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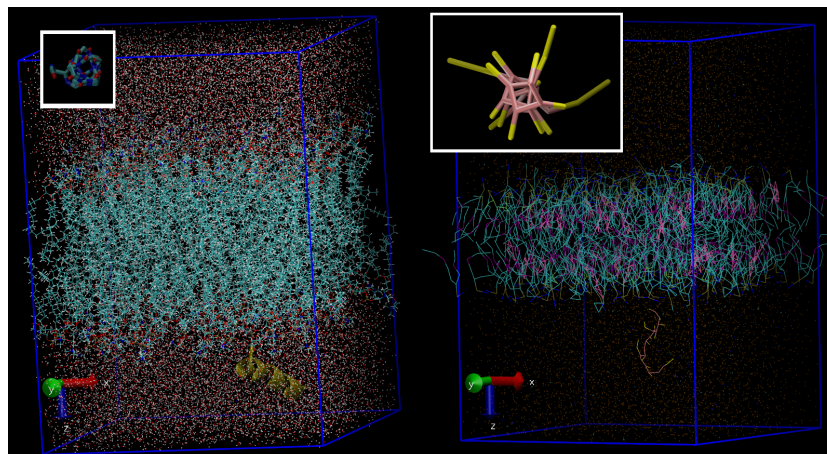
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Simulations Moléculaires des fissions membranaires médiées par la protéine BARS

Par: Thomas LE MÉTAYER



Soutenu à Rennes, le 11 Juin 2019

Devant le jury composé de : (Nom, Qualité)

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Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST

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Résumé long

Parce qu'elle permet la formation de transporteurs moléculaires entre les différents compartiments cellulaires, la fission membranaire est un des processus indispensables à tout évènement de trafic cellulaire. C'est particulièrement le cas au niveau de l'appareil de Golgi, qui constitue le centre majeur de transfert et de tri des protéines à l'échelle de la cellule. Cependant, les bicouches lipidiques qui composent les membranes sont des structures thermodynamiquement stables, et leur réarrangement implique de vaincre des états intermédiaires très énergétiques. Très souvent, cette énergie est fournie lors de l'hydrolyse de molécules de GTP par les protéines de la superfamille des Dynamines, mais il a été montré que la protéine CtBP1/BARS peut causer la fission de membranes indépendamment des Dynamines. Cette fission s'observe en présence de la protéine BARS entière et du domaine C-terminal seul, *in vivo* et dans des liposomes. Elle pourrait être causée, comme c'est le cas dans d'autres mécanismes de fission, par l'insertion dans les membranes d'une hélice α amphiphile de 16 acides aminés, dont la structure a été observée par Dichroïsme Circulaire par l'équipe du Dr. Luini (IBP, Italie). Nous nous proposons donc d'étudier par Simulation Moléculaire d'une part les propriétés structurales de ce segment protéique, et d'autre part ses interactions avec des modèles membranaires.

Tout d'abord, nous avons utilisé plusieurs outils de prédiction structurale à partir de la séquence protéique. Nous avons ensuite utilisé deux échelles complémentaires de modélisation pour les simulations : l'échelle atomistique permet une représentation plus précise des molécules et une structure secondaire flexible. Le gros-grain permet des simulations plus longues, car moins lourdes en calcul, mais les protéines ont une structure secondaire figée. Deux modèles de membranes ont également été considérés : un modèle homogène de bicouche de Phosphatidylcholine et un modèle complexe reproduisant la fraction lipidique des membranes golgiennes. Nous avons donc utilisé les simulations atomistiques pour observer la structure secondaire via l'algorithme DSSP, en partant d'un peptide en hélice α ou en pelote libre. Pour les simulations gros-grain, la structure secondaire en hélice a été imposée au peptide. Les simulations gros-grain ont permis d'observer les interactions entre les peptides et membranes sur des temps d'échantillonnage plus longs, et de quantifier si et comment les deux entités interagissent.

La prédiction de structure indique que le peptide séparé du reste du domaine C-terminal ne serait pas une hélice mais désordonné. De plus, ce fragment ne présenterait pas de comportement amphipathique marqué, c'est à dire une distinction claire entre face hydrophobe et face hydrophile. Les simulations atomistiques confirment cette prédiction car les fragments en hélice α au début de la simulation se déplient en structure désordonnée. On observe cependant bien un contact entre les peptides et membranes, et plus avec les membranes golgiennes que les membranes contenant uniquement des Phosphatidylcholines. Dans les simulations gros-grain, les peptides se collent aux membranes pendant toute la durée de la simulation. La quantification des contacts entre les acides aminés et les lipides ne montre pas de distinction entre une face avec beaucoup de contacts et une avec peu, ce qui indiquerait un comportement amphiphile. En revanche, on observe que les contacts se font principalement entre les acides aminés chargés positivement et les lipides au groupes de tête chargés négativement. On peut donc émettre l'hypothèse que les interactions entre ce fragment de BARS et les modèles de membranes golgiennes seraient électrostatiques.

Ces résultats et hypothèses, transmis à l'équipe du Dr. Luini, pourraient être vérifiées et affinées avec des simulations gros-grain plus longues, qui sont en cours de calcul. Il serait également intéressant de faire varier la concentration de peptide dans le système ou de réaliser ces observations sur le domaine C-terminal entier, afin d'observer comment certaines propriétés physiques des membranes varient. On pourrait par exemple mesurer la diffusion des lipides, l'aire par lipide ou l'épaisseur de la membrane qui sont trois indicateurs de réarrangement de membranes, ou encore les *compressibility modulus* et les profils de pression latérale, indicateurs de la courbure de la membrane nécessaire à l'évènement de fission.

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Molecular Simulations of membrane fission mediated by BARS protein

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Introduction

Membrane fission is a fundamental process in membrane transport, allowing the formation of vesicular or tubular carriers by budding and fission from donor compartment [1]. It is a ubiquitous process that results in the division of one continuous bilayer into two distinct membranes, proceeding through steps where the membrane warps and loses continuity [2]. However, because the whole mechanism is awfully thermodynamically unfavorable, it requires force generated by enzymes and structural cooperation between lipids and proteins to happen[2, 3].

