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Research Article

Lipid-dependent conformational landscape of the ErbB2 growth factor receptor dimers

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ABSTRACT

Altered lipid metabolism has been linked to cancer development and progression. Several roles have been attributed to the increased saturation and length of lipid acyl tails observed in tumors, but its effect on signaling receptors is still emerging. In this work, we have analyzed the lipid dependence of the ErbB2 growth factor receptor dimerization that plays an important role in the pathogenesis of breast cancer. We have performed coarse-grain ensemble molecular dynamics simulations to comprehensively sample the ErbB2 monomer-dimer association. Our results indicate a dynamic dimer state with a complex conformational landscape that is modulated with increasing lipid tail length. We resolve the native N-terminal "active" and C-terminal "inactive" conformations in all membrane compositions. However, the relative population of the N-terminal and C-terminal conformers is dependent on length of the saturated lipid tails. In short-tail membranes, additional non-specific dimers are observed which are reduced or absent in long-tailed bilayers. Our results indicate that the relative population as well as the structure of the dimer state is modulated by membrane composition. We have correlated these differences to local perturbations of the membrane around the receptor. Our work is an important step in characterizing ErbB dimers in healthy and diseased states and emphasize the importance of sampling lipid dynamics in understanding receptor association.

1. Introduction

A marked increase in degree of saturation and lipid tail lengths have been reported in breast cancers and it has been suggested that reduced membrane fluidity is a feature of advanced disease conditions (Baenke et al., 2013; Bernardes and Fialho, 2018; Maulucci et al., 2016; Peetla et al., 2013; Zalba and Ten Hagen, 2017). Several consequences of the altered lipid metabolism have been suggested, such as a reorganization of microdomains and signaling molecules. Its effect on membrane receptors is relatively less explored, especially in the context of those receptors that are upregulated in cancer. There are emerging reports of lipid modulation of receptor organization (Rath and Deber, 2012; Pawar and Sengupta, 2018; Kharche and Sengupta, 2020) by both direct interactions or indirect changes in membrane physical properties (Paila et al., 2009; Pawar et al., 2015a). Previous studies have focused on increased receptor association with increasing membrane thickness (hydrophobic mismatch) and have reported an increasing number of

dimer states (Cymer et al., 2012; Mall et al., 2001; Mitchell et al., 1992; Sparr et al., 2005). However, molecular details of these dimer states are still emerging and effects of altered membrane composition on conformational dynamics are still unclear.

The growth factor receptor ErbB2, is a receptor tyrosine kinase whose association and subsequent activation leads to cell proliferation and migration (Schlessinger, 2000). ErbB2 dimers are currently the main targets in the treatment of several breast cancers, including metastatic breast cancer (Baenke et al., 2013). The lipid dependence of the ErbB2 dimers is noteworthy in light of its upregulation and increased association in breast cancers (Llorente et al., 2013). ErbB2 dimerization and activation has been analyzed by several direct and indirect methods and revealed a highly dynamic monomer/dimer equilibrium (Chung et al., 2010; He and Hristova, 2012; Jura et al., 2009; Li et al., 2012; Schlessinger, 2000). Additionally, the dimerization of the transmembrane helices in the absence of the extracellular and intracellular domains has been reported (Beevers et al., 2012; He and Hristova, 2012),

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although quantitative measurements in lipid bilayers are limited (Fink et al., 2012; Hong and Bowie, 2011). Two structures of the ErbB2 transmembrane helix dimer have been resolved by NMR studies that correspond to an active N-terminal and inactive C-terminal states (Bocharov et al., 2008, 2016). The active/inactive states are distinguished by increased interactions at the N-terminal and C-terminal SmXXXSm (GxxxG) motifs, respectively. The activation of the receptor has been linked to the interconversion of these dimer states (Bell et al., 2000; Fleishman et al., 2002; Mendrola et al., 2002). Interestingly, in the ErbB2 oncogenic mutants a modulation of these dimer states has been suggested by specific interactions with charged lipids (Maeda et al., 2018; Matsushita et al., 2013). A role of cholesterol in modulating association in rat ErbB2 (Neu) has been reported (Hasan et al., 2019), suggesting a role of lipid chain order.

Molecular dynamics simulations provide a valuable complement to experimental studies, especially to explore the molecular details of transmembrane helix association (Arkhipov et al., 2013; Lemmin et al., 2013; Zhang et al., 2013; Ende et al., 2004). Coarse-grain models have been successful in investigating the dimerization of several transmembrane helices (Chng and Tam, 2011; Hall et al., 2012; Prasanna et al., 2013, 2014; Sharma et al., 2013; Wei et al., 2013). These studies were able to describe protein-protein contacts (We et al., 2013), "hydrophobic" mismatch (Benjamini and Smit, 2013; Schmidt et al., 2008) and membrane perturbations (Morozova et al., 2012). Improved sampling methods such as umbrella sampling have been used to estimate the potential of mean force (PMF) of dimerization (Castillo et al., 2013; Henin et al., 2005; Janosi et al., 2010; Prasanna et al., 2013; Sengupta et al., 2010). In the particular case of ErbB2, the alternate dimer conformations of ErbB2 dimers that were experimentally observed were not distinguished in the early free-energy profiles (Janosi et al., 2010; Prasanna et al., 2013). However, we were able to identify these experimentally-resolved conformers (Pawar and Sengupta, 2019) using an ensemble of coarse-grain unbiased simulations, i.e. the DAFT ensemble simulation methodology (Wassenaar et al., 2015). In fact, in all four members of the ErbB family the predicted homodimer conformers were observed to be consistent with structures resolved by NMR (Pawar and Sengupta, 2019). Such an ensemble simulation approach coupled with exhaustive sampling of lipid dynamics have been demonstrated to accurately describe the energy landscape for a model polyaniline peptide (Pawar et al., 2015b), and may prove useful to analyze lipid-effects.

