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

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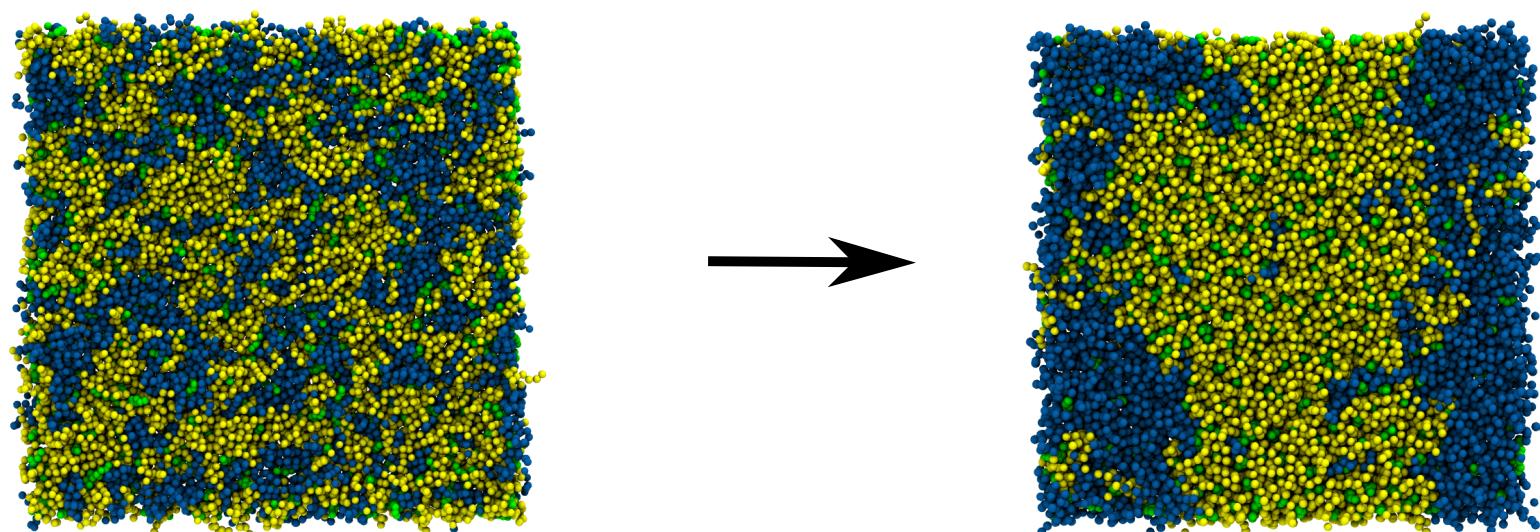
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Abstract

Liquid-liquid phase separation and the resultant formation of biological condensates play an essential role in cell homeostasis. One early evidence for the phenomenon was in lipid membranes, where the preference for specific lipids to separate into ordered regions led to the so-called "lipid raft" hypothesis. Despite considerable evidence for the formation of biological condensates and their speculated role in disease states, we poorly understand the molecular grammar behind phase separation. While standard molecular dynamics (MD) simulations can reproduce the phenomenon in silico with high spatial resolution, we lack an effective way to determine the exact thermodynamics of these systems. Here, we present our efforts to use MD simulations to understand phase separation thermodynamics in model membranes, combining coarse-grained (CG) modeling with enhanced sampling protocols. With this pipeline, we can assess not just whether the system favors the formation of the co-existing phases or not as a function of temperature and membrane composition but also quantify the system's propensity to phase separate in the first place. Our work demonstrates that the efficiency of these simulations is highly dependent on the choice of collective variables used to sample and analyze the data.

