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# Accurate Estimation of Free Energy Landscape of Phase Separation in Lipid Bilayers

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## Abstract

Liquid-liquid phase separation and the subsequent biological condensate formation help maintain cell homeostasis. Cell membranes have previously been shown to undergo a similar process of domain formation, where certain lipids separate into ordered and disordered regions, which led to the "lipid-raft" hypothesis. Fully understanding the thermodynamics of lipid phase separation is not trivial as it involves multiple variables. Here, we attempt to construct the free energy surface (FES) of phase separation events in model lipid bilayers using molecular dynamics (MD). Even though the current status of standard MD can realize a separating mixed lipid system, we lack an effective way to sample multiple such transitions to construct FES with statistical rigor. The relative timescale and system size needed for simulating phase separation events pose a challenge for adequate sampling. To tackle this, we use coarse-grained model membranes with enhanced sampling protocols. Using this pipeline, we can quantify the propensity of the lipid system to phase separate. We also evaluate the system behavior using auxiliary variables that track phase separation and compare it with the principal collective variable that drives the enhanced sampling. We also discuss our plans to extend the current pipeline to construct phase separation FES as a function of different compositions and environments.

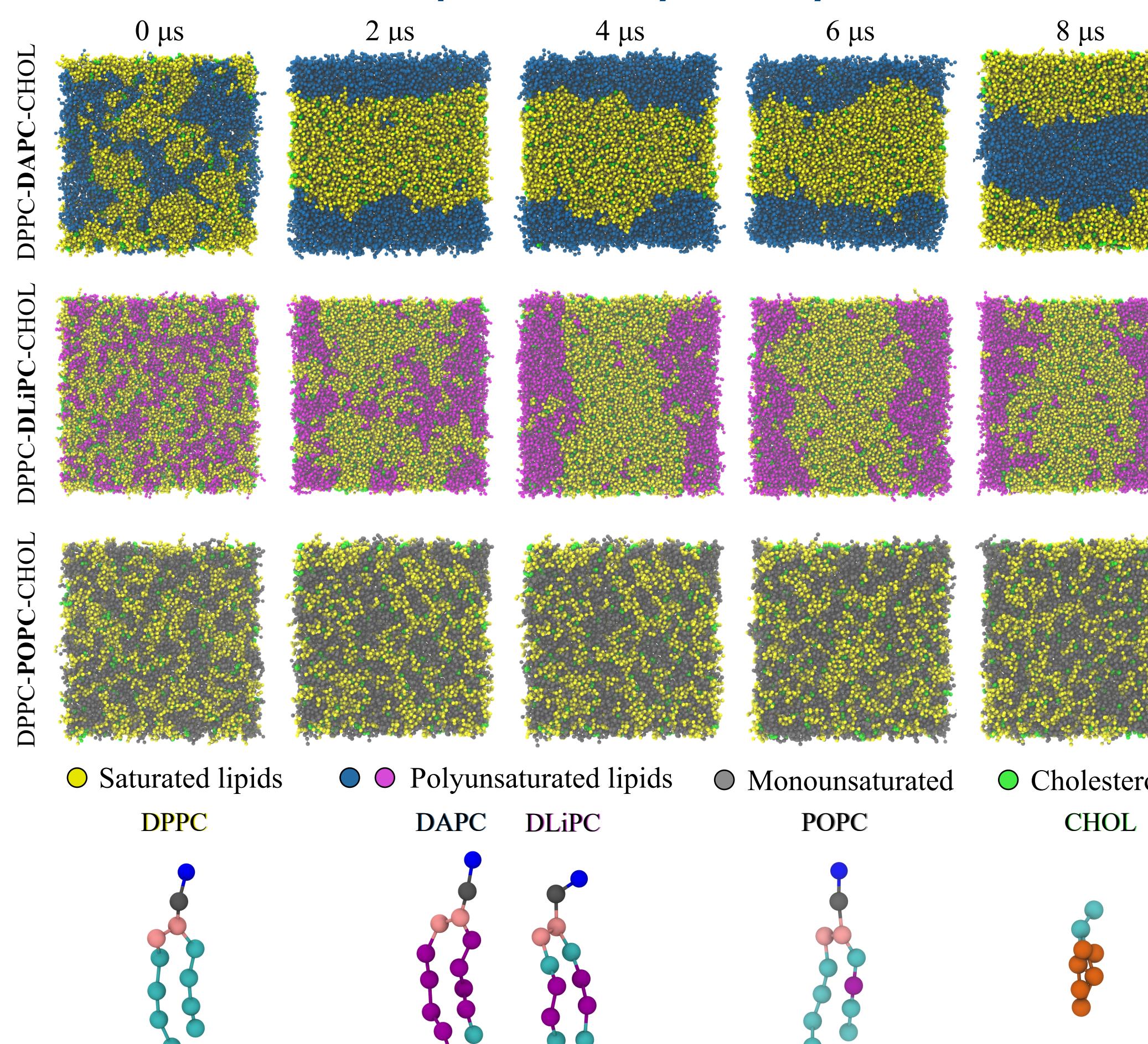
## Simulation Details

### Standard MD Simulations

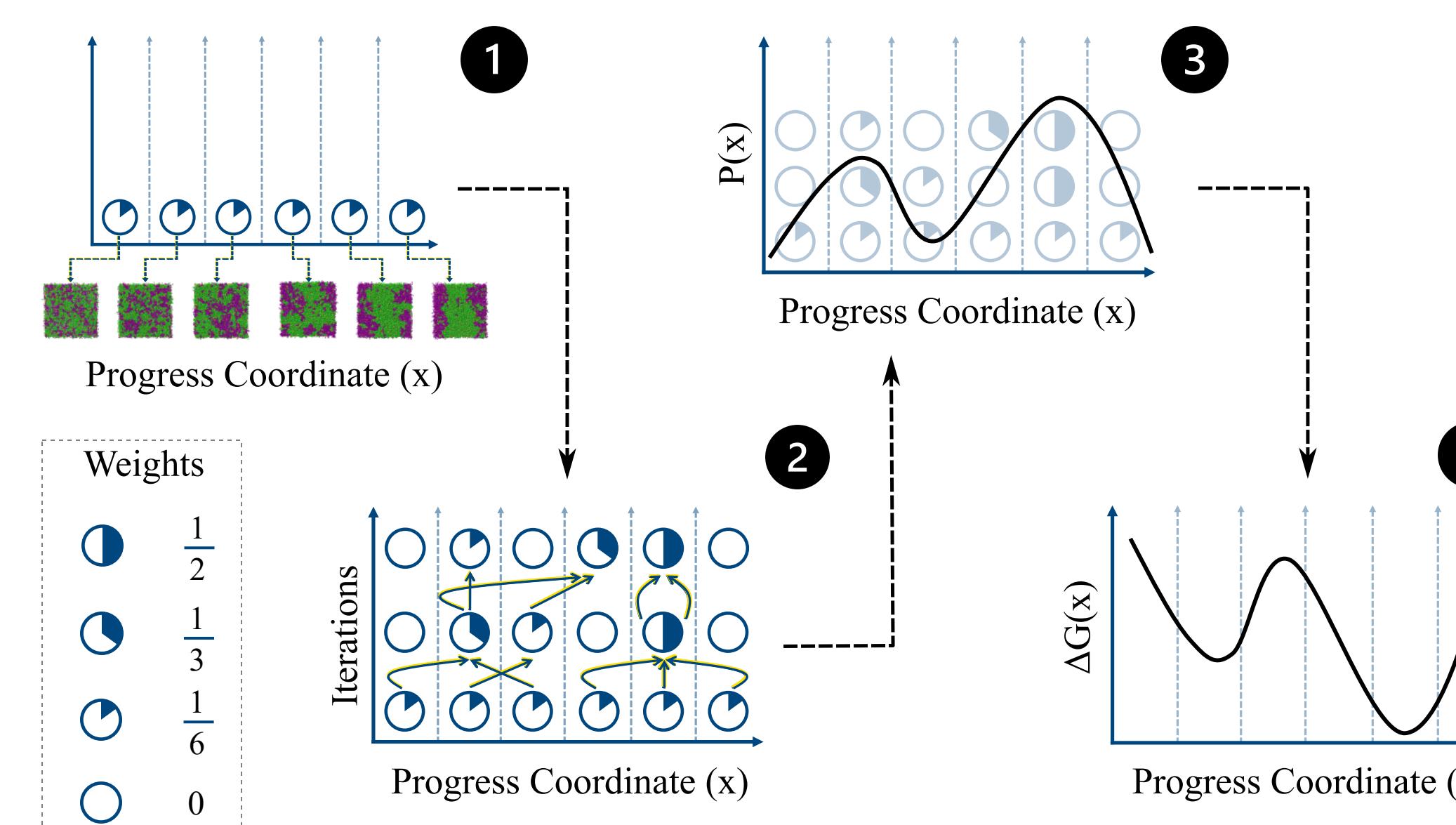
Bilayer construction : CHARMM-GUI  
Force Field : CG - MARTINI 2  
MD Engine : GROMACS 2021  
Timestep : 20 fs