Many fission events involve a wide range of structurally diverse GTPases forming the Dynamin superfamily [4]. However not all fission events are reliant on Dynamin: several viruses can enter mammalian cells by macropinocytosis, a Dynamin-independent pathway [5]. The transport of light Golgi-to-membrane exocytic vesicles in budding yeasts also appears unaffected by deletion of the only Dynamin-like protein they express [6].

The Carboxy-terminal Binding Protein / Brefeldin ADP-Ribosylated Substrate (CtBP/BARS) protein family was identified as controlling the fission in the Golgi-to-plasma-membrane transport pathway [7, 8], where Dynamin is not involved [9]. It is required for several intracellular membrane trafficking steps and the partitioning of the Golgi apparatus during mitosis [10] but its mechanism resmain uncertain [11, 12, 13].

The complete CtBP1 sequence (Uniprot accession number Q9Z2F5) weighs 50kDa and is 430 residues long. The protein includes a 10 *kDa* C-terminal sub-unit that has the same fission-inducing effect as the whole protein in liposomes [14]. This domain has a 16-residue helix that is predicted to be amphipathic. Its helical secondary structure was confirmed by circular dichroism experiments performed in the group of A. Luini (IBP, Italy) and the was only crystallized recently [15]. Insertion of amphipathic helices have been shown to promote membrane fission before [16] but it is not known whether it is the case for BARS.

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In this project, we use atomistic and coarse-grain Molecular Dynamics simulations to study the interactions of the above mentioned 16-residue peptide with lipid membranes. We show how the secondary structure of BARS protein varies over time and investigate if and how protein membrane interact. Several experiments are being carried out in parallel by A. Luini's team in order to study the structure and mechanisms of CtBP1/BARS.

Common Abbreviations

Membrane lipids:

DOPC = 1,2-dioleoyl-sn-glycero-3-phosphocholine

DOPE = 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine

DOPS = 1,2-dioleoyl-sn-glycero-3-phospho-L-serine

POPI = 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoinositol

PLPA = 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphate

PSM = Palmitoyl-sphingomyelin

CHOL = Cholesterol

Material and Methods

We use All-Atom and Coarse-Grained Molecular Dynamics simulations to inspect both the structure of the putative 16-residue amphipathic helix and its interactions with a membrane bilayer.

Secondary Structure prediction tools

We used several prediction tools available online to predict CtBP1/BARS' secondary structure : JPred4 [17] and HHPred [18], as well as the HELIQUEST tool [19] for predicting amphipathic-helices.

Simulation systems setup

Several tools were used for building simulation systems. 100x100 Å All-Atom lipid bilayers were designed using the online tool CHARMM-GUI [20] and are either pure POPC or a Golgi-like composition (Table 1) [21]. We equilibrated both alone in water + 0.15 mM NaCl to have a stable reference system.

Lipid	mol%
DOPC	49
DOPE	16
DOPS	5
PSM	6
CHOL	17
POPI	6
PLPA	1

Table 1: Golgi-like lipid bilayer composition

The All-Atom protein model for the R326-N341 sequence was built using the PyMol Builder tool [22] and forced into either a α -helix or random coil secondary structure. Termini were neutralized by addition of a N-terminal acetyl group and a C-terminal N-methyl group.

The Coarse-Grained membranes were built using the `insane` Python script [23] and the protein model using the `Martinize` Python script [24] to convert the All-Atom model to Coarse-Grain.

One problem we faced was that the GROMACS function `solvate` used to fill the simulation box with solvent molecules was unreliable. It inserted highly polar water molecules into the apolar bilayer core. To make minimization and equilibration steps faster and more stable, we used a Python script using the `gro.io` Python package to compute the z-coordinate of membrane leaflets, assign lipids into either leaflet, and remove any water located inbetween. Running the system through another energy minimization-equilibration cycle after the water was removed turned out to be sufficient to obtain stable initial systems for simulations.

All-Atom Molecular Dynamics parameters

All-atom force-field parameters

All-atom simulations were performed using the CHARMM-36 force field for lipid and protein molecules [25, 26] and the TIP3P model for water [27]. Hydrogen bonds were constrained using the LINCS algorithm [28]. The Particle Mesh Ewald algorithm [29] was used to handle electrostatics with a 10 Å cutoff for the short-range part and a grid spacing of 1.2 Å for the long-range contribution in reciprocal space. The Verlet buffer scheme [30] was used for non-bonded interactions, the neighbor list was updated every 20 steps. Two baths (protein and lipids, water and ions) were coupled to a temperature of 300 K using the Bussi Velocity-rescaling thermostat [31] with a time constant $\tau = 1$ ps. Pressure in the x/y dimensions was scaled isotropically with a Parrinello-Rahman barostat [32] and coupled to a reference pressure of 1 bar, while pressure in the z dimension was coupled independently to a reference pressure of 1 bar. For all dimensions, the same pressure coupling parameters were used: $\tau = 10$ ps and compressibility of $4.5 \cdot 10^{-5}$ bar⁻¹.

All-atom integration parameters

The GROMACS 2016.4 software [33] was used to run the simulations using a 2 fs integration time step. All systems were minimized until $F_{max} < 10^{-3}$ kJ.mol⁻¹.nm⁻¹ with a steepest descent algorithm and equilibrated for 1 ns. All-atom production runs lasted 1 μ s where coordinates and velocities were written every 100 ps for a total of 10,000 frames. Production runs were run twice with a different seed for velocity generation and a slightly different starting state, making a total of eight runs to enhance statistical sampling

All-Atom trajectory analysis

All eight 1 μ s trajectories were collected. They were fitted onto the first one frame from each trajectory to ensure that the membrane is in the middle of the simulation box. We then ran the XSSP version [34] of the DSSP algorithm for assigning secondary structure [35] and plotted the results using Matplotlib [36] and the GROMACS `xpm2ps` function.

