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

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Abstract

For a long time, the major focus of membrane simulations was laid on rectangular membrane patches based on the fluid mosaic model. Because of the computational performance of today's computer hardware, it is now possible to generate and simulate larger structures, such as vesicles or micelles. Yet, there are no approaches available to generate these partly complex structures in a convenient and interactive way using WYSIWYG methods and exporting it to PDB format.

The CELLmicrocosmos 2.2 MembraneEditor was originally developed for the interactive computation of heterogeneous rectangular membrane patches, solving 2.5D packing problems. Now, its packing capabilities were extended into the third dimension by introducing the Vesicle Builder which is optimized for the computation of vesicular mono- or bilayer membranes. The shape computation is based on an ellipsoid formula enabling the generation of vesicles featuring different lipid compositions, shapes and sizes. More complex shapes can be generated by combining different shapes. Moreover, extended shape customization is possible by modifying and extending the algorithm.

Three application cases are discussed: 1) Different potential vesicular configurations including wavy, ellipsoid, enclosing and modular structures are modelled and shortly discussed; 2) To evaluate the compatibility of the Vesicle Builder with simulation tools, a three-component vesicle was modelled and successfully simulated. 3) To show the capability to generate large structures, a vesicle with a radius of 370 Å was generated, consisting of approx. 50,000 lipids and 2 million atoms, respectively.

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. Introduction

1.1. Vesicles

Lipid vesicles are small organelles which form a spherical component. Their size can vary from 25 nm up to 1 μ m in diameter, depending on their function and cellular location. Vesicles are composed of different phospholipids, glycolipids, sterols, free fatty acids and proteins. Phospholipids consists of a polar headgroup (e.g. cholin) and apolar fatty acids, which are both bound to glycerin. The apolar fatty acids aggregate in order to minimize contact to the surrounding water while the headgroups are presented to the solvated sides of the liposome. The components show a high lateral diffusion and a high rotation rate. A changing of the membrane side, also called a flip-flop, occurs rather rarely for phospholipids but quite often for cholesterols [AJL*17]. Vesicle membranes can also contain lipid rafts, which are cholesterol-rich membrane parts involved in protein sorting. Moreover, lipid rafts are often thicker than non-raft regions. The spherical form makes vesicles very stable, so that they can be purified by dialysis, chromatography or centrifugation [VVP06]. Since vesicles can fuse specifically with other bilayer membranes, they play an important role in the intracellular transport system and are connected with many essential cell functions. These properties make lipid vesicles very interesting for artificial, specific drug delivery systems. Using artificial vesicles with specific surface proteins allows the binding and fusion with characteristic target cells, minimizing the required drug concentration, the side effects and the delay of the effect.

Since the behaviour of vesicles strongly depends on different factors – such as the lipid and protein composition, environment factors like heat, pressure, pH, charge, etc. – a realistic model for hy-

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pothesis testing is required. This model can be developed by computationally creating molecular systems of vesicles and performing molecular simulations under desired conditions [CWNS08, SS97, JP75].
The plasma membrane of a typical animal cell is also a lipid bilayer which can be described by a circular and/or vesicular shape. This fact allows us to use vesicles as a small model for whole cell membranes. With such models, it would be possible to simulate inter- and intracellular mechanisms. In comparison to the simulation of rectangular membrane patches, vesicle simulations require additional computational resources.

1.2. Vesicle Generation

There exist only a small number of tools with the capabilities to generate vesicles based on the *PDB (Protein Data Bank)* format [BWF*00]. We provided an overview of membrane packing algorithms for 2.5D membrane packing problems [Som13]. Basically, the most important tools discussed there are nowadays also capable for generating vesicles.

