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Supplement for: The Vesicle Builder – A Membrane Packing Algorithm for the CELLmicrocosmos MembraneEditor

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Abstract

This supplementary Material contains an advanced protocol of the Molecular Dynamic Simulation discussed in the paper "The Vesicle Builder - A Membrane Packing Algorithm for the CELLmicrocosmos MembraneEditor". The MembraneEditor as well as the Vesicle Builder plugin can be downloaded from `https://Cm2.CELLmicrocosmos.org`

CCS Concepts

• ***Software and its engineering*** → *Software prototyping;* • ***Theory of computation*** → *Packing and covering problems;* • ***Human-centered computing*** → *Visualization toolkits;*

1. MD Simulation

The capability of the Vesicle Builder to create structures compatible to *Molecular Dynamic (MD)* simulations was evaluated by creating a vesicle and performing a MD simulation with GROMACS. Here, the protocol is described in more detail than in the original manuscript.

1.1. System Generation and Setup

The vesicle was generated with the Vesicle Builder plugin for the CmME using the PDB files for cholesterol, DPPC and POPE, which were extracted from a simulated membrane (the phospholipids are based on [KF08], cholesterol is based on the dataset "ffgmx_lipids.tar.gz" [Ros15] and "ffgmx.rtp" from an older GROMACS version) and imported to the local CmME database. The Vesicle Builder was started with a random seed of 70 and a radius of 75 Å. Since membranes can contain up to 30% cholesterol [CKM*13, Bar05], the lipid distribution was chosen the following way: 30% cholesterol, 70% for phospholipids, more precisley: 40% DPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine) and 30% POPE (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine).

When the desired lipid numbers (1300 for EL and 700 for IL) were reached, the PDB file was exported. For this purpose, the MembraneEditor provides PDB export settings compatible to Gromacs. In order to perform an MD simulation, a 21*21*21 nm^3 box was defined around the vesicle using *editconf* and filled with water using *genbox* with the SPC-water model [Som13]. Since genbox places water all over the system, some water molecules were placed inside the membrane. This would make the system very unstable, so these molecules were removed using a small TCL script, which uses the *atomsel* module of VMD [HDS96].

In order to describe the energy of the system, the GROMOS96 forcefield [SDVG01, Hei13] with ffG45a3 parameters was used.

1.2. Simulation

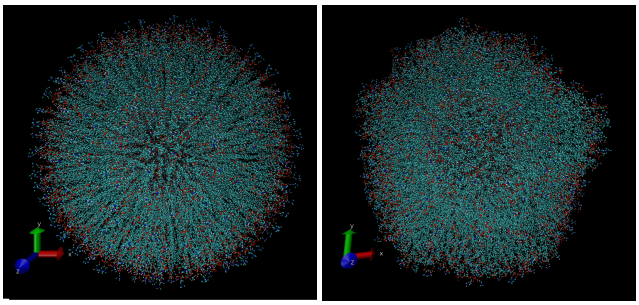

Figure 1: *MD Simulation. Left image shows the minimized and equilibrated starting structure of the vesicle, the right one the shape of the vesicle after 10 ns.*

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The MD production run was started with the structure which can be seen in Figure 1 left. The vesicle remained stable over 10 ns, see Figure 1 right. It was simulated on a cluster at the RWTH Aachen using 120 cores and an approximate runtime of 10 days. For this system the change of volume, density and energy was recorded. Over time, the density of the system increased (Figure 2 and the volume of the system decreased (Figure 3. For the total energy, a decreasing for 0.5 ns can be observed, after that, the value remains relative stable at -1.0812*10 7 kJ/mol (Figure 4).

Furthermore, the occurrence of rotations of molecules and the movement as well as the temporal size variations were analysed. As one can see in Figures 6 and 7, the maximal distance both for the EL and the IL increases during the simulation. The diameter of the IL becomes stable around 100 Å, the diameter of the EL becomes relatively stable around 180 Åand shows high fluctuations during the simulation.

