

Systematic Parameterization of Flory–Huggins Models from Molecular Dynamics Simulations for Ternary Lipid Mixtures

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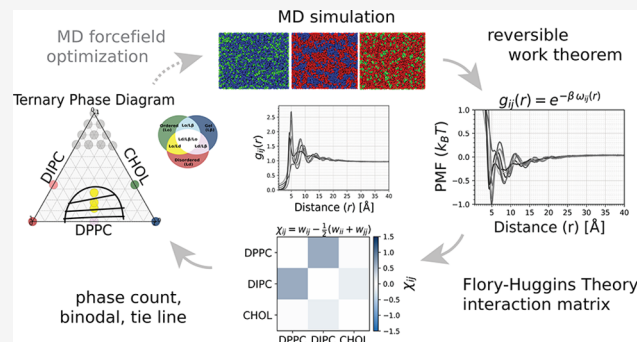


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ABSTRACT: Ternary lipid mixtures with saturated and unsaturated lipids and cholesterol serve as widely used model systems to study coexisting liquid-ordered (L_o) and liquid-disordered (L_d) domains in biomembranes. Despite advances in both experiments and simulations, a computationally efficient systematic approach to extract effective pairwise lipid–lipid and lipid–cholesterol interactions from molecular dynamics (MD) simulations for mesoscale models remains limited. Moreover, parametrizing Flory–Huggins (FH) or lattice Monte Carlo (MC) models becomes especially challenging for multicomponent systems due to the size and complexity of the pairwise interaction matrix. To address this, we aim to bridge molecular-level interactions and continuum mean-field models by implementing a multiscale framework that integrates coarse-grained (CG-MD) and all-atom (AA-MD) simulations with FH theory and lattice MC models. Pairwise interaction energies (w_{ij}) are extracted from radial distribution functions using the reversible work theorem and formulated into FH interaction parameters (χ_{ij}). We apply this multiscale framework to determine phase count and analyze tie-line and bistable phase behavior of (L_d/L_o) domains for ternary lipid mixtures, specifically using the FH model. The extracted χ_{ij} values from MD reproduce domain formation in both MC and FH models. Comparisons across AA-MD, CG-MD, MC, and FH frameworks reveal both quantitative differences and similarities, as well as conserved trends in phase separation behavior. This study establishes a systematic approach to parametrize mesoscale models for multicomponent lipid membranes directly from MD simulations. Furthermore, our approach can serve as a tool for system-specific mesoscale biomembrane modeling studies that embed molecular-level detail and bridge top-down and bottom-up perspectives for future studies.



INTRODUCTION

Phase separation is ubiquitous across soft matter and living systems, from simple oil–water demixing to the emergent compartmentalization of cells. Within cells, phase separation gives rise to biomolecular condensates such as nucleoli, P bodies, Balbiani bodies, and membrane rafts, enabling spatial and temporal organization beyond membrane-bound compartments.^{1–8} Liquid–liquid phase separation leads to lipid raft formation in the cell membrane.^{9,10} Functional lipid rafts have been associated with various biological processes, including immune responses, the assembly of specialized structures such as virus budding, apoptosis, endocytosis, cytoskeleton organization, protein transport, and cellular signaling.^{9–12} These domains differ in key structural properties. Liquid-ordered (L_o) domains have smaller area per lipid, increased bilayer thickness, and more ordered lipid tails compared to liquid-disordered (L_d) domains. This lateral heterogeneity plays crucial roles in signaling, trafficking, protein sorting, and membrane remodeling.^{13–15}

The study of lipid rafts has been driven by both theoretical and experimental approaches, which have been used to build a comprehensive understanding of membrane organization and

its thermodynamics and kinetics.^{16–25,25–47} Lipid rafts are small-scale organizations in a cell membrane that are difficult to characterize experimentally. Their nanoscale size and the compositional complexity of the plasma membrane have made it difficult to establish the relevance or even existence of rafts in living cells, which has been a famously controversial topic of interest.^{36,44}

The development of far-field STED nanoscopy now provides the spatial precision needed to uncover these complex processes.⁴⁸ Several methods, which are locally sensitive, have been successful in detecting heterogeneities on the scale of a small collection of molecules, including fluorescence techniques, such as lifetime, anisotropy,⁴⁹ quantum yield,³⁵ and quenching by spin-labels.⁵⁰ Despite a wealth of experimental

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data, a detailed atomic-level description of nanoscale domains in the L_o/L_d bistable phase regime is incomplete. Molecular-scale interactions and structure drive the partitioning of membrane components, which helps establish connections between the L_o and L_d in model membranes and rafts in living cells. These observations underscore the need for the integration of bottom-up and top-down approaches, to study phase separation in model membranes and the related formation of rafts in living cells, through synergizing experiments, computations, and theory.

To bridge this gap, model membrane systems—particularly ternary lipid-cholesterol mixtures—have become a critical experimental and theoretical platform for studying lipid phase behavior.^{9,17,21,27,33,51–56} Ternary mixtures comprising a high-melting saturated lipid such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or sphingomyelin, a low-melting unsaturated lipid such as 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DIPC, also known as DUPC or DLiPC) or dioleoylphosphatidylcholine (DOPC), and cholesterol (CHOL) have been widely used to recapitulate the L_o/L_d phase coexistence.^{57–59} In these systems, phase separation emerges from a balance between mixing entropy and favorable enthalpic interactions, particularly attractive and repulsive interactions between cholesterol and lipids that promote demixing. Cholesterol preferentially interacts with saturated lipids, stabilizing L_o domains and enabling the formation of coexisting L_o and L_d regions. These ternary systems provide essential insights into the fundamental thermodynamics of membrane organization and serve as a proxy for studying raft-like behavior in biological membranes. Some fundamental questions continue to provoke reflection. For instance, what underlying principles drive lipid–lipid phase separation in complex multicomponent biomembranes? How can we quantitatively characterize lipid interactions to better understand membrane phase behavior?

The current state-of-the-art in molecular dynamics (MD) simulations is characterized by a multiscale framework that utilizes both all-atom (AA) and coarse-grained (CG) resolutions to simulate complex biomolecular systems on length scales of up to submicrons and on time scales ranging from milliseconds to seconds. These advances have resulted from the efficient use of GPUs and high-performance supercomputers. CG models, like MARTINI and many others, simplify the system by grouping atoms into beads. Notably, the MARTINI3 CG force field yields sampling efficiency that is roughly 50 times that of the CHARMM36 atomistic force field.⁴⁷ Furthermore, advanced sampling techniques for lipid raft thermodynamics, including weighted ensemble method,⁶⁰ thermodynamic integration,⁶¹ and umbrella sampling,⁶² help extend the accessible phase space beyond what traditional MD can capture. However, challenges persist due to limitations in sampling; many biologically realistic systems still cannot reliably reach equilibrium at an atomistic resolution.

Several studies have attempted to bridge molecular simulations with theoretical models using Monte Carlo simulations of lattice models of lipids^{63,64} and Flory–Huggins theory.^{65–67} Features such as lipid domain formation and critical behavior observed in simulations or experiments can be mapped onto phenomenological models like the Flory–Huggins regular solution (FH) theory or lattice-based Monte Carlo (MC) simulations. In such frameworks, the phenomenological system-specific mixing parameter captures the effective interaction strengths between components and helps

identify conditions that promote phase separation.⁶⁸ These frameworks have provided insight into the phase behavior of multicomponent systems. However, many of these studies rely on Flory interaction parameters derived either from analytical solutions⁶⁹ of Flory–Huggins theory, which is feasible primarily for binary or ternary lipid mixtures, or from experimental estimates, such as the phenomenological Flory parameters reported by Almeida and co-workers.⁶³ This reliance becomes increasingly limiting in the context of multicomponent lipid mixtures due to the vast number of lipid–lipid interaction pairs. Consequently, estimating such parameters analytically becomes highly challenging for systems beyond two or three components, which restricts the direct applicability of these models to complex biomembranes.

