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Nonpolar interactions between trans-membrane helical EGF peptide and phosphatidylcholines, sphingomyelins and cholesterol. Molecular dynamics simulation studies‡

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Abstract: A molecular dynamics simulation study of four lipid bilayers with inserted trans-membrane helical fragment of epithelial growth factor (EGF) receptor (EGF peptide) was performed. The lipid bilayers differ in their lipid composition and consist of (i) unsaturated phosphatidylcholine (palmitoyloleoylphosphatidylcholine, POPC), (ii) POPC and 20 mol% of cholesterol (Chol), (iii) sphingomyelin (SM) and 20 mol% of Chol, and (iv) SM and 50 mol% of Chol. Only 1 out of 26 residues in the EGF-peptide sequence is polar (Thr). The hydrophobic thickness of each bilayer is different but shorter than the length of the peptide and so, due to hydrophobic mismatch, the inserted peptide is tilted in each bilayer. Additionally, in the POPC bilayer, which is the thinnest, the peptide loses its helical structure in a short three-amino acid fragment. This facilitates bending of the peptide and burying all hydrophobic amino acids inside the membrane core (Figure 1(b)). Bilayer lipid composition affects interactions between the peptide and lipids in the membrane core. Chol increases packing of atoms relative to the peptide side chains, and thus increases van der Waals interactions. On average, the packing around the peptide is higher in SM-based bilayers than POPC-based bilayers but for certain amino acids, packing depends on their position relative to the bilayer center. In the bilayer center, packing is higher in POPC-based bilayers, while in regions closer to the interface packing is higher in SM-based bilayers. In general, amino acids with larger side chains interact strongly with lipids, and thus the peptide sequence is important for the pattern of interactions at different membrane depths. This pattern closely resembles the shape of recently published lateral pressure profiles [Ollila *et al* J. Struct. Biol. DOI:10.1016/j.jsb.2007.01.012]. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: membrane heterogeneity; lipid rafts; hydrophobic mismatch; van der Waals interactions; membrane packing; EGF receptor

INTRODUCTION

Biological membranes are highly complex mixtures of many lipid species. These lipids belong to three major classes: glycerol-based lipids, sphingolipids, and sterols. Glycerol-based lipids are phosphatidylcholines (PC), phosphatidylethanolamines, phosphatidylserines, phosphatidylglycerols, and phosphatidylinositols; sphingolipids are most commonly sphingomyelins (SMs) (with a phosphatidylcholine headgroup) and glycosphingolipids (with a sugar headgroup). In the animal kingdom, the most common sterol is cholesterol (Chol). A typical biological membrane is composed of a hundred or more lipid species; its composition is regulated by various cellular mechanisms to ensure a proper environment for membrane proteins [1] and the fulfillment of specific membrane functions.

One of the consequences of lipid heterogeneity is the formation of lipid domains within the membrane. The best known type of lipid domains are rafts, membrane compartments rich in Chol and saturated phospholipids and glycolipids [2]. In unstimulated resting cells rafts are very small (so-called precursor rafts) but they can enlarge and stabilize after stimulation, when their constituents are cross-linked (large raft platforms) [3]. In a common view, raft lipids form liquid-ordered nano-size domains within the bulk liquid-disordered environment composed of unsaturated PC molecules and smaller amounts of Chol. Rafts are involved in numerous cellular processes mainly by acting as platforms for certain membrane proteins [4] and localized signal transduction [5]. Formation of domains within biomembranes, crucial for the membranes to carry out

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their biological functions, is controlled by the membrane molecular composition. Studies of the correlation between the membrane composition and the properties of the membrane and its constituents will get us closer to the understanding of basic regulatory and controlling processes in the membrane, and are of high importance.

In this study, we applied the molecular dynamics (MD) simulation method to examine how atomic-level interactions and the 3D structure of a membrane protein depend on the lipid composition of the membrane. We used a trans-membrane helical fragment of epithelial growth factor (EGF) receptor, a raft-specific protein, as the membrane protein [4,5]. This trans-membrane peptide (EGF peptide) was inserted into four bilayers of varying composition that modeled two membrane environments: raft, composed of SM and Chol, and nonraft composed of unsaturated PC or unsaturated PC and Chol. Molecular modeling has not been commonly used to study a single trans-membrane EGF peptide in a lipid bilayer. To our knowledge, only a few modeling studies have been performed, focusing mostly on different aspects such as dimerization, signaling, and systems biology [6–8]. This study is, to our knowledge, the first one to focus on EGF lipid–peptide interactions. Among other trans-membrane peptides studied by MD simulation are polyalanine [9], RAS peptide [10], K channel helix [11], KALP peptide [12], and cell signaling peptide [13]. The main concerns of these studies were the peptide secondary structure, peptide tilt, ordering of lipids surrounding the peptide, and peptide–lipid and peptide–water hydrogen bonding.

In this study, we concentrate on the details of interactions between the EGF-peptide and lipid molecules in the core of bilayers of different lipid compositions by analyzing the packing of atoms in the core.

METHODS

Four model bilayers with a trans-membrane peptide were constructed. The bilayers consisted of (i) 68 palmitoyloleoylphosphatidylcholine (POPC); (ii) 50 POPC and 16 Chol molecules (POPC-Chol); (iii) 68 *N*-stearoyl-D-erythrosphingosylphosphorylcholine (SSM) and 18 Chol molecules (SM-Chol20); and (iv) 68 SSM and 68 Chol molecules (SM-Chol50) and the trans-membrane peptide. The amino acid sequence of the peptide (Ile-Met-Ile-Ala-Thr-Gly-Met-Val-Gly-Ala-Leu-Leu-Leu-Leu-Leu-Val-Val-Ala-Leu-Gly-Ile-Gly-Leu-Phe-Met-Arg) was the same as the trans-membrane fragment of the EGF receptor [14]. Ace (acetamide) and Nme (*N*-methyl) groups were added at the C and N termini of the peptide, respectively. All models were constructed by replacing four lipid molecules in previously well-equilibrated bilayers by the peptide molecule. Details concerning the construction, equilibration, and parameterization of the bilayers can be found in our previous papers, i.e., POPC [15], POPC-Chol [16], SM-Chol20, and SM-Chol50 [17] (the number associated with 'Chol' indicates the Chol concentration). The final configurations of those simulations were used as the initial membrane structures for the current study.

