7.4: Designing Molecular Membranes Models with VMD

Introduction

While modern experimental techniques are able to resolve membrane-bound protein function, structural determination is still a challenge. To provide insight into the behavior of membranes and the proteins residing within them, computational biologists utilize molecular dynamics (MD) simulations. Since the early 2000's there has been a remarkable increase in the number of computational methods, software, and visualization aids, reinforcing the need for MD [Ref. 1]. This tutorial is for users to become familiar with producing and visualizing atomistic membranes for MD simulations. This tutorial will use Visual Molecular Dynamics (VMD), a molecular visualization program of large biomolecular systems, to visualize membrane models. Additionally, the tutorial will discuss how CHARMM-GUI, a molecular systems generation web interface, can be used to build membrane models. Completion of this tutorial will allow the user to:

1. Design, visualize, and select certain lipid sections using the VMD command interface.
2. Visualize homogenous phosphatidylethanolamine (POPE) and phosphatidylcholine (POPC) membranes using VMD's *MembraneBuilder* extension tool.
3. Create solvated, heterogenous lipid membrane models using the CHARMM-GUI *Membrane Builder* extension tool.

This tutorial uses a Linux 64 version of VMD (ver. 1.9.3 OpenGL). Other system downloads can be found here: www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD [Ref. 2]. Other versions may result in an inexact rendition of the tutorial, however, the concepts remain valid and should be practiced. Experimentation is encouraged. The CHARMM-GUI *Membrane Builder* extension can be found here: http://www.charmm-gui.org/?doc=input/membrane.bilayer [Ref. 3-8].

Note: text in *italics* indicates verbatim text/tools/commands that can be found/typed on your screen when going through the tutorials. User exercises are also indicated in *italics.* Figure references are highlighted in **bold** to indicate helpful visuals.

1. Basic VMD visualization of POPE

The Protein Data Bank (PDB) is the largest online repository containing more than 150,000 three-dimensional biological structures to date [Ref. 9-11]. Computational biologists frequently use the PDB as a starting point for their molecular models, either implementing existing PDB structures (as .pdb file formats) or formatting their own PDB template for molecular dynamics (MD). However, most PDB files do not contain membrane structure components; computational biologists typically design their own membranes and insert protein structures within them. Thus, this section is an introduction for new users on how to navigate Visual Molecular Dynamics (VMD) software before building membranes. This section will use the lipid phosphatidylethanolamine (POPE) as the example structure. For a review on lipid types and their properties, visit the BPH 241 Lipids webpage: https://phys.libretexts.org/Courses/University_of_California_Davis/UCD%3A_Biophysics_241_-_Membrane_Biology/Lipids

1.1. Contents of a .pdb file and loading the POPE model

Phosphatidylethanolamine (POPE) is a glycerophospholipid and one of the most abundant lipid types in bacteria. In the downloadable files section (bottom of page), you will find the file *1.1_single_POPE.pdb*. A .pdb file is a Protein Data Bank file containing all of the experimental and three-dimensional coordinate information needed to visualize the biomolecule **(Figure 1.1)**. More information on .pdb files and their syntax can be found here: http://www.wwpdb.org/documentation/file-format [Ref. 12-13].

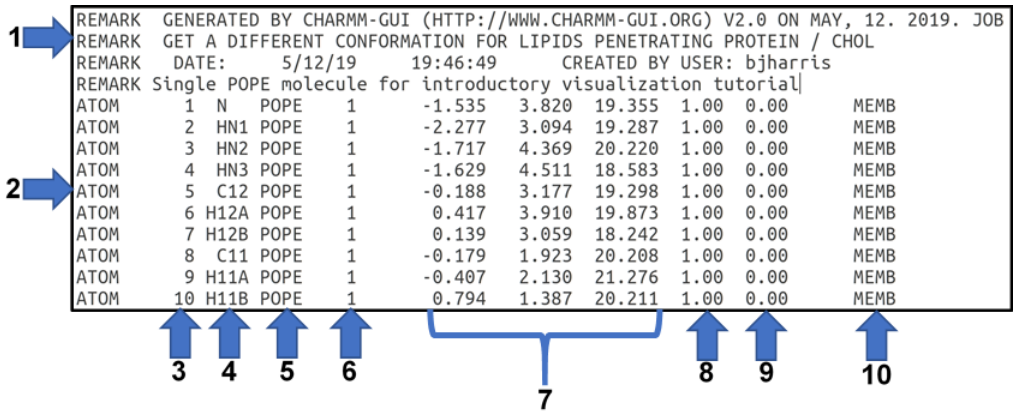

Figure 7.4.1.1. *Example text from a .pdb file.* ***1)****The REMARK section is for user comments.* ***2)*** *The ATOM section contains coordinate and atom type information.* ***3)*** *The index (serial number) of each atom. Note while PDB files start at 1, the index for VMD starts at 0.* ***4)*** *The atom name.* ***5)*** *The residue/molecule name* ***6)*** *The residue/molecule sequence number.* ***7)*** *The orthogonal X, Y, and Z coordinates in Angstroms.* ***8)*** *The occupancy.* ***9)*** *The temperature factor.* ***10)*** *Unread information stating that this is part of a membrane. Further explanation of .pdb syntax can be found in [Ref. 12].*

After downloading the VMD software, redirect to the VMD folder and open the README file for installation instructions. In addition to installation, Linux users will have to add a VMD path to their .bashrc file to call from the terminal. On the last line of your .bashrc file, add *export PATH=/type/your/directory/here/vmd:$PATH* **(Figure 1.2)**. Now, the keyword *vmd* can be typed in the Linux terminal to launch VMD. Note: Some Linux distributions, Windows, and Mac devices as well as VMD software distributions may differ; always read the README file for the best installation guidance.

```
export PATH=/usr/local/bin/vmd:$PATH
```

Figure 7.4.1.2. *An example of adding VMD to your .bashrc file, located in your home directory.*

After launching VMD, you will see two windows: the *VMD Main* and the *VMD Display*. The *VMD Main* window contains the executable commands and options to manipulate the molecule on the *VMD Display*. Next, from the *VMD main* window, load the *single_POPE.pdb* file using *File → New Molecule...* A new window called the *Molecule File Browser* will appear. From here, click *Browse* and navigate to the folder containing *single_POPE.pdb*. After selecting, click *Load* in the *Molecule File Browser* window to load the POPE molecule to the *VMD Display* **(Figure 1.3)**.

Figure 7.4.1.3. *How to load a PDB file.* ***1)*** *The VMD main window contains all commands and options for manipulating models.*

To create a new session, click File → New Molecule... ***2)*** *In the Molecule File Browser, files can be selected with the Browse button.*

Once a file is selected the Molecule File Browser will determine the file type. A file is loaded by clicking the Load button.