CHARMM-GUI Micelle Builder is able to generate all-atom micelles compatible to different forcefields - such as CHARMM, NAMD and Gromacs - using a web interface. CHARMM-GUI Micelle Builder has a number of advantages: the micelle layer can be constructed of ca. 130 molecules which are available from the integrated library, a protein from the PDB database can be inserted, and the simulation files can be prepared using a solvation and energy-minimization process. However, it is only possible to generate micelles (no bilayers possible) and only a single protein can be added into the micelle layer. To construct vesicular bilayer membranes, the *CHARMM-GUI CG Vesicle Builder* can be used. This web tool is able to generate vesicular structures of different sizes, but the options are restricted to spherical shapes, and the maximum size is currently limited to 3 million atoms (personal correspondence with Yifei Qi, April 26, 2020). In addition, only coarse-grained systems can be generated based on 15 different lipid types, and supported force fields are NAMD and MARTINI (as of April 25, 2020) [CJL*13, QCH*14].

MemBuilder is an alternative web tool to the CHARMM-GUI tools for GROMACS users, offering also solvation and energy minimization of the generated spherical micelles or liposomes. MemBuilder is restricted to a maximum inner radius of 30 Å and provides only four different lipid types [GAA*14].

In contrast to the previously discussed tools, *PackMol* is a standalone software which supports custom lipid types. It is a command-line tool which can be locally used to generate vesicles. It is open source and has to be used in conjunction with scripts which define the composition of the molecular structures based on basic shapes: plane, cube/box, sphere, ellipsoid, and cylinder [MABM09].

None of the previously discussed functionality provides a fully interactive visualization in combination with the option to add custom lipids and proteins into the membrane. CHARMM-GUI and MemBuilder only provide spherical vesicle shapes. PackMol requires to visualize all vesicles offline and is limited to the vesicle configurations definable by the script file. However, a small number of different shapes can be combined in PackMol. In contrast to these tools, the *CELLmicrocosmos 2.2 MembraneEditor (CmME)* combines WYSIWYG, a number of different packing algorithms and interactive functionalities with support for custom lipid and protein files. But up to now, it was not supporting vesicle-like structures [SDG*11].

Here, a membrane packing algorithm plugin is presented which extends the capabilities of CmME. Moreover, by combining the multi-layer and raft functionalities of the CmME with the 3D packing of the Vesicle Builder, the generation of more complex 3D shapes is now possible, covering the whole range of the previously discussed tools and even more.

To support the previous statement, the first application case shows a number of shapes which can be generated by using the Vesicle Builder. Then, the structure of a vesicle model is evaluated by performing molecular dynamics simulations with GROMACS. Finally, the generation of a relatively large vesicle containing more than 50,000 lipids is discussed.

2. Methods

We start by introducing CELLmicrocosmos MembraneEditor (CmME). Then, the new methods in context of the Vesicle Builder will be discussed.

2.1. MembraneEditor

CmME is a software approach to model heterogeneous membrane systems. The user interface of CmME is shown in Figure 1. It allows to import different PDB files of lipids and proteins and the creation of lipid bilayers with predefined lipid distributions. It is also possible to add microdomains/rafts or multiple membrane layers. Moreover, tools for analysing the effective number of lipids and distribution percentages for the *internal layer (IL)* and *external layer (EL)* are integrated. The standard visualization simplifies the lipid structures to crystal-like shapes surrounding the original atomic structures of the original molecules. But it is also possible to examine and edit the atomic structure of each lipid in context of its environment [SDG*11].

One of the 2.5D lipid packing algorithms included in CmME is the *Random Placing (RP)* algorithm: molecules are randomly inserted into the 2D layer. The placing process is based on a random seed and therefore reproducible. The collision detection operates on the atomic structures of the molecules. The final structures can be exported to PDB format which can be optimized for the external tool or simulation package which should be used for visualization or further manipulations. For simulation purposes, CmME leaves the solvation and equilibration process to the applied simulation package which has been proven to be a reliable approach approach [SDG*11, RSP*12, LKS13, TBS*12, RSB*14, AAM*13, MAR*13].

The lipid filling process is accompanied by many iterations of shaking, twisting and approaching to the centre of the membrane (or the neighbouring protein). Meanwhile, the lipid internal atomic structures remain stiff. This method can be applied to an empty membrane area or to an already generated membrane for adding additional lipids. A few more complex packing algorithms build

on the RP algorithm: 1) The Advanced Random Placing algorithm uses RP to subdivide the membrane area into different small equal patches which are then subsequently filled with lipids; 2) The Distributor can be used to define an average area per lipid and uses RP to distribute lipids.

