

***In silico* study of the interaction of the Myelin Basic Protein C-terminal α -helical peptide with DMPC and mixed DMPC/DMPE lipid bilayers**

Kyrylo Bessonov ¹

University of Guelph, 50 Stone Road East, Guelph, Canada

ABSTRACT

Biological membranes continue to be extensively investigated in different ways. This paper presents the benefits of Molecular Dynamics (MD) approaches to study the properties of biological membranes and proteins using the freely available GROMACS package, in the context of the Myelin Basic Protein (MBP) C-terminal α -helical peptide. A mixed membrane consisting of 2-Dimyristoyl-*sn*-Glycero-3-phosphocholine/1,2-Dimyristoyl-*sn*-Glycero-3-phosphoethanolamine (DMPC/DMPE), and pure DMPC membranes, composed of 188 and 248 lipids, respectively, were simulated for 200 ns at 309 K. The DMPC membrane was approximately three times more fluid compared to the DMPC/DMPE system, with the diffusion coefficients (D) being 0.0207×10^{-5} cm²/s and 0.0068×10^{-5} cm²/s, respectively. In addition, the 14-residue peptide representing the C-terminal α -helical region of murine Myelin Basic Protein (MBP), with amino acid sequence ^{NH}₂-A₁₄₁YDAQGTLISKIFKL₁₅₄-^{COOH}, was simulated in both membrane systems for 200 ns. The peptide penetrated further into the DMPC bilayer compared to the mixed DMPC/DMPE bilayer, potentially because of the reduced accessibility of the charged peptide amino acid side chains to the formal positive charge of the amine N atom surrounded by methyl and methylene groups in DMPC, that might have resulted in greater overall peptide mobility [3]. These findings are significant in their implication that membrane composition affects the behavior of MBP, providing further insights into myelin structure. Our preliminary results suggest that local changes in membrane composition (e.g. enrichment in DMPE molecules), as well as, electrostatic nature of primary amino acid sequence could cause localized denaturation / instability of external MBP α -helices possibly augmenting the degradation of myelin in multiple sclerosis (MS), resulting in a subsequent decrease of nerve impulse propagation efficiency.

Keywords: myelin, MBP, GROMACS, Multiple Sclerosis, DMPC, DMPE

1 INTRODUCTION

Myelin Basic Protein (MBP) is an important protein in the central nervous system. The protein is found in various isoforms with a predominant splice isoform of 18.5 kDa in an adult brain. The main physiological role of MBP is to maintain the myelin sheath that wraps around neurons by holding together both cytoplasmic sides of oligodendrocyte membranes, thus facilitating the compaction of the myelin sheath and allowing efficient signal propagation [7].

Recent studies have demonstrated that the severity of MS is correlated with post-translational modifications of MBP, such as citrullination [8]. Due to its central role, MBP is thought to be connected with myelin degradation. MS attacks the myelin-wrapped nerves of the central nervous system. Molecular Dynamics (MD) provides a nice, quick way to study the behavior and interaction patterns of MBP with lipid membranes that could provide insights into molecular details of myelin structure, and pathogenic mechanisms in MS.

The main focus of this article is to provide both a practical and methodological approach to MD using GROMACS [10], as well as to introduce possible applications of such simulations to real biological problems. The supplementary website provides additional information, key files and additional programs that facilitate the setup of MD simulations. Here, the simulations of DMPC and mixed DMPC/DMPE (1:1 ratio) membranes and MBP C-terminal peptide were performed on SHARCNET cluster revealing importance of membrane composition on MBP behavior useful to further knowledge on MS pathogenesis.

¹ Corresponding author. E-mail: kbessonov@uoguelph.ca Website: <http://www.uoguelph.ca/~kbessonov/main.html>

2 METHODS

2.1 Purpose

To investigate how both DMPC and DMPC/DMPE membranes affect the behavior of a 14-residue peptide representing one of three α -helical regions of the classical murine Myelin Basic Protein (MBP). The sequence $^{\text{NH}}_2\text{-A}_{141}\text{YDAQGTLISKIFKL}_{154}\text{-COOH}$ was modeled as 14-residue α -helical peptide using Molecular Operations Environment 2008 (MOE, Chemical Computing Group, Montreal).