3) *Once a file is loaded, it will appear in the VMD Display window. In this example, a single POPE molecule is displayed as lines.*

1.2 Basic VMD visualization features: orientation, rotation, and representation

The *VMD Display* has two primary mouse manipulation modes: rotate (press *r* on keyboard) and translate (press *t* on keyboard). More mouse mode commands can be found here: www.ks.uiuc.edu/Research/vmd/vmd-1.9.3/ug/node32.html [Ref. 14-15].

For rotate mode, the following mouse commands are:

- Left click and hold = free three-dimensional rotation
- Right click hold = rotation parallel to computer screen
- Mouse wheel = zoom in/out of center

For translate mode, the following mouse commands are:

- Left click and hold = translate molecule parallel to computer screen
- Right click and hold mouse wheel = zoom in/out of center

On the *VMD Display*, you should see the *Lines* representation of a POPE molecule; *Lines* representation is when atom types are represented by colored lines indicating the element type. In this model, the colors for each element are:

- cyan = carbon
- white = hydrogen

- red = oxygen
- orange = phosphorous
- blue = nitrogen

The *Lines* representation is helpful to visualize the entire molecule, but there are other representations that can be used. From the *VMD Main*, go to *Graphic → Representations…* A new window called *Graphical Representations* will appear **(Figure 1.4)**. Here you will see a selected molecule tab where you can visualize different molecules. You will also see *Draw style*, *Selections*, *Trajectory*, and *Periodic* tabs that tweak the visual representation of each molecule.

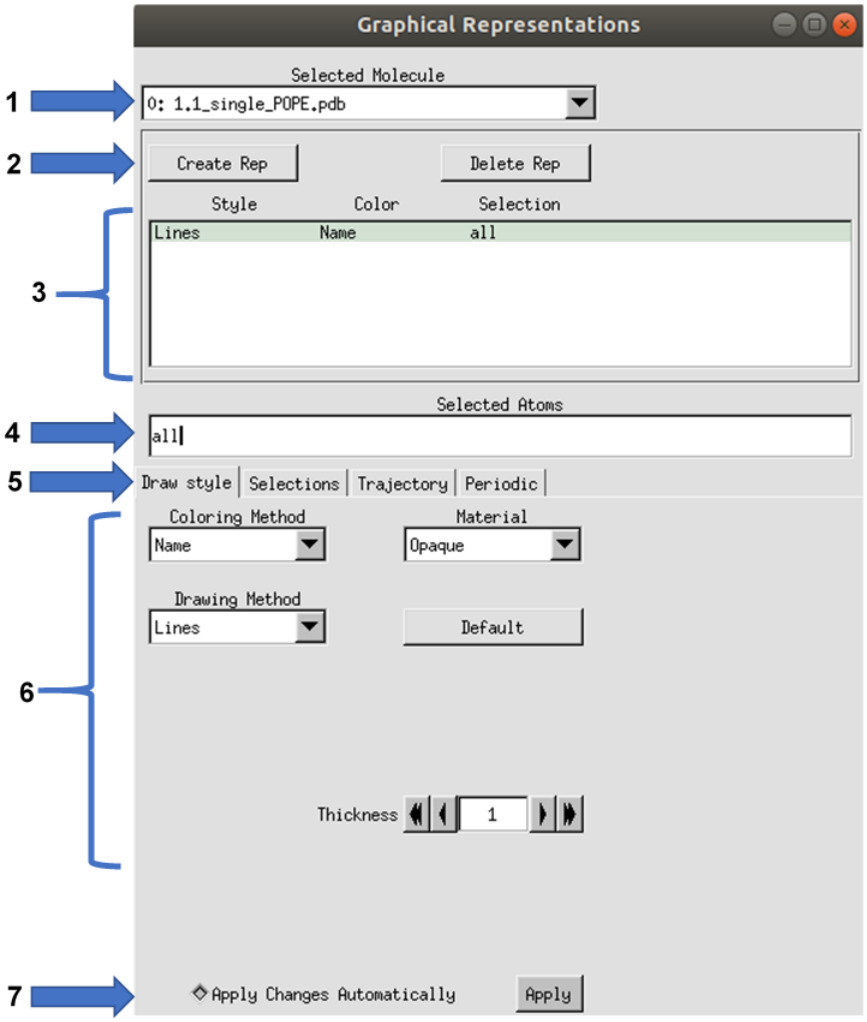

Figure 7.4.1.4. *The Graphical Representations tab allows you to modify the visual representation of your model.* ***1)*** *Select a molecule for editing.* ***2)*** *Create representations of the selected molecule. This is useful for representing the same model in different graphical styles.* ***3)*** *A window of all created representations. Highlight a representation to enable it for editing.* ***4)*** *A command window that can be used to select specific parts of an atom for visualization.* ***5)*** *The tab selection menu with the Draw Style tab open. The Draw Style tab controls visual representation of the model. The Selections tab allows precise selection of specific atoms within the model. The Trajectory tab controls animation features while the Periodic tab controls periodic image display; both will not be discussed in this tutorial but are explained in the VMD User's Guide [Ref. 15].* ***6)*** *All controls in the Draw Styles tab; the Coloring Method controls how atoms are colored, the Drawing Method how the model is drawn, the Material method how the model is shaded, and the Thickness controlling the model line thickness. Other options may appear as different Drawing Methods are selected.* ***7)*** *Once a graphical representation is complete, changes must be applied and can be toggled to apply automatically.*

Focus on the *Draw style* tab. In it, you can change the *Coloring Method*, the *Drawing Method*, the *Material* shading, and the *Thickness* of representation. Change the *Thickness* of the line from 1 to 5. It should be easier now to visualize the molecule. Reset the *Thickness* to 1.

In the *Drawing Method* selection pane you will find different molecule visualization schema. Some common styles are *Lines*, *VDW*, and *CPK* **(Figure 1.5)**, however there are other styles available **(Table 1.1)**. The *Lines* style is the oldest style which uses

lines as bonds and points as atoms. The *VDW* is a Van der Waals style and is a helpful space-filling representation. The *CPK* style can be thought of as a combination of *Lines* and *VDW* by providing scaled atoms as spheres linked by cylindrical bonds.

In the *Coloring Method* selection pane you will find different coloring schemes depending on atom typing, molecule collection, residue ID, and some physical properties. The effectiveness of these schemes will be dependent on your .pdb file parameters. For now, the *single_POPE.pdb* file is only useful with the *Name* method. Experiment with the various drawing/coloring methods. Afterwards, reset the molecule with *Coloring Method: Name* and *Drawing Method: CPK*.

