Original Research

Structure of the Myelin Sheath Proteolipid Plasmolipin (PLLP) in a Ganglioside-Containing Lipid Raft

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Abstract

Background: Plasmolipin (PLLP) is a membrane protein located in lipid rafts that participates in the formation of myelin. It is also implicated in many pathologies, such as neurological disorders, type 2 diabetes, and cancer metastasis. To better understand how PLLP interacts with raft components (gangliosides and cholesterol), we undertook a global study combining in silico simulations and physicochemical measurements of molecular interactions in various PLLP-ganglioside systems. Methods: In silico studies consisted of molecular dynamics simulations in reconstructed membrane environments. PLLP-ganglioside interaction measurements were performed by microtensiometry at the water-air interface on ganglioside monolayers. Results: We have elucidated the mode of interaction of PLLP with ganglioside GM1 and characterized this interaction at the molecular level. We showed that GM1 induces the structuring of the extracellular loops of PLLP and that this interaction propagates a conformational signal through the plasma membrane, involving a cholesterol molecule located between transmembrane domains. This conformational wave is finally transmitted to the intracellular domain of the protein, consistent with the role of PLLP in signal transduction. Conclusions: This study is a typical example of the epigenetic dimension of protein structure, a concept developed by our team to describe the chaperone effect of gangliosides on disordered protein motifs which associate with lipid rafts. From a physiological point of view, these data shed light on the role of gangliosides in myelin formation. From a pathological point of view, this study will help to design innovative therapeutic strategies focused on ganglioside-PLLP interactions in various PLLP-associated diseases.

Keywords: lipid rafts; ganglioside; cholesterol; neurological disorder; disease; signal transduction; plasmolipin

1. Introduction

Proteolipids are a subclass of membrane proteins soluble in a chloroform–methanol mixture [1]. The proteolipid plasmolipin (PLLP), also known as plasma membrane proteolipid (PMLP) or transmembrane 4 superfamily, member 11 (TSF11) [2], is a main component of the myelin sheath [3, 4]. Moreover, the human PLLP (encoded by the PLLP gene) [5], an 18-kD protein, is presumably involved in various pathological conditions such as Alzheimer’s disease [6], type 2 diabetes [7], and cancer metastasis [8]. PLLP is involved in intracellular transport [9], lipid raft formation [10], and Notch signaling [2]. Finally, PLLP was also identified as a virus entry receptor [11]. An intriguing aspect of proteolipids such as PLLP is that most of the protein structure is embedded in the membrane [2], leaving very few surfaces of contact for the polar headgroups of glycosphin-golipids such as gangliosides, which are key lipid raft components [12, 13]. The predicted topology of human PLLP is a tetraspan protein with both N-terminal and C-terminal domains located intracellularly [2]. Correspondingly, the extracellular areas of PLLP are limited to two loops, encompassing amino acid residues 125–144 (AVDLTSLRGRTPYNQRAAAS), referred to as loop 1 (the largest one) and 57–64 (DTPYHLYP), referred to as loop 2 (the smallest one). Alpha-Fold has not satisfactorily resolved the structure of these loops [14, 15], consistent with secondary structure predictions that consider these regions as disordered domains [16]. Indeed, apart from the transmembrane (TM) domains, the AlphaFold predictions for human PLLP that are available through the UniProt database (entry Q9Y342, https://www.uniprot.org/uniprotkb/Q9Y342/entry) have either low (70 > pLDDT > 50) or very low (pLDDT < 50) confidences indexes.

Gangliosides are important components of the myelin sheath [17]. Knockout mice lacking major brain gangliosides display decreased central myelinlation and axonal degeneration in both the central and peripheral nervous systems [18]. However, although these data suggest that gangliosides are functionally important for myelin biogenesis [19], the molecular basis of this concept remains poorly understood. Solving this problem requires understanding the molecular interactions between PLLP and gangliosides. However, disordered loops in the proteolipid make this analysis particularly difficult. Since PLLP is found within
lipid rafts [10,20], these membrane domains may be a privileged site of ganglioside-PLLP interactions. The present study aimed to assess whether the disordered loops of PLLP could be structured upon binding to the sugar head group of gangliosides, according to the lipid chaperone theory [21,22]. To this end, we studied the interaction of a synthetic peptide derived from the largest extracellular loop of PLLP with ganglioside monolayers using the Langmuir microtensiometry technology. Then we performed a series of molecular dynamics simulations of human PLLP in a lipid raft environment. Our results give new insights into how proteolipids interact with lipid rafts and how gangliosides control the folding of this intriguing class of membrane proteins through a limited set of molecular interactions at the plasma membrane-water interface.