2.2 Preparation of DMPC and mixed DMPC/DMPE membranes for MD simulation

The peptide was carefully positioned slightly above the lipid bilayer using both Visual Molecular Dynamics (VMD) and self-written *gro_mover* programs. DMPC and DMPC/DMPE membranes were neutralized with Na^+ and Cl^- ions inserted into the aqueous layer using *genion* and giving an overall system charge of zero, and an overall system pH of 7.0. To prevent overlap between atoms and increase stability of the system, energy minimization (EM) using the steepest descents method was done for both membranes. EM finds the system local potential energy minimum by using a specified force field. EM is usually required to be done before any MD run, because the solvation of lipid membranes in water usually introduces some bad contacts/atom clashes that need to be relaxed before being given kinetic energy (i.e., MD). The assembled membrane systems were simulated on the SHARCNETTM computer cluster using 96 processors for a total trajectory time of 200ns.

2.3 The $^{\text{NH}}_2\text{-A}_{141}\text{YDAQGTLISKIFKL}_{154}\text{-COOH}$ properties

The peptide had an overall +1 charge and displayed 38% hydrophilicity based on its primary sequence (Fig. 1). The negatively-charged aspartic acid (D) residue confers -1 charge to peptide's N-terminus, while two lysine (K) residues ensure a +2 charge at the C-terminal end. The overall peptide pI is 9.6

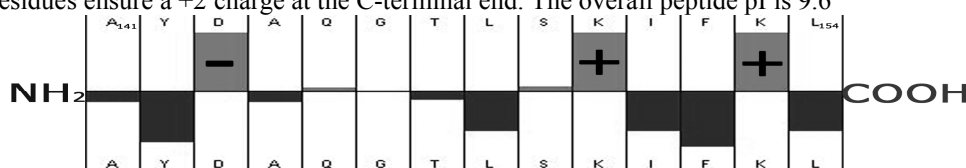


Figure 1: Hydrophilicity plot of the $^{\text{NH}}_2\text{-A}_{141}\text{YDAQGTLISKIFKL}_{154}\text{-COOH}$ showing N- and C-termini. The Y-axis represents the Hopp-Woods hydrophilicity scale while + and - signs refer to amino acid R-side chain charge [11].

3 RESULTS and DISCUSSION

3.1 Measuring DMPC and DMPC/DMPE lipid bilayer parameters

Membrane dynamics simulations provide a powerful means for studying how temperature, protein, cholesterol content, and numerous other parameters affect membrane characteristics such as fluidity and lipid velocity.

The use of computer clusters through SHARCNETTM significantly diminished the total trajectory computation time. The assembled DMPC and DMPC/DMPE systems were simulated for 200 ns. The obtained DMPC and DMPC/DMPE trajectory files were analyzed against diffusion coefficients, total kinetic energies, pressure, temperature, and other parameters (see **Table 1**) using *g_energy* [10] and an *InflateGro* Perl script [4]. Other parameters, such as solvent accessibility, were not successfully measured due to *g_sas*'s difficulty [10] in recognition of the hydrophobic parts of the DMPC molecule.

Table 1. Summary of measured parameters for DMPC and DMPC/DMPE membrane systems used in MD simulations.

Membrane characteristic	DMPC	DMPC/DMPE
Total number of atoms	62,613	30,416
Total number of lipid molecules	248	94/94 (188)
Diffusion coefficient (D) 10 ⁻⁵ cm ² /s	0.0207	0.0068
Kinetic Energy (J/mol)	161,870	78,602
Total Energy (J/mol)	-891,474	-477,439
Heat Capacity Cv (J/mol*K)	12.4721	12.4724
Temperature (K)	309	309
Pressure (bar)	1.66	1.097
Average Area per lipid (Å ² /lipid)	67.26	55.24
Membrane Thickness (Å)	33.9 – 35.72	35.8-38

Diffusion coefficients (D) describe the mobility of the molecules. The higher D values are indicative of greater mobility. The simulation results (**Table 1**) indicate that a DMPC bilayer has three times greater fluidity as compared to the mixed DMPC/DMPE bilayer at 309 K, as suggested by the diffusion coefficients (D) of $0.0207 \times 10^{-5} \text{ cm}^2/\text{s}$ and $0.0068 \times 10^{-5} \text{ cm}^2/\text{s}$, respectively. The difference in fluidity between both membranes could be partially explained by the difference in the density of lipid packing. The DMPC membrane was found to be more loosely packed as compared to the DMPC/DMPE membrane system, with average areas per lipid of 67 \AA^2 and 55 \AA^2 , respectively. It is expected that membranes with a higher density of lipid packing will restrict movement of freely diffusible molecules such as peptides.

Membrane thickness was calculated by labeling phosphate atoms of lipid molecules on opposite sides of the bilayer with the help of VMD 1.8.6 software. The thickness did not change significantly during simulation, indicating stability of the membrane. Accurate membrane thickness determination was hindered by constant random lipid motion in both bilayers (data not shown).