In this work, we have used the coarse-grain ensemble simulations using the DAFT methodology to characterize the lipid effects on ErbB2 dimerization. We have analyzed the effect of lipid tail length and charged head-group moieties. Consistent with previous studies we observe the native dimer states, although with a more complex lipid-dependent conformational landscape. A distinct lipid chain length modulation of the N-terminal and C-terminal conformers is observed. The convergence and statistical properties of the method are explored, exemplifying the importance of adequate sampling when studying protein-protein interactions in membranes.

2. Methods

2.1. The DAFT approach

Simulations were performed with the Martini force-field (Marrink et al., 2007; Monticelli et al., 2008) using the protocol, Docking Assay For Transmembrane proteins (DAFT) (Wassenaar et al., 2015). In this approach, isotropic distributions of relative orientations between two transmembrane helices are generated and multiple simulations performed in parallel. The two monomers of ErbB2 that were modeled as ideal helices were embedded in a membrane and solvated. Molecular dynamics simulations are performed using the automated coarse-grain simulation workflow (Wassenaar et al., 2013). The initial distance between the center of mass of two protein monomers was set to 4.0 nm. The helices were built from sequence using Pymol and aligned to the z-

Table 1

Overview of protein association dynamics and lipid thickness. The median association time is reported. Error estimates were obtained from resampling the original data (100 bootstraps). The bilayer thickness reported is the average of the global thickness measured as distance between the upper and lower leaflet PO4 beads (600 - 1000 ns). The standard deviation for membrane thickness is indicated.

Bilayer	Dimers (%)	Association time (ns)	Bilayer thickness (nm)
DLPG	77.1	199.5 $\pm$ 7.5	3.3 $\pm$ 0.1
DLPC	83.7	165.5 $\pm$ 7.4	3.4 $\pm$ 0.1
DLPG/PC	87.0	134.5 $\pm$ 6.7	3.4 $\pm$ 0.1
DPPC	87.9	150.0 $\pm$ 5.2	4.1 $\pm$ 0.1

axis. The sequence corresponding to the ErbB2 transmembrane domain studied here : ⁶⁵¹LTSHSAVVGILLVVVLGVVFGILI⁶⁷⁵.

2.2. System setup and simulation parameters

Ensemble simulations were set up with varying protein and lipid composition, as summarized in Table 1. For lauroyl bilayers, DLPC (1,2-dilauroyl-sn-glycero-3-phosphocholine), DLPG (1,2-dilauroyl-sn-glycero-3-phosphorylglycerol) and mixed DLPC/DLPG bilayers were considered. For the long-tailed lipids, DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DBPC (1,2-dibehenoyl-sn-glycero-3-phosphocholine) and DXPC (1,2-dihexacosanoyl-sn-glycero-3-dilignoceroyl) were considered. In the Martini force-field these lipids are represented with 3–6 beads in each lipid tail, respectively. For each system, two peptide monomers were embedded in approximately 85 lipid molecules and solvated with 1000 water molecules. In the first set of lauroyl and palmitoyl bilayers, a set of 500 simulations of 1 μ s each were performed. In the second set of DXPC and DBPC bilayers, a set of 300 simulations of 1 μ s each were performed. In total, the simulations presented here correspond to 3.2 ms of effective atomistic simulation time considering a speed-up factor of 4 in coarse-grain simulations (Marrink et al., 2007; Monticelli et al., 2008).

To set up the simulations, 500 steps of steepest descent energy minimization was performed, followed by 10 ps of constant volume simulation with restraints on the protein structure. During this stage, a time step of 2 fs was used to allow proper relaxation of the protein and the lipids. Subsequent equilibration and production run were carried out using the standard Martini parameters (Marrink et al., 2007; Monticelli et al., 2008): Non bonded interactions were truncated at 1.2 nm using a shifted LJ potential with switch function from 0.9 to 1.2 nm and electrostatic interactions with a switch function from 0.0–1.2 nm. For the final runs, a time step of 20 fs was used. The temperature was controlled using a stochastic thermostat with a coupling time 1 ps and the pressure was maintained using the Berendsen barostat with a coupling time of 1 ps and compressibility of 310^{-4} bar⁻¹. The production simulations were performed under NPT conditions (T = 310 K and P = 1 bar) for 1 μ s, saving frames every 500 ps. All simulations were performed with GROMACS (Hess et al., 2008) version 4.5.5.

2.3. Mapping the dimer conformations

Helix association was defined by an inter-helical distance cut-off of 1.2 nm (Fig. S1). The time of association is reported as the first association time, i.e. the start time of the period 50 ns of stable dimer formation. As the distribution of first association times is non-normal (Fig. S2), the median values of the association time are reported in Table 1. The interaction energies were calculated over the trajectory to test for convergence, as described by Wassenaar and co-workers (Wassenaar et al., 2015). To capture the ensemble statistics, the mean (black line) and median (red line) of the distribution, together with the vigintile points (5%) away from the median are represented.

