

# In or out?

## Accumulation of the DLin-MC3-DMA (MC3)

### Lipid in POPC, POPE, DOPC, and DOPE

### Bilayers According to the CHARMM36 and

### SLipids Force Fields

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## Introduction

Ionizable lipids play an integral role in the drug delivery systems like the lipid nanoparticle (LNP) formulation of the COVID-19 mRNA vaccines developed by BioNTech and Moderna. These lipids respond to the pH by changing their protonation state ensuring that the LNPs become active only in certain body tissues. As these lipids can vary in size, charge, and their response to pH, they can be used with other lipids to engineer LNPs to have required properties. Such an engineering process would require a large set of experiments to be run for every different available composition of the LNPs. Molecular dynamics simulations (MD)

can model different LNPs compositions in computers, allowing one to assess the properties of them faster and cheaper than the experiments. This would require high accuracy computational models (force fields, FF) of the lipid components. Two recent computational force fields, namely CHARMM36<sup>1</sup> and SLipids,<sup>2</sup> have predicted contradicting results for the DLin-MC3-DMA (referred as MC3 henceforth) ionizable lipid's behavior in PE and PC lipid bilayers: CHARMM36 FF predicts an accumulation of the MC3 lipids in the center of the POPE and POPC bilayers whereas Slipid FF predicts no such accumulation. Here, we compare the predictions of these two FFs by running MD simulations of small membrane patches. Our results show that MC3 lipids with CHARMM36 FF accumulates in the bilayer center whereas no such accumulation is observed with SLipids, confirming the published results. We further show that the accumulation behavior of the MC3 lipids does not significantly depend on the lipid tail saturation, as POPE/POPC and DOPE/DOPC bilayers predicted similar behavior. We hope that our results will help solving the discrepancies between the available MC3 FFs. Our entire data set including the MD trajectories can be found on public repositories.

Please note that this is not a peer-reviewed publication nor a preprint. We are making our preliminary results publicly available in the hope that our results (with not-so-pretty figures) will be useful to the researchers without disclosing any data nor waiting months for a peer-reviewed publication. Therefore, use the data at your own risk. If you have any questions or comments, you can reach us via GitHub or email.

## Literature Results

### CHARMM36

The original publication for the CHARMM36 FF of the ionizable cationic lipids can be found in Ref.<sup>1</sup>

Simulated bilayers for this study are MC3-POPC (number of lipids in both leaflets 30:570 and 90:510) and MC3-POPE (number of lipids in both leaflets 34:646 and 110:578). All systems contain  $\approx 160K$  atoms and 150 mM NaCl. Each system is simulated for 2  $\mu$ s and the last 600 ns are used for the analysis. The time step is 4 fs with hydrogen mass repartitioning.

The below results are copied almost verbatim from the original publication.

1. Neutral MC3 molecules accumulate in the bilayer center,
2. MC3 accumulates more in POPE bilayers than in POPC bilayers,
3. The accumulation of MC3 was not observed in the study with the SLipids. The difference in accumulation is attributed to the different force fields,
4. The difference between the accumulation in POPC and POPE bilayers can be attributed to a negative spontaneous curvature of POPE and a smaller spontaneous curvature of POPC, which could enable POPE monolayers to better accommodate domains of neutral MC3 in the bilayer center.

## SLipids

The original publication for the SLipids FF of the ionizable cationic lipid DLin-MC3-DMA (MC3) can be found in Ref.<sup>2</sup>

Simulated bilayers for this system are MC3-DOPC and MC3-DOPE (the total number of lipids is 10:190 and 30:170). Salt was most likely not included as the publication does not give this detail. Total simulation time per system is 600 ns of which the last 300 ns is used for analysis.

The below results are copied almost verbatim from the SLipids paper and email correspondences with the authors.

1. In DOPE bilayers, the head group of the MC3 lipid prefers to be located in the region of the phospholipid head groups,
2. In DOPC bilayers, a small amount of MC3 lipid was detected even in the center of the bilayer,
3. MC3 lipids have a tendency to aggregate if they are placed in the initial configuration close to each other, that is the initial configuration of the system affects its accumulation behavior (unpublished results obtained from personal correspondence with the authors),
4. POPE/POPC and DOPE/DOPC bilayers behave differently as the saturation of the lipid tails affect the accumulation of the MC3 lipids (unpublished results obtained from personal correspondence with the authors).

## Simulation Details

We simulated the following systems: MC3-POPC, MC3-POPE, MC3-DOPC, and MC3-DOPE bilayers at 5 mol% and 15 mol% of MC3 concentration. Each bilayer contains 40 lipids in total of which 2 (for the 5 mol% systems) and 6 (for the 15 mol% systems) of are the MC3 lipids. We obtained the initial structures from CHARMM-GUI and renamed the lipid atoms for SLipids. Therefore, both CHARMM36 and SLipid simulations start from the exact same configuration. Total simulation time per system is 1  $\mu$ s. We use V-rescale thermostat and Parinello-Rahman barostat to keep the system temperature at 303 K and 1 bar, respectively. We also apply the semi-isotropic pressure coupling. We constrain the lengths of the hydrogen containing bonds via SHAKE. We apply 1.2 nm real-space cutoff for the Coulomb interactions. We smoothly switch van der Waals interactions to zero between 1.0 nm to 1.2 nm. We calculate the density profiles using the last 500 ns of the simulations. We investigate the flip-flop and aggregation behavior of the MC3 lipids using the full trajec-

tory length. Further details of the simulation setups can be found in the Zenodo repositories.

To be consistent with SLipids paper,<sup>2</sup> MC3 lipid head group is defined as the terminal N- and 2 C atoms. MC3 lipid tail group is defined as the two terminal -CH<sub>3</sub> groups. For the choice of atom groups, please see Fig. 2.

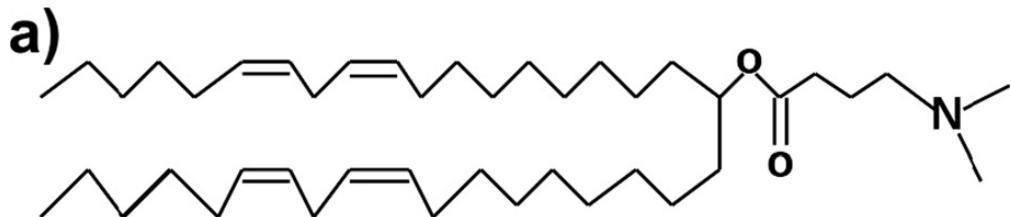


Figure 1: structure of the MC3 lipid

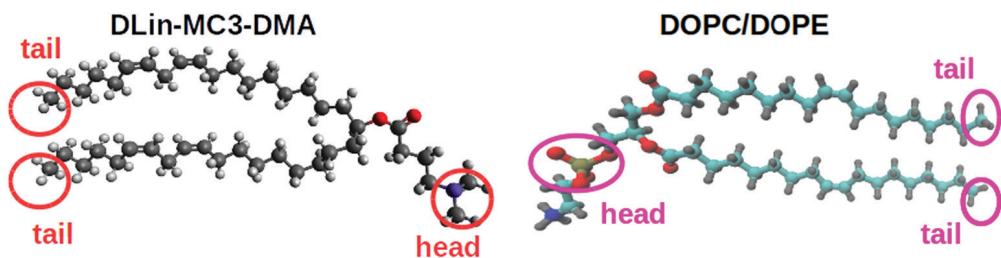


Figure 2: Visual depiction of the MC3 tail and head groups used in the density profiles.

## Aim

Based on the literature results, our aims for this project are to answer the following questions:

1. Do MC3 lipids go into the POPC, POPE, DOPC, and DOPE bilayers?
2. Does the concentration of the MC3 lipids affect the accumulation behavior?
3. Does the saturation of the lipid tails affect the accumulation behavior?
4. Do the MC3 lipids aggregate in the bilayers?

# Conclusions

Based on our simulation results using two different force fields, we can draw the following conclusions:

1. Do MC3 lipids go into the POPC, POPE, DOPC, and DOPE bilayers?

MC3 head groups will go into the bilayer center in PE lipids, if one uses CHARMM36.

With the SLipids force field, no accumulation of the MC3 head groups will be observed.

2. Does the concentration of the MC3 lipids affect the accumulation behavior?

For SLipids, no. For CHARMM36, only with the PE lipids the MC3 head group concentration at the bilayer center surpasses the one at the lipid surface.

