The impact of a simple α-helical transmembrane peptide on lipid membrane dynamics

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Proteins form an important part of cellular membranes but their capacity to influence membrane organization and dynamics remains poorly understood. Here, we provide evidence that the rough surface of a helical transmembrane peptide, representing a simple integral protein, reduces lateral mobility of membrane constituents by trapping lipid acyl chains and increasing local viscosity. This effect is further pronounced by the presence of cholesterol which segregates from the vicinity of transmembrane peptides to avoid contact with their rough surface.

Cellular membranes are formed by lipid bilayers densely populated with proteins (up to 50% of total mass).[1] Integral proteins span a lipid bilayer via single or multiple transmembrane domains (TMDs). Protein TMDs are sequences of 15-30, prevalently non-polar, amino acids. They form α-helix or β-sheet to stably integrate into the hydrophobic core of membranes and are in direct contact with lipids. TMDs differ in their length and hydrophobicity, properties which were shown to influence, for example, protein sorting between cellular membranes.[2,3] In addition, specific protein-lipid interactions influencing the structure and function of proteins frequently occur in, or close to, the membrane[4] thus pointing to the impact of TMDs on membranes and associated cellular processes.

Previous studies investigated membrane proteins mainly in the context of existing theories of plasma membrane organization and dynamics, e.g. investigations of the effects of obstacles (crowding and cytoskeleton), hydrophobic mismatch and formation of protein clusters or domains.[5-10] Here, we have adapted simple proteo-lipid model membranes of only two components, transmembrane peptide and phospholipids (Supplementary Fig 1 and Supplementary Discussion, Section 1), to investigate a direct impact of membrane proteins in absence of the phenomena mentioned above. The experiments were performed in the absence and presence of 25 mol% cholesterol, an important component of plasma membrane in mammals. The synthetic, highly purified, transmembrane peptide (LW21 variant: GLLDSKKWWLLLLLLLALLLLLLLWWKKFSRS) adapts a monomeric, α-helical structure in the tested membranes (Supplementary Fig 2).[6] The flanking residues (not underlined) stabilize its transbilayer orientation, as shown previously.[11] Good surface expression of the LW21-GFP fusion protein in human cells supports the LW21 peptide as a viable model for the TMD of plasma membrane proteins (Supplementary Fig. 3). Dioleoylphosphatidylcholine (DOPC) glycerophospholipid, of low melting temperature (-18.3°C)[12], was selected to preserve the fluid character of membranes under all tested conditions and avoid formation of lipid domains.[13] Moreover, LW21 peptide incorporates into membranes composed of DOPC with and without cholesterol with comparable efficiency (Supplementary Fig. 4). Highly fluid character of membranes with homogenously distributed monomeric peptides lacking bulky non-membranous parts was prerequisite to investigate a direct impact of integral proteins on membranes.

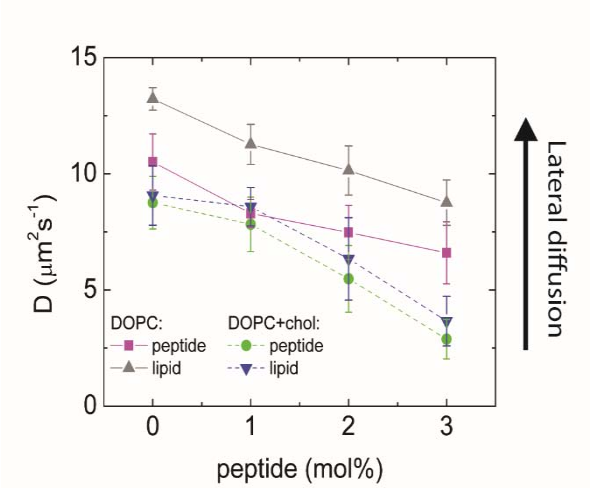

Figure 1. Impeded lateral diffusion in membranes with peptide. Lateral diffusion coefficients of lipid tracer (DiD; grey and blue lines) and fluorescently labelled LW21 peptide (magenta and green lines) were measured in GUVs composed of DOPC (full lines) and DOPC:cholesterol (3:1; dashed lines), in the presence of increasing concentration of unlabeled peptide. Each presented diffusion coefficient (*D*) was measured for at least 10 vesicles in three independent experiments using callibration-free z-scan FCS (fluorescence correlation spectroscopy) technique. Error bars indicate standard deviations (SD).