However, the key challenge persists in a systematic approach to extract the phenomenological effective lipid–lipid interaction matrix from molecular dynamics simulation; the vastness of this matrix also imposes challenges in estimation from experiments. Recent work by Thalmann and co-workers⁷⁰ have attempted to embark on this quest through Kirkwood–Buff integrals from statistical mechanics, which relate to thermodynamic quantities such as the second virial coefficient or the pairwise interaction parameter. Their framework represents an important step toward quantitative mapping between microscopic correlations and macroscopic interaction parameters.

The focus of this study is to bridge this gap by utilizing a bottom-up and top-down approach, which offers a path to connect microscopic dynamics to emergent mesoscale phenomena relevant to biological membranes. We employ a combined approach of CG-MD, AA-MD, lattice MC, and mean-field modeling to investigate lipid phase separation in ternary mixtures of DPPC (saturated lipid), DIPC (unsaturated lipid), and cholesterol. By integrating simulations across resolutions and force fields and employing backmapping techniques, we systematically analyze phase behavior, with a quantitative and qualitative comparison to the experiments. Notably, our findings demonstrate that despite resolution differences, the fundamental thermodynamic basis of liquid–liquid phase separation is conserved.

METHODS

This section outlines the methodology used in this study to investigate the impact of a change in cholesterol concentration on the phase separation of ternary lipid mixtures using coarse-grained molecular dynamics (CG-MD) simulations with MARTINI3 force field, All-atom Molecular Dynamics (AA-MD) Simulations using CHARMM36m force field, as well as lattice and mean-field modeling approaches.

Coarse-Grained and All-Atom Molecular Dynamics Simulations. Modeling complex biological systems at fully atomistic resolution presents significant challenges due to the extensive temporal and spatial scales involved. To overcome these limitations, CG force fields have become increasingly important in the study of micro- to mesoscale systems. For each binary lipid mixture composed of an equimolar ratio of DPPC and DIPC (50:50), DPPC and CHOL (50:50), and DIPC and CHOL (50:50), three replicas were performed. Further, for each ternary lipid mixture composed of DPPC:DIPC:CHOL at compositions of 39:39:22, 35:35:30, and 43.5:43.5:13, three replicas were also performed at 300.15 K. The simulation box dimensions were approximately 25 nm × 20 nm × 10 nm. The simulations were conducted using a

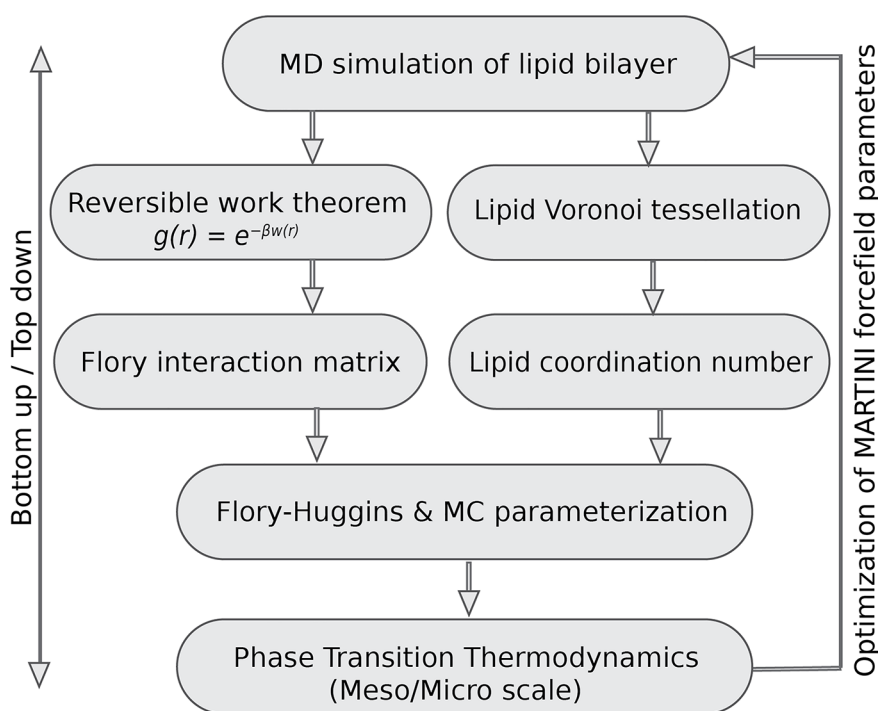


Figure 1. Schematic representation of the workflow of the proposed methodology to parametrize the Flory–Huggins model and the Monte Carlo simulation of Flory parameters from molecular dynamics.

recent reparameterized version of MARTINI3 (martini3001_NLT) force fields⁷¹ in GROMACS 2021.5.⁷² An ionic concentration of 150 mM was maintained using Na⁺ and Cl[−] ions. The initial lateral positions of the lipids were randomized in each system, and approximately 1700 lipids were present in all systems to ensure that at appropriate cholesterol concentrations the system will undergo phase separation.⁵⁷ Subsequently, after preparing the system in a randomized state, the system's energy was minimized using the steepest descent algorithm. Afterward, the system was equilibrated for 10 ns at 300.15 K using the Berendsen thermostat and a semi-isotropic barostat at 1 bar with a compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$. Finally, a production run was performed for 10 μs at 300.15 K with the Bussi velocity-rescale thermostat.⁷³ The pressure was maintained at 1 bar with a Parrinello–Rahman barostat⁷⁴ in a semi-isotropic pressure coupling scheme, coupled at a 12 ps interval with a compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$. Furthermore, a 100 ns production run was carried out for DPPC and DIPC (50:50), DPPC and CHOL (50:50), and DIPC and CHOL (50:50). These binary mixture trajectories are used in RDF and PMF analysis to extract effective w_{ij} interaction parameters from the binary simulations summarized in Figure 1.

Furthermore, all-atom molecular dynamics simulations were performed using the CHARMM36m⁷⁵ force field to extract pairwise interaction energetics for DPPC, DIPC, and cholesterol. Three binary mixtures (DPPC–DIPC, DPPC–CHOL, and DIPC–CHOL) were simulated at 50:50 molar ratios using GROMACS 2021.5 at 300.15 K. Each system contained approximately 1204 lipid/sterol molecules, $\sim 52,500$ TIP3P water molecules, and 140 Na⁺/140 Cl[−] ions to maintain 150 mM ionic strength. The simulation box dimensions were approximately 20 nm $\times$ 20 nm $\times$ 10 nm. After steepest descent energy minimization, a six-step NPT equilibration protocol totaling 2 ns was performed with gradually released positional

restraints. Temperature and pressure were maintained using the velocity-rescaling thermostat and Parrinello–Rahman barostat (1 bar, semi-isotropic coupling, compressibility $4.5 \times 10^{-5} \text{ bar}^{-1}$). A 200 ns production run was carried out under fully unrestrained conditions. Trajectories were saved every 100 ps for RDF and PMF analysis to extract effective w_{ij} as summarized in Figure 1.

Analysis of the MD Simulation Trajectory. We examine various order parameters that provide insight into the phase behavior and lipid interactions within the membrane.

Lateral Lipid Mixing Entropy. The lateral lipid mixing entropy quantifies the degree of phase separation in lipid bilayers by measuring the probability of nearest neighbor contacts between different lipid types. Following our early studies of phase separation in ternary lipid bilayers,⁵⁷ we employ mixing entropy as a measure of the extent of phase separation. The mixing entropy is defined in terms of the probability of contacts formed by the same lipid type (p_1) or different lipid types (p_2), with $p_1 + p_2 = 1$:

$$S_{\text{mix}}(t) = -p_1(t) \ln p_1(t) - p_2(t) \ln p_2(t) \quad (1)$$

Local lipid contacts were computed using nearest neighbors determined via Voronoi tessellations on the xy -plane for both upper and lower leaflets separately. The Voronoi tessellation was performed using the C2A and C2B beads of DPPC, the D2A and D2B beads of DIPC, and the R2 bead of CHOL. This method offers advantages over definitions of mixing entropy based on coarse-grained measurements of lipid composition within a space-fixed grid, whose resolution influences the entropy estimate. The lateral lipid mixing entropy provides a measure of phase separation extent, offering insights into lipid organization and interactions within the membrane. The full 10 μs duration of trajectory is considered for order-parameter analysis in Figure 3.