3. Does the lipid tail saturation affect the accumulation behavior?

For SLipids, no. For CHARMM36, it depends. at 15% MC3 concentration, we observe more accumulation in POPE than in DOPE. At 5% MC3 concentration, the opposite is true. DOPC and POPC results are rather similar. Yet, with the CHARMM36m force field we see more flip-flopping of the MC3 lipids in PE bilayers than in PC.

4. Do the MC3 lipids aggregate?

For both force fields, we see aggregation of the MC3 lipids at 15% mol concentration.

For the SLipids, the aggregation occurs around the bilayer surface, for the CHARMM36 the aggregation occurs at the bilayer center.

For the 5% mol concentration, MC3 lipids do not form any contacts with the SLipids force field, mostly due to the lack of flip-flop of the MC3 lipids. WIth the CHARMM36 force field, we observe interactions among the MC3 lipids.

## Results

For all the systems, we calculate the mass density profiles of the lipids alongside the membrane normal, plot the z-coordinate of the MC3 lipid head groups as a function of time to see if the MC3 lipids change their initial leaflet (flip-flop), and number of contacts between the MC3 non-hydrogen atoms to check possible MC3 aggregation.

### Flip-flop of the MC3 Lipids

Figs. 3- 10 shows the z-coordinates of the MC3 lipid head groups (Fig. 2) as a function of the simulation time. Our main observation from this data is that MC3 lipids change their initial leaflet with the CHARMM36 FF but not with the SLipids FF. For the CHARMM36 FF, we see that the MC3 lipids spend more time in the PE bilayer centers and flips more often compared to the PC bilayers, in agreement with the density profiles in Fig. 11- 18. This ability of the MC3 lipids to "flip-flop" within the bilayer yields significant MC3 lipid presence in the bilayer center.

### Accumulation of the MC3 Lipids

From the density profiles along the membrane normal (Fig. 11- 18), we observe the following:

1. In POPC bilayers with 5% MC3 lipids (Fig. 11):
  - (a) MC3 lipid head groups stay in the bilayer surface with both the CHARMM36 and SLipids force fields,
  - (b) MC3 lipid head groups are located further out from the bilayer center with the SLipids compared to CHARMM36,
  - (c) MC3 lipid tail groups are found in the bilayer center with CHARMM36,
  - (d) MC3 lipid tail groups do not go into the bilayer center with SLipids.

2. In POPC bilayers with 15% MC3 lipids (Fig. 12):
  - (a) Overall, same profiles as the POPC bilayers with 5% MC3 lipids.
  - (b) MC3 lipid head groups are getting closer to the bilayer center with SLipids compared to the 5% concentration.
3. In POPE bilayers with 5% MC3 lipids (Fig. 13):
  - (a) MC3 lipid head groups can be found both in the bilayer surface and the center with CHARMM36,
  - (b) MC3 lipid tail groups highly accumulate in the bilayer center with CHARMM36,
  - (c) MC3 lipid head groups stay in the bilayer surface with SLipids,
  - (d) MC3 lipid tail groups are not located in the bilayer surface with SLipids.
4. In POPE bilayers with 15% MC3 lipids (Fig. 14):
  - (a) MC3 lipid head group concentration around the bilayer center increases with CHARMM36,
  - (b) Density profiles are qualitatively the same as in 5% MC3 concentration.
5. In DOPC bilayer with 5% MC3 lipids (Fig. 15):
  - (a) MC3 lipid head groups slightly accumulate in the bilayer center but stay mostly at the surface with CHARMM36,
  - (b) MC3 lipid tail groups are found mostly in the bilayer center with CHARMM36,
  - (c) MC3 lipid head groups are found exclusively in the bilayer surface and none are detected in the bilayer center with SLipids,
  - (d) MC3 lipid tail groups are not found in the bilayer center.
6. In DOPC bilayer with 15% MC3 lipids (Fig. 16):

- (a) Density profiles are quantitatively the same as in 5% MC3 concentration.

7. In DOPE bilayer with 5% MC3 lipids (Fig. 17):

- (a) MC3 lipid head groups can be found both in the bilayer center and surface with CHARMM36,
- (b) MC3 lipid tail groups are found mostly in the bilayer center with CHARMM36,
- (c) MC3 lipid head groups are located at the bilayer surface with SLipids,
- (d) MC3 lipid tail groups are not located at the bilayer center with SLipids.

8. In DOPE bilayer with 15% MC3 lipids (Fig. 18):

- (a) Density profiles are quantitatively the same as in 5% MC3 concentration.

## Aggregation of the MC3 Lipids

The number of contacts between the MC3 lipids as a function of time is given in Fig. 19-26. Here, we define a contact whenever the distance between two non-hydrogen atoms is less than 0.35 nm. For the 15 mol% systems, we separate the contacts into two distinct cases: i) the contacts between the MC3 lipids that were initially in the same leaflet, and ii) the contacts between the MC3 lipids that were initially not in the same leaflet.

Our main results are:

1. For the 5 mol% systems, MC3 lipids with the SLipids FF do not form any contacts,
2. For the 5 mol% systems, MC3 lipids with the CHARMM36 FF form contacts. The number of contacts is not affected by the lipid type,
3. For the 15 mol% systems, MC3 lipids in the same leaflet form aggregates with the SLipids FF. As SLipids FF does not predict any significant flip-flop of the MC3 lipids, contacts between the MC3 lipids that belonged initially to the opposite leaflets do not form any contacts,

4. For the 15 mol% systems, MC3 lipids, due to the flip-flop predicted by the CHARMM36 FF, form significant contacts both within the same and different leaflets,
5. For the 15 mol% systems with the CHARMM36 FF, the number of contacts between the MC3 lipids that belonged initially to the different leaflets (and the density profiles) indicate that these lipids form aggregates at the bilayer center.

