Molecular Dynamics Simulation of Melittin in a Dimyristoylphosphatidylcholine (DMPC) Bilayer Membrane

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Contents

Abstract

Molecular dynamics(MD) trajectories of melittin inserted into DMPC bilayer membrane are generated using MD simulation for a better understanding of protein-membrane interactions, especially interactions involved in the anchoring and stabilization of membrane-bound proteins. Four different systems were constructed. Temperature scan was adopted for DMPC only system and DMPC+Melittin system for phase transition comparison and the effects melittin and Cholesterol havce on the dynamics of DMPC at both liquid and gel phases were observed by measuring some properties of our systems, area per lipid, order parameter, gauche fraction and RMSF value, for instance. Some relative experimental results were also provided here to verify our simulation trajectories.

1 Preparation

workshops Attend some required workshop related to our research work, for instance: Introduction to Linux command, vi usage, make file usage, High performance Computing(HPC) introduction, mpi and OpenMp parallel computing, graphics processing unit (gpu) computing (CUDA language).

Cooperation and discussion Discuss with our supervisor in Oak Ridge Laboratory, Dr. Xiaolin Cheng and our cooperators, who have much experience with neutron scattering experiment to decide the simulation systems we are going to build. Some experimental results about DMPC and Melittin theyve already obtained are also covered in our discussion. Their experiments are conducted under two temperatures: 7 C (280K) for Gel phase and 37 C (310K) for liquid phase.

2 Background

Melitin (structure is displayed in Appendix) is the major protein component of the bee venom that has a pronounced effect on the lysis of membranes [1]. The amino-terminal region (residues 1 to 20) is predominantly hydrophobic whereas the carboxy-terminal region (residues 21 to 26) is hydrophilic due to the presence of a stretch of positively charged amino acids. This amphiphilic property of melittin makes it water soluble and yet it spontaneously associates with natural and artificial membranes. [2]

The majority of phospholipids belong to the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) families.(ref 1) Although phospholipid bilayers containing PC headgroups express fewer functionalities, it is central in maintaining membrane integrity. (ref 8)

The structure and iteractions of membrane-bound form is still difficult to be characterized by some advanced techniques like x-ray crystallography [3], electron microscopy [4], and nuclear magnetic resonance [5] due to the complexity of membrane environment. Thus the detailed computer simulation can be valuable for a better understanding of interactions like anchoring and stabilization of membrane-bound proteins. Simulations can be used to provide additional information on an atomic level, in a resolution that cannot be obtained experimentally. However, simulations may suffer from uncertainties in the forcefield and relatively short simulation times. Since simulations result from a modeling of the actual interactions and forces in a bilayer, they

should be validated by comparing to experimental data.

In fact different mechanisms about lytic activity of melittin has been proposed based on observations. Some suggested that membrane melittin increases membrane permeability by partial penetration of the bilayer.[6] Some models involved the formation of a canal structure by the aggregation of four transbilayer melittin molecules.[7][8] Others proposed that aggregated melittin could lead to cell lysis by leaving large holes.[9] What's more, other membrane protein may also be involved in membrane lytic process.[10]

The detailed understanding of interaction of melittin with lipid bilayer membrane is of significance since the amphiphilic a-helical conformation resembles those of signal peptides[11], and the envelope glycoprotein gp41 from the human immunodeficiency virus (HIV) [12]. What's more, melittin mimics the N-terminal of HIV-1 virulence factor Nef1-25.[13]

3 Introduction to our system

Four systems We have four systems, the following CHLs means cholesterol.