To analyze the main clusters sampled for each system, the last 400

ns of each trajectory were combined, giving a total set of 4×105 structures for each system. The normalized population as a function of the distance and the inter-helical angle between the peptides was calculated from the combined trajectories. Distances were binned using a bin-size of 0.05 nm and angles were binned using a bin-size of 2° , yielding a populations histogram, which was subsequently normalized. The confidence interval was determined using the bootstrap method (Efron, 1979), drawing 1000 random sets of data comprising of 4×105 structures from the combined trajectory (sampling with replacement). To calculate the protein-protein contact maps, all structures from all simulations corresponding to a given cluster were extracted. A cutoff distance of 0.55 nm was used to assign the presence (value = 1) or absence of a contact (value = 0). The contacts were then averaged with respect to number of structures yielding values between 0 and 1. The analysis of structural properties of lipid was performed on the trajectory omitting the first 250 ns.

3. Results

3.1. Capturing the pathways and energetics of protein association

To probe the molecular determinants of lipid-dependent ErbB2 dimerization, we used the DAFT coarse-grain simulation framework. In the first step, we focus on the lauroyl and palmitoyl lipids and test the effect of head-group and acyl chain length. Independent unbiased coarse-grain molecular dynamics simulations of 1 μ s were performed for an ensemble of 500 randomly placed monomer pairs, corresponding to 0.5 ms simulation time for each bilayer. During the course of the simulations, the monomer pairs diffuse and encounter each other in the membrane. In most of the simulations the monomers associated to stable dimers although a few unassociated monomers were present in the ensemble (Table 1). In addition, unique association/dissociation events were sampled in a few trajectories. The percentage of dimers formed in the simulations was relatively high (Table 1). After 1 μ s, approximately 80 % dimer species were observed in each bilayer. The lowest percentages of dimers were observed in DLPG bilayers and the largest in DPPC bilayers. The association times range between 130–200 ns, and the differences between the bilayers is small. The values of association times reported here should be considered as a qualitative description of association due to the nature of the coarse-grain force-field.

To analyze the dimerization events, the interaction energy between the transmembrane helices was plotted as a function of time for the ensemble simulations. The evolution of the distributions over time for DLPC and DPPC bilayers is shown in Fig. 1. The interaction energies for the other bilayers is shown in Fig. S3. To capture the ensemble statistics, the mean (black line) and median (red line) of the distribution, together with the vigintile points (5%) away from the median are represented. Such a representation has been previously shown to well represent the convergence of dimer assemblies in membranes (Wassenaar, 2015). At the start of the simulations the transmembrane helices are separated with zero interaction energy. During the simulations, the transmembrane helices diffuse toward each other and interact, such that the distribution of interaction energies becomes more favorable. Toward the end of the trajectories, the ensemble of simulations converges to an equilibrium. The equilibrium interaction energies is -250 kJ/mol for DLPC and -280 kJ/mol for the DPPC bilayers. The protein-protein interaction energies in DLPG and mixed DLPC/DLPG bilayers is the same as DLPC bilayers. Interestingly, the protein-protein interaction energetics is dependent on the lipid tail length, suggesting a difference in the dimer conformations sampled in these bilayers. The interaction energy between the membrane and protein is similarly plotted in the lower panel of Fig. 1(C and D). An initial relaxation of the transmembrane helices is observed to account for the transmembrane helices being fully solvated compared to the initial state generated. The lipid-protein interaction energies converge at the same time-scales as dimer

formation. Surprisingly, the lipid-protein interactions are similar for all bilayers, irrespective of head-group and acyl tail moieties. Counter-intuitively, the lipid bilayer composition appears to modulate protein-protein, but not protein-lipid energetics.

3.2. Conformational landscape of ErbB2 dimers is lipid dependent

The normalized population distribution of ErbB2 dimers in DLPC and DPPC bilayers as a function of the inter-helical distance is shown in Fig. 2. Interestingly, the dimer conformers observed were dependent on the lipid composition. Two main clusters (I and II) were resolved that are present in all bilayers, corresponding to an inter-helical distance 0.6 nm and 0.8 nm. An additional lipid-dependent dimer state (cluster III) was resolved with larger inter-helical distances (1.1 nm). Clusters I and II are observed in DPPC bilayers, but all three clusters were resolved in DLPC bilayers. In all lauroyl bilayers, the three clusters were resolved despite the differences in the head-group moieties (Fig. S4). In addition, the relative population of cluster I appears to be dependent on lipid type. The convergence of the clusters was tested by varying the time intervals (Fig. S4). Taken together, we observe an important modulation of the conformational landscape of the ErbB2 dimer by the lipid-tail length.

The 2D population distribution of ErbB2 dimerization was calculated as a function of the inter-helical distance and inter-helical angle (shown in Fig. 3, Fig. S5). As expected, the right-handed dimer conformer with negative inter-helical angles was observed to be the most favorable, irrespective of the membrane composition. Again, the three clusters (I-III) were observed of which cluster I and II, are present in all the bilayers and cluster III is observed only in DLPC/DLPG bilayers. In general, cluster I appears to be a narrow distribution, and the relative population is dependent on lipid tail length. Cluster II and III are relatively broad and reduce in population in the long-tailed lipids. Taken together, we observe that the dimer conformers in lauroyl bilayers are more flexible compared to the close-packed, tight dimer interface observed in DPPC bilayers. The convergence of the 2D normalized population distribution was tested (Fig. S6) and our results suggest that the 2D normalized population are robust and able to distinguish between specific and non-specific association.

