan residues are highlighted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

for water and the membrane bilayer (see Fig. S3). A distinct difference was observed between the peptide in water and in the presence of POPC bilayers, with the latter displaying lower and therefore more favorable interaction energy. To further break down the peptide energetics, we calculated the residue wise electrostatic interaction energy maps of the initial structures in water and POPC bilayers (Fig. 6a,b). Interestingly, the plots indicate a difference in interaction energies in the two cases, suggesting that the inter-residue energetics is modulated by long-range electrostatic effects of the bilayer. This trend persists in all simulation sets over the simulation time (Figs. 6c,d and S4). The off-diagonal values correspond to the secondary structure character of the peptide, and as expected, more off-diagonal elements are observed in the membrane due to its folded state. Taken together, these results show that the POPC bilayer helps induce a folded compact state enabling high β-sheet propensity.

3.4. Effect of a scrambled sequence peptide

To analyze the specificity of membrane-dependent folding, we considered a peptide with a scrambled sequence, *i.e.,* the same residues in a randomly varying sequence. The peptide was modeled in an extended conformation, and simulations were performed in water and in the presence of POPC bilayers. The secondary structure plots of the peptide in water and membrane are shown in Fig. 7a,b. As the figure shows, the peptide remains essentially unstructured in water and in presence of POPC bilayer, although a few folding/unfolding events are sampled. The average secondary structure propensity confirms low β-sheet content (Fig. 7c,d). Similar to the wild type peptide, the scrambled peptide interacts transiently with the bilayer (Fig. 7e). The main interactions are at the central part of the peptide that contains the tryptophan residues (positions 19, 24 and 26 in the scrambled sequence). Similar to the wild type peptide, the intra-peptide interaction energies are less favorable in water (Figs. S5 and S6), although the difference between interaction energetics in bilayer and water is less for the scrambled peptide relative to the wild type peptide (Figs. S3 and S5). This indicates that electrostatics plays a predominant role in the interaction between the N-terminal domain and the membrane. Taken together, our results suggest that the membrane bilayer confers a high β-sheet propensity to the wild type N-terminal domain of CXCR1, but not to the scrambled peptide.

4. Discussion

In this work, we have used microsecond time scale unbiased molecular dynamics simulations to sample the dynamics of the CXCR1 N-terminal domain in the presence of membrane bilayers. Molecular dynamics simulations have been shown to accurately represent aspects of folding dynamics of water soluble peptides (Piana et al., 2014; Lane et al., 2013). However, much less is known about the structural organization of peptides and protein domains that interact with membranes. For relatively small antimicrobial peptides, molecular dynamics simulations have been shown to be successful in capturing key aspects of membrane organization (Cirac et al., 2011; Mika et al., 2011). More recently, it has been demonstrated that long time scale atomistic simulations with the CHARMM force-field appears to be well suited to analyze interactions of peptides at membrane interfaces (Sandoval-Perez et al., 2017). Although, the spontaneous binding of a small peptide from solution to the membrane surface has been suggested to be a slow process (Lin and Grossfield, 2014), molecular dynamics has been able to reproduce key experimental features (Bajaj et al., 2016; Farrotti et al., 2015). Counter-intuitively, it has been shown that for a small soluble peptide, unbiased molecular dynamics can sample the energy landscape in significantly shorter times than enhanced sampling methods such as replica exchange molecular dynamics (REMD) or simulated annealing, but the population ensembles differ (Marzinek et al., 2016). Longer time scale simulations could provide a more accurate sampling of the structural energetics, and help discover new facets of how the membrane modulates the dynamics of membrane protein domains.

In conclusion, we have performed multi-μs atomistic molecular dynamics simulations to analyze the conformational dynamics of the N-terminal region of the CXCR1 chemokine receptor. Although the N-terminal domain of chemokine receptors in general, and CXCR1 in particular, is known to play an important role in the overall function of the receptor, the structure and dynamics of the domain remain elusive.