Coarse-Grained Molecular Dynamics parameters

The peptide has been confirmed by circular dichroism to be largely helical. Therefore we decided to carry out Coarse-Grain Molecular Dynamics simulations assuming a perfect α -helical structure to explore the interactions of this putative helix with model lipid membranes.

Although the principle for these is the same as before, there are some differences to note.

Coarse-Grain force-field and integration parameters

Simulations were performed using the MARTINI force field for lipid [24] and protein molecules [37] and the 4-to-1 definition of CG water. We used a 20 *fs* integration time step and switched to Reaction-field electrostatics [38] with a 1.1 *nm* Verlet Potential Shift for short range interactions [39]. Temperature and pressure coupling used the same thermostats and semiisotropic barostats as the All-atom systems.

Coarse-grained production runs first lasted 5 μ s where coordinates and velocities were written every 10 *ns* for a total of 500 frames. We then extended runs to 50 μ s but these simulations are not finished yet.

Coarse-Grain trajectory analysis

The GROMACS function `mindist` allowed us to monitor the system behavior by measuring the minimum distance between peptides and membrane. It also counts contacts between the protein and membrane to assess whether the helix exhibits amphipathicity.

Results

Coarse-Grain simulations analysis

Interesting properties to observe on the Coarse-Grain simulations are the localization of the peptide and which residues from the protein interact with which lipids from the membrane.

BARS helix docks to both membrane models

The system is built with the protein floating in water 1 *nm* away from the membrane. We can see in Figure 1 that it tends to stick to both membranes for the whole simulation. The average minimum distance computed with the GROMACS `mindist` function over the run is very close in both systems and close to the expected value of twice the Van der Waals radius of 0.235 *nm* used in the MARTINI force-field [24].

Electrostatic forces drive helix-membrane interaction

Using the GROMACS `mindist` function with a radius of 8Å, we plotted the number of contacts between the protein and the membrane, and between each amino acid residue and each lipid type in the membrane. We also computed the distance between Centers of Mass of the protein and membrane *z*-coordinate using the GROMACS `traj` function.

We find that some residues in the peptide have more contacts with the membrane than others (Figure 2.A). When applying the average number of contact for each amino acid onto the putative secondary structure of the α -helix [19], we find there is no obvious repartition of contacts on one side of the helix that would be lipophilic (Figure 2.B).

When plotting contacts between the protein and each lipid type, we can see that the protein interacts almost exclusively with POPI and DOPC molecules (Figure 3.A). These interactions can be explained by the fact that POPI has a negatively charged headgroup and DOPC is the most common in the membrane composition. We then plotted contacts between each residue and both lipid types (Figure 3.B & 3.C).

There does not appear to be any favored side of contact with DOPC molecules, probably because of how overwhelming the molecule is in the membrane composition (49%, Table 1). However, the amino acids that interact more with POPI are Arginine and Lysine which are both positively charged at pH = 7.

Finally to inspect which parts of the lipids interact with positive residues, we measured contacts between positive residues and each bead in the head of DOPC and POPI. Positive residues interact more with the choline than the phosphate of DOPC (Figure 4.A & 4.B), probably because cholines are the most protruding groups. However, we observe in Figures 4.C & 4.D that this is not the case for POPI. Positive residues actually have more contacts with the C1 inositol bead although it is not the most protruding one. Its number of contacts with PO4 is also on average equal to C2 & C3 beads, which are the most protruding.

Preferential contacts between negative headgroups and positive residues suggest that the interaction between the peptide and membranes may be driven by electrostatic forces.

All-Atom secondary structure analysis

Structure prediction tools

Figures 5.A & 5.B show that both tools predict the 16-residue sequence we are interested in to be mostly random coil. HELIQUEST predicts an average mean hydrophobicity ($\langle H \rangle = 0.271$ on a scale from -1.01 to 2.25 [40]) with a low mean helical hydrophobic moment ($\langle \mu H \rangle = 0.569$ on a scale from 0 to 3.26 [41]). These values do not allow us to place the protein onto the graph in Figure 5.D, which could have been another prediction of the peptide's amphipathicity.

These values mean that the 16-residue sequence has both hydrophobic and hydrophilic residues but that the helix has a low amphipathicity because there is no hydrophobic and hydrophilic face.

However, the secondary structure prediction tools are designed for and most accurate on soluble proteins and they should not be considered very reliable on membrane proteins, including peripheral ones.

All-atom simulations also exhibit electrostatic attraction

When plotting the Minimum distance between peptides and membranes in both 1 μ s All-atom runs, we can see that the peptide tends to spend more time in contact with the Golgi membranes (Figure 6). This could be explained by the fact that the protein has an overall charge of +2. In POPC membranes, all lipid headgroups are zwitterionic phosphatidylcholines while Golgi

membranes are comparatively more negatively charged, and is coherent with Coarse-grain observations.

Secondary structure of the peptide is not stable in water

Considering the uncertainty of secondary structure predictions, we decided to use All-atom simulations since they have already been used to predict secondary structure [42]. For both 1 μ s atomistic runs we plotted the DSSP results over time. Figures 7.A & 7.B show the ratio of amino acids from the peptide in α -helix conformation.

Peptides that are initially helical start unfolding during equilibration and tend towards a random coil conformation as the run progresses. There seems to be a recurring value of 0.3, meaning 5 amino acids assigned as α -helix. To know whether this is a full helix turn or disconnected bends, we plotted the secondary structure of each individual amino acid over time for the folded peptide simulations. Figures 7.C to 7.F show that residues from I7 to K12 are the ones which tend to be in the α -helical conformation. These residues are also the ones that had the most contacts with membranes in the CG simulations in Figure 2, meaning they could either be stabilized into helix by membrane contact or their helical conformation allows them to interact the membrane more.