2. Materials and Methods

2.1 Determination of the Minimal Ganglioside-Binding Domain of Human PLLP

Ganglioside binding domain (GDB) predictions were performed according to the algorithm developed for amyloid proteins [23]. This allowed us to identify the RGTRPYNQR motif in the second extracellular loop of human PLLP. A synthetic peptide encompassing this motif (SLRGTRPYNQRA) was synthesized by Schafer-N (Copenhagen, Denmark) at a purity > 95%.

2.2 Langmuir Microtensiometry

The synthetic peptide SLRGTRPYNQRA was dissolved in pure water at a concentration of 1 mM and used at a final concentration of 10 µM in Langmuir microtensiometry studies. Surface pressure measurements were recorded with an automated Langmuir trough (Microtrough X, Kibron, Helsinki, Finland) [24–26]. The peptide was added in the aqueous phase underneath a monolayer of the indicated ganglioside (GM1, GD1a, or GT1b, all purchased from Matreya) at the initial surface pressure \( \pi_i \) indicated. The interaction of the peptide with the gangliosides was measured as an increase in the surface pressure of the monolayer [27]. Both kinetics (\( \Delta \pi = f(t) \)) and specificity experiments (measurements at \( \pi_i \) various values, (\( \Delta \pi = f(\pi_i) \)) were performed [28]. Data are representative of triplicate experiments with SD <15%.

2.3 Molecular Modeling Studies

The three-dimensional structure of the human PLLP was modeled via an \textit{ab initio} method using the protein structure prediction service Robetta (https://robetta.bakerlab.org/) [29]. The first thirty-three residues corresponding to an intrinsically disordered region exposed to the intracellular environment were removed from the structure. We simulated PLLP in two different settings, (i) PLLP inserted into a POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) bilayer and (ii) PLLP inserted into a lipid raft environment consisting of an equimolar mixture of ganglioside and cholesterol [30,31] as previously described [32–34]. Both environments were solubilized with TIP3P water (the TIP3P water model as implemented in CHARMM specifies a 3-site rigid water molecule with charges and Lennard-Jones parameters assigned to each of the 3 atoms) [35] and neutralized with Na\(^+\) and Cl\(^-\) ions at a final concentration of 0.15 mol/L using the tools “Add solvation box” and “Add ions” in VMD software [36]. The molecular dynamics simulations were performed using NAMD 2.14 (Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign, Urbana-Champaign, IL USA) for Windows 10 and the force field CHARMM36m [37]. Before production runs, each system was minimized for 5000 steps and equilibrated at constant temperature (310 K) and constant pressure (1 atm). The cutoff for calculating non-covalent intermolecular interactions was set at 12 Å, and the PME algorithm was used to calculate long-range interactions.

3. Results

3.1 Physicochemical Studies of Ganglioside-Plasmolipin Interactions

As a preliminary analysis, we submitted the extracellular domains of human PLLP to our dedicated algorithm for the prediction of ganglioside binding domains [23]. This algorithm has been previously validated with numerous endogenous and microbial proteins [38]. The largest extracellular loop of human PLLP (loop 1) fulfills the algorithm with the RGTRPYNQR motif [39]. A synthetic peptide encompassing this predicted ganglioside binding domain was thus tested for its ganglioside binding properties. In these experiments, a ganglioside cluster was reconstituted as a monolayer at the air-water interface, and the interaction of the loop 1-derived peptide with this ganglioside monolayer was measured by surface pressure measurements [28]. Although consisting of a single lipid leaflet, lipid monolayer systems, including reconstituted lipid rafts coupled with surface pressure measurements, have been validated as valuable models for the study of membrane-protein interactions [40–44]. Most importantly, lipid raft-like microdomains have been observed in ganglioside monolayers by fluorescence imaging [45], consistent with the use of these systems for studying protein binding to lipid rafts [46]. As shown in Fig. 1, the synthetic loop 1 recognized the three gangliosides tested: GM1 (the main ganglioside of myelin) [47], GD1a, and GT1b (both known to interact with myelin-associated glycoprotein) [48]. The kinetics of interaction were similar with all three gangliosides (Fig. 1a). However, the data in Fig. 1b indicate that the interaction of the synthetic loop 1 still occurs with GM1 at higher surface pressure values (> 50 mN·m\(^{-1}\)), which is a criterion of higher specificity [28] compared with GD1a and GT1b. In these experiments, a series of monolayers prepared at dif-
different initial surface pressure values are probed with the synthetic peptide added in the aqueous phase. At high surface pressure values, the monolayer consists of densely packed gangliosides; thus, the insertion of the peptide loop between the polar head groups of gangliosides is more difficult. Indeed, this parameter reflects the avidity of the peptide for the monolayer. It is generally considered that the interaction is biologically consistent when it is still detected at values of the surface pressure $> 30 \text{mN/m}^{-1}$, which is the mean value measured for a typical plasma membrane [49]. The critical pressure of insertion, which corresponds to the value of the initial surface pressure at which the peptide no longer induces an increase in pressure, thus makes it possible to evaluate its avidity for the different gangliosides [23,50], as illustrated in Fig. 1b. Overall, these physicochemical experiments fully confirmed the data obtained in silico and formally identify the extracellular loop 1 of human PLLP as a canonical and functional ganglioside-binding domain.