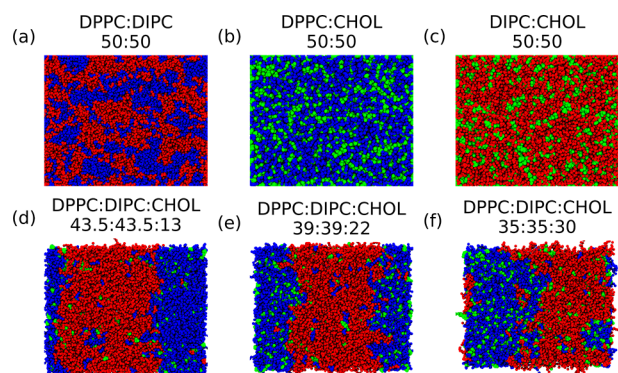


Figure 2. Binary and ternary bilayer membrane systems composed of the lipids DPPC and DIPC and the sterol cholesterol, simulated using coarse-grained molecular dynamics (CG-MD) with the MARTINI3 force field. Top views of the final simulation frames for the upper leaflets are shown for trajectories with different compositions: (a) DPPC:DIPC (50:50), (b) DPPC:CHOL (50:50), (c) DIPC:CHOL (50:50), (d) DPPC:DIPC:CHOL (43.5:43.5:13), (e) DPPC:DIPC:CHOL (39:39:22), and (f) DPPC:DIPC:CHOL (35:35:30). DPPC, DIPC, and cholesterol are shown in blue, red, and green, respectively. Visualizations were generated using VMD 1.9.4.

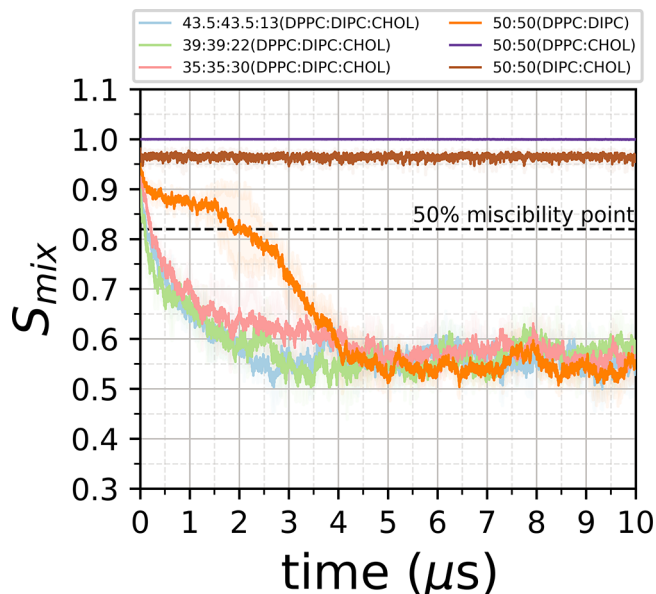


Figure 3. Time evolution of the lipid mixing entropy (S_{mix}) for different binary and ternary membrane compositions, computed from MARTINI3 simulations. Compositions include ternary mixtures of DPPC:DIPC:CHOL at 43.5:43.5:13, 39:39:22, and 35:35:30 molar ratios, as well as binary mixtures DPPC:DIPC (50:50), DPPC:CHOL (50:50), and DIPC:CHOL (50:50). Solid lines represent the mean S_{mix} over three independent simulations; shaded regions denote the standard deviation. The horizontal dashed line marks the 50% miscibility threshold. Compositions exhibiting strong phase separation display a more rapid and pronounced decline in mixing entropy.

Lateral Radial Distribution Functions. We computed the two-dimensional radial distribution functions, $g(r)$, to quantify lateral organization and spatial correlations between lipid species within the membrane plane. The RDFs for six lipid pair interactions are evaluated: DPPC–DPPC, DPPC–DIPC, DIPC–DIPC, DPPC–CHOL, DIPC–CHOL, and CHOL–CHOL. All simulations were performed using the MARTINI3 coarse-grained force field. Beads were selected according to

their chemical identity: saturated tail beads included C1A, C2A, and C3A; unsaturated tail beads included C1A, D2A, and D3A; glycerol backbone beads were represented by GL2; and cholesterol ring groups were represented by R1, R2, and R3. The bilayer midplane was estimated using the average z -coordinate of the PO4 beads from DPPC and DIPC, and all lipids with bead positions above this plane were considered part of the upper leaflet. For cholesterol, which lacks a clear headgroup marker, leaflet assignment was based on a 15 Å neighbor search around the lipid headgroups. Pairwise distances were computed in the xy -plane for each simulation frame using periodic boundary conditions. These lateral RDF profiles reveal key insights into lipid clustering behavior, phase separation, and preferential interactions among different lipid species.

The all-atom binary production runs analyzed for 200 ns and were used for local structural comparison (RDFs/PMFs) for homogeneous binary systems. The membrane patches simulated in this study were larger; therefore, shorter simulations were sufficient to obtain converged RDFs, consistent with prior observations by Voth and co-workers.^{76,77} The binary coarse-grained (CG) simulations used for parametrization were analyzed over the final 100 ns of a 10 μs trajectory. It was found that due to faster diffusion of lipid in MARTINI, less sampling was required to obtain converged RDFs. AA simulations are employed to validate local structure, while mesoscale demixing is examined through CG and mean-field modeling.

Furthermore, RDF convergence tests were performed for both the all-atom (AA) and coarse-grained (CG) systems. Using a block size of 10 ns, we computed the xy -projected RDFs, PMFs, and their decompositions for each block. Each AA and CG trajectory was further divided into separate trajectories corresponding to individual lipid leaflets; the xy -RDFs were calculated for each leaflet and subsequently averaged to obtain a converged RDF for the entire simulation, as reported in all figures. Comparison between the two leaflets also served as an additional convergence check, since both leaflets of a symmetric bilayer are expected to exhibit similar behavior. Here we exploit the reversible work theorem, which connects the radial distribution function to the potential of mean force (PMF) of association between two CG sites as $g_{ij}(r) = e^{-\beta w_{ij}(r)}$.

Lateral Voronoi Tessellation and Coordination Number Analysis. Voronoi tessellation was applied to the xy -coordinates of PO4 atoms (DPPC, DIPC) and ROH groups (CHOL) in the upper leaflet, identified relative to the bilayer midplane. The coordination number z was computed per molecule as the number of Voronoi neighbors and averaged across a 5 μs trajectory (with sampling every 10 frames).