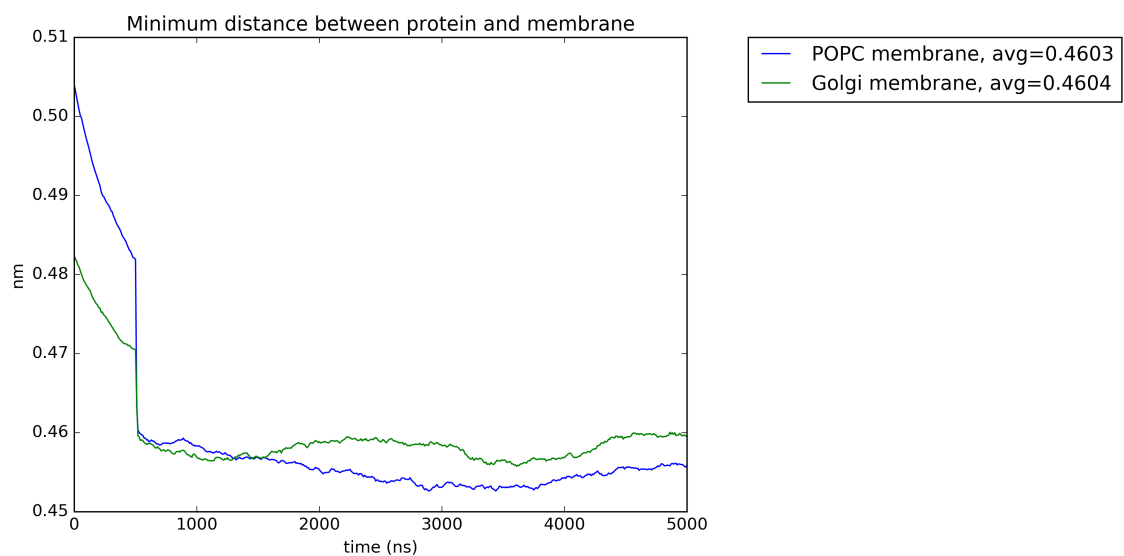


Figure 1: **Minimum distance between protein and membranes in both Coarse-Grain simulations.**

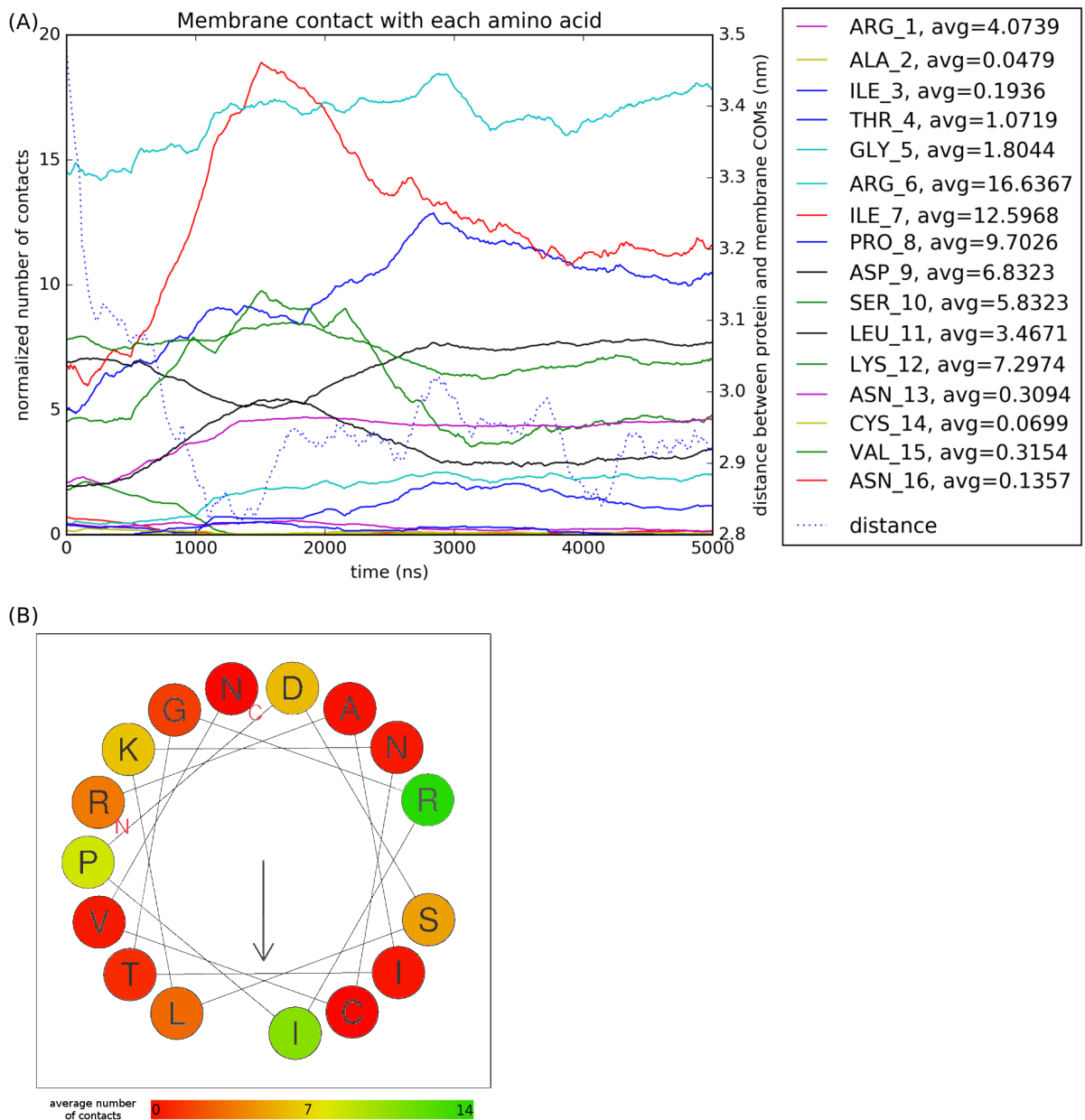


Figure 2: Contacts between the membrane and protein residues in Coarse-Grain models. (A) Contacts $< 8 \text{ \AA}$ between each residue and the membrane. (B) Average number of contacts of the putative 16-residue α -helix (adapted from [19]). The arrow indicates the predicted hydrophobic face.

