

Lipid Bilayer Phase Diagrams and Phase Diagram Evaluation Methods

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I'd like to primarily credit the entirety of Rowlinson and Swinton's *Liquids and Liquid Mixtures*[1] text for informing me about the thermodynamics and statistical mechanics of fluid mixtures and chapters 7 and 8 of Frenkel and Smit's *Understanding Molecular Simulation*[2] for an introduction to basic methods for sampling phase coexistence. The notation attempts to be as faithful as possible to that used by Rowlinson and Swinton, and that used by Benjamin Widom.

1 Background and motivation

Certain compositions of lipid bilayers can spontaneously experience a liquid-liquid phase coexistence often referred to as lipid phase separation lipid, lipid domain formation, or lipid raft formation. Lipid bilayers can be composed of literally hundreds of different lipids and protein, and so very simplified mixtures that exhibit lipid phase separation have been used to understand these phase separations. Phase diagrams of ternary lipid bilayer mixtures have been estimated via fluorescence,[3, 4, 5, 6, 7, 8] and x-ray scattering [9, 10, 11] measurements.

The most commonly considered lipid phase separation involve the formation of a liquid ordered (L_o) phase which coexists with a liquid disordered (L_d) phase, which differ in chemical composition. L_o domains consist of saturated lipids, L_d domains consist of unsaturated lipids, and L_o domains contain more cholesterol (CHOL) than L_d domains. These past works have identified that lipid mixtures phase separate in a concave region of the composition space, defined by mole fractions of each molecular species in the membrane, resulting in a general concept of bilayer phase diagrams at physiological temperature which visually presented in Fig. 1.

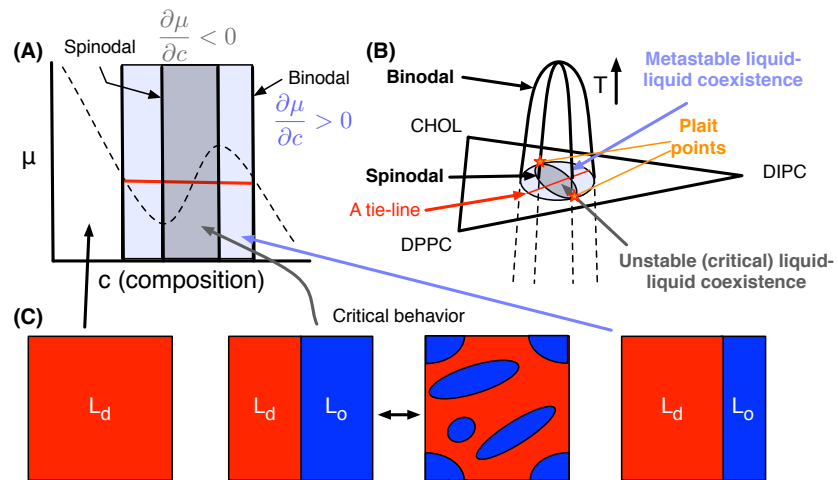


Figure 1: (A) Derivative of chemical potential in system composition, showing a liquid-liquid phase transition. The red line corresponds to a tie-line, for which the difference in chemical potentials of L_o and L_d phases are equal. (B) Hypothetical phase diagram of a phase-separating ternary mixture, demonstrating the shape change of the binodal and spinodal as dependent on temperature and composition, as well as hypothetical tie lines and plait points (where tie lines just become a point). (C) Illustrations of lack of phase coexistence, unstable phase coexistence, and metastable phase coexistence.

The aforementioned work with fluorescence techniques all use fluorescence probes which can only label one chemical species in the membrane, not the L_o state itself. Additionally, these fluorescence methods have a limited resolution of about ~ 25 nm. Experiments employing x-ray scattering experiments have experienced substantial difficulty in obtaining spectra in which amounts of each phase are easily separated, and have sometimes been interpreted using previous, fluorescence experiments to attempt to inform their analysis.

