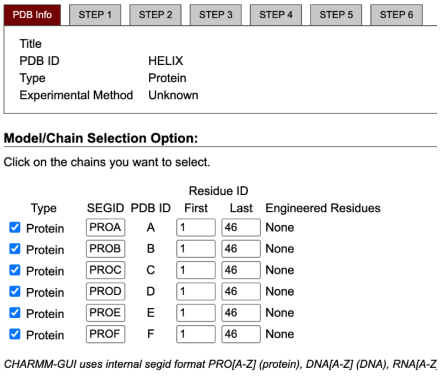
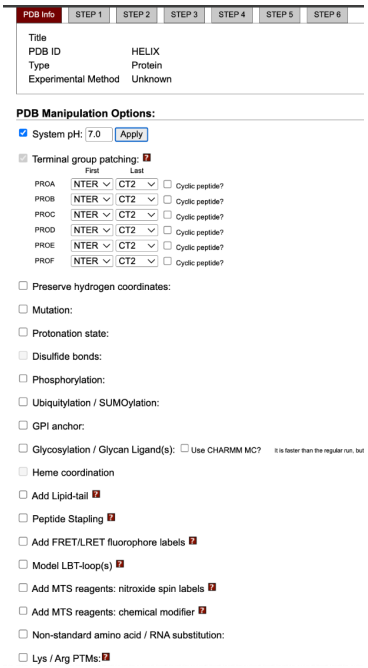
# Building the membrane-protein system in CHARMM-GUI

## PDB Info

1. Upload PDB file
2. Selected all chains



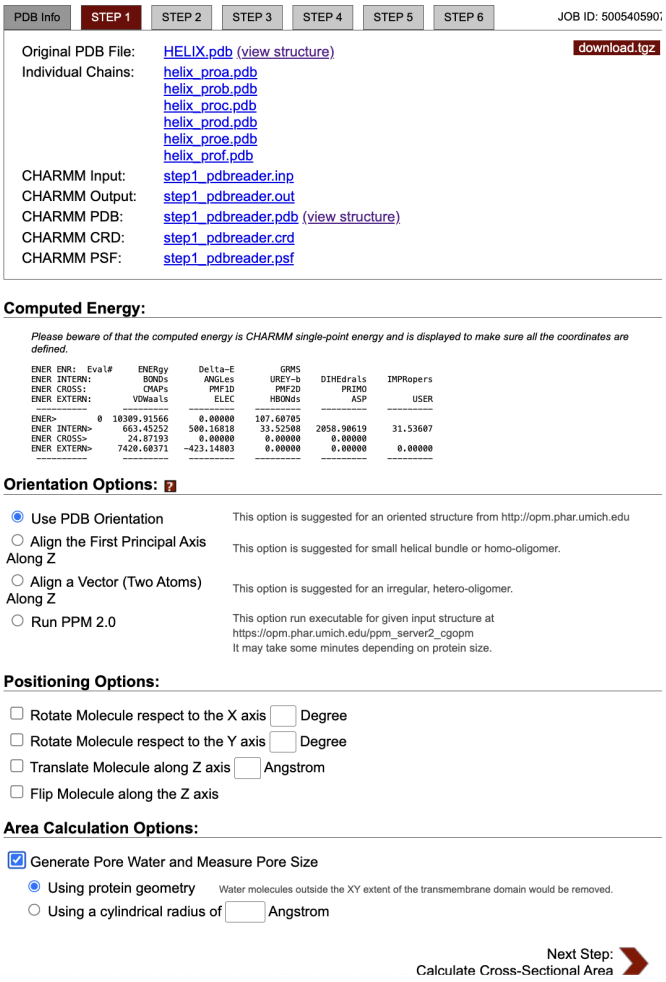

3. System pH: 7
4. Terminal group patching (all): NTER *(standard N-terminus)*, CT2 *(amidated C-terminus)*




## 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)*




# 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: Rectangular (Currently, only CHARMM, NAMD, and GROMACS support

2. Length of Z based on:
   - ◉ Water thickness 25 (Minimum water height on top and bottom of the system)
   - ○ Hydration number 50 (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: 75 (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 | | [Image] | 1 | 1 | 40.0 |

