

# Building the membrane-protein system in CHARMM-GUI

## PDB Info

1. Upload PDB file
2. Selected all chains

**PDB Info** STEP 1 STEP 2 STEP 3 STEP 4 STEP 5 STEP 6

|                     |         |  |  |  |  |
|---------------------|---------|--|--|--|--|
| Title               |         |  |  |  |  |
| PDB ID              | HELIX   |  |  |  |  |
| Type                | Protein |  |  |  |  |
| Experimental Method | Unknown |  |  |  |  |

**Model/Chain Selection Option:**

Click on the chains you want to select.

| Type  | SEGID | PDB ID | Residue ID |      | Engineered Residues |
|---|-------|--------|------------|------|---------------------|
|   |       |        | First      | Last |                     |
| <input checked="" type="checkbox"/> Protein | PROA  | A      | 1          | 46   | None                |
| <input checked="" type="checkbox"/> Protein | PROB  | B      | 1          | 46   | None                |
| <input checked="" type="checkbox"/> Protein | PROC  | C      | 1          | 46   | None                |
| <input checked="" type="checkbox"/> Protein | PROD  | D      | 1          | 46   | None                |
| <input checked="" type="checkbox"/> Protein | PROE  | E      | 1          | 46   | None                |
| <input checked="" type="checkbox"/> Protein | PROF  | F      | 1          | 46   | None                |

CHARMM-GUI uses internal segid format PRO[A-Z] (protein), DNA[A-Z] (DNA), RNA[A-Z]

3. System pH: 7
4. Terminal group patching (all): NTER (*standard N-terminus*), CT2 (*amidated C-terminus*)

**PDB Info** STEP 1 STEP 2 STEP 3 STEP 4 STEP 5 STEP 6

|                     |         |  |  |  |  |
|---------------------|---------|--|--|--|--|
| Title               |         |  |  |  |  |
| PDB ID              | HELIX   |  |  |  |  |
| Type                | Protein |  |  |  |  |
| Experimental Method | Unknown |  |  |  |  |

**PDB Manipulation Options:**

☒ System pH: 7.0

☒ Terminal group patching:

|      | First | Last |  |
|------|-------|------|--|
| PROA | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |
| PROB | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |
| PROC | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |
| PROD | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |
| PROE | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |
| PROF | NTER  | CT2  | <input type="checkbox"/> Cyclic peptide? |

☐ Preserve hydrogen coordinates:

☐ Mutation:

☐ Protonation state:

☐ Disulfide bonds:

☐ Phosphorylation:

☐ Ubiquitylation / SUMOylation:

☐ GPI anchor:

☐ Glycosylation / Glycan Ligand(s): ☐ Use CHARMM MC? It is faster than the regular run, but

☐ Heme coordination

☐ Add Lipid-tail

☐ Peptide Stapling

☐ Add FRET/LRET fluorophore labels

☐ Model LBT-loop(s)

☐ Add MTS reagents: nitroxide spin labels

☐ Add MTS reagents: chemical modifier

☐ Non-standard amino acid / RNA substitution:

☐ Lys / Arg PTMs:

## Step 1

1. Orientation options: Use PDB orientation

2. Generate pore water and measure pore size: Using protein geometry (*water molecules outside the XY extent of transmembrane domain will be removed*)

PDB Info
STEP 1
STEP 2
STEP 3
STEP 4
STEP 5
STEP 6

JOB ID: 500540590;

Original PDB File: [HELIX.pdb \(view structure\)](#) [download.tgz](#)  
Individual Chains: [helix\\_proa.pdb](#)  
[helix\\_prob.pdb](#)  
[helix\\_proc.pdb](#)  
[helix\\_proe.pdb](#)  
[helix\\_prof.pdb](#)  
CHARMM Input: [step1\\_pdbreader.inp](#)  
CHARMM Output: [step1\\_pdbreader.out](#)  
CHARMM PDB: [step1\\_pdbreader.pdb \(view structure\)](#)  
CHARMM CRD: [step1\\_pdbreader.crd](#)  
CHARMM PSF: [step1\\_pdbreader.psf](#)


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**Computed Energy:**

Please beware of that the computed energy is CHARMM single-point energy and is displayed to make sure all the coordinates are defined.