Phase Separation in Lipid Bilayers



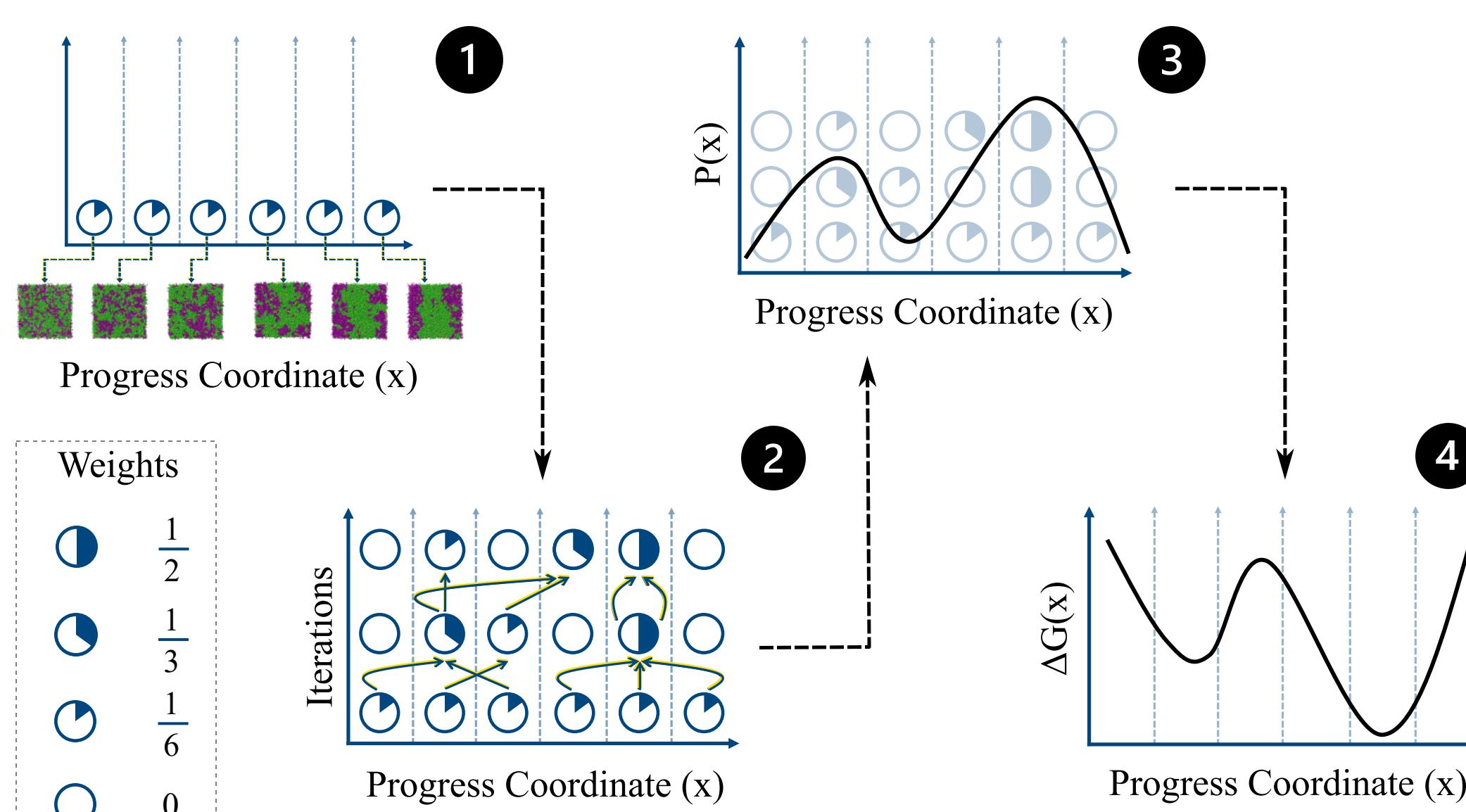
- Experimental evidence of "Lipid Rafts"
- Physiologically relevant
 - Induces phase-specific conformational changes to bound proteins
 - Phase-specific concentration of signaling molecules at membranes
- Computational studies are less expensive
 - Bilayer dynamics is mainly planar - Thin layer approximation
 - Well-parameterized coarse grained (CG) models are available