Monte Carlo Simulations. To investigate lateral phase separation in multicomponent lipid bilayers, 2D Monte Carlo (MC) simulations were performed on triangular lattices using the Flory–Huggins interaction model. Membrane configurations were generated for binary and ternary lipid mixtures consisting of DPPC, DIPC, and cholesterol. The simulated systems included the following compositions: DPPC:DIPC (50:50), DPPC:CHOL (50:50), DIPC:CHOL (50:50), and ternary mixtures DPPC:DIPC:CHOL at 43.5:43.5:13, 39:39:22, and 35:35:30 ratios, respectively. For each composition, the top view of the final lattice configuration was visualized. The simulations were carried out on a triangular

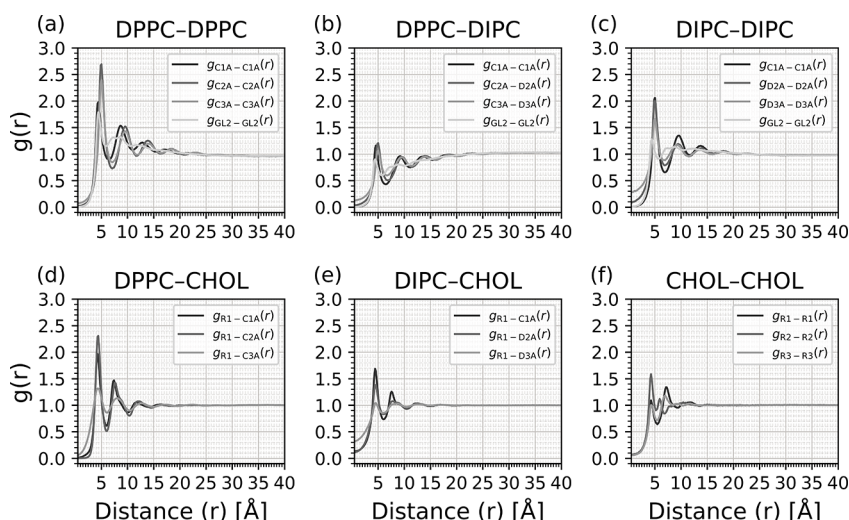


Figure 4. Radial distribution functions (RDFs) for homogeneous and heterogeneous interactions between lipid pairs, as labeled: (a) DPPC–DPPC, (b) DPPC–DIPC, (c) DIPC–DIPC, (d) DPPC–CHOL, (e) DIPC–CHOL, and (f) CHOL–CHOL. Coarse-grained (CG) beads are labeled according to the following scheme: saturated tail beads—C1A, C3A, and C4A; unsaturated tail beads—D2A; glycerol groups—GL2; and cholesterol ring groups—R1, R2, and R3. Simulations were performed using the MARTINI3 force field. CG MARTINI3 binary mixture trajectories are used in RDF analyses.

lattice with periodic boundary conditions. Each site on the lattice was initialized by randomly assigning a lipid identity based on the prescribed composition. Lipid–lipid interactions were governed by a Flory–Huggins-type interaction matrix χ_{ij} , expressed in units of $k_B T$. These parameters were selected based on estimates of pairwise interaction energies obtained from coarse-grained molecular dynamics simulations using the MARTINI3 force field. Each MC simulation was performed for 10^6 steps using the Metropolis algorithm. At each step, a pair of randomly chosen lattice sites with different lipid identities was selected, and a trial swap was attempted. The local energy change $\Delta G/k_B T$ associated with the swap was computed based on the interactions with the six nearest neighbors in the triangular lattice. The move was accepted with a probability $\min(1, \exp(-\Delta G/k_B T))$, ensuring proper Boltzmann sampling of configurations. All simulations were performed at a temperature of 300.15 K, consistent with Flory–Huggins, AA-MD, and CG-MD simulation temperatures. The interaction matrices are parametrized from the MD simulation data with a systematic method proposed in this work. Here, we exploit the reversible work theorem, which connects the radial distribution function to the potential of mean force (PMF) of association between two CG sites, as explained previously and summarized in Figure 1.

Mean-Field Modeling. Thermodynamics Principle of Multicomponent Lipid–Lipid Phase Separation. We consider an isothermal, incompressible system composed of $N = 3$ different lipids. In equilibrium, in principle such a system can form at most N phases based on the Gibbs phase rule.⁷⁸ These phases are homogeneous regions with distinct compositions. However, in realistic scenarios, fewer phases typically emerge due to some components being miscible. Since the phases are homogeneous, their composition is fully described by the particle counts $N_i^{(n)}$ for each component $i = 1, \dots, N$, or the associated volume fractions $\phi_i^{(n)}$:

$$\phi_i^{(n)} = \frac{v N_i^{(n)}}{V^{(n)}} \quad (2)$$

where $V^{(n)}$ is the volume of each of the M coexisting phases for $n = 1, \dots, M$ and v is the molecular volume. In this study molecular volume ratio of DPPC:DIPC:CHOL is 2:2:1.

Furthermore, we can express the associated free energy density functional of each homogeneous region using the Flory–Huggins formalism:^{65,66}

$$f[\phi_i] = \frac{k_B T}{v} \left[\sum_{i=1}^N \phi_i \ln \phi_i + \sum_{i,j=1}^N \frac{1}{2} \chi_{ij} \phi_i \phi_j \right] \quad (3)$$

The pairwise interaction between components i and j is represented by χ_{ij} . Here, $\chi_{ij} > 0$ corresponds to an effective repulsive interaction, and $\chi_{ij} < 0$ corresponds to an effective attractive interaction. In this study, the χ_{ij} parameters are given in units of $k_B T$ (where k_B is the Boltzmann constant and T is the temperature).

The interaction Flory matrix elements or can be interpreted as second virial coefficients, χ_{ij} can be calculated from the interaction energies, w_{ij} between components i and j using

$$\chi_{ij} = \frac{z_{ij}(2w_{ij} - w_{ii} - w_{jj})}{2k_B T}$$

where $z_{ij} = \frac{z_i + z_j}{2}$ is the lattice coordination number and z_i is the effective coordination number of component i on the lattice.

The first term of the free energy density given by eq 3 accounts for translational entropy, whereas the second term captures enthalpic contribution. We consider our system to be thermodynamically large, with homogeneous phases and negligible interfaces; equilibrium states are achieved through the minimization of the average free energy density $\overline{f}[\phi_i]$. The entire system can be characterized by the Flory matrix χ and the average volume fraction $\overline{\phi}_i$. The number of coexisting phases can be calculated by minimizing the total free energy of all compartments while ignoring the interfaces. The equilibrium phase count can be estimated by clustering all compartments based on the similarity of their final

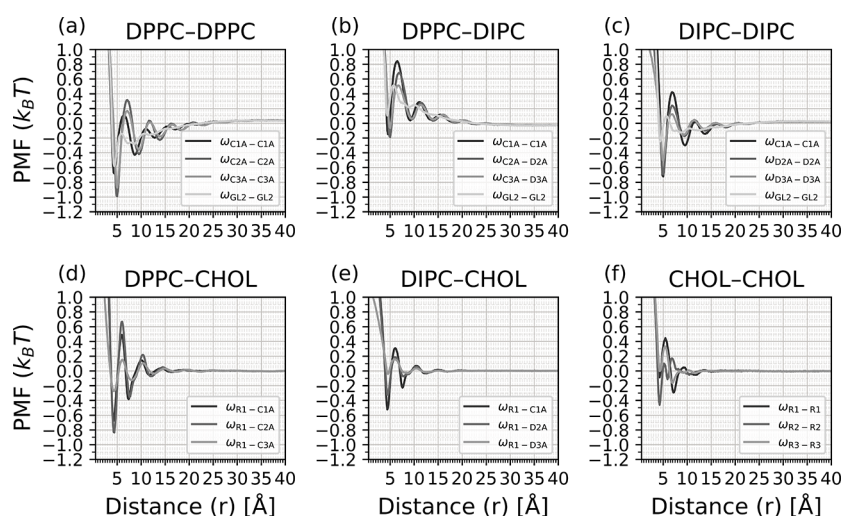


Figure 5. Potentials of mean force (PMFs) for homogeneous and heterogeneous interactions between lipid pairs, as labeled: (a) DPPC–DPPC, (b) DPPC–DIPC, (c) DIPC–DIPC, (d) DPPC–CHOL, (e) DIPC–CHOL, and (f) CHOL–CHOL. Coarse-grained (CG) beads are labeled according to the following scheme: saturated tail beads—C1A, C3A, and C4A; unsaturated tail beads—D2A; glycerol groups—GL2; and cholesterol ring groups—R1, R2, and R3. Simulations were performed using the MARTINI3 force field. CG MARTINI3 binary mixture trajectories are used in RDF and PMF analyses to extract effective w_{ij} interaction parameters.

compositions. A detailed theoretical and numerical scheme of the Flory–Huggins formalism is presented in the SI. In this work, we explored interaction matrices with a range of initial compositions. The interaction Flory matrices are parametrized from the MD simulation data with a systematic method proposed in this work as summarized in Figure 1. Here we exploit the reversible work theorem, which connects the radial distribution function to the potential of mean force (PMF) of association between two CG sites.