| ENER ENR:    | Eval# | ENERgy      | Delta-E    | GRMS      |            |           |
|--------------|-------|-------------|------------|-----------|------------|-----------|
| ENER INTERN: |       | BONDs       | ANGLEs     | UREY-b    | DIHEDrals  | IMPRopers |
| ENER CROSS:  |       | CHAPs       | PPF1D      | PPF2D     | PRIND      |           |
| ENER EXTERN: |       | VMDwals     | ELEC       | HBDWds    | ASP        | USER      |
| ENER:        | 0     | 10309.91566 | 0.00000    | 107.60705 |            |           |
| ENER INTERN: |       | 663.45252   | 500.16818  | 33.52508  | 2058.90619 | 31.53607  |
| ENER CROSS:  |       | 24.87193    | 0.00000    | 0.00000   | 0.00000    |           |
| ENER EXTERN: |       | 7420.60371  | -423.14093 | 0.00000   | 0.00000    | 0.00000   |

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**Orientation Options:** 

☒ Use PDB Orientation This option is suggested for an oriented structure from <http://opm.phar.umich.edu>

☐ Align the First Principal Axis  
Along Z This option is suggested for small helical bundle or homo-oligomer.

☐ Align a Vector (Two Atoms)  
Along Z This option is suggested for an irregular, hetero-oligomer.

☐ Run PPM 2.0 This option run executable for given input structure at [https://opm.phar.umich.edu/ppm\\_server2\\_cgopm](https://opm.phar.umich.edu/ppm_server2_cgopm)  
It may take some minutes depending on protein size.

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**Positioning Options:**

☐ Rotate Molecule respect to the X axis  Degree

☐ Rotate Molecule respect to the Y axis  Degree

☐ Translate Molecule along Z axis  Angstrom

☐ Flip Molecule along the Z axis


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**Area Calculation Options:**

☒ Generate Pore Water and Measure Pore Size

☒ Using protein geometry Water molecules outside the XY extent of the transmembrane domain would be removed.

☐ Using a cylindrical radius of  Angstrom

Next Step: 
Calculate Cross-Sectional Area

## Step 2: Calculate cross-sectional area

1. Box type: rectangular
2. Length of Z based on: water thickness 25 (*minimum water height on top and bottom of the system*)
3. Length of XY based on: ratios of lipid components
4. Length of XY: 75 (initial guess) (*the system size along the X and Y must be the same*)

option even for homogeneous lipid bilayer building.

☒ **Heterogeneous Lipid**

1. Box Type:  (Currently, only CHARMM, NAMD, and GROMACS support

2. Length of Z based on:

☒ Water thickness  (Minimum water height on top and bottom of the system)

☐ Hydration number  (Number of water molecules per one lipid molecule)

3. Length of XY based on:

☒ Ratios of lipid components

☐ Numbers of lipid components

Length of X and Y:  (initial guess)

(The system size along the X and Y must be the same)

5. Lipid membrane composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol (CHL), ratio 2:1

| Lipid Type  | Charge [e] | Tail Info. [sn1/sn2] | Images                  | Upperleaflet Ratio (Integer)   | Lowerleaflet Ratio (Integer)   | Surface Area                      |
|-------------|------------|----------------------|-------------------------|--------------------------------|--------------------------------|-----------------------------------|
| ▼ Sterols   |            |                      |                         |                                |                                |                                   |
| Cholesterol | 0          |                      | <a href="#">[Image]</a> | <input type="text" value="1"/> | <input type="text" value="1"/> | <input type="text" value="40.0"/> |
| -           | -          |                      |                         |                                |                                |                                   |

### ▼ PC (phosphatidylcholine) Lipids

|      |   |             |                         |                                |                                |                                   |
|------|---|-------------|-------------------------|--------------------------------|--------------------------------|-----------------------------------|
| DDPC | 0 | 10:0 / 10:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="64.1"/> |
| DCPC | 0 | 11:0 / 11:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="64.1"/> |
| DLPC | 0 | 12:0 / 12:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="64.1"/> |
| DMPC | 0 | 14:0 / 14:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="64.1"/> |
| DPPC | 0 | 16:0 / 16:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="63.0"/> |
| DSPC | 0 | 18:0 / 18:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="65.6"/> |
| PSPC | 0 | 16:0 / 18:0 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="68.3"/> |
| PYPC | 0 | 16:0 / 16:1 | <a href="#">[Image]</a> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="64.1"/> |
| POPC | 0 | 16:0 / 18:1 | <a href="#">[Image]</a> | <input type="text" value="2"/> | <input type="text" value="2"/> | <input type="text" value="68.3"/> |