In addition, CmME contains a plugin manager which allows to import different membrane packing algorithms and tools for editing and improving the packing, distribution and properties of the membrane components. This plugin manager was used in a number of student projects to develop the Vesicle Builder. The basic idea of the lipid distribution process is taken from the previously-mentioned Random Placing algorithm. CmME leaves the minimization and equilibration process to the simulation package of choice. That this is a reasonable approach, was shown before [SDG*11] and will be also evaluated for the VesicleBuilder in chapter MD Test System (Section 3.2).

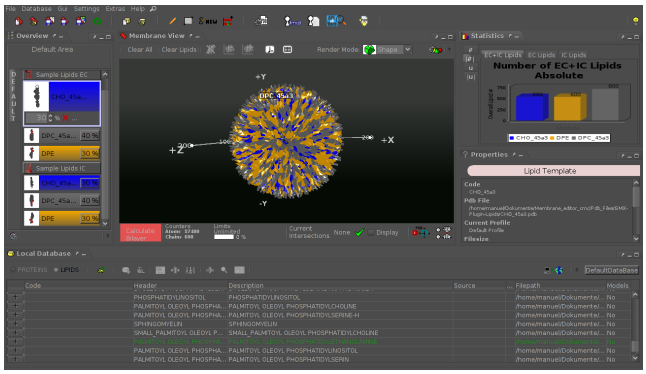

Figure 1: *The MembraneEditor GUI. Left: the lipid distribution. Bottom: the lipid database. Top: the 3D window showing vesicles in shape visualization. Right: the effective number of lipids after the membrane generation process has finished.*

2.2. 3D Membrane Packing Problems

As previously mentioned, the CmME was originally developed to solve 2.5D membrane packing problems [SDG*11]. Basically this means that the lipids are placed onto a 2D area, but the collision detection is operating on three dimensions, taking into account the complete three-dimensional atomic structure of the lipids. In case of vesicles, this is not sufficient anymore. Here, a 3D membrane packing problem has to be solved: the collision detection is operating in three dimensions, plus the placement of the lipids is occurring in three-dimensional space.

2.3. New Membrane Packing Algorithm: Vesicle Builder

To solve 3D membrane packing problems, the Vesicle Builder was developed. This is a plug-in implemented for CmME with the aim to create heterogeneous ellipsoid single- or double-layered membranes. The workflow consists of a few simple steps. In the CmME database, lipids are stored in the PDB format. Here, also custom PDB files can be imported to be used for the lipid placement. After adding one or more lipid types to the membrane model, the relative percentage distribution has to be set. When the "Calculate Bilayer" procedure is started, a GUI appears and the user can modify the X, Y, Z semi-axis dimensions and propose a desired number of lipids. Figure 4 shows the GUI. There is a simple version, where only the shape of the vesicle can be changed and the number of lipids is automatically predicted by the program. In addition, it is possible to switch to the PRO GUI (Figure 4 right), enabling to change a number of different algorithm settings.

2.4. The Ellipsoid

For the positioning of lipids on the surface, a coordinate system has to be defined. The Vesicle Builder follows two main principles based on the geometry: 1) the vesicle is modelled as an ellipsoid, and 2) lipids are placed following a three-dimensional polar coordinate system.

The ellipsoid (Figure 2) is a three-dimensional surface where the semiaxes on the X, Y and Z axis (respectively a, b, c) can assume different values. Varying these values, the shape will change its characteristics: the ellipsoid becomes a sphere if $a = b = c$, it becomes a spheroid, if only two sides are equal, and the ellipsoid becomes a cylinder if one of the variables is set to ∞.