In this work, the effective pairwise interaction energy (w_{ij}) was estimated as the depth of the first minimum of the potential of mean force (PMF), Figure 5. This first minimum represents the most probable contact separation and captures the dominant attractive contribution responsible for lateral association. Although higher-order oscillations in the PMF—reflecting packing effects and secondary solvation-shell structuring—are present, they are less pronounced in our results and are effectively averaged out in mean-field representations. Therefore, the first minimum provides a physically meaningful descriptor of first-shell lipid–lipid affinity that is sufficient to reproduce the essential physics driving phase separation.

The present FH model represents a single-leaflet mean-field treatment and therefore neglects explicit interleaflet coupling and hydrophobic mismatch effects. Consequently, phenomena such as domain registration or antiregistration are not captured. Future extensions could incorporate coupling terms between corresponding lattice sites of opposing leaflets to model symmetric as well as asymmetric bilayer registration energetics explicitly.

Numerical Simulation of the Flory–Huggins Model. In this work, all numerical simulations for the Flory–Huggins model are performed using the *flory* Python package,^{68,79,80} which uses the above-mentioned formalism to calculate the phase count.

RESULTS AND DISCUSSION

Ternary phase diagrams of saturated lipid (DPPC), unsaturated lipid (DIPC), and cholesterol (CHOL) form a widely

used model system for lipid raft studies, both in experimental^{16,20,33,55} and theoretical^{56,57,81–84} contexts. This study aims to bridge molecular-level interactions with macroscopic phase behavior by reproducing the ternary phase diagram of this model system. We first employ coarse-grained molecular dynamics (CG-MD) simulations to extract parameters for phenomenological mean-field-based Flory–Huggins (FH) models and lattice-based Monte Carlo (MC) simulations. This multiscale top-down and bottom-up framework is schematically summarized in Figure 1, which outlines the stepwise parametrization workflow—from particle-based MD observables to lattice-based and field-based models.

By grounding the MC and FH simulations in Flory interaction parameters derived from potential of mean force (PMF) profiles in CG-MD simulations, we ensure that the emergent mesoscopic behavior retains molecular underpinnings consistent with bilayer physics. This modeling framework is especially well-suited to investigate how variations in lipid composition, cholesterol content, or hybrid lipid fraction modulate domain formation and phase boundaries—phenomena directly relevant to biological membranes. In this way, CG simulations not only provide molecular-level insights into lipid domain structure and kinetics but also serve as quantitative building blocks for predictive mesoscale modeling of membrane heterogeneity and function.

Lipid bilayers can be studied using various particle-based simulation techniques, each offering different levels of molecular detail. In this work, we employed both MARTINI coarse-grained (CG) models and all-atom (AA) models, both of which have successfully captured structural and dynamical properties of lipid bilayers in previous studies. These properties include inter- and intraleaflet lipid interactions, phospholipid–cholesterol interactions, and the thermodynamics of phase separation as a function of cholesterol content.^{57,81,82,85–87}

While AA simulations on high-performance computing platforms are capable of characterizing lipid rafts in model membranes, they remain computationally intensive due to the slow diffusion of lipids and cholesterol and the vast chemical

diversity of the lipidome.²⁷ As a result, systematic parameter scans remain a significant challenge. Several CG models have been developed to overcome this bottleneck—including MARTINI,^{88–91} SPICA,⁹² ELBA,⁹³ and Cooke models⁹⁴—with varying degrees of resolution and lipid specificity. Among these, the MARTINI model offers a well-balanced trade-off between computational efficiency and molecular level spatial organization, making it particularly suitable for probing physical mechanisms of phase separation in ternary lipid–cholesterol systems. MARTINI CG models have proven especially useful for investigating coexistence between liquid-ordered (L_o) and liquid-disordered (L_d) phases at different cholesterol concentrations.⁵⁷

The overarching goal of this study is to propose a systematic pipeline to extract second virial coefficients (i.e., Flory–Huggins interaction parameters) for lipid–lipid and lipid–cholesterol interactions from MARTINI simulations of the ternary membrane system (Figure 1). These interaction parameters form the effective parameter space used to study the thermodynamics of lipid phase separation using FH theory and lattice-based models for multicomponent systems. To this end, we simulated both binary and ternary lipid mixtures using the MARTINI3 force field. Final snapshot frames from CG-MD simulations (Figure 2) reveal distinct trends in lateral lipid organization and homogeneous mixing across different compositions. Among the binary mixtures (Figures 2a–c), the DPPC:DIPC system shows clear domain formation with 50:50 molar ratio, indicating a tendency to demix. In contrast, the other two binary mixtures—DPPC:CHOL and DIPC:CHOL—remain largely homogeneous, suggesting weak phase separation under similar conditions. The ternary mixtures (Figures 2d–f) with compositions DPPC:DIPC:CHOL of 39:39:22, 35:35:30, and 43.5:43.5:13, respectively, exhibit increasingly pronounced lateral phase separation. These systems show spatial segregation into CHOL-rich and CHOL-poor domains, underscoring cholesterol's critical role in modulating phase behavior reported in previous studies.⁵⁷

To quantify the phase separation kinetics, we tracked the temporal evolution of the mixing entropy, $S_{\text{mix}}(t)$, and identified the time to reach the 50% miscibility threshold, consistent with earlier studies⁵⁶ (Figure 3). All CG-MD simulations were conducted using the MARTINI3 force field, which showed notably faster demixing time scales than those reported for MARTINI2 and MARTINI3 in prior studies.^{47,56,95} For ternary mixtures, the compositions 43.5:43.5:13, 39:39:22, and 35:35:30 (DPPC:DIPC:CHOL) reached the 50% miscibility point at approximately 120, 100, and 200 ns, respectively. These rapid transitions suggest a strong intrinsic driving force toward lateral segregation at intermediate cholesterol concentrations. By contrast, the binary DPPC:DIPC mixture (50:50) crossed the miscibility threshold at 1.81 μs . Notably, such demixing behavior of DPPC and DIPC has not been observed in MARTINI2 or MARTINI3 and appears to be a unique feature of the reparameterized MARTINI3 force field,⁷¹ which captures the bistable coexistence of ordered DPPC and disordered DIPC domains. This feature may result from the effective repulsive interaction between DPPC and DIPC. Quantification of these interaction free energies is tedious within the framework of MD simulation of lipid bilayers; one has to rely on enhanced sampling techniques.^{60–62} However, challenges persist due to limitations in sampling, computational cost, and systematic characterization of a vast lipidome database. Meanwhile, the binary

mixtures DPPC:CHOL and DIPC:CHOL remain mixed within the simulation time scale, consistent with their high miscibility and lack of strong demixing tendencies.

These observations reaffirm the role of cholesterol as a regulator of domain formation and suggest that ternary compositions containing both saturated and unsaturated lipids at intermediate CHOL levels are optimal for rapid phase separation. The $S_{\text{mix}}(t)$ analysis thus identifies an optimal cholesterol range that kinetically favors the onset of domain segregation.

Mapping Coarse-Grained Interactions to Flory–Huggins Parameters via the Reversible Work Theorem.