#### Calculated Number of Lipids:

| Lipid Type  | Upperleaflet Number | Lowerleaflet Number |
|-------------|---------------------|---------------------|
| Cholesterol | 26                  | 26                  |
| POPC        | 52                  | 52                  |

#### Calculated XY System Size:

|                  | Upperleaflet | Lowerleaflet |
|------------------|--------------|--------------|
| Protein Area     | 1141.98769   | 1135.9864    |
| Lipid Area       | 4591.6       | 4591.6       |
| # of Lipids      | 78           | 78           |
| Total Area       | 5733.58769   | 5727.5864    |
| Protein X Extent | 27.89        |              |
| Protein Y Extent | 27.77        |              |
| Average Area     | 5730.59      |              |
| A                | 75.70        |              |
| B                | 75.70        |              |

#### Determined System Size:

|               |            |                                       |                                |
|---------------|------------|---------------------------------------|--------------------------------|
| Box Type      | Rectangle  |                                       |                                |
| Crystal Type  | TETRAGONAL |                                       |                                |
| System Size   | A          | 75.7006384                            | Dimension along the A (X) axis |
|               | B          | 75.7006384                            | Dimension along the B (Y) axis |
|               | C          | 115.125                               | Dimension along the C (Z) axis |
| Crystal Angle | Alpha      | 90.0                                  | Angle between the axis B and C |
|               | Beta       | 90.0                                  | Angle between the axis A and C |
|               | Gamma      | 90.0                                  | Angle between the axis A and B |
| # of Lipids   | on Top     | 78                                    |                                |
|               | on Bottom  | 78                                    |                                |
| Z Center      | 11.1575    | Center of the system along the Z axis |                                |

## Step 3:

1. Replacement method: Check lipid ring (and protein surface) penetration (*build system using replacement method*)
2. Include ions
3. Ion placing method: Distance
4. Cation: Ammonium (NH<sub>4</sub><sup>+</sup>)
5. Anion: Chloride (Cl<sup>-</sup>)
6. Concentration: 1

## 7. Neutralizing

### System Building Options:

- ☐ Insertion method Build system using insertion method
- ☒ Replacement method Build system using replacement method
- ☒ Check lipid ring (and protein surface) penetration

**For this system, insertion method can not be used. Replacement method will be used instead.**

### Component Building Options:

- ☒ Include Ions

Ion Placing Method: Distance

☐ Basic Ion Types

☒ More Ion Types

Ammonium (NH<sub>4</sub><sup>+</sup>) Cl<sup>-</sup> Add This Ion

| Formula            | Cation                                   | Anion           | Concentration | Neutralizing                       |
|--------------------|--|-----------------|---------------|------------------------------------|
| NH <sub>4</sub> Cl | Ammonium (NH <sub>4</sub> <sup>+</sup> ) | Cl <sup>-</sup> | 1             | <input checked="" type="radio"/> - |

Calculate Solvent Composition

| Ion                          | Count |
|------------------------------|-------|
| NH <sub>4</sub> <sup>+</sup> | 224   |
| Cl <sup>-</sup>              | 224   |

Please note that the ion count is an approximation based on geometry. The real number will be calculated in the next step.


### Pore Water Options:

The addition of pore water may result in water molecules placed outside the protein. Please download the structure file and open it using your molecular visualization software. In the list below, click water molecules that are outside the pore and should be removed.

Water filled protein structure: [step2.1\\_pore.pdb](#)