For the lipids and peptide, the OPLS (optimized parameters for liquid simulations) parameters [18] and for water, TIP3P parameters [19] were used. The united-atom approximation was applied to the CH, CH_2, and CH_3 groups of POPC, SSM, Chol, and peptide molecules.

Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm [20] was used to preserve the bond lengths of the OH and NH groups of water, Chol, SSM, and peptide molecules. The time step was set to 2 fs. The bilayers were simulated for 20 ns, and the particle-mesh Ewald (PME) summation method [21] with a real cut-off of 12 Å, β-spline interpolation order of 5, and direct sum tolerance of 10^{-6} was used for electrostatics. It has been shown that proper treatment of electrostatics is crucial in membrane systems [22,23], as well as in those containing peptides [24] and small molecules [25].

The simulations were performed with AMBER 5.0 [26] simulation suite. The first 5 ns were considered as the equilibration period [17], and the next 15 ns were used for analysis. Data were stored every 1 ps, and thus a total of 15 000 frames were analyzed. The list of nonbonded pairs was updated every 25 steps. Restraints of a flat-bottom harmonic potential as defined and implemented in the AMBER package [26] were imposed on the double bonds to prevent *cis–trans* isomerization. During the first nanosecond of each simulation, restrains were also imposed on the peptide π and χ angles to preserve the helical structure.

The simulations were carried out at a constant temperature of 310 K (37 °C), and constant pressure (1 atm). The temperatures of the solute and solvent were controlled independently. Both the temperatures and pressure of the systems were controlled by the weak coupling method [27]. The relaxation times for temperatures and pressure were set at 0.4 and 0.6 ps, respectively. The applied pressure was controlled anisotropically, each direction being treated independently with the trace of the pressure tensor kept constant at 1 atm. In the conditions used in these studies, phase separation should occur in both the POPC-Chol and the SM-Chol20 bilayers [28]. The short timescales (in terms of experimental systems) and the small sizes of the bilayers do not allow us to observe phase separation, but on the basis of previously published analysis of these bilayers [15–17] we can conclude that in both cases the bilayers are in the Lo phase.

RESULTS AND DISCUSSION

Hydrophobic Mismatch

In an energetically favorable state, the hydrophobic thicknesses of the membrane and the membrane protein, or peptide, match each other [29–32]. The situation when they do not match is called *hydrophobic mismatch* [29–32]. Hydrophobic mismatch can lead to structural changes in the peptide and reorganization of lipids in the bilayer. The bilayer thickness is thus an important parameter characterizing bilayer systems with trans-membrane proteins and peptides. In practice, however, membrane thickness is not an easily measurable or a well-defined parameter [33,34]. Here, we define the membrane thickness to be the average P–P distance, i.e. the average distance between positions of phosphorus atoms in the opposite leaflets.

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Owing to the different compositions, the bilayers used in this study differ in their thicknesses. The P–P distances in peptide-free bilayers are 35.5, 37.9, 46.7, and 46.5 Å in POPC, POPC-Chol, SM-Chol20, and SM-Chol50 bilayers, respectively [15–17]. Because of these rather large differences, the trans-membrane EGF peptide inserted into the bilayers experiences various hydrophobic matching conditions. To illustrate the effect of the bilayer thickness on the peptide 3D structure, snapshots of POPC and POPC-Chol bilayers with the inserted peptide are shown in Figure 1. One can easily see that in both bilayers the peptide structure is essentially helical, but in the pure POPC bilayer, which is thinner than the POPC-Chol bilayer, a fragment of the helix at amino acids Leu19-Gly20-Ile21 is disturbed. This local structural defect allows the peptide to bend in such a way that its upper part, instead of sticking into the interface, remains buried inside the bilayer core. This bend of the helix appeared already in the early stage of the simulation, after the restrains on the π and χ angles were released, and remained for the subsequent 19 ns of the simulation. Such a bend was not observed in the case of the thicker POPC-Chol bilayer.

Figure 2(a) shows a close-up of the peptide bend region together with the nearest lipid molecules. As can be seen, the amide group of Gly20 forms hydrogen bonds with POPC carbonyl oxygen atoms and water. These hydrogen bonds were observed during the whole simulation and it is likely that they are responsible for stabilizing the defect in the helical structure.

The other effect of the hydrophobic mismatch observed in our study is the tilting of the peptide long axes relative to the bilayer normal. This is demonstrated in Figure 2(b), which shows the inclination of the peptide relative to the bilayer normal in POPC-Chol, SM-Chol20, and SM-Chol50 bilayers. The peptide tilt angle is approximately 10°, and it is slightly larger in the POPC-Chol than in the SM-Chol bilayer. The data obtained in these simulations do not allow us to give precise values of the peptide tilt angle. As was shown by Sansom *et al.* [35] in their coarse-grained simulations, the peptide tilt is a dynamic parameter which oscillates in a broad range (±10°) with a period of about 5–10 ns, which likely corresponds to membrane thickness oscillations [36].