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)

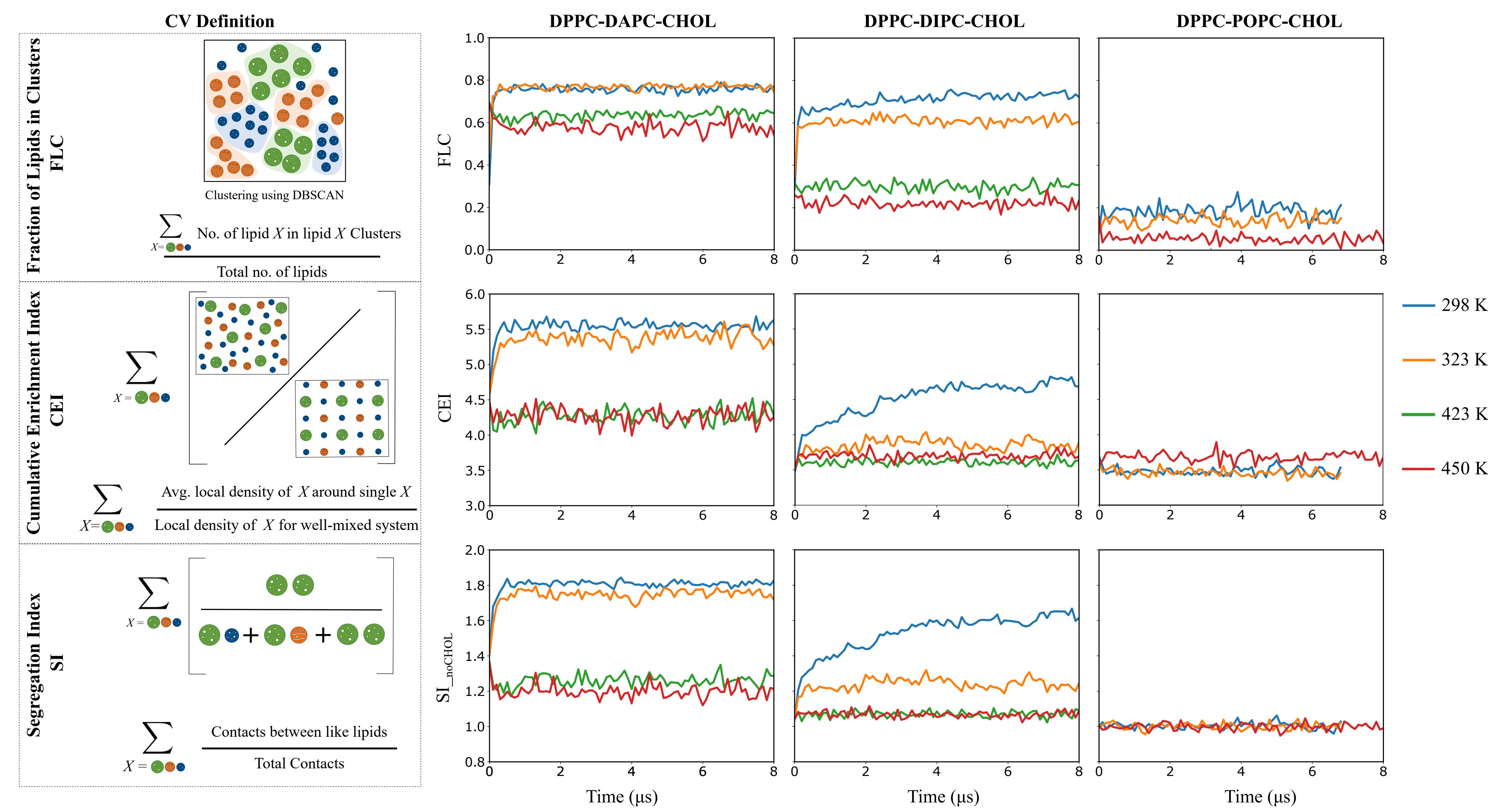