1 Pure DMPC, DMPC concentration 5 (w/w) %, Melittin to DMPC ratio: 1:500 (Temperature scan, from 275K to 315K, A total of 9 sets of temperatures with a 5 K difference)

2 DMPC + CHL, CHL to DMPC ratio: 1:4, Melittin to DMPC ratio: 1:500 (Temperature scan, from 275K to 315K, A total of 9 sets of temperatures with a 5 K difference)

3 DMPC + Melittin, DMPC concentration 5 (w/w) %, Melittin to DMPC ratio: 1:500 (7 centigrade and 27 centigrade)

4 DMPC + CHL + Melittin, CHL to DMPC ratio: 1:4, Melittin to DMPC ratio: 1:500 (7 centigrade and 27 centigrade)

4 Cooperator's experimental results

1. At 7 C and 37 C, DMPC dynamics are very different from each other.

2. Adding Melittin to DMPC, the dynamics are affected at both 7 C and 37 C.

3. At 7 C and 37 C, DMPC+CHL dynamics are different from each other.

4. Adding Melittin to DMPC+CHL, the dynamics at liquid phase (37 C) is not affected.

5. Temperature scan for DMPC, there is phase transition. Adding Melittin to DMPC, there is no phase transition for temperature scan.

5 Method

5.1 Building system

Generate membrane Build the DMPC membrane on website `http://www.charmm-gui.org/`. Select Membrane only system, set the water thickness to be 27 and select the DMPC lipid. Set DMPC number to be 250 (or DMPC to be 200 and cholesterol to be 50) for both upper leaflet and lower leaflet. Ions are not included in this step. We only generate water box here. Record the box size information displayed here for later usage.

Obtain polypeptide Download pdb file of Melittin from protein bank website `http://www.rcsb.org/pdb/home/home.do`. Dow2nload the protein with acess code 2MLT. There are two chains inside it and we will only take one chain of melittin (one melittin monomer).

Generate protein-membrane system Open the .pdb file of 'Melittin' and 'DMPC' using software Visual Molecular Dynamics (VMD). Move the Melittin into proper position (inside the water layer of membrane without contacting with lipid) of DMPC membrane by using VMD. Prepare all pdb files. System get built here.

Discussion about proper protein position Based on some previous work about simulation on melittin and DMPC, our supervisor, Dr Cheng suggested that it may take a long simulation time for the melittin to automatically get inserted into membrane by just putting melittin on lipid-solution region. After reading more iterature we find a possible better and more advanced way to construct the microscopic model. In this approach melittin is supposed to be deeply inserted into the upper layer of bilayer membrane

and corresponding lipid molecules should be deleted to aviod heavy atoms overlap. The helix should still be oriented parallel to membrane-water interface, leaving the hytrophilic residues contacting with water and hytrophobic residues in contact with lipid acyl chains.

5.2 Preparing for gromacs

Gromacs version We choose to use gromacs 5.0.1 installed on cluster darter and nanoheat. We used single precision to run the simulation.

Preparing .gro file for simulation Download force field charmm 36 to current directory and choose to use it in next step. Use 'pdb2gmx' (may take a long time) in gromacs to get .gro format for system. Then modify the name of atoms, box size recorded in gro file. For systems with melittin, considering the melittin is of +5 charge, we change five water molecules to be chlorine atoms to neutralize this system.

Preparing .top file for simulation Then we generate .itp files separately for DMPC and cholesterol and melittin and create top file ourselves to include them. And we will modify the atom number recorded in it to make it corrsponds to our system.

Preparing .ndx file for simulation Use make_ndx command to select and rename coupling atom groups. Name of groups need to be corresponding to what recorded inside the .mdp file we are going to use for minimization, equilibration and production.

5.3 Runing simulation

5.3.1 Minimization

Run minimization without any coupling.

5.3.2 Equilibration

Run several steps of equilibrations at certain temperature (290K, for instance). Since the initial structure of our system is not well equilibrated, first of all we need to set the time step to be quite small and gradually increase it so that gromacs won't give error report.

5.3.3 Set different temperature

After system gets well equilibrated, difference in temperature happens before the production run. Generate velocities at different desired temperatures and run simulation for around 1 or 2 ns. Starting from this step all simulations are done in clusters using at least 64 cores for better performance.