Simulation Details

Standard MD Simulations

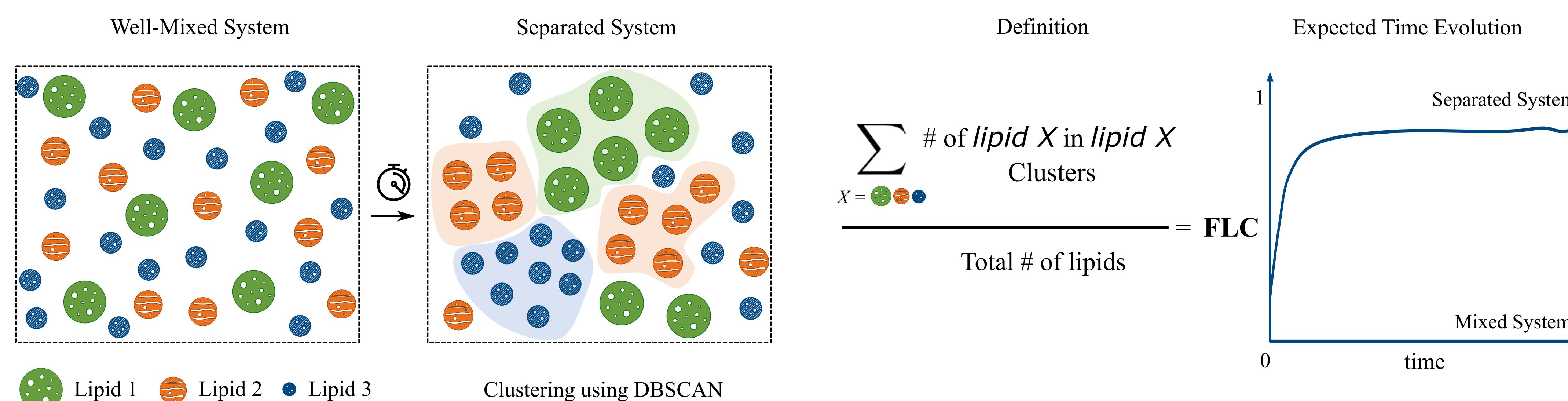
- Systems :
 - DPPC - DLiPC - CHOL (0.42/0.28/0.3)
 - DPPC - DAPC - CHOL (0.5/0.3/0.2)
 - DPPC - POPC - CHOL (0.4/0.4/0.2)
- Temperatures :
 - 298 K, 323 K, 333 K, 343 K, 353 K,
 - 423 K, 450 K
- MD Engine : GROMACS 2021
- Bilayer construction : CHARMM-GUI
- Force Field : CG - MARTINI
- Barostat : 1 bar (Parrinello-Rahman)
- Thermostat : v-rescale
- Electrostatics cutoff : 1.1 nm (RF)
- van der Waals cutoff : 1.1 nm (PS-verlet)
- Timestep : 20 fs

Weighted Ensemble* (WE)