Specifically, we exploit the connection between pairwise radial distribution functions (RDFs) derived from CG simulations and the potential of mean force (PMF) via the reversible work theorem, allowing estimation of effective pair interaction energies w_{ij} between lipid types. These interaction energies can be mapped onto Flory–Huggins χ_{ij} parameters, which serve as inputs for mean-field free energy-based FH and lattice-based MC simulations, bypassing the computational cost of long-time scale all-atom MD simulations and the need for enhanced sampling techniques. This multiscale framework enables the exploration of nanodomain stability and collective phase behavior in large membranes while preserving chemical specificity encoded by CG force fields.

For each lipid pair—DPPC–DPPC, DPPC–DIPC, DIPC–DIPC, DPPC–CHOL, DIPC–CHOL, and CHOL–CHOL—we computed RDFs between representative CG beads (e.g., saturated: C1A, C3A, C4A; unsaturated: D2A; glycerols: GL2; cholesterol rings: R1–R3) and converted them to PMFs using $w_{ij}(r) = -k_B T \ln g_{ij}(r)$. RDFs were computed from binary mixture CG simulations (Figures 2a–c), each containing only the two species of interest to isolate pairwise interactions. The depth of each PMF minimum relative to its asymptotic baseline was recorded as the effective pair interaction energy $w_{ij}(r)$, and averaged across chemically equivalent beads and replicates to obtain $w_{ij} \pm \sigma$ (Figures 4 and 5), here σ represents standard deviation.

To validate this CG-based approach, we compared the resulting w_{ij} values against all-atom (AA) CHARMM36m simulations, where atomistic RDFs were mapped to CG-level by defining chemically consistent non-hydrogen atom groups. The PMFs obtained from these mapped AA RDFs (Figure S1) provide a physically grounded benchmark. A comparative analysis (Figure S2 and Table S1) shows strong agreement between CG and AA models, particularly for DPPC–DPPC, DPPC–CHOL, and CHOL–CHOL, which exhibit consistently attractive interactions. The agreement also extends to literature values from Almeida et al.,⁶³ validating the magnitude and sign of key interactions. Notably, CG simulations tend to yield slightly more attractive values for disordered lipid interactions (e.g., DIPC–CHOL, DPPC–DIPC), a known limitation due to the smoother potentials and reduced entropic resolution of CG models.^{76,96} Despite these limitations, the consistency across CG, AA, and experimental interaction energies supports the reliability of PMF-derived w_{ij} as input for mesoscale models.

While the PMF-derived w_{ij} values capture effective pairwise interactions, an additional input required for Flory–Huggins modeling is the local coordination environment—i.e., the average number of neighbors each lipid species experiences in the membrane. To quantify this spatial packing information, we performed a Voronoi tessellation on the tail bead positions

of DPPC, DIPC, and CHOL in the upper leaflet for the ternary mixture DPPC:DIPC:CHOL (35:35:30). Figure 6a illustrates

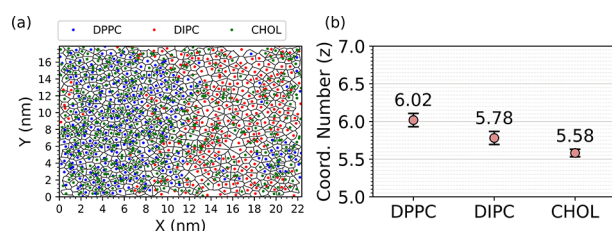


Figure 6. (a) Voronoi tessellation of lipid and cholesterol tail positions in the upper leaflet of the membrane at the final frame of the DPPC:DIPC:CHOL (35:35:30) simulation. DPPC (blue), DIPC (red), and cholesterol (green) dots represent tail bead positions, and Voronoi edges are shown in black. (b) Average coordination number (z) of DPPC, DIPC, and cholesterol, computed over a 5 μ s trajectory for the 35:35:30 composition. Error bars represent standard deviations.

a representative tessellation from the final simulation frame, where each polygon corresponds to the local neighborhood of a lipid or cholesterol molecule. Visually, the system exhibits clear heterogeneity in domain texture and lipid clustering, with tightly packed DPPC-rich regions and more loosely arranged DIPC environments.

We calculated the average coordination number z for each component over the final 5 μ s of the trajectory (Figure 6b). DPPC displayed the highest average coordination number ($z \sim 6.1$), indicating its tendency to form tightly packed, hexagonal-like arrangements, consistent with its saturated chain structure and propensity to organize into ordered L_o -like regions. In contrast, DIPC had a lower coordination number ($z \sim 5.2$), reflective of the disorder and increased free area associated with its unsaturated chains. Cholesterol exhibited an intermediate coordination number ($z \sim 5.7$), reflecting its dual role: fitting into ordered domains with DPPC and modulating fluidity in disordered environments.

These coordination trends are in qualitative agreement with experimental observations from fluorescence quenching, NMR, and X-ray scattering studies, which show that cholesterol preferentially associates with saturated lipids and enhances packing density in L_o domains.^{9,17,21,33,51–55} As such, Voronoi-based coordination analysis provides a physically grounded link between CG simulations and mesoscale FH model parametrization, supplying the local coordination numbers z needed to scale interaction energies in the FH free energy expression.

Monte Carlo Simulations and Validation of Flory Interaction Parameters. The extracted w_{ij} parameters outlined in the previous section were validated using 2D lattice-based Monte Carlo (MC) simulations (Figure 7). In this model, each lattice site is occupied by one molecule and interacts with its six nearest neighbors, representing a triangular lattice. For these simulations, the upper bounds of the w_{ij} ranges were employed—corresponding to the most repulsive interactions for DPPC–DIPC and DIPC–CHOL, and the most attractive interaction for DPPC–CHOL (Figure S2 and Table S1). These values were chosen to represent the most distinctly phase-separating regime, appropriate for lattice models that do not account for fluctuations in local concentration and coordination number z of lipid and cholesterol. Final frames from the MC simulations highlight

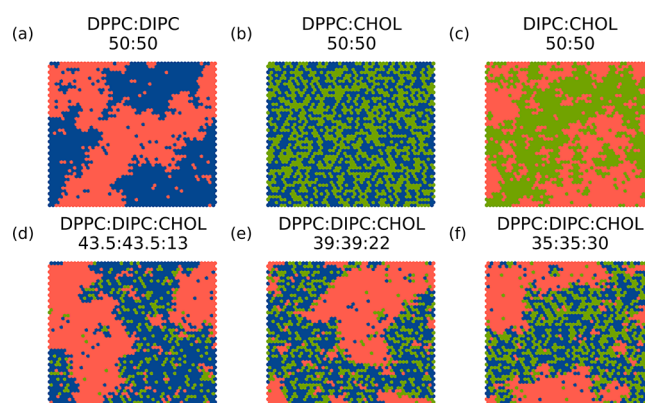


Figure 7. Binary and ternary membrane configurations composed of DPPC, DIPC, and cholesterol obtained from 2D Monte Carlo (MC) simulations using the Flory–Huggins interaction parameter. Shown are top views of the final simulation frames for different compositions: (a) DPPC:DIPC (50:50), (b) DPPC:CHOL (50:50), (c) DIPC:CHOL (50:50), (d) DPPC:DIPC:CHOL (43.5:43.5:13), (e) DPPC:DIPC:CHOL (39:39:22), and (f) DPPC:DIPC:CHOL (35:35:30). DPPC, DIPC, and cholesterol are represented in blue, red, and green, respectively. Flory parameters χ_{ij} are in units of per $k_B T$. The Flory–Huggins interaction parameters used in the simulations are presented in Figure S3, which were extracted from the CG simulations (Figure S2 and Table S1).

that among the binary mixtures (Figures 7a–c), the DPPC:DIPC system shows domain formation, indicating a tendency to demix. In contrast, the other two binary mixtures—DPPC:CHOL and DIPC:CHOL—remain largely homogeneous. Ternary systems exhibited clear demixing transitions (Figures 7d–f), in agreement with coarse-grained MD observations.