5.3.4 Production

Run the final simulation for 5 nanoseconds and start analysis. Using VMD to check the structure of systems in Gel phase. After some analysis based on the simulation trajectory we got, the system under relatively low temperature (Gel phase) takes longer time to get stable. Thus we run simulation for 50ns altogether (we run double precision gromacs on cluster 'darter' with 192 cores and wall time 24 hours, it can generate around 35 ns trajectories per day).

5.4 Analysis

5.4.1 Area per lipid

Use 'g_energy' command in gromacs and select to record x and y in .xvg file. Then plot area per lipid (x length * y length /lipid number) versus time figure.

This result will generally increase with temperature increasing. And the increasing rate can be larger for systems in liquid phase. The result may indicate whether our system has reached equilibrated or not.

5.4.2 Density

Use 'g_density' command in gromacs to record density value in .xvg file. Then plot density versus z coordinate figure.

The result reveals the change in density along the normal direction. By such analysis the location and thickness of different layers of component can be determined.

5.4.3 Order parameter

Generate .ndx file to select carbon atoms in two tails of DMPC lipid (C22 to C214 for tail 2 and C32 t0 C314 for tail 3). Use 'g_order' command in gromacs to record the order parameter of two tails for the last nanosecond. The orientational order parameter is defined as:

$$S = \frac{1}{2}(3 < cos^2\beta > -1)$$

where β is the instantaneous angle between the director of the C-D bond and the bilayer normal. The angular brackets denote a time and ensemble average.

The order parameter, which is often used to validate or calibrate molecular dynamics simulations, can measure the rigidity and orderliness of tails in DMPC lipid.

5.4.4 g_angle

In stereochemistry, The term gauche refers to conformational isomers(conformers) where two vicinal groups are separated by a 60 torsion angle. We calculated the proportion of gauche in all dihedrals in two lipid acyl chains.

6 Result

6.1 Area Per Lipid

Just as the name implies, Area Per Lipid is the average area each lipid possesses in the leaflet of DMPC bilayer. The equation below is how we calculated it.

$$Area\ per\ lipid = \frac{Area\ of\ the\ laeflet}{Lipid\ number\ per\ leaflet} = \frac{X * Y}{250}$$

In figure 1, for systems with temperatures from 275K to 305K, the area per lipid drops down significantly, which means the systems are still not well equilibrated. While for 310K and 315K, the curves are kind of stable, which means the systems might have already equilibrated.

Besides, from 275K to 285K and from 295K to 315K, the area per lipid will increase as temperature increases, while from 285K to 295K, on the contrary, the area per lipid will decrease as temperatures increases. It is easy to understand that area per lipid will increase with temperature. As temperature goes up, the molecules will move faster and possess more space, therefore the area per lipid will increase. According to a related paper, the transition temperature of DMPC is about 296K, which is in consistent with this anomalism.