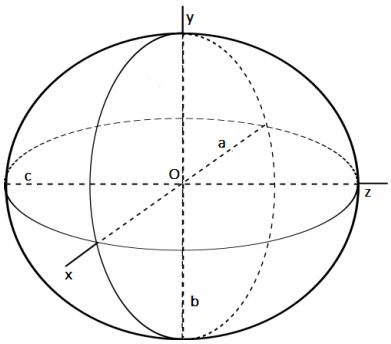

Figure 2: *Schema of a three-axial ellipsoid.*

Lipids have to be placed on the surface of this ellipsoid. The polar coordinate system (Figure 3) is used for the determination of the coordinates for each molecule of the vesicle. The position on the sphere is determined by r, θ and ϕ. The radius r is the Euclidean distance between the molecule and the origin O, the angle θ represents the longitude of the molecule (movement on the $X-Z$ plane, called also azimuthal angle) and the angle ϕ the latitude (the distance from the pole, also polar angle).

The equation of a ellipsoid can be defined as:

$$\begin{cases} x = x_0 + a * \cos\theta * \sin\phi \\ z = z_0 + b * \sin\theta * \sin\phi \\ y = y_0 + c * \cos\phi \end{cases} \quad (1)$$

where $(0 \leq \theta \leq 2\pi)$ and $(0 \leq \phi \leq \pi)$. Each molecule on the ellipsoid is determined by the three coordinates (x, y, z), where the centre of the vesicle is in (x_0, y_0, z_0). The radius r in Figure 3 is derived from the semiaxes a, b and c in Equation 1. The computation

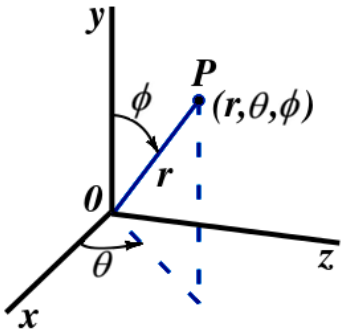

Figure 3: *Polar coordinate system. The point on the spherical surface is defined by radius r and the angles θ and ϕ. Image redrawn based on Kreyszig 2011 [Kre11]*

of the area per lipid (see Section 2.5) needs the arithmetic formula for obtaining the scalene ellipsoid surface. An appropriate formula is provided by the Knud Thomsen expression which approximates the surface area S with [Kla71, Kla76]:

$$S \approx 4\pi\left(\frac{a^p b^p + a^p c^p + b^p c^p}{3}\right)^{\frac{1}{p}} \quad (2)$$

where a, b, c represent the ellipsoid's semiaxes. The relative error is $\pm 1.061\%$ in the worst case, when p in Equation 2 is ≈ 1.6075 [Mic13].

Figures 4 and 5 show the predicted vesicle properties in numbers, and the 3D view visualizes a simple preview of the spheroid using three circles . To be able to predict the size of the vesicle, a number of aspects have to be taken into account.

2.5. The Vesicle Builder Workflow

The first task is to propose adequate values for the vesicle dimensions. The proposed vesicle size depends 1) on the size of the membrane area, 2) on the heights of the different lipid types and 3) on the size of the internal cavity. In fact, as the reader can see in Figure 6, on the same portion of ellipsoid (green), different numbers of lipids can be added on the internal and external layer. The volume of each head group is a bottleneck for the placing, so in the internal side lipid tails are less packed than their head groups. For predicting the more accurate number of lipids, different areas have to be considered.

The second problem to be solved is to predict the number of lipids to be placed onto both sides of the vesicle together with the expected area per lipid, depending on the chosen semiaxes. In Figure 5 the resulting area per lipid is computed, based on the X-Z dimensions of the bounding box of each lipid type. In case of heterogeneous vesicles, all the lipid types extensions have to be taken into account.

