FINITE-SIZE EFFECT ON THE FREE ENERGY LANDSCAPE OF PHASE SEPARATION IN LIPID BILAYERS ESTIMATED USING MOLECULAR DYNAMICS

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Abstract

The "lipid raft" hypothesis posits that cell membranes contain domains with distinct lipid compositions, with "rafts" of ordered lipids and cholesterol floating in a sea of disordered lipids. This behavior is readily recapitulated in experiments with model membranes, where phase diagrams show regions with multiple coexisting phases. Molecular dynamics (MD) simulations can also be used to observe spontaneous phase separation. The existence of coexisting phases demonstrates that the free energy to separate is negative but does not report on the amplitude of the free energy change; historically, this value was only accessible from theoretical treatments. We recently developed a new technique to compute the free energy change associated with phase separation using MD simulations with the weighted ensemble method. However, to compare the results with theoretical or experimental measurements, we need to understand the effects of finite simulation size in contrast to the far larger experimental systems and "infinite" theoretical models. We investigate this by computing free energy profiles as a function of a lipid-clustering based coordinate for systems ranging from 324 to 10110 lipids. The results indicate that only very large systems even begin to approach the thermodynamic limit..

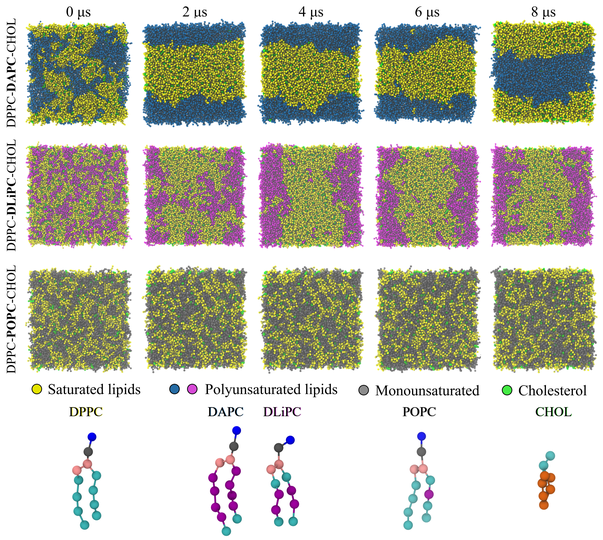
Simulation Details

Standard MD Simulations

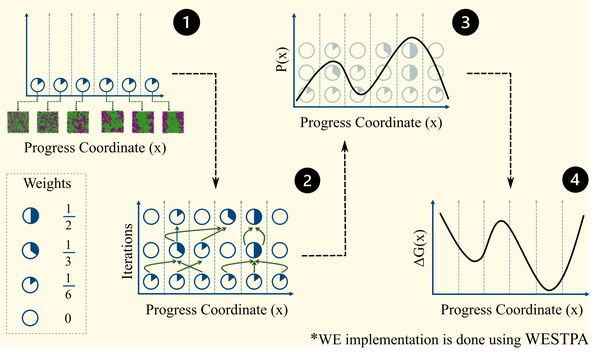
Bilayer construction : CHARMM-GUI
Force Field : CG - MARTINI 2
MD Engine : GROMACS 2021
Timestep : 20 fs

Barostat : 1 bar (Parrinello-Rahman)
Thermostat : 298 K - 450 K (v-rescale)
Electrostatics cutoff : 1.1 nm (RF)
van der Waals cutoff : 1.1 nm (PS-verlet)

Model Lipid Bilayer Systems

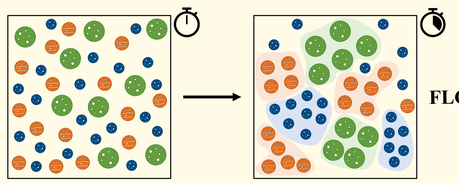


Weighted Ensemble* (WE)