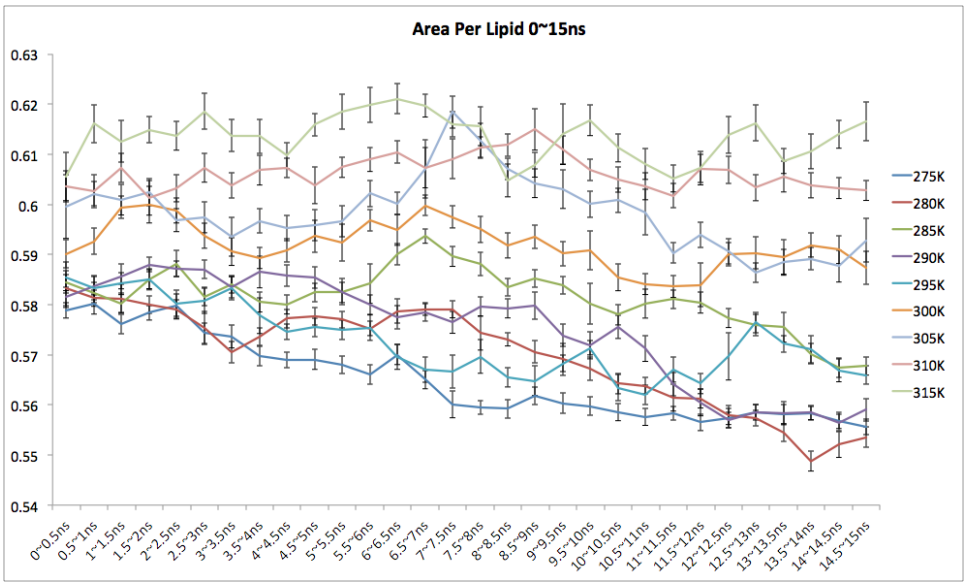

Figure 1: Area per lipid

6.2 Order Parameter

Lipid order parameter is a measure of the orientational alignment of the CD (carbon-deuterium) bond and is defined previously. By observing the order parameter of the two tails of DMPC, we can have a general idea of how the DMPCs are aligned in the DMPC bilayer.

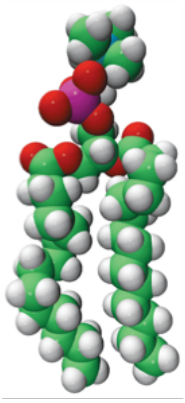

Figure 2: Structure of DMPC

The four charts in 3 separately plot out the order parameters of the first tail of DMPC in different time intervals. In 0 1 ns, the curves are very close to each other, while in 24 25 ns, it is obvious that the curves are divided into

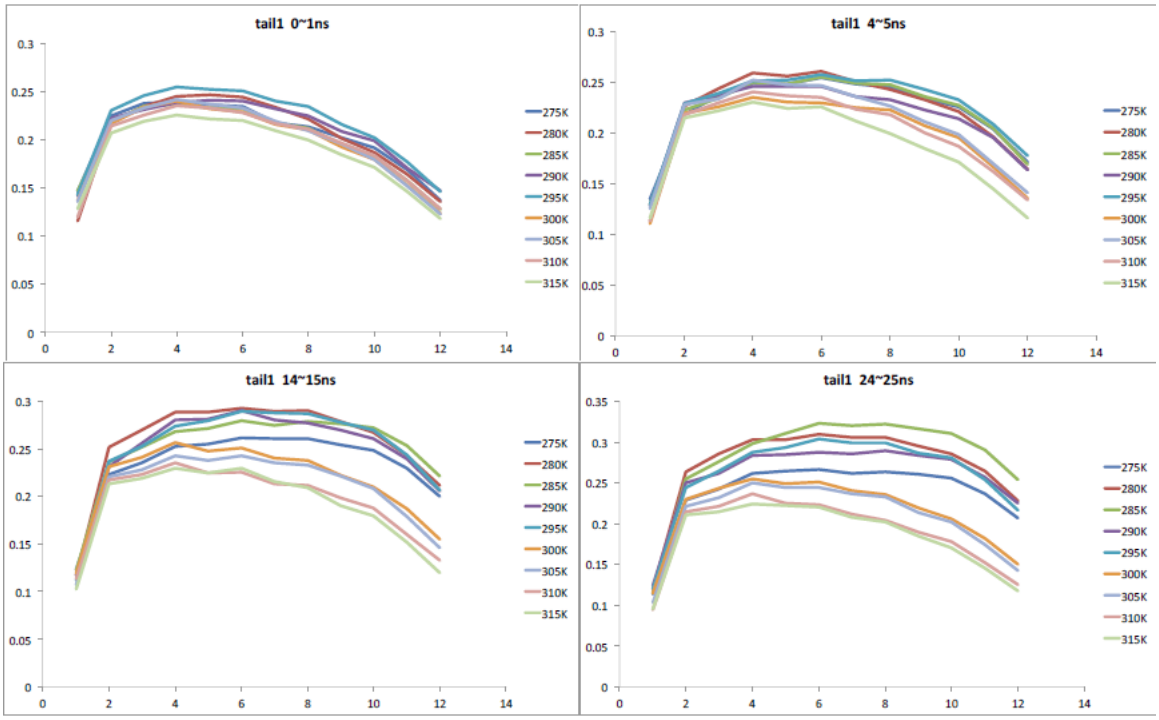

Figure 3: Order parameter in tail1

two groups. The upper group has lower temperatures, namely, from 275K to 295K. While the lower group has a higher temperature, or from 300K to 315K. The reason why these curves are divided into two groups is because of phase transition too. Meanwhile, the gap is in well consistent with the experiment result that the melting point of DMPC is 296K.