Fig. 1. A synthetic peptide derived from loop 1 of human plasmolipin interacts with gangliosides. (a) Kinetics of peptide SLRGTRPYNQRA interaction with gangliosides GM1, GD1a, and GT1b. (b) Interaction of peptide SLRGTRPYNQRA with ganglioside monolayers prepared at various values of the initial pressure.

3.2 Molecular Dynamics Simulations of Plasmolipin in Membrane Systems

In this study, we present two molecular dynamics simulations which consist of human PLLP inserted into a phosphatidylcholine (POPC) bilayer or a lipid raft environment. To ensure our structure does not go through unstable 3D conformations, we measured its root mean square fluctuation (RMSF). The RMSF is a measure of the degree of flexibility or mobility of atoms or groups of atoms in a protein or other biomolecular systems [36]. The RMSF of the PLLP inserted into a POPC bilayer (black line) or in a lipid raft environment (orange line) are plotted in Fig. 2. The results show that the transmembrane domains of PLLP in either POPC or lipid raft transient around 0.5 Å. In comparison, the flexible loops reach a maximal value of 2 Å. Overall, the small RMSF value of the structured regions of PLLP suggests that our protein is stable over the trajectory.

Fig. 2. RMSF of plasmolipin in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer (black line) and lipid raft environment (orange line).

3.3 Plasmolipin is More Stable in a Lipid Raft Environment

Lipid rafts are cholesterol-rich plasma membrane microdomains also enriched in sphingolipids, including sphingomyelin and gangliosides [51]. Among the different classes of gangliosides expressed by human cells, GM1 is of high interest because it is the main brain myelin ganglioside [52]. This study aimed to evaluate if lipid raft gangliosides could have a conformational impact on the flexible loops of PLLP. To this end, we first plotted the total number of hydrogen bonds (H-bonds) formed over time between (i) PLLP and each type of membrane lipid (POPC bilayer or lipid raft), (ii) loop 1 of PLLP and each membrane or (iii) loop 2 of PLLP with each membrane. More H-bonds were formed in the lipid raft environment with a total of 8303 for the whole protein, 2040 for loop 1, and 772 for loop 2, compared with 6521, 1605, and 395 for the POPC bilayer, respectively (Fig. 3). The fact that more H-bonds were formed over time in the lipid raft environment suggests that these bonds are more stable and thus more
durable. In the case of loop 1, the main residues that interact with the membrane component are R132 and R135. To evaluate the stability of these residues interacting with their lipid partner, we plotted the distances between them and their partner identified at the end of the trajectory. We can consider that two atoms enter a non-covalent interaction between 1.5 and 5 Å. The plots presented in Fig. 4 show that the distance between the guanidinium group of R132 and an oxygen atom belonging to the phosphate group of a POPC molecule is very unstable, in contrast with the intermolecular interactions of the guanidinium group of R132 with the COO− group of the sialic acid (purple line) and the OH group of the sphingosine part of GM1 (black line). In the case of the guanidinium group of R135, the data show that the intermolecular interaction with the oxygen atom of the phosphate group of a POPC molecule is unstable between 5 and 35 ns. However, it stabilizes between 35 and 50 ns. In the lipid raft environment, the intermolecular interaction between the guanidinium group of R135 and the OH group of the sphingosine part of GM1 (black line) is very stable. This is not the case for the cholesterol molecule (yellow line) between 5 and 25 ns, although, after this initial phase, a stabilization occurs between 25 and 50 ns. Altogether, our computational results suggest that the intermolecular interaction between R132 and R135 of loop 1 is optimized in the lipid raft environment.