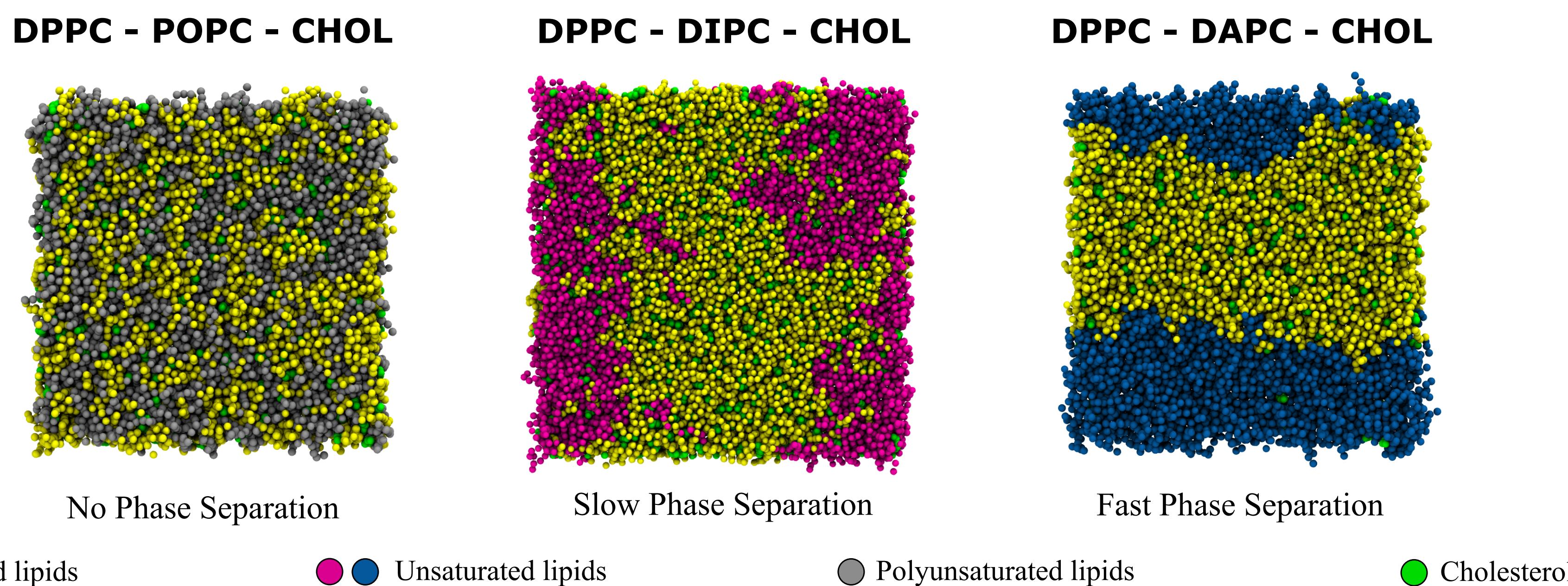


*WE implementation is done using WESTPA

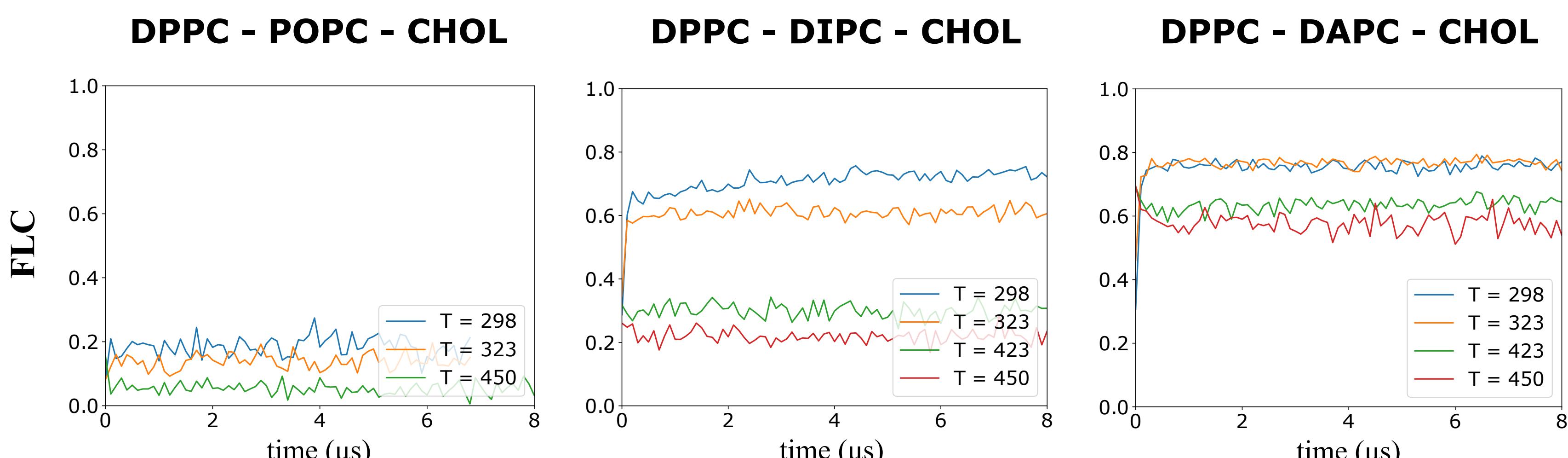
Fraction of Lipids in Clusters (FLC) as Collective Variable (CV)



