Report 04: Equilibration

June 21, 2022

1 Initial System

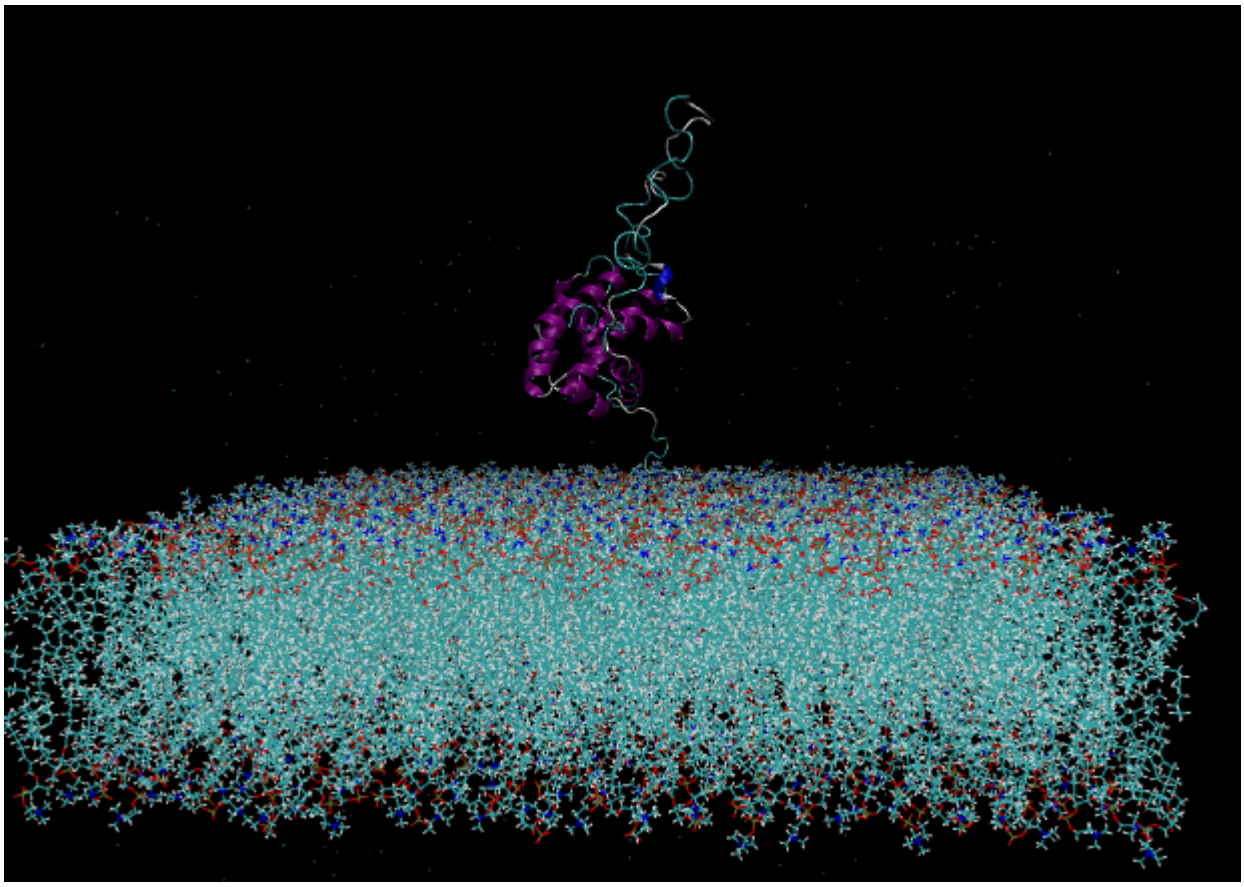

Figure 1: Initial protein-membrane system.