To elucidate the dimer interface of the structures corresponding to each of the dimer clusters, we constructed residue-residue contact maps. The contact maps corresponding to each of the clusters are similar in all bilayers (Fig. 4 and Fig. S7). In cluster I, specific contacts are observed at the N-terminal region, including the SmXXXSm-like motif (also called the GxxxG motif). In addition, several contacts are observed along the transmembrane helices, corresponding to a tightly packed dimer interface. This conformational state corresponds to the N-terminal active state of ErbB2 dimer (Bocharov et al., 2008). In cluster II, the protein-protein contacts are mainly observed at the C-terminal motif, that has been suggested to correspond to the inactive dimer state (Bocharov et al., 2016). Representative structures from the cluster I are superimposed with the NMR resolved structures (PDB ID:2JWA). We observe a low deviation between the aligned structures giving a larger confidence on the predicted conformational landscape (Fig. 4C). Further, we calculated the contact map for the structures corresponding to cluster III in lauroyl bilayers (Fig. S8). Interestingly, the contact map is very disperse and does not display specific residue-residue contacts. Several non-native contacts are observed. This dimer state is only seen in the shorter tailed lipids and the state is suggestive of a very flexible and dynamic state, in contrast to the well packed states (cluster I and II). A representative example of each of the three clusters is shown in Fig. S9. In general, the dimers sample the active N-terminal conformation (cluster I), together with the inactive C-terminal conformation (cluster II). In the more fluid DLPC/DLPG bilayers, additional non-specific inactive conformations are observed (cluster III).

Fig. 1. Interaction energy between peptides over time shown for (A) DLPC and (B) DPPC bilayers. The protein-lipid interaction energy is plotted for (C) DLPC and (D) DPPC bilayers. The mean and median of the distribution are drawn in black and red respectively. The dashed white lines show the fitted model for the above measures. Also shown are the vigintile points (5%) away from the median drawn using rainbow gradient. The maximum and minimum of the distribution is shown as small pink dots. In the top panel, the dashed black lines indicate the estimated mean and median.

Fig. 2. Population density distributions of inter-helical distances in ErbB2 dimers. The normalized population for the transmembrane helix in lauroyl bilayers: DLPC (blue), and palmitoyl bilayer DPPC (red).

3.3. Membrane effects in modulating dimer conformations

Membrane perturbations have been previously related to transmembrane dimerization (Andersen and Koeppe, 2007; Jensen and Mouritsen, 2004; Morozova et al., 2011). We observe two types of lipid modulations: absence of cluster III and increased sampling of cluster I.

As a reporter of membrane perturbations, the average membrane thickness was calculated from the head-group PO4 beads in the two leaflets of the bilayer (Fig. 5 and Fig. S10). Local bilayer thickness variations were observed around the dimer in all bilayers and the thickness of the annular lipids was reduced. The perturbations appeared to be lower and localized around the protein for bilayers with the lauroyl tails, compared to the DPPC bilayer. To quantify the local perturbations, we calculated the normalized thickness that accounts for the differences in the global thickness of the palmitoyl and lauroyl tailed bilayers (Fig. 5C and D). The normalized perturbations in the lauroyl-tailed bilayer are small and localized. In contrast, the normalized perturbations around the dimer in DPPC are strong and extend up to 2 nm² around the dimer. It appears that the lower membrane perturbations in DLPC/DLPG bilayers allow non-specific clusters with less well packed dimer structures and larger inter-helical distances. In contrast, to minimize the perturbations in DPPC bilayers, a tighter dimer structure is observed. Thus, the changes in membrane thickness and hydrophobic mismatch appear to be correlated to the absence of cluster III in DPPC bilayers.

To analyze how lipid tail length affects the relative populations of cluster I and II, we considered additional longer tail bilayers. We performed simulations with long-tailed lipids DBPC and DXPC. The right-handed dimer conformer is dominant in all membrane compositions and cluster III is absent in both bilayers (Fig. S11). Interestingly, there are notable changes in the relative probability of clusters I and II that characterize the dimer conformational ensemble. In particular, there is a shift in populations of cluster I and II with increasing lipid tail length

Fig. 3. Conformational landscape of the ErbB2 dimers, as a function of the inter-helical distance and angle. The plot shows the normalized population density distribution for the transmembrane helix in (A) DLPC, (B) DPPC bilayers. Contour lines are drawn between at the interval of 0.1.

Fig. 4. Contact maps of protein-protein interface corresponding to (A) Cluster I, and (B) Cluster II of the ErbB2 in DPPC bilayers. Only alternate residues are shown on the axes. A value of 1.0 correspond to a very well preserved contact where as 0.0 correspond to no contacts. (C) The structural alignment of representative structures from Cluster I (Red) and NMR model (Grey) N-terminal interactions.

(Fig. 6). The relative population of the N-terminal “active” conformer, corresponding to cluster I, increases with increasing tail length. In larger bilayers, the ErbB2 transmembrane dimer adopts more tightly packed states with lower inter-helical distances that corresponds to the active state in the receptor.

