

Kinetics and Mechanism of Phase Separation in Ternary Lipid Mixtures Containing APP C99: Atomistic vs Coarse-Grained MD Simulations

George A. Pantelopulos,[#] Sangram Prusty,[#] Asanga Bandara, and John E. Straub*



Cite This: *J. Chem. Theory Comput.* 2025, 21, 5291–5303



Read Online

ACCESS |



Metrics & More

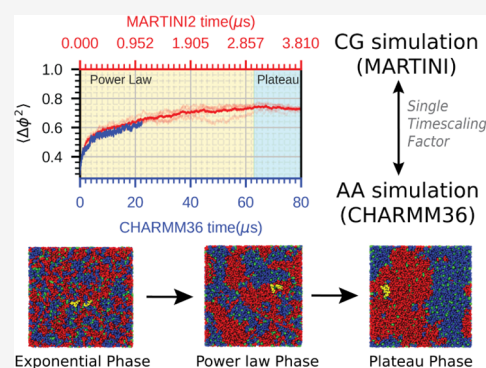


Article Recommendations



Supporting Information

ABSTRACT: The phase separation of lipid bilayers, composed of mixtures of saturated and unsaturated lipids and cholesterol, is a topic of fundamental importance in membrane biophysics and cell biology. The formation of lipid domains, including liquid-disordered domains enriched in unsaturated lipids and liquid-ordered domains enriched in saturated lipids and cholesterol, is believed to be essential to the function of many membrane proteins. Experiment, theory, and simulation have been used to develop a general understanding of the thermodynamic driving forces underlying phase separation in ternary and quaternary lipid mixtures. However, the kinetics of early events in lipid phase separation in the presence of transmembrane proteins remain relatively understudied. Using large-scale all-atom and coarse-grained simulations, we explore the kinetics and phase separation of ternary lipid mixtures of saturated lipid, unsaturated lipid, and cholesterol in the presence of transmembrane proteins. Order parameters employed in the Cahn–Hilliard theory provide insight into the kinetics and mechanism of lipid phase separation. We observe three distinct time regimes in the phase separation process: a shorter exponential time phase, followed by a power-law phase, and then a longer plateau phase. Comparison of lipid, protein, and lipid–protein dynamics between all-atom and coarse-grained models identifies both quantitative and qualitative differences and similarities in the phase separation kinetics. Moreover, timescaling of the dynamics of the AA and CG simulations yields a similar kinetic mechanism of phase separation. The findings of this study elucidate fundamental aspects of membrane biophysics and contribute to ongoing efforts to define the role of lipid rafts in the structure and function of the cellular membrane.



INTRODUCTION

Lipid mixtures composed of saturated and unsaturated lipids, in conjunction with cholesterol in appropriate proportions, are observed to undergo phase separation.^{1–4} Cholesterol has a higher affinity for associating with saturated lipids compared to unsaturated lipids.^{5,6} Domains formed by the association of predominantly saturated lipids and cholesterol adopt a liquid-ordered (L_o) phase when the cholesterol mole fraction exceeds 0.2 (20 mol %).^{7–10} The L_o domains are characterized by a smaller area per lipid, increased membrane thickness, and more ordered lipid tails.^{11,12} The higher density observed in liquid-ordered domains leads to hexagonal packing of lipid tails at lower mol % cholesterol^{9,13} and the formation of cholesteric gels at very high concentrations, characteristic of ocular membranes.¹⁴

“Lipid rafts” are evidenced to be principally defined by the L_o phase and have been hypothesized to play a functional role in cellular metabolism.^{15,16} It has been conjectured that certain membrane proteins are recruited to raft domains, at times facilitated by post-translational lipidation, and colocalized with related proteins or substrates in a way that facilitates function.^{17–19} One example is found in the amyloid cascade

associated with Alzheimer’s disease. Evidence suggests that the enzymes β -secretase and γ -secretase are colocalized in raft domains and carry out correlated cleavage of the amyloid precursor protein (APP) substrate, C99.^{20–23}

The phenomenon of lipid phase separation and raft domain formation has received a great deal of attention in experimental and theoretical studies.^{1,24–29} The seminal work of Keller and Veatch³⁰ established a minimal experimental model exhibiting binary phase separation, where the formation of L_o lipid domains involves a ternary mixture including saturated lipid, unsaturated lipid, and cholesterol. In addition, simple statistical models of binary phase separation, such as Ising models^{31,32} and Flory–Huggins models,^{24,33} have been used to capture key features of the observed process of phase separation. However, phase separation may also be observed in more complex mixtures,

Received: January 6, 2025

Revised: April 22, 2025

Accepted: April 28, 2025

Published: May 15, 2025



including sphingophospholipid, sphingomyelin, glycolipids GM1 and GM3, and even proteins.^{34–41}

Computer simulations of lipid phase separation employing coarse-grained (CG) and all-atom (AA) models have been used to study the nature of the L_d and L_o phases. Spontaneous phase separation from ternary lipid mixtures was first observed in computational studies employing CG models.^{42,43} An early study of L_d and L_o states using AA models consisted of a single-phase system that was initially phase-separated.⁸ A more recent study using an AA model observed the early stages of phase separation from an initially mixed state.⁴⁴

Theoretical arguments, based on Flory–Huggins models of binary phase separation, and systematic computational studies employing CG models, have demonstrated that systems on the order of a thousand lipids are required to create thermodynamically stable phase-separated states.³³ The mixed state is favored by entropy through the entropy of mixing. The phase-separated state is favored by enthalpy, where saturated lipids engage in favorable van der Waals interactions following dissociation from unsaturated lipids and association with cholesterol, transitioning from the L_d phase to the L_o phase. Additional contributions to stabilizing entropic changes in lipid tail⁴⁵ and lipid–water conformations may also contribute to stabilizing the phase-separated state.⁴⁶ There is a free energy cost to creating an interface between the L_o and L_d domains. The system size must be large enough such that the enthalpic gain of forming the L_o domains can compensate for the cost of demixing and formation of the domain interface.^{33,47}

While the fundamental thermodynamic driving forces responsible for lipid phase separation into L_d and L_o domains are well established, relatively little is known about the early stages of lipid phase separation kinetics. The time scales, presumed to be tens of milliseconds, and length scales, estimated to be on the order of a tenth of a micron, for phase separation into L_o domains pose challenges for experiments.^{48,49} These length scales are at the limit of super-resolution microscopy. Chromophores, like amide bands in sphingophospholipids, are sensitive to hydrogen bonding with solvent or protein, but they show limited sensitivity to the membrane phase, resulting in the absence of reliable spectroscopic indicators for membrane phase detection.⁵⁰

Relatively little insight into the kinetics and mechanism of lipid phase separation has been provided by simulations using CG or AA models. The AA and CG simulation study by Bennett et al.⁵¹ observed phase separation in a ternary lipid mixture involving cholesterol on a time scale of tens of microseconds. While the study included a nonequilibrium simulation of phase separation, the primary focus of the work was thermodynamics. They observed that the CG model was able to capture the enthalpic stabilization on forming the L_o phase but failed to capture the loss of entropy associated with lipid tail ordering. However, the cost in free energy associated with establishing the domain interface, found to be critical in other studies, was not discussed. Free energies as a function of lipid mixing have been determined for the MARTINI2 coarse-grained model.⁵² A barrier between miscible and phase-separated states of approximately 1 kcal/mol was observed, similar to estimates based on lipid domain line tensions.^{33,47}

A significant number of studies have employed free energy functional approaches to explore the kinetics of phase separation in lipid mixtures.^{53–58} These studies have often represented the state of the system in terms of a space- and time-dependent field variable representing the local lipid composition. Many

important observations have been made regarding the nature of the kinetics of lipid phase separation, including the roles of nucleation and growth, domain ripening, coalescence, and budding,⁵⁴ as well as the role of hydrodynamics in the membrane and solvent.⁵⁹

Of particular importance are considerations of the role of line tension. Though the line tension on the nanoscopic scale has not been estimated experimentally, it was noted that lower line tensions would be consistent with the formation of lipid raft microdomains that exist in a quasi-equilibrium with larger-scale domains.^{59–62} In the event that the line tension is larger, lipid rafts were proposed to form through a process involving the wetting of proteins by saturated lipids and cholesterol. The coalescence of similarly lipid-wetted proteins could lead to the larger scale formation of raft domains containing multiple proteins.^{63–65} Alternatively, it has been proposed that nanoscopic raft domains could be formed through the processes of exocytosis or endocytosis.⁵⁹

A notable recent study of the kinetics of lipid phase separation using AA molecular dynamics included simulations of bilayers formed by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine:1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DPPC:DOPC) and DPPC:DOPC:cholesterol lipid mixtures over a range of temperatures from 270 to 310 K.⁴⁴ Over the course of 10-microsecond-long simulations, the phase separation process was observed to continue, indicating that the time scale for phase separation was on the order of tens of microseconds. In ternary mixtures, phase separation into gel- L_o phases was observed at 270 K and L_o – L_d phases at 280 and 290 K, while a single L_d phase was observed at 310 K. However, no particular insight into the mechanism of phase separation was noted.

In this work, we perform large-scale molecular dynamics simulations of a pair of C99 protein conjugates in a ternary lipid mixture consisting of 37 mol % saturated lipid, DPPC, 37 mol % unsaturated lipid, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DUPC), and 26 mol % cholesterol (CHOL) at atomistic and coarse-grained resolution. Our study explores the kinetic mechanism of lipid phase separation in the presence of transmembrane proteins and provides a comparative analysis between AA and CG models. Notably, we find that, after appropriate timescaling, the kinetic mechanisms of phase separation are similar in both models, despite some differences in the early stages of phase separation. Furthermore, we assess the monomeric and dimeric states, as well as the localization of the protein in both models. Overall, our findings provide insight into the kinetic mechanism of lipid phase separation and microdomain formation in ternary lipid mixtures in the presence of APP C99 protein, while also highlighting the importance of timescaling when comparing dynamics at different levels of resolution.

METHODS

All-Atom Simulation on ANTON2. We performed a single simulation of a 496,452-particle system containing a lipid bilayer using the NPT ensemble for 22.38 μ s of sampling. The system was composed of two 40-residue transmembrane proteins, 640 DPPC lipids, 640 DUPC lipids, and 480 CHOL in a symmetric lipid bilayer. The membrane and protein system was solvated in 96800 TIP3P waters and 139 Na^+ and 149 Cl^- ions. The initial conditions of the system consisted of a random lateral mixture of lipids and two copies of APP-C99_{16–55} proteins (C99) separated by a distance equivalent to one lipid solvation shell, with the juxtamembrane domains of each C99 structured into an α -helix

and oriented toward each other based on PDB: 2LLM.⁶⁶ The CHARMM36⁶⁷ force field family of parameters was used for all components of the system. The initial conditions were prepared and equilibrated using CHARMM-GUI⁶⁸ protocols.