6.3 Gauche Conformation Fraction

In stereochemistry, The term gauche refers to conformational isomers(conformers) where two vicinal groups are separated by a 60 torsion angle.

Figure 4 below shows the trans(left) and gauche(right) rotamers of butane. The two methyl groups can be in an anti-bonding relationship, or offset at sixty degree dihedral angles.

There are both 14 carbon atoms in the two tails of DMPC, and every four adjacent carbon atoms can form a dihedral. Because trans and gauche conformations are two stable structure, the dihedral angles will be close to

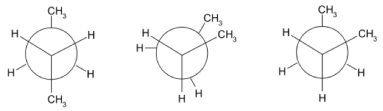

Figure 4: rotamers of butane

either 60 degree or 180 degree. Therefore, we divided all the dihedrals into two groups. One is from -120 degree to 120 degree, which is named gauche. The other group trans is from 120 to 180 degree or -120 to -180 degree. Then we calculated the fraction of gauche over all the dihedrals in one tail of all the 500 DMPC in the DMPC only system.

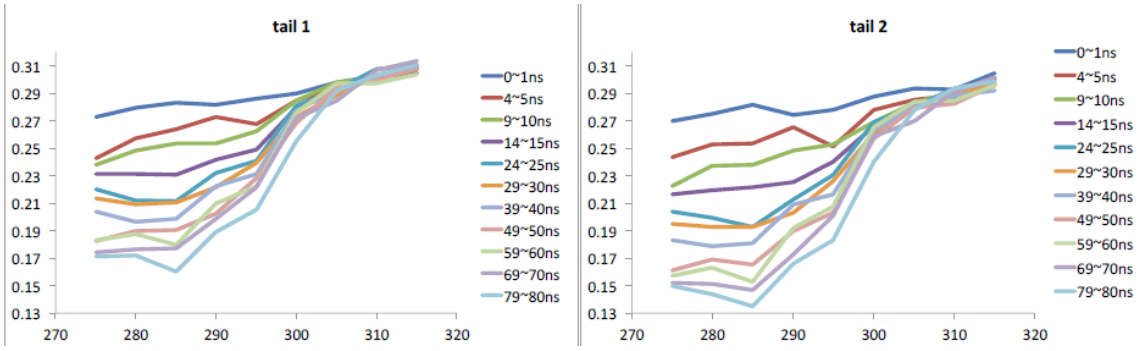

Figure 5: gauche fraction

In 5, for both tails, generally the gauche fraction will increase as temperature increases. This is what we should expect because trans conformation has a little higher energy then gauche conformation. Besides, in higher temperatures, the gauche fraction curves already converge, which means the systems are already well equilibrated. However, in lower temperatures, the gauche conformation fractions are still dropping down, which might imply us to extend our simulations to a longer time. Again, the difference happens around the transition temperature 296K.

In 6, there are two plots which aim to compare the gauche fraction between the systems with different starting structures. However, the results seem to conflict with each other. So due to the lack of sufficient data, no conclusion might be drawn from these two figures. We can only say that in the time interval 59 60ns, it seems that the first tail of DMPCs in the DMPC only system has a higher level of gauche fraction, which means the DMPC + melittin system is more ordered in this specific situation.