## Figures

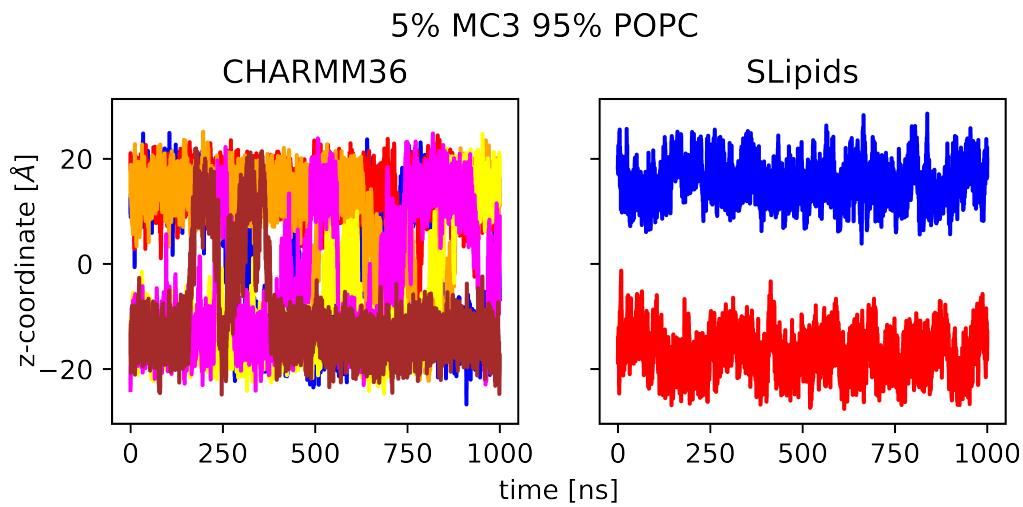


Figure 3: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

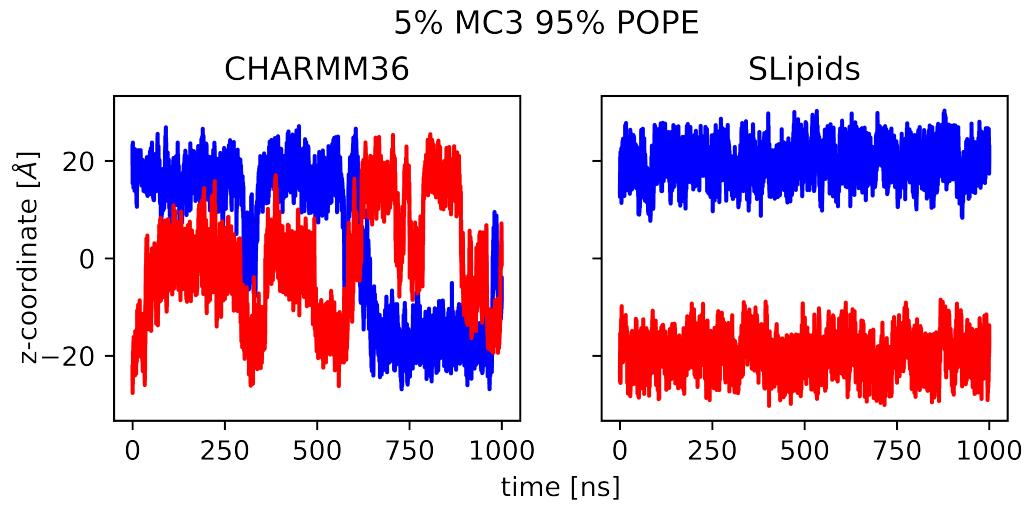


Figure 4: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

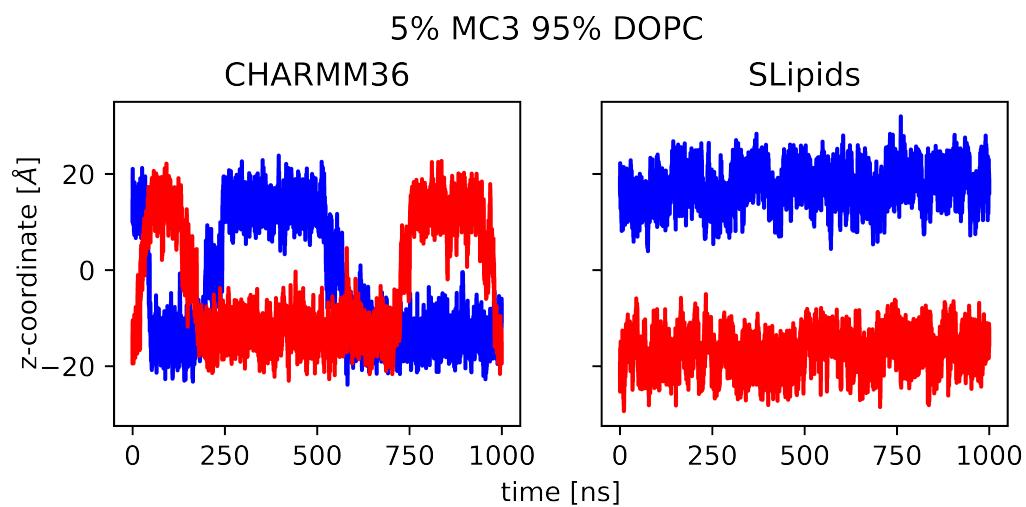


Figure 5: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

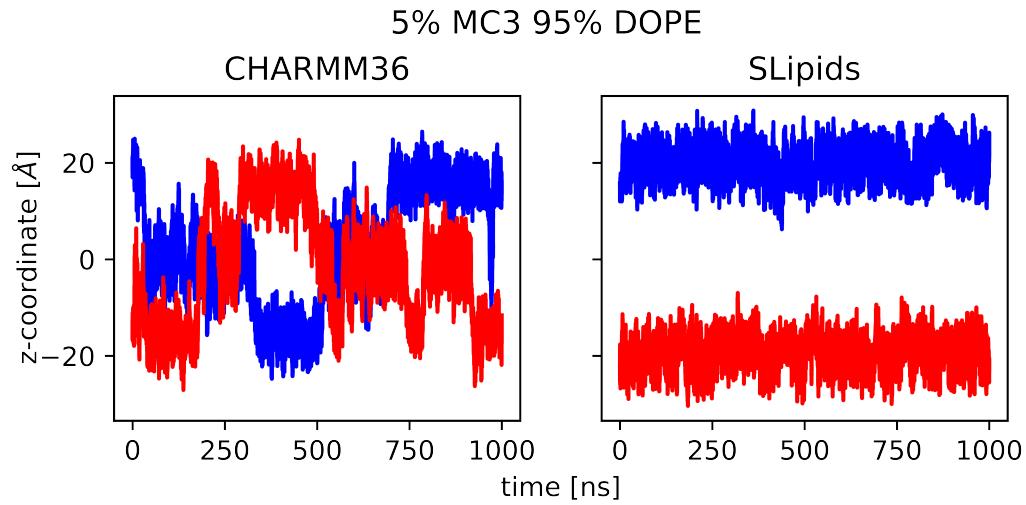


Figure 6: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

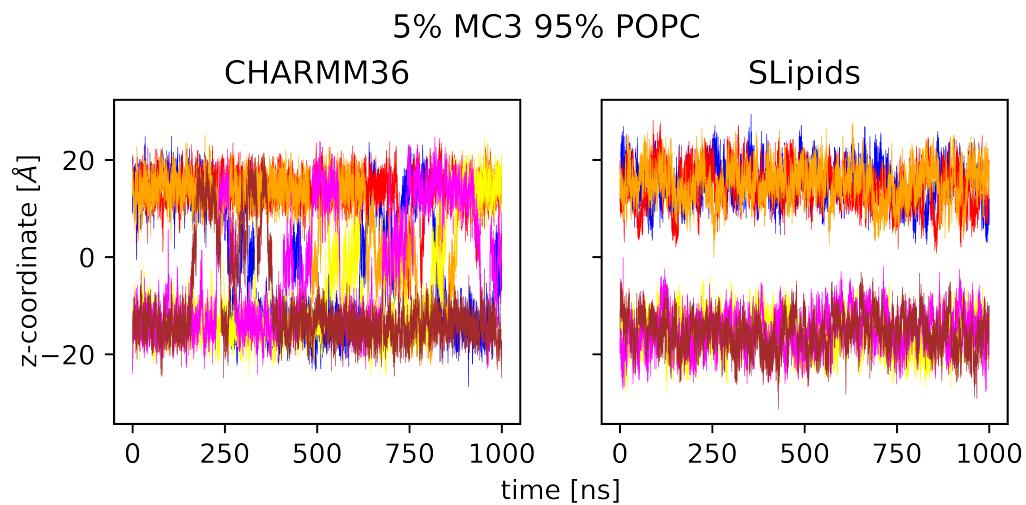