3.2 Simulation of 14-residue MBP C-terminal peptide [$\text{NH}_2\text{-A}_{141}\text{YDAQGTLISKIFKL}_{154}\text{-COOH}$]

In addition to bare membrane simulations, the simulation of the 14-residue long MBP C-terminal peptide in DMPC and mixed DMPC/DMPE bilayers was done for the first time. Interesting trends of the two systems related to membrane composition and protein secondary structure preservation were observed over the course of the 200 ns simulation. The depth of penetration and α -helical structure stability were successfully measured (data not presented here). Overall, the peptide penetrated further into the DMPC membrane as compared to the mixed DMPC/DMPE membrane. Helical secondary structure retention was stronger in the DMPC bilayer system (**Fig. 2**). This might be due to DMPC and DMPE N atom formal positive charge distribution and accessibility differences as explained in Section 3.3. Thus, the electrostatic environment of the two membranes might partially dictate stability of the peptide amongst other factors (i.e., localized pH, lipid-peptide thermodynamics, lipid density) [12].

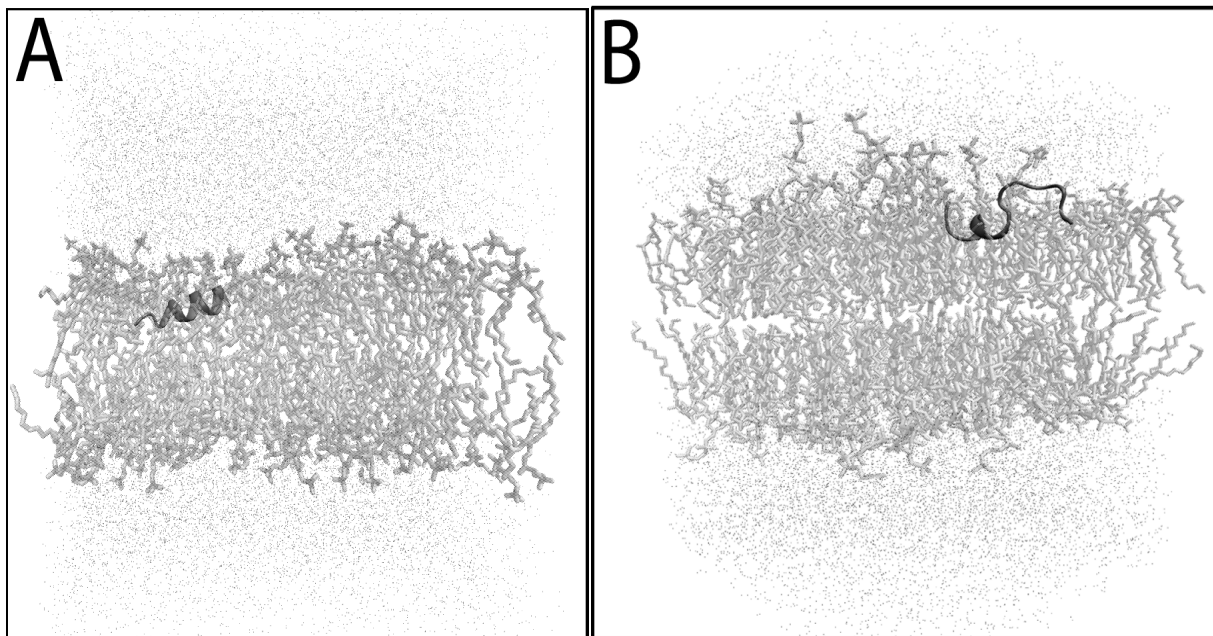


Figure 2: **A)** DMPC lipid bilayer showing 14-residue C-terminal MBP peptide after an 80 ns simulation. Note that the N-terminus of the peptide has deeply penetrated at least halfway into the leaflet. The helical structure of the peptide is preserved, compared to the initial state; **B)** DMPC/DMPE mixed bilayer showing 14-residue C-terminal MBP peptide. Observe that the helical structure has been greatly compromised after 80 ns. The peptide penetrated the DMPC/DMPE bilayer less significantly deeper compared to the DMPC bilayer possibly due to electrostatic interactions and a decrease in steric hindrance (see **Fig. 2A**).