The production simulation was run on an ANTON2 ASIC19⁶⁹ in an NPT ensemble using a 2.4 fs time step and a RESPA⁷⁰ scheme with the default bonded interval of 1 time step, far nonbonded interval of 3 time steps, and nonbonded near interval of 1 time step using Desmond.⁷¹ The pressure was maintained at 1 bar and a compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$ with an MTK barostat⁷² semi-isotropic pressure coupling scheme. The temperature was coupled to a Nosé-Hoover thermostat^{73,74} at a reference temperature of 299 K, which was required to ultimately achieve an average simulation temperature of 295 K. The temperature shift was likely a consequence of the temperature evaluation method used in Desmond while employing this aggressive multiple time step scheme.⁷⁵ Thermostats and barostats were each coupled at 24 ps intervals. Long-range interactions were computed using the u-series method with the default parameters. Center-of-mass motion removal was applied to prevent incidental drift. Thermodynamic variables were saved every 2.4 ps, and coordinates were saved every 240 ps. The Anton2 trajectory reached 18 μs . The trajectory was extended to 22.38 μs in GROMACS using the leapfrog integrator with a 2 ps time step, and the semi-isotropic Parrinello–Rahman barostat for pressure coupling,⁷⁶ a Nosé-Hoover thermostat for temperature coupling, and Particle Mesh Ewald⁷⁷ for long-range electrostatics.

MARTINI2 and MARTINI3 Simulations on CPUs+GPU.

We performed five 12 μs simulations of 43,846-particle systems of identical membrane compositions to the all-atom systems described in the previous subsection, with the MARTINI2,^{78,79} MARTINI3,^{80,81} and the recent update to MARTINI3 bonded parameters for DPPC and DUPC (renamed to DLiPC) “MARTINI-openbeta”⁸² coarse-grained force fields. Due to the MARTINI 4-to-1 all-atom to coarse-grained mapping of water, we represent the solvent with 24,200 water particles, 10% of which are “anti-freeze” water to prevent spontaneous freezing in MARTINI2 simulations, along with 139 Na^+ and 145 Cl^- ions. The same initial configuration, spacing, and relative orientations of the two C99_{16–55} proteins as in the all-atom systems were used in all coarse-grained simulations. The initial coordinates of each of the 5 replicate systems in MARTINI2 and MARTINI3 used the same initial lipid and protein positions, generated from 5 well-mixed representations of these systems in MolPainter.⁸³ Each system was minimized and then annealed with a 10 fs time step over 2 ns, from 200 to 295 K, and equilibrated for 1 ns at 295 K using the Berendsen thermostat and semi-isotropic barostat at 1 bar and $3 \times 10^{-4} \text{ bar}^{-1}$ compressibility, using the same nonbonded pair interaction calculation scheme as in the production simulations.

Production simulations were run on the Boston University SCC cluster with 7 Intel Xeon E5-2680v4 CPUs and 1 P100 GPU for each replica, using a 24 fs time step and a leapfrog integration scheme using GROMACS 2021.5.⁸⁴ The pressure was maintained at 1 bar with a Parrinello–Rahman barostat semi-isotropic pressure coupling scheme, coupled at a 12 ps interval and a compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$.⁷⁶ The temperature was maintained at 295 K with the Bussi velocity-rescale thermostat, coupled at a 1 ps interval for separate groups of the membrane, including the protein and the solvent.⁸⁵ Lennard-Jones pair interactions were computed and shifted to zero at 1.1 nm. Electrostatic pair interactions were computed

using the reaction-field scheme with a 1.1 nm cutoff and a dielectric constant of $\epsilon = 15$.

Analysis. Mixing Entropy. Following our early studies of phase separation in ternary lipid bilayers,³³ the mixing entropy was defined in terms of the probability of contacts formed by the same lipid type (p_1) or different lipid type (p_2)

$$S_{\text{mix}}(t) = -p_1(t) \ln p_1(t) - p_2(t) \ln p_2(t) \quad (\text{S1})$$

The local lipid contacts were computed using nearest neighbors determined via Voronoi tessellations. This method provides advantages over definitions of mixing entropies based on coarse-grained measurements of lipid composition within a space-fixed grid, the resolution of which influences the estimate of mixing entropy. Atoms closest to the lipid leaflet midplane, two from lipids and one from cholesterol, were selected within each leaflet in each frame, from which a Voronoi tessellation was performed.

Cahn–Hilliard Local Composition Order Parameter. At temperatures below the miscibility temperature, the miscible state is thermodynamically unstable and undergoes spinodal decomposition into the thermodynamically stable phase-separated state. The dynamics of this process can be modeled using the compositional order parameter $\phi(\mathbf{r}, t)$, which provides a measure of the local composition of the lipid bilayer as a function of space and time. It is defined as

$$\phi(\mathbf{r}, t) = x_o(\mathbf{r}, t) - x_d(\mathbf{r}, t) = 2x_o(\mathbf{r}, t) - 1 \quad (\text{S2})$$

where $x_o(\mathbf{r}, t)$ and $x_d(\mathbf{r}, t)$ are the mole fractions of saturated and unsaturated lipids, respectively. For a miscible state, we expect $\phi \approx 0$, while for liquid-ordered or liquid-disordered regions, we expect $\phi \approx 1$ and $\phi \approx -1$, respectively. During the process of phase separation, the probability distribution $p(\phi)$ reflecting the range of observed values of $\phi(\mathbf{r}, t)$ at a given time, evolves from a distribution initially peaked near $\phi \approx 0$ to a bimodal distribution with peaks near $\phi \approx -1$ and $\phi \approx 1$.

Equivalently, we can express the state of the system in terms of a local concentration $c(\mathbf{r}, t)$ of the liquid-ordered phase that can be related to the $\phi(\mathbf{r}, t)$ as

$$c(\mathbf{r}, t) = x_o(\mathbf{r}, t) = \frac{1}{2}(\phi(\mathbf{r}, t) + 1) \quad (\text{S3})$$

To compute the local lipid compositional order parameter $\phi(\mathbf{r}, t)$, we define a 2D plane on both the upper and lower membrane leaflet. In MARTINI simulations, we use C2A and C2B beads for DPPC, D2A, and D2B beads for DUPC, and R2 beads for CHOL to define the 2D plane. In atomistic simulations, C27 and C37 atoms define the 2D membrane plane for DPPC and DUPC, while the C8 atom defines it for CHOL. These 2D planes are then divided into local areas using either a grid-based definition or Voronoi tessellations.

For the grid-based definition, we divide the total area into square grids with N grids along the x - and y -dimensions. The compositional order parameter $\phi(\mathbf{r}, t)$ is determined based on the chemical identities of neighbors within the same grid or nearest neighbors of each tessell within the same leaflet by determining the local mole fraction x_o of DPPC (16:0 PC) and the local mole fraction x_d of DUPC (18:2 PC). The time evolution of the variance of $\phi(\mathbf{r}, t)$ across all points, denoted as $\langle \Delta\phi^2 \rangle(t)$ is used as a measure of the progress of phase separation,

$$\langle \Delta \phi^2 \rangle(t) = \frac{1}{N} \sum_i^N (\phi(\mathbf{r}_i, t) - \langle \phi(\mathbf{r}_i, t) \rangle)^2 \quad (\text{S4})$$

where $\phi(\mathbf{r}_i, t)$ is the local composition order parameter at a particular position $\mathbf{r}_i$ at time t and $\langle \phi(\mathbf{r}_i, t) \rangle = \frac{1}{N} \sum_i^N \phi(\mathbf{r}_i, t)$, and N is the number of cells or tessellation tiles.

Nelson–Halperin 2D Bond-Orientational Order Parameter. The lateral packing of lipids around the protein was calculated using the Nelson–Halperin 2D bond-orientational order parameter, ψ_6 defined as

$$\psi_6^k = \frac{1}{6} \sum_{l \in nn(k)}^6 e^{i6\theta_{kl}} \quad (\text{S5})$$

where θ_{kl} represents the angle between a reference vector and the vector connecting the particles k and l in the plane. The positions of the k^{th} tail particle and its six nearest neighbors ($l \in nn(k)$) are used to determine a plane of best fit, onto which the particles were projected. The reference vector is chosen as the projection of the positive X-axis onto this plane. The average bond-orientational order parameter $|\psi_6^k|$ achieves its maximum value of 1 when the nearest neighbors form a perfectly ordered hexagonal pattern.

P_2 Liquid Crystal Order Parameter. The tail ordering of DPPC was calculated as

$$P_2 = \frac{1}{2} (3 \langle \cos^2 \theta_{ij} \rangle - 1) \quad (\text{S6})$$

where θ_{ij} represents the angle between the vector formed from the first and last carbon or coarse-grained bead in each lipid tail and the membrane normal vector. The values of P_2 lie between -0.5 and 1 , with $P_2 = 1$ signifying a straight tail parallel to the membrane normal.

RESULTS AND DISCUSSION

Both AA and CG Models Display Spontaneous Phase Separation to Form L_o and L_d Domains. Instantaneous configurations depicting the spatiotemporal evolution of lipid phase separation are presented for the AA model simulation in Figure 1. Over the course of the $22 \mu\text{s}$ simulation, the lipid phase separation spontaneously proceeds from a miscible state to small

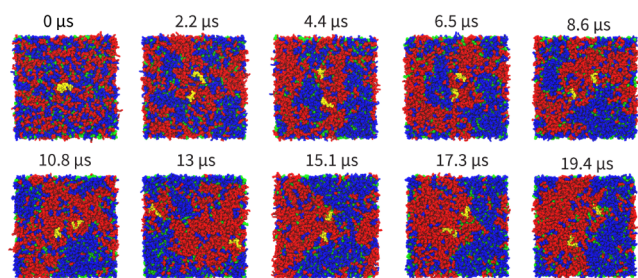


Figure 1. Ten configurations illustrating lipid phase separation in CHARMM36 in a ternary lipid mixture composed of DPPC (blue), DUPC (red), and cholesterol (green). The liquid-ordered (L_o) phase, mainly composed of DPPC, appears blue, while the liquid-disordered (L_d) phase, rich in DUPC, appears to be more red. The yellow C99 proteins are found to be predominantly positioned within the L_d phase at the domain interface. While DPPC is enriched in the L_o phase and DUPC is enriched in the L_d phase, both lipid species are present in each phase as minority components. Cholesterol (green) is distributed in both phases, but preferentially associates with DPPC.

island-like L_o and L_d aggregates, which eventually merge to form completely phase-separated L_o and L_d domains. The two C99 proteins are found to be localized at the domain interface and are primarily solvated by unsaturated lipids. Moreover, on the time scale sampled, the C99 proteins are observed to remain monomeric in the AA simulation.