▼ **PC (phosphatidylcholine) Lipids**

| | | | | | | |
|---|---|---|---|---|---|---|
| DDPC | 0 | 10:0 / 10:0 | [Image] | 0 | 0 | 64.1 |
| DCPC | 0 | 11:0 / 11:0 | [Image] | 0 | 0 | 64.1 |
| DLPC | 0 | 12:0 / 12:0 | [Image] | 0 | 0 | 64.1 |
| DMPC | 0 | 14:0 / 14:0 | [Image] | 0 | 0 | 64.1 |
| DPPC | 0 | 16:0 / 16:0 | [Image] | 0 | 0 | 63.0 |
| DSPC | 0 | 18:0 / 18:0 | [Image] | 0 | 0 | 65.6 |
| PSPC | 0 | 16:0 / 18:0 | [Image] | 0 | 0 | 68.3 |
| PYPC | 0 | 16:0 / 16:1 | [Image] | 0 | 0 | 64.1 |
| POPC | 0 | 16:0 / 18:1 | [Image] | 2 | 2 | 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 (NH4+)
5. Anion: Chloride (Cl-)
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 ($NH_4^+$) $Cl^-$ Add This Ion

| Formula | Cation | Anion | Concentration | Neutralizing | |
|---|---|---|---|---|---|
| $NH_4Cl$ | Ammonium ($NH_4^+$) | $Cl^-$ | 1 | ◉ | - |

Calculate Solvent Composition

| Ion | Count |
|---|---|
| $NH_4^+$ | 224 |
| $Cl^-$ | 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