In addition, a small deformation is visible; the shape changed from a nearly perfect sphere to a slightly egg-shaped one. Figure 7 shows for the internal layer, that the maximal start diameter is about 80 Å. During the simulation, this value increases for 4ns and than gets stable around 100 Å. In the external layer in Figure 6, the maximal start diameter is about 180 Å. One can see high fluctuations during the simulation, but it seems to get relatively stable around 180 Åafter 6 ns.

In this System, the cholesterol of the IL showed a movement of 57.77 Å/ns in average, the cholesterol of the EL a movement of 56.72 Å/ns. The phospholipids moved 49.19 Å/ns in average on the EL and 41.28 Åon the IL.

Eighteen EL phospolipid flip flops could be detected by the script, but an optical inspection showed, that none of these were real flip flop but just turns of the headgroup. The count of flip-flops for cholesterol was measured 38 for the IL and 27 for the EL. The cholesterol flip-flop rate for IL is 5.5 events/ns, and for EL it is 10.2 events/ns.

1.3. Discussion

The increasing of the layers depicted in Figures Figure 6 and 7 was not expected but can be backtraced to the lipid packing and the deformation. Analyzing the trajectory, the spherical form in the beginning deforms to a more ellipsoid-like form. The result is the enlargement of one axis, which can be seen in the maximal diameter. The high fluctuations in the EL during this process can be explained by the fact that some phospholipids tend to leave the membrane and then are pulled back to the membrane, which can be seen in the trajectory. Whereas the lipid movement of the IL has two constraints – the internal water and the EL, creating a cavity – the EL has basically one constraint: the external water.

Reasons for the slight deformation of the vesicle might be the too low temperature (300 K) or the unphysiologically solvation in water only. Another reason for the unstable EL could be the lipid distribution. In biologically realistic bilayers, the EL and the IL are asymmetric [Bre72]. Since both the EL and the IL have the exact same lipid distribution, this could lead to the unstable EL side of the vesicle. Also, the cholesterol rate may be too high, even if there

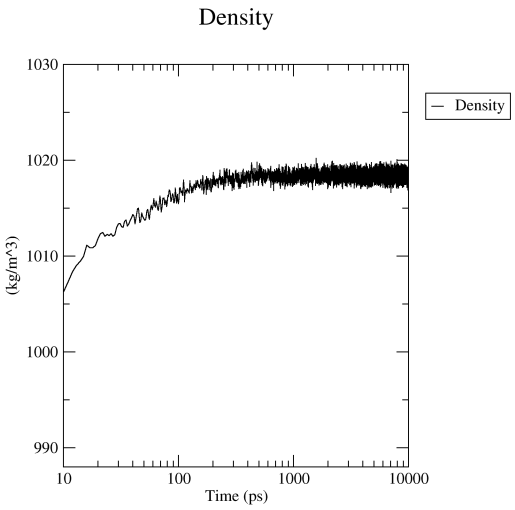

Figure 2: *Density in kg/m^3 of the system (logarithmic scale). Over time the density increases and after about 0.5 ns the density stabilizes at $1,019kg/m^3$.*

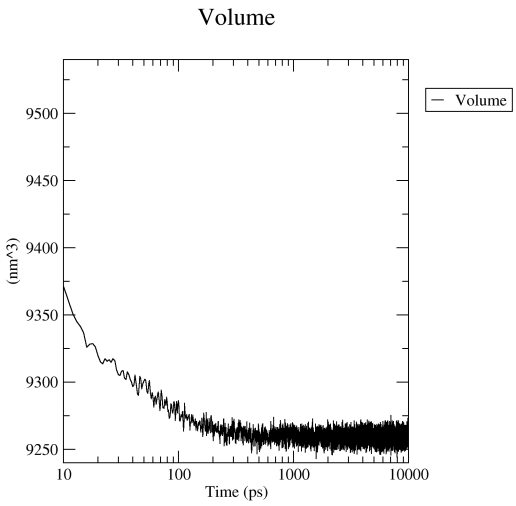

Figure 3: *Volume in nm^3 of the system (logarithmic scale). After approximately 0.5 ns the volume stays stable around 9,275 nm^3.*

exist membranes with this rate. In addition, realistic vesicle have a much more complex lipid distribution at all, containing much more different phospholipids, sterols and proteins. The size of the vesicle (15 nm in diameter) is also unrealistic, since vesicles in nature are at least around 25 nm in diameter [AJL*17]. This value could be reached with the CmME, but a high computing power is required to simulate such a big amount of atoms. An alternative is