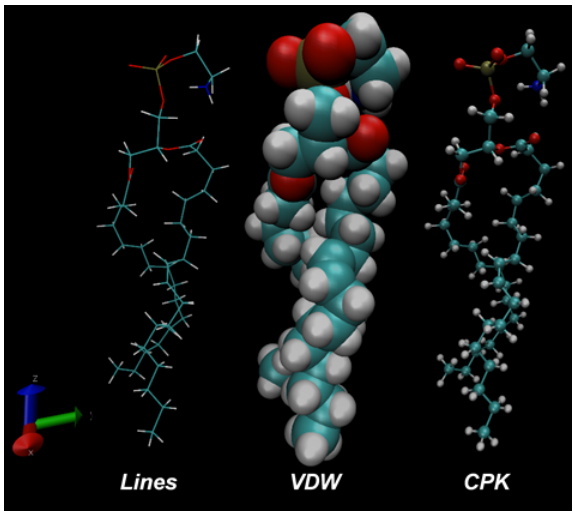

Figure 7.4.1.5. *POPE visualization using (Left) Lines, (Middle) Van Der Waals, and (Right) CPK draw styles.*

The Lines style is useful for understanding structure, the VDW style for space-filling, and the CPK style as a mixed style utilizing both Lines structure and CPK space-filling.

Table 1.1. *Molecular draw styles from VMD User's Guide Version 1.9.3 [Ref. 15]*

Representation styles	Description
Lines	simple lines for bonds, points for atoms
Bonds	lighted cylinders for bonds
DynamicBonds	dynamically calculated distance-based bonds
HBonds	display hydrogen bonds
Points	just points for atoms, no bonds
VDW	solid van der Waal spheres for atoms, no bonds
CPK	scaled VDW spheres, with cylinders for bonds
Licorice	spheres for atoms, cylinders for bonds, same radius
Polyhedra	polyhedra connecting atoms within a cutoff radius
Trace	connected cylindrical segments through C_α atoms
Tube	smooth cylindrical tube through the C_α atoms
Ribbons	flat ribbon through the C_α atoms
NewRibbons	smooth ribbon through the C_α atoms
Cartoon	cartoon diagram (cylinders and ribbons) based on secondary structure
NewCartoon	smooth cartoon diagram (smooth ribbons) based on secondary structure
PaperChain	display ring structures as polygons, colored by ring pucker
Twister	flat ribbon tracing glycosidic bonds, with twists oriented by sugar residues
QuickSurf	molecular surface (Gaussian density surface)
MSMS	molecular surface as determined by the program MSMS
Surf	molecular surface as determined by SURF
VolumeSlice	display a texture mapped slice from a volumetric data set
Isosurface	display an isovalue surface from a volumetric data set
FieldLines	field lines generated by integrating particles by volume gradient vectors
Orbital	molecular orbital selected by wavefunction type, spin, excitation, and orbital ID
Beads	per-residue approximate bounding spheres
Dotted	dotted van der Waals spheres for atoms, no bonds
Solvent	dotted representation of the solvent accessible surface

1.3. Identification of atom types

To label specific atoms, go to the *VMD Main* window and select *Mouse* → *Label* → *Atoms*. Clicking on an atom will show the molecule name followed by the atom name. From the color scheme, label the phosphorous atom followed by the nitrogen. To manage the labels, go to the *VMD Main* window and select *Graphics* → *Labels…* Here you can find a collection of the labels you have made. You can click on each label and show, hide, delete, and reposition them in the *VMD Display* **(Figure 1.6)**. For example,

select the *POPE1:P* (the phosphorous) atom label. Under the *Picked Atom* tab you will find the naming information (*ResName*, *ResID*, etc). In the *Properties* tab you can drag the crosshair in the *Offset* chart to reorient the label. In the *Global Properties* tab you can adjust your text size and thickness.

In addition to visualizing atom labels, you can visualize bond lengths and angles. Again, from the *VMD Main* window, select *Mouse → Label → Bonds*. To visualize a bond length or distance between atoms, you must click on the two atoms you are interested in measuring from. You can find the labeled distances from *Graphics → Label* in the *VMD Main* window. Similarly, you can calculate angles by clicking on three atoms **(Figure 1.7)**. It is important to note that VMD cannot display double bonds since the .pdb file does not contain bond information. Indeed, PDB files contain coordinate information only. VMD compensates for this by using a distance measure algorithm to determine which atoms are bonded. Protein Structure Files can also be used to explicitly state bonding information but are beyond the scope of this tutorial. Therefore, an intuitive understanding is sometimes needed for the viewer to fully understand the model.

Figure 7.4.1.6. *The labels window where you can identify atom naming schema and adjust visual labels.* ***1)*** *Select between atoms, bonds, angles, dihedrals, and springs labeling.* ***2)*** *The selection of atoms currently labeled. Those shown on the VMD Display are in black text while hidden labels are shown in red text.* ***3)*** *The Tab selection menu.* ***4)*** *The Picked Atom tab displays coordinate and naming information of the selected atom.* ***5)*** *The Graph tab plots data for the selected labels and will not be discussed in this tutorial.* ***6)*** *The Properties tab allows the user to move the label Offset portrayed on the VMD Display as well as rename the label Format.* ***7)*** *The Global Properties tab allows adjustments to label Text Size and Thickness.*

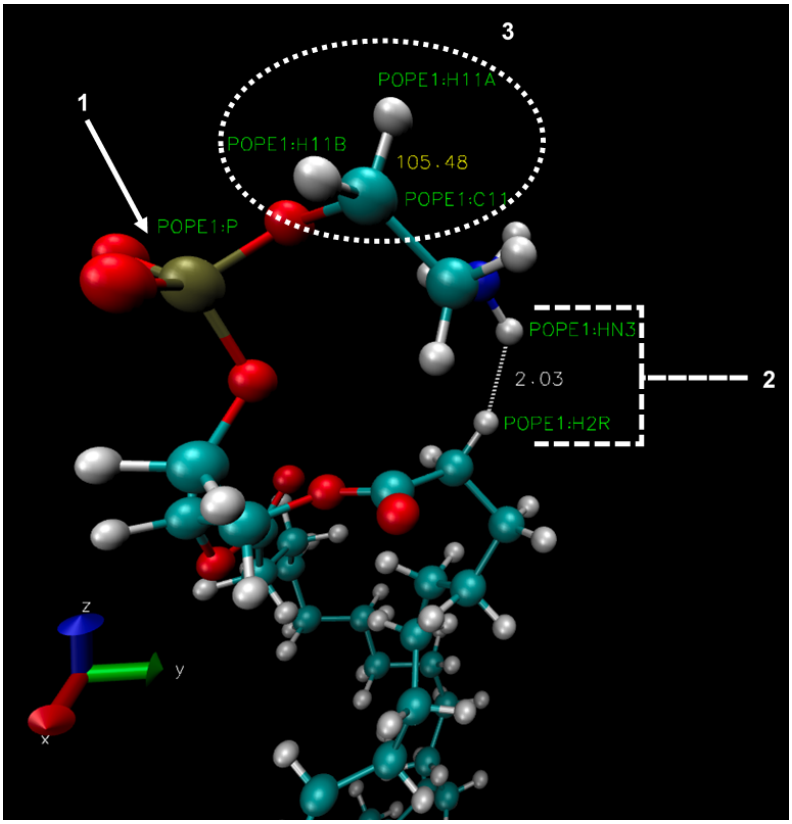

Figure 7.4.1.7. A POPE molecule demonstrating: **1)** The atom label of a phosphorous atom "POPE1:P".