Changes in the peptide secondary structure in response to hydrophobic mismatch have been discussed by Killian [29] in the light of transitions between different helical conformations (α-helix, π-helix, and 3–10 helix). Such changes were observed in MD simulation studies [37] but have not been confirmed, to our knowledge, experimentally [38–40]. In this study, the structural changes of the peptide are of different nature: continuity in the helical structure of the EGF peptide inserted into the POPC bilayer is partly lost as a bend in the helix appears. To our knowledge, there is no other study showing a similar change in the trans-membrane peptide structure. Thus, our results suggest the possibility of a new structural response to hydrophobic mismatch.

In the literature, two other responses to hydrophobic mismatch have been described: change of the peptide tilt and local changes of the membrane curvature [29–32]. In the latter case, lipids neighboring a protein can adopt a more disordered (ordered) conformation which may locally decrease (increase) the membrane thickness when the protein's hydrophobic profile is shorter (longer) than that of the membrane. In our simulations, we observed mainly changes of the peptide tilt; small changes of the curvature were observed only in the initial part of simulation of the POPC bilayer.

Hydrophobic Interactions in the Membrane Core

In our previous studies on PC–sterol interactions [41,42], we have demonstrated that the radial distribution function (RDF) can be used to quantify how different molecular groups and atoms order relative to

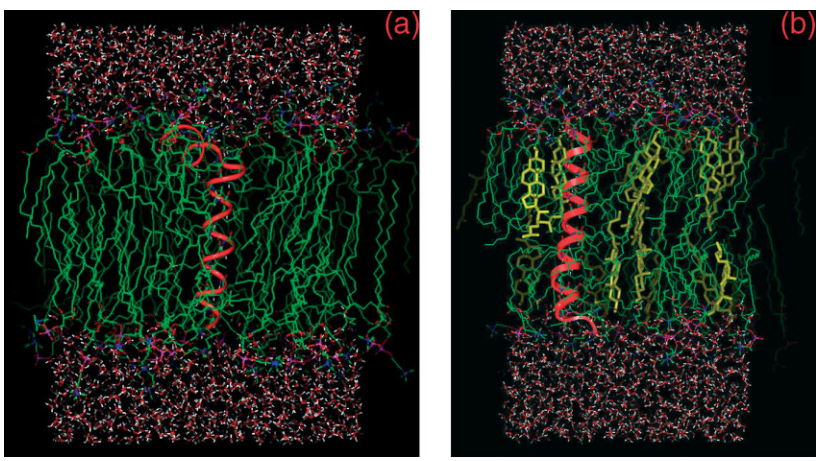

Figure 1 (a) Snapshots of the structure of POPC and (b) POPC-Chol bilayers at the end of simulation run. Color scheme: carbon – green, oxygen – red, hydrogen – white, nitrogen – blue, phosphorus – purple. Chol molecules are shown in yellow.

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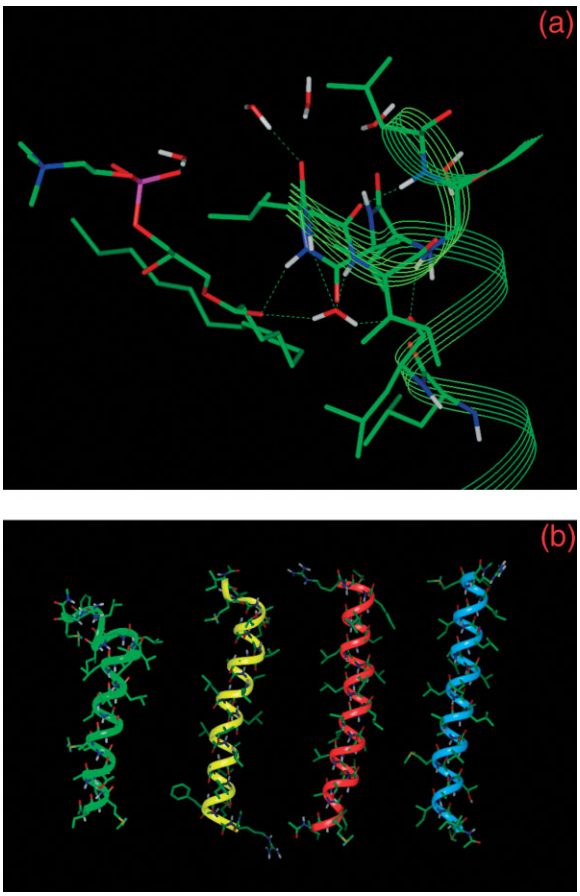

Figure 2 (a) Snapshot of peptide fragment which adopts nonhelical structure in POPC bilayer with surrounding lipid and water, hydrogen bonds are marked. (b) Snapshots of the peptide structure at the end of simulation, green – POPC, yellow – POPC-Chol, red – SM-Chol20, blue – SM-Chol50 bilayer.

each other, and to provide essential information about hydrophobic interactions in the membrane core. The RDF describes the probability of finding a particle β at a distance between r and $r + dr$ away from a particle α in a simulation box of volume V containing N particles:

$$RDF = \frac{V}{N}\left\langle \frac{n(r)}{4\pi r^2 dr} \right\rangle,$$

where $n(r)$ is the number of particles β in a spherical ring of radius r and width dr around the particle α, $4\pi r^2\ dr$ is the ring volume, and $\langle\rangle$ denotes an ensemble average. Pairs of atoms belonging to the same molecule were omitted when calculating the RDF.