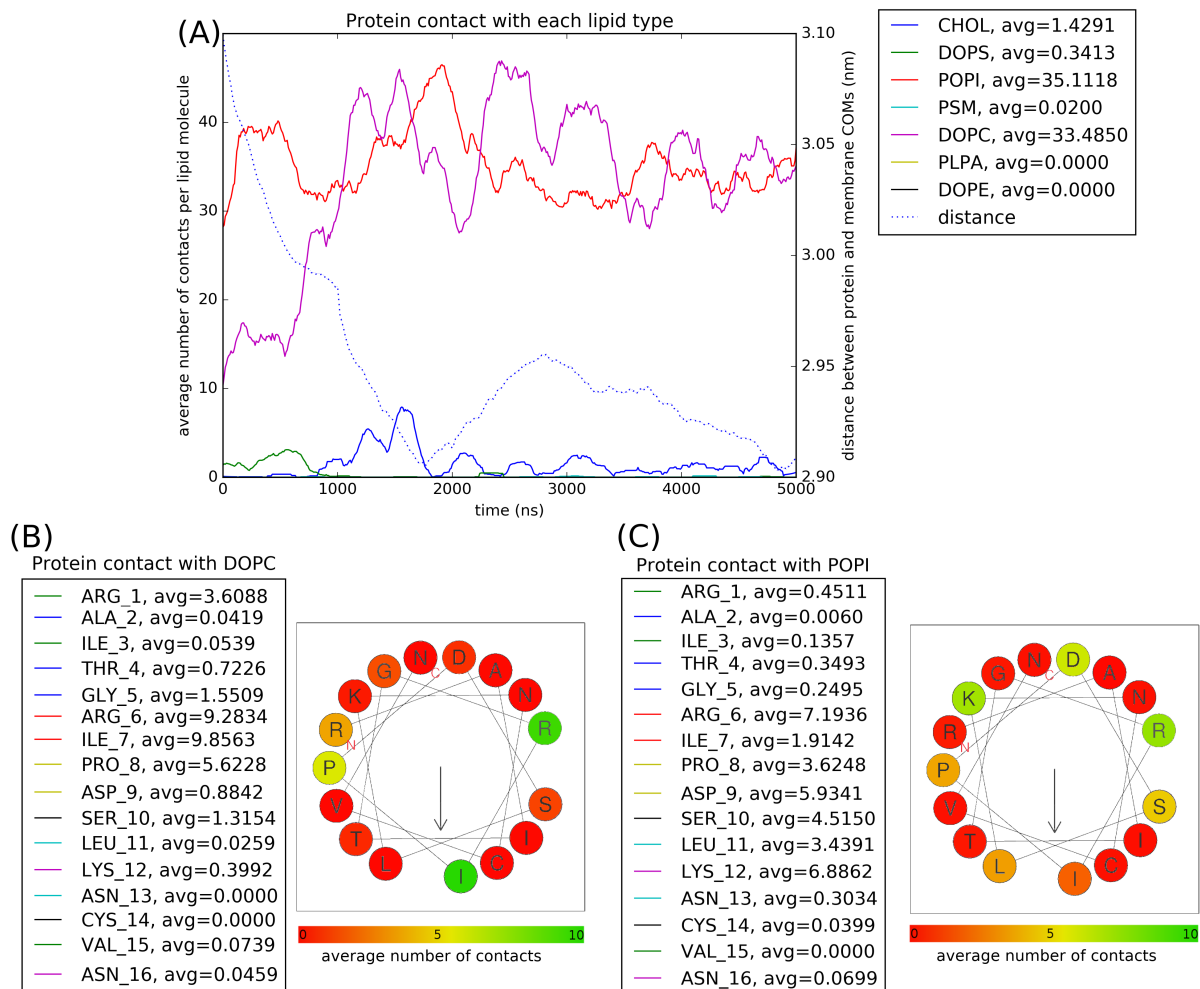


Figure 3: Contacts between the peptide and lipid types in Coarse-Grain models. (A) Contacts $< 8 \text{ \AA}$ between each residue and the membrane. (B) Average number of contacts of the putative 16-residue α -helix with DOPC. (C) Average number of contacts of the putative 16-residue α -helix with POPI.