2) *The atom distance between two hydrogen atoms as 2.03 Angstroms.*

3) *The angle between a carbon atom and two of its hydrogen bonding partners as 105.48 degrees.*

Exercises (section 1.3)

Exercise 1.1: Identify one atom of each element: Carbon, hydrogen, oxygen, nitrogen, and phosphorous. Rearrange each label so that they are all visible while clearly indicating the atom.

Exercise 1.2: How many double bonds are there in POPE? Where would you expect double bonds?

1.4. Advanced selection of specific regions

As you create more complex models you'll want to be able to specify a viewing region. This can be done by using the *Graphical Representations* window **(Figure 1.4)**. Again, from the *VMD Main*, select *Graphics → Representations…* to open the *Graphical Representations* window. First, create a new representation of the selected molecule by clicking *Create Rep*. In the representation box you'll now see two representations. By double-clicking you can hide the representation; hidden representations are in red text while visible representations in black text. Highlight the visible representation to enable it for editing.

In the selected atoms box, you can utilize certain keywords to enable or disable parts of your model. You can even use more complex strings as well as boolean or numeric values to specify your selection criteria; VMD's expression system is based on PERL (a computing language) syntax. Examples of these regular expressions can be found in section 6.3 of the VMD User's Guide [Ref. 15]. Back in the *Labels* window (*Graphics → Label)* you may have noticed certain labels having type information in the *Picked Atom* tab **(Figure 1.6)**. Keywords such as *ResName*, *ResID*, *Name*, *Type*, *Chain*, *SegName*, and *Index* can be used to specify a selection **(Table 1.2)**; descriptions of all keywords can be found in section 5.48 of the VMD User's Guide [Ref. 15]. For now, since there is only one POPE molecule labeled as *ResName: POPE* and specified as *Chain: X*, sort through the atom types for practice. To visualize all carbon atoms, use a regular expression such as **(Figure 1.8)**:

- *name "C.*"*

where the *C* represents the canonical carbon atom naming convention and the .* indicates any carbon atoms in the model. In an expression, this example means to search for anything classified as a *name* that starts with *C*. Another way to visualize carbon only atoms is by typing:

- *not name "O.*" and not name "P.*" and not name "H.*" and not name "N.*"*

Your representation should not have changed as you'll only see carbon atom types. In more complex models, you can specify single molecule chains or even molecule types:

- *resname POPE*
- *chain X*

In both of these cases you should see the entire phosphatidylethanolamine (POPE) molecule. More selection expressions can be found in Chapter 6.3 of the VMD 1.9.3 User's Guide [Ref. 15]

Table 1.2. *Examples of commonly found keywords for selection of specific model regions. Argument identifiers are: str=string and num=number. More information on other keywords can be found in [Ref. 15].*

Keyword	Arg	Description
resname	str	residue name
resid	num	residue id
name	str	atom name
type	str	atom type
chain	str	the one-character chain identifier
segname	str	segment name
index	num	the atom number, starting at 0

Figure 7.4.1.8. *Understanding atom selection.* ***1)*** *A window of all created representations. Highlight a representation to enable it for editing. Red text indicates hidden representations.* ***2)*** *Expressions can be input in the Selected Atoms command window. In this example, only carbon atoms are being selected. More examples can be found in the VMD User's Guide [Ref. 15].* ***3)*** *VMD provides a list of all expressions that can be used to specify selections.* ***4)*** *An example of all POPE carbon atoms displayed from the input (name "C.*").*

Exercises (section 1.4)

Exercise 1.3. Visualize all atoms but carbon in the single_POPE.pdb file.

Exercise 1.4. Using the index notation, visualize the polar head group. Hint: index notation for VMD starts at zero.

1.5 Saving and loading your session

From *VMD Main*, select *File → Save Visualization State…* Here you can specify which folder and what file name you would like using traditional file path syntax: *user/home/my_folder/my_file_name.vmd* Make sure you save as a .vmd or you will be unable to load the file! If you forget, rename your file so that you include the .vmd file type. To load your session, start VMD and from *VMD Main* select *File → Load Visualization State…* Note: *File → New Molecule…* is for importing new molecule file types only. It is better to use the *Load Visualization State* if you want to preserve your model representation settings **(**Figure 1.9).

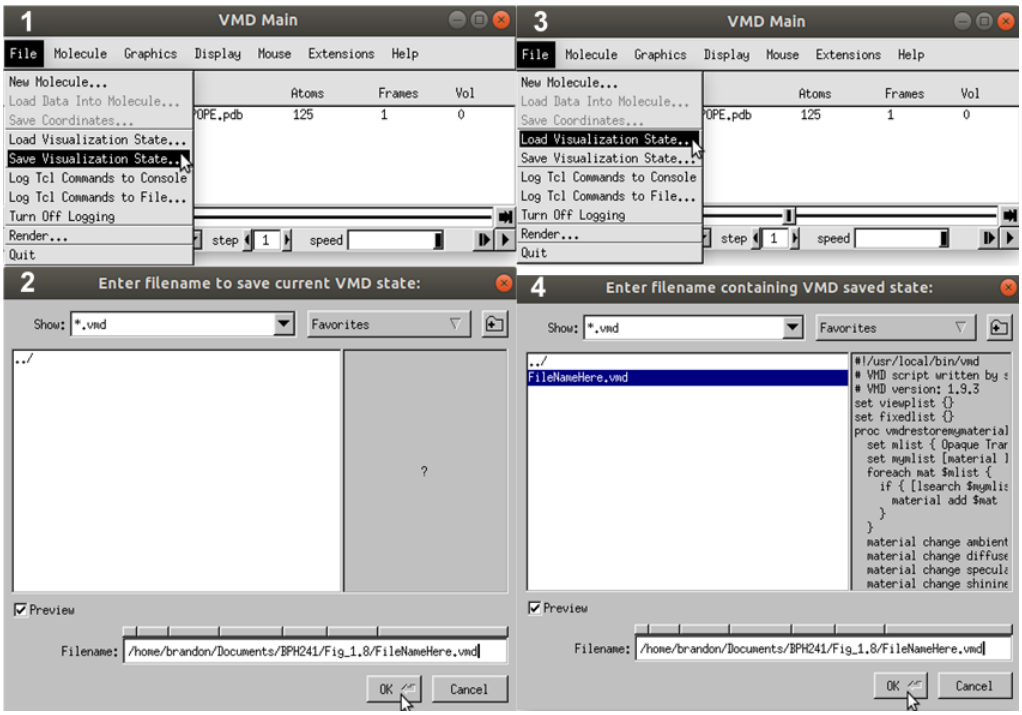

Figure 7.4.1.9. *Saving and loading sessions. **1)** To save a session, navigate to File → Save Visualization State… **2)** The save window will appear. VMD sessions are saved as .vmd files. **3)** To load a session, navigate to File → Load Visualization State… **4)** The load window will appear. Load a .vmd file to resume a session.*

2. Homogenous membranes using VMD's *MembraneBuilder* tool

The purpose of the Visual Molecular Dynamics (VMD) *MembraneBuilder* tool is to generate a membrane model surrounded by water which a protein can then be placed into. This complete lipid/protein/water model can then be used to run molecular dynamics simulations after generation of force field components and simulation parameters; a review on force fields can be found at [Ref. 16]. For this tutorial, the *MembraneBuilder* tool extends our interpretation of individual phosphatidylethanolamine (POPE) molecules to a homogenous POPE membrane. Unfortunately, the *MembraneBuilder* tool can 1) only generate homogenous POPE and phosphatidylcholine (POPC) membranes and 2) generate only water surrounding molecules. For more information on how *MembraneBuilder* executes its algorithm, visit here: www.ks.uiuc.edu/Research/vmd/plugins/membrane/ [Ref. 17]. For homogenous or heterogeneous membranes with the option of ion solvation, skip this section and proceed to Section 3 which uses the CHARMM-GUI interface to generate membranes.