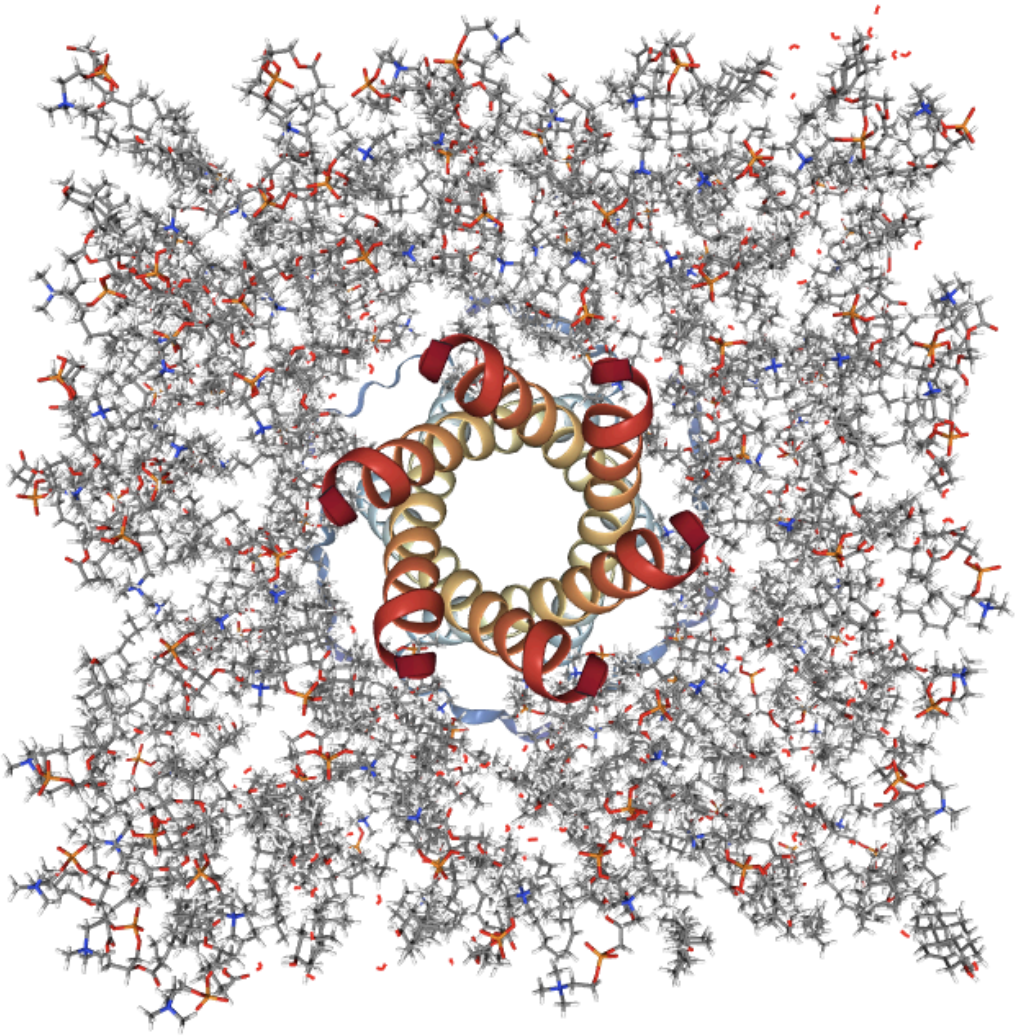
Check atom number of OH2 to remove

| | 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

Compnent PDB:



## Check lipid pentration

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.
```

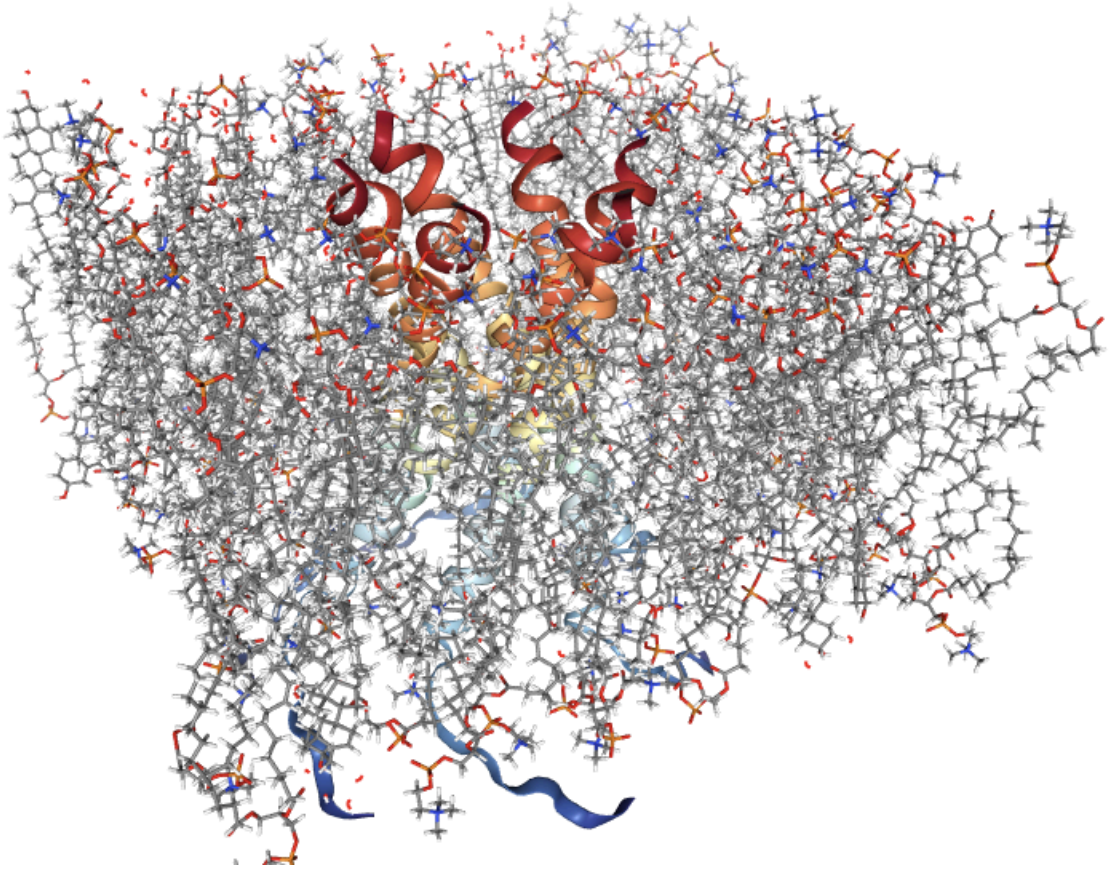
Lipid ring penetration:

```
No lipid ring penetration is found.
```

## Building Ion and Waterbox

Membrane components are generated. Due to time constrains, 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



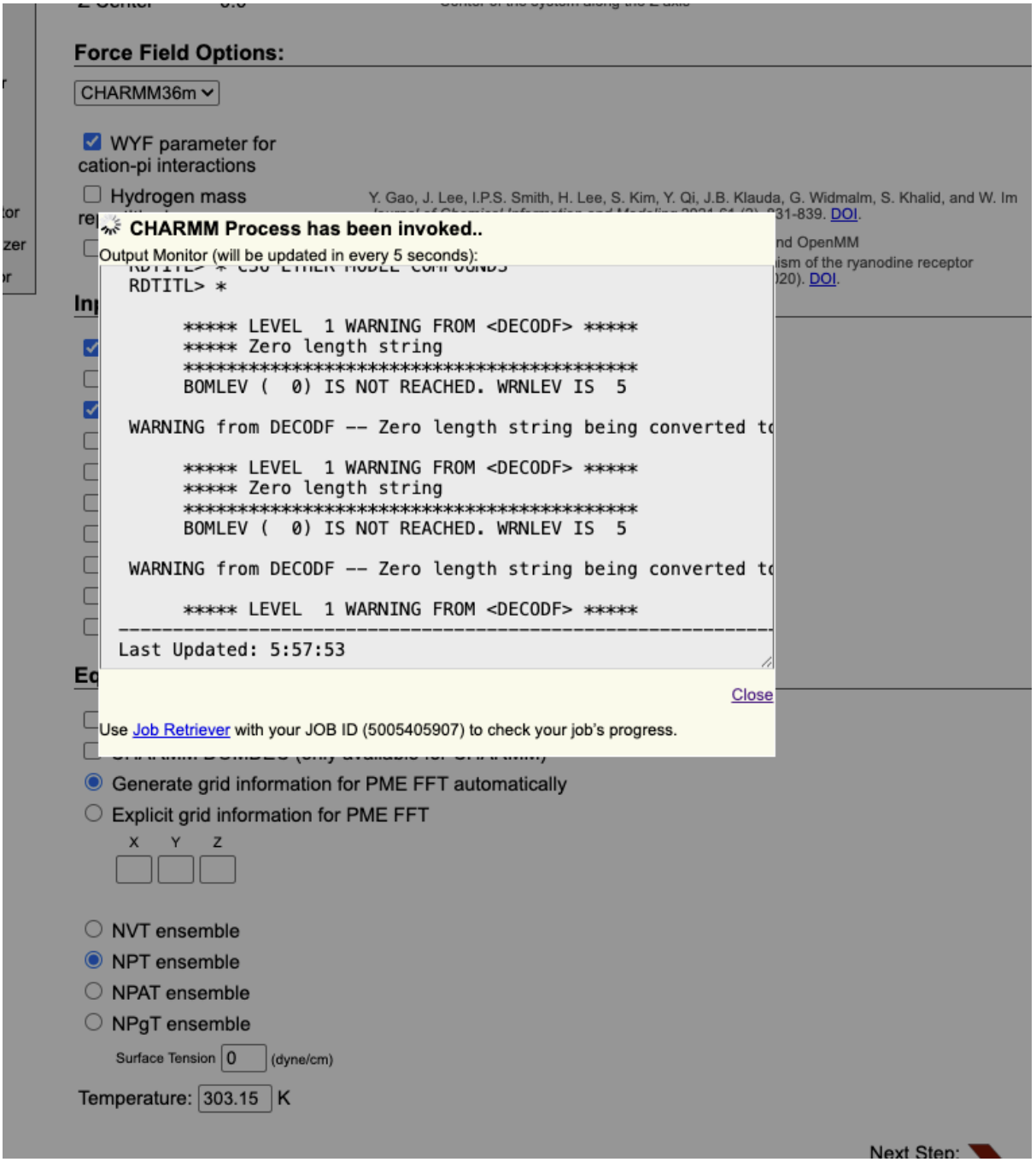