The surface area is computed based on Equation 2. Each membrane side refers to the virtual sphere between the inner and outer membrane layer. The *area per lipid (APL)* of the different lipid

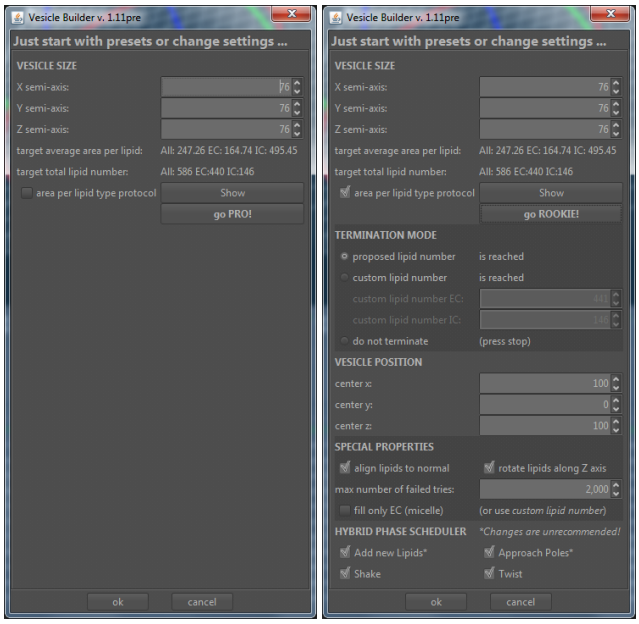

Figure 4: *Vesicle Builder GUI. Left: the simplified ROOKIE GUI can be used to directly start the vesicle generation process without the need to change much settings. Only the ellipsoid properties of the vesicle's shape has to be set, whereas the optimal number of lipids is internally predicted. Right: the PRO GUI of the Vesicle Builder providing full control over all settings.*

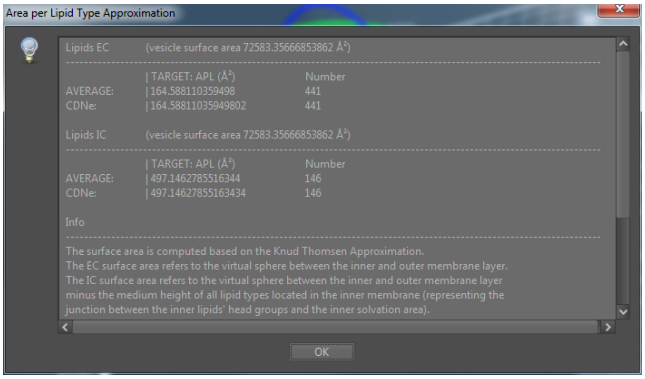

Figure 5: *Vesicle Builder: Area per lipid type approximation. Here, the area for each lipid is roughly predicted.*

types (*APLT* in Figure 5) is pre-computed based on 1) the radius of a lipid type as computed by CmME (which is based on the projection of the lipid size onto the membrane surface area), or 2) the sum of the 2D bounding box X and Z values as computed by CmME and divided by 2 [SDG*11]. The smaller value has the higher priority.

The generation of the APL values listed here is based on three values: 1) the pre-computed APL by CmME, 2) the predicted number of lipids based on 1. in combination with the lipid type percentages, and 3) the surface area based on Equation 2. All APL values are computed in Å^2.

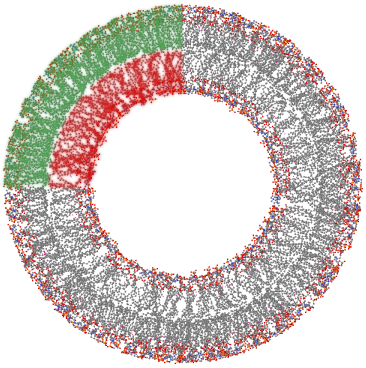

Figure 6: *Ellipsoid Cut. For the same portion of the ellipsoid, different numbers of lipids are added to the internal (red) and external side (green). The structure was generated with the Vesicle Builder.*

The placing of lipids is done in two steps. In the initial phase of the algorithm, lipids are positioned choosing random coordinates. Then, in the hybrid phase, three steps alternate cyclically: adding of new lipids, approaching, shaking and twisting (Figure 7). The idea is taken from the previously-mentioned Random Placing algorithm for the production of rectangular membrane patches. With this mechanism, the new free space is filled with new additional lipids at each iteration.