A similar phase separation process is also observed for CG simulations, as shown in Figure S1. However, a significant difference is observed in the protein localization and oligomeric state between the AA and CG simulations. In the MARTINI2 simulations, the protein is predominantly localized at the domain interface. In contrast, in MARTINI3 simulations, the protein shifts toward the L_d domain bulk, suggesting a difference in lipid–protein interactions between the two models. Furthermore, in contrast to AA simulations, where C99s persist as monomers, C99s dimerize in simulations with both the MARTINI2 and MARTINI3 force fields. The lack of observation of C99 dimerization in AA simulations is likely due to differences in dynamics and the time scale of phase separation introduced by coarse-graining, as well as inherent limitations in the CHARMM36 force field.⁸⁶

Timescaling of Dynamics in AA and CG Simulations Yields Similar Time Evolution. A useful quantitative measure of the progression of phase separation is the mixing entropy $S_{\text{mix}}(t)$ as shown in Figure 2 for the AA and CG dynamics. Figure 2a demonstrates a comparison of the time evolution of S_{mix} in CHARMM36 (AA) and MARTINI2 (CG) simulations. The time axes of MARTINI2 (red) have been scaled with respect to the CHARMM36 (blue) time scale to provide the best fit of the two time series. Similarly, Figure 2b provides the comparison between CHARMM36 (AA) and MARTINI3 (CG) simulations. The scaling factor has been calculated using a χ^2 analysis as demonstrated in Figure S2. For CHARMM36 and MARTINI2 simulations, the scaling factor has been calculated to be 22, and for CHARMM36 and MARTINI3 simulations, it has been calculated to be 29. The phase separation process in MARTINI3-openbeta was found to be 49 times faster than that in the CHARMM36 simulation. This is a rather striking change that can only be attributed to the change in the bonded parameters of the updated MARTINI3 lipids.

The mixing entropy, S_{mix} , is 1 in the ideal mixture and 0 in the bulk phase. However, in a phase-separated lipid bilayer, the L_o phase contains a minority component of DUPC at equilibrium, and vice versa for the L_d phase. Thus, for a finite-size system, the value of S_{mix} for the phase-separated state must be greater than zero. As is evident from Figure 2 S_{mix} reaches >0.5 . Moreover, the time dependence of $S_{\text{mix}}(t)$ is qualitatively similar in the AA and CG simulations after time scaling. The 50% miscibility point⁹ is reached in approximately $5 \mu\text{s}$ in CHARMM36, 230 ns in MARTINI2, and 170 ns in MARTINI3 simulations.

Furthermore, lipid phase separation is accompanied by an increase in the proportion of DPPC molecules with higher values of $|\psi_6|$, indicating a more ordered packing of DPPC molecules, as shown in Figures S3 and S4. In the MARTINI2 simulations, approximately 10% of the DPPC molecules, and in the MARTINI3 simulations, around 5%, exhibit $|\psi_6| > 0.85$ (Figure S3), demonstrating the formation of gel-like nanoscopic domains. Furthermore, the time evolution of the fraction of DPPC molecules that exhibit $|\psi_6| > 0.85$ reveals that the formation of these gel-like domains begins in the early stages of phase separation. A similar trend is observed for DPPC molecules with $0.5 < |\psi_6| < 0.85$, as shown in Figure S4. Moreover, the time evolution of the proportion of DPPC

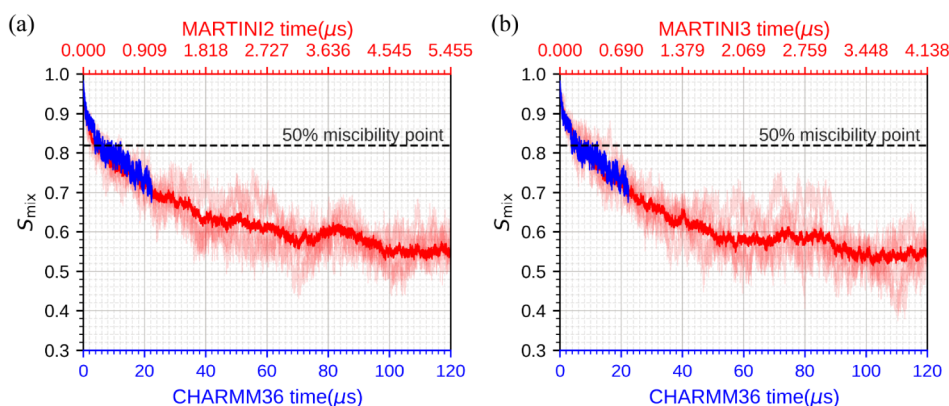


Figure 2. Time evolution of the mixing entropy S_{mix} for the AA CHARMM36 (blue) and CG (red) (a) MARTINI2 and (b) MARTINI3 simulations. The time axes depict the evolution of the time in each simulation where the CHARMM36 time is approximately 22 times longer than the MARTINI2 time and 29 times longer than the MARTINI3 time at a similar degree of phase separation.

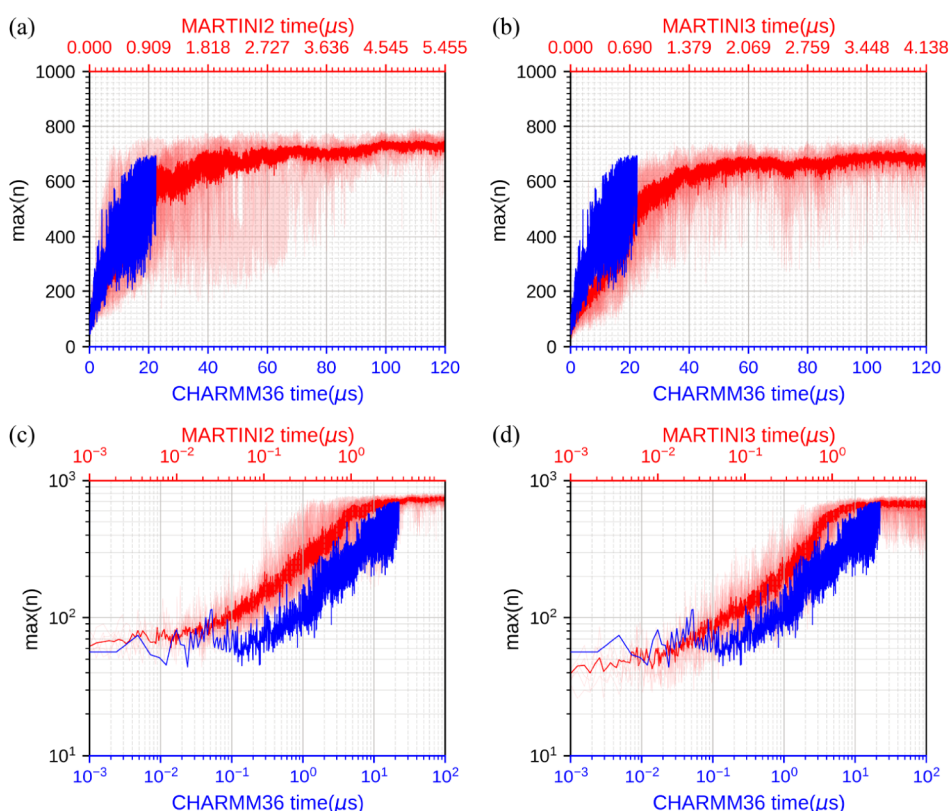


Figure 3. Size of the largest lipid cluster observed in the simulation as a function of time for AA and CG trajectories. The time axis is scaled by using the same scaling factor as that of S_{mix} . The top panel demonstrates the comparison between the time evolution of maximum cluster size between CHARMM36 and (a) MARTINI2, (b) MARTINI3 simulations. (c, d) The bottom panel demonstrates the time evolution on a log–log scale for the top panel comparisons. Following time scaling, general agreement between the AA and CG results is observed (top) however, on a log–log scale (bottom), a greater rate of initial growth of the maximum cluster size is observed in the CG simulation.

molecules with $P_2 > 0.9$ (Figure S5) increases and saturates as phase separation progresses, reflecting a transition to a more ordered phase.

Another useful measure of the progress of phase separation is the largest cluster size, $\text{max}(n)$, among spatial clusters of DPPC and cholesterol, representing the growth of the largest L_0 domain.³³ This measure is expected to approach a constant value representing the phase-separated state. Figure 3 depicts the maximum cluster size as a function of time. The time axes have been scaled based on the scaling factor obtained for S_{mix} . Figure 3a,b illustrates the qualitative similarity observed

between the evolution of the maximum cluster size in AA (CHARMM36) and CG (MARTINI2 and MARTINI3) dynamics upon time scaling, with the CG dynamics reaching convergence. However, a greater difference between the two time series is evident on the log–log plot, as shown in Figure 3c,d. Even after the time scaling, the CG dynamics is observed to have a faster early stage of growth of the maximum cluster size, while the AA dynamics presents a noticeable lag phase.

The size of the maximum cluster increases as a power-law after 20 ns in the MARTINI2 time scale until the convergence point:

$$\max(n(t)) \propto t^\beta \quad (\text{S7})$$

where fitting after scaling to the MARTINI2 time scale leads to exponents of $\beta_{AA} = 0.52$, $\beta_{M2} = 0.54$, and $\beta_{M3} = 0.55$. These exponents are indicative of diffusion-limited coalescence being the dominant mechanism for domain growth over the course of the simulation. A coalescence mechanism of domain growth is expected as (1) the composition of lipids forming L_o and L_d domains is roughly equal, and thus their domain areas are approximately equal, and (2) the time scale for evaporation of lipids from domains is large compared with the simulation time scale.

Origin of Timescaling in Terms of Higher Diffusion and Sticking Probabilities. To understand the origin of the empirical scaling factor between the time evolution of AA and CG simulations, we develop a simple model for the nucleation and growth of a growing cluster. Let i be the number of monomers in a cluster n . The reversible reaction for the addition of another monomer to the cluster n can be written as



where $c(i)$ is the rate of condensation of a monomer onto a cluster of i monomers and $e(i+1)$ is the rate of evaporation of a monomer from a cluster of $i+1$ monomers. If condensation occurs through diffusion-controlled encounter, the rate of addition of monomers is approximately

$$c(i) = \alpha 4\pi D \sigma n_1(t) \quad (\text{S9})$$

where $D = D_i + D_1$ is the relative rate of diffusion of the cluster and monomer, and $\sigma = r_i + r_1$ is the encounter radius for contact between the cluster and monomer. The parameter α is a sticking probability that represents the fraction $0 \leq \alpha \leq 1$ of collisions that lead to the addition of a monomer to the cluster. Therefore, the ratio of the rate of growth of a cluster in AA and CG simulations can be given by

$$\frac{\tau_{AA}}{\tau_{CG}} = \frac{D_{AA}\alpha_{AA}}{D_{CG}\alpha_{CG}} \quad (\text{S10})$$

Thus, the emergence of a scaling factor can be attributed to differences in the diffusion coefficients and the sticking probability for the AA and CG dynamics.