Three Systems to Study Phase Separation

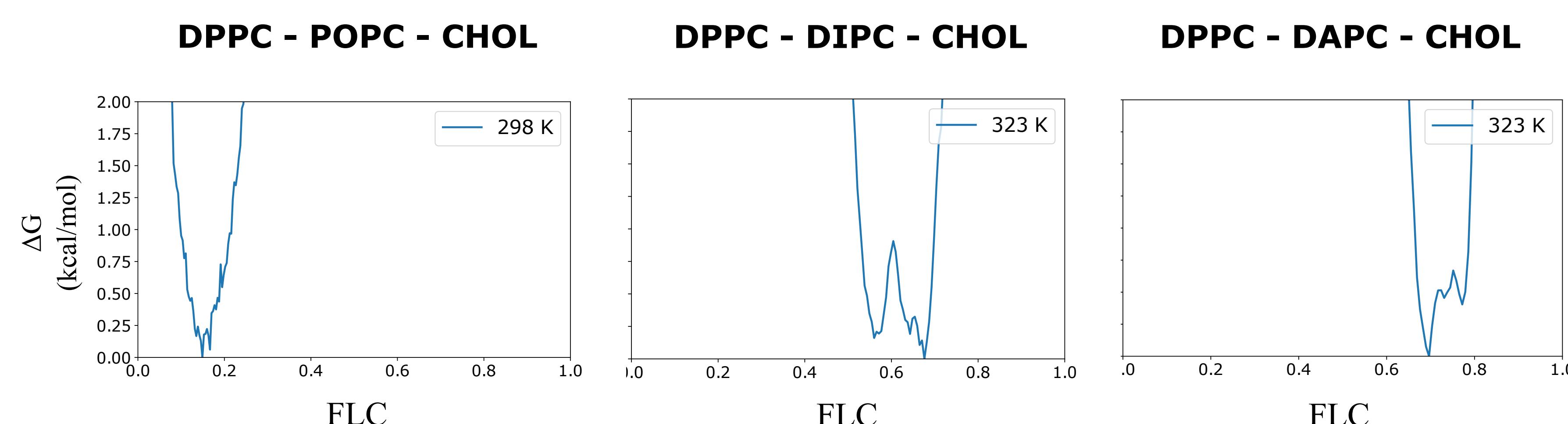


Standard MD is Not Enough to Estimate Free Energy Landscape



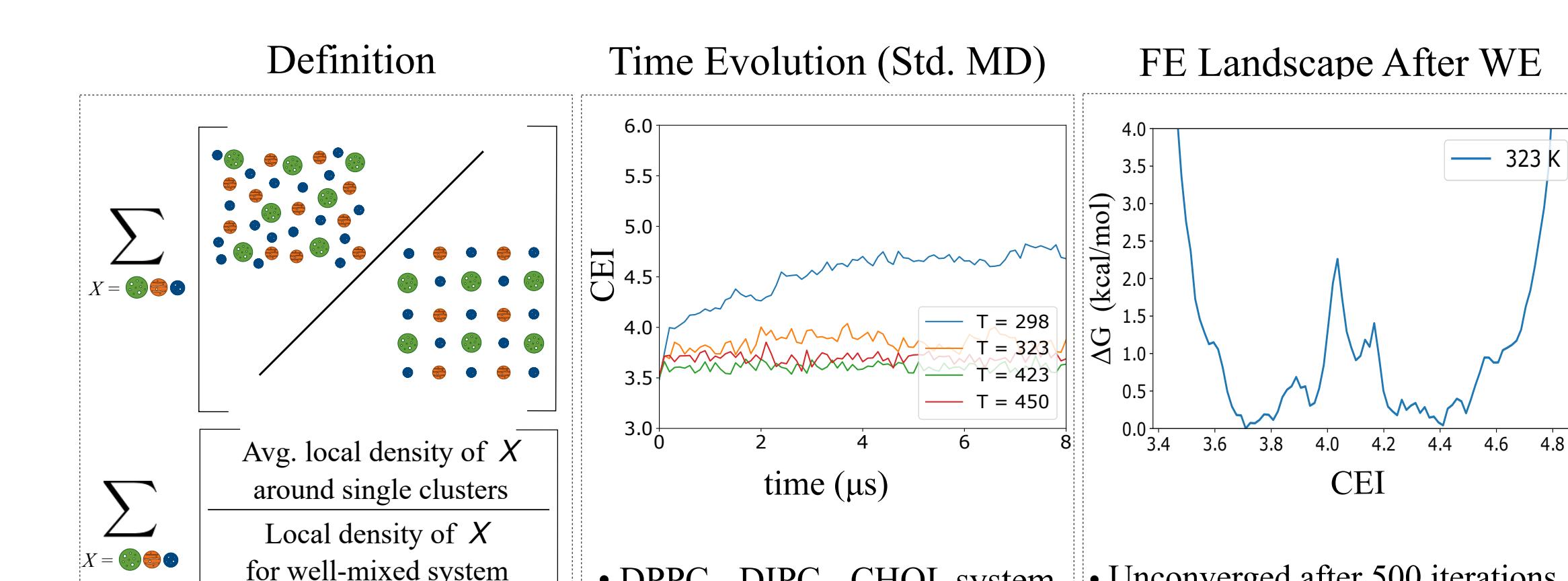
- FLC clearly distinguishes between mixed and separated states
- Long standard simulation does not realize multiple transitions