### Model Lipid Bilayer Systems



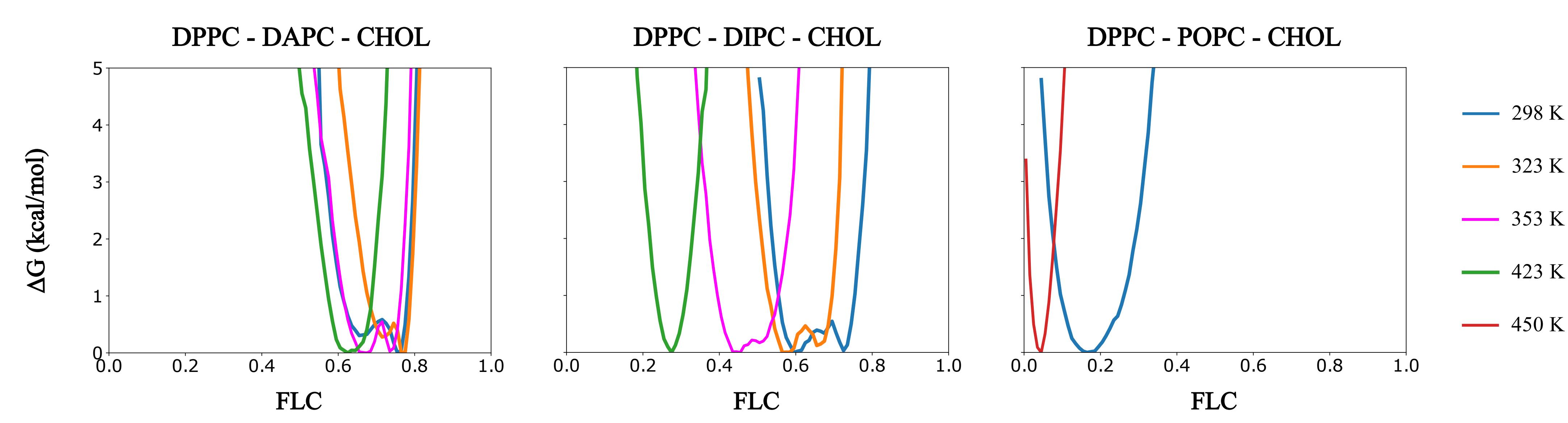
### Weighted Ensemble\* (WE)