4. Discussion

The organization of ErbB2 into active dimers plays a central role in cellular signaling, and is implicated in neurodegenerative diseases, development defects as well malignancy in tumors (Schlessinger et al., 2000). Receptor association has been probed for the ErbB2 receptor previously, but mainly to characterize the protein-protein contacts (Beevers et al., 2012; He and Hristova, 2012; Mendrola et al., 2002). Recent studies combining solid state NMR with fluorescence spectroscopy have highlighted the importance of the membrane composition (Matsushita et al., 2013; Dixon et al., 2019), that assume increased significance in light of altered lipid metabolism in several cancers. Here, we have used coarse-grain simulations to achieve a comprehensive unbiased sampling of the association between human ErbB2 receptors in varying membrane composition. The conformational landscape of ErbB2 association has been elucidated in different membrane compositions, from simulations aggregating to 3.2 ms. Our results demonstrate a unique modulation of the energetics of association of ErbB2 transmembrane helices by membrane composition. The most striking feature is the presence of multiple clusters, whose relative populations, is dependent on the lipid composition. The results highlight the importance of lipid composition dependent membrane effects in transmembrane helix association.

An important aspect of our results is that the relative population as well as the structure of the dimer state is modulated by membrane composition. We observe two main lipid-dependent effects: the absence of cluster III and the increase in cluster I in the longer tail bilayers. It is important to note here the significant role of long-tailed saturated lipids in cancer cell types (Llorente et al., 2013). A comparison of increased tail-length bilayers reveals increased membrane perturbations indicative of hydrophobic mismatch that lead to differences in the conformational landscape of ErbB dimers. We have previously related membrane perturbations around transmembrane helices to a lipophobic effect (Dubey et al., 2017), and it appears that the tighter dimer structures are sampled in long-tailed bilayers to minimize the lipophobic penalty. The reduced perturbations in DLPC bilayers allow the sampling of flexible “inactive” dimers with larger inter-helical distances. With increasing membrane thickness, the relative population shifts to lower inter-helical distances, such that cluster III is completely absent and cluster I increases. The effect of the head-group moiety is observed to be relatively small in this study, although long-tailed charged lipids have been reported to modulate ErbB2 association

Fig. 5. Thickness profile of the bilayer around the dimer state of ErbB2. The top panel shows the membrane thickness profile in the xy plane for (A) DLPC and (B) DPPC. The normalized thickness i.e., the deviation from the average membrane thickness is shown in the bottom panel for (C) DLPC and (D) DPPC bilayers.

Fig. 6. The relative population of dimer states in all membrane composition with increasing lipid tail length (DLPC, DPPC, DBPC, DXPC). The ratio of the relative population for the N-terminal (Cluster I) and C-terminal (Cluster II) dimer states.

(Matsushita et al., 2013). Importantly, cluster I corresponds to the N-terminal active dimer state and could be related to increased function and cell signaling.

The importance of the transmembrane domain of the ErbB2 receptor is supported by both, structural and mutational data. For instance, the crystal structures of the non-membrane domains show that the asymmetric intracellular domain dimer required for function (Kovacs et al., 2015) is directly related to the orientation and conformational dynamics of transmembrane domain (Valley et al., 2017; Purba et al., 2017). Importantly, transmembrane regions are known to

interact with similar affinities to that of the full-length ErbB receptors (Bennasroune et al., 2004; Sharpe et al., 2002; Tzahar et al., 1996). Oncogenic mutations, especially in the SmXXXSm-like motif of the transmembrane has reported to modulate the association of the receptors (He and Hristova, 2008; Placone et al., 2014).

The ErbB2 transmembrane dimer has been extensively characterized in palmitoyl-tailed bilayers, by various computational methods (Aller et al., 2005; Bryan et al., 2004; Soumana et al., 2006). Surprisingly, the population density distributions calculated from ensemble simulations reveals multiple clusters in the dimer state (Pawar and Sengupta, 2019) that had not been resolved by enhanced sampling approaches (Prasanna et al., 2013; Janosi et al., 2010). The main advantage of the current method over enhanced sampling approaches such as umbrella sampling is the extensive sampling of lipid dynamics. We have previously shown that in the case of polyaniline peptides, the population distribution sampled by similar approaches can be related to the underlying energy landscape (Pawar et al., 2015b). In the current case, the low number of association/dissociation events makes it difficult to estimate the binding free energies in the different bilayers. We believe however, that the current framework captures the essential features of the transmembrane helix association and allows us to compare the modulation of transmembrane dimerization by varying membrane composition (Kharche and Sengupta, 2020).

In conclusion, we have performed multiple unbiased simulations to resolve the conformational landscape of ErbB2 transmembrane helices in different membranes. Using this approach, we have characterized the most populated ErbB2 dimer conformations in bilayers of varying head-group and acyl chains. The results show that the approach allows the construction of robust 2D population density distributions that can be used to investigate lipid-mediated effects. The results exemplify the importance of sufficient sampling of lipid dynamics when studying protein-protein interactions in membranes. Membrane perturbations due to hydrophobic mismatch between the protein and the lipid is shown to be related to additional cluster corresponding to non-native

dimer structures. To the best of our knowledge, this is one of the few studies that is able to capture such a modulation of the conformational landscape by the lipid environment of a process that was considered to be mainly protein-driven. Our work demonstrates the importance of a comprehensive understanding of the membrane environment to re-interpret physiologically-relevant membrane protein association.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.chemphyslip.2020.104911>.