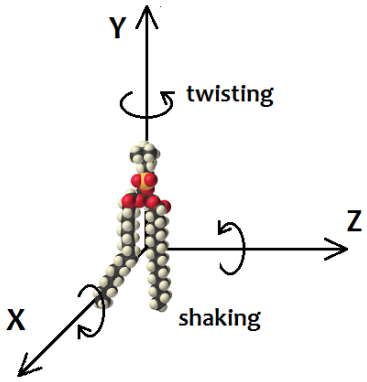

Figure 7: *Shaking and Twisting. The shaking is the rotation around the X and Z axis, the twisting along the Y axis.*

3. Application Cases

3.1. Vesicle Shapes

Based on the previously discussed methods it is possible to generate vesicular membranes with different shapes:

- spheres with $a = b = c$ (Figure 9 left and 10),
- ellipsoid with $a \neq b \vee a \neq c$ (Figure 8.1),
- cylinders with one of $a \vee b \vee c = \infty$ (Figure 8.5).

Moreover it is possible to generate more complex shapes by using the Vesicle Builder in combination with the raft and multilayer support of CmME. Figure 8 shows these shapes.
To generate liposome models containing e.g. differing membrane compositions on the left and right side of the vesicle, or to define rafts with a specific lipid composition it is possible to define microdomains (Figure 8.1). To illustrate vesicle fusion processes, it is possible to combine and merge two different vesicles (Figure 8.2). Strongly deformed membranes - undergoing for example the process of vesicle formation - can also be computed. As an example, a tilde-like bilayer patch was generated by combining two sphere segments (Figure 8.3). If a patch-like structure is required which does not follow a simple rectangular shape – which are often used for conventional MD simulations – then the mircodomain tool of CmME can be used to define the outer boundaries of the patches. Elliptical, rectangular as well as free-hand patches (such as the small raft in Figure 8.1) are possible. One example is the circular patch featuring a slight curvature (Figure 8.4). Also tube-like structures – which are known from the prokaryotic capsule – are possible. Here, a tube with two hemispheres as endings was generated (Figure 8.5). It is also possible to place e.g. a number of micelles into a liposome (Figure 8.6). This approach can be used to model multilamellar vesicles which are often used as drug carriers. By using microdomains, any of these structures can be cut. For example, the illustration in Figure 6 – showing a cut through a sphere structure resulting in a ring structure – was also modelled with the Vesicle Builder. The microdomains are basically used to define the limiting area in which the molecular structure should be created. The ellipsoid vesicle in Figure 8.1 requires a single packing process in which all lipids are placed; two microdomains are required: the one for the small raft and one to define the limiting area for the right hemisphere. Figure 8.2,3,5 and 6 require different packing stages, depending on the number of different geometrical shapes to create. Moreover, the resulting models might have to be manually optimized by using CmME. For example in Figure 8.2, the packing algorithm will place a few lipids in the centre area where both vesicles fuse. These redundant lipids have to be manually deleted by using CmME.

3.2. MD System

The Vesicle Builder was evaluated by creating a vesicle and simulating it with GROMACS. Here, the protocol is briefly described.
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 30% for cholesterol and 70% for phospholipids; in this case 40% DPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine) and 30% POPE (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine). When the desired number of lipids (1300 for EL and 700 for IL) was reached, the PDB file was exported with properties described in the appendix. 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 gen-

