Bcl-xL Membrane Building Process

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October 29, 2022

1 Membrane Builder

- Bilayer Builder
  - Protein/Membrane System
    - PDB Manipulation Options:

PDB Manipulation Options:

- [x] Terminal group patching:

	First	Last	
PROA	NTER	CTER	[ ] Cyclic peptide?

- [ ] Preserve hydrogen coordinates:
- [ ] Mutation:
- [ ] Protonation state:
- [ ] Disulfide bonds:
- [ ] Phosphorylation:
- [ ] GPI anchor:
- [ ] Glycosylation / Glycan Ligand(s):
- [ ] 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:

∗ Bilayer Builder

Computed Energy:

Please beware of that the computed energy is CHARMM single-point energy and is displ

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ENER ENR:  Eval#     ENERgy      Delta-E         GRMS
ENER INTERN:          BONDs       ANGLes       UREY-b    DIHEdrals    IMPRopers
ENER CROSS:           CMAPs        PMF1D        PMF2D        PRIMO
ENER EXTERN:        VDWaals         ELEC       HBONds          ASP         USER
 ----------       ---------    ---------    ---------    ---------    ---------
ENER>         0   3109.46476     0.00000    15.08280
ENER INTERN>      650.13647     300.47018    68.08528   2516.95562      3.00847
ENER CROSS>       -39.48373       0.00000     0.00000      0.00000
ENER EXTERN>     1484.26025   -1873.96779     0.00000      0.00000      0.00000
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Orientation Options:

- (•) Use PDB Orientation — This option is suggested for an
- ( ) Align the First Principal Axis Along Z — This option is suggested for sma
- ( ) Align a Vector (Two Atoms) Along Z — This option is suggested for an i
- ( ) Run PPM 2.0 — This option run executable for gi
  It may take some minutes deper

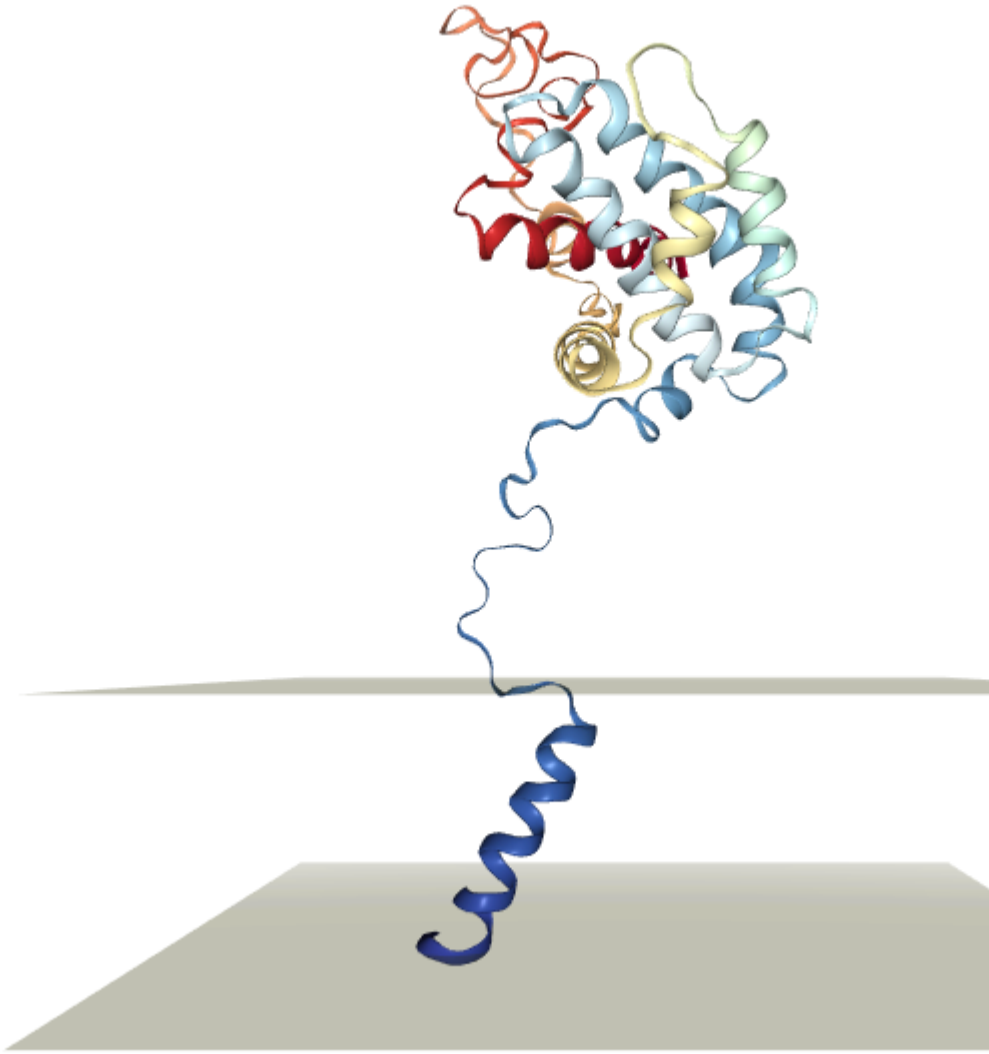
Positioning Options:

- [ ] 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

Area Calculation Options:

- [ ] Generate Pore Water and Measure Pore Size

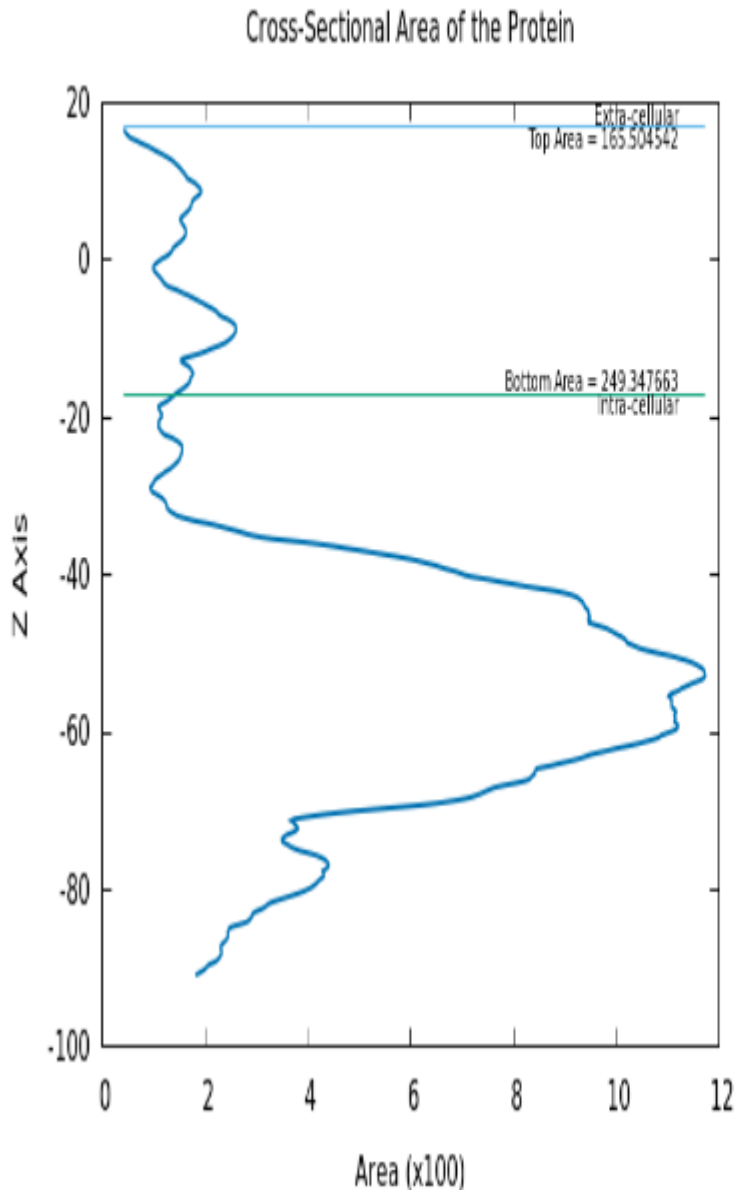
* Calculate Cross-Sectional Area:
  - Oriented PDB:



· Calculated Cross Sectional Area:

Calculated Cross Sectional Area:

Protein Top Area: 1
Protein Bot Area: 2
X Extent: -25 to 21
Y Extent: -27 to 43
Z Extent: -91 to 17



1.1 Determine the system size

Simulation systems:

Table 1. Summary of microsecond-timescale simulations run on Anton2.