Proteins account for approximately for 2-3 mol% of cellular membranes (see Supplementary Discussion, Section 2 for more details). Therefore, we measured the lateral diffusion of fluorescent lipid tracer, DiD, and peptide in membranes (giant unilamellar vesicles; GUVs) containing 1-3 mol% of LW21 using calibration-free *z*-scan FCS technique.[14] In Fig 1, we demonstrate that increasing the concentration of monomeric α-helical peptide (LW21) reduced lateral diffusion of both lipid tracer and labelled peptide (grey and red lines, respectively). At the highest tested peptide concentration, 3 mol%, diffusion coefficients of both molecules were reduced by approximately 35% in DOPC membranes. In agreement with the literature,[7] diffusion of lipid molecules was somewhat faster compared to the peptide and this was preserved for all tested peptide concentrations. In contrast, lateral diffusion of lipids and peptides was indistinguishable in the presence of 25 mol% cholesterol (Fig 1; dark blue and green lines). The impact of transmembrane helical peptide on the mobility of membrane components was more pronounced in the presence of cholesterol than in its absence. At the highest peptide concentration, 3 mol%, we observed 2-3 fold decrease in the diffusion coefficients for both tested molecules (Fig 1). Quantitative analysis (D_{25}/D_0 values) supports the enhanced effect of peptide on the mobility of membrane components in the presence of sterol compared to its absence (Supplementary Table 1).

In order to better understand the effect of simple α-helical transmembrane peptides, we characterized properties of such membranes using experimental and *in silico* approaches. First, a higher local viscosity was detected in membranes with increasing concentration of peptides using time-resolved emission spectra (TRES) analysis

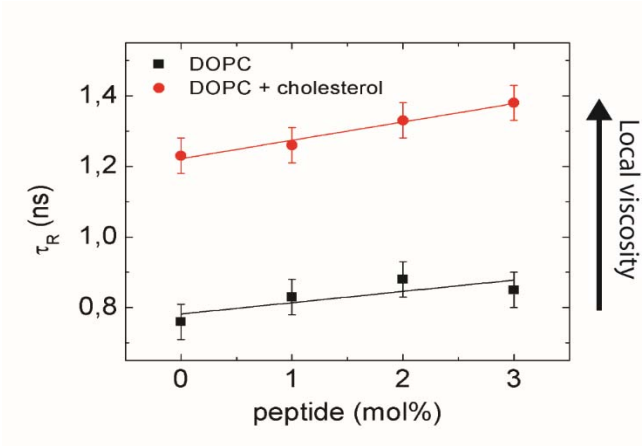

Figure 2. Increased local membrane viscosity in the presence of peptide. Local lipid mobility (viscosity) as a function of increasing LW21 peptide concentration was determined in the absence (black squares) or presence (red circles) of 25 mol% cholesterol using Laurdan fluorescent probe. Error bars represent intrinsic uncertainty of the method.

of the environment-sensitive probe Laurdan (Fig 2). The impact was slightly stronger in the presence of cholesterol compared to its absence (Fig 2; linear regression slope of 0.05 versus 0.03, respectively). Similar effect was observed when probing fluorescence anisotropy of diphenylhexatriene (DPH) in LW21-containing membranes (Supplementary Table 2, Supplementary Discussion, Section 3 and ref. [15]).

The experimental data using Laurdan and DPH probes indicate restricted mobility of lipids in the vicinity of TMD of proteins, phenomena which was reported in a recent computational study with large, multi-spanning proteins.[16] Here, we have performed fully atomistic MD simulations (Supplementary Movies 1-3) with a simple transmembrane peptide (LW21) incorporated into lipid bilayers composed of DOPC with and without cholesterol to get more molecular insight into our experimental data. We confirm considerably impeded mobility of lipids close to the peptide in both absence and presence of cholesterol (Supplementary Fig 5). Moreover, we observed that this is caused by the trapping of acyl chains of annular lipids in the grooves of the rough surface of the peptide, which is formed by amino acid side chains (Fig 3a,b). Such lipid-peptide contacts are non-specific and exhibit substantial stability (Supplementary Figs. 6 and 7). On the contrary, cholesterol was excluded from the annulus, thus exhibiting fewer contacts with the peptide compared to phospholipids (Fig. 3c-e and Supplementary Fig 8). This is probably caused by the incompatibility between the planar shape of cholesterol molecule and the roughness of transmembrane peptides (ref. [17], Supplementary Fig. 9 and Supplementary Discussion, Section 4).

Altogether our data demonstrate that proteins can influence membrane dynamics and organization by exposing the rough surface of their TMDs to the surrounding lipids. This causes lipid acyl chain trapping, cholesterol segregation and, as a consequence, reduced lateral diffusion of membrane molecules. Importantly, this effect is observed in the absence of hydrophobic mismatch, detectable domains and the presence of obstacles or peptide aggregates, thus supporting a direct effect of proteins on lipid membranes. It is also not restricted to the peptide tested in this work since trapping of lipid acyl chains in the grooves of the surface of a membrane protein was recently observed in 2D crystals of aquaporin-0.[18] A rough surface of transmembrane domains, with potential for acyl chain trapping, was shown for a number of proteins (for example PDB ID: 5EE7[19] or 2NA8[20], Supplementary Discussion, Section 5).