Aside from somewhat arbitrary definitions of phase transition temperatures, the liquid phase separation phase transition in lipid membranes has only directly been quantified via fluorescence experiments at compositions near an apparent “plait” point (Fig 1.B).[12] In this work, the authors attempt to characterize the phase transition by interpreting their measurements as if the system were a 2D Ising model, and along the way make two major assumptions: (1) That the Onsager’s direct relation between correlation length (ξ) and the line tension (λ) applies here as if this were a 2D Ising model and (2) that the γ critical exponent (describing $-(\partial p/\partial V)_T$ above T_c) is the same as in the Ising model ($\gamma = 7/4$). They show that a measurement interpreted to be similar to the 2D Ising model spin pair correlation function appears to conveniently collapse to $\gamma = 7/4$, however, mixture liquid-liquid phase separations quantified directly using thermodynamic variables have not generally been reported to behave the same as the 2D Ising model, for example, the γ exponent being 1, $5/4$, or $6/4$. [1]

These authors later inferred the dynamical critical exponents not by direct determination of exponents from measuring thermodynamic variables, but by trying to convert fluorescence signals which give information about the state of mixing of one molecular species to 2D conserved spin models following Kawasaki dynamics.[13]

However, just looking at the lateral composition of one molecular species in the membrane is far from sufficient for understanding phase coexistence in lipid membranes. Very recently, in mixtures which do not exhibit this kind of spatially-separated liquid-liquid phase coexistence, Javanainen et. al.[14] and Katira et. al.[15] have demonstrated via atomistic and coarse-grained simulations that different liquid phases can coexist in pure lipid membranes at physiological temperature. In addition to this, we recently have shown that in systems which are too small to experience spatial phase separation on the ~ 25 nm scale still have some amount of L_o phase coexisting with L_d phases,[16] and we have observed that a newly observed liquid “cholesteric” phase can form on nanometer scale (manuscript in preparation). These very recent works have shown that experimentally-obtained concepts of lipid phases can break down on the nanome-

ter scale, where phase coexistence can still very much influence the environment in which membrane proteins exist.

Because the time scale and system sizes necessary for observing the formation of some lipid phase separations are so large, we currently can not use conventional MD to get near equilibrium for these mixtures without prior information. Methods that enhance lipid diffusion have made some strides forward, but fail to achieve a large enough enhancement to reach equilibrium.[17, 18, 19] Additionally, a semi-grand ensemble method developed in the group of James Kindt around 2006[20, 21, 22] cannot truly determine phase co-existence of ternary mixtures, because it only swapped the identities of *lipids* (not cholesterol) and not *phases*. As we will discuss later, proper semigrand ensemble simulations are extremely expensive to perform.

2 Phase coexistence

Phase coexistence is the case where two or more phases of a system (not necessarily separated by a easily-defined interface) all have the same chemical potential (μ), temperature (T), and pressure (p) (e.g. $(\mu, T, p)^I = (\mu, T, p)^{II} = (\mu, T, p)^{III}$). Typically, when we consider an experiment in the lab or on the computer, T and p are equal throughout the system, and so differences in μ are the chief way by which phases are defined. μ_i is defined as change in energy of a phase in response to the change in the number of components in the phase by

$$\mu_i = \left(\frac{\partial U}{\partial N_i} \right)_{S, V, N_{j \neq i}} = \left(\frac{\partial G}{\partial N_i} \right)_{T, P, N_{j \neq i}} = \left(\frac{\partial H}{\partial N_i} \right)_{S, P, N_{j \neq i}} = \left(\frac{\partial A}{\partial N_i} \right)_{T, V, N_{j \neq i}}, \quad (1)$$

where the i phase is changing in number of components. This means that, as a system equilibrates, μ can be thought of as the *gradient* which molecular components follow to change phases, resulting in a $\Delta\mu = 0$ between all phases once equilibrium has been achieved, assuming there are any coexisting phases left by the end of this process.

Chemical potentials for real molecules are considered in terms of the excess beyond that of ideal molecules by

$$\mu_i(p, T, X_i) = \mu_i(p, T)^o + RT \ln(a_i), \quad (2)$$

where a_i is the thermodynamic activity of phase i and $\mu_i(p, T)^o$ is the chemical potential of a phase of ideal molecules. $a_i = X_i\gamma_i$, where X_i is the fraction of the system in phase i and γ_i is the activity coefficient of phase i .

Following the change of potential with change in molar fraction (molecular composition) or phase fraction, is the obvious, and in context of thermodynamic theory, most correct way to evaluate the coexistence of phases and draw phase diagrams.

To express the fraction of phases present in a two-phase coexistence on phase diagrams, tie lines are used as a visual representation. Tie lines define the amount of each of the two phases present within a system via the “lever rule”, visually demonstrated in Fig 2. Tie lines can represent a curved, rather than simply flat surface as they orthogonally intersect both sides of the spinodal when connecting two compositions on the binodal.