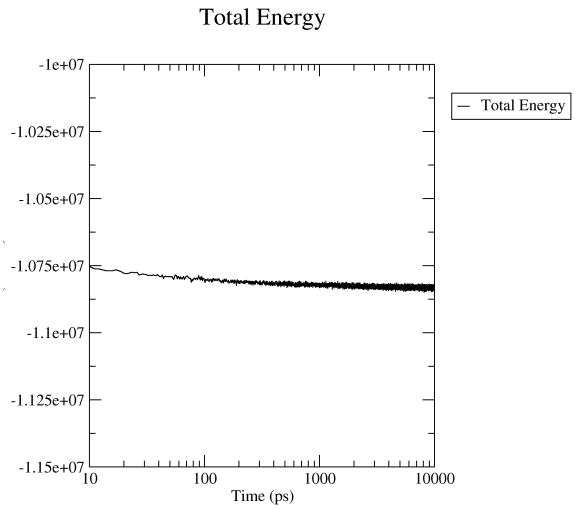

Figure 4: *Total Energy of the whole system (logarithmic scale). At t = 0, the energy has a value of approximately -1.075*107 kJ/mol, but after 5 ns the energy reaches a value of -1.083*107 kJ/mol which stays stable for the rest of the simulation.*

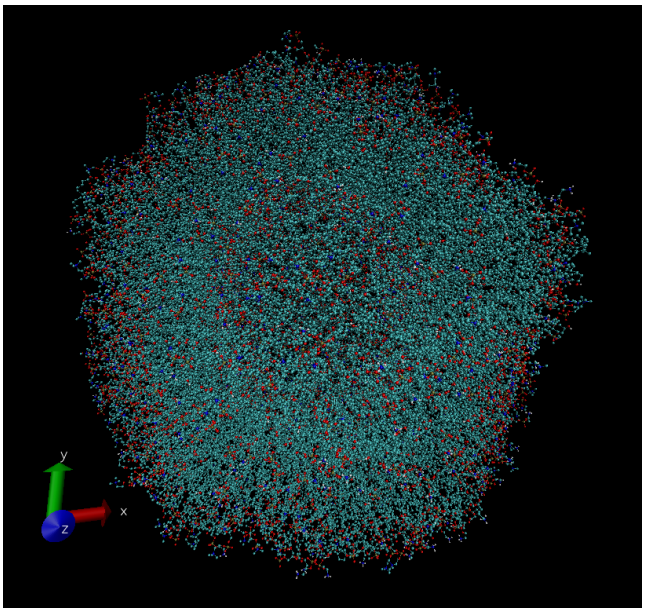

Figure 5: *Shape of the vesicle after 10 ns.*

too use coarse-grained approaches, such as the MARTINI force-field [MRY*07].

Another factor which could lead to deformations is the unrealistic environment. The fluid in which the vesicle is simulated consists only of water, and especially for the intravesicular fluid this is not the correct solvent [GM88]. For a realistic environment, the pH and

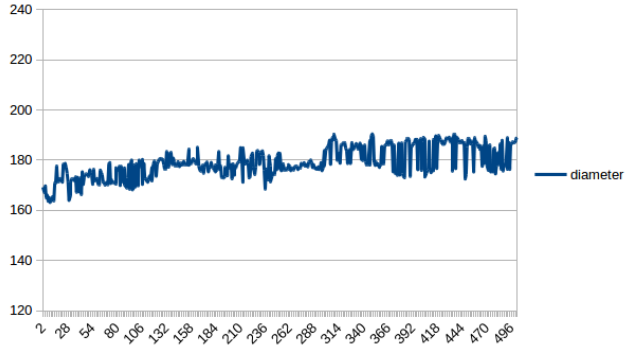

Figure 6: *Maximal diameter of the EL. Big fluctuations are visible, but the tendency shows an increase until frame 121, at which a relative relatively stable value of 180 Åis reached.*