2 Metodology

[Jo2007] "After the protein/membrane complex is assembled, the equilibration must be performed to relax the uncorrelated initial system before MD production simulations. However, due to the significant amount of computing power that cannot be hosted in our server, Membrane Builder provides six consecutive CHARMM input files for equilibration, which can be modified for continual production simulations. As shown in Table 1, to assure gradual equilibration of the initially assembled system, various restraints are applied to the protein, water, ions, and lipid molecules during the equilibration [27]: (1) harmonic restraints to ions and heavy atoms of the protein, (2) repulsive planar restraints to prevent water from entering into the membrane hydrophobic region, and (3) planar restraints to hold the position ofhead groups of membranes along the Z-axis. These restraint forces are slowly reduced as the equilibration progresses. To warrant the successful equilibration, i.e., to avoid instability of dynamics integrations

during equilibration, the NVT dynamics (constant volume and temperature) is used for the first and second steps, and the NPAT (constant pressure, area, and temperature) dynamics for the rest at 303.15 K (DMPC and POPC) and 323.15 K (DPPC)"

Table 1. Detailed information on each equilibration step

Step	Ensemble[1]	Timesteps	Equilibration Time	Force Constants for Harmonic Restraint[2]				
				Protein Backbone[3]	Protein Sidechain[3]	Water[4]	Lipid[5]	Ion[3]
1	NVT	1 fs	25 ps	10.0	5.0	2.5	2.5	10.0
2	NVT	1 fs	25 ps	5.0	2.5	2.5	2.5	0.0
3	NPAT	1 fs	25 ps	2.5	1.0	1.0	1.0	0.0
4	NPAT	2 fs	100 ps	1.0	0.5	0.5	0.5	0.0
5	NPAT	2 fs	100 ps	0.5	0.1	0.1	0.1	0.0
6	NPAT	2 fs	100 ps	0.1	0.0	0.0	0.0	0.0

[1]NVT stands for constant volume and temperature, and NPAT for constant pressure, area, and temperature.
[2]Force constants are in kcal/(mol·Å^2).
[3]Positional harmonic restraints.
[4]Harmonic restraints to keep water molecules away from the membrane hydrophobic region.
[5]Harmonic restraints to keep the lipid tail in -5 Å$<Z<5$ Å, and lipid head groups close to the membrane surface ($Z=\pm17$ Å for DMPC and $Z=\pm19$ Å for DPPC and POPC).
doi:10.1371/journal.pone.0000880.t001

Figure 2: Equilibration steps.

3 Results

3.1 Equilibration

Step	Ensemble[1]	Timesteps	Equilibration Time	Force Constants for Harmonic Restraint[2]				
				Protein Backbone[3]	Protein Sidechain[3]	Water[4]	Lipid[5]	Ion[3]
1	NVT	1 fs	25 ps	10.0	5.0	2.5	2.5	10.0
2	NVT	1 fs	25 ps	5.0	2.5	2.5	2.5	0.0
3	NPAT	1 fs	25 ps	2.5	1.0	1.0	1.0	0.0
4	NPAT	2 fs	100 ps	1.0	0.5	0.5	0.5	0.0
5	NPAT	2 fs	100 ps	0.5	0.1	0.1	0.1	0.0
6	NPAT	2 fs	100 ps	0.1	0.0	0.0	0.0	0.0

[1] NVT stands for constant volume and temperature, and NPAT for constant pressure, area, and temperature.
[2] Force constants are in kcal/(mol·Å^2).
[3] Positional harmonic restraints.
[4] Harmonic restraints to keep water molecules away from the membrane hydrophobic region.
[5] Harmonic restraints to keep the lipid tail in −5 Å$<Z<$5 Å, and lipid head groups close to the membrane surface ($Z = \pm 17$ Å for DMPC and $Z = \pm 19$ Å for DPPC and POPC).