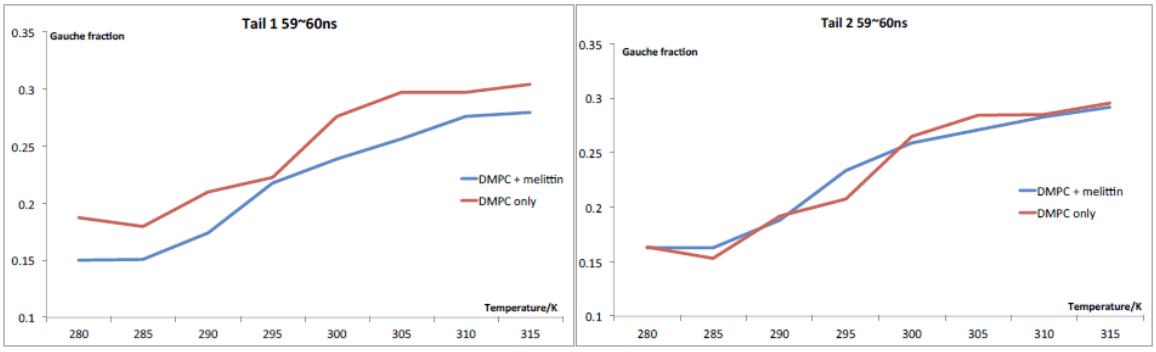

Figure 6: gauche fraction comparison

6.4 RMSF

RMSF stands for root mean square fluctuation, which shows the mobility of atoms. The RMSF of all the hydrogen atoms attached to the carbon in the two tails are calculated.

$$RMSF = \sqrt{\frac{1}{T}\sum_{t_j=1}^{T}(x_i(t_j) - \bar{x}_i)^2}$$

In figure 7, from the head to the tail, RMSF significantly increases, which is consistent with the model experimentalists used. A big gap around 295K appears, which represents the phase transition.

7 Future Development

After all current systems get analyzed, we intend to build a larger system with more than one melittin molecules and run a much longer simulation. In this system melittins should be inserted carefully in correct positions in order to reduce simulation time. We believe if the structure is relatively close to reality and simulation lasts long enough, it's possible for to observe melittin aggregation as well as lytic process (deformation of the membrane, canal or hole on membrane) in our trajectory.