2.1 Generate a POPE membrane

From *VMD Main*, select *Extensions → Modeling → Membrane Builder* to open the *Membrane* window. In this window, select phosphatidylethanolamine (POPE) as the lipid, and generate a 50x50 Angstrom^2 area. Change the output prefix to *POPE_membrane*. The *topology* section provides two CHARMM force fields that can be used: CHARMM27 and CHARMM36. Generally, CHARMM27 is for nucleic acids and lipids while CHARMM36 is for proteins and lipids; CHARMM36 is noted to have superior lipid parameters and thus should be used for most membrane biology models [Ref. 18]. After selecting CHARMM36 (c36), click *Generate Membrane*. You should now see a POPE membrane that is 50x50 Angstrom^2 with the leaflets surrounded by water. You will also note some patterns of randomness and discontinuity; perfect membranes are physiologically impossible thus stochasticity (randomness) is modeled. Visualize the membrane using a *CPK Drawing Method* as described in Section 1.2 **(Figure 2.1)**.

Figure 7.4.2.1. *Designing a membrane with MembraneBuilder.* ***1)*** *Access the MembraneBuilder window from the Extensions menu.* ***2)*** *The Membrane*

window allows users to generate a specific area (in Angstroms) of membrane. The membrane options are POPE and POPC with generation force fields

using either CHARMM27 (c27) or CHARMM36 (c36) ***3)*** *A side view of the generated POPE membrane with surrounding water from the tutorial instructions.*

Next, verify the generated membrane x-y length. From the *Graphical Representations* window **(Figure 1.4)**, remove the water by typing in the *Selected Atoms* text box:

- *not water*

In the *VMD Main*, select *Mouse → Label → bonds*. Using the x-, y-, and z-planes, take 5 rough measurements and record the average; a review on how to do this is in Section 1.3. You'll notice that the membrane area will be less than 50 Angstrom^2 (around 45 Angstrom^2 average). This is likely due to the close packing arrangement performed after initial generation of the model. You can save these models as discussed in Section 1.5 or save the membrane as a PDB coordinate file. From the *VMD Main*, *File → Save Coordinates…* will open a *Save Trajectory* window where you can select the molecule, atoms, and file format you wish to save as.

Exercises (section 2.1)

Exercise 2.1. Take an average of 5 measurements to determine the thickness of the membrane bilayer. Does this match experimental values? Section 1.3 describes how to measure distances.

Exercise 2.2. Take an average of 5 measurements to determine the water thickness. Why does the model need water? Should thickness be lower? Should thickness be higher?

3. Heterogeneous membrane building using CHARMM-GUI *Membrane Builder*

The CHARRM (Chemistry at HARvard Macromolecular Mechanics) force field was initially developed for proteins and nucleic acids but has since been extended to feature a robust set of lipid types [Ref. 19-20]. The most recent force field, CHARMM36, supports proteins, nucleic acids, carbohydrates, lipids, and small molecules [Ref. 21]. CHARMM-GUI is an online graphical user interface that allows the generation of multiple MD models and preliminary input files for the start of simulation [Ref. 4-8]. This section will cover the *Membrane Builder* tool that allows users to develop membrane models with the option of including protein complexes. For this tutorial, only the bilayer builder section, without protein insertion, and without preliminary simulation building will be discussed. While there are other membrane building options, the bilayer builder is the simplest all-atom tool to work through for beginners. Alternatively, some membrane building tools do not utilize all-atom modeling; for an alternative atom modeling schema see Course Grained Simulations:

https://phys.libretexts.org/Courses/University_of_California_Davis/UCD%3A_Biophysics_241_-_Membrane_Biology/Extra_Credit/Coarse_Grain_Simulations_of_Membranes.

Note : should you want to try protein-membrane model building in the future, make sure your protein PDB has the correct orientation relative to the membrane or you will improperly insert your protein. Improper protein insertion will result in simulations not reflecting real-life predictions since the protein is not in a physiological state. A tutorial on protein insertion can be found here: www.ks.uiuc.edu/Training/Tutorials/science/membrane/mem-tutorial.pdf [Ref. 22].

3.1. Determine lipid components, size, and solvation

Click on the following link to reach the CHARMM-GUI *Membrane Builder Bilayer Builder* webpage: http://www.charmm-gui.org/?doc=input/membrane.bilayer [Ref. 3]. Scroll down to find a brief explanation of the workflow, an option to include a Protein Data Bank (.pdb) file, and an option to generate a membrane only system. Click the *Membrane Only System* to be redirected to the *System Size Determination Options* page **(Figure 3.1)**.

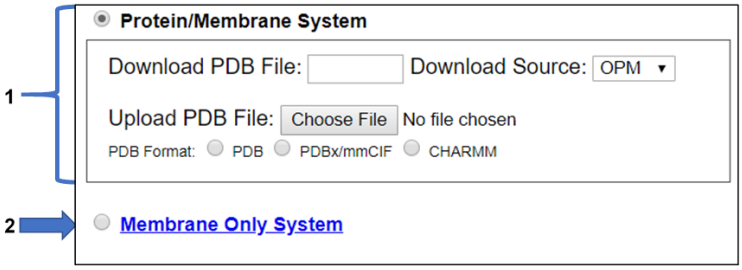

Figure 7.4.3.1. The start of the CHARMM-GUI Membrane Builder webpage.

1) *An option to create a protein/membrane system with a .pdb is provided.*

2) *For this tutorial, select "Membrane Only System."*

For this tutorial, users will recreate a model that has been used to study the effects of lipid tail saturation [Ref. 6]. The model is the CPRΔ1 model, a yeast-like model that contains a relatively high concentration of unsaturated lipids. In the *System Size Determination Options*, click the *Heterogeneous Lipid*, the *Water thickness*, and the *Ratios of lipid components* options. Next, input 20 Angstroms as the water thickness, and input 80 Angstroms as the initial X-Y guess **(Figure 3.2).** The initial X-Y guess is dependent upon the problem being solved; for example, a larger membrane protein would require a larger membrane area in order to be embedded. Below the system size is the *lipid components* options **(**Figure 3.3). Fill in the lipid ratios as indicated in **Table 3.1**. When finished, click the *Show the System Info* button and verify the generated dimensions. Ideally, both leaflets should have close to the same surface area; uneven areas can induce membrane bending artifacts during simulations and thus would be non-physiological. Once the system dimensions calculate near identical leaflet areas, click *Next Step: Determine the System Size* located in the lower right of the CHARMM-GUI webpage. This step may take some time. Do not close or refresh the CHARMM-GUI webpage.