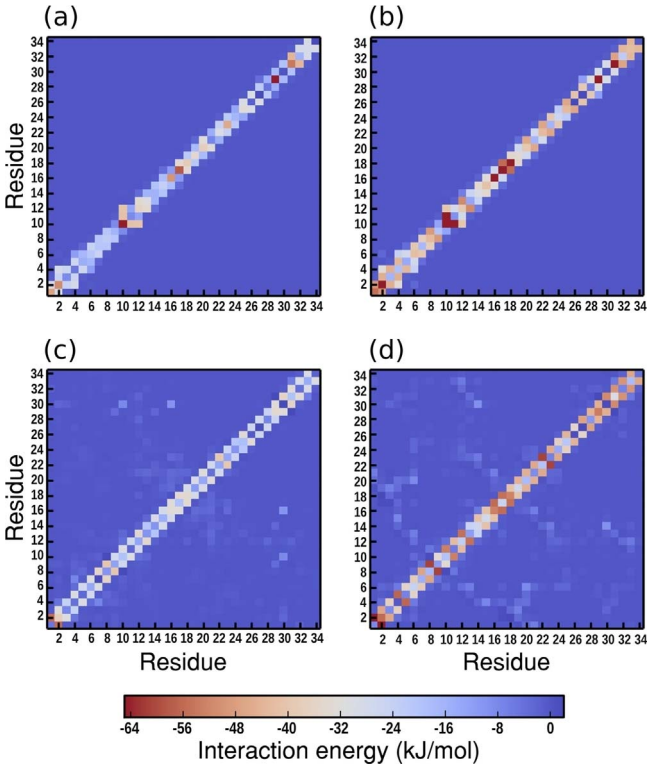

Fig. 6. Residue-wise electrostatic component of interaction energy of the N-terminal peptide at the start of the simulation in water (a) and POPC bilayers (b). The residue-wise electrostatic component of interaction energies, averaged over simulation time and three independent simulations, are shown for water (c) and POPC bilayers (d). The color scale bar corresponding to electrostatic component of interaction energies is shown below the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

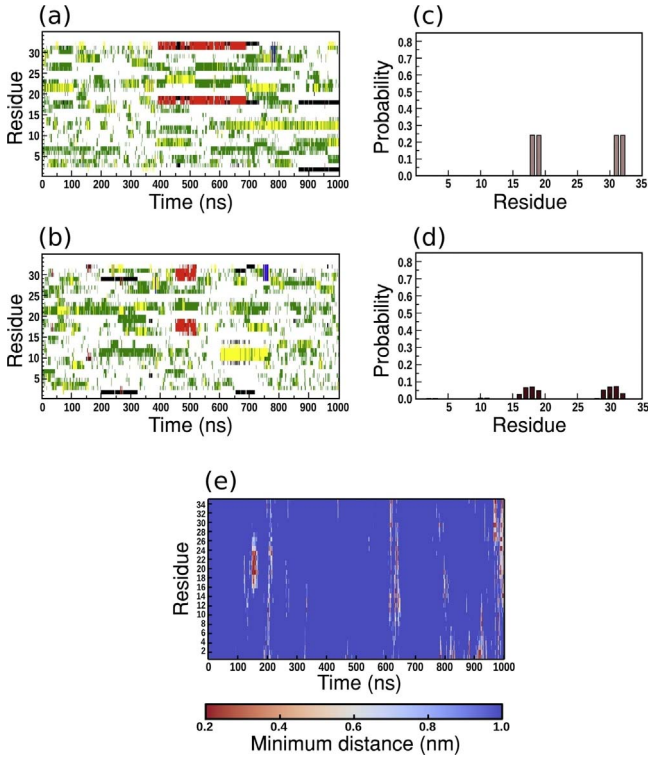

Fig. 7. Secondary structural characteristics and membrane binding propensity of the scrambled peptide. Plots of the secondary structural elements of the scrambled peptide in (a) water and (b) in presence of POPC bilayers are shown. β-sheet propensity per residue of the scrambled peptide is shown in (c) water and (d) presence of POPC bilayers. Residue-wise interaction of the scrambled peptide with POPC bilayers depicted as minimum distance as a function of time (e) is also shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Recent NMR structure of CXCR1 showed that its N-terminal domain is highly dynamic and for this reason, most of the N-terminal amino acids could not be resolved (Park et al., 2012). Our results show that the peptide prefers to be folded in the presence of POPC bilayers and prefers to adopt a β-sheet structure. These results assume greater relevance in light of the fact that IL-8 has been shown to bind the N-terminal domain with higher affinity in micellar environment relative to aqueous solution (Rajagopalan and Rajarathnam, 2004), thereby suggesting the role of membrane interactions in imparting ligand specificity. In a broader perspective, our results reaffirm the emerging paradigm that cellular membranes could be important modulators of chemokine receptors in particular, and GPCRs in general.

Conflict of interest

The authors declare no conflict of interest.

Supporting Information

Supporting information contains six figures (Figs. S1–S6). Fig. S1 shows snapshots of the initial systems, Fig. S2 shows interactions between the N-terminal peptide and POPC bilayer, Figs. S3 and S5 show protein-protein interaction energy, Figs. S4 and S6 show residue-wise interaction energies.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2017.09.003.

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