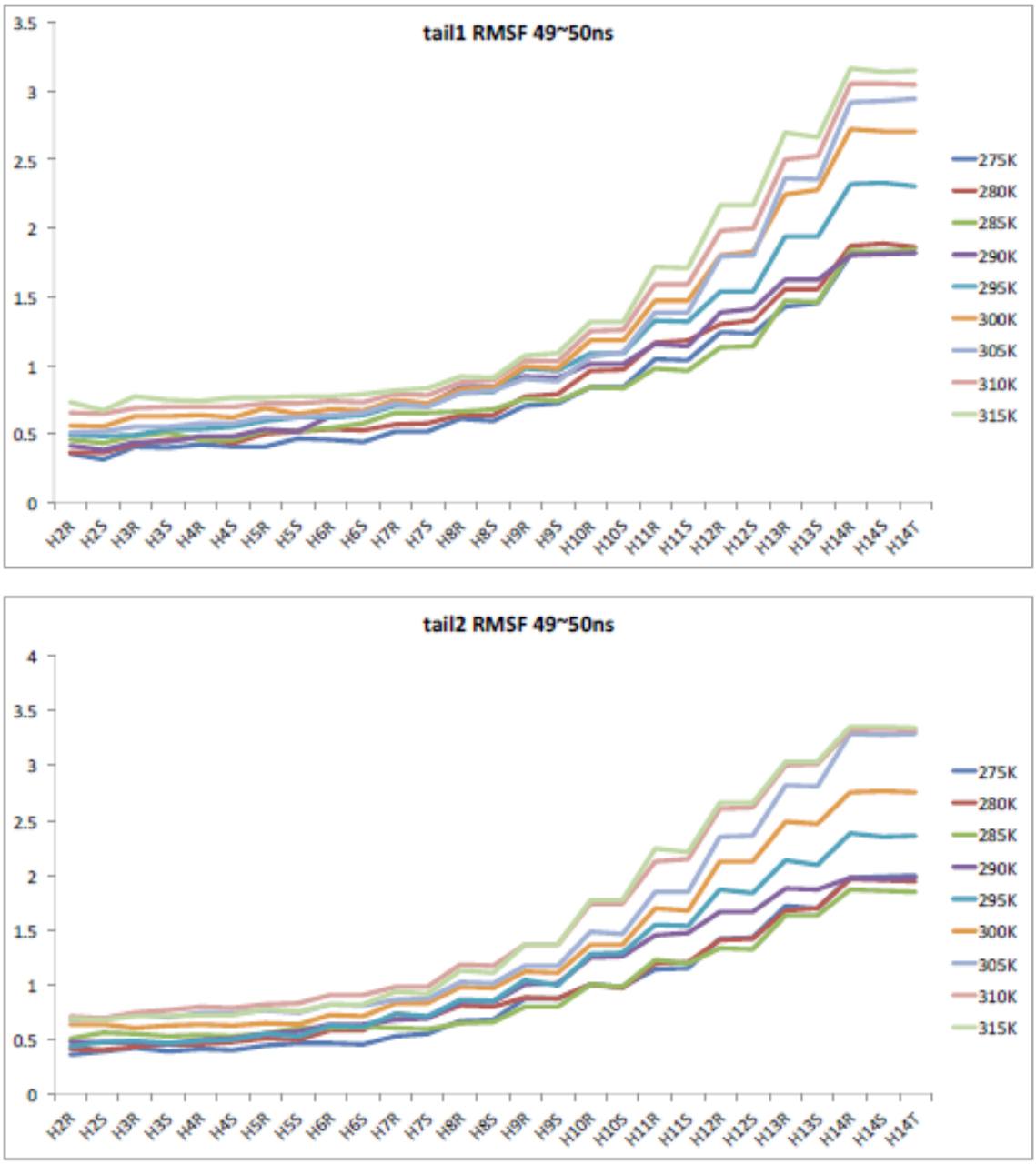

Figure 7: RMSF

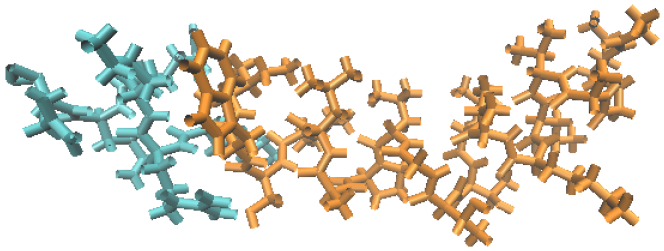

Figure 8: Melittin structure

8 Appendix

8.1 Structure of melittin

Figure 8 demonstrates the structure of melittin, residues 1-20 (painted to orange) make up the amino-terminal region, which is predominantly hydrophobic and was deeply inserted into lipid membrane whereas carboxy-terminal region (residues 2126 painted to blue) is hydrophilic and in contact with bulk

8.2 Structure of DMPC

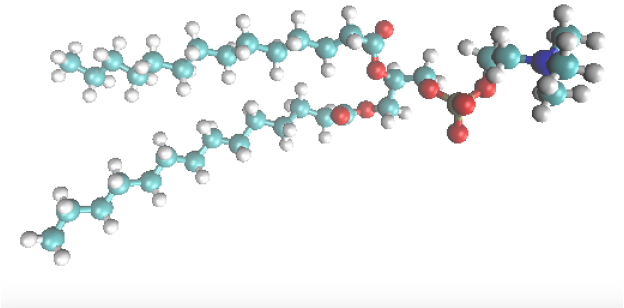

Figure 9: DMPC structure

Figure 9 demonstrates the structure of DMPC generated by software 'VMD', we can observe its two lipid acyl tails and lipid head structure.