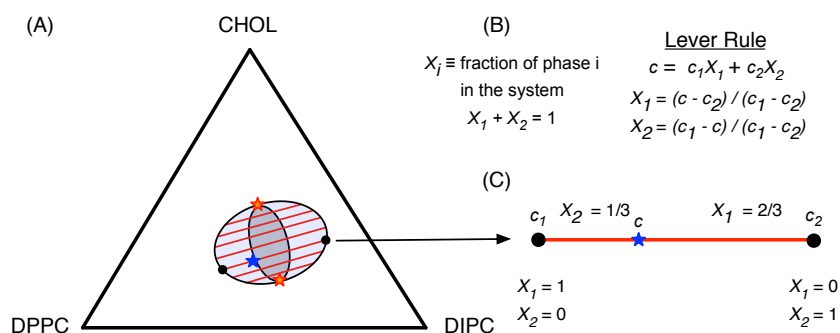


Figure 2: (A) “Flat” view of the ternary phase diagram imagined in Fig 1.B, showing several example tie lines, and a blue star for the example composition in (C). (B) The lever rule. (C) Example of the lever rule for a tie line connecting two compositions where c is one-third the distance from c_1 to c_2 , corresponding to the blue star in (A).

3 Obtaining the chemical potential via Statistical Mechanics

As stated earlier, phase coexistence is most correctly characterized by measuring the μ of each phase present in the system, that μ is defined by changes in the energy with changes in the number of molecules, and that we measure μ as an

“excess” of chemical potential above what is expected of ideal molecules (Eq. 2). Benjamin Widom derived how this excess μ can be obtained in statistical mechanics in 1963,[23] developing what he called “Potential Distribution Theory,” for which he provided an updated discussion in 1982.[24] Slightly modifying his equations to make our later discussion clearer, the canonical partition function of N particles, Q_N , is written as

$$Q_N = \frac{1}{N!} \int_V \dots \int_V e^{-U_N \beta} d\tau_1 \dots d\tau_N \quad (3)$$

where $\beta = \frac{1}{k_B T}$, the thermodynamic beta, U_N is the potential energy of the system, and $d\tau$ are infinitesimal elements of volume of each particle. To a system, a $N+1^{\text{th}}$ “test” particle can be inserted, described by the Q_{N+1} partition function

$$Q_{N+1} = \frac{1}{(N+1)!} \int_V \dots \int_V e^{-(U_N + \Psi) \beta} d\tau_1 \dots d\tau_{N+1} \quad (4)$$

where Ψ is the interaction energy of the inserted particle with the rest of the system. Eq. 4 can be redefined

$$Q_{N+1} = \frac{1}{(N+1)!} V(N)! Q_N \langle e^{-\Psi \beta} \rangle \quad (5)$$

where $\langle \dots \rangle$ is the ergodically sampled average of insertions to the ensemble of configurations of the system at equilibrium, independent of the influence the $N+1^{\text{th}}$ particle would have if it were truly part of the system. It should be obvious that the chemical potential can then be written as $\mu = -k_B T (Q_{N+1}/Q_N)$, given its definition in Eq. 1. We can separate the ideal and excess parts of μ by taking a few steps, starting with

$$Q_{N+1}/Q_N = \frac{V}{N+1} \langle e^{-\Psi \beta} \rangle = \rho_{N+1} \langle e^{-\Psi \beta} \rangle, \quad (6)$$

where ρ_{N+1} is the density of the system after insertion of the test particle. We can take the of log this, and then multiply by $-k_B T$ obtaining

$$\mu = -k_B T \ln(Q_{N+1}/Q_N) = -k_B T \ln(\rho_{N+1}) - k_B T \ln \langle e^{-\Psi \beta} \rangle, \quad (7)$$

where $-k_B T \ln(\rho_{N+1})$ is chemical potential of an ideal mixture (μ^{id}), which can be determined analytically. This means that $\mu^{ex} = -k_B T \ln \langle e^{-\Psi\beta} \rangle$, the excess chemical potential.

Widom also adapted this treatment to spatially inhomogeneous systems, such that the chemical potential is written as dependent on position, \vec{r} . [24] All of the computational methods devised for determining phase coexistence are based in these concepts developed by Widom and his predecessors, such as John G. Kirkwood who very nearly almost exactly presented Widom's theorem in 1935. [25]

4 Classic simulation methods for efficient determination of phase diagrams

Because of experimental short-comings in directly evaluating phase coexistence, a vast number of theoretical studies and models have been developed to try to understand the phase coexistence and character of phase transitions mixtures of two- three- and more numbers of components. In the case of lipid membranes, we are considering coexistence between at least two liquid phases in a system composed of at least three components... however phase diagrams are much easier to determine when there is a single component in the system, and coexistence is only considered between two phases, like between a gas and liquid phase, and so the vast majority of work has been done on this simpler topic.