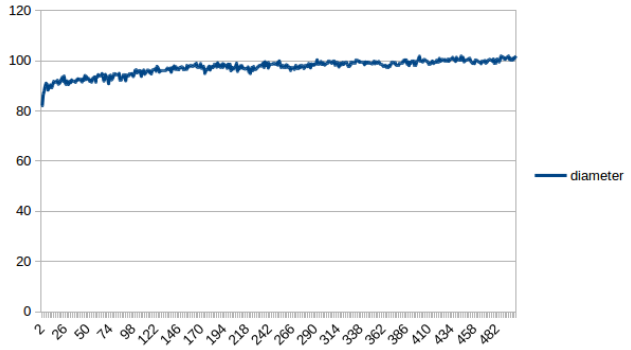

Figure 7: *Maximal diameter of the IL. The diameter seems to become stable around 100 Å.*

the amount of ions has to be considered. Besides, the temperature chosen for the simulation was 300 K, which is too low for mammals. Since the simulation under simple conditions was stable, the next step would be to choose more realistic conditions for a further run.

The flip-flop analysis showed, that neither in the EL layer nor in the IL layer a flip-flop event for a phospholipid happened. This was expected, since flip-flops are a very rare event for phospholipids (about once per month for a given lipid). The cholesterol flip-flop rates are very low. Since this vesicle has a very high cholesterol concentration, it may show a lipid raft like behaviour. This could explain the low flip flop rate, as shown by Marrink et al. [RM08]. Even if the flip flop rate calculated in this simulation is lower than the rates by Marrink et al., this simulation provides additional evidence that cholesterol flip flop can occur in very small timescale. It also has been shown, that the hydroxy group of cholesterol in polyunsaturated lipid membranes is often located at the centre of the bilayer [MdVH*08]. This can be also observed in this simulation (Figure 8. However, a flip-flop would be considered the change of the membrane side and not just a turn, not all of these events can be called flip-flop, but it shows the difference in mobility between cholesterol and phospholipids. This difference is also supported by

the movement of the lipids. The average of distance travelled by cholesterol is about 16% higher in the IL and 39% higher in the EL than the distance travelled by phospholipids. This can be explained by the structure of the molecules. Cholesterol consists mainly of 4 hydrophobic carbonrings with only one small hydrophilic hydroxy group. In contrast, phospholipids have 2 hydrophobic fatty acid tails, but also a large hydrophilic headgroup. A flip-flop requires the head group to move through the region with mainly hydrophobic structure to reach the other side of the membrane, this is extremely unlikely especially in such a short simulation time. Cholesterol has a hydrophilic group, but this group is so small that the energy barrier for the hydroxy group to move into the hydrophobic core of the membrane is not very high [CKM*13]. However, in this experiment a rectangular membrane patch and not a vesicle was used. Also the position of cholesterol in the core of the membrane (Figure 8) is consistent with recent research results [MdVH*08], since both fatty acids in the phospholipids are unsaturated.

Cholesterol has a very similar mobility in both the internal and the external layer. In case of the phospholipids, the movement in the external layer is about 19% higher than in the internal layer. In comparison, IL cholesterol moves 39% more than IL phospholipids, and EL cholesterol moves 15% more than EL phospholipids. The higher movement of cholesterol in comparison with the phospholipids can be explained by the structure of the cholesterol. Cholesterol is much smaller and more hydrophobic. Since the major part of the membrane (the core) is also hydrophobic, the movement of cholesterol has not such a high energy barrier as the one of the phospholipids. The same movement for cholesterol in the IL and EL verifies this measurement and shows, that both layers may have similar properties, which may result from the same lipid composition.