Figure 3 shows the RDFs of all lipid carbon atoms, only POPC carbon atoms, and only Chol carbon atoms relative to the EGF-peptide atoms in the membrane core of the POPC-Chol bilayer. The POPC–peptide RDF have a well-resolved maximum at 5 Å and a minimum at 7 Å. The maximum and minimum of the Chol–peptide RDF are barely resolved indicating weak sterol–peptide van der Waals interactions in the membrane core region

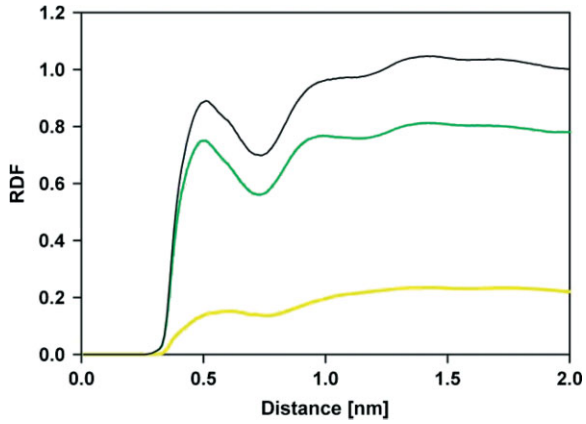

Figure 3 Radial distribution functions (RDF) of the acyl tail carbon atoms (green line), Chol carbon atoms (yellow line), and sum of both (black line) in the POPC-Chol bilayer core relative to peptide atoms.

[42]. Similar shapes of RDFs were obtained for the remaining three bilayers (data not shown).

The RDFs in Figure 3 are averages over all peptide atoms. To have a deeper insight into the hydrophobic interactions, we calculated RDFs of lipid carbon atoms relative to the atoms of four selected amino acids in the POPC-Chol bilayer (Figure 4). As can be seen from Figure 4, the main contribution to the maxima in the RDFs in Figure 3 comes from the side-chain atoms. The peptide main chain is shielded by the side chains, and thus its interactions with lipid atoms are weak. Also the main chain of glycine, the amino acid with practically no side chain, is weakly interacting with lipids due to the side chains of neighboring residues. Unfortunately, the data obtained in these simulations are not of sufficient quality to provide a detailed quantification of how the side-chain structure (e.g. leucine and isolecine) influences the interactions with lipids. In our previous studies of bilayers without the peptide [41], we were able to explain the shapes of PC-Chol RDFs. In those studies, however, the number of Chol molecules was 16, while here we have just 1 peptide molecule. To address the above questions, longer simulations starting from various initial structures are needed. Work is in progress.

Packing of Atoms in the Membrane Core

The packing of atoms in the bilayer core can be estimated by calculating the number of neighbors according to the method described in References 41 and 42. A neighbor is defined as an atom belonging to a different molecule and located not further than 7 Å (the position of the first minimum in the corresponding RDF) from an arbitrary chosen atom in the hydrophobic core of the bilayer. The average numbers of neighbors of the peptide atoms are 16.8 ± 0.1, 17.2 ± 0.1, 18.7 ± 0.1, and 20.8 ± 0.1 in POPC, POPC-Chol, SM-Chol20, and SM-Chol50 bilayers, respectively. To visualize the peptide neighborhood, Figure 5 shows a snapshot of the peptide

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with neighboring POPC and Chol molecules. Profiles of the number of neighbors of the peptide atoms in all four bilayers are shown in Figure 6. The atom index refers to the position of a given peptide atom in the corresponding PDB (Protein Data Bank) file (see supplementary materials). For clarity, only a part of the profile for the amino acids located in the bilayer center is shown. The shape of the profile reflects the peptide atoms' accessibility to lipids. The number of neighbors of the peptide main chain atoms is around 10, which is much smaller than that of the side chain's atoms (up to 40). This quantifies the fact that in a helical structure the main chain is buried among the side chains and is shielded from the lipids (Figure 4).

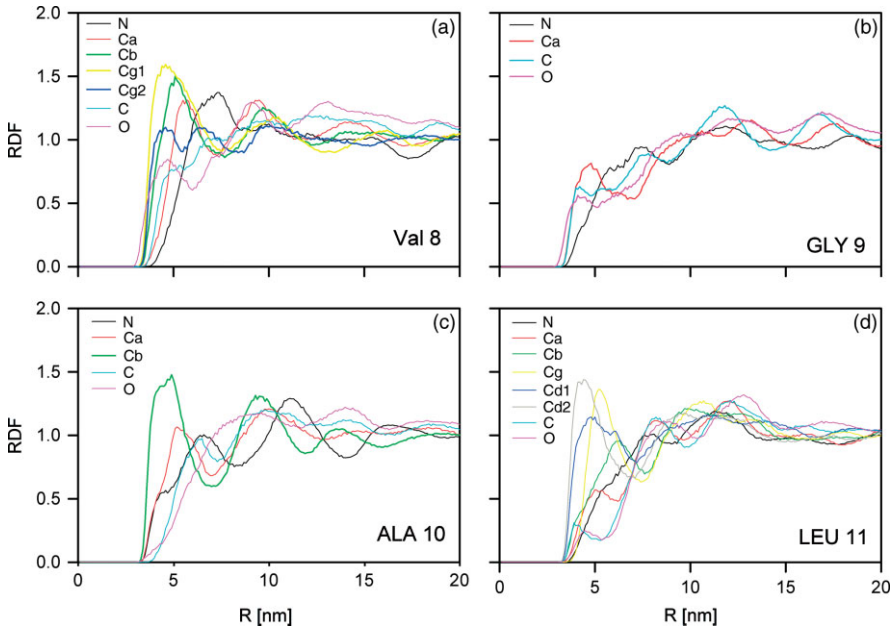

Figure 4 Radial distribution functions (RDFs) of lipid carbon atoms in the POPC-Chol bilayer core relative to (a) Val 8, (b) Gly 9, (c) Ala 10, and (d) Leu 11 atoms. RDF for each atom is shown separately.