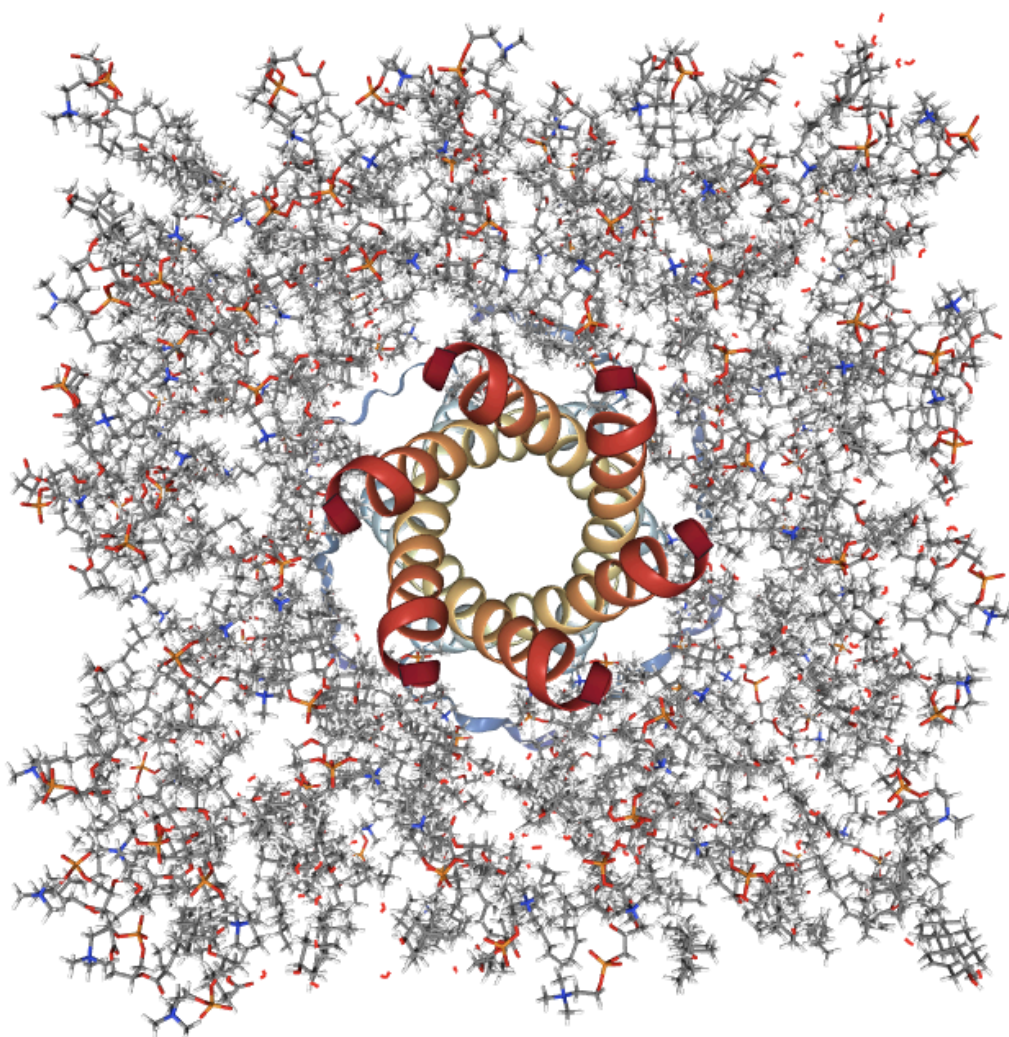
Check atom number of OH2 to remove

|    | 1    | 2    | 3    | 4    | 5    | 6  | 7    | 8    | 9    | 10   |      | 1  | 2    | 3    | 4    | 5    | 6    | 7  | 8    | 9    | 10   |      |      |
|----|------|------|------|------|------|----|------|------|------|------|------|----|------|------|------|------|------|----|------|------|------|------|------|
| 1  | 3979 | 3982 | 3985 | 3988 | 3991 | 6  | 3994 | 3997 | 4000 | 4003 | 4006 | 11 | 4009 | 4012 | 4015 | 4018 | 4021 | 16 | 4024 | 4027 | 4030 | 4033 | 4036 |
| 21 | 4039 | 4042 | 4045 | 4048 | 4051 | 26 | 4054 | 4057 | 4060 | 4063 | 4066 | 31 | 4069 | 4072 | 4075 | 4078 | 4081 | 36 | 4084 | 4087 | 4090 | 4093 | 4096 |
| 41 | 4099 | 4102 | 4105 | 4108 | 4111 | 46 | 4114 | 4117 | 4120 | 4123 | 4126 | 51 | 4129 | 4132 | 4135 | 4138 | 4141 | 56 | 4144 | 4147 | 4150 | 4153 | 4156 |
| 61 | 4159 | 4162 | 4165 | 4168 | 4171 | 66 | 4174 | 4177 | 4180 | 4183 | 4186 | 71 | 4189 | 4192 | 4195 | 4198 | 4201 | 76 | 4204 | 4207 | 4210 | 4213 | 4216 |
| 81 | 4219 | 4222 | 4225 | 4228 | 4231 | 86 | 4234 | 4237 | 4240 | 4243 | 4246 | 91 | 4249 |      |      |      |      |    |      |      |      |      |      |

Next Step:   
Build Components

## Step 4: Build components

Component PDB:



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### Check lipid penetration

The protein surface penetration check finds the lipid tails that go beyond the protein surface, and the lipid ring penetration check detects the lipid tails that pass through the cyclic groups (e.g., cholesterol ring) in the simulation systems. Energy minimization can resolve many of these bad contacts, but one might need to visually check the following lipid molecules to ensure the following contacts are resolved. The user can regenerate the lipid bilayer if necessary.