Figure 7: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

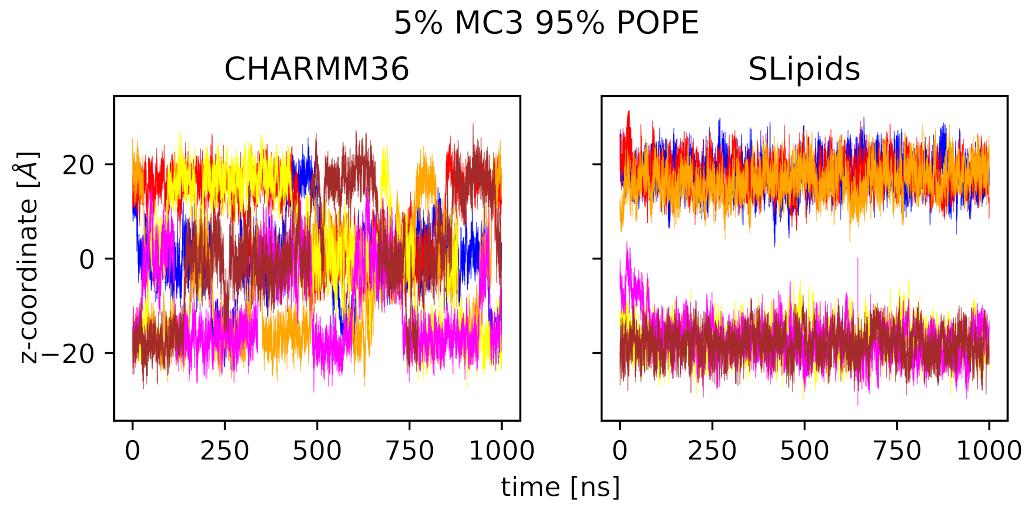


Figure 8: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

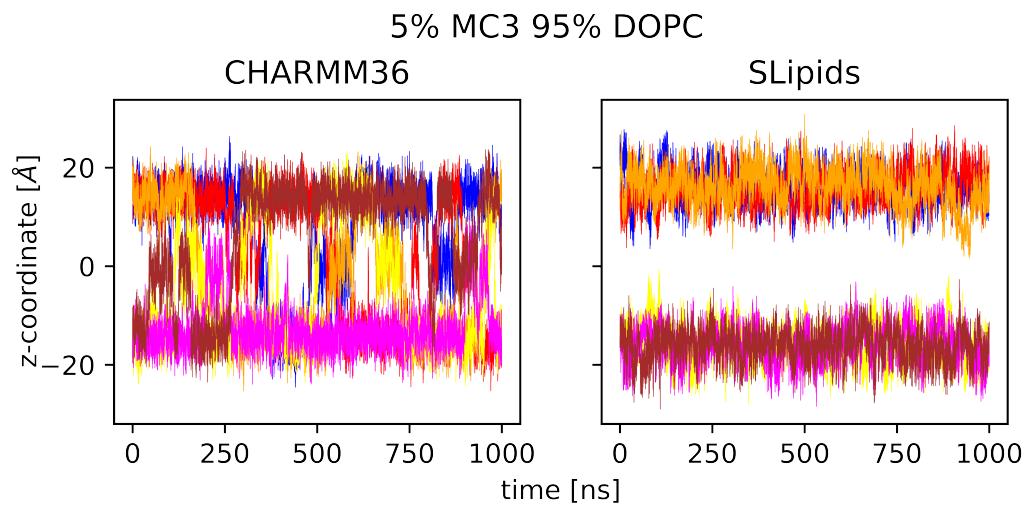


Figure 9: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

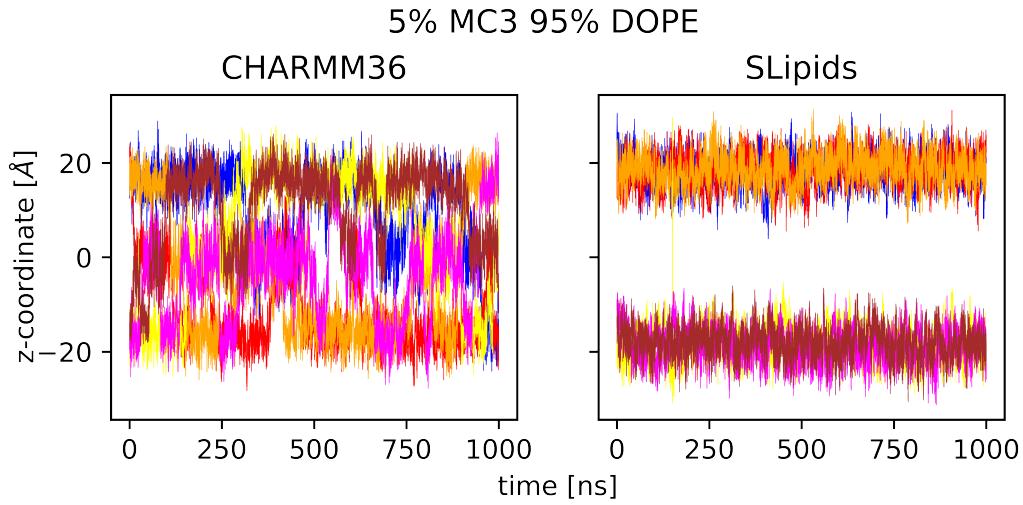


Figure 10: The z-coordinates of the MC3 lipid head groups (per definition in Fig. 2) as a function of simulation time.