The herein reported association of lipids with membrane proteins is reminiscent of the lipid shell hypothesis proposed by Anderson and Jacobson in 2002[21] in which it is predicted that membrane proteins can associate with sphingolipids (and/or cholesterol) and co-migrate as distinct proteo-lipid platforms. Together with our observation that phospholipids are trapped by a rough surface of TMDs it implicates that proteins can form two kinds of lipid shells: i) based on specific protein-lipid interactions for long-term complex formation[4], and ii) non-specific interactions of lipids with a rough protein surface for highly dynamic, transient assemblies (this work and refs. [16,18]). Evi-

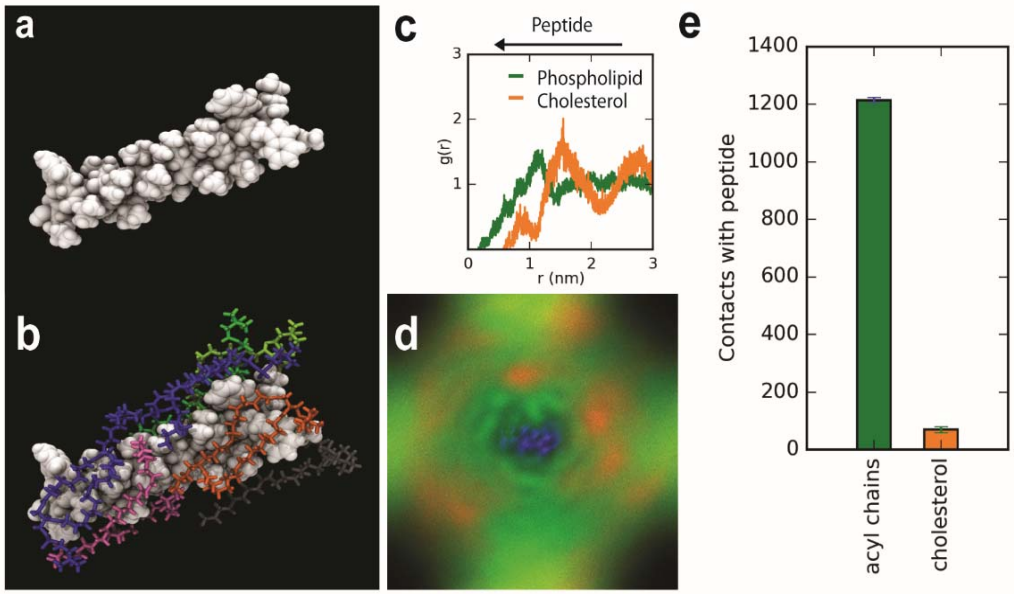

Figure 3. Non-specific lipid acyl chain trapping on the LW21 peptide and cholesterol segregation – MD simulations. a and **b.** A typical snapshot from MD simulation of LW21 in DOPC bilayer indicating trapping of lipid acyl chains in the grooves formed by peptide side chains. Peptide surface (a) is shown in white. Interacting lipids are shown in different colors using licorice representation (b). Non-interacting lipids and water were removed for clarity. **c**. Pair correlation function (g(r)) of phospholipids and cholesterol from the centre of mass of the peptide. This function quantifies the probability of intermolecular distances between the peptide and lipids with respect to those in an ideally mixed system. **d**. Distribution map of cholesterol (orange) and phospholipids (green) in membrane with peptide (blue). The peptide was centered and rotations were removed by data postprocessing. **e**. Quantification of phospholipid and cholesterol contacts with the peptide calculated in MD simulations. Error bars represent error of the mean estimated by the block averaging method.

dently, both types of protein-lipid interactions influence membranes.

The direct effect of helical TMDs on membrane dynamics and organization demonstrated in this work may have previously unexpected consequences for cell membranes and associated processes (see also Supplementary Discussion, Section 6). For example, it implies that reorganization of proteins (e.g. receptor clustering by ligands) might cause a local reduction in the mobility of membrane molecules. Inverse correlation between protein density and lateral diffusion that has been observed in both model and cell membranes in past might not be exclusively due to crowding.[22,23] In addition, a tendency of cholesterol to segregate from TMDs might help formation and/or stabilization of cholesterol-enriched, protein-low domains. Such changes will locally affect intermolecular interactions or reaction kinetics of cellular processes associated with membranes.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

M.O. and M.C. conceived the project; M.O., P.J., J.Sy., L.C. and M.H. designed the experiments, M.O., P.J., J.Sa. and L.C. performed the experiments and M.O., P.J., L.C. and M.C. written and edited the paper.

Competing financial interests

The authors declare no competing financial interests.

Additional information

Any supplementary information is available in the online version of the paper. Correspondence should be addressed to M.C.