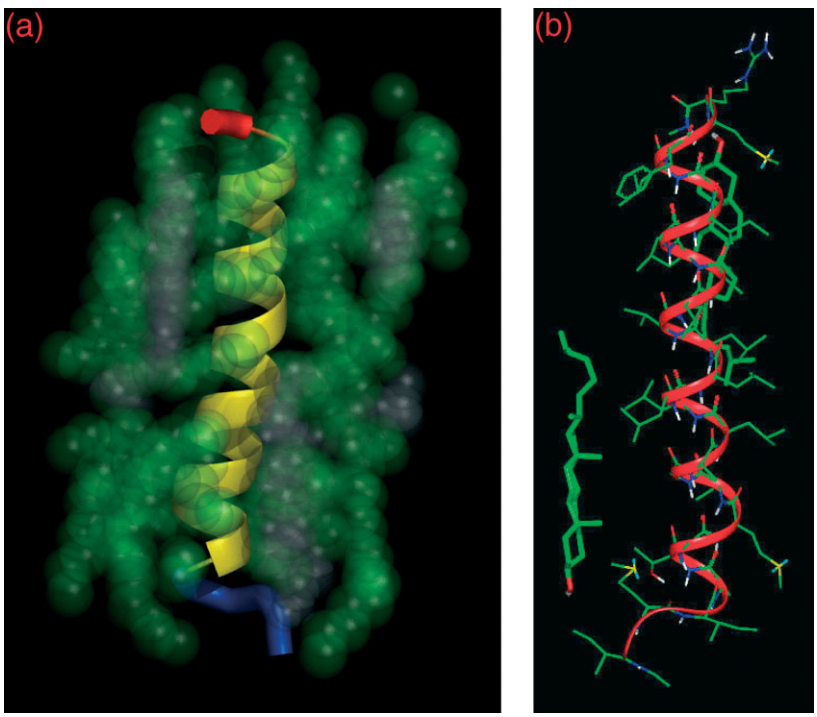

Figure 5 Snapshots of the peptide and neighboring lipids at the distance 0.7 nm – lipids are represented by van der Waals spheres: (a) POPC – green, Chol – yellow, (b) snapshot of the peptide and one of neighboring Chol molecule.

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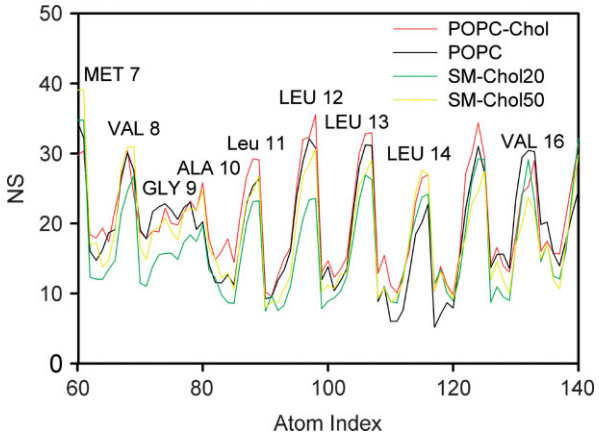

Figure 6 Profile of the number of neighbors of peptide atoms. Atom index refers to the atom position in PDB file (see suppl. material). Maxima corresponding to a given amino acid side chain are marked.

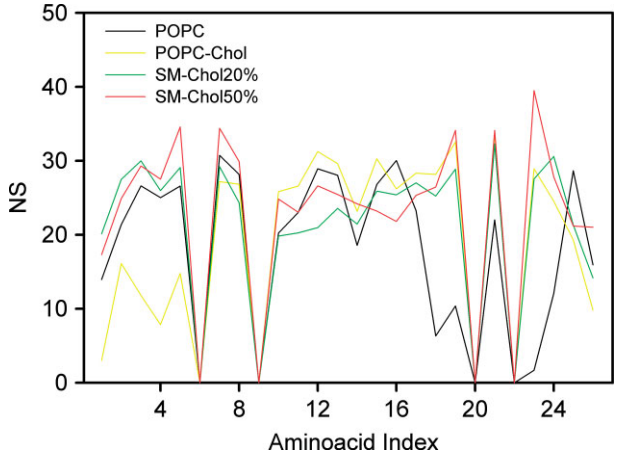

Figure 7 Profile of the average number of neighbors of the side chains.

Since the side chains are the most important in determining the nature of lipid–peptide interactions, in the following analysis we concentrate on the side-chain–lipid interactions. Figure 7 shows the average number of neighbors per side-chain atom in all four bilayers. Data presented in Figure 7 show two trends: (i) Chol promotes tighter packing around the peptide – the number of peptide side-chain neighbors is higher in the POPC-Chol than in the POPC bilayer, and higher in the SM-Chol50 than in the SM-Chol20 bilayer; (ii) packing relative to the peptide side-chains in the center of POPC and POPC-Chol bilayers is tighter than in SM-Chol20 and SM-Chol50 bilayers, but close to the water–membrane interface the situation is reversed. This unexpected observation is likely a result from the presence of the *cis* unsaturated bond in the POPC β-chain – the SM molecule has two saturated tails (with the exception of a *trans*-double bond at the beginning of the tail). A *cis* double bond affects the shape of the density profile of the bilayer; the minimum observed in the center of the bilayer is less deep in *cis* unsaturated bilayers [43] and that is most likely reflected in the trend described above. Owing to the comparatively narrow minimum, the effect on the packing observed in the center of the bilayer is very sensitive to the exact location of the peptide along the bilayer normal, and thus some amino acids do not follow this trend perfectly. In addition, to explain the above, we observed that the motional freedom of the chains is larger in POPC-Chol bilayer center. To put this in perspective, studies of the free volume properties of PCs and SMs [43] strongly support this conclusion. It has been established [43] that packing of SMs is distinctly different, and much tighter, in comparison to PCs. Although it is not possible to establish it quantitatively, this may have important consequences for signaling and packing of proteins and peptides in rafts (SM is a well-known raft lipid).