Protein surface penetration:

No protein surface penetration is found.

Lipid ring penetration:

No lipid ring penetration is found.

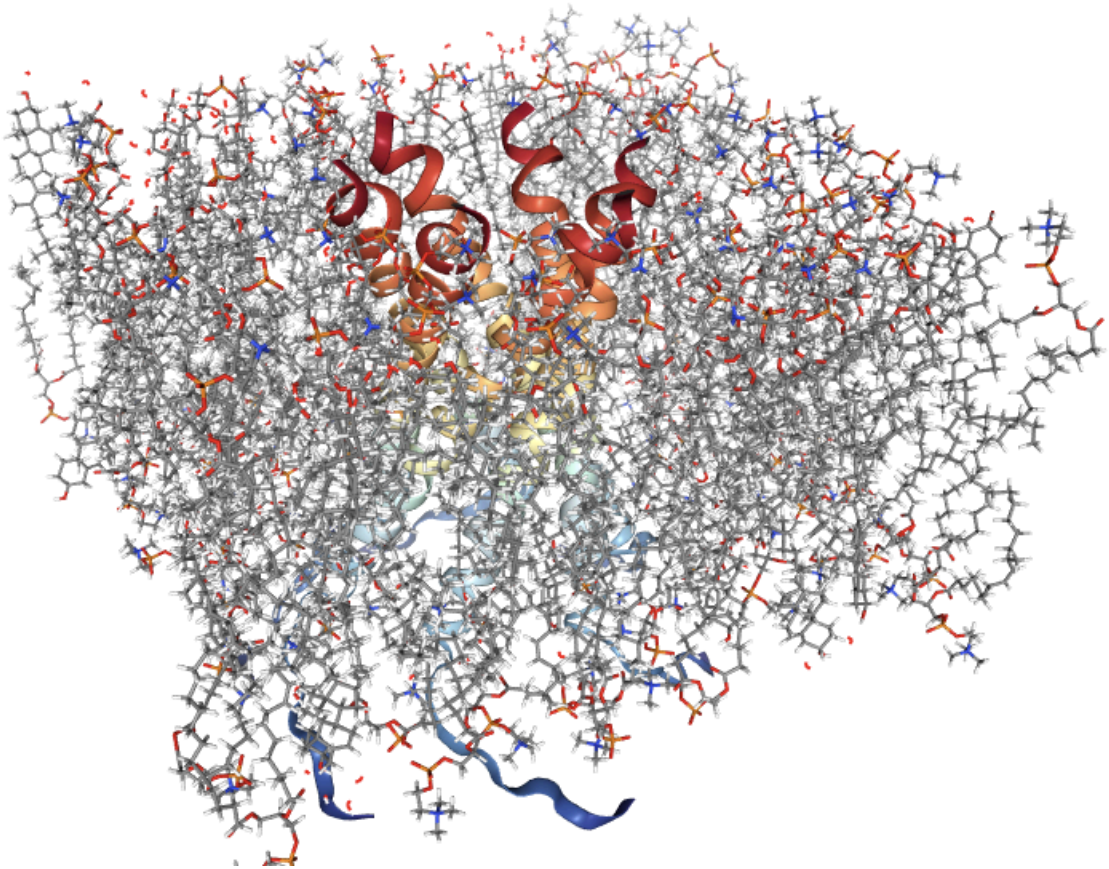
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### Building Ion and Waterbox

Membrane components are generated. Due to time constraints, we first generate the lipid bilayer then generate ions and the water box. Click "Next Step" to generate ions and the water box.



## Step 5



## Step 6

1. Force field: CHARMM36m
2. WYF parameter for cation-pi interactions
3. Input generation: More CHARMM minimization during input generation, GROMACS
4. Generate grid information for PME FFT automatically
5. NPT ensemble