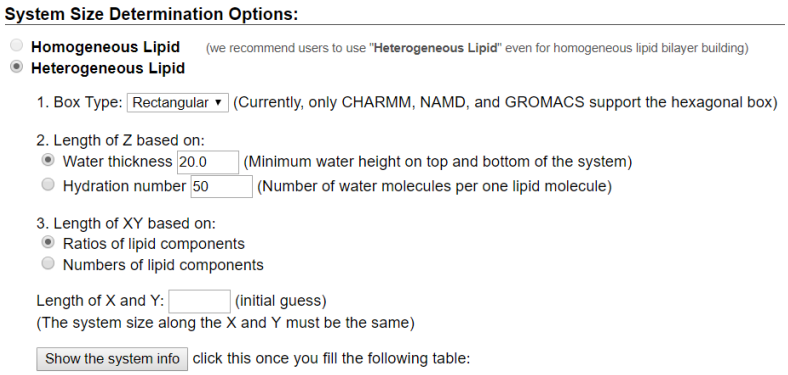

Figure 7.4.3.2. *System size determination options for use in the tutorial. Size values will vary depending on desired physiological parameter being studied, desired computational processing speed, and MD software used. For the tutorial, use 80 Angstroms as the initial X-Y guess.*

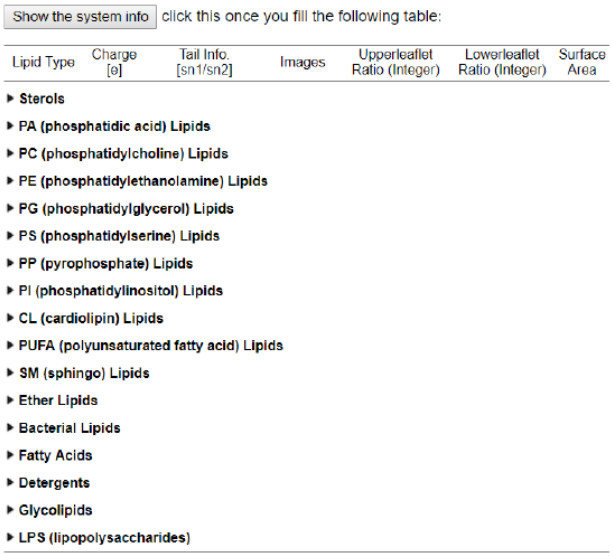

Figure 7.4.3.3. *Lipid components options in CHARMM-GUI Membrane Builder*

Make sure you click "Show the system info" after you input your desired lipids to verify near identical leaflet areas!

Table 3.1. *Representative model components from supplemental Table 2 in So S. et al, 2009 [Ref. 6]. These lipid components will be used for the tutorial section to demonstrate how CHARMM-GUI can be used to model biophysical problems.*

Model	System Size[b]	Molecules of Each Component									# Atoms
		POPA	POPS	POPE	DOPC	DPPC	Chol	K^+	Cl^-	H_2O	
CPRΔ1[c]	79.2 x 79.2 x 76.7	20	10	60	100	20	60	46	16	11885	67007
CPRΔ1[sat d]	86.9 x 86.9 x 84	20	10	60	20	100	60	46	16	11826	66830
Hypo[e]	89.3 x 89.3 x 84	14	14	84	28	112	28	46	18	12836	72970

[a] Both top and bottom leaflets have the same lipid compositions
[b] Each initial system size (in Å) is given by $L_X \times L_Y \times L_Z$, where L_X, L_Y, and L_Z correspond to the system size along the *X*, *Y*, and *Z*-axes, respectively.
[c] POPA : POPS : POPE : DOPC : DPPC : Chol = 2 : 1 : 6 : 10 : 2 : 6
[d] POPA : POPS : POPE : DOPC : DPPC : Chol = 2 : 1 : 6 : 2 : 10 : 6
[e] POPA : POPS : POPE : DOPC : DPPC : Chol = 1 : 1 : 6 : 2 : 8 : 2

In the next web page, you will have a system output box showing you the various CHARMM parameter files generated from the previous step **(Figure 3.4)**. You can download the system parameter files at any step during the tutorial by clicking *download.tgz* in the top right of the CHARMM-GUI webpage. You can view the *Generated Packed System* by clicking *view structure*; this will open an additional window that can be manipulated similar to the Visual Molecular Dynamics (VMD) display. Viewing the structure after each step is helpful in understanding how CHARMM-GUI constructs the model. In this step, only the packing arrangement has been determined **(Figure 3.4)**. Below the system output box you will see the *Determined System Size* section with size parameters for building the system. Further down you will see the *System Building Options* section **(Figure 3.5)**. A key component of system building is deciding if the insertion or replacement method of adding model components should be used. Generally, insertion methods are meant for protein insertions into bilayers while membrane-only models should use the replacement method. Additionally, enable the *Check lipid ring penetration* box to verify that lipid replacement does not produce positional artifacts. Next, in the *Component Building Options* section, ions are included in the system to generate a charge neutral model for electrostatic calculations during the simulation. For this case, we want to mimic the number of ions listed in **Table 3.1**. Adjust the ion concentration until you have exactly the correct number of ions. For this tutorial, use the *Monte Carlo* ion placement method **(Figure 3.5)**. Once finished, click *Next Step: Build Components* located in the lower right of the CHARMM-GUI webpage. This step may take some time. Do not close or refresh the web page.

Figure 7.4.3.4. *The CHARMM parameter files that can be downloaded at each step are useful for understanding how the model is built.*

__1)__ Click on the step3_packing.pdb (blue arrow) to view the structure. __2)__ A top-down view of the packing arrangement indicated as orange spheres.

The pink and blue squares indicate the upper and lower leaflet faces, respectively. This model view can be rotated similar to the VMD Display.