In order to get quantitative insight into the magnitude of contribution of diffusion coefficient to that of the scaling factor, we have compared the lateral diffusion constant of DPPC, DUPC, and Cholesterol obtained from AA and CG simulations in this section. We have calculated the lateral diffusion constant from the slope of the average mean square displacement vs time plots (Figure S6), excluding the first and last 10% of the trajectory. For the CG simulations, we used five replicas each of the MARTINI2 and MARTINI3 simulations to calculate the average mean square displacement.

Figure 4 shows a comparison of the diffusion constant for each of the lipids involved in the AA and CG simulations. We observe an enhancement in the diffusion constant for CG simulations relative to the AA simulations. Table 1 presents the ratio of the diffusion coefficient of CG dynamics to that of AA dynamics for DPPC, DUPC, and cholesterol. We note that the highest diffusion rates are observed for cholesterol. Considering the highest ratios of the diffusion constant of 8.4 and 12.6 for MARTINI2 and MARTINI3 simulations, respectively, we obtain a missing factor of ~ 2.6 and ~ 2.3 for MARTINI2 and

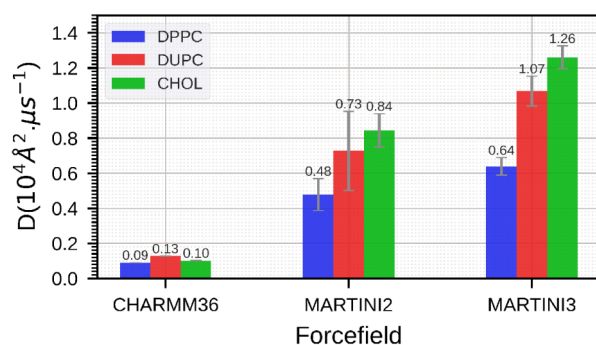


Figure 4. Mean diffusion constant for DPPC, DUPC, and Cholesterol as obtained from a single AA (CHARMM36) trajectory and 5 replicas of CG (MARTINI2 and MARTINI3) trajectories. MARTINI3 exhibits the highest diffusion constant for all lipids, which can be attributed to the fastest rate of phase separation.

Table 1. Ratio of Diffusion Constant for Lipids in CG Simulations to AA Simulations

Lipid	D_{M2}/D_{AA}	D_{M3}/D_{AA}
DPPC	5.3	7.1
DUPC	5.6	8.2
CHOL	8.4	12.6

MARTINI3 simulations. These factors can be attributed to the sticking probability ratios, as per the model we developed above.

Three-Stage Mechanism of Phase Separation. Several studies have utilized the Cahn–Hilliard theory extensively in order to understand phase separation kinetics. However, most of the studies have employed hydrodynamic approaches that are only applicable to larger length and time scales and thus cannot capture the microscopic picture. Bagchi and coworkers^{87,88} were the first to apply concepts from the Cahn–Hilliard theory to MD simulations to understand the microscopic origin of the different stages involved in the kinetics of spinodal decomposition in binary mixtures. In their analysis, three kinetic phases were observed during phase separation from a miscible fluid mixture: an initial short-time exponential phase, followed by a power-law phase, and a long-time logarithmic convergence. Building upon Bagchi’s work, we developed the Cahn–Hilliard local composition order parameter ($\phi(r,t)$) to study the different stages involved in the kinetics of lipid phase separation in ternary lipid mixtures in the presence of the transmembrane protein APP C99.

The effectiveness of the local composition order parameter ϕ in tracking the progress of the phase separation process was evaluated by analyzing the probability distributions $P(\phi)$ at the initial and final phases of the phase separation process for both the AA and CG trajectories, as illustrated in Figure 5. ϕ was calculated by using an 8×8 grid. Initially, the distribution displayed an approximately Gaussian distribution centered at zero, indicating a miscible lipid mixture. However, as the system evolves toward the final phase-separated state, the distribution develops sharp peaks at $\phi \approx \pm 1$, signifying the formation of L_o and L_d domains.

Figure 6 illustrates the instantaneous values of $\phi(r,t)$ associated with each lipid in the upper leaflet of the membrane as a function of the lipid coordinate, calculated at timestamps labeled in the figure. The calculation of ϕ in this case and the subsequent analysis are based on Voronoi tessellation. Initially, at $t = 0$, different values of ϕ are scattered throughout the plane,

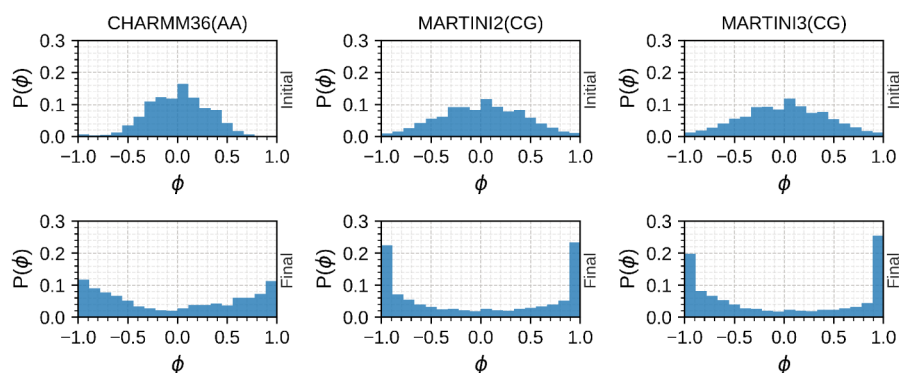


Figure 5. Probability distribution of ϕ at the initial and final stages of lipid phase separation for AA (CHARMM36) and CG (MARTINI2 and MARTINI3) simulations. Initial phases demonstrate a Gaussian distribution centered at 0 and the final phases exhibit a bimodal distribution with a peak at $\phi \approx \pm 1$.

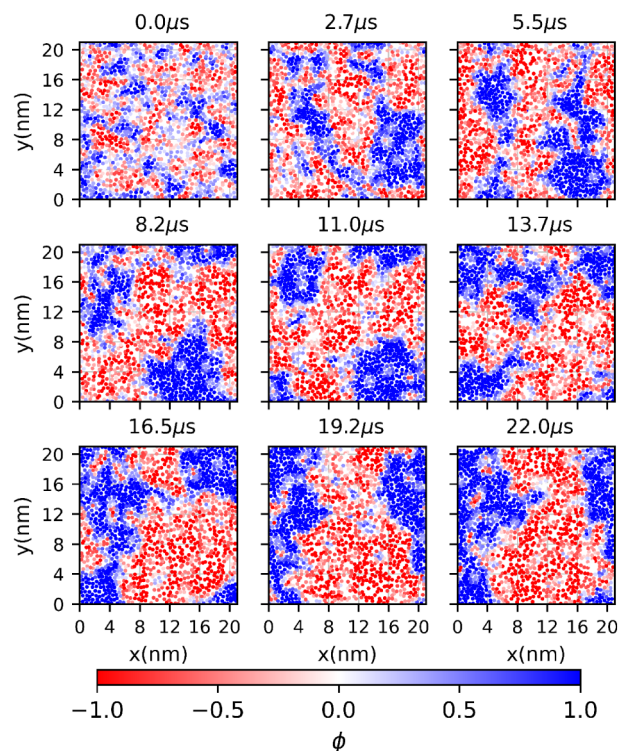


Figure 6. Instantaneous spatial distribution of ϕ as a function of coordinates in the XY plane in CHARMM36. Each point represents a lipid on the upper leaflet of the membrane. Red-colored points represent a negative value of ϕ , whereas blue points represent positive values, as per the colorbar given below the plots. At $t = 0$, both higher as well as lower values of ϕ are observed to be scattered throughout the plane, representing a miscible state. As phase separation progresses, higher and lower values of ϕ start to cluster, forming larger phase separated domains, with blue representing the L_o domain and red representing the L_d domain.

and a larger proportion of the plane is occupied with ϕ values near zero, indicating a miscible state. As phase separation progressed, points of similar ϕ values aggregated, analogous to crystal nucleation. Subsequently, we observed the merger of these small aggregates to form larger blue ($\phi \sim +1$) and red ($\phi \sim -1$) domains, signifying the formation of L_d and L_o phases.

Subsequently, the different stages of the phase separation process are evaluated from the time evolution of the variance of ϕ , $\langle \Delta \phi^2 \rangle$. The time evolution of $\langle \Delta \phi^2 \rangle$ can be divided into three stages, as shown in Figure 7a.

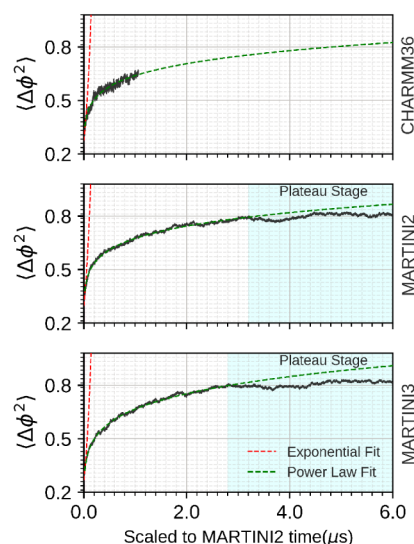


Figure 7. Time evolution of variance of ϕ , $\langle \Delta \phi^2 \rangle$ averaged over all space with respective fitting curve for exponential phase (red-dashed line) and power law phase (green-dashed line). In the case of MARTINI2 and MARTINI3 simulations, the final plateau phase is shaded cyan.