3.3 Membrane penetration differences by MBP C-terminal peptide – Hypothesis

Synthetic DMPC and DMPE molecules represented phosphocholine (PC) and phosphoethanolamine (PE). Choline has three CH_3 groups attached to an N atom, while ethanolamine has three H atoms (see **Fig. 3**). Both choline and ethanolamine have the same formal charge of +1, but behave differently [3]. Nevertheless, substitution of ethanolamine for choline in the bacterial cell wall significantly alters important biological functions, such as cellular adhesion and bacterial transformation [9]. Zull and Hopfinger [3] measured the

accessibility of a negative test charge to a positively-charged N atom, concluding that ethanolamine interacts more strongly with anions. Even though choline lipids have a three times stronger partial positive charge on an N atom, the positive formal charge is sterically poorly accessible, which could result in poorer interactions with anions. The positive charge of ethanolamine on an N atom is more diffused and more accessible [3]. The negative N-terminus of the peptide could be thought of as a negative charge. Indeed, our results indicate that the peptide interacted more strongly with DMPE lipids in the mixed DMPC/DMPE membrane, as compared to the pure DMPC membrane, probably due to differences in the N positive charge accessibility. Thus, the negatively-charged N-terminus of the peptide stayed attached to the surface of DMPC/DMPE membrane, not being able to penetrate the membrane further. The opposite was observed in simulations with a DMPC bilayer. The partially shielded positive N charge of the DMPC bilayer was not as effective in capturing the negative N-terminus of the peptide, resulting in deeper penetration into the bilayer.

3.4 Comparison of our results to external evidence – Significance

The above results highlight the importance of the membrane composition, in conjunction with an array of other membrane properties, on final protein structural stability and behavior that is ultimately reflected in its biological function. These findings further support evidence from other studies that protein-membrane interactions and α -helical protein stability are governed by combination of factors including: hydrogen bonds, ion pairs, favorable surface van der Waals interactions, and thermodynamic parameters [12]. We were able to confirm that the interactions of the individual amino acids of a peptide with each other and with the surrounding medium (e.g., membrane lipids and polar water) determine the peptide's final structure, stability, and interaction behavior [13].

4 CONCLUSIONS

This study shows the usefulness of computational MD approaches in studying the conformations of biological membranes, particularly the effects of various factors affecting their fluidity and protein stability. The DMPC membrane showed a greater degree of fluidity at 309K compared to the mixed DMPC/DMPE membrane, with diffusion coefficients of $0.0207 \times 10^{-5} \text{ cm}^2/\text{s}$ and $0.0068 \times 10^{-5} \text{ cm}^2/\text{s}$. The DMPC membrane was more strongly penetrated by a 14-residue α -helical MBP C-terminal peptide compared to the mixed DMPC/DMPE bilayer. The peptide showed a greater stability retaining more of its α -helical structure in the DMPC membrane system compared to the mixed DMPC/DMPE one. These findings highlight possible dependence of MBP structure on membrane and sequence compositions, providing further insights into myelin structure. Enrichment of in DMPE molecules caused localized denaturation / instability of MBP C-terminal peptide α -helix. This finding suggests localized denaturation of solvent accessible MBP α -helices could possibly augment the degradation of myelin in MS, resulting eventually in subsequent decrease of nerve impulse propagation efficiency.

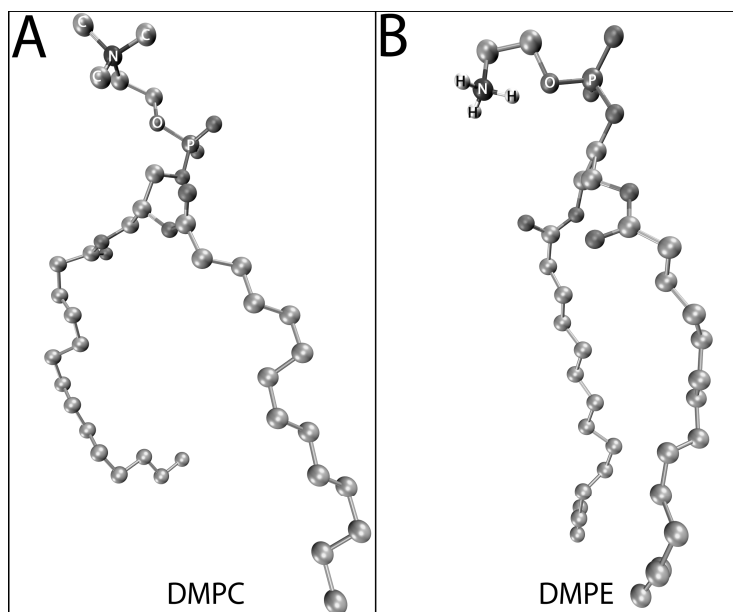


Figure 3: A) DMPC to B) DMPE lipid structural comparison explaining differences in accessibility to formal positive charge of the N atom. Some important atoms around the N atom are labeled with white letters.

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