Figure 8 shows the total number of side-chain neighbors, the total number of side-chain neighbors divided by the number of atoms in the side chain, and the number of atoms per side chain in the POPC-Chol bilayer. This analysis differs from the previous one: in Figure 8, a lipid atom is counted as a neighbor of the side chain only once, while in Figure 7 a lipid atom can be a neighbor of more than one atom of the side chain. The profiles in Figure 8 have certain specific features. First, they are symmetric with the symmetry point at the 14th amino acid. This reflects the symmetry of the two membrane leaflets. The central part of the profile (amino acids 10–19), is characterized by comparatively high number of neighbors, while the flanking regions (amino acids 1–9 and 20–26) are characterized by large oscillations in the number of neighbors. This is mainly due to different sets of amino acids in these regions. Amino acids in the central part are only leucines and valines with alanines at the edges, while the flanking regions contain variety of residues: small glycine and alanine residues, larger aromatic phenylalanine, sulfur-containing methionine, polar threonine, and others. This difference between the central and flanking parts of the peptide can be better understood in the context of the recently published lateral pressure profile of a lipid bilayer containing saturated and unsaturated PCs and sterols [44] and mixture of POPC, SM, and Chol [45]. The shape of the lateral pressure profile depends on the membrane composition but in all cases the central region of the bilayer is characterized by a constant value of pressure, while in the regions closer to the interface pressure oscillates ([44] Figures 3 and 4, the hydrophobic part of the bilayer relevant to this discussion is from −1.7 to 1.7 nm). The correlation between the amino acid sequence and the shape of the lateral profile pressure may result from evolution of the system which has optimized the physicochemical properties of both peptide and membrane to create the

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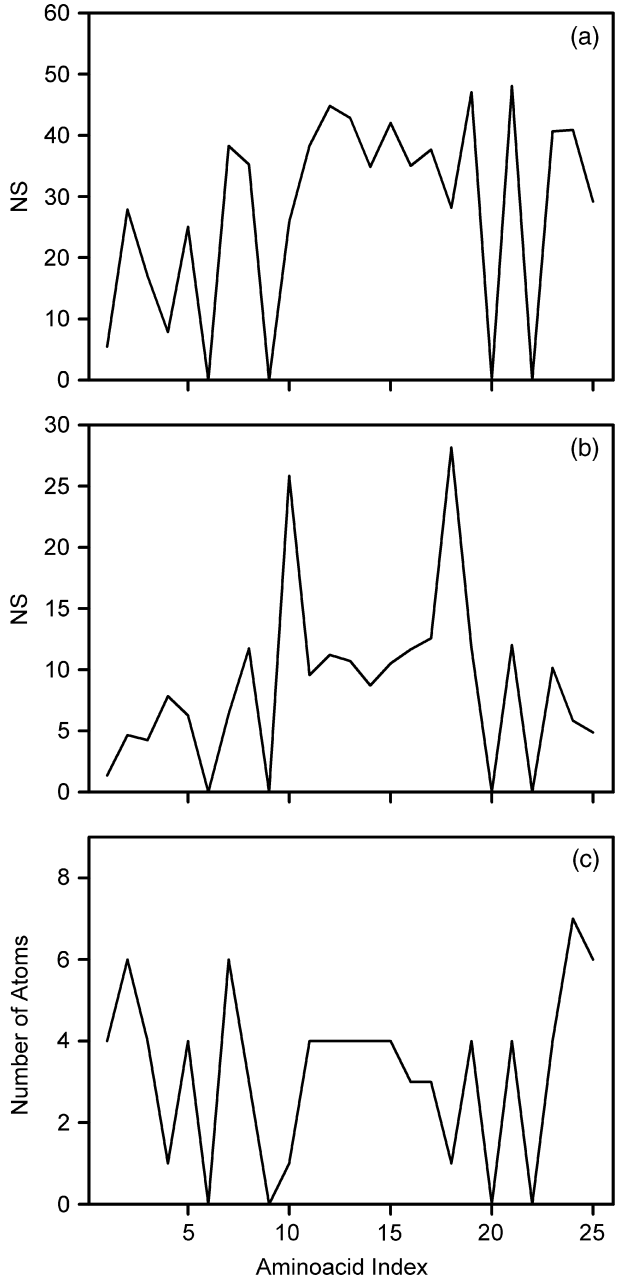

Figure 8 (a) Profile of the total number of neighbors of side chain, (b) the same number divided by the number of atoms in side chain, (c) number of atoms in side chain in POPC-Chol bilayer.

most efficient molecular system, although we stress that this suggestion is purely speculative.

CONCLUSIONS

In this article, we present the results of MD simulations of four compositionally different lipid bilayers with the trans-membrane helical fragment of EGF receptor. The results of our study can be divided into two categories: nonspecific effects of the hydrophobic mismatch and specific effects of the membrane composition and the peptide sequence.

The effect of matching of the peptide hydrophobic length with membrane hydrophobic thickness is one of the most discussed topics in the peptide-membrane literature [29–32]. The bilayers used in this study differ in thickness, and thus we can observe the effect of thickness on the peptide properties and interactions. First, we observed titling of the peptide relative to the bilayer normal and local deformation of the peptide helical structure. They enable the whole peptide to be buried inside the membrane core. The first effect is widely discussed in the literature [29–32], while the second has not been postulated so far. Local changes of the membrane curvature were observed only during the initial stage of the simulations.