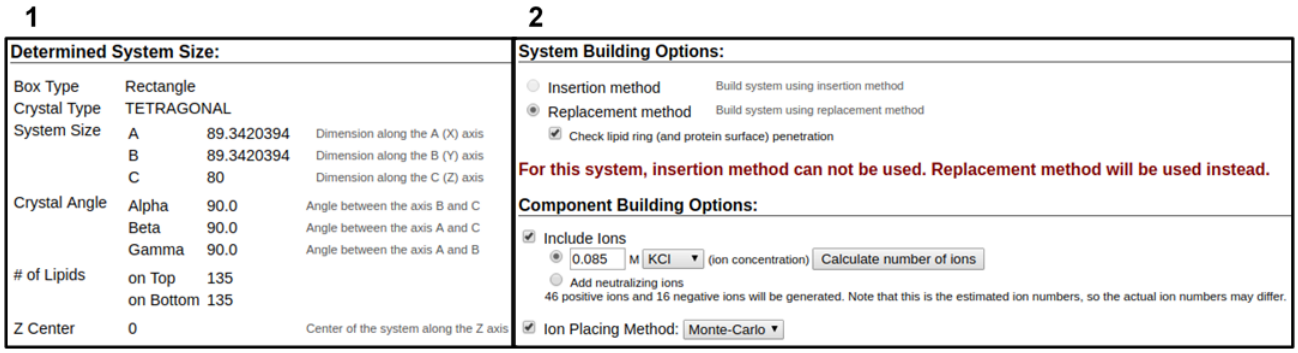

Figure 7.4.3.5. *System Size, System Build, and Component Build options for this tutorial. __1)__ The determined system size is a good self-check to verify the correct dimensions before building further. __2)__ For membranes, replacement method (the replacement of lipids) is used instead of insertion method (the insertion of a molecule such as a protein). The system build verifies that lipids do not have positional clashes. The component building options allows ion solvation, with either a distance or Monte-Carlo placement method. For this tutorial, 0.085 M of KCl will be used with the Monte-Carlo placement method.*

3.2. Final assembly, export, and visualization

Step 4 of the CHARMM-GUI *Membrane Builder* provides a self-check for lipid ring penetration as well as ion/water box building. In the system output box at the top of the page you can find the *step4_lipid.pdb* structure for viewing **(Figure 3.6)**. Click *Next Step: Assemble Components*.

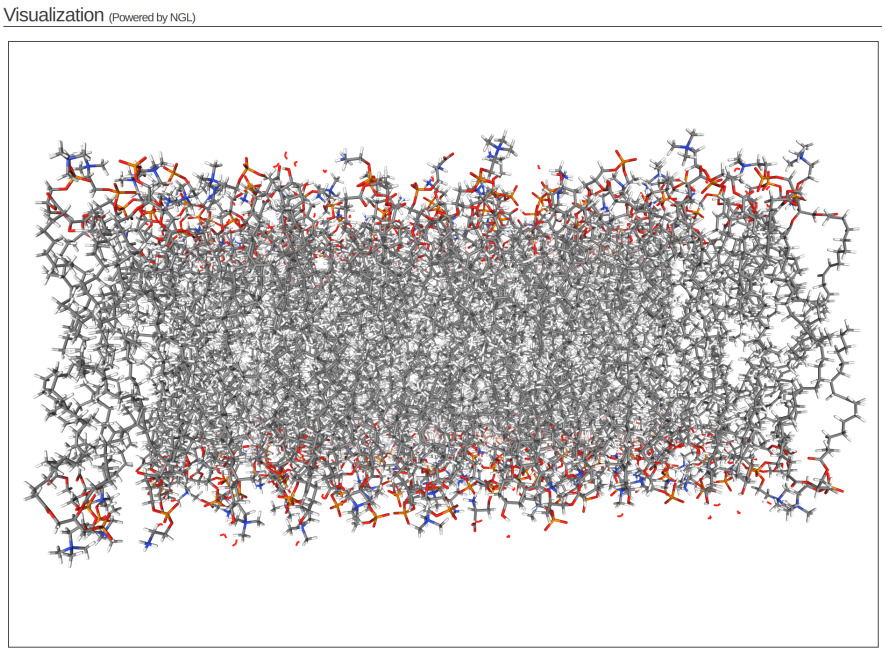

Figure 7.4.3.6. *Side perspective of lipid membrane from tutorial after completing Step 3: Determine the System Size.*

This model is the step before solvation.

Step 5 of the CHARMM-GUI *Membrane Builder* provides force field options, input generation options, and equilibration options for generating M.D. simulation parameters. Input options can be generated for various M.D. force fields such as GROMACS, AMBER, and CHARMM, among others. The use of a force field will largely be dependent upon your experimental question and your lab's computational expertise. A review describing the necessity for force fields and some common types can be found at [Ref. 16].

At the top of the system output box you will see the assembled .pdb file labeled as *step5_assembly.pdb*. You can download this .pdb file or the entire CHARMM-GUI folder by clicking the *download.tgz* icon at the top right of the CHARMM-GUI webpage. After downloading, the final model can be visualized using VMD as described in Section 1 **(Figure 3.7)**.

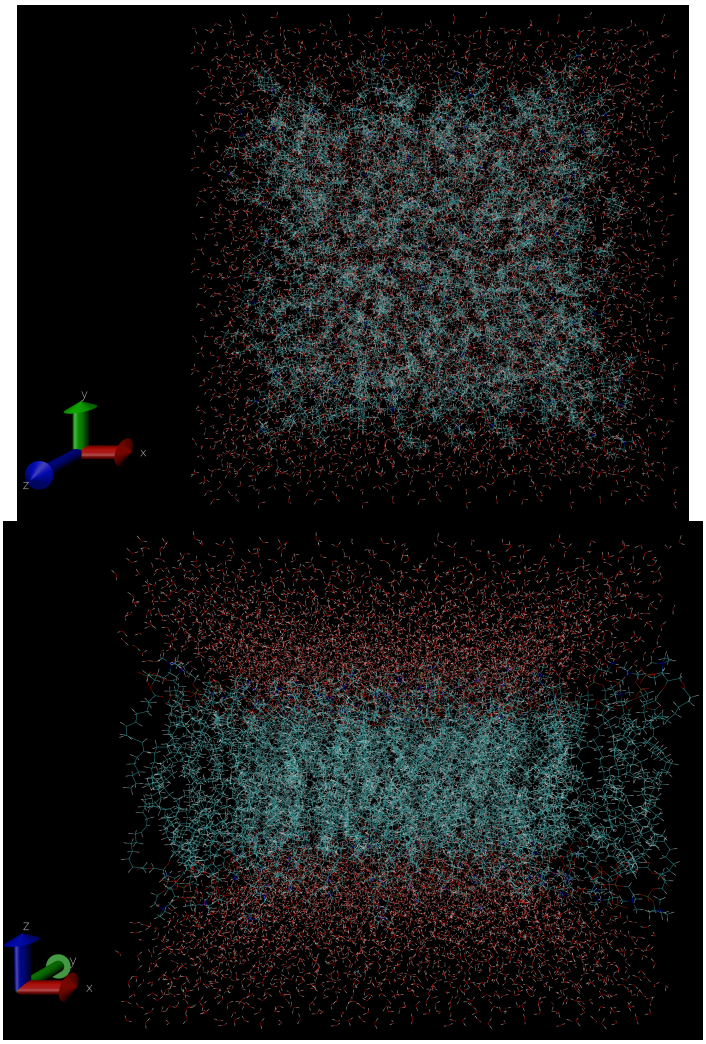

Figure 7.4.3.7. *(Left) top view of generated membrane model from tutorial (Right) side view of generated membrane model from tutorial.*

Exercises (section 3.2)

Exercise 3.1. From the complete model **(**Figure 3.7), isolate each class of lipid, ion, and water atoms as their own representation. Hint: you may need to look at the .pdb file in a text editor or in the VMD *Labels* feature to find common naming schemes.