References

- Aller, P., Voiry, L., Garnier, N., Genest, M., 2005. Molecular dynamics (MD) investigations of preformed structures of the transmembrane domain of the oncogenic Neu receptor dimer in a DMPC bilayer. *Biopolymers* 77, 184–197.
- Andersen, O.S., Koeppe, R.E., 2007. Bilayer thickness and membrane protein function: an energetic perspective. *Annu. Rev. Biophys. Biomol. Struct.* 36, 107–130.
- Arkipov, A., Shan, Y., Das, R., Endres, N., Eastwood, M.P., Wemmer, D.E., Kuriyan, J., Shaw, D.E., 2013. Architecture and membrane interactions of the EGF receptor. *Cell* 152 (557–), 569.
- Baenke, F., Peck, B., Miess, H., Schulze, A., 2013. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. *Dis. Model. Mech.* 6, 1353–1363.
- Beevers, A.J., Nash, A., Salazar-Cancino, M., Scott, D.J., Notman, R., Dixon, A.M., 2012. Effects of the oncogenic V664E mutation on membrane insertion, structure, and sequence-dependent interactions of the neu transmembrane domain in Micelles and model membranes: an integrated biophysical and simulation study. *Biochem.* 51, 2558–2568.
- Bell, C.A., Tynan, J.A., Hart, K.C., Meyer, A.N., Robertson, S.C., Donoghue, D.J., 2000. Rotational coupling of the transmembrane and kinase domains of the Neu receptor tyrosine kinase. *Mol. Biol. Cell* 11, 3589–3599.
- Benjamini, A., Smit, B., 2013. Lipid mediated packing of transmembrane helices- a dissipative particle dynamics study. *Soft Mat.* 9, 2673–2683.
- Bennasroune, A., Fickova, M., Gardin, A., Dirrig-Grosch, S., Aunis, D., Cremel, G., Hubert, P., 2004. Transmembrane peptides as inhibitors of ErbB receptor signaling. *Mol. Biol. Cell* 15, 3464–3474.
- Bernardes, N., Fialho, A.M., 2018. Perturbing the dynamics and organization of cell membrane components: a new paradigm for cancer-targeted therapies. *Int J Mol Sci* 19 3871.
- Bocharov, E.V., Mineev, K.S., Volynsky, P.E., Ermolyuk, Y.S., Tkach, E.N., Sobol, A.G., Chupin, V.V., Kirpichnikov, M.P., Efremov, R.G., Arseniev, A.S., 2008. Spatial structure of the dimeric transmembrane domain of the growth factor receptor ErbB2 presumably corresponding to the receptor active state. *J. Biol. Chem.* 283 (6950–), 695.
- Bocharov, E.V., Lesovoy, D.M., Pavlov, K.V., Pustovalova, Y.E., Bocharova, O.V., Arseniev, A.S., 2016. Alternative packing of EGFR transmembrane domain suggests that protein-lipid interactions underlie signal conduction across membrane. *Biochimica et Biophysica Acta-Biomembranes* 1858, 1254–1261.
- Castillo, N., Monticelli, L., Barnoud, J., Tieleman, D.P., 2013. Free energy of WALP23 dimer association in DMPC, DPPC, and DOPC bilayers. *Chem. Phys. Lip.* 169, 95–105.
- Chng, C.P., Tam, S.M., 2011. Leukocyte integrin αLb2 transmembrane association dynamics revealed by coarse-grained molecular dynamics simulations. *Proteins* 79, 2203–2213.
- Chung, I., Akita, R., Vandlen, R., Toomre, D., Schlessinger, J., Mellman, I., 2010. Spatial control of EGF receptor activation by reversible dimerization on living cells. *Nature* 464 783–710.
- Cymer, F., Veerappan, A., Schneider, D., 2012. Transmembrane helix-helix interactions are modulated by the sequence context and by lipid bilayer properties. *Biochim. Biophys. Acta-Biomembr.* 1818, 963–973.
- Dubey, V., Prasanna, X., Sengupta, D., 2017. Estimating the lipophobic contributions in model membranes. *The Journal of Physical Chemistry B* 121 (9), 2111–2120.
- Efron, B., 1979. Bootstrap methods: another look at the jackknife. *Ann. Stat.* 7, 1–26.
- Ende, B.M., Sharom, F.J., Davis, J.H., 2004. The transmembrane domain of Neu in a lipid bilayer: molecular dynamics simulations. *Eur. Biophys. J.* 33.
- Fink, A., Sal-Man, N., Gerber, D., Shai, Y., 2012. Transmembrane domains interactions within the membrane milieu: principles, advances and challenges. *Biochim. Biophys. Acta* 1818, 974–983.
- Fleishman, S.J., Schlessinger, J., Ben-Tal, N., 2002. A putative molecular-activation switch in the transmembrane domain of erbB2. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15937–15940.
- Hall, B.A., Armitage, J.P., Sansom, M.S.P., 2012. Mechanism of bacterial signal transduction revealed by molecular dynamics of trimeric dimers and trimers of dimers in lipid vesicles. *PLoS Comp. Biol.* 8, e1002685.
- Hasan, M., Patel, D., Ellis, N., Brown, S.P., Lewandowski, J.R., Dixon, A.M., 2019. Modulation of transmembrane domain interactions in neu receptor tyrosine kinase by membrane fluidity and cholesterol. *J. Membr. Biol.* 252, 357–369.
- He, L., Hristova, K., 2012. Physical-chemical principles underlying RTK activation, and their implications for human disease. *Biochim. Biophys. Acta* 1818 (995–), 1005.
- He, L., Hristova, K., 2008. Pathogenic activation of receptor tyrosine kinases in mammalian membranes. *J. Mol. Biol.* 384, 1130–1142.
- Henin, J., Pohorille, A., Chipot, C., 2005. Insights into the Recognition and Association of Transmembrane α-Helices. The Free Energy of Helix Dimerization in Glycophorin A. *J. Am. Chem. Soc.* 127, 8478–8484.