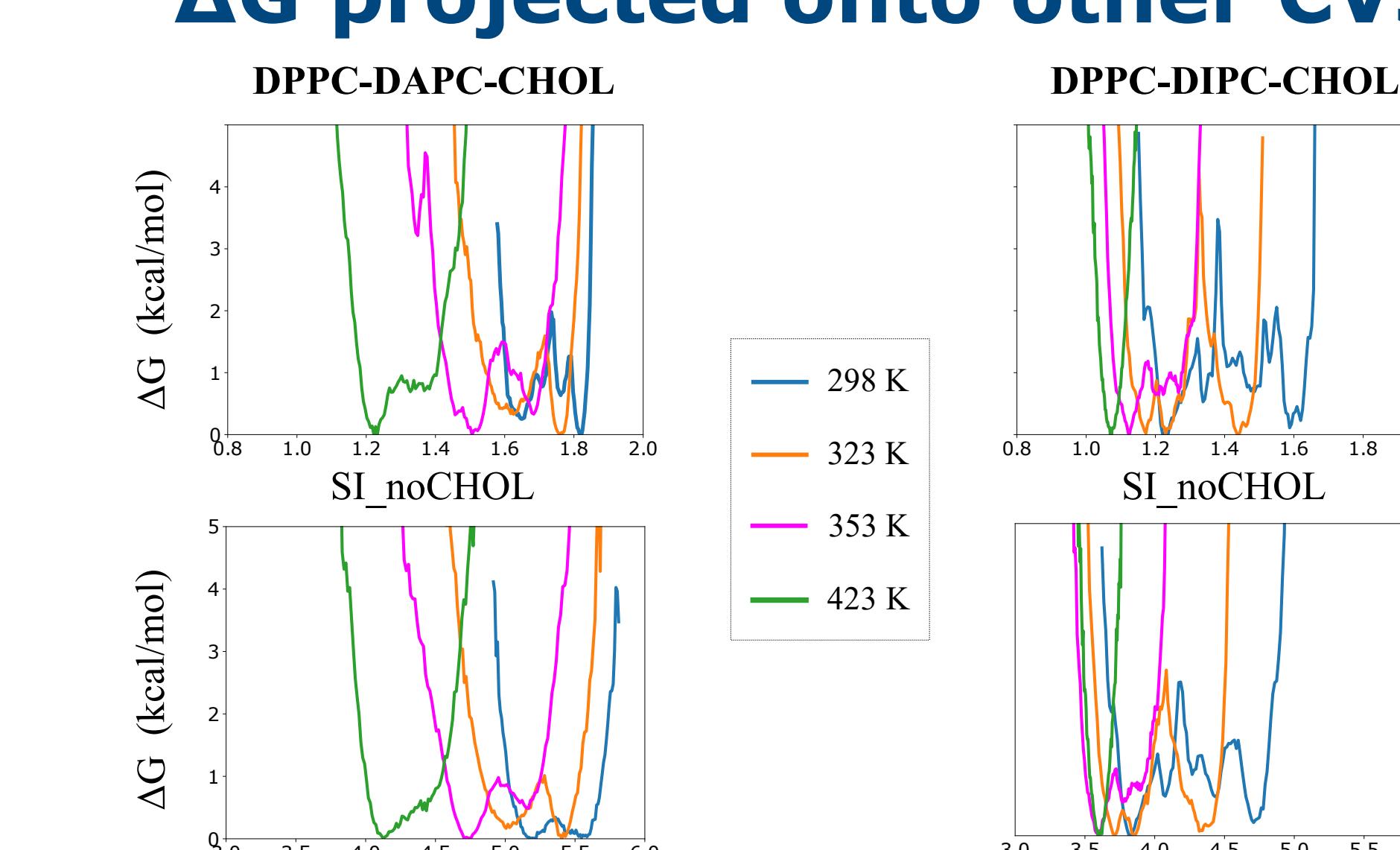
## Collective Variables Track Phase Separation