The bilayers used in this study differ not only in the membrane thickness but also in the properties of their cores. Among them are ordering of the acyl tails, atom density profiles, and atom packing [46]. These differences are further modulated by the presence of Chol and unsaturation. An interesting question arises – how do these differences influence lipid–peptide interactions? As can be seen from Figures 5 and 6, both factors influence the packing of lipid atoms around the peptide side chains. Thus, they modulate the van der Waals interactions between the lipids and the peptide. The presence of Chol increases the van der Waals interactions in both SM and PC bilayers. Unsaturation has the opposite effect: on average, in an unsaturated bilayer there are fewer interactions between the lipids and the peptide than in a saturated bilayer. However, the number of interactions depends on the depth of an amino acid location – in the center of the bilayer, the packing around the peptide is denser in POPC-based bilayers, while in the regions closer to the interface, the packing is denser in SM-based bilayers. These differences can be of biological importance as stronger and more numerous interactions are likely to better stabilize protein structures and reduce fluctuations which can affect protein functions.

The second specific question is how a particular amino acid sequence influences the interactions with the lipids? First, amino acids with larger side chains have stronger interactions with lipids. This is a non-specific effect, independent of the side-chain structure. Thus the peptide sequence generates a characteristic pattern of interactions along the peptide chain with stronger interactions in the central part of the peptide and of oscillating strength in the regions closer to the interface. This pattern resembles the shape of the lateral pressure profile observed in MD simulations [44,45]. The peptide used in this study is the trans-membrane fragment of EGF receptor, and thus its sequence is nonrandom and reflects protein evolutionary adaptation to the membrane properties. We

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believe that this arrangement also increases protein stability.

Supplementary Material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/1075-2617/suppmat/

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REFERENCES

1. van Meer G. Cellular lipidomics. *EMBO J.* 2005; **24**: 3159.
2. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997; **387**: 569–572.
3. Subczynski WK, Kusumi A. Dynamics of raft molecules in the cell and artificial membranes: approaches by pulse EPR spin labeling and single molecule optical microscopy. *Biochim. Biophys. Acta* 2003; **1610**: 231–243.
4. Pike LJ, Han X, Gross RW. Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids: a shotgun lipidomics study. *J. Biol. Chem.* 2005; **280**: 26796–26804.
5. Hur EM, Park YS, Lee BD, Jang IH, Kim HS, Kim TD, Suh PG, Ryu SH, Kim KT. Sensitization of epidermal growth factor-induced signaling by Bradykinin is mediated by c-Src. Implications for a role of lipid microdomains. *J. Biol. Chem.* 2004; **279**: 5852–5860.
6. Mayawala K, Vlachos DG, Edwards JS. Computational modeling reveals molecular details of epidermal growth factor binding. *BMC Cell Biol.* 2005; **6**: 41.
7. Wiley HS, Shvartsman SY, Lauffenburger DA. Computational modeling of the EGF-receptor system: a paradigm for systems biology. *Trends Cell Biol.* 2003; **13**: 43–50.
8. Hoeller R, Hoeller D. Current challenges in quantitative modeling of epidermal growth factor signaling. *FEBS Lett.* 2005; **579**: 6289–6294.
9. Shen L, Bassolino D, Stouch T. Transmembrane helix structure, dynamics, and interactions: multi-nanosecond molecular dynamics simulations. *Biophys. J.* 1997; **73**: 3–20.
10. Gorfe AA, Pellarin R, Caflisch A. Membrane localization and flexibility of a lipidated ras peptide studied by molecular dynamics simulations. *J. Am. Chem. Soc.* 2004; **126**: 15277–15286.
11. Capener CE, Shrivastava IH, Ranatunga KM, Forrest LR, Smith GR, Sansom MSP. Homology modeling and molecular dynamics simulation studies of an inward rectifier potassium channel. *Biophys. J.* 2000; **78**: 2929–2942.
12. Kandasamy SK, Larson RG. Molecular dynamics simulations of model trans-membrane peptides in lipid bilayers: a systematic investigation of hydrophobic mismatch. *Biophys. J.* 2006; **90**: 2326–2343.
13. Ramamoorthy A, Kandasamy SK, Lee DK, Kidambi S, Larson RG. Structure, topology, and tilt of cell-signaling peptides containing nuclear localization sequences in membrane bilayers determined by solid-state NMR and molecular dynamics simulation studies. *Biochemistry* 2007; **46**: 965–975.
14. Rigby AC, Grant CWM, Shaw GS. EGF Helix Solution and solid state conformation of the human EGF receptor transmembrane region. *Biochim. Biophys. Acta – Biomembr.* 1998; **1371**: 241–253.
15. Murzyn K, Róg T, Jezierski G, Kitamura K, Pasenkiewicz-Gierula M. Effects of phospholipid unsaturation on the membrane/water interface: a molecular simulation study. *Biophys. J.* 2001; **81**: 170–183.
16. Róg T, Pasenkiewicz-Gierula M. Cholesterol effects on the unsaturated phosphatidylcholine bilayer: a molecular dynamics simulation study. *Biochimie* 2006; **88**: 449–460.
17. Róg T, Pasenkiewicz-Gierula M. Cholesterol-sphingomyelin interactions: a molecular dynamics simulation study. *Biophys. J.* 2006; **91**: 3756–3767.
18. Jorgensen WL, Tirado-Rives J. The OPLS potential functions for proteins. Energy minimizations for crystals of cyclic peptides and crambin. *J. Am. Chem. Soc.* 1988; **110**: 1657–1666.
19. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 1983; **79**: 926–935.
20. Ryckaert JP, Ciccotti G, Berendsen HJC. Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comput. Phys.* 1977; **23**: 327–341.
21. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald method. *J. Chem. Phys.* 1995; **103**: 8577–8593.
22. Patra M, Karttunen M, Hyvönen MT, Falck E, Lindqvist P, Vattulainen I. Molecular dynamics simulations of lipid bilayers: major artifacts due to truncating electrostatic interactions. *Biophys. J.* 2003; **84**: 3636–3645.
23. Patra M, Karttunen M, Hyvönen MT, Falck E, Vattulainen I. Lipid bilayers driven to a wrong lane in molecular dynamics simulations by truncation of long-range electrostatic interactions. *J. Phys. Chem. B* 2004; **108**: 4485–4494.
24. Smith PE, Pettitt BM. Peptides in ionic solutions: a comparison of the Ewald and switching function techniques. *J. Chem. Phys.* 1991; **95**: 8430–8441.
25. Falck E, Hautala JT, Karttunen M, Kinnunen PKJ, Patra M, Saaren-Seppala H, Vattulainen I, Wiedmer SK, Holopainen JM. Interaction of fusidic acid with lipid membranes: Implications to the mechanism of antibiotic activity. *Biophys. J.* 2006; **91**: 1787–1799.
26. Case DA, Pearlman DA, Caldwell JW, Cheatham TE III, Ross WS, Simmerling C, Darden TA, Merz KM, Stanton RV, Cheng AL, Vincent JJ, Crowley M, Ferguson DM, Radmer RJ, Seibel GL, Singh UC, Weiner PK, Kollman PA. *AMBER 5.0*. University of California: San Francisco, 1997.
27. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* 1984; **81**: 3684–3690.
28. Almeida RFM, Fedorov A, Prieto M. Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts. *Biophys. J.* 2003; **85**: 2406–2416.
29. Killian JA. Hydrophobic mismatch between proteins and lipids in membranes. *Biochim. Biophys. Acta* 1998; **1376**: 401–416.
30. Killian JA. Synthetic peptides as models for intrinsic membrane proteins. *FEBS Lett.* 2003; **555**: 134–138.
31. Dumas F, Lebrun MC, Tocanne J-F. Is the protein/lipid hydrophobic matching principle relevant to membrane organization and functions? *FEBS Lett.* 1999; **458**: 271–277.
32. Gil T, Ipsen JH, Mouritsen OG, Sabra MC, Sperotto MM, Zuckermann MJ. Theoretical analysis of protein organization in lipid membranes. *Biochim. Biophys. Acta* 1998; **1376**: 245–266.
33. Armen RS, Uitto OD, Feller SE. Phospholipid component volumes: determination and application to bilayer structure calculations. *Biophys. J.* 1998; **75**: 734–744.
34. Patra M, Salonen E, Terama E, Vattulainen I, Faller R, Lee BW, Holopainen J, Karttunen M. Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers. *Biophys. J.* 2006; **90**: 1121–1135.