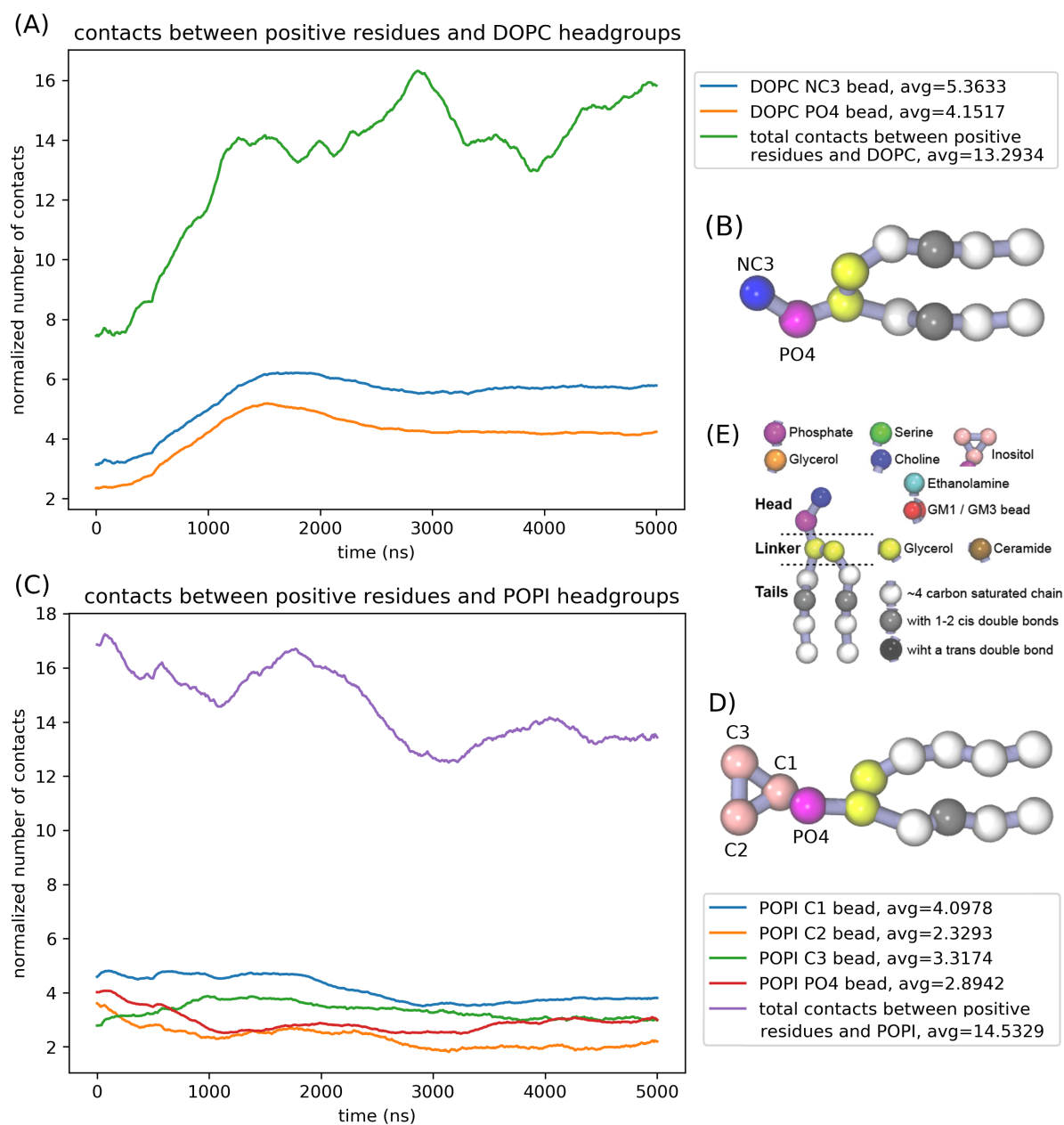
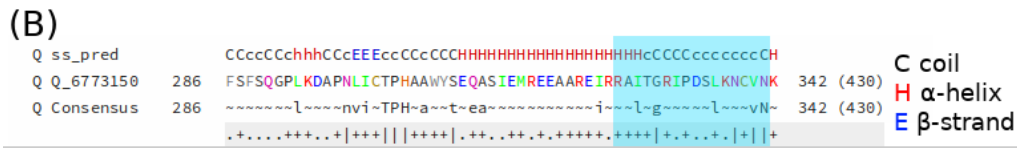
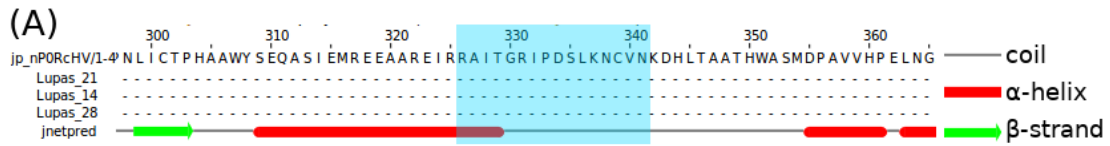


Figure 4: **Preferential contacts between positive residues and lipids in Coarse-Grain models.** (A) Contacts $< 8 \text{ \AA}$ between DOPC headgroup beads and positive residues. (B) MARTINI mapping of DOPC molecules. (C) Contacts $< 8 \text{ \AA}$ between POPI headgroup beads and positive residues. (D) MARTINI mapping of POPI molecules. (E) MARTINI bead types (adapted from the MARTINI website).



(C)

1RAITGRIPDSLKNCV16

Physico-chemical properties	Polar residues + GLY	Nonpolar residues
Hydrophobicity $\langle H \rangle$ 0.271	Polar residues + GLY (n / %) 9 / 56.25	Nonpolar residues (n / %) 7 / 43.75
Hydrophobic moment $\langle \mu H \rangle$ 0.569	Uncharged residues + GLY SER 1, THR 1, ASN 2, GLY 1	Aromatic residues
Net charge z 2	Charged residues LYS 1, ARG 2, ASP 1	Special residues CYS 1, PRO 1
	Hydrophobic face : none	

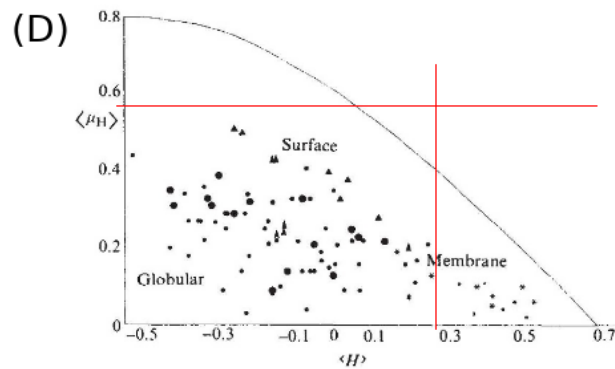


Figure 5: **Algorithmic prediction of the peptide's properties.** (A,B) Secondary Structure prediction plots for CtB1/BARS using Jpred4 or HHPred, resp. The blue window indicates the putative 16-residue helix. Capitalization in (B) is proportional to amino acid volume. (C) HELIQUEST prediction of amphipathicity. (D) Placement of the 16-residue peptide onto an empiric clustering plot of proteins as a function of $\langle H \rangle$ and $\langle \mu H \rangle$ (adapted from [41]).