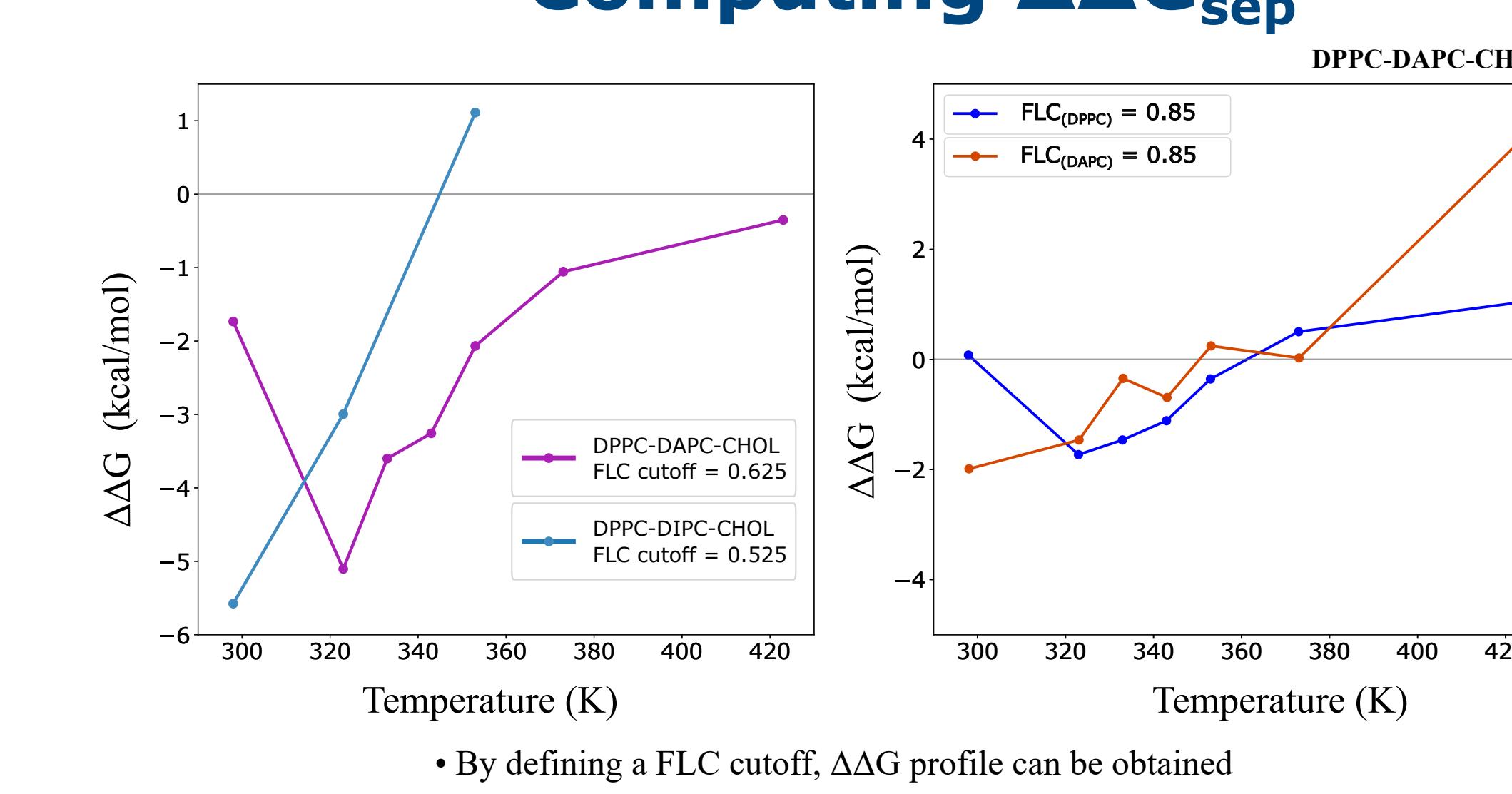
## Free Energy Landscapes of Lipid Bilayer Systems