35. Bond PJ, Holyoake J, Ivetac A, Khalid S, Sansom MSP. Coarse-grained molecular dynamics simulations of membrane proteins and peptides. *J. Struct. Biol.* 2007; **157**: 593–605.
36. Murtola T, Róg T, Falck E, Karttunen M, Vattulainen I. Transient ordered domains in pure phospholipid bilayers. *Phys. Rev. Lett.* 2006; **97**: 238102.
37. Shen L, Bassolino D, Stouch T. Transmembrane helix structure, dynamics and interactions: multi-nanosecond molecular dynamics simulation. *Biophys. J.* 1997; **73**: 3–20.
38. Zhang YP, Lewis RN, Hodges RS, McElhaney RN. Peptide models of helical hydrophobic transmembrane segments of membrane proteins. 2. Differential scanning calorimetric and FTIR spectroscopic studies of the interaction of Ac-K2-(LA)12-K2-amide with phosphatidylcholine bilayers. *Biochemistry* 1995; **34**: 2362–2371.
39. Katsaras J, Prosser RS, Stinson RH, Davis JH. Constant helical pitch of the gramicidin channel in phospholipids bilayers. *Biophys. J.* 1992; **61**: 827–830.
40. Killian JA, Prasad KU, Hains D, Urry DW. The membrane as an environment of minimal interconversion. A circular dichroism study on the solvent dependence of the conformational behavior of gramicidin in diacylphosphatidylcholine model membranes. *Biochemistry* 1988; **27**: 4848–4855.
41. Róg T, Pasenkiewicz-Gierula M. Cholesterol-phospholipid hydrophobic interactions: a molecular simulation study. *Biophys. Chem.* 2004; **107**: 151–164.
42. Róg T, Pasenkiewicz-Gierula M. Cholesterol effect on the phospholipid condensation and packing in the bilayer: a molecular simulation study. *FEBS Lett.* 2001; **502**: 68–71.
43. Róg T, Murzyn K, Gurbiel R, Kitamura K, Kusumi A, Pasenkiewicz-Gierula M. Effects of phospholipid unsaturation on the structure and dynamics of the hydrocarbon core of the membrane. A molecular simulation study. *J. Lipid Res.* 2004; **45**: 326–336.
44. Ollila S, Róg T, Karttunen M, Vattulainen I. Role of sterol type on lateral profiles of lipid membranes affecting membrane protein functionality: comparison between cholesterol, desmosterol, 7-dehydrocholesterol and ketosterol. *J. Struct. Biol* 2007; **159**: 311–323. DOI:10.1016/j.jsb.2007.01.012.
45. Niemelä PS, Ollila S, Hyvönen MT, Karttunen M, Vattulainen I. Assessing the nature of lipid raft membranes. *PLoS Comput. Biol.* 2007; **3**: e34.
46. Kupiainen M, Falck E, Ollila S, Niemelä P, Gurtovenko AA, Hyvönen MT, Patra M, Karttunen M, Vattulainen I. Free volume properties of Sphingomyelin, DMPC, DPPC, and PLPC bilayers. *J. Comput. Theor. Nanosci.* 2005; **2**: 401–413.