- Exponential stage: this stage is characterized by the rapid exponential growth, $\langle \Delta \phi^2 \rangle = a_0 e^{\alpha t}$ of $\langle \Delta \phi^2 \rangle$, where α is the exponential factor and a_0 is the constant prefactor of the exponential function, stemming from the initial relaxation of the system leading to the formation of small aggregates of similar phases, as observed in Figure 6 (Figure 7). Notably, even during this early stage, the distribution of $|\psi_6|$ for DPPC reveals the presence of a finite fraction of molecules with $|\psi_6| > 0.85$, indicating the formation of hexagonally packed, gel-phase-like nanoscopic aggregates of DPPC (Figure S7), although the overall density of highly ordered DPPC molecules remains low. This suggests that the onset of hexagonal packing occurs alongside the initial phase-separation process. This stage lasts until 20 ns in the MARTINI2 time scale. The fitting parameters are provided in Table S1.
- Power law stage: following the exponential phase, the phase separation slows and transitions to a power-law stage, $\langle \Delta \phi^2 \rangle(t) = a_1 t^\beta$ that persists up to 3.2 μ s in MARTINI2 simulations and 2.8 μ s in MARTINI3 simulations (Figure 7). It is characterized by exponents

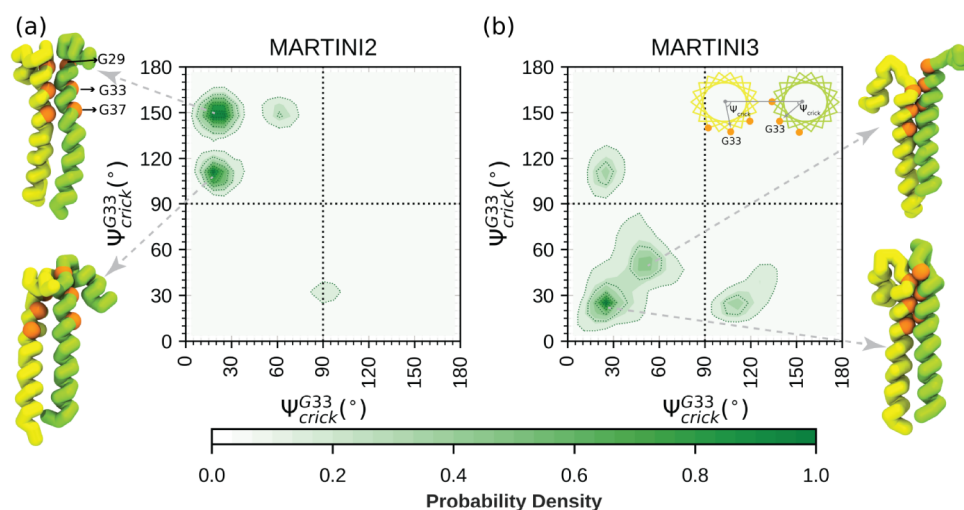


Figure 8. G33–G33 crick angle distribution for (a) MARTINI2 and (b) MARTINI3 simulations. The MARTINI2 simulations demonstrate predominance of the Gly-side conformation, as shown by the structure on the left of the plot. The MARTINI3 simulations demonstrate predominance of Gly-in conformations, as shown by the structure on the right of the plot.

of $\beta_{AA} = 0.125$, $\beta_{M2} = 0.123$, and $\beta_{M3} = 0.154$. In this stage, lipids of similar phases coalesce to form one large L_o or L_d domain, leaving behind nanoscopic impurities in opposing phases. In addition, there is an increase in the proportion of DPPC molecules exhibiting $|\psi_{\phi}| > 0.85$, indicating greater hexagonal ordering of DPPC (Figure S7). Fitting the cluster size with a power-law function, we obtained an exponent of $\frac{1}{2}$, similar to that from time-dependent spectroscopic analysis of domain growth in near-critical lipid mixtures.^{89,90} However, as the domain size and $\langle \Delta\phi^2 \rangle$ measured here are microscopic quantities, we simply consider these exponents to be measures of the similarity of kinetics between the coarse-grained and all-atom models. The complete set of fitting parameters can be found in Table S2.

- (c) Plateau stage: after the formation of larger domains, the phase separation kinetics transitions to a plateau stage, as illustrated in the shaded cyan region in Figure 7. This stage has also been observed by Bagchi and coworkers,^{87,88} who found this logarithmic phase to correspond to the smoothing of domain boundaries. Here, nanoscopic domains of the opposite phase to the major domains gradually merge with the major domain of the corresponding phase.

The same three regimes of phase separation and corresponding exponents observed in the MARTINI3-openbeta model were found to be similar to those of CHARMM36, MARTINI2, and MARTINI3 models (Figures S8 and S9 and Tables S1 and S2).

Discrepancies in APP C99 Dimer Propensity and Binding Modes. In CHARMM36 simulations, the protein predominantly remains in the monomeric state, whereas MARTINI2 and MARTINI3 simulations show a greater tendency for dimerization. In MARTINI2, four out of five replicas form a dimer within the first 5 μ s (Figure S10), while in MARTINI3, all replicas dimerized within 2.1 μ s. These discrepancies between atomistic (AA) and coarse-grained (CG) simulations may arise from differences in the time scale of phase separation and force field-specific limitations. In particular, Domanski et al.⁸⁶ have demonstrated that the

CHARMM36 force field insufficiently stabilizes Glycophorin A homodimers, which are stabilized by a glycine zipper motif similar to that of the C99 transmembrane domain. To reproduce the experimentally observed dimer stability, comparable to results from MARTINI2 CG simulations, it was necessary to scale down the lipid–protein interaction strength by a factor of 0.9 in CHARMM36. Without this correction factor, the force field is expected to inherently disfavor glycine zipper homodimeric states. However, because dimerization events in CG simulations occur within 2.1–5 μ s, whereas the timescaling factors for AA relative to MARTINI2 and MARTINI3 are approximately 22 and 29, respectively. The 22 μ s of sampling in AA simulations corresponds to only 0.7–1.0 μ s in CG time scales and would thus be unlikely to exhibit homodimerization.

To further investigate the modes of dimerization, we analyzed the Crick angle (Figure 8) and crossing angle distributions (Figure S11) in the MARTINI2 and MARTINI3 simulations. The Crick angle measures the relative orientation of the $G^{29}xxxG^{33}xxxG^{37}$ Gly zipper between the two monomers. It is defined as the angle between the vector connecting the centers of mass of this motif in both monomers and the vector joining the center of mass of the motif to G33 (illustrated in the inset of Figure 8b). Because C99 contains this Gly zipper, its canonical dimerization state features the glycines of both C99 facing “in” toward each other to form $Ca-H\cdots O=C$ Gly–Gly hydrogen bonds characteristic of this sequence motif.^{20,91,92}

In the dimer state, the Crick angle distribution of G33 differs notably between MARTINI2 and MARTINI3. In MARTINI2, the distribution shows distinct populations corresponding to a Gly-side conformation, characterized by one helix having an acute Crick angle and the other an obtuse Crick angle, as shown in Figure 8a. In contrast, MARTINI3 predominantly favors a Gly-in conformation, where both helices exhibit acute Crick angles (Figure 8b).

Additionally, the crossing angle, which defines the handedness of the dimer superhelix, indicates a preference for a right-handed configuration in both models. However, MARTINI3 dimers never exhibited left-handed configurations, though MARTINI2 dimers exhibited a minor population of left-handed dimers (Figure S11). Simulations of the full-length C99 protein in POPC bilayers, similar to the DUPC and cholesterol L_d

domains to which C99 is associated, observed the Gly-in state at high propensity, and the crossing angle has been observed with a slight preference for the left-handed superhelix.⁹² As such, both models exhibited significant discrepancies from AA dimers; however, properly capturing the Gly-in state in MARTINI3 is an important improvement over MARTINI2.

Similar Ordering but Distinct Composition of Lipids Surrounding the Protein. Differences in protein–lipid interaction parameters across force fields are known to influence the local environment of proteins within membranes.^{93–97} In complex bilayers, two key aspects can be affected: the local lipid composition surrounding the protein and the ordering of lipids around it. To investigate lipid ordering, we calculated the $|\psi_6|$ parameter for lipids in the first Voronoi shells around the protein. The results, shown in Figure 9, indicate similar $|\psi_6|$

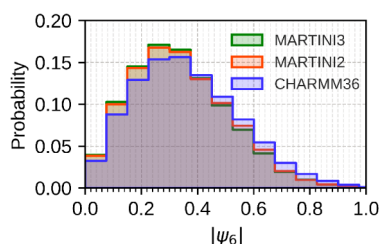


Figure 9. Probability distribution of $|\Psi_6|$ values of first voronoi neighbors around the protein.

distributions for both all-atom (AA) and coarse-grained (CG) force fields, with peaks in the range of 0.2–0.4. These low $|\psi_6|$ values suggest disordered lipid packing around the protein, which is consistent with its localization in the L_d domain of the membrane.

In contrast, the lipid composition surrounding the protein differs significantly for varying force fields, as shown in Figure 10. In CHARMM36 and MARTINI3 simulations, the protein is predominantly surrounded by DUPC, with peak fractions of 0.8 and 1.0, respectively. However, in MARTINI2 simulations, while DUPC is still the major lipid, substantial proportions of DPPC and CHOL are also present around the protein. This compositional difference stems from the protein's preferential localization within the membrane. In MARTINI2, the protein is localized in the L_o – L_d domain interface, an often-observed phenomenon in the MARTINI2 force field.^{47,98} In MARTINI3 and CHARMM36, C99 is more embedded within the bulk L_d domain, in line with the $\sim 90\%$ preference for C99 to partition to L_d domains.^{20,23}

Despite differences in protein colocalization and oligomeric states, the mechanism and kinetics of phase separation in the presence of the protein are not affected. This suggests that while local lipid composition, protein organization, and lipid–protein

interactions vary between force fields, the larger-scale phase behavior of the membrane remains robust. The thermodynamic driving forces for phase separation appear to dominate over the local variations in the protein environment, as well as the differences in lipid–lipid interactions and lipid diffusion, establishing consistent phase separation kinetics across different scales of simulations.

CONCLUSIONS

In this work, molecular dynamics simulations of *de novo* phase separation in ternary lipid mixtures consisting of 37% DPPC, 37% DUPC, and 26% cholesterol are performed for both AA (CHARMM36) and CG (MARTINI2 and MARTINI3) models. By analyzing the dynamics of lipid phase separation from a miscible state to a distinctly phase-separated state, we gained insight into the time scale and mechanism of lipid phase separation. Comparison of the dynamics for the AA and CG models provides insight into the origin of the differences in domain formation rates observed for the two models.

The time scale for phase separation in AA models is observed to be on the order of 20 μ s, while the time scale for phase separation in simulations based on CG models is found to be faster by a factor of 22 for the MARTINI2 simulation and 29 for the MARTINI3 simulations. The faster rate of domain formation is partly explained by an enhanced rate of diffusion in CG models as opposed to AA models. However, an additional difference is observed in the greater lipid–lipid sticking probability—the fraction of monomer collisions with a cluster that result in a monomer joining the cluster—for CG models in the process of nucleation and growth of domains.