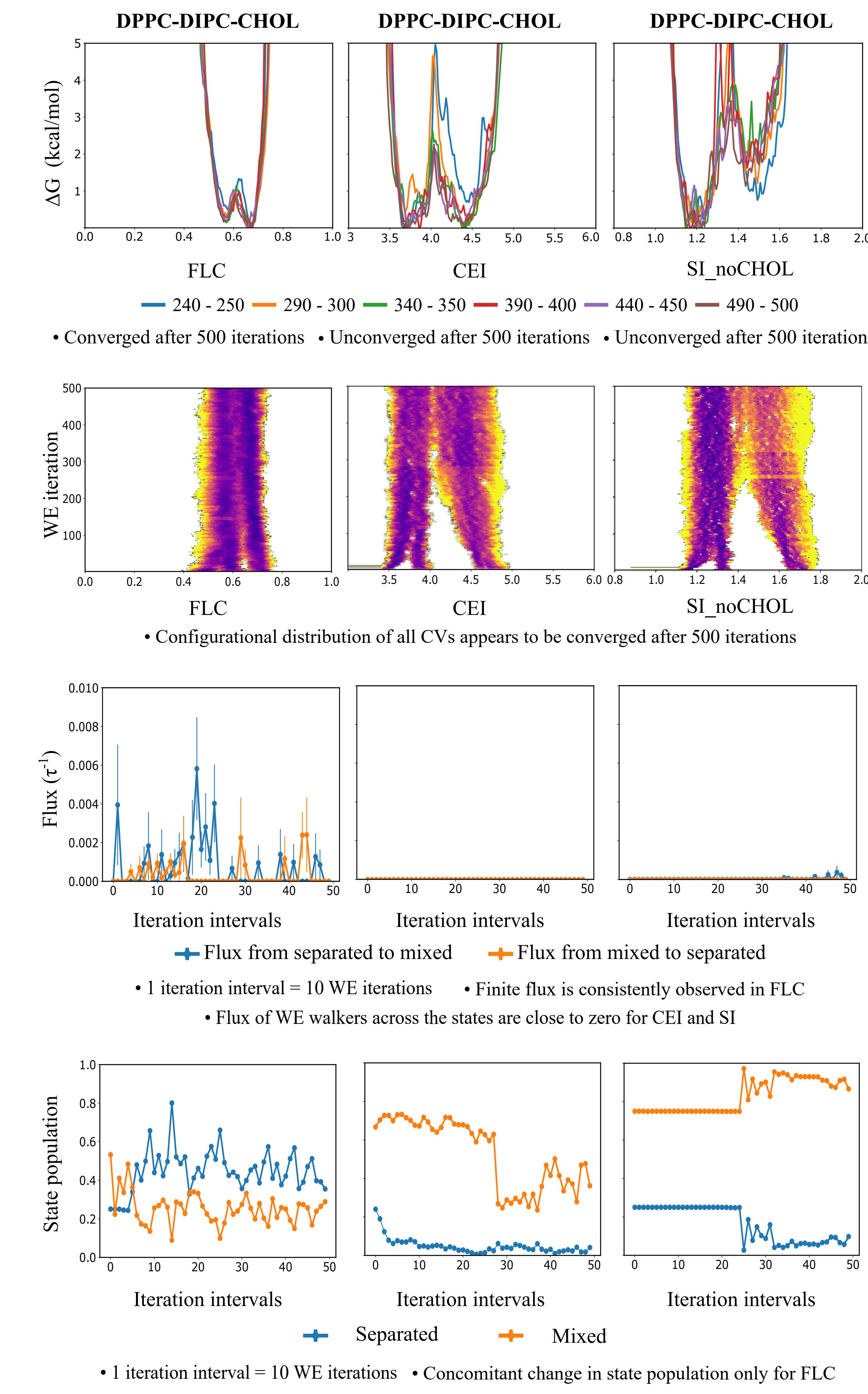
## ΔG projected onto other CVs



## Computing ΔΔG<sub>sep</sub>



## CV Choice is Critical



## Conclusions

- Constructing phase separation free energy landscape is possible with WE MD
- Collective variable based on the clustering is promising
- Right choice of collective variable leads to success

## Future Directions

- Switch to all-atom models
- Molecular interaction deciding Phase separation
- Increase the efficiency
- Include replica exchange with WE protocol