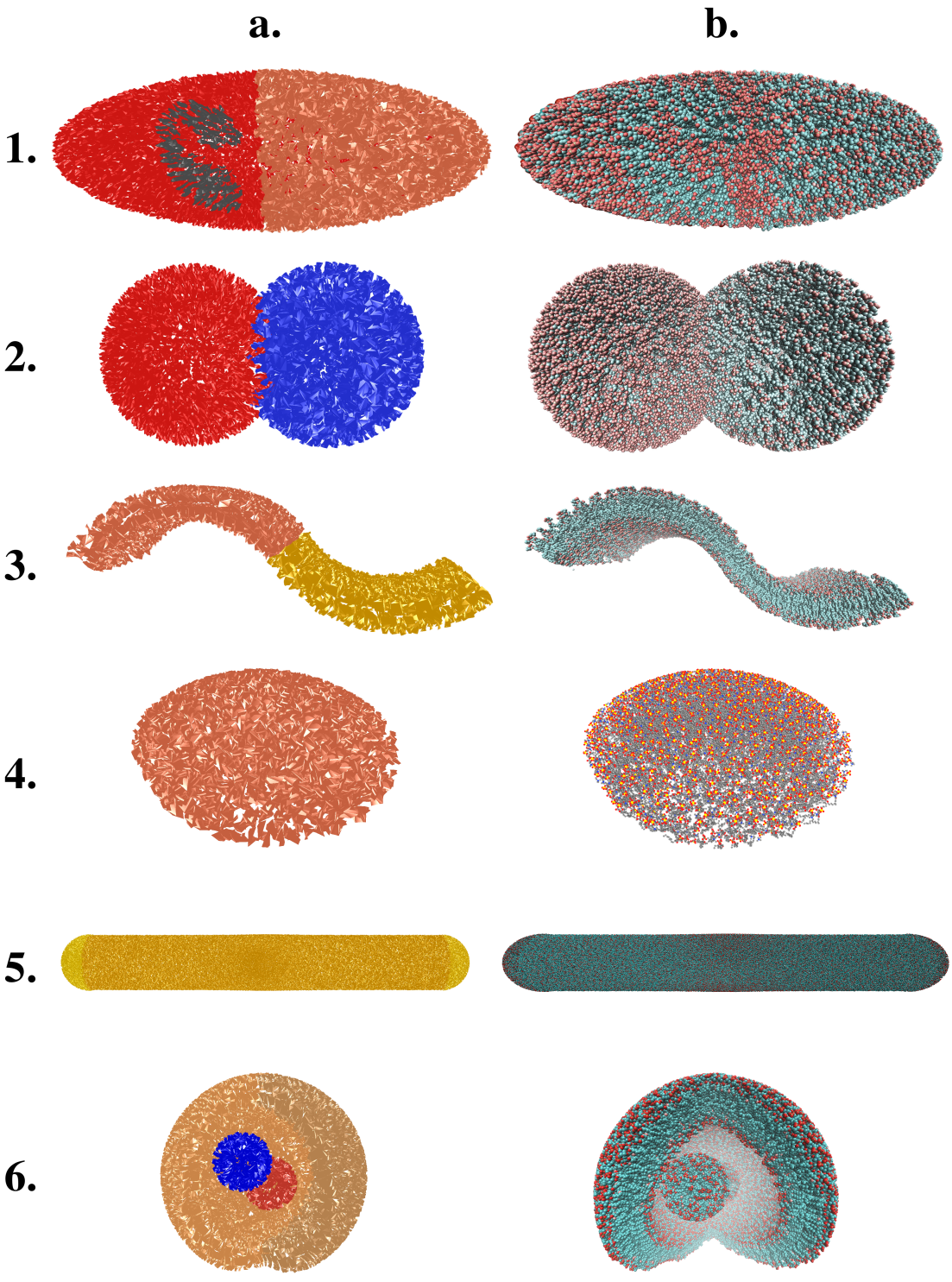

Figure 8: *Different vesicle shapes as shown with CmME and VMD. Left images shows the structures as generated in CmME in shape visualization, right images show the same structures imported to VMD (except 4b which is taken from the covalent radius visualization in CmME, 1-3 and 5-6 show the Van der Waals visualization of VMD).*

box 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 with ffG45a3 parameters was used [SDVG01, Hei13].

The MD run was started with the structure which can be seen in Figure 9 left. The vesicle was stable over the simulation time of 10 ns, see Figure 9 right. It was simulated on a cluster at the RWTH Aachen using 120 cores and an approximate runtime of 10 days.

In short it can be stated that the simulation remained stable after 10 ns, but also that a small deformation is visible; the shape changed from a nearly perfect sphere to a slightly egg-shaped one.

More details on the simulation setup and the analysis are found in the Supplementary Material.