- Hess, B., Kutzner, C., Van Der Spoel, D., Lindahl, E., 2008. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J. Chem. Theor. Comp.* 4, 435–447.
- Hong, H., Bowie, J.U., 2011. Dramatic destabilization of transmembrane Helix interactions by features of natural membrane environments. *J. Am. Chem. Soc.* 133, 11389–11398.
- Janosi, L., Prakash, A., Doxastakis, M., 2010. Lipid-modulated sequence-specific association of glycophorin A in membranes. *Biophys. J.* 99, 284–292.
- Jensen, M.O., Mouritsen, O.G., 2004. Lipids do influence protein function-the hydrophobic matching hypothesis revisited. *Biochim. Biophys. Acta* 1666, 205–226.
- Jura, N., Endres, N.F., Engel, K., Das, R., Deindl, S., Lamers, M.H., Wemmer, D.E., Zhang, X., Kuriya, J., 2009. Mechanism for activation of the egf receptor catalytic domain by the juxtamembrane segment. *Cell* 137, 1293–1307.
- Kharache, S.A., Sengupta, D., 2020. Dynamic protein interfaces and conformational landscapes of membrane protein complexes. *Current Opinion in Structural Biology* 61, 191–197.
- Kovacs, E., Zorn, J.A., Huang, Y., Barros, T., Kuriyan, J., 2015. A structural perspective on the regulation of the epidermal growth factor receptor. *Annu. Rev. Biochem.* 84, 739–764.
- Lemmin, T., Soto, C.S., Clinthorne, G., DeGrado, W.F., Peraro, M.D., 2013. Assembly of the transmembrane domain of E. Coli PhoQ histidine kinase: implications for signal transduction from molecular simulations. *PLoS Comp. Biol.* 9, e1002878.
- Li, E., Wimley, W.C., Hristova, K., 2012. Transmembrane helix dimerization: beyond the search for sequence motifs. *Biochim. Biophys. Acta* 1818, 183–193.
- Llorente, A., Skotland, T., Sylvänne, T., Kauhanen, D., Róg, T., Orłowski, A., Vattulainen, I., Ekroos, K., Sandvig, K., 2013. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim. Biophys. Acta (BBA)-Molec. Cell Biol. Lip.* 1831, 1302–1309.
- Maeda, R., Sato, T., Okamoto, K., Yanagawa, M., Sako, Y., 2018. Lipid-protein interplay in dimerization of juxtamembrane domains of epidermal growth factor receptor. *Biophys. J.* 114, 893–903.
- Mall, S., Broadbridge, R., Sharma, R.P., East, J.M., Lee, A.G., 2001. Self-association of model transmembrane alpha-helices is modulated by lipid structure. *Biochemistry.* 40, 12379–12386.
- Marrink, S.J., Risselada, H.J., Yefimov, S., Tieleman, D.P., de Vries, A.H., 2007. The MARTINI force field: coarse grained model for biomolecular simulations. *J. Phys. Chem. B* 111, 7812–7824.
- Matsushita, C., Tamagaki, H., Miyazawa, Y., Aimoto, S., Smith, S.O., Sato, T., 2013. Transmembrane helix orientation influences membrane binding of the intracellular juxtamembrane domain in Neu receptor peptides. *Proc. Natl. Acad. Sci.* 110 (1646–), 1651.
- Maulucci, G., Cohen, O., Daniel, B., Sansone, A., Petropoulou, P.I., Filou, S., Spyridonidis, A., Pani, G., De Spirito, M., Chatgililoglu, C., Ferreri, C., Kypreos, K.E., Sasson, S., 2016. Fatty acid-related modulations of membrane fluidity in cells: detection and implications. *Free Radic. Res.* 50, S40–S50.
- Mendrola, J.M., Berger, M.B., King, M.C., Lemmon, M.A., 2002. The single transmembrane domains of ErbB receptors self-associate in cell membranes. *J. Biol. Chem.* 277, 4704–4712.
- Mitchell, D.C., Straume, M., Litman, B.J., 1992. Role of sn-1-saturated, sn-2-polyunsaturated phospholipids in control of membrane receptor conformational equilibrium: effects of cholesterol and acyl chain unsaturation on metarhodopsin I. *Autm. Metarhodopsin II equilibrium. Biochemistry* 31, 662–670.
- Monticelli, L., Kandasamy, S.K., Periole, X., Larson, R.G., Tieleman, D.P., Marrink, S.J., 2008. The MARTINI coarse-grained force field: extension to proteins. *J. Chem. Theor. Comp.* 4, 819–834.
- Morozova, D., Guigas, G., Weiss, M., 2011. Dynamic structure formation of peripheral membrane proteins. *PLoS Comput. Biol.* 7, e1002067.
- Morozova, D., Weiss, M., Guigas, G., 2012. Shape as a determinant of membrane protein cluster formation. *Soft Mat.* 8, 11905–11910.
- Paila, Y.D., Tiwari, S., Chattopadhyay, A., 2009. Are specific nonannular cholesterol binding sites present in G-protein coupled receptors? *Biochim. Biophys. Acta (Biomembranes)* 1788, 295–302.
- Pawar, A.B., Sengupta, D., 2018. Effect of membrane composition on receptor association: implications of Cancer lipidomics on ErbB receptors. *J. Membr. Biol.* 251, 359–368.
- Pawar, A.B., Sengupta, D., 2019. Resolving the conformational dynamics of ErbB growth factor receptor dimers. *J. Struct. Biol.* 207, 225–233.
- Pawar, A.B., Deshpande, S.A., Gopal, S.M., Wassenaar, T.A., Athale, C.A., Sengupta, D., 2015a. Thermodynamic and kinetic characterization of transmembrane helix association. *Phys. Chem. Chem. Phys.* 17, 1390–1398.
- Pawar, A.B., Prasanna, X., Sengupta, D., 2015b. Effect of lipid bilayer composition on membrane protein association. *Adv. Pl. Lip. Bil. Lipos.* 22, 43–63.
- Peetla, C., Vijayaraghavalu, S., Labhasetwar, V., 2013. Biophysics of cell membrane lipids in cancer drug resistance: implications for drug transport and drug delivery with