However, the movement of phospholipids in the IL and the EL are not the same. This behaviour is not as expected, but can be explained by the trajectory. The fluctuations of the diameter of the EL are much higher than in the internal layer, which necessarily results in a higher movement of the phospholipids in the external layer. The increasing density (Figure 2) can be explained by the decreasing volume (Figure 3). Since density is defined as mass/volume and the mass is constant (NPT ensemble), the volume has to decrease.

1.4. Conclusions

The original publication discusses three application cases. Here, we presented our second application case; a system which was simulated using GROMACS (Figure 1). The simulated system shows an increase in density, which can be interpreted as a sign for a too relaxed packing of the lipids. The measurement of the maximal diameter in the IL layer becomes stable around 100 Åand for the EL 180. Whereas cholesterol has a very similar mobility in both layers, the one of the phospholipids is about 19% higher in EL than in IL. IL cholesterol moves 39% more than IL phospholipids, and EL cholesterol moves 15% more than EL phospholipids. Whereas flip-flops for phospholipids did not occur, there was flip-flop rate for IL cholesterol of 5.5 events/ns and for EL cholesterol of 10.2 events/ns. This relatively low rates are confirmed by recent research which come to the conclusion that cholesterol flip flop can happen on a very short time scale [RM08, BMH*09]. However, the flip flop rate in microsecond scale as calculated here, might not be correct as Kamp et al. (1995) [KZZ*95] and Choubey et al. [CKM*13] suggested. Additionally, the scripts used to calculate the flip-flop rate and the lipid movement underlay relative naive assumptions providing simple estimations. For the flip flop script, one could consider a second condition besides the turn, which checks if the centre of mass made a movement along the normal vector of the lipid. The movement script could be improved by calculating the along the vesicle surface. This would require detailed knowledge about the surface of the vesicle, for example by precalculating an isosurface, which could be computed with the marching cubes algorithm [LC87]. However, the implementation of this algorithm would have been too time consuming for this project. In addition, for future analysis, it would be interesting to extend tools such as APL@Voro to analyse and visualize the area per lipid also for vesicular structures [LKS13]. Finally it can be stated that the simulation showed that it is possible to generate vesicles by using the Vesicle Builder in conjunction with the CmME which can be used for MD simulations.

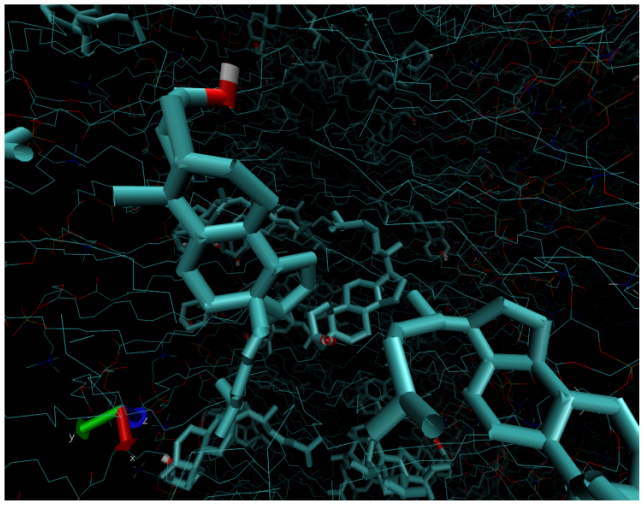

Figure 8: *Position of cholesterol molecules. It can be seen, that some are located in the core of the membrane (center) and some are in the EL or IL (left and right).*

The MembraneEditor as well as the Vesicle Builder plugin can be downloaded and installed from https://Cm2.CELLmicrocosmos.org

2. Acknowledgement

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3. Author Contributions

JZ developed a first simple prototype of the Vesicle Builder. BG extended his work towards a functional prototype during her ERASMUS project and her Master thesis under guidance from AG and

BS. MK used the Vesicle Builder to create a starting structure for MD simulations during a short internship: he performed the simulation under guidance from BS, did the analysis and wrote part of the supplementary material and the main publication. MK used methodologies established by AH and RR during their diploma thesis supervised by JK and BS. TD was the first main developer of the MembranEditor. BG, MK, JZ, JK, AG and BS wrote and revised the manuscript. BS lead the overall project.

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