A three-stage mechanism was observed for lipid phase separation in the AA and CG models. At short times, we observe an exponential growth leading to the formation of nanoscopic liquid-ordered domains. This behavior closely resembles the initial phase separation kinetics observed in binary fluids,^{87,88} where a temperature quench below the miscibility temperature triggers spinodal decomposition. The exponential phase also resembles the early-time nucleation process observed in protein fibrillization⁹⁹ and protein liquid–liquid phase separation.¹⁰⁰

Following the exponential phase, the system undergoes a crossover to a slower power-law regime where nanodomains coalesce to form larger bulk domains. This slowdown in dynamics seems to arise, in part, due to the substantial decrease in the self-diffusion of the L_o phase domains. Protein liquid–liquid phase separations have also been observed to exhibit a power-law regime.^{101–103}

At long times, phase separation enters a plateau stage, where large domains stabilize and coarsening slows significantly. Bagchi and coworkers^{87,88} characterized this final stage as a

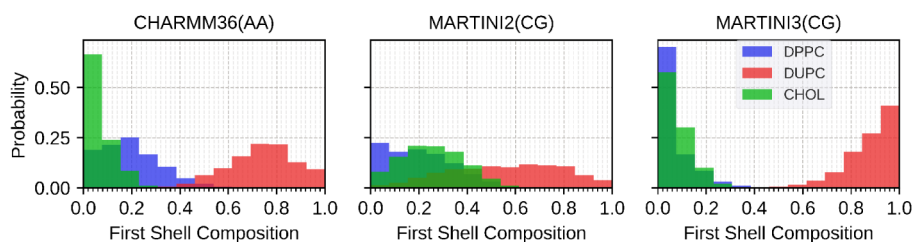


Figure 10. Probability distribution of DPPC (blue), DUPC (red), and CHOL (green) in the first voronoi neighboring shell of APP C99 for AA (CHARMM36) and CG (MARTINI2 and MARTINI3) force fields.

domain coarsening following a logarithmic function. Our coarse-grained simulation seems to suggest this long-time scale phase may be logarithmic, though longer time scales would be necessary to investigate this exceptionally slow process.

While the emergence of this three-stage kinetics is similar to binary fluid phase separation, it is important to note that our study does not propose a universal mechanism for phase separation. However, the extent to which this mechanism extends to other phase-separating systems depends on the underlying thermodynamic conditions and model-specific parameters.

Despite discrepancies in protein–lipid interactions among CHARMM36, MARTINI2, and MARTINI3 force fields—including the well-known overestimation of lipid–lipid interactions, issues with balancing interactions due to significant differences in Lennard-Jones radii, and solute–solvent imbalances in interaction strength in MARTINI, as well as limitations of CHARMM36 in accurately capturing the interfacial properties of lipid bilayers, which may impact phase separation dynamics^{43,104–109}—simulations using all three force fields produce consistent phase separation kinetics when the time scales of the order parameters are appropriately rescaled. While coarse-grained models exaggerate enthalpic contributions to phase separation and all-atom models may misrepresent interfacial behavior, the emergence of similar kinetic profiles across models of differing resolution suggests that the fundamental mechanism of phase separation is robust. This consistency across models implies that even more simplified representations, such as the Cooke model,¹¹⁰ may be sufficient to produce the same mechanism and kinetics of phase separation.

Taken together, our results provide insight into the time scale and mechanism of phase separation in ternary lipid mixtures in the presence of a transmembrane protein. The findings of this study provide insight into the mechanism of nanodomain formation, contributing to our understanding of the size and time scale of transient nanodomain formation in a membrane.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jctc.5c00016>.

Supplemental analyses are presented in the included Supporting Information. The all-atom trajectory at 2.4 ns resolution, simulation input and parameter files for GROMACS and Desmond, and analysis scripts are available in the public archive at doi:10.5281/zenodo.15041214. Additional data are available upon request (PDF)

■ AUTHOR INFORMATION

Corresponding Author

John E. Straub — Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States; orcid.org/0000-0002-2355-3316; Email: straub@bu.edu

Authors

George A. Pantelopulos — Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States; Present Address: Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases,

NIH, Bethesda, MD, USA; orcid.org/0000-0002-4373-1677

Sangram Prusty — Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States; orcid.org/0000-0003-3582-1792

Asanga Bandara — Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.jctc.5c00016>

Author Contributions

#G.A.P. and S.P. contributed equally to this work. G.A.P., A.B., J.E.S., and S.P. designed the research; S.P., G.A.P., A.B., and J.E.S. performed the research; G.A.P. and S.P. analyzed the data; and S.P., J.E.S., and G.A.P. wrote the paper.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the generous support of the National Science Foundation (CHE-1900416), the National Institutes of Health (R01 GM107703), and the high-performance computing resources of the Boston University Shared Computing Cluster (SCC). Anton 2 computer time was provided by the Pittsburgh Supercomputing Center (PSC) through Grant R01GM116961 from the National Institutes of Health. The Anton 2 machine at PSC was made available by D.E. Shaw Research. The authors thank Ayan Majumder, Biman Bagchi, and Sarmistha Sarkar for their helpful discussions.

■ REFERENCES

- (1) Veatch, S. L.; Keller, S. L. Organization in Lipid Membranes Containing Cholesterol. *Phys. Rev. Lett.* **2002**, *89*, 268101.
- (2) Baumgart, T.; Hess, S. T.; Webb, W. W. Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **2003**, *425*, 821–824.
- (3) Dietrich, C.; Bagatolli, L. A.; Volovyk, Z. N.; Thompson, N. L.; Levi, M.; Jacobson, K.; Gratton, E. Lipid Rafts Reconstituted in Model Membranes. *Biophys. J.* **2001**, *80*, 1417–1428.
- (4) Romanauska, A.; Köhler, A. Lipid saturation controls nuclear envelope function. *Nat. Cell Biol.* **2023**, *25*, 1290–1302.
- (5) Silvius, J. R. Role of cholesterol in lipid raft formation: lessons from lipid model systems. *Biochim. Biophys. Acta - Biomembr.* **2003**, *1610*, 174–183.
- (6) Engberg, O.; Hautala, V.; Yasuda, T.; Dehio, H.; Murata, M.; Slotte, J. P.; Nyholm, T. K. M. The Affinity of Cholesterol for Different Phospholipids Affects Lateral Segregation in Bilayers. *Biophys. J.* **2016**, *111*, 546–556.
- (7) Vist, M. R.; Davis, J. H. Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: deuterium nuclear magnetic resonance and differential scanning calorimetry. *Biochemistry* **1990**, *29*, 451–464.
- (8) Sodt, A. J.; Sandar, M. L.; Gawrisch, K.; Pastor, R. W.; Lyman, E. The molecular structure of the liquid-ordered phase of lipid bilayers. *J. Am. Chem. Soc.* **2014**, *136*, 725–732.
- (9) Pantelopulos, G. A.; Straub, J. E. Regimes of Complex Lipid Bilayer Phases Induced by Cholesterol Concentration in MD Simulation. *Biophys. J.* **2018**, *115*, 2167–2178.
- (10) Javanainen, M.; Martinez-Seara, H.; Vattulainen, I. Nanoscale Membrane Domain Formation Driven by Cholesterol. *Sci. Rep.* **2017**, *7* (1), 1143.
- (11) Quinn, P. J.; Wolf, C. The liquid-ordered phase in membranes. *Biochim. Biophys. Acta - Biomembr.* **2009**, *1788*, 33–46.
- (12) Sarkar, T.; Farago, O. Characterizing the heterogeneity of membrane liquid-ordered domains. *Eur. Phys. J. E* **2023**, *46* (10), 99.