- nanoparticles. *Adv. Drug Deliv. Rev.* 65, 13–14.
- Placone, J., He, L., Del Piccolo, N., Hristova, K., 2014. Strong dimerization of wild-type ErbB2/Neu transmembrane domain and the oncogenic Val664Glu mutant in mammalian plasma membranes. *Biochim. Biophys. Acta* 1838, 2326–2330.
- Prasanna, X., Praveen, P.J., Sengupta, D., 2013. Sequence dependent lipid-mediated effects modulate the dimerization of ErbB2 and its associative mutants. *Phys. Chem. Chem. Phys.* 15, 19031–19041.
- Prasanna, X., Chattopadhyay, A., Sengupta, D., 2014. Cholesterol modulates the dimer interface of the beta-adrenergic receptor via cholesterol occupancy sites. *Biophys. J.* 106, 1290–1300.
- Purba, E.R., Saita, E.I., Maruyama, I.N., 2017. Activation of the EGF receptor by ligand binding and oncogenic mutations: the “rotation model”. *Cells* 6, E13.
- Rath, A., Deber, C.M., 2012. Protein structure in membrane domains. *Ann. Rev. Biophys.* 41, 135–155.
- Schlessinger, J., 2000. Cell signaling by receptor tyrosine kinases. *Cell* 103 (211), 225.
- Schmidt, U., Guigas, G., Weiss, M., 2008. Cluster formation of transmembrane proteins due to hydrophobic mismatching. *Phys. Rev. Lett.* 101, 128104.
- Sengupta, D., Marrink, S., 2010. Lipid-mediated interactions tune the association of glycoporphin: a helix and its disruptive mutants in membranes. *J. Phys. Chem. Chem. Phys.* 12, 12987–12996.
- Sharma, S., Juffer, A.H., 2013. An atomistic model for assembly of transmembrane domain of t cell receptor complex. *J. Am. Chem. Soc.* 135 (2188-), 2197.
- Sharpe, S., Barber, K.R., Grant, C.W., 2002. Interaction between ErbB-1 and ErbB-2 transmembrane domains in bilayer membranes. *FEBS Lett.* 519, 103–107.
- Soumana, O.S., Aller, P., Garnier, N., Genest, M., 2006. Transmembrane peptides from tyrosine kinase receptor. Mutation-related behavior in a lipid bilayer investigated by molecular dynamics simulations. *J. Biomol. Str. Dyn.* 23, 91–100.
- Sparr, E., Ash, W.L., Nazarov, P.V., Rijkers, D.T.S., Hemminga, M.A., Tieleman, D.P., Killian, A., 2005. Self-association of transmembrane alpha-helices in Model membranes. *J. Biol. Chem.* 280, 39324–39331.
- Tzahar, E., Waterman, H., Chen, X., Levkowitz, G., Karunakaran, D., Lavi, S., Ratzkin, B.J., Yarden, Y., 1996. A hierarchical network of inter-receptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol. Cell. Biol.* 16, 5276–5287.
- Valley, C.C., Lewis, A.K., Sachs, J.N., 2017. Piecing it together: unraveling the elusive structure-function relationship in single-pass membrane receptors. *Biochim. Biophys. Acta* 1859, 1398–1416.
- Wassenaar, T.A., Ingólfsson, H.I., Prieß, M., Marrink, S.J., Schaefer, L.V., 2013. Mixing MARTINI: electrostatic coupling in hybrid atomistic-coarse-Grained biomolecular simulations. *J. Phys. Chem. B* 117 (3516), 3530.
- Wassenaar, T.A., Pluhackova, K., Moussatova, A., Sengupta, D., Marrink, S.J., Tieleman, D.P., Böckmann, R.A., 2015. High-throughput simulations of dimer and trimer assembly of membrane proteins. The DAFT Approach. *Journal of Chemical Theory and Computation*. 11, 2278–2291.
- Wei, P., Zheng, B.K., Guo, P.R., Kawakami, T., Luo, S.Z., 2013. The association of polar residues in the DAP12 homodimer: TOXCAT and molecular dynamics simulation studies. *Biophys. J.* 104, 1435–1444.
- Zalba, S., Ten Hagen, T.L., 2017. Cell membrane modulation as adjuvant in cancer therapy. *Cancer Treat. Rev.* 52, 48–57.
- Zhang, L., Sodt, A.J., Venable, R.M., Pastor, R.W., Buck, M., 2013. Prediction, refinement, and persistency of transmembrane helix dimers in lipid bilayers using implicit and explicit solvent/lipid representations: microsecond molecular dynamics simulations of ErbB1/B2 and EphA1. *Prot. Struc. Func. Bioinf.* 81, 365–376.