Exercise 3.2. Generate the other two models from Jo, S. et al 2009 ***(Table 3.1)*** *[Ref. 6]. What do you expect will be different compared to the CPRΔ1 model?*

Exercise 3.3. Find another biomembrane of interest and generate the model. What parameters must you know to ensure that it is physiologically relevant?

Exercise 3.4. Temperature can be a critical factor during M.D. system equilibration. As seen in CHARMM-GUI Membrane Builder step 5, there is an option to adjust the temperature. What are reasonable/unreasonable temperatures? What is a good rule of thumb? Hint: Membrane fluidity.

Exercise 3.5. Review the following article [Ref. 23] for examples on how molecular simulations have helped validate and promote experimental findings. What stood out to you? Which experiment did you find most interesting?

Acknowledgments

Some images were made with VMD/NAMD/BioCoRE/JMV/other software support. VMD/NAMD/BioCoRE/JMV/ is developed with NIH support by the Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign.

Some images were made with the online CHARMM-GUI visualization tool during Section 3: Heterogeneous membrane building using CHARMM-GUI *Membrane Builder*.

References

[1] Hospital, A., Goñi, J. R., Orozco, M., Gelpí, J. L. (2015). Molecular dynamics simulations: advances and applications. *Adv Appl Bioinform Chem.* **8**(1): 37-47. doi: 10.2147/AABC.S70333

[2] Software downloads download VMD. Available at www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD

[3] CHARMM-GUI Membrane Builder. Available at http://www.charmm-gui.org/?doc=input/membrane.bilayer

[4] Jo, S., Kim, T., Iyer, V. G., Im, W. (2008). CHARMM-GUI: a web-based graphical user interface for CHARMM. *J Comput Chem.* **29**(11): 1859-1865. doi: 10.1002/jcc.20945

[5] Wu, E. L., Cheng, X., Jo, S., Rui, H., Song, K. C., Dávila-Contreras, E. M., Qi, Y., Lee, J., Monje-Galvan, V., Venable, R. M., Klauda, J. B., Im, W. (2014). CHARMM-GUI Membrane Builder toward realistic biological membrane simulations. *J Comput Chem.* **35**(27): 1997-2004. doi: 10.1002/jcc.23702

[6] Jo, S., Lim, J. B., Klauda, J. B., Im, W. (2009). CHARMM-GUI Membrane Builder for mixed bilayers and its application to yeast membranes. *Biophys J.* **97**(1): 50-58. doi:10.1016/j.bpj.2009.04.013

[7] Jo, S., Kim, T., Im, W. (2007) Automated builder and database of protein/membrane complexes for molecular dynamics simulations. *PLoS One* **2**(9): e880. doi: 10.1371/journal.pone.0000880

[8] Lee, J., Patel, D. S., Ståhle, J., Park, S. J., Kern, N. R., Kim, S., Lee, J., Cheng, X., Valvano, M. A., Holst, O., Knirel, Y. A., Qi, Y., Jo, S., Klauda, J. B., Widmalm, G., Im, W. (2019). CHARMM-GUI Membrane Builder for complex biological membrane simulations with glycolipids and lipoglycans. *J Chem Theory Comput.* **15**(1): 775-786. doi: 10.1021/acs.jctc.8b01066

[9] PDB statistics: overall growth of released structures per year. (2019). Available at: www.rcsb.org/stats/growth/overall

[10] Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., Bourne, P. E. (2000). *The Protein Data Bank. Nucleic Acids Res.* **28**(1): 235-242. doi: 10.1093/nar/28.1.235

[11] Burley, S. K., Berman, H. M., Bhikadiya, C., Bi, C., Chen, L., Di Costanzo, L., Christie, C., Dalenberg, K., Duarte, J. M., Dutta, S., Feng, Z., Ghosh, S., Goodsell, D. S., Green, R. K., Guranovic, V., Guzenko, D., Hudson, B. P., Kalro, T., Liang, Y., Lowe, R., Namkoong, H., Peisach, E., Periskova, I., Prlic, A., Randle, C., Rose, A., Rose, P., Sala, R., Sekharan, M., Shao, C., Tan, L., Tao, Y. P., Valasatava, Y., Voigt, M., Westbrook, J., Woo, J., Yang, H., Young, J., Zhuravleva, M., Zardecki, C. (2019). RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucleic Acids Res.* **47**(D1): D464-D474. doi: 10.1093/nar/gky1004

[12] PDB file format documentation. Available at: http://www.wwpdb.org/documentation/file-format

[13] Berman, H., Henrick, K., Nakamura, H. (2003). Announcing the worldwide Protein Data Bank. *Nat Struct Biol.* **10**(12): 980. doi: 10.1038/nsb1203-980

[14] Mouse modes. (2019). Available at: www.ks.uiuc.edu/Research/vmd/vmd-1.9.3/ug/node32.html

[15] VMD user's guide version 1.9.3. (2016). Available at www.ks.uiuc.edu/Research/vmd/vmd-1.9.3/ug.pdf

[16] González, M. A. (2011) Force fields and molecular dynamics simulations. *Collection SFN* **12**(1): 169-200. doi: 10.1051/sfn/201112009

[17] Balabin, I. Membrane plugin, version 1.1. Available at www.ks.uiuc.edu/Research/vmd/plugins/membrane/

[18] Huang, J., MacKerell, A. D. Jr. (2013) CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data. *J Comput Chem.* **34**(25): 2135-2145. doi: 10.1002/jcc.23354

[19] Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., Karplus, M., (1983). CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J Comput Chem.* **4**(2) 187-217. doi: 10.1002/jcc.540040211

[20] Brooks, B. R., Brooks III, C. L., MacKerell Jr., A. D., Nilsson, L., Petrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R. W., Post, C. B., Pu, J. Z., Schaefer, M., Tidor, B., Venable, R. M., Woodcock, H. L., Wu, X., Yang, W., York, D. M., Karplus, M. (2010). CHARMM: the biomolecular simulation program. *J Comput Chem.* **30**(10): 1545-1614. doi: 10.1002/jcc.21287

[21] Kessel, A. and Ben-Tal, N. (2018) Introduction to proteins structure, function, and motion second edition. Boca Raton, FL: CRC Press, Taylor & Francis Group.

[22] Aksimentiev, A. Sotomayor, M., Wells, D., Mahinthichaichan, P. (2016) Membrane Proteins Tutorial. Available at http://www.ks.uiuc.edu/Training/Tutorials/science/membrane/mem-tutorial.pdf

[23] Hollingsworth S. A., Dror, R. O. (2018) Molecular dynamics simulation for all. *Neuron* **99**(6): 1129-1143. doi: 10.1016/j.neuron.2018.08.011