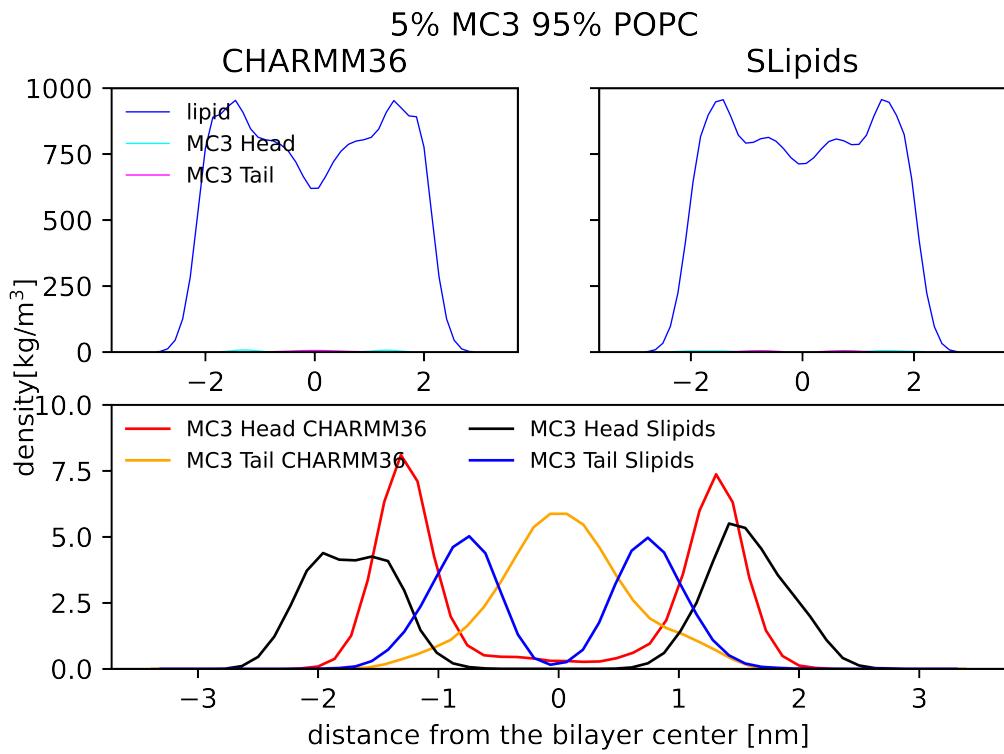


Figure 11: Mass density profile for the 5% MC3 lipid in a POPC bilayer

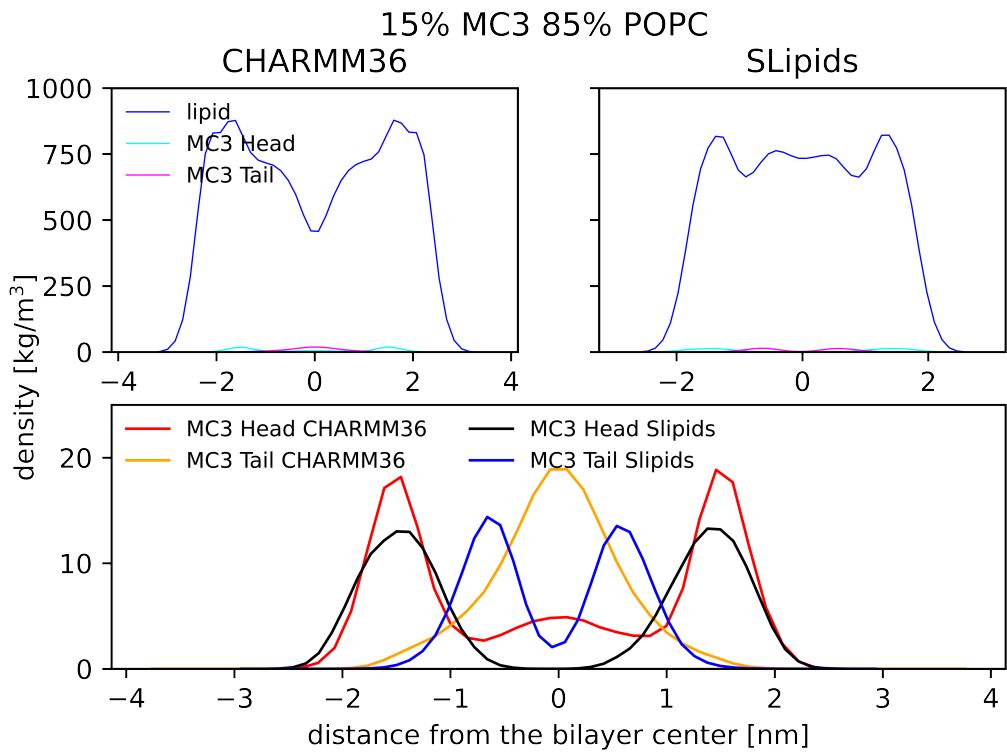


Figure 12: Mass density profile for the 15% MC3 lipid in a POPC bilayer

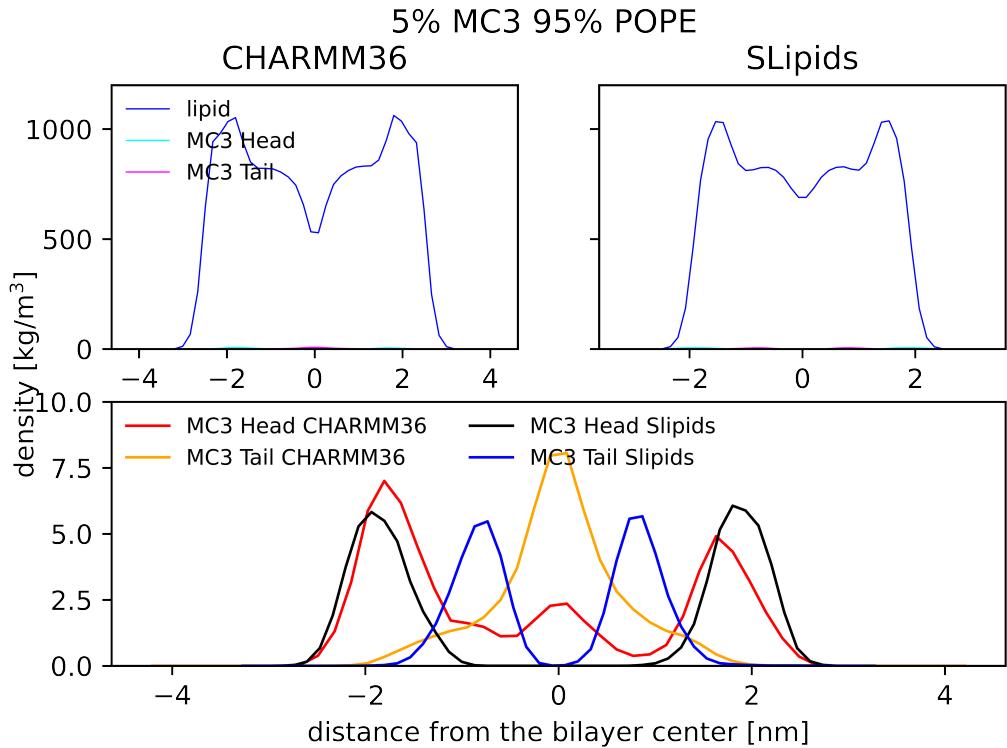


Figure 13: Mass density profile for the 5% MC3 lipid in a POPE bilayer

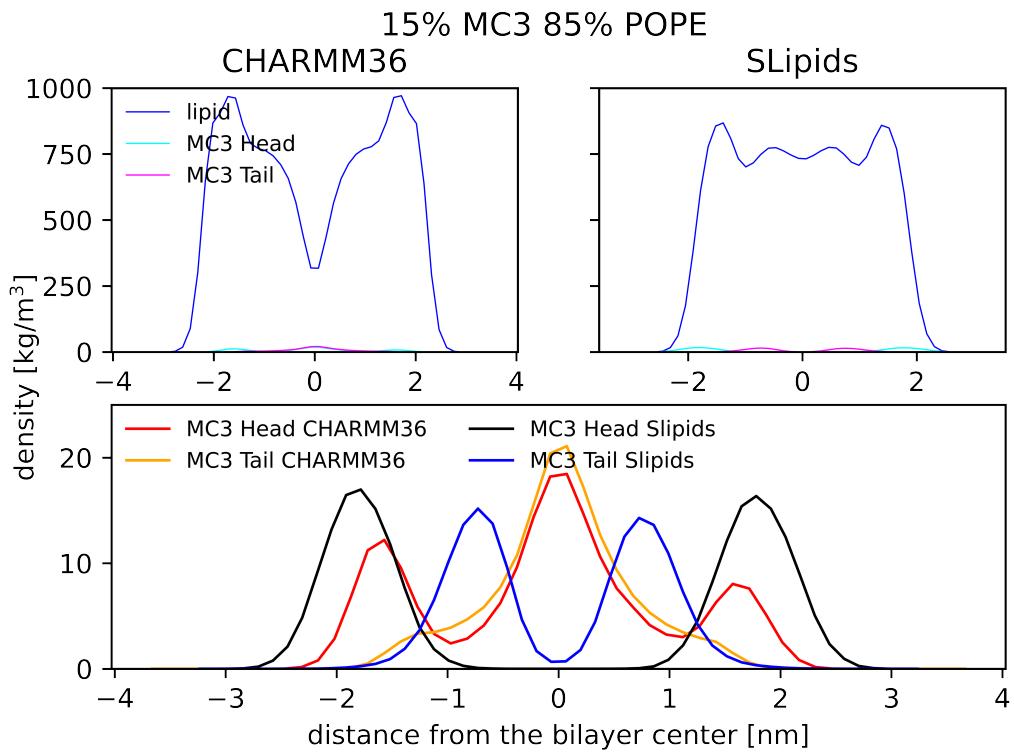


Figure 14: Mass density profile for the 15% MC3 lipid in a POPE bilayer

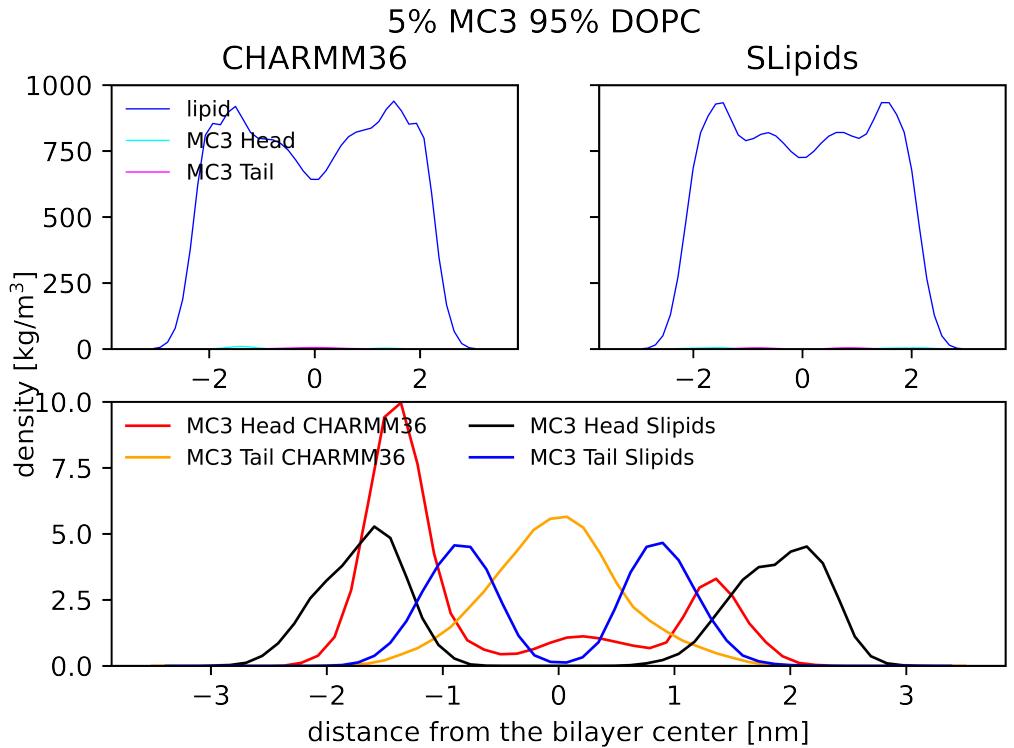