Simulated System	Protonation State	Memb
Bcl-xL with 50 mM NaCl	Protonated Bcl-xL	
Bcl-xL with 50 mM NaCl	Unprotonated Bcl-xL	
Bcl-xL with neutralizing NaCl	Protonated Bcl-xL	2
Bcl-xL with neutralizing NaCl	Unprotonated Bcl-xL	2

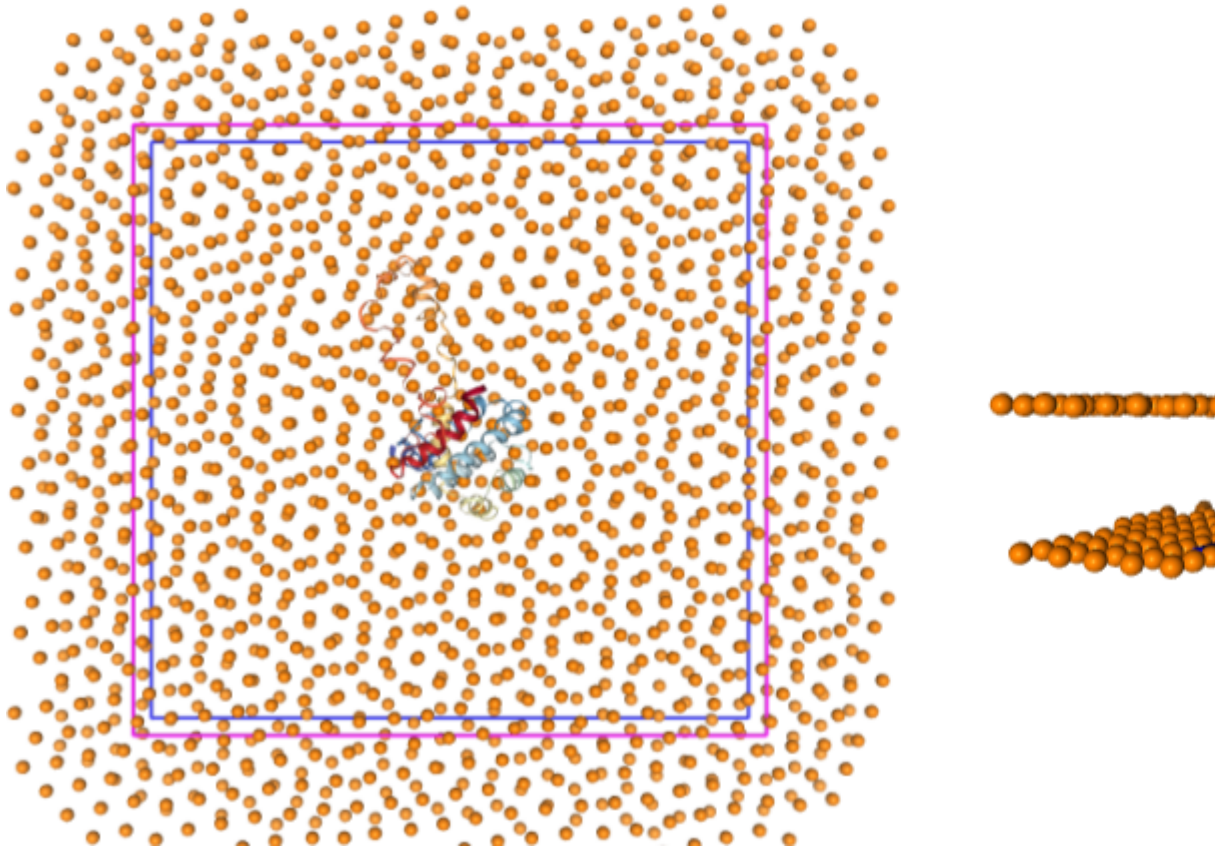
1.1.1 System 01: Bcl-xL with 50 mM NaCl, Protonated Bcl-xL, POPC

- For the protonated 100% POPC simulation,
- Bcl-xL was embedded in a lipid bilayer consisting of:
  - 412 POPC (Paper)
    - System : 412 upperleaflet, 411 lowerleaflet)
  - Both solvated with 36,094 water molecules and neutralized,
  - And with 50 mM excess NaCl (32 Na+, 56 Cl−),
  - For a total of 167,206 atoms and an initial simulation cell size of 130.0 Å × 128.1 Å × 125.0 Å
- Calculated XY System Size:

Determined System Size:

Box Type	Rectangle		
Crystal Type	TETRAGONAL		
System Size	A	168.264303	Dimension along the A (X
	B	168.264303	Dimension along the B (Y
	C	155.952	Dimension along the C (Z
Crystal Angle	Alpha	90.0	Angle between the axis B a
	Beta	90.0	Angle between the axis A a
	Gamma	90.0	Angle between the axis A a
# of Lipids	on Top	412	
	on Bottom	411	
Z Center	-35.476		Center of the system along

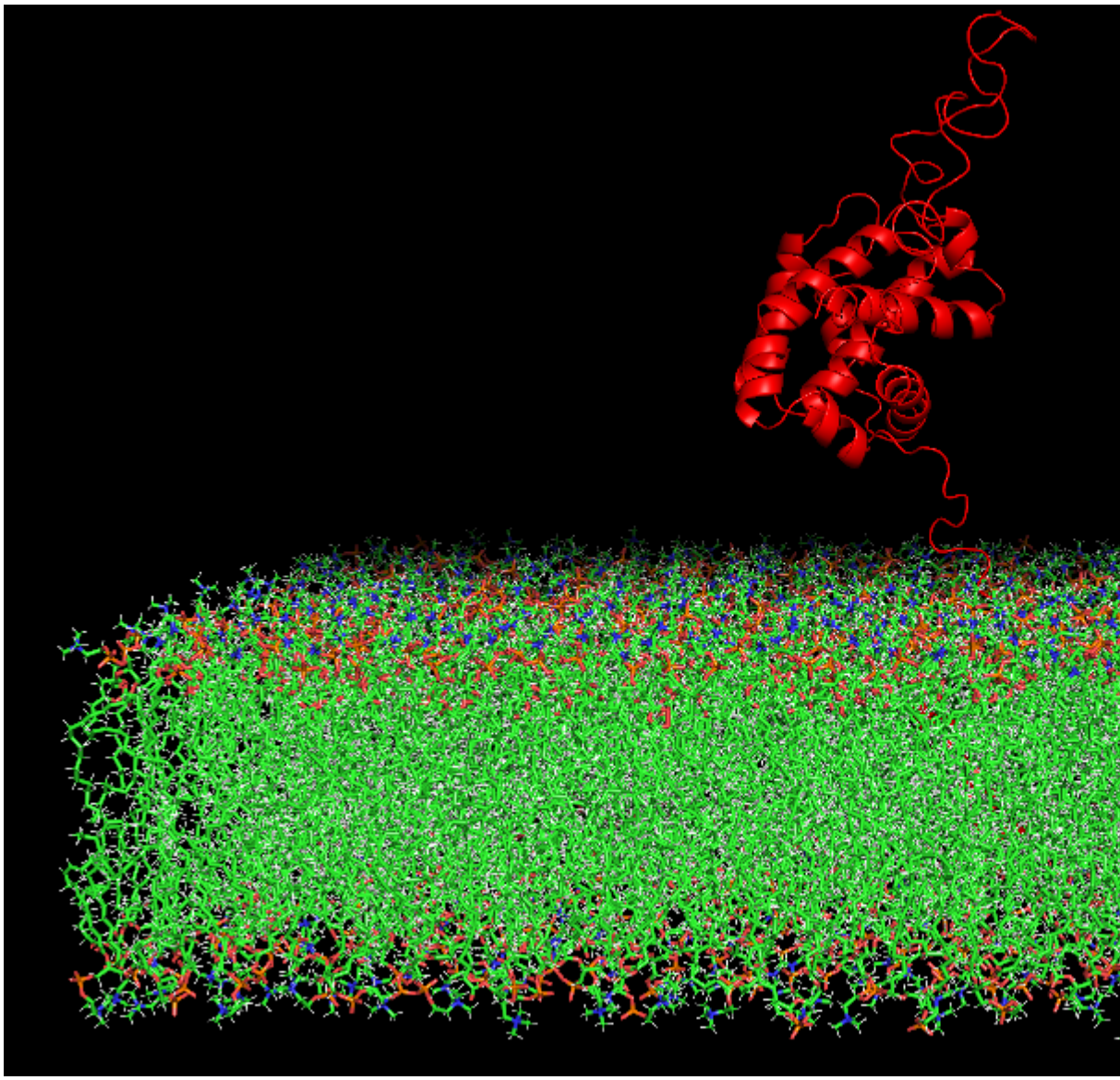
- Generated Packed System:



1.2 Build Components

On the basis of the system size, the generation of individual components for the system, including the membrane, bulk water, and counter ions will be completed in this step.