MC simulation results from this study are consistent with the experimentally grounded Monte Carlo simulation by Almeida et al.⁶³ and Sarkar et al.⁹⁷ It has been observed that phase separation in ternary lipid mixtures composed of saturated phospholipids, unsaturated phospholipids, and cholesterol can be effectively captured using lattice-based simulations parametrized by thermodynamic data.⁶³ In their approach, pairwise interaction energies (w_{ij}) were inferred from calorimetric and fluorescence resonance energy transfer (FRET) measurements and subsequently incorporated into a Flory–Huggins interaction matrix for Monte Carlo simulations. Strong DPPC–CHOL attraction, moderate repulsion between DOPC–CHOL, and a significant DPPC–DOPC demixing tendency were reported—trends that align closely with the interaction hierarchy derived from coarse-grained simulations in the present work, refer to Table S1. The close agreement between the CG-derived w_{ij} values and those inferred from calorimetry in Almeida's study lends strong support to the validity of this multiscale modeling framework, which bridges molecular simulations with mesoscale lattice models of membrane organization.

Despite the broad agreement, several key distinctions between the two approaches can be identified. In the Almeida study, w_{ij} values were derived phenomenologically by fitting experimental phase diagrams and thermodynamic observables. In contrast, in the present work the parameters were extracted directly from coarse-grained molecular dynamics simulations using the reversible work theorem. This bottom-up approach allows for molecular specificity to be encoded into mesoscale models without empirical fitting. The CG-based approach is

also more readily extensible to vast lipidome parameter scans and can be systematically updated to accommodate new simulation data without assumptions of interleaflet compositional symmetry or ideal mixing. Nevertheless, the strong quantitative and qualitative agreement with the calorimetry-derived interaction matrix from Almeida and co-workers⁶³ underscores the robustness and predictive utility of the current multiscale framework. By grounding mesoscale simulations in physically interpretable and transferable interaction parameters obtained from coarse-grained MD, the approach enables efficient and realistic modeling of lipid domain formation and membrane heterogeneity across a broad compositional space.

Flory–Huggins Solution Theory: Ternary Phase Diagram and Tie-Line Construction. In this section, we explore thermodynamic phase boundaries and spinodal decomposition kinetics using Flory–Huggins solution theory simulations.⁶³ The FH model represents a further coarse-grained, field-based continuum approach. Here, we parametrize the model using the lower bounds of the w_{ij} ranges to capture effective, mean-field interactions and validated against AA-derived parameter set. These values correspond to the minimum repulsive or attractive interactions: i.e., minimum repulsion for DPPC–DIPC and DIPC–CHOL, and minimum attraction for DPPC–CHOL. The coordination number z_{ij} derived from CG-MD simulations is used in the FH free energy formulation to compute the Flory interaction parameters $\chi_{ij} = z_{ij} \left(w_{ij} - \frac{1}{2}(w_{ii} + w_{jj}) \right)$. The FH framework is suitable for identifying phase coexistence regions and for estimating free energy minima, phase boundaries, and critical miscibility points.

The typical phase diagram of ternary lipid mixtures depends on both the temperature and the specific lipid components. Temperature plays a crucial role, as pure saturated lipids in the gel phase (L_β) and pure unsaturated lipids in the fluid phase (L_d) undergo temperature-dependent first-order phase transitions. These diagrams typically exhibit a general form reported in several studies.^{17,20,21,28,33,35,98,99}

Cholesterol is miscible with saturated DPPC, and together they form a liquid-ordered (L_o) phase—except when cholesterol content exceeds the solubility limit of approximately 66%, beyond which cholesterol precipitates. The presence of the third component, cholesterol, opens up the possibility of a diverse array of bistable (L_o/L_d , L_o/L_β , L_d/L_β) and tristable ($L_o/L_d/L_\beta$) regimes, in addition to the three pure phases (L_d , L_β , and crystalline cholesterol) at the edges of the Gibbs triangle. As cholesterol concentration increases, the two-phase coexistence region between L_o and L_d becomes increasingly narrow, with gradually smaller and more tilted tie-lines. The merger of L_o and L_d occurs at the critical point. In equilibrium, such a ternary system can support at most three coexisting phases, based on the Gibbs phase rule.⁷⁸ These phases are homogeneous regions with distinct compositions. This demonstrates that monostable, bistable, and tristable phases are inherently sensitive to temperature, lipid composition, and molecular-level interactions. If the temperature is such that the saturated lipid exists in a fluid state, the gel–gel phase coexistence region vanishes from the phase diagram, along with the three-phase triangle (i.e., the tristable $L_o/L_d/L_\beta$ regime). The only remaining coexistence region is the bistable L_o/L_d phase.

This study proposes a systematic and transferable approach to constructing binary, ternary, quaternary, and, more generally, multicomponent phase diagrams with computational efficiency. To test the proposed framework, we construct a ternary phase diagram (Figure 8) using the FH model with

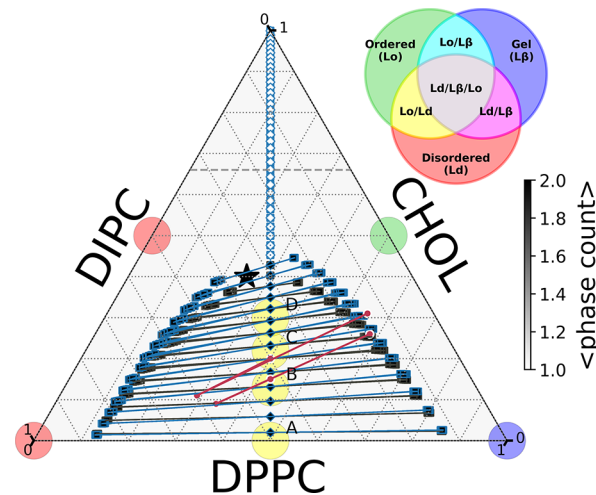


Figure 8. Ternary compositional phase diagram of DPPC, DIPC, and CHOL computed with a three-component Flory–Huggins (FH) model at fixed overall composition using symmetric pairwise interaction parameters χ_{ij} . Diamond markers denote initial homogeneous states; systems that lower free energy via demixing are shown as end point markers and are connected by tie-lines of corresponding colors. Larger yellow background markers (labeled A, B, C, D) correspond to MARTINI3-openbeta CG reference compositions (DPPC:DIPC = 1:1 with CHOL = 0, 13, 22, 30 mol %), exhibiting L_o/L_d coexistence and using the same color scheme as the Venn inset. Overlays show FH results from the AA-derived parameter set (tie-lines in blue) and the lower-bound CG-derived parameter (tie-lines in black). The Flory–Huggins interaction parameters used in the numerical simulation are presented in Table S1 and Figure S4–S6. Red markers and connecting lines denote specific ternary compositions corresponding to 15% and 20% CHOL cases used for quantitative comparison of tie-line slopes and tilt angles. These selected points align with experimental conditions from DOPC:DPPC:CHOL deuterium NMR studies by Veatch et al.,⁹⁹ conducted at $T = 298.15$ K. In the FH model, DOPC is functionally mapped to DIPC. Critical point position based on Veatch et al. indicated as black star. The larger dots are colored according to phase behavior, as classified in the accompanying Venn diagram inset. The dashed gray line highlights a constant CHOL = 0.66 mole fraction threshold.

fixed χ_{ij} values and conservation of total composition. Each diamond-shaped dot denotes an initially homogeneous state. Some compositions remain mixed upon energy minimization, while others spontaneously phase-separate into two distinct phases, indicated by black dot pairs connected by tie-lines. The extent of phase separation and the angle of the tie-lines reveal dominant partitioning tendencies.