Figure 15: Mass density profile for the 5% MC3 lipid in a DOPC bilayer

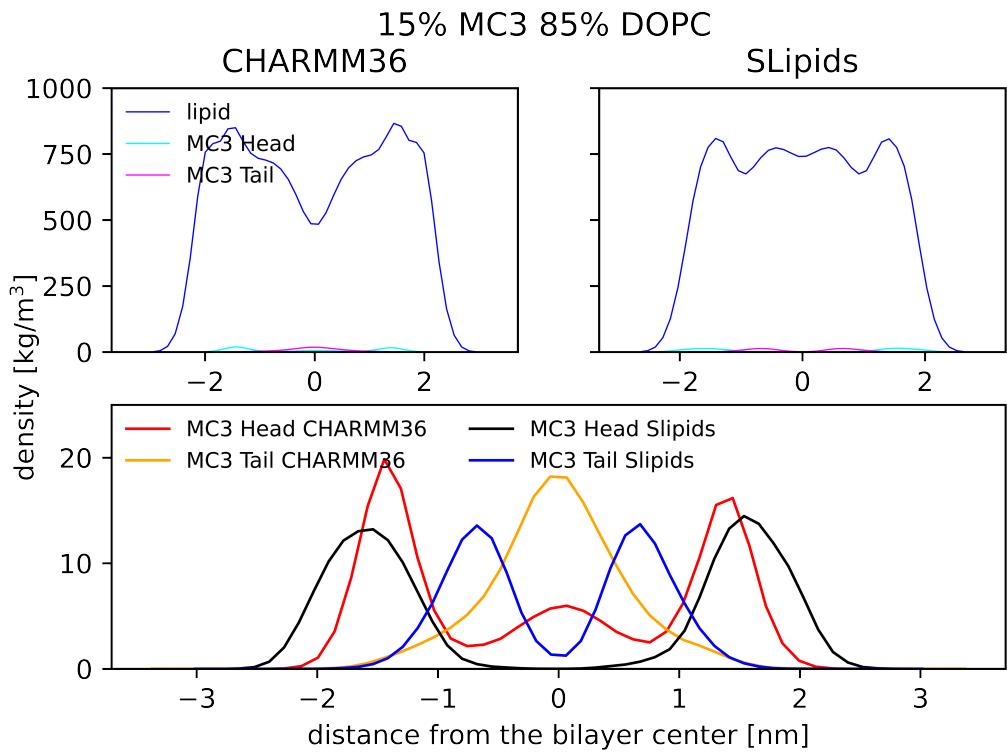


Figure 16: Mass density profile for the 15% MC3 lipid in a DOPC bilayer

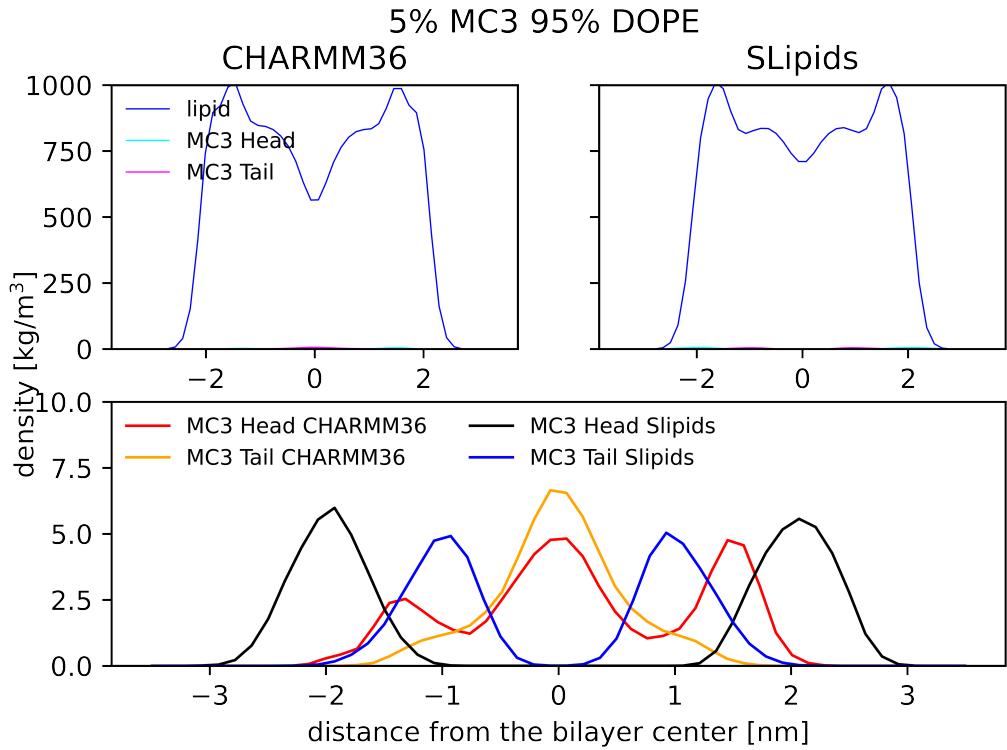


Figure 17: Mass density profile for the 5% MC3 lipid in a DOPE bilayer

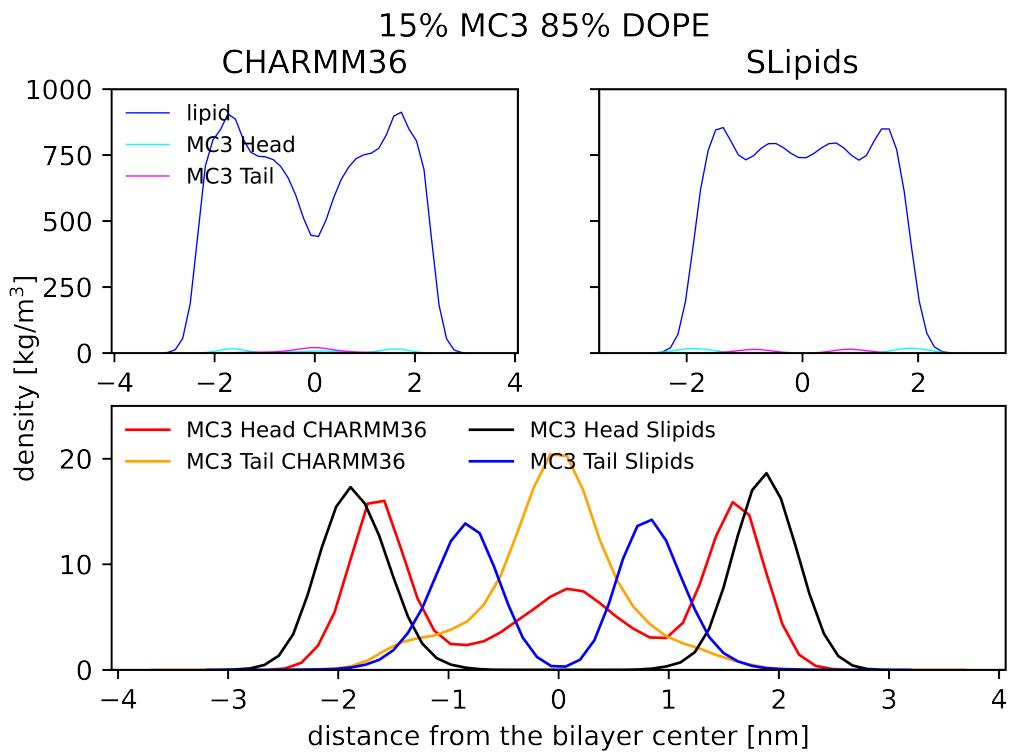


Figure 18: Mass density profile for the 15% MC3 lipid in a DOPE bilayer

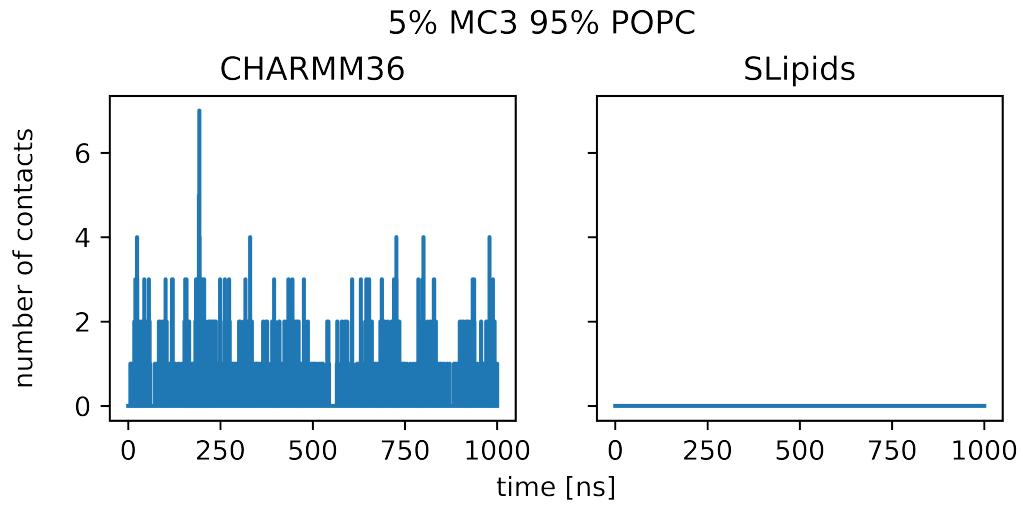


Figure 19: Number of contacts between the MC3 lipids. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