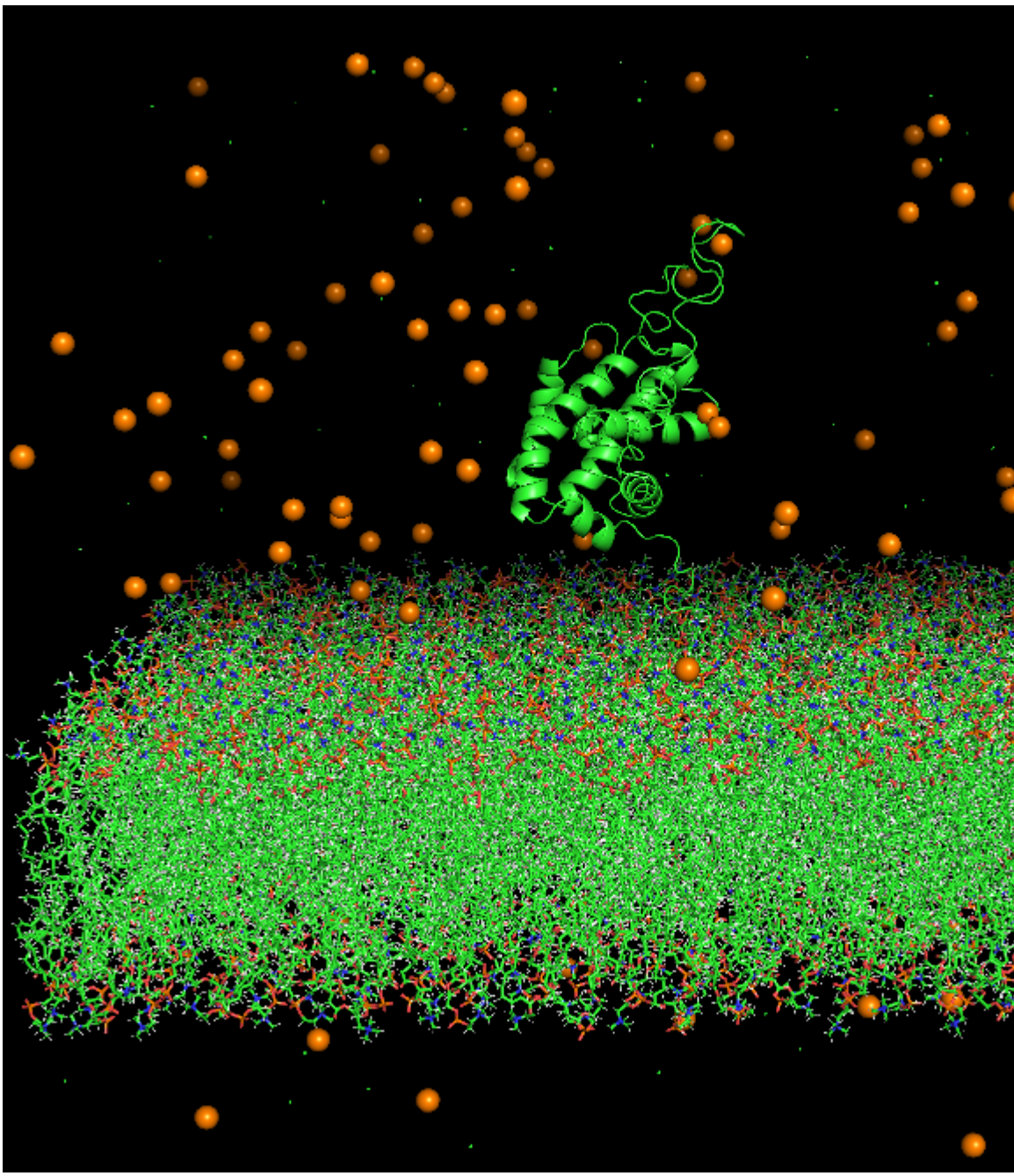
- Neutralized with 50mM excess NaCl (103Na+, 90Cl-)
- PDB Structure:



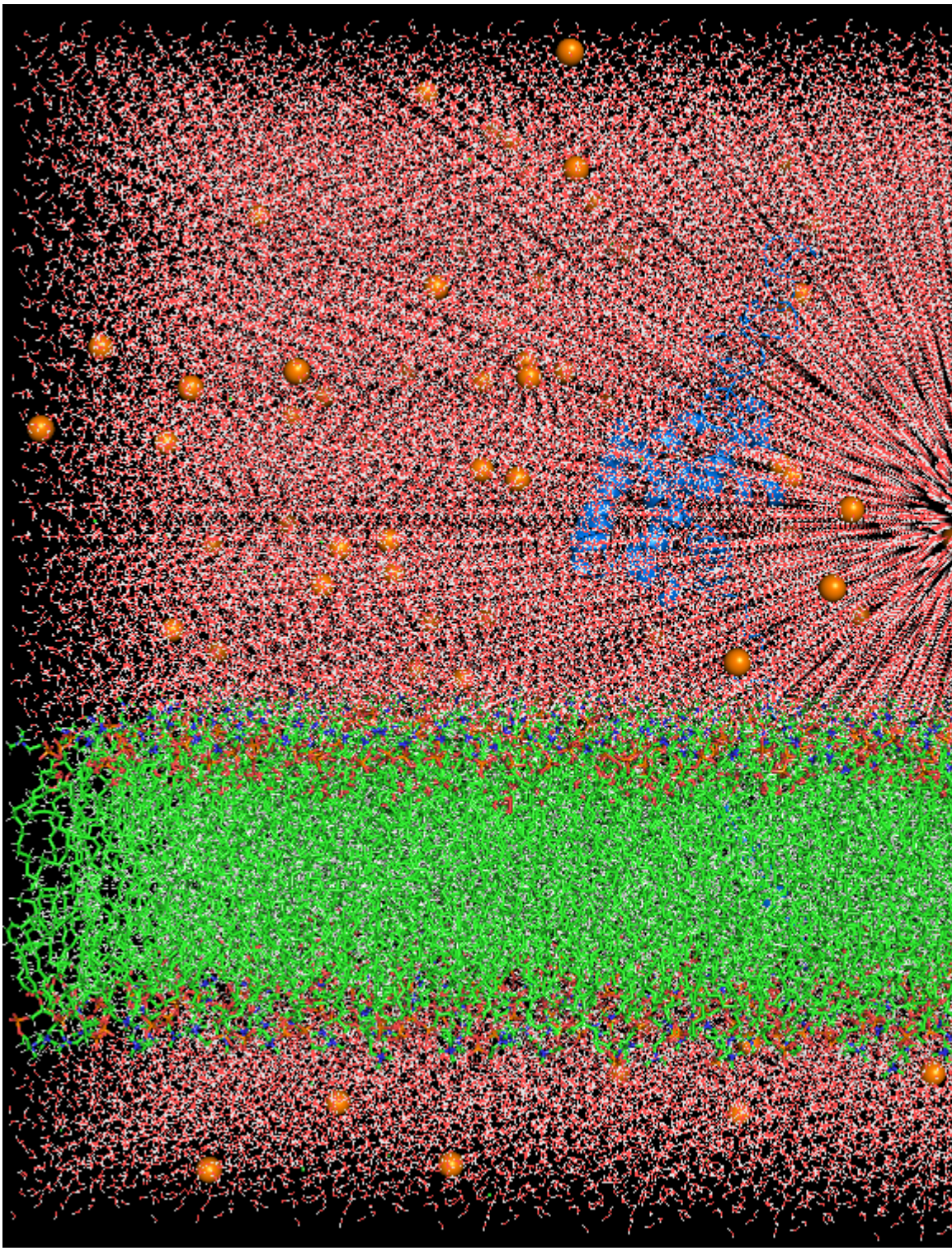
1.3 Assemble the components

So for now, the entire system containing protein, lipid bilayer, bulk water, and ions is generated completely through user-specified parameters and options in CHARMM-GUI.