I will very briefly review three "basic" methods for computationally efficient determination of phase equilibria and measurements of chemical potential which address the problem in significantly different ways. Other, ensemble-specific or problem-specific versions have been built on top of these methods many times.

4.1 Widom Insertion

The Widom insertion method essentially involves performing MC or MD simulations and introduction of a test particle/molecule to different regions of the simulated system at many different frames of simulation sampled at equilibrium, determining μ^{ex} directly by evaluating $-k_B T \ln \langle e^{-\Psi\beta} \rangle$. In principle, this is the best way to determine μ^{ex} . μ^{ex} can be determined in terms of changes in number of different chemical species in the system too, say, finding μ_α^{ex} for species α at some system composition, and also provides for the determination of other partial molar quantities, like partial molar enthalpy (h_α).

However, Widom Insertion is of extremely limited practicality. For a dense system, like a fluid or a solid, it takes an incredibly long time to find points in the fluid where particle insertions have favorable interaction energies, and yet these regions contribute the overwhelming majority of energy to the system, so they must be found to sample $\langle e^{-\Psi\beta} \rangle$. Many people have tried to get around this using crafty sampling methods, but it would be preferable to avoid using particle insertions when determining phase coexistence if possible. We do not need to obtain *absolute* values of partial molar thermodynamic variables for individual chemical species to determine phase diagrams and characterize phase transitions.

For a phase separating binary mixture, it would be possible to use *swaps* of chemical species (say, *A* and *B*) in the system to quickly determine the chemical potential difference between species, $\Delta\mu^{ex}$, at a composition by evaluating

$$\Delta\mu^{ex} = -k_B T \ln \left\langle \frac{N_B}{N_{A+1}} e^{-\beta\Delta U^{A-B}} \right\rangle, \quad (8)$$

resulting from sufficiently sampling the energy difference of many swaps of a particle *B* in to a particle *A* at equilibrium (see Ref. [26] for derivation). This method can be used to determine phase diagrams for binary mixtures, where two phases are pure, but not for cases where phases are composed of many different chemical species, like in lipid raft formation.

4.2 Semigrand ensemble

The Semigrand ensemble method, pioneered by David A. Kofke,[27] involves (1) permitting the *chemical identity* of molecules in the system to interchange and (2) considering the chemical potential of a number of species of interest (*q*) in the system as relative to the chemical potential of one reference species in the system, here conveniently referred to as μ_1 .

Semigrand ensemble is written using fugacities (*f*) rather than chemical potentials, related by

$$\ln f_\alpha = \beta\mu_\alpha - \beta\mu_\alpha^o(T) \quad (9)$$

where $\beta\mu_\alpha^o(T)$ is the reference state ($P = 1$ atm) ideal gas chemical potential. Semigrand ensemble also uses fugacity fractions

$$\xi_\alpha = \frac{f_\alpha}{\sum_\alpha^q f_\alpha}, \quad (10)$$

making differences in fugacities bounded from 0 to 1. The canonical semigrand ensemble is written as

$$d(\beta Y) = U_\mu d\beta - \beta P dV + \beta \mu_1 dN - \sum_{\alpha=2}^q \left[\frac{N_\alpha}{\xi_\alpha} + \frac{N - N_\alpha}{\xi_\alpha} \right] d\xi_\alpha \quad (11)$$

where U_μ contains the temperature dependence of reference-state chemical potentials for chemical species other than $\alpha = 1$,

$$U_\mu = U + \sum_{\alpha=2}^q N_\alpha \frac{d}{d\beta} [\beta(\mu_\alpha^o - \mu_1^o)]. \quad (12)$$

The partition function of the canonical semigrand ensemble can be written as

$$Y = \sum_{\alpha=1}^q \frac{z_\alpha^N}{N!} \prod_{\alpha=1}^z \left(\frac{z_\alpha}{z_1} \right)^{N_\alpha} \exp[\beta(\mu_\alpha - \mu_1) N_\alpha] Q_N N! \quad (13)$$

where $Q_N N!$ is the un-normalized configurational partition function (see Eq. 3) and z_α describes just the kinetic contribution to the molecular partition function of species α . You can see that the phase space is truly of a “grand” size... there’s nothing “semi-” about how large it is.

In semigrand ensemble simulations, movements in composition space are randomly proposed by evaluating a Metropolis exchange criterion

$$\min\left(1, \frac{f_i}{f_j} \exp(-\beta[U_i - U_j])\right), \quad (14)$$

preserving detailed balance.