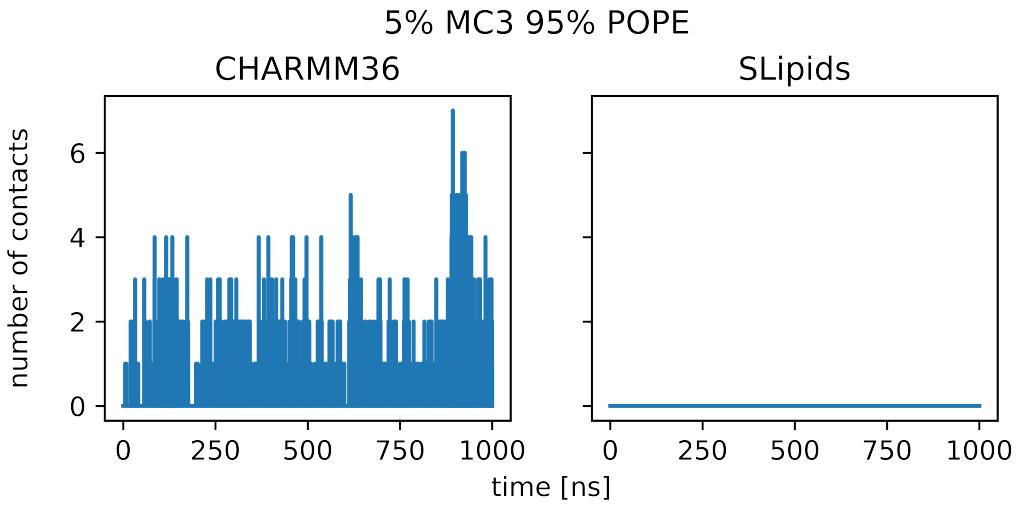


Figure 20: Number of contacts between the MC3 lipids. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

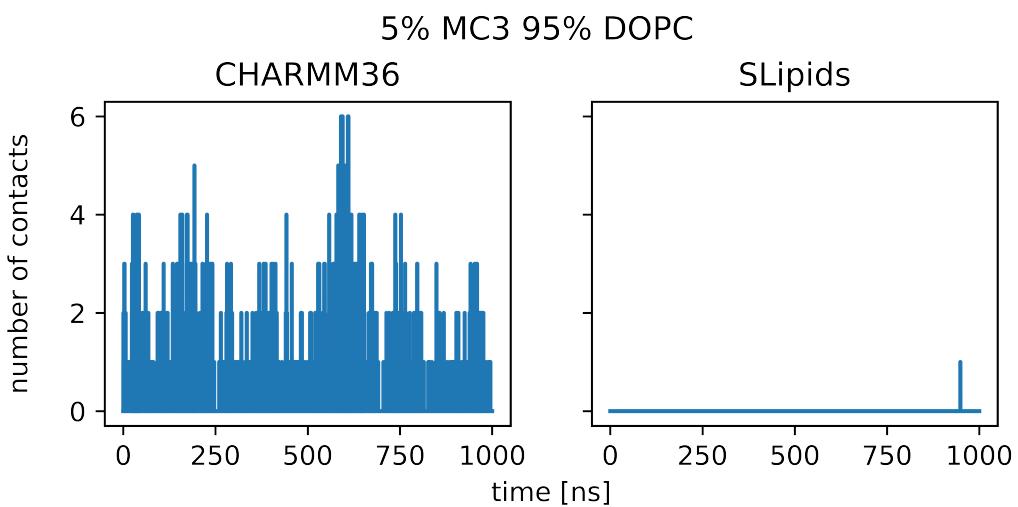


Figure 21: Number of contacts between the MC3 lipids. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

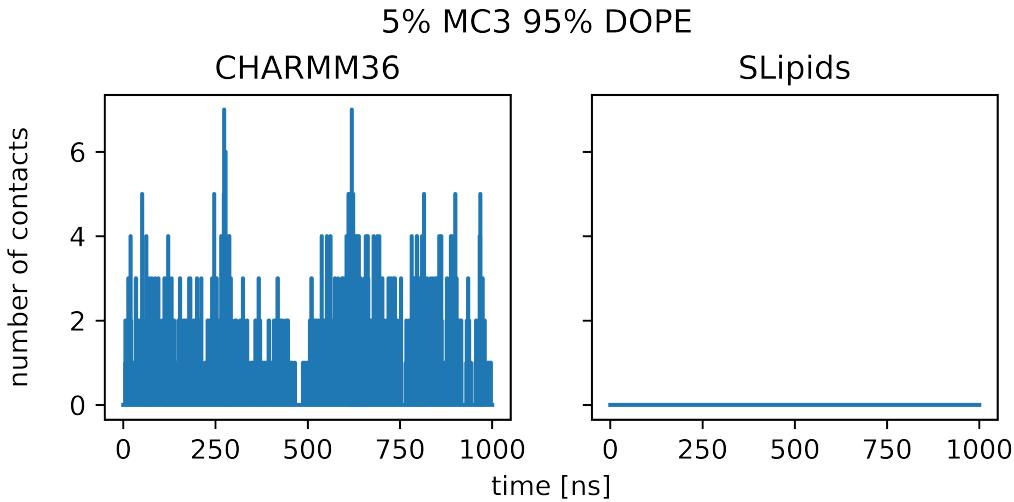


Figure 22: Number of contacts between the MC3 lipids. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

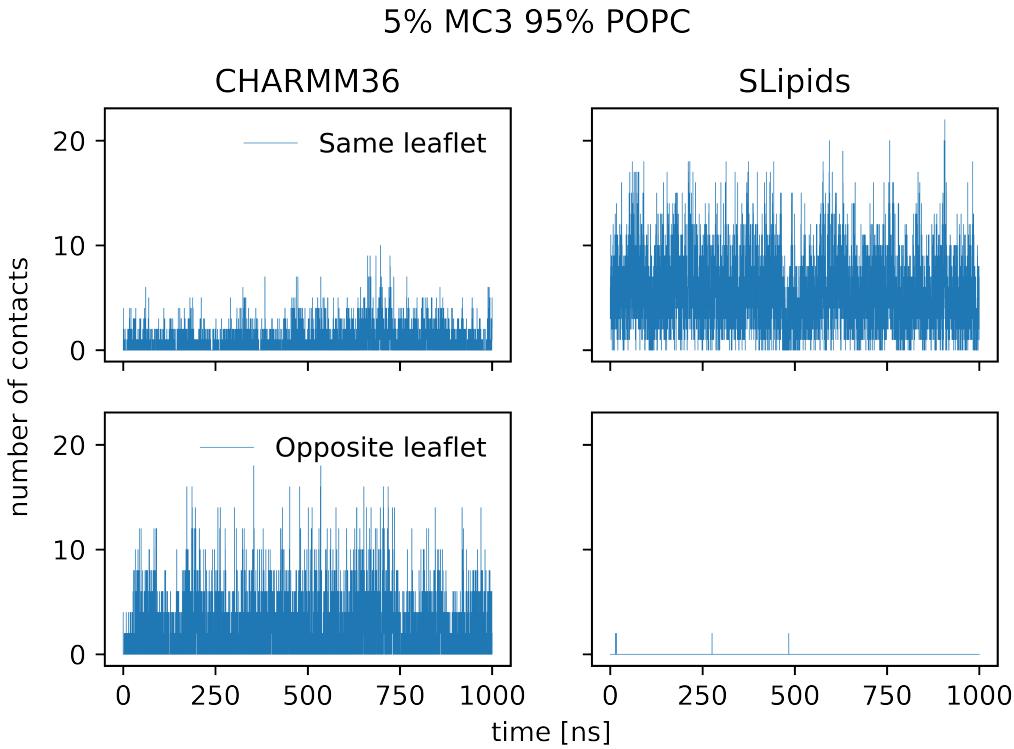


Figure 23: Number of contacts between the MC3 lipids. The first row is the contacts between the lipids that have been initially in the same leaflet. The second row is the number of contacts between the lipids that have been initially in the opposite leaflet. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