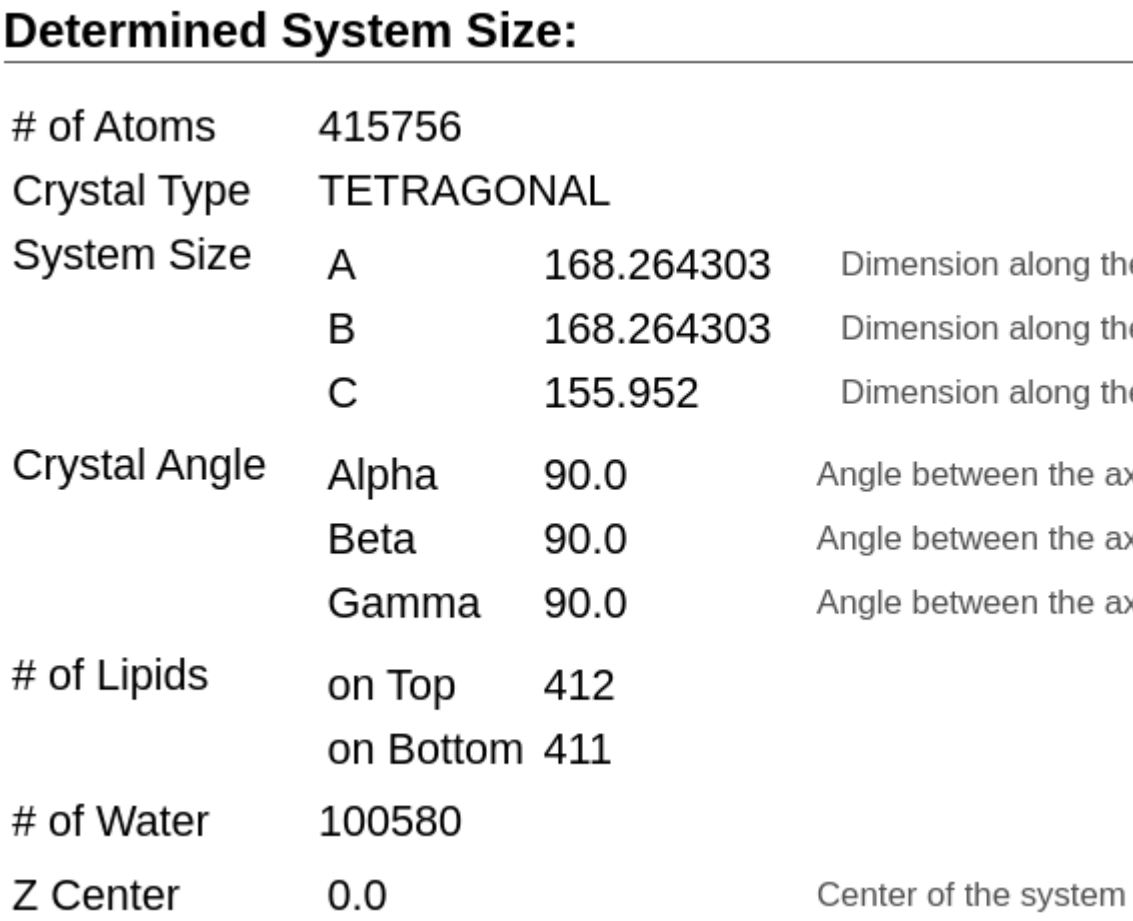
– PDB file for the ion:



– PDB file for the final assembly: lipids + ions + water:



– Final summary:



Determined System Size:

# of Atoms	415756		
Crystal Type	TETRAGONAL		
System Size	A	168.264303	Dimension along the
	B	168.264303	Dimension along the
	C	155.952	Dimension along the
Crystal Angle	Alpha	90.0	Angle between the ax
	Beta	90.0	Angle between the ax
	Gamma	90.0	Angle between the ax
# of Lipids	on Top	412	
	on Bottom	411	
# of Water	100580		
Z Center	0.0		Center of the system

2 System equilibration in NAMD

We equilibrate the system in four steps:

- (1) melting lipid tails,
- (2) relaxing the membrane and water with the protein constrained,
- (3) relaxing side chains with protein backbone constrained, and
- (4) relaxing the whole system.

We equilibrate this multiphase system step by step to speed up the equilibration process. The entire equilibration involves several minimization-equilibration cycles, fixing parts of molecules and relaxing the remaining components gradually.

Releasing the whole system at once results in a rapid change of the system size as well as unfavorable conformations, typically causing the simulation to fail.

2.1 Melting of lipid tails

- **Goal:** "In this step, the complete membrane-protein system excluding lipid tails will be fixed for the first simulation. Because the membrane is built in a nearly crystalline state, the aliphatic tails must be "melted" to achieve a more fluid- like state."
- **Method:** We modified a tcl script (getcnst_S1.tcl to bclxl_getcnst_S1.tcl) that uses PMPE lipid to work with a POPC lipid as both lipids have the same tail.