doi:10.1371/journal.pone.0000880.t001

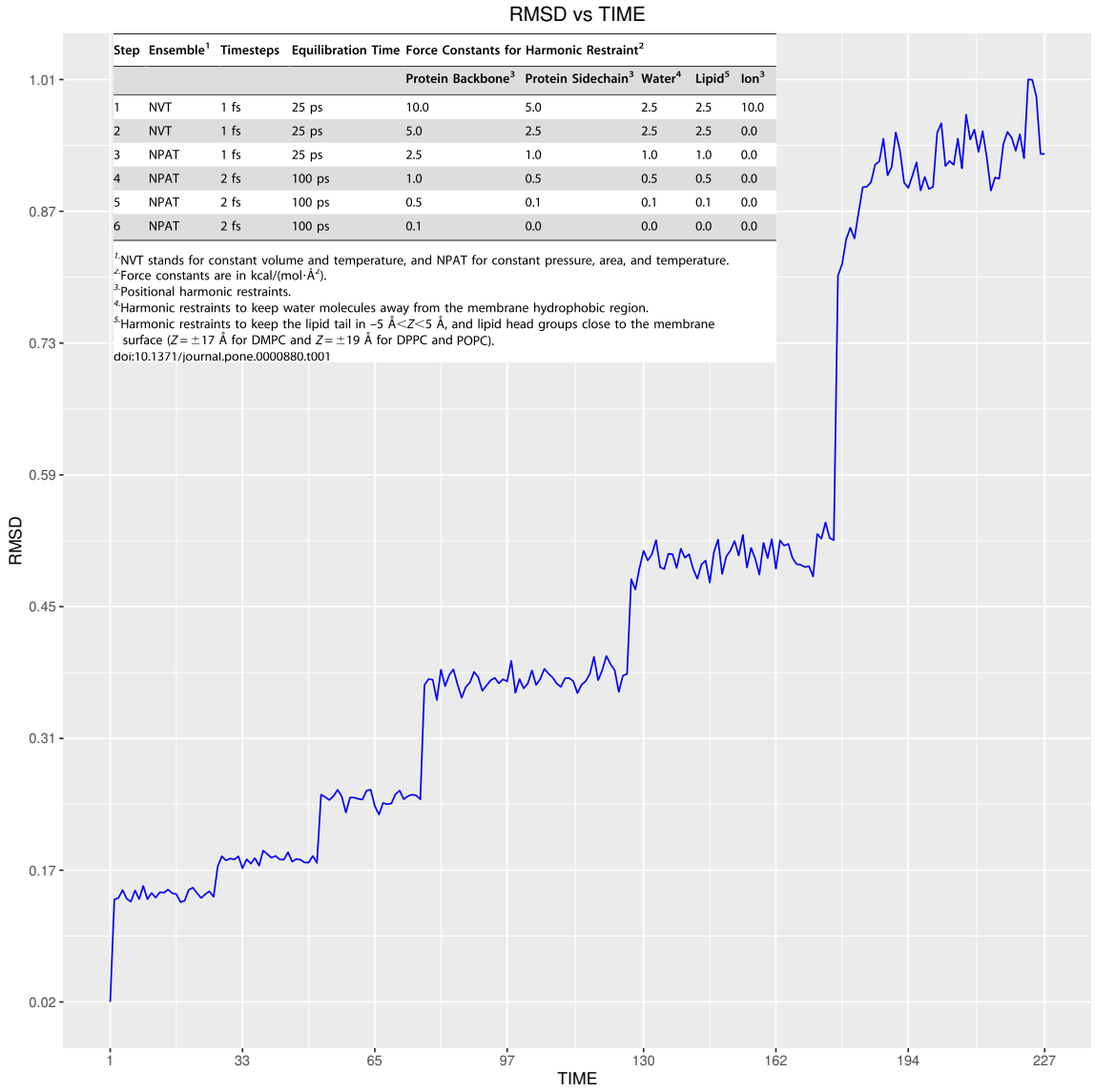

Figure 3: RMSD for equilibration steps.