Similar measurements were completed for the extracellular loop 2. The main residues of loop 2 that interact with the membrane components are Y60 and H61. The distances of the intermolecular interactions between these residues and their final partner are plotted in Fig. 5. The distance between the OH group of Y60 and an oxygen atom of the phosphate group of a POPC molecule is mostly superior to 5 Å, indicating that the intermolecular interaction between both chemical groups is not stable over the trajectory. It is the same for H61, which was found to interact with two molecules of POPC via van der Waals (black line) and CH-π (red line) interactions [53]. For the lipid raft system, we found that Y60 predominantly interact with the OH group of the sphingosine part of the GM1 molecule during the first 40 ns of the simulation (black line). Then it switches to interact with a hydroxyl group of the glucose residue of the same ganglioside molecule at the end of the trajectory (red line). H61 was found to interact with both the glucose and galactose residues of a GM1 molecule, and it is predominantly interacting with those sugars between 15 to 25 ns and 40 to 50 ns. As for loop 1, these results suggest that the intermolecular interaction between loop 2 and raft gangliosides is more stable than with POPC molecules.

To gain more accuracy in the evaluation of the differences in stability of the PLLP loops in the two different lipidic environments, we studied the confinement of the mass center of the backbone of these structures along the x, y, and z-axis. The more the mass center of a structure is confined, the less the conformation of this structure varies. Thus, this parameter indicates the conformational freedom.
Fig. 4. Snapshots showing the interaction of residues R132 and R135 belonging to loop 1 in interaction with their lipid/glycolipid partner after 50 ns of simulation. The plots below each panel show the variation of the distances between both partners over the trajectory.

Fig. 5. Snapshots showing the interaction of residues Y60 and H61 belonging to loop 2 in interaction with their lipid/glycolipid partner after 50 ns of simulation. The plots below each panel show the variation of the distances between both partners over the trajectory.

of the protein surrounded by membrane lipids. In lipid rafts, the confinement of a protein is expected to be stronger in the central area of the raft, where cholesterol and sphingolipids are densely packed, compared with the more flexible peripheral zones [54]. The mass centers for the backbone of the full protein, the backbone of loop 1, and the backbone of loop 2 in the POPC bilayer (black lines) and the lipid raft (orange lines) are plotted in Fig. 6. The data show that for the full protein, loop 1 or loop 2, the mass center of all these structures fluctuates less along the x, y, and z axis in the lipid raft environment. Our in silico data suggest that the different structures of the protein are more stable in the
presence of gangliosides and cholesterol molecules, which appear to be key membrane components with which PLLP can durably interact over time.

3.4 The Intracellular Flexible Loop of Plasmolipin Undergoes Long-Range Stabilization by the Lipid Raft

Next, we speculated if the lipid raft, which is a microdomain formed by the clustering of gangliosides and cholesterol in the upper leaflet of the plasma membrane, has an impact on the conformation of the intracellular loop of PLLP that we coined “loop 3” (Fig. 7). In any case, the only partners loop 3 has in both systems are POPC molecules. We performed the same measurements as for loops 1 and 2 to confirm. The histogram presented in Fig. 7 reveals that more H-bonds were formed between loop 3 and the POPC molecules in the lipid raft system. This result suggests that these bonds are more stable in the lipid raft environment, which may therefore help to stabilize the conformational landscape of loop 3. The plots presenting the variation of the center of mass of the backbone of loop 3 in both environments along the x, y, and z axis suggest that the conformation of loop 3 varies less along the y-axis with a standard deviation of 1.79 Å in the lipid raft system against 5.5 Å in the POPC bilayer. It varies slightly less along the z-axis with a standard deviation of 1.38 Å in the lipid raft system against 1.86 Å in the POPC bilayer. The difference in variation of both systems along the x-axis is unimportant, with a standard deviation of 2 Å in the lipid raft against 2.1 Å in the POPC bilayer. Together, our computational results suggest that the intracellular flexible loop of PLLP, which is not in direct contact with the lipid raft components of the exofacial membrane leaflet, is stabilized by the global lipid raft environment.