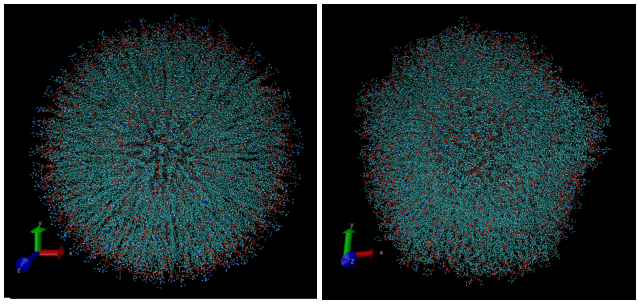

Figure 9: *MD Simulation. Left image shows the minimized and equilibrated starting structure of the vesicle, the right one the shape of the vesicle after 10 ns.*

3.3. Large Vesicle Generation

To test the capabilities of the Vesicle Builder, a large test system was created. The lipid composition is the same as the previously discussed MD test system; 3:4:3 (Cholesterol:DPPC:POPE). For membrane computation a reasonable desktop computer was used with an Intel® i7-4790 Processor (3.6 GHz) and 32 GB Ram. For visualization, the computer was equipped with a NVIDIA Quadro K4200. A vesicle with a radius of 270 Å and more than 50,000 lipids (EL 27,784, IL 24,073) was created, featuring 2,266,154 atoms. The creation of the vesicle with shape-based visualization required 1 h, 28 m and 7 s. The computed average area per lipid was approx. 62 $Å^2$ for EL and 71 $Å^2$ for IL.

4. Conclusions

It was shown that the Vesicle Builder can be used to create both vesicles and more complex shapes, such as membrane fragments, tubes, wavy structures, merging vesicles etc. (Figure 8). The different systems can be used as illustrations, can help predicting area per lipid/packing densities, or to generate starting structures for MD simulations. The only related tool which might be able to reproduce some of our examples given in Figure 8 is PackMol. But especially CmME's capability to combine different multilayer structures with the freehand microdomain drawing tool enable more complex forms than PackMol allows.

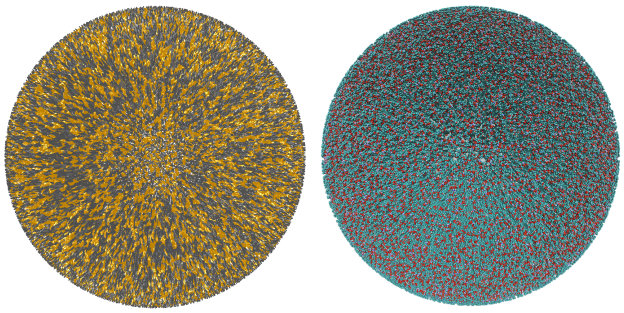

Figure 10: *The large vesicle as shown in CmME and VMD. Left image shows the vesicle as generated in CmME (shape visualization), right image shows the same structure imported to VMD (Van der Waals visualization).*

The second application case presented a system which was simulated using GROMACS (Figure 9). 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 Å and for the EL 180 Å. 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 phospholipid flip-flops did not occur as expected, there was a flip-flop rate for IL cholesterol of 5.5 events/ns and for EL cholesterol of 10.2 events/ns. These 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, there are alternatives to calculate the flip-flop rate which might be tested in the future [KZZ*95, CKM*13]. .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 lipid's path along the vesicle's curved 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 MD simulation-compatible vesicles by using the Vesicle Builder. In terms of micelles, CHARMM-GUI Micelle Builder provides a good alternative, but is very limited in size. Larger sizes plus vesicular structures are possible with the CHARMM-GUI CG Vesicle Builder, but it is currently limited to 3 million atoms and supports only coarse-grained structures. The MemBuilder tool supports only four different lipid types and the generation of very small vesicles. In contrast to these tools, CmME enables the user to import nearly every PDB lipid file into the database and use it for packing.

As shown by our third application case, this tool can be used now to model larger mono- or bilayered membranes (Figure 10), with and without proteins, investigating different vesicular shapes to observe the functional behaviour of these membrane structures. In case of larger structures like vesicles, also coarse-grained molecular dynamic approaches based on, e.g., the MARTINI forcefield can be used [MRY*07]. CmME also supports the generation of coarse-grained membranes.

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

5. Acknowledgement

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