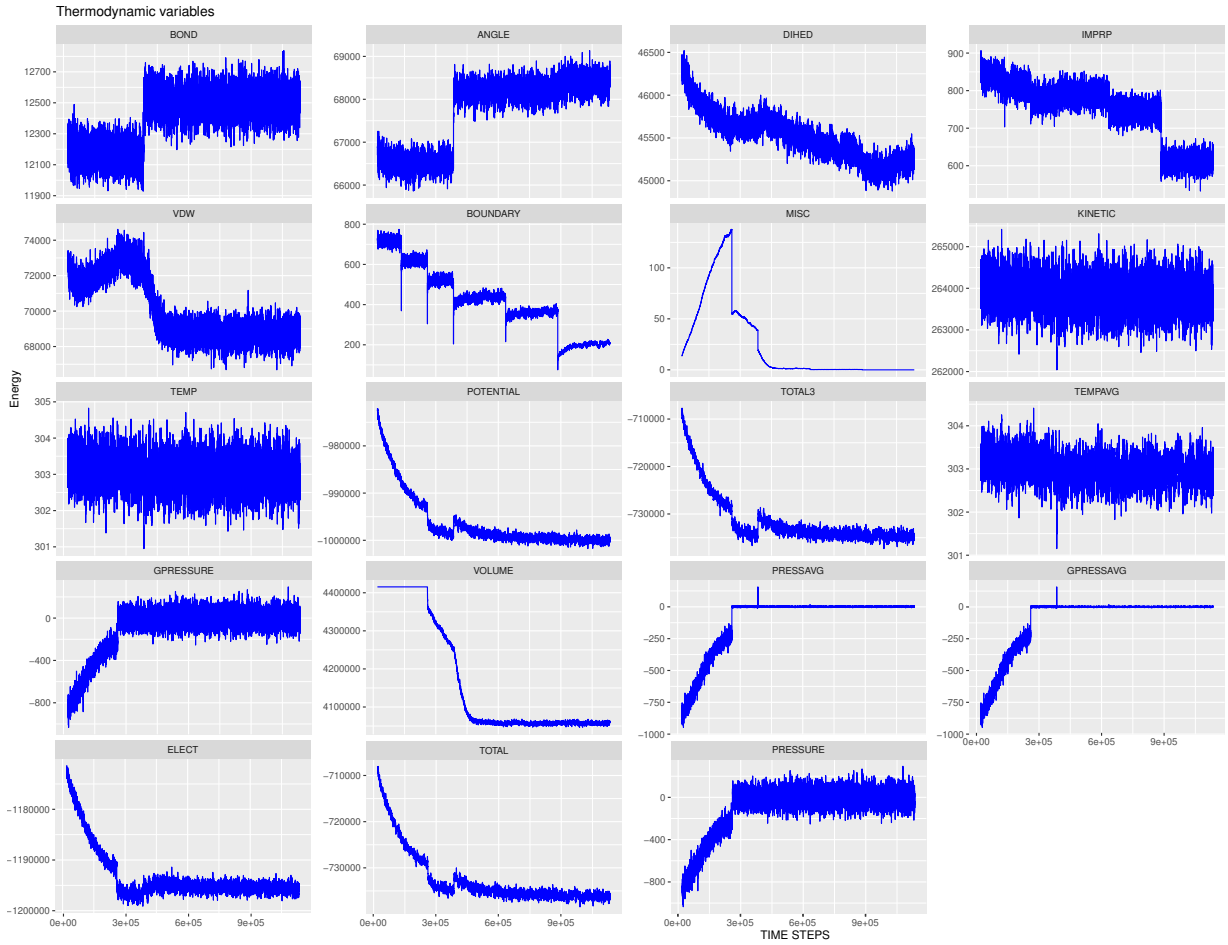

Figure 4: Thermodynamic variables describing the thermodynamic state of the whole system.