# Step 6

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

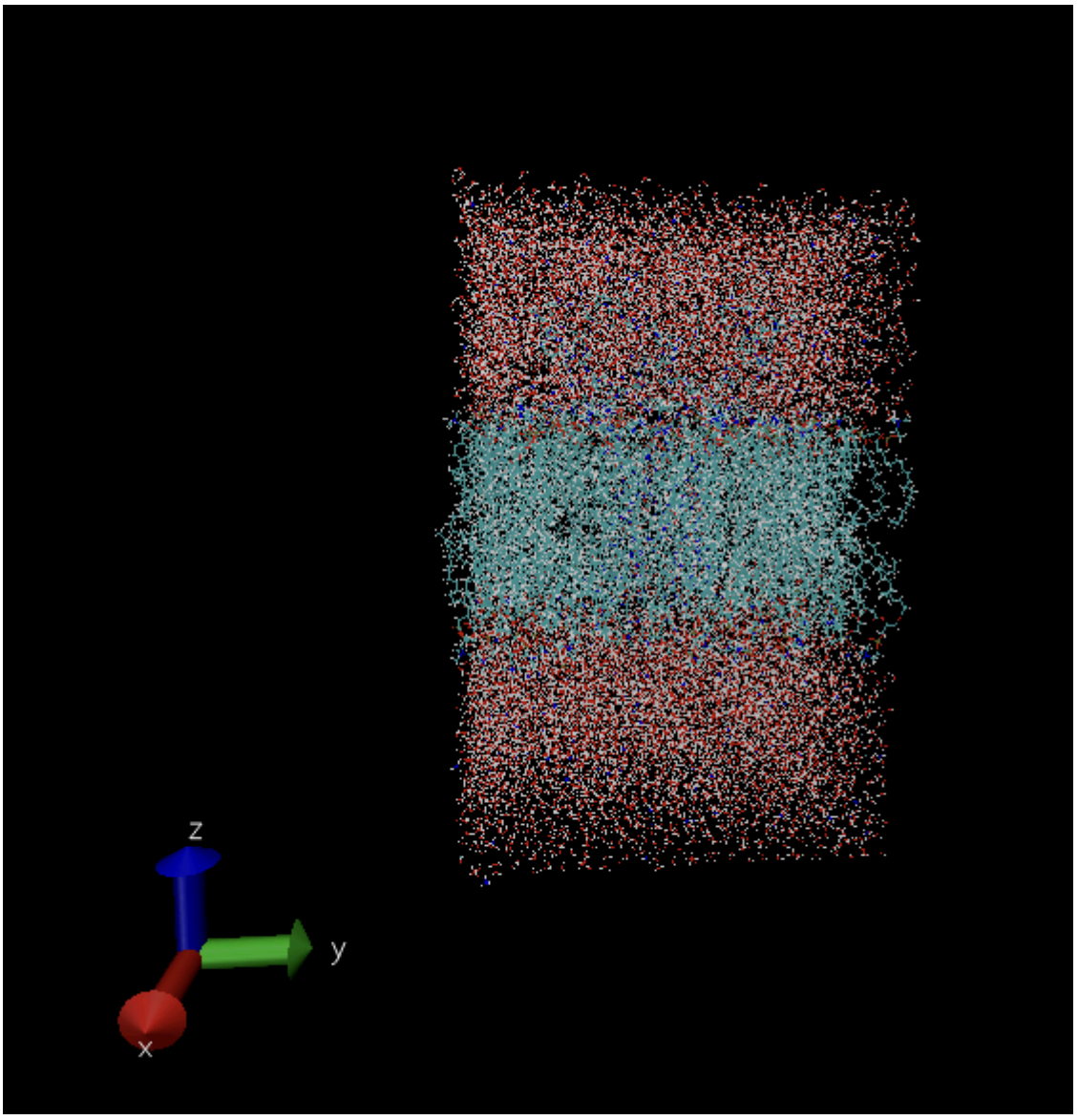
**Determined System Size:**

| | | | |
|---|---|---|---|
| # 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 |




Checking the system in VMD

1. Lines



2. Cartoon