- (13) Schachter, I.; Paananen, R. O.; Fábián, B.; Jurkiewicz, P.; Javanainen, M. The Two Faces of the Liquid Ordered Phase. *J. Phys. Chem. Lett.* **2022**, *13*, 1307–1313.
- (14) Fernandes, J. B.; Yu, Y.; Klauda, J. B. Molecular dynamics simulations of the human ocular lens with age and cataract. *Biochim. Biophys. Acta, Biomembr.* **2022**, 1864.
- (15) Sezgin, E.; Levental, I.; Mayor, S.; Eggeling, C. The mystery of membrane organization: Composition, regulation and roles of lipid rafts. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 361–374.
- (16) Levental, I.; Levental, K. R.; Heberle, F. A. Lipid Rafts: Controversies Resolved, Mysteries Remain. *Trends Cell Biol.* **2020**, *30*, 341–353.
- (17) Levental, I.; Lingwood, D.; Grzybek, M.; Coskun, U.; Simons, K. Palmitoylation regulates raft affinity for the majority of integral raft proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 22050–22054.
- (18) Lorent, J. H.; Levental, I. Structural determinants of protein partitioning into ordered membrane domains and lipid rafts. *Chem. Phys. Lipids* **2015**, *192*, 23–32.
- (19) Bodoso, J.; Iyer, S. S.; Srivastava, A. Preferential Protein Partitioning in Biological Membrane with Coexisting Liquid Ordered and Liquid Disordered Phase Behavior: Underlying Design Principles. *J. Membr. Biol.* **2020**, *253*, S51–S62.
- (20) Pantelopulos, G. A.; Abraham, C. B.; Straub, J. E. Cholesterol and Lipid Rafts in the Biogenesis of Amyloid- β Protein and Alzheimer's Disease. *Annu. Rev. Biophys.* **2024**, *53*, 455–486.
- (21) Ehehalt, R.; Keller, P.; Haass, C.; Thiele, C.; Simons, K. Amyloidogenic processing of the Alzheimer β -amyloid precursor protein depends on lipid rafts. *J. Cell Biol.* **2003**, *160*, 113–123.
- (22) Hur, J.-Y. γ -Secretase in Alzheimer's disease. *Exp. Mol. Med.* **2022**, *54*, 433–446.
- (23) Capone, R.; Tiwari, A.; Hadziselimovic, A.; Peskova, Y.; Hutchison, J. M.; Sanders, C. R.; Kenworthy, A. K. The C99 domain of the amyloid precursor protein resides in the disordered membrane phase. *J. Biol. Chem.* **2021**, *296*, 100652.
- (24) Idema, T.; Van Leeuwen, J. M.; Storm, C. Phase coexistence and line tension in ternary lipid systems. *Phys. Rev. E* **2009**, *80* (4), 041924.
- (25) Elson, E. L.; Fried, E.; Dolbow, J. E.; Genin, G. M. Phase Separation in Biological Membranes: Integration of Theory and Experiment. *Annu. Rev. Biophys.* **2010**, *39*, 207–226.
- (26) Paez-Perez, M.; Vyšniauskas, A.; López-Duarte, I.; Lafarge, E. J.; López-Ríos De Castro, R.; Marques, C. M.; Schroder, A. P.; Muller, P.; Lorenz, C. D.; Brooks, N. J.; Kuimova, M. K. Directly imaging emergence of phase separation in peroxidized lipid membranes. *Commun. Chem.* **2023**, *6* (1), 15.
- (27) Marsh, D. Cholesterol-induced fluid membrane domains: A compendium of lipid-raft ternary phase diagrams. *Biochim. Biophys. Acta - Biomembr.* **2009**, *1788*, 2114–2123.
- (28) Garvey, C. J.; Bryant, S. J.; Elbourne, A.; Hunt, T.; Kent, B.; Kreuzer, M.; Strobl, M.; Steitz, R.; Bryant, G. Phase separation in a ternary DPPC/DOPC/POPC system with reducing hydration. *J. Colloid Interface Sci.* **2023**, *638*, 719–732.
- (29) Hishida, M.; Yanagisawa, R.; Yamamura, Y.; Saito, K. Phase separation of a ternary lipid vesicle including n-alkane: Rugged vesicle and bilayer flakes formed by separation between highly rigid and flexible domains. *J. Chem. Phys.* **2019**, *150* (6), 064904.
- (30) Veatch, S. L.; Keller, S. L. Separation of Liquid Phases in Giant Vesicles of Ternary Mixtures of Phospholipids and Cholesterol. *Biophys. J.* **2003**, *85*, 3074–3083.
- (31) Lee, I. H.; Imanaka, M. Y.; Modahl, E. H.; Torres-Ocampo, A. P. Lipid Raft Phase Modulation by Membrane-Anchored Proteins with Inherent Phase Separation Properties. *ACS Omega* **2019**, *4*, 6551–6559.
- (32) Reigada, R.; Buceta, J.; Gómez, J.; Sagués, F.; Lindenberg, K. Phase separation in three-component lipid membranes: From Monte Carlo simulations to Ginzburg-Landau equations. *J. Chem. Phys.* **2008**, *128* (2), 025102.
- (33) Pantelopulos, G. A.; Nagai, T.; Bandara, A.; Panahi, A.; Straub, J. E. Critical size dependence of domain formation observed in coarse-grained simulations of bilayers composed of ternary lipid mixtures. *J. Chem. Phys.* **2017**, *147* (9), 095101.
- (34) He, S.; Maibaum, L. Identifying the Onset of Phase Separation in Quaternary Lipid Bilayer Systems from Coarse-Grained Simulations. *J. Phys. Chem. B* **2018**, *122*, 3961–3973.
- (35) Basu, I.; Mukhopadhyay, C. In silico phase separation in the presence of GM1 in ternary and quaternary lipid bilayers. *Phys. Chem. Chem. Phys.* **2015**, *17*, 17130–17139.
- (36) Konyakhina, T. M.; Wu, J.; Mastroianni, J. D.; Heberle, F. A.; Feigenson, G. W. Phase diagram of a 4-component lipid mixture: DSPC/DOPC/POPC/chol. *Biochim. Biophys. Acta - Biomembr.* **2013**, *1828*, 2204–2214.
- (37) Ackerman, D. G.; Feigenson, G. W. Multiscale modeling of four-component lipid mixtures: Domain composition, size, alignment, and properties of the phase interface. *J. Phys. Chem. B* **2015**, *119*, 4240–4250.
- (38) Goh, S. L.; Amazon, J. J.; Feigenson, G. W. Toward a better raft model: Modulated phases in the four-component bilayer, DSPC/DOPC/POPC/CHOL. *Biophys. J.* **2013**, *104*, 853–862.
- (39) Liu, Y.; Barnoud, J.; Marrink, S. J. Gangliosides Destabilize Lipid Phase Separation in Multicomponent Membranes. *Biophys. J.* **2019**, *117*, 1215–1223.
- (40) Puff, N.; Watanabe, C.; Seigneuret, M.; Angelova, M. I.; Staneva, G. Lo/Ld phase coexistence modulation induced by GM1. *Biochim. Biophys. Acta - Biomembr.* **2014**, *1838*, 2105–2114.
- (41) Manna, M.; Javanainen, M.; Monne, H. M.-S.; Gabius, H.-J.; Rog, T.; Vattulainen, I. Long-chain GM1 gangliosides alter transmembrane domain registration through interdigitation. *Biochim. Biophys. Acta - Biomembr.* **2017**, *1859*, 870–878.
- (42) Baoukina, S.; Mendez-Villuendas, E.; Bennett, W. F. D.; Tieleman, D. P. Computer simulations of the phase separation in model membranes. *Faraday Discuss* **2013**, *161*, 63–75.
- (43) Davis, R. S.; Sunil Kumar, P. B.; Sperotto, M. M.; Laradji, M. Predictions of Phase Separation in Three-Component Lipid Membranes by the MARTINI Force Field. *J. Phys. Chem. B* **2013**, *117*, 4072–4080.
- (44) Gu, R. X.; Baoukina, S.; Peter Tieleman, D. Phase Separation in Atomistic Simulations of Model Membranes. *J. Am. Chem. Soc.* **2020**, *142*, 2844–2856.
- (45) Carpenter, T. S.; Lopez, C. A.; Neale, C.; Ingolfsson, H. I.; Montour, C.; Gnanakaran, S.; Lightstone, F. C. Accurate phase separation of complex lipid mixtures (DPPC/DOPC/CHOL) with a refined coarse grained martini model. *Biophys. J.* **2018**, *114*, 102a.
- (46) Frenkel, D. Entropy-driven phase transitions. *Phys. A: stat. Mech. Appl.* **1999**, *263*, 26–38.
- (47) Bandara, A.; Panahi, A.; Pantelopulos, G. A.; Nagai, T.; Straub, J. E. Exploring the impact of proteins on the line tension of a phase-separating ternary lipid mixture. *J. Chem. Phys.* **2019**, *150* (20), 204702.
- (48) Heberle, F. A.; Feigenson, G. W. Phase separation in lipid membranes. *Cold Spring Harbor Perspect. Biol.* **2011**, *3*, a004630.
- (49) Javanainen, M.; Hammaren, H.; Monticelli, L.; Jeon, J. H.; Miettinen, M. S.; Martinez-Seara, H.; Metzler, R.; Vattulainen, I. Anomalous and normal diffusion of proteins and lipids in crowded lipid membranes. *Faraday Discuss* **2013**, *161*, 397–417.
- (50) Shirota, K.; Yagi, K.; Inaba, T.; Li, P.-C.; Murata, M.; Sugita, Y.; Kobayashi, T. Detection of Spingomyelin Clusters by Raman Spectroscopy. *Biophys. J.* **2016**, *111*, 999–1007.
- (51) Bennett, W. F. D.; Shea, J.-E.; Tieleman, D. P. Phospholipid Chain Interactions with Cholesterol Drive Domain Formation in Lipid Membranes. *Biophys. J.* **2018**, *114*, 2595–2605.
- (52) Poruthoor, A. J.; Sharma, A.; Grossfield, A. Understanding the free-energy landscape of phase separation in lipid bilayers using molecular dynamics. *Biophys. J.* **2023**, *122*, 4144–4159.
- (53) Wallace, E. J.; Hooper, N. M.; Olmsted, P. D. The kinetics of phase separation in asymmetric membranes. *Biophys. J.* **2005**, *88*, 4072–4083.
- (54) Frolov, V. A.; Chizmadzhev, Y. A.; Cohen, F. S.; Zimmerberg, J. “Entropic traps” in the kinetics of phase separation in multicomponent membranes stabilize nanodomains. *Biophys. J.* **2006**, *91*, 189–205.