8.3 Figure of DMPC bilayer membrane

Figure 10 demonstrates the overall structure of our most complicated system with cholesterol in green and melittin in red color. The melittin is generally

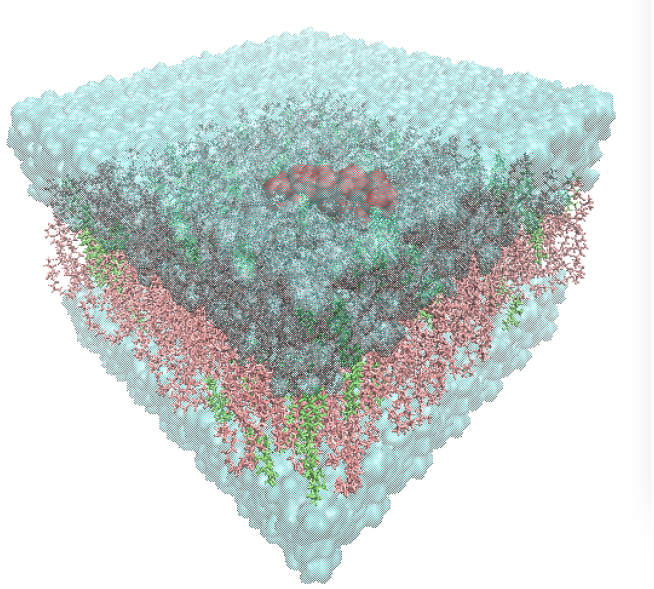

Figure 10: overall structure

parallel to membrane-solution interface.

8.4 File location

Our initial systems and force feild files are in cluster 'nanoheat'. Later priduction simulation results are in cluster 'darter'(/luster/medusa/zhiyao).

9 Acknowledgement

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References

[1] E. Habermann, *Bee and wasp venoms.*, vol. 177. 1972.

[2] Dempsey and C. E., *The actions of melittin on membranes.*, vol. 1031. 1990.

[3] Deisenhofer, J., and H. Michel, *The photosynthetic reaction center from the purple bacterium rhodopseudomonas viridis.*, vol. 245. 1989.

[4] Henderson, J. M. Baldwin, T. A. Ceka, F. Zemlin, E. Beckmann, and K. H. Downing, *Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy.*, vol. 213. 1990.

[5] Cross, T. A., and S. J. Opella., *Protein structure by solid-state nuclear magnetic resonance: residues 40 to 45 of bacteriophage fd coat protein.*, vol. 182. 1985.

[6] Ash, P. S., A. S. Bunce, C. R. Dawson, and R. C. Hider, *Study of vesicle leakage induced by melittin*, vol. 510. 1978.

[7] Smith, R., F. Separovic, T. J. Milne, A. Whittaker, F. M. Bennett, B. A.Cornell, and A. Makriyannis, *Structure and orientation of the pore-forming peptide, melittin, in lipid bilayer*, vol. 241. 1994.

[8] Tosteson, M. T., and D. C. Tosteson, *Melittin forms channels in lipid bilayers*, vol. 36. 1981.

[9] Dufourc, E. J., I. C. P. Smith, and J. Dufourcq, *Molecular details of melittin-induced lysis of phospholipid membranes as revealed by deuterium and phosphorus NMR*, vol. 25. 1986.

[10] Werkmeister, J. A., A. Kirkpatrick, J. A. McKenzie, and D. E. Rivett, *The effect of sequence variations and structure on the cytolytic activity of melittin peptides*, vol. 1157. 1993.

[11] Golding, C., and P. OShea, *The interactions of signal sequences with membranes*, vol. 23. 1995.

[12] Rabenstein, M., and Y. K. Shin, *A peptide from the heptad repeat of human immunodeficiency virus gp41 shows both membrane binding and coiled-coil formation*, vol. 34. 1995.

[13] Barnham, K. J., S. A. Monks, M. G. Hinds, A. A. Azad, and R. S. Norton, *Solution structure of polypeptide from the N terminus of the HIV protein Nef*, vol. 36. 1997.