3.5 Interaction of a Cholesterol Molecule with Plasmolipin

Finally, we observed that one cholesterol molecule specifically interacts with PLLP. The interaction is depicted in Fig. 8, in which we present a snapshot of the cholesterol-PLL complex at 0, 15, 35, and 50 ns. First, the cholesterol molecule interacts with the residues F119, I120, and A125, which belong to the 3rd transmembrane domain (TM3), via van der Waals and CH-π interactions [55]. Throughout the simulation, the cholesterol molecule diffuses and adapts its conformation to the TM domains of PLLP to maintain its interactions with the residues identified above and to interact with the residues F145 and L149, which belong to the 4th...
Loop 3 (89 to 100) 89-FQLHMKLYMVPW-100

Fig. 7. 3D structure of plasmolin. Loops 1, 2, and 3 are highlighted in blue, purple, and red, respectively. The structure is accompanied by a histogram showing the total number of hydrogen bonds (H-bonds) formed between loop 3 and the lipids in the POPC bilayer (black bar) or loop 3 and the lipids in a lipid raft environment (orange bar). The colors for the variations of the mass center of the backbone are the same as in Fig. 6 (black lines for the POPC bilayer, orange lines for the lipid raft).

TM domain (TM4). This cholesterol molecule could serve as a bridge to maintain the transmembrane domains TM3 and TM4 at a closer distance. To evaluate, we measured and compared the distances between the center of mass of the backbone of these transmembrane domains in the POPC bilayer and lipid raft systems over the trajectory. The variation of the distance over time plotted in Fig. 8 reveals that the differences in the distance between the mass center of the backbone of the TM domains TM3 and TM4 in the POPC bilayer (black line) and lipid raft (orange line) are small but significant throughout the trajectory, with a mean value of 8.1 Å in POPC system against 7.9 Å in the lipid raft system (Fig. 8, histogram). This difference in cholesterol behavior suggests that the raft environment optimizes the interaction of cholesterol with the TM domains of PLLP. Thus, cholesterol is likely to play a role in the conformational changes initiated by the gangliosides on the extracellular loops of the proteolipid.

4. Discussion

PLLP is a special case of a membrane protein associated with rafts but has only a small surface area available to anchor itself to the lipids of these microdomains. PLLP is a major component of myelin [3]. It is also involved in signal transduction, which implies that its extracellular domains must be able to transmit conformational information to the other side of the plasma membrane. One of the major results of our study is the identification, in one of the two extracellular loops of PLLP, of a functional ganglioside binding motif displaying a high specificity for the ganglioside GM1. This motif corresponds to a canonical domain of interaction with gangliosides, which we identified 20 years ago [39]. It has long been part of the list of potential ganglioside-binding domains identified in brain proteins [56]. The experimental confirmation of this domain’s functionality is required to correlate its binding capacities to raft gangliosides with the 3D structure of the protein in its membrane environment. Our team’s recent progress in obtaining realistic 3D structures of membrane proteins in various plasma membrane environments has made the task possible, which motivated the present study [27,33,57–59].

The data obtained in silico showed that human PLLP interacts with ganglioside GM1 in a reconstituted lipid raft system. The interaction is driven by the two extracellular
loops of PLLP, which undergo a specific structuration that does not occur in a POPC system. The largest loop (loop 1) displays a canonical ganglioside binding motif whose ganglioside binding properties can be demonstrated with a synthetic peptide in a ganglioside monolayer assay. Considering the results, we can conclude that this ganglioside binding motif does not have a pre-established structure. On the contrary, this extracellular loop is globally disordered. In fact, it is precisely the role of gangliosides to induce the structuring of this loop when the protein reaches a raft. In this respect, our data may explain why knocked-out mice lacking major brain gangliosides show decreased central myelination and axonal degeneration [18]. The key point is that in the liquid disordered (Ld) phase of the plasma membrane containing chiefly glycerophospholipids such as POPC [51], the disordered loops of PLLP are not sufficiently constrained to acquire a functional fold. In contrast, the liquid-ordered (Lo) phase of lipid rafts contains gangliosides [51] which act as membrane chaperones for PLLP. In the raft environment, PLLP can acquire a functional structure that is mandatory for the biogenesis of the myelin sheath. Hence, the lack of gangliosides leads to a non-functional PLLP, explaining the demyelination phenotype of ganglioside-negative mice.

The localization of PLLP in lipid rafts is also consistent with its function in signal transduction [10]. Our in silico study suggests that the acquisition of a functional 3D structure of PLLP is first initiated by the binding of the proteolipid to raft gangliosides. Then, this conformational information propagates through the plasma membrane, relayed by a cholesterol molecule controlling the relative position of the TM domains. This conformational wave then reaches the intracellular (cytosolic) area of the protein, which reacts by adopting and stabilizing a particular structure.