Free Energy (FE) Landscape After WE

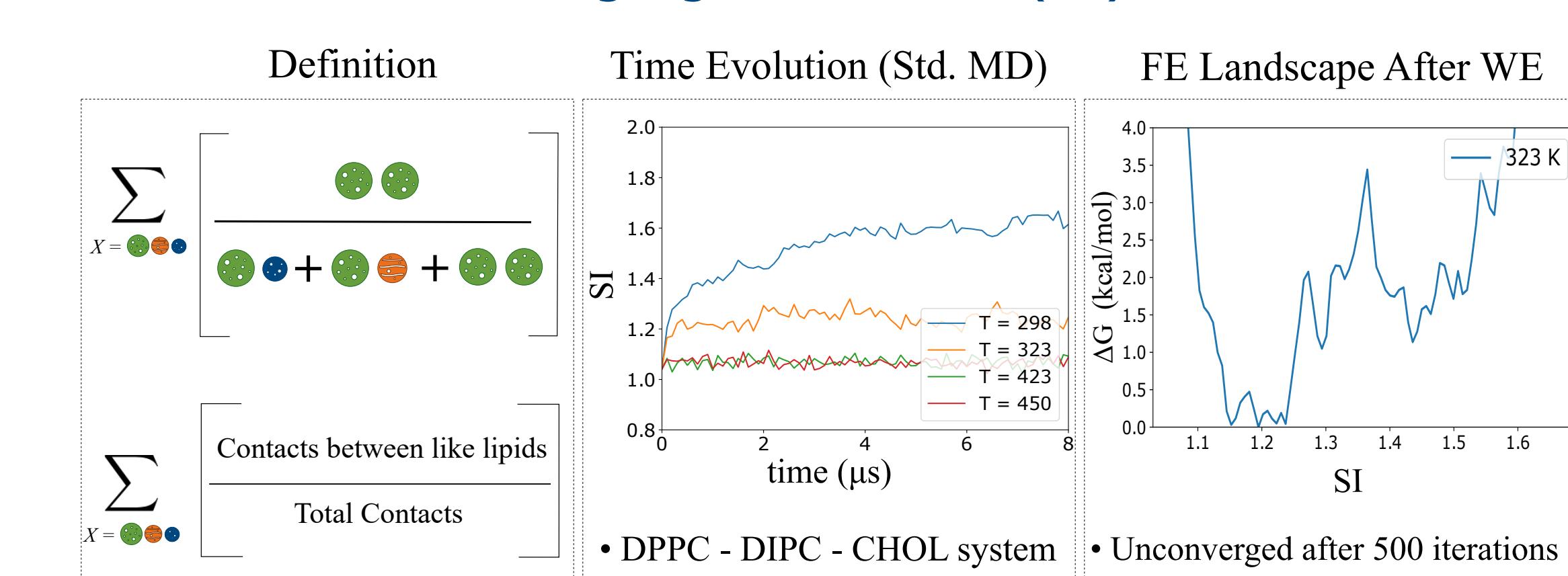


Other CVs

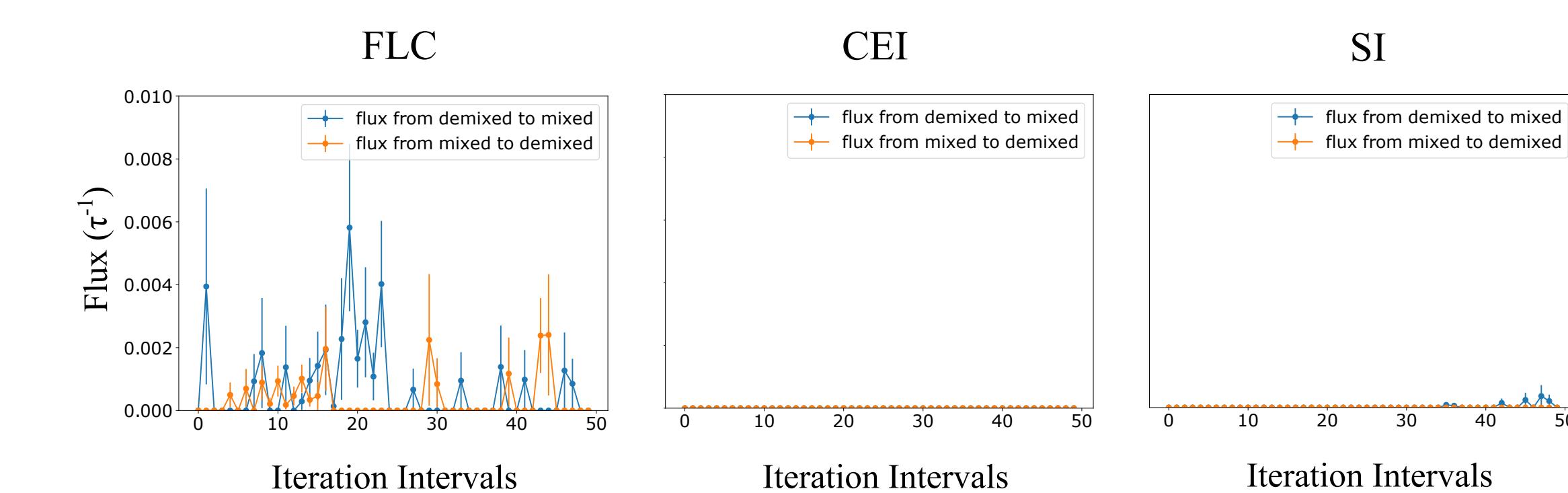
Cumulative Enrichment Index (CEI)



Segregation Index (SI)



State Crossing Depends on CV



- One iteration interval = 10 WE iterations
- Flux of WE walkers across the states are close to zero for CEI and SI
- Finite flux is consistently observed in FLC

Conclusions

- Constructing free energy landscape is possible with "right" collective variable
- Collective variable-based on the clustering is promising
- Choice of "right" collective variable → Success of WE protocol

Future Directions

- Structural and thermodynamic profiling
- More control experiments with toy systems
- Introducing new components:
 - Peptides
 - More lipid species
- Box size effects on Free energy landscape
- Switch to All-Atom models
 - Molecular interaction deciding Phase separation
- Increase the efficiency
 - Include replica exchange with WE protocol



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