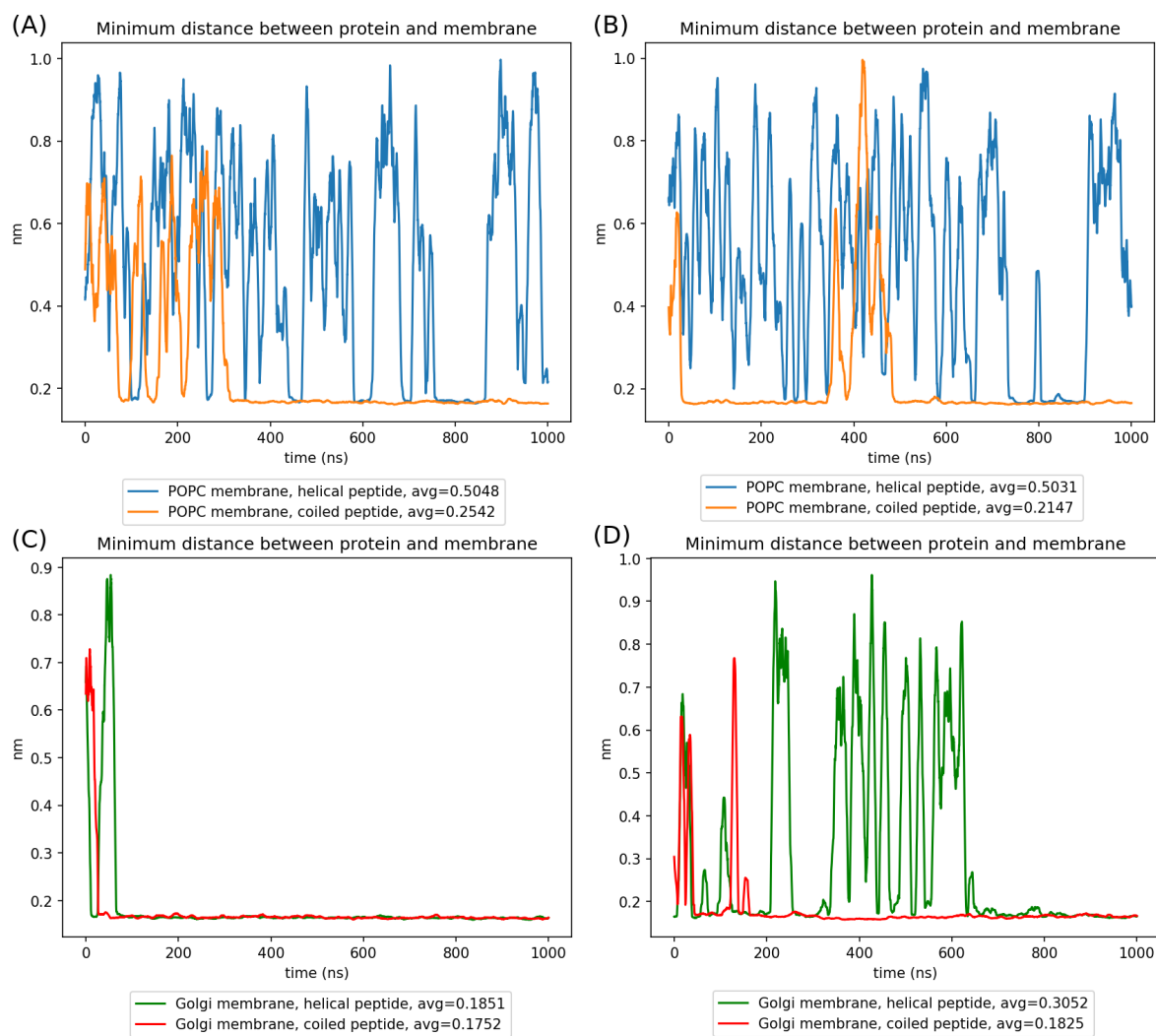


Figure 6: Minimum distance between peptides and membranes in both 1 μ s All-atom runs (left vs. right). (A,B) Pure POPC membranes. (C,D) Golgi-like membranes.