- (55) Kuzmin, P. I.; Akimov, S. A.; Chizmadzhev, Y. A.; Zimmerberg, J.; Cohen, F. S. Line Tension and Interaction Energies of Membrane Rafts Calculated from Lipid Splay and Tilt. *Biophys. J.* **2005**, *88*, 1120–1133.
- (56) Stepanova, M. Reversible Formation of Nanodomains in Monolayers of DPPC Studied by Kinetic Modeling. *Biophys. J.* **2009**, *96*, 4896–4905.
- (57) Garcke, H.; Kampmann, J.; Rätz, A.; Röger, M. A coupled surface-Cahn-Hilliard bulk-diffusion system modeling lipid raft formation in cell membranes. *Math. Models Methods Appl. Sci.* **2016**, *26*, 1149–1189.
- (58) Zhiliakov, A.; Wang, Y.; Quaini, A.; Olshanskii, M.; Majd, S. Experimental validation of a phase-field model to predict coarsening dynamics of lipid domains in multicomponent membranes. *Biochim. Biophys. Acta - Biomembr.* **2021**, *1863*, 183446.
- (59) Fan, J.; Han, T.; Haataja, M. Hydrodynamic effects on spinodal decomposition kinetics in planar lipid bilayer membranes. *J. Chem. Phys.* **2010**, *133* (23), 235101.
- (60) Fan, J.; Sammalkorpi, M.; Haataja, M. Formation and regulation of lipid microdomains in cell membranes: Theory, modeling, and speculation. *FEBS Lett.* **2010**, *584*, 1678–1684.
- (61) Hancock, J. F. Lipid rafts: contentious only from simplistic standpoints. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 456–462.
- (62) Usery, R. D.; Enoki, T. A.; Wickramasinghe, S. P.; Weiner, M. D.; Tsai, W.-C.; Kim, M. B.; Wang, S.; Torng, T. L.; Ackerman, D. G.; Heberle, F. A.; Katsaras, J.; Feigenson, G. W. Line Tension Controls Liquid-Disordered + Liquid-Ordered Domain Size Transition in Lipid Bilayers. *Biophys. J.* **2017**, *112*, 1431–1443.
- (63) Hoferer, M.; Bonfanti, S.; Taloni, A.; La Porta, C. A. M.; Zapperi, S. Protein-driven lipid domain nucleation in biological membranes. *Phys. Rev. E* **2019**, *100*, 042410.
- (64) Akimov, S. A.; Frolov, V. A. J.; Kuzmin, P. I.; Zimmerberg, J.; Chizmadzhev, Y. A.; Cohen, F. S. Domain formation in membranes caused by lipid wetting of protein. *Phys. Rev. E* **2008**, *77*, 051901.
- (65) Molotkovsky, R. J.; Galimzyanov, T. R.; Batishchev, O. V.; Akimov, S. A. The Effect of Transmembrane Protein Shape on Surrounding Lipid Domain Formation by Wetting. *Biomolecules* **2019**, *9* (11), 729.
- (66) Nadezhdin, K. D.; Bocharova, O. V.; Bocharov, E. V.; Arseniev, A. S. Structural and Dynamic Study of the Transmembrane Domain of the Amyloid Precursor Protein. *Acta Nat.* **2011**, *3*, 69–76.
- (67) Best, R. B.; Zhu, X.; Shim, J.; Lopes, P. E. M.; Mittal, J.; Feig, M.; MacKerell, A. D. J. Optimization of the Additive CHARMM All-Atom Protein Force Field Targeting Improved Sampling of the Backbone ϕ , ψ and Side-Chain χ_1 and χ_2 Dihedral Angles. *J. Chem. Theory Comput.* **2012**, *8*, 3257–3273.
- (68) Lee, J.; et al. CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. *J. Chem. Theory Comput.* **2016**, *12*, 405–413.
- (69) Shaw, D. E., et al. Anton 2: Raising the Bar for Performance and Programmability in a Special-Purpose Molecular Dynamics Supercomputer. *SC'14: proceedings Of The International Conference For High Performance Computing, Networking, Storage And Analysis*. IEEE: New Orleans, LA, USA, 2014, 41–53.
- (70) Tuckerman, M.; Berne, B. J.; Martyna, G. J. Reversible multiple time scale molecular dynamics. *J. Chem. Phys.* **1992**, *97*, 1990–2001.
- (71) Bowers, K. J.; Chow, E.; Xu, H.; Dror, R. O.; Eastwood, M. P.; Gregersen, B. A.; Klepeis, J. L.; Kolossváry, I.; Moraes, M. A.; Sacerdoti, F. D., et al. Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters. *ACM/IEEE SC 2006 Conference (SC'06)*. Association for Computing Machinery, 2006, 43–43..
- (72) Lippert, R. A.; Predescu, C.; Ierardi, D. J.; Mackenzie, K. M.; Eastwood, M. P.; Dror, R. O.; Shaw, D. E. Accurate and efficient integration for molecular dynamics simulations at constant temperature and pressure. *J. Chem. Phys.* **2013**, *139* (16), 164106.
- (73) Nosé, S. A unified formulation of the constant temperature molecular dynamics methods. *J. Chem. Phys.* **1984**, *81*, 511–519.
- (74) Hoover, W. G. Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A* **1985**, *31*, 1695–1697.
- (75) Jung, J.; Kobayashi, C.; Sugita, Y. Optimal Temperature Evaluation in Molecular Dynamics Simulations with a Large Time Step. *J. Chem. Theory Comput.* **2019**, *15*, 84–94.
- (76) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **1981**, *52*, 7182–7190.
- (77) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An Nlog(N) method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089–10092.
- (78) Wassenaar, T. A.; Ingólfsson, H. I.; Böckmann, R. A.; Tieleman, D. P.; Marrink, S. J. Computational Lipidomics with insane: A Versatile Tool for Generating Custom Membranes for Molecular Simulations. *J. Chem. Theory Comput.* **2015**, *11*, 2144–2155.
- (79) Melo, M.; Ingólfsson, H. I.; Marrink, S. J. Parameters for Martini sterols and hopanoids based on a virtual-site description. *J. Chem. Phys.* **2015**, *143* (24), 243152.
- (80) Souza, P. C.; Alessandri, R.; Barnoud, J.; Thallmair, S.; Faustino, I.; Grünwald, F.; Patmanidis, I.; Abdizadeh, H.; Bruininks, B. M.; Wassenaar, T. A.; et al. Martini 3: a general purpose force field for coarse-grained molecular dynamics. *Nat. Methods* **2021**, *18*, 382–388.
- (81) Borges-Araújo, L.; Borges-Araújo, A. C.; Ozturk, T. N.; Ramirez-Echemendia, D. P.; Fábán, B.; Carpenter, T. S.; Thallmair, S.; Barnoud, J.; Ingólfsson, H. I.; Hummer, G.; Tieleman, D. P.; Marrink, S. J.; Souza, P. C. T.; Melo, M. N. Martini 3 Coarse-Grained Force Field for Cholesterol. *J. Chem. Theory Comput.* **2023**, *19*, 7387–7404.
- (82) Pedersen, K. B. et al. The Martini 3 Lipidome: Expanded and Refined Parameters Improve Lipid Phase Behavior. *ChemRxiv*, **2024**.
- (83) Pantelopulos, G. A.; Liberatore, A. MolPainter: A Tool for Painting and Solvating Layered Molecular Systems. *J. Open Source Softw* **2023**, *8*, 5128.
- (84) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1–2*, 19–25.
- (85) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **2007**, *126* (1), 014101.
- (86) Domański, J.; Sansom, M. S. P.; Stansfeld, P. J.; Best, R. B. Balancing Force Field Protein–Lipid Interactions To Capture Transmembrane Helix–Helix Association. *J. Chem. Theory Comput.* **2018**, *14*, 1706–1715.
- (87) Hazra, M. K.; Sarkar, S.; Bagchi, B. Three-stage phase separation kinetics in a model liquid binary mixture: A computational study. *J. Chem. Phys.* **2019**, *150* (14), 144501.
- (88) Sarkar, S.; Bagchi, B. Inhomogeneous phase separation kinetics in liquid binary mixtures: Sensitivity to initial local composition †. *J. Indian Chem. Soc.* **2022**, *99*, 100346.
- (89) Stanich, C.; Honerkamp-Smith, A.; Putzel, G.; Warth, C.; Lamprecht, A.; Mandal, P.; Mann, E.; Hua, T.-A.; Keller, S. Coarsening Dynamics of Domains in Lipid Membranes. *Biophys. J* **2013**, *105*, 444–454.
- (90) Laradji, M.; Sunil Kumar, P. Domain growth, budding, and fission in phase-separating self-assembled fluid bilayers. *J. Chem. Phys.* **2005**, *123* (22), 224902.
- (91) Dominguez, L.; Foster, L.; Straub, J. E.; Thirumalai, D. Impact of membrane lipid composition on the structure and stability of the transmembrane domain of amyloid precursor protein. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (36), No. E5281–E5287.
- (92) Pantelopulos, G. A.; Matsuoka, D.; Hutchison, J. M.; Sanders, C. R.; Sugita, Y.; Straub, J. E.; Thirumalai, D. Formation of extramembrane β -strands controls dimerization of transmembrane helices in amyloid precursor protein C99. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (52), No. e2212207119.
- (93) Majumder, A.; Straub, J. E. Addressing the Excessive Aggregation of Membrane Proteins in the MARTINI Model. *J. Chem. Theory Comput.* **2021**, *17*, 2513–2521.

- (94) Siu, S. W. I.; Vácha, R.; Jungwirth, P.; Böckmann, R. A. Biomolecular simulations of membranes: Physical properties from different force fields. *J. Chem. Phys.* **2008**, *128*, 125103.
- (95) Sandoval-Perez, A.; Pluhackova, K.; Böckmann, R. A. Critical Comparison of Biomembrane Force Fields: Protein-Lipid Interactions at the Membrane Interface. *J. Chem. Theory Comput.* **2017**, *13*, 2310–2321.
- (96) Borges-Araújo, L.; Pereira, G. P.; Valério, M.; Souza, P. C. T. Assessing the Martini 3 protein model: A review of its path and potential. *Biochim. Biophys. Acta, Proteins Proteomics* **2024**, 1872, 141014.
- (97) Soni, J.; Gupta, S.; Mandal, T. Recalibration of MARTINI-3 Parameters for Improved Interactions between Peripheral Proteins and Lipid Bilayers. *J. Chem. Theory Comput.* **2024**, *20*, 9673–9686.
- (98) Kwon, S.; Pantelopulos, G. A.; Straub, J. E. Efficient calculation of the free energy for protein partitioning using restraining potentials. *Biophys. J.* **2023**, *122*, 1914–1925.
- (99) Wei, J.; Meisl, G.; Dear, A. J.; Michaels, T. C.; Knowles, T. P. Kinetics of Amyloid Oligomer Formation. *Annu. Rev. Biophys.*, **2025**, 54. .
- (100) Babinchak, W. M.; Surewicz, W. K. Liquid-Liquid Phase Separation and Its Mechanistic Role in Pathological Protein Aggregation. *J. Mol. Biol.* **2020**, *432*, 1910–1925.
- (101) Lee, D. S. W.; Choi, C.-H.; Sanders, D. W.; Beckers, L.; Riback, J. A.; Brangwynne, C. P.; Wingreen, N. S. Size distributions of intracellular condensates reflect competition between coalescence and nucleation. *Nat. Phys.* **2023**, *19*, 586–596.
- (102) Da Vela, S.; Braun, M. K.; Dörr, A.; Greco, A.; Möller, J.; Fu, Z.; Zhang, F.; Schreiber, F. Kinetics of liquid-liquid phase separation in protein solutions exhibiting LCST phase behavior studied by time-resolved USAXS and VSANS. *Soft Matter* **2016**, *12*, 9334–9341.
- (103) Van Lindt, J.; Bratek-Sklicki, A.; Nguyen, P. N.; Pakravan, D.; Durán-Armenta, L. F.; Tantos, A.; Pancsa, R.; Van Den Bosch, L.; Maes, D.; Tompa, P. A generic approach to study the kinetics of liquid–liquid phase separation under near-native conditions. *Commun. Biol.* **2021**, *4*, 77.
- (104) Carpenter, T. S.; López, C. A.; Neale, C.; Montour, C.; Ingólfsson, H. I.; Di Natale, F.; Lightstone, F. C.; Gnanakaran, S. Capturing Phase Behavior of Ternary Lipid Mixtures with a Refined Martini Coarse-Grained Force Field. *J. Chem. Theory Comput.* **2018**, *14*, 6050–6062.
- (105) Jarin, Z.; Newhouse, J.; Voth, G. A. Coarse-Grained Force Fields from the Perspective of Statistical Mechanics: Better Understanding of the Origins of a MARTINI Hangover. *J. Chem. Theory Comput.* **2021**, *17*, 1170–1180.
- (106) Alessandri, R.; Souza, P. C. T.; Thallmair, S.; Melo, M. N.; de Vries, A. H.; Marrink, S. J. Pitfalls of the Martini Model. *J. Chem. Theory Comput.* **2019**, *15*, 5448–5460.
- (107) Sharma, P.; Desikan, R.; Ayappa, K. G. Evaluating Coarse-Grained MARTINI Force-Fields for Capturing the Ripple Phase of Lipid Membranes. *J. Phys. Chem. B* **2021**, *125*, 6587–6599.
- (108) Ollila, O. S.; Pabst, G. Atomistic resolution structure and dynamics of lipid bilayers in simulations and experiments. *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858*, 2512–2528.
- (109) Ferreira, T. M.; Coreta-Gomes, F.; Ollila, O. H. S.; Moreno, M. J.; Vaz, W. L. C.; Topgaard, D. Cholesterol and POPC segmental order parameters in lipid membranes: solid state 1H - 13C NMR and MD simulation studies. *Phys. Chem. Chem. Phys.* **2013**, *15*, 1976–1989.
- (110) Varma, M.; Khuri-Makdisi, F.; Deserno, M. Raft-like lipid mixtures in the highly coarse-grained Cooke membrane model. *J. Chem. Phys.* **2024**, *161*, 114103.