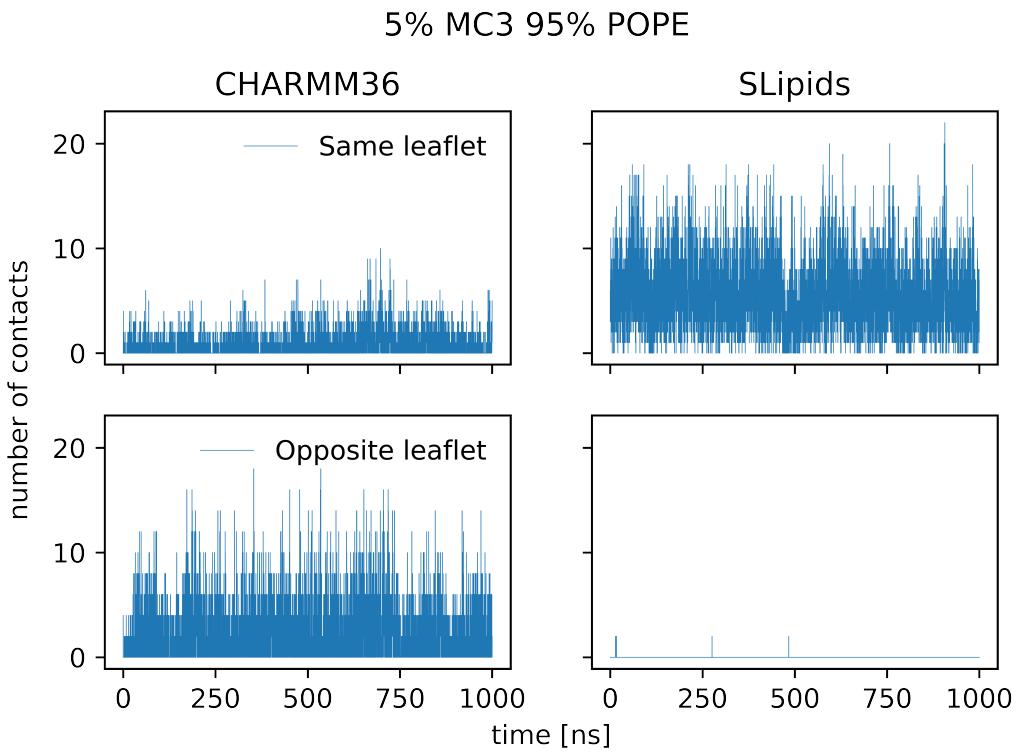


Figure 24: Number of contacts between the MC3 lipids. The first row is the contacts between the lipids that have been initially in the same leaflet. The second row is the number of contacts between the lipids that have been initially in the opposite leaflet. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

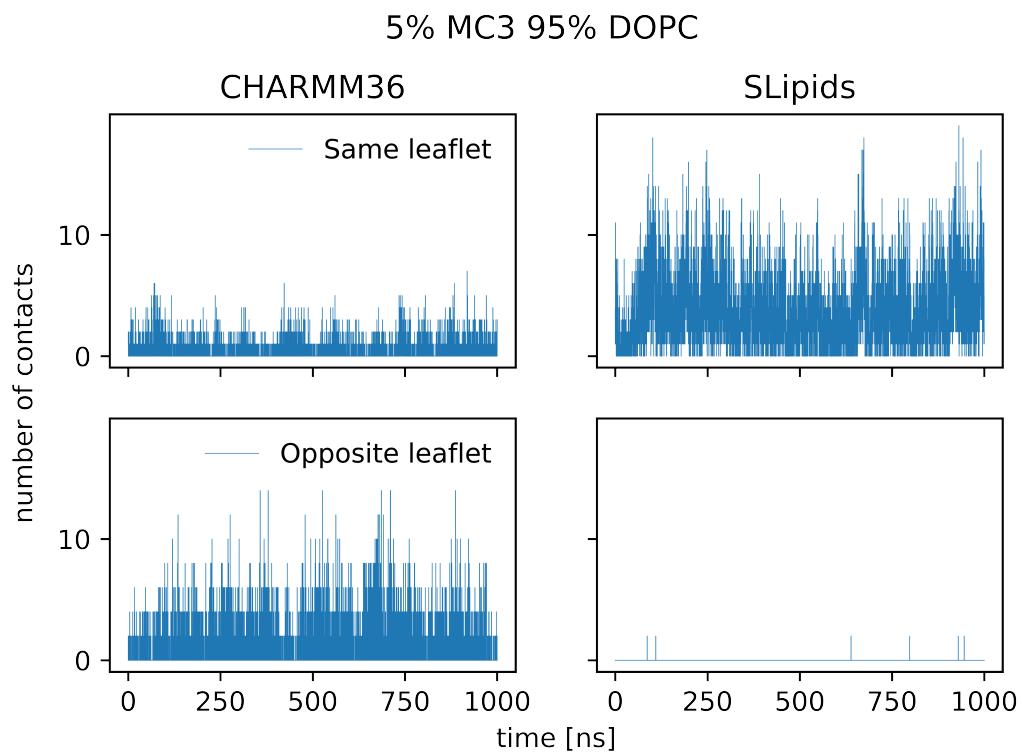


Figure 25: Number of contacts between the MC3 lipids. The first row is the contacts between the lipids that have been initially in the same leaflet. The second row is the number of contacts between the lipids that have been initially in the opposite leaflet. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

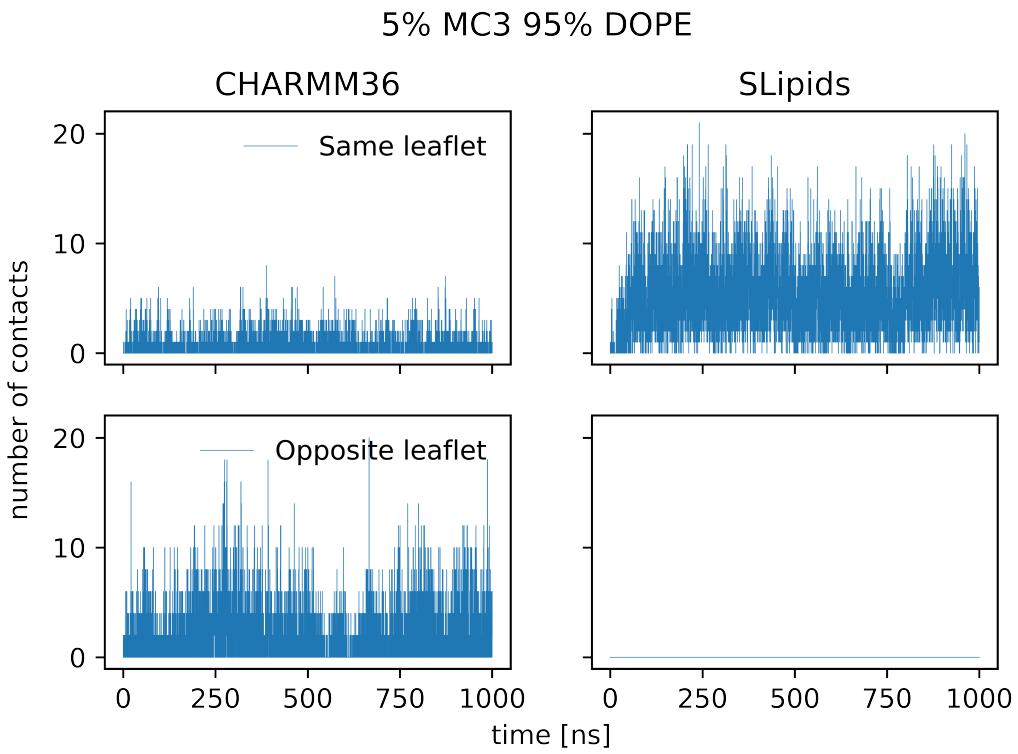


Figure 26: Number of contacts between the MC3 lipids. The first row is the contacts between the lipids that have been initially in the same leaflet. The second row is the number of contacts between the lipids that have been initially in the opposite leaflet. If the distance between two non-hydrogen atoms is less than 0.35 nm we count this as one contact.

## References

- (1) Park, S.; Choi, Y. K.; Kim, S.; Lee, J.; Im, W. CHARMM-GUI Membrane Builder for Lipid Nanoparticles with Ionizable Cationic Lipids and PEGylated Lipids. *Journal of chemical information and modeling* **2021**, *61*, 5192–5202.
- (2) Ermilova, I.; Swenson, J. DOPC versus DOPE as a helper lipid for gene-therapies: molecular dynamics simulations with DLin-MC3-DMA. *Physical Chemistry Chemical Physics* **2020**, *22*, 28256–28268.