Importantly, three larger yellow dots mark CG-MD compositions with DPPC:DIPC in a 1:1 ratio and CHOL contents of 0, 13, 22, and 30 mol %, labeled as points A, B, C, and D. These correspond to systems that exhibit liquid-ordered (L_o) and liquid-disordered (L_d) phase coexistence. The tie-line orientation and critical point phase behavior observed from MD simulations qualitatively match the FH-predicted two-phase regions, providing strong cross-validation between the multiscale approaches. The color coding of the

large dots reflects phase classification based on the Venn diagram shown in the figure inset, distinguishing bistable from monostable lipid organization. Tie-lines run roughly left to right in the bistable region with a tilt toward more cholesterol in the DPPC-rich phase. The comparison is made for the 1:1 (DPPC:DOPC) mixtures with 15% and 20% CHOL, the FH model qualitatively reproduces the experimentally observed tie-line orientations, capturing the gradual increase in tilt angle with increasing cholesterol content, as reported by Veatch and co-workers.⁹⁹ The quantitative difference at intermediate CHOL concentrations suggests that further refinement of the interaction parameters χ_{ij} —or the incorporation of temperature-dependent forms $\chi_{ij}(T)$ —may be necessary. The quantitative differences in binodal points and tie-line tilt angles may stem from differences in lipid composition (DIPC instead of DOPC), temperature discrepancies, significant reduction in degrees of freedom in the FH model, and other variations in the NMR experimental conditions.

Nevertheless, the qualitative and quantitative agreement in the phase coexisting between liquid-ordered (L_o) and liquid-disordered (L_d), as indicated by end points of tie-lines, critical miscibility point, and the approximate estimation of the bistable phase boundary, supports the validity of mapping CG-MD-derived parameters onto a mean-field FH framework. Taken together, this multiresolution approach—from bead-level interactions to lattice models and continuum thermodynamics—highlights the consistency and predictive power of using CG-MD simulations to parametrize phenomenological models of membrane phase separation.

Moreover, this systematic approach has broader utility for force-field refinement by integrating both top-down and bottom-up strategies. It can be applied to target adjustment of specific bead–bead cross-interactions whose w_{ij} values generate systematic FH/MC discrepancies with respect to experimental observables (e.g., tie-line orientation, critical points). Furthermore, it facilitates a reproducibility assessment across coarse-grained force-field families (e.g., MARTINI, SPICA, SIRAH)¹⁰⁰ by mapping $w_{ij} \rightarrow \chi_{ij}$ and projecting the resulting interaction matrices onto a common set of FH/MC observables. The CG-derived FH parameters can then be cross-validated against AA-mapped PMFs, yielding an internally consistent and transferable route to parameter optimization. This approach can also be integrated into programmatically available atomistic repositories¹⁰¹ to explore the vast lipidomics complex phase separation properties.

The FH model derived from molecular simulations provides a transferable thermodynamic representation that connects microscopic pairwise interactions to macroscopic phase equilibria. While CG-MD simulations directly capture molecular level spatial organization, the FH formulation allows rapid, quantitative exploration of large compositional spaces and prediction of tie-lines, critical points, and miscibility gaps—quantities that are otherwise difficult to extract systematically from molecular trajectories. Thus, the FH framework complements, rather than replaces, CG simulations by enabling predictive and analytical insight into the phase behavior of multicomponent lipid mixtures across multiple compositions and vast lipidomics chemical phase space. Importantly, the proposed systematic approach establishes a transferable link between molecular energetics and emergent phase behavior that enables high-throughput prediction of phase separation tendencies across large combinatorial mixtures. Once the interaction matrix is parametrized from a

minimal set of binary MD simulations, the FH formulation allows rapid evaluation of ternary or higher-order lipid systems at minimal additional computational expense. This provides a complementary approach to atomistic or coarse-grained simulations, for rapid investigation of membrane phase behavior for multicomponent lipid mixtures across diverse lipidomics composition space.

CONCLUSIONS

This study utilizes a bottom-up and top-down approach, offering a path to connect microscopic dynamics to emergent mesoscale phenomena relevant to biological membranes. We employ a combined multiscale modeling approach of CG-MD, AA-MD, lattice MC, and mean-field models to investigate lipid phase separation in ternary mixtures of DPPC (saturated lipid), DIPC (unsaturated lipid), and cholesterol. We implement mean-field modeling approaches to investigate the equilibrium phase behavior and morphology of N -component liquid mixtures within the framework of the Flory–Huggins theory of regular solutions. Specifically, we focus on the matrix of second virial coefficients, which represent the pairwise interactions in a ternary lipid mixture with $N = 3$ components, and use an $N \times N$ Flory matrix extracted from CG-MD simulation with a comparison to AA-MD. We explore how effective attractive and repulsive interactions among lipids can shift the system into homogeneous and phase-separated states. Interestingly, CG-MD, MC lattice, and mean-field simulations reveal similar tendencies for phase separation.

All models consistently show phase separation at optimal cholesterol concentrations, forming distinct liquid-ordered (L_o) and liquid-disordered (L_d) phases with characteristic strip morphologies observed in CG-MD due to the finite size effect, in contrast to lattice MC simulation. Mean-field modeling further highlights the impact of lipid–lipid and lipid–cholesterol interactions on phase behavior, offering insights into the equilibrium phase count and the formation of bistable regions in ternary phase diagrams, along with quantitatively determining the bistable phase boundary, critical miscibility point, and tie-lines connecting binodal points. By integrating simulations across resolutions and force fields and employing backmapping techniques, we systematically analyze phase behavior, quantifying the contributions of cholesterol concentration in domain formation.

Notably, our findings demonstrate that despite differences in resolution, the fundamental thermodynamic mechanisms underlying liquid–liquid phase separation remain conserved. Although this approach is practically expedient, we would like to emphasize that it also reflects good physics. As we often observe, large-scale phenomena typically rely on a limited number of effective parameters, with much of the intricate finer detail becoming less relevant. In the context of this study, a number of related questions remain. Do three-body or higher-order lipid interactions contribute meaningfully to phase separation in complex biomembranes? How can we best project the interaction parameter space into a handful of effective parameters? Addressing these questions will necessitate identify and retain relevant microscopic details, thus preventing oversimplification and optimizing the generality of the model of a given complexity. Our integrated framework provides a comprehensive and quantitative method for studying lipid phase separation, offering valuable insights into lipid organization in biomembranes and serving as a tool for system-specific mesoscale biomembrane modeling in future

investigations. Moreover, this systematic approach has broader applications in force-field refinement, as it is complemented by both top-down and bottom-up approaches.

A future extension of this study can explore bilayer coupling parameters¹⁰² such as rate of cholesterol flip-flop extracted for MD simulation, incorporate interleaflet coupling effects beyond pairwise in FH regular solution theory. Incorporating such effects into a phenomenological extension of the FH framework represents an exciting future direction for bridging mean-field thermodynamics with membrane bilayer physics. The findings in this study, resulting from the combination of computations and theory, advance our understanding of membrane phase behavior and establish a multiscale foundation for future studies of complex biological membranes.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jctc.5c01550>.

Methodological details on cholesterol flip-flop correction and leaflet assignment, lipid order parameter analysis, and the CHARMM-to-MARTINI mapping scheme and the derivation and application of the Flory–Huggins formalism for ternary mixtures, together with comparative pairwise interaction energies from MARTINI3 and CHARMM36m simulations, potential of mean force profiles, interaction matrices, and ternary phase diagrams (Figures S1–S8) (PDF)

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Author Contributions

S.P.N., J.E.S., and D.Z. designed the research; S.P.N. performed the research; S.P.N. and J.E.S. analyzed the data; S.P.N. and J.E.S. wrote the original draft; and S.P.N., J.E.S., and D.Z. reviewed and edited the manuscript.

Notes

The authors declare no competing financial interest. MD simulation input and parameter files for GROMACS and analysis scripts for order parameter analysis are available in the public archive at <https://github.com/SonaliPNayak/TernarylipidFHparameter>.

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