3.2 Production

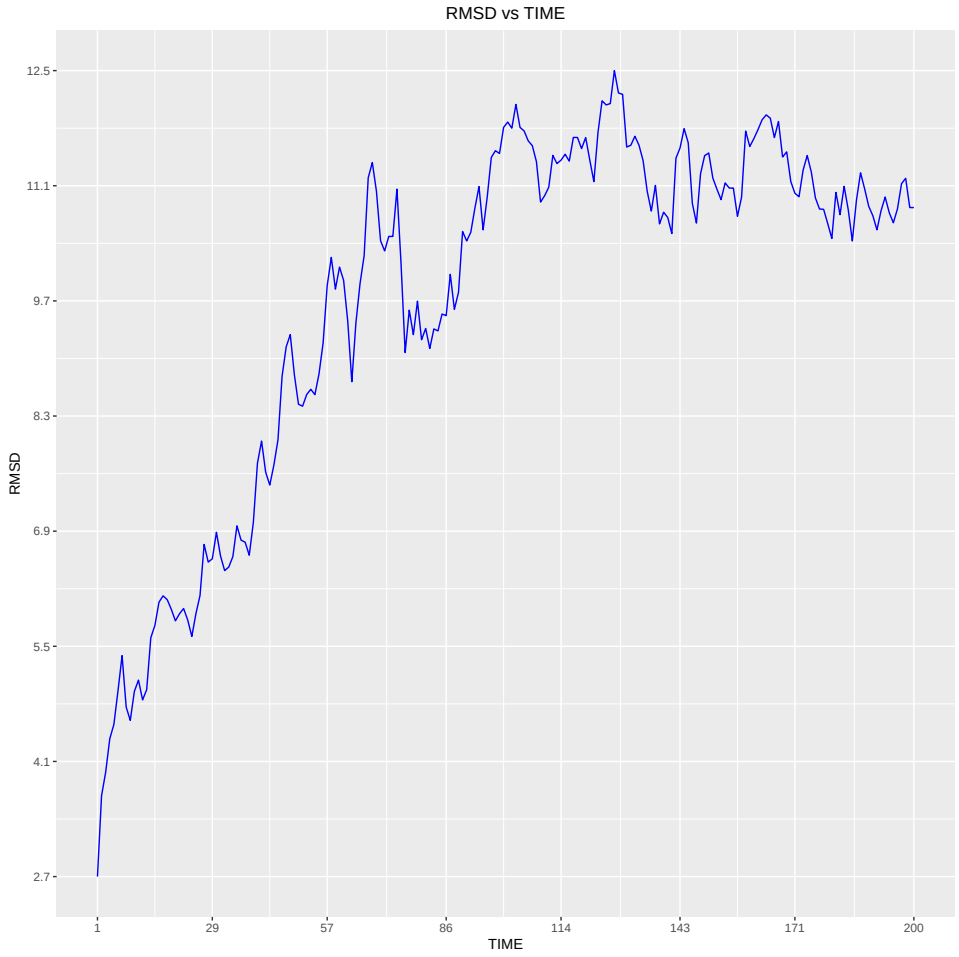

Figure 5: RMSD for 20ns of MD simulation.

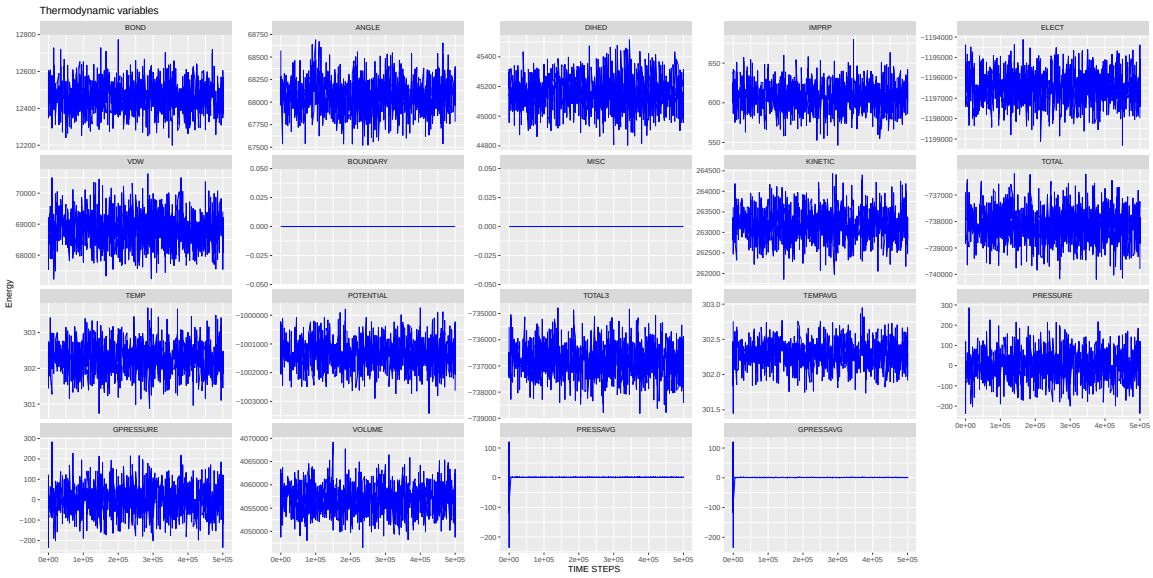

Figure 6: Thermodynamic variables for 20ns MD simulations.