The concept of conformational coupling across the plasma membrane during receptor activation has been recognized for a long time [60]. The propagation of a conformational wave has also been proposed to explain how the transient receptor potential vanilloid 1 (TRPV1) ion channel is activated by capsaicin [61]. The data presented here not only confirm the interest in these concepts but explains for the first time how raft lipids may control this complex mechanism. We suggest that a conformational wave rep-
Fig. 9. The conformational wave model. (a) When surrounded by phosphatidylcholine (POPC) molecules, plasmolipin oscillates between several conformers. The intracellular loop, which is not constrained by the plasma membrane, remains flexible. (b) The interaction of the extracellular loops with GM1 induces a conformational change in the extracellular domains of plasmolipin. This initial interaction triggers a conformational wave which is transmitted to the TM domains under the control of cholesterol and finally reaches the intracellular loop. (c) The conformational wave starts in the extracellular loops, propagates throughout the thickness of the plasma membrane, and ends in the intracellular loop. Overall, the stabilization of the extracellular loop by raft gangliosides induces the structuration of the intracellular loop through a ganglioside-cholesterol-dependent mechanism.

represents a long-distance transfer of energy throughout the thickness of the lipid bilayer. This could also be interpreted as a long-range chaperone effect. In the case of PLLP, the wave is triggered by the interaction of the extracellular loops with the headgroups of GM1 gangliosides in the raft environment (Fig. 9). Outside the raft, phosphatidylcholine molecules (POPC) have little impact on PLLP structure which has the freedom to oscillate between several closely related conformers (Fig. 9a). The structuring of the extracellular loops by raft gangliosides then generates coordinated movements of the TM helices (Fig. 9b). The role of cholesterol would be to control the conformational flexibility of the TM domains and determine their tilt during the energy transfer of the wave. In this model, the four-helix bundle structure of PLLP is optimally tuned by cholesterol, according to the conformational information transmitted by raft gangliosides. Overall, the conformational wave generated by the extracellular loops propagates through the TM domains and reaches the intracellular loop, which eventually acquires a stabilized structure (Fig. 9c). By clustering gangliosides and cholesterol, lipid rafts can be viewed as transducer platforms able to propagate such controlled conformational waves throughout the plasma membrane. The tail-to-tail organization of cholesterol molecules [62], which conformationally links the exofacial and intracellular leaflets of the plasma membrane through a mirror code, allowing the same TM domain to accommodate two cholesterol molecules in opposite orientations [63], seems particularly well adapted for propagating these conformational waves. Finally, it is important to note that in addition to the mechanical waves described here, this concept also includes the “swipe card” model based on an electron transfer wave across the TM domains of membrane receptors, as proposed for odorant recognition (see Figs. 2 and 3 in ref. [64]). In this respect, our model may reconcile two distinct scenarios of signal transduction: (i) the classic transmembrane conformational coupling [65], based on the complementary shape theory and thus viewed as a “mechanical” wave, and (ii) the more iconoclast “quantum” wave model that includes a molecular vibration frequency as additional information to explain how odorant molecules activate their receptors [64,66].

5. Conclusions

The chaperone effect of raft lipids in the 3D folding of membrane proteins is one of the fundamental elements of the theory we have recently developed and called the epigenetic dimension of protein structure [57]. The
epigenetic dimension of protein structure, which considers the chaperone-like conformational regulation of raft lipids (gangliosides and cholesterol), is a major limitation of the AlphaFold algorithm [58]. This concept applies both to membrane proteins such as PLLP and to extracellular proteins that trigger toxic effects in brain cells and cause neurodegenerative diseases such as Alzheimer’s disease [67] and Parkinson’s disease [68]. By understanding the molecular mechanisms that control the interaction of these proteins with rafts, we have already begun to design innovative therapeutic agents for the prevention and treatment of neurodegenerative diseases [69,70]. These new drugs are rationally designed to target raft gangliosides, which play a central role in the changes in raft homeostasis associated with several pathological conditions, including cancer, Alzheimer’s disease, and type 2 diabetes [54].

PLLP is among the potential targets of such therapeutic agents specifically designed to block the interaction of selected proteins with raft gangliosides. The involvement of PLLP in numerous pathologies (Alzheimer’s disease, type 2 diabetes, cancer metastasis, and viral infections) [2] makes this protein a new target of choice to improve our therapeutic arsenal against these diseases. Finally, the results of our study shed light on the molecular mechanisms associating the raft components with the physiological functions of PLLP, in particular signal transduction and myelin formation [2].

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

JF, HC, NY and CDS designed the research study. FA performed the molecular modeling studies. MM and CDS performed the monolayers studies. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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