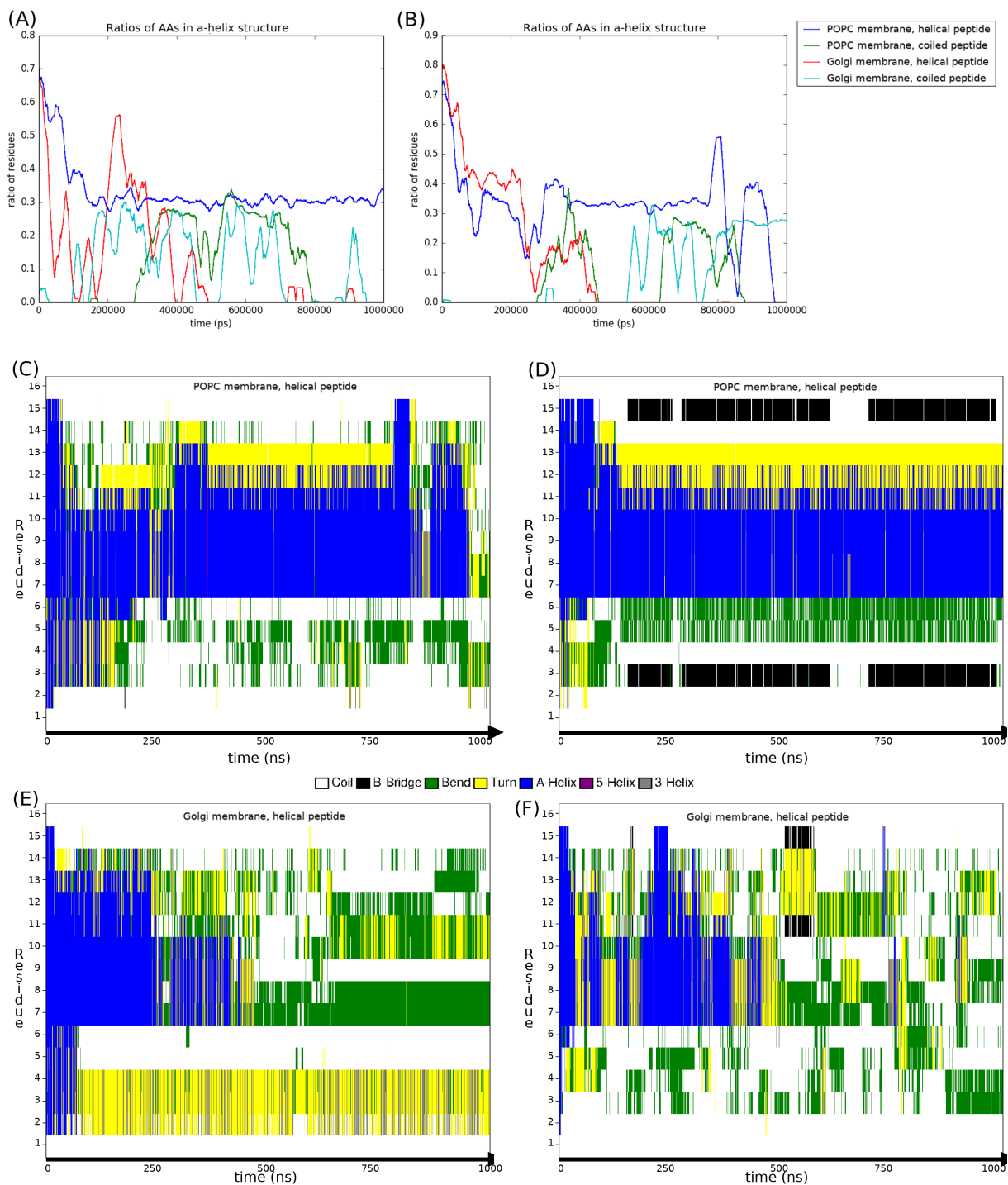


Figure 7: Assignment of secondary structure by the DSSP algorithm over the two 1 μ s All-Atom runs (left vs. right). (A,B) Ratio of residues in the peptide assigned as α -helix. (C,D) DSSP plot for each residue in the POPC membrane-helical peptide runs. (E,F) DSSP plot for each residue in the Golgi membrane-helical peptide runs.

Discussion

Molecular Dynamics simulations validate the hypothesis that the peptide and the membrane interact. The study of contacts between amino acids and lipids suggests that the interaction might be driven by electrostatic forces between negatively charged lipids and basic residues bearing a positive charge at $\text{pH} = 7$. From predictive tools and the study of secondary structure in All-Atom simulations, we cannot confirm that the 16-residue sequence is helical when separated from the rest of the protein.

There are more physical properties of membranes we could study on longer simulations and with increasing peptide concentration : lipid diffusion, area per lipid and membrane thickness can all indicate conducting to the rearrangement of lipids. Compressibility modulus and lateral pressure profiles are also useful for measuring membrane curvature [43], another mandatory step for membrane fission. All of these measurements have been planned and will be carried out in the future, using the present work as a starting point.

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This report will be shared with Dr. Luini's team at the Institute of Protein Biochemistry in Naples, Italy.

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Many thanks to Luca for taking the time and trusting me to work on this project. It has been a really enriching experience and I met a lot of great people at IBCP. I'll really miss coffee and lunch with the team!

I would also like to thank my teachers Jean-Marc and Sandrine for always being available for us students.

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Titre français : Simulations Moléculaires des fissions membranaires médiées par la protéine BARS Titre anglais : Molecular Simulations of membrane fission mediated by BARS protein	
<p>Résumé : Il a été montré que la protéine CtBP1/BARS cause la fission de membranes golgiennes et de liposomes sans consommation d'ATP. Dans ce mémoire, nous analysons comment un fragment de 16 résidus supposément α-hélicoïdal issu du domaine C-terminal de BARS interagit avec les membranes en utilisant la simulation moléculaire.</p> <p>Pour observer ces interactions, nous avons utilisé des simulations de Dynamique Moléculaire à échelle atomistique et gros-grain. Plusieurs modèles membranaires variant de simples bicouches lipidiques homogènes à des modèles simulant la composition des membranes golgiennes ont été considérés et confrontés à des modèles du fragment de BARS dans adoptant différentes structures secondaires.</p> <p>Nos résultats montrent que la structure du fragment protéique varie fréquemment. Ce fragment interagit avec les modèles membranaires en se plaquant à leur surface via des interactions électrostatiques entre les résidus positifs et les lipides membranaires négatifs. Il n'est pas possible de confirmer que sa structure ou ses propriétés lorsqu'il est séparé du reste de la protéine sont celles observées <i>in vivo</i> et <i>in vitro</i>.</p> <p>Produire plus de simulations avec de plus grands domaines de la protéine ou avec des concentrations croissantes du peptide pourrait permettre d'observer plus en détail comment les propriétés physiques de la membrane sont influencées par la protéine et aider à comprendre le mécanisme de fission engendré par BARS.</p>	
<p>Abstract : The CtBP1/BARS protein has been shown to cause ATP-hydrolysis-independent membrane fission both in Golgi membranes and liposomes. This memoir analyzes how one putative amphipathic 16-residue α-helical fragment from the C-terminal domain of BARS interacts with membranes using <i>in silico</i> Molecular Simulations.</p> <p>To observe these interactions, we used Molecular Dynamics simulations at atomistic and coarse-grained scales. We used several membrane models ranging from very simple homogeneous lipid bilayers to ones mimicking the composition of the Golgi membranes. These membranes were confronted to several models of the protein fragment varying in secondary structure.</p> <p>Our results show that the protein fragment undergoes a lot of structural change. It interacts with model membranes by sticking to them, mostly via electrostatic interactions between positive residues and negative lipids. We cannot confirm that its structure or properties when detached from the rest of the protein are the same as observed <i>in vivo</i> or <i>in vitro</i>.</p> <p>Running more simulations with bigger domains of the protein or with an increasing concentration of the peptide in the future could provide more insight on how physical properties of membranes are influenced by the protein and help unveil the mechanism behind membrane fission.</p>	
Mots-clés : Molecular Dynamics, fission membranaire, interactions proteines-membranes Key Words: Molecular Dynamics, membrane fission, protein-membrane interactions	

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