6. Temperatre 303.15K

**Determined System Size:**

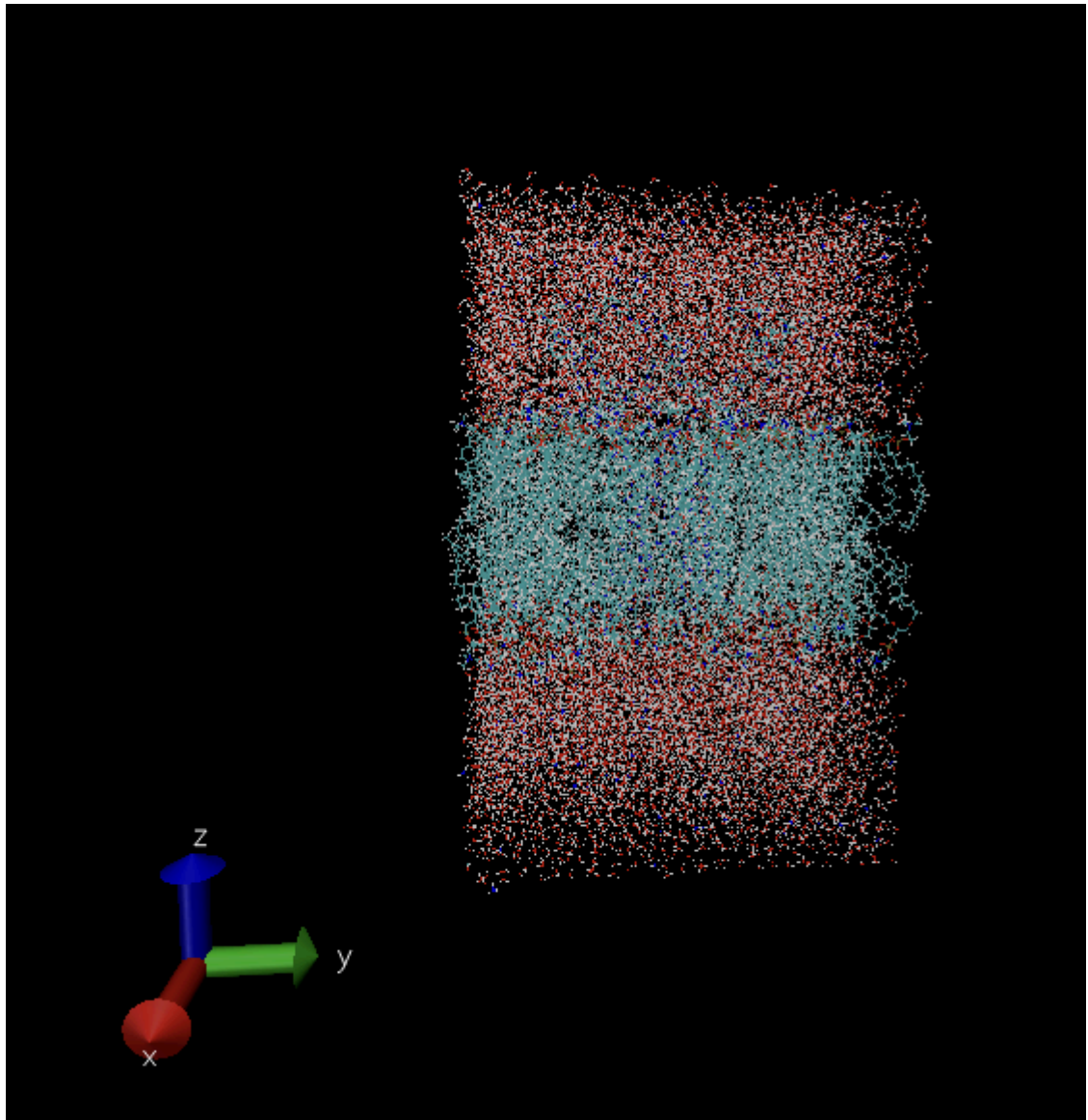
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|               |            |            |                                       |
|---------------|------------|------------|---------------------------------------|
| # of Atoms    | 61449      |            |                                       |
| Crystal Type  | TETRAGONAL |            |                                       |
| System Size   | A          | 75.7006384 | Dimension along the A (X) axis        |
|               | B          | 75.7006384 | Dimension along the B (Y) axis        |
|               | C          | 115.125    | Dimension along the C (Z) axis        |
| Crystal Angle | Alpha      | 90.0       | Angle between the axis B and C        |
|               | Beta       | 90.0       | Angle between the axis A and C        |
|               | Gamma      | 90.0       | Angle between the axis A and B        |
| # of Lipids   | on Top     | 78         |                                       |
|               | on Bottom  | 78         |                                       |
| # of Water    | 12679      |            |                                       |
| Z Center      | 0.0        |            | Center of the system along the Z axis |





## 1. Lines



2. Cartoon