*WE implementation is done using WESTPA

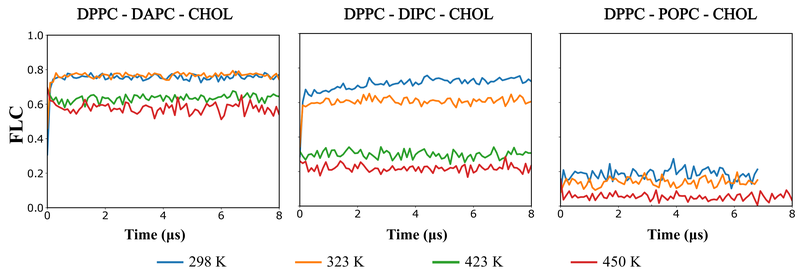
Fraction of Lipids in Clusters (FLC)



$$FLC = \frac{\sum_{X=\bullet\bullet\bullet} \text{No. of lipid } X \text{ in lipid } X \text{ Clusters}}{\text{Total no. of lipids}}$$

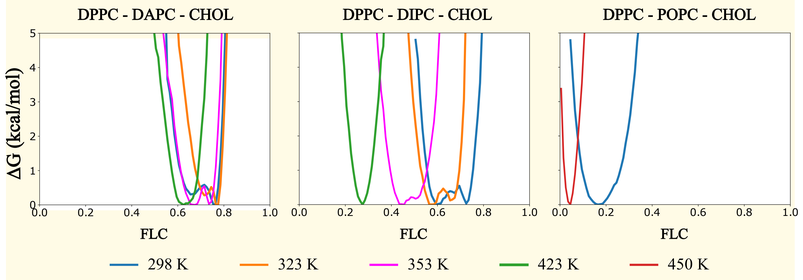
*Clustering using DBSCAN

FLC Tracks Phase Separation



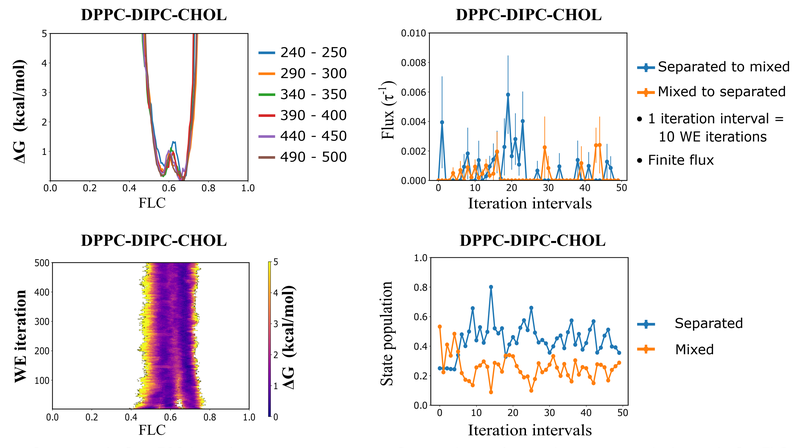
• FLC distinguish system composition and temperature • FLC clearly distinguish between mixed and separated states
• Long standard simulations does not realize multiple transitions

Free Energy Landscapes



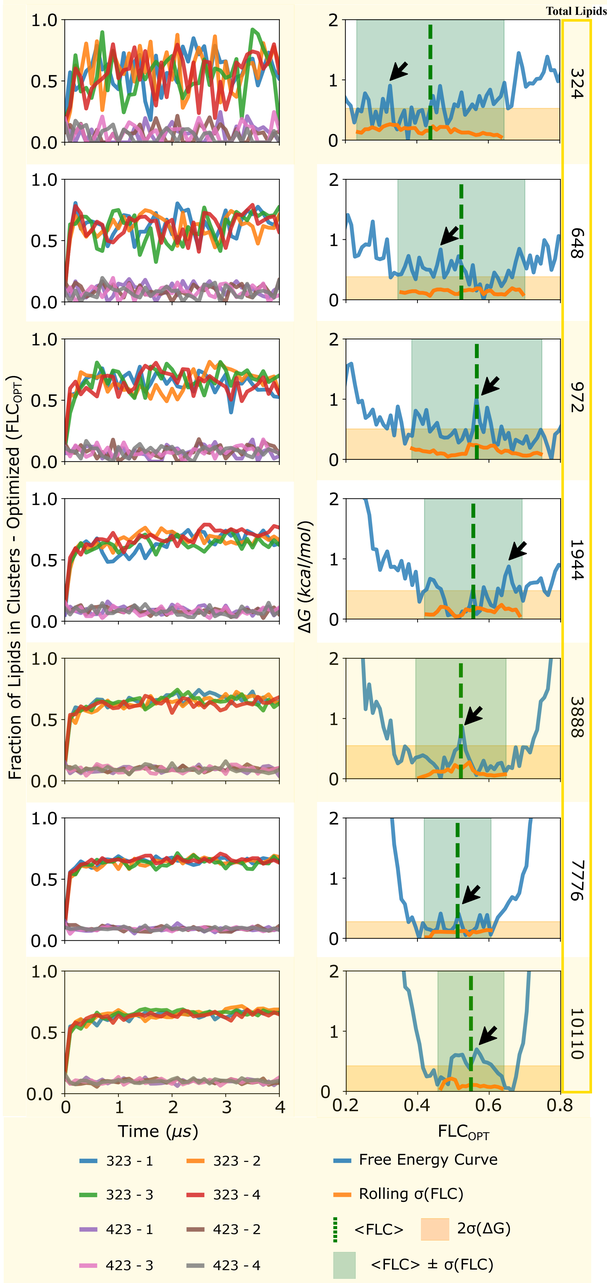
• Free energy landscape captures the role of system composition and temperature in phase separation propensity

Convergence & State Transitions



• Converged after 500 iterations
• Concomitant change in state population only for FLC

System Size Effects on Free Energy Landscape



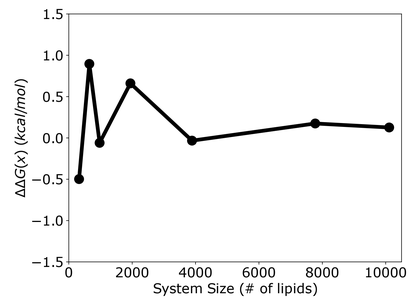
• Standard MD simulations often gets trapped in one of the free energy basins
• Smaller systems have large σ(FLC) in both Standard MD and WE MD
• As system size ↑ WE free energy curves narrows and basins are well-defined, including the barrier between basins

System Size Simulation Details

System	DPPC (0.42)	DIPC (0.28)	CHOL (0.3)	Total Lipids	Total CG Water
1	138	90	96	324	2430
2	276	180	192	648	4860
3	414	270	288	972	7290
4	828	540	576	1944	14580
5	1656	1080	1152	3888	29160
6	3312	2160	2304	7776	58320
7	4306	2808	2996	10110	75825

- Systems 1-5 completed 1000 WE iterations
- Systems 6, 7 completed 600 and 500 WE iterations
- All WE MD simulations at 323K

$\Delta\Delta G_{sep}$ Values Converges for >4k Lipid Systems



- Divide ΔG curve to obtain $\Delta\Delta G_{sep}$
- FLC cutoff = Max ΔG* within <FLC>± σ(FLC)

Conclusions

- Converged free energy landscape for phase separation is possible with WE MD using FLC
- Finite size effects can lead to potential errors in interpreting free energy landscapes
- ΔG and ΔΔG estimation agrees for >3888 lipid systems

Future Directions

- Structural and thermodynamic profiling
- More control experiements with toy systems
- Introducing new components:
  - Peptides
  - Asymmetry
- Switch to all-atom models
  - Molecular interactions deciding phase separation
- Increase the efficiency
  - Include replica exchange with WE protocol
  - Fine tuning WE parameters

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