This all sounds nice, having an ensemble in which relative fugacities of all species are used to equilibrate in composition space... there is still a momentous hindrance to the use of semigrand ensemble, however. For semigrand ensemble

simulations to work, $f_1(f_2\dots f_q)$ must somehow be known. $f_1(f_2\dots f_q)$ must be found using Widom insertions some way or another. Kofke, realising how expensive this is, proposed using Widom insertions along *pathways* of interest in composition space to parameterize semigrand ensemble simulations to search for phase boundaries.

Using pathways for semigrand ensemble is interesting, and the Kindt group did something like this between 2006 and 2011 which maintained the chemical potential difference of two kinds of lipids at some unknown constant.[20, 21, 22]

4.3 Gibbs ensemble

The aforementioned methods consider the chemical potential of individual chemical species (μ_α), however, to evaluate phase coexistence at equilibrium, we just need to know the relative difference of the chemical potentials of *phases* in the system. Phases could have many different molecular compositions, as is the case with L_d and L_o phases in lipid membranes, and we certainly would like to avoid determining μ_α at every single point in the phase diagram, and even precise determination of μ_1 at every point on the phase diagram to perform semigrand ensemble simulation seems frighteningly expensive to obtain.

The Gibbs ensemble method, proposed by Athanassios Z. Panagiotopoulos in 1987,[28] accomplishes the evaluation of the $\Delta\mu$ between two phases in an almost embarrassingly simplistic way. In the canonical Gibbs ensemble, the system is split into two separate boxes ($i=1$ and $i=2$), composed of $N = N_1 + N_2$ molecules and $V = V_1 + V_2$. Particles and an amount of volume are proposed to be exchanged between these two boxes, described by the partition function

$$Q_G(N, V, T) = \frac{1}{V} \sum_{N_1=0}^N \int dV_1 Q(N_1, V_1, T) Q(N - N_1, V - V_1, T). \quad (15)$$

As can be seen in the partition function, the Gibbs ensemble allows for one box to become completely emptied of molecules while the other is completely filled i.e. the case where there is truly no phase coexistence. In the original Gibbs ensemble presentation, considering a single molecular species, the simulation randomly attempts to (1) make a particle displacement MC move of a random particle in a box, (2) make a volume exchange move between boxes, and (3) make a particle exchange move between boxes, all three of these moves preserving detailed balance.

MC particle displacement of a random particle within a randomly chosen box is accepted by

$$\min(1, \exp(-\beta [U_{N_1}^{new} - U_{N_1}^{old}])) \quad (16)$$

identical to that of MC displacements in conventional canonical ensemble simulations. The volume exchange of a discrete volume ΔV , proposing $V_1^{new} = V_1^{old} + \Delta V$ is accepted by

$$\min\left(1, \frac{(V_1^{new})^{N_1} (V - V_1^{new})^{N-N_1}}{(V_1^{old})^{N_1} (V - V_1^{old})^{N-N_1}} \exp(-\beta [U_N^{new} - U_N^{old}])\right). \quad (17)$$

The particle exchange from box 1 to box 2, placing the exchange particle to a randomly chosen new location in the target box, is accepted by

$$\min\left(1, \frac{N_1(V - V_1)}{(N - N_1 + 1)V_1} \exp(-\beta (U_N^{new} - U_N^{old}))\right), \quad (18)$$

and labels “1” and “2” on this equation would be switched for the reverse exchange attempt. As recent as October of 2017, work has been done using MC Gibbs ensemble to examine liquid-vapour phase equilibria in a six-component mixture.[29]

Works following Panagiotopoulos’s original paper with MC Gibbs ensemble have tried to make improvements, but I will not discuss those. In the next section I will discuss a MD Gibbs ensemble method which appears to be suited to determining liquid-liquid phase coexistence in a dense liquid.

5 Gibbs Ensemble Molecular Dynamics (GEMD)

A MD version of the Gibbs ensemble method was proposed in 1994 by Palmer and Lo in which molecules in a system are coupled to a continuous parameter that scales intermolecular interactions such that, within a single simulation box, the system is effectively allowed to separate into two separate phases.[30] This approach has most recently been “updated” (to my knowledge) in 2016 by Gartner et al.,[31] and GEMD will be discussed using their notation.

In GEMD, a fourth degree of freedom, ξ , is introduced, bounded between 0 and 1, which governs whether a particle exists in one of two phases ($\xi=0$ or $\xi=1$) (Fig. 3). This pairwise potential energy is written as

$$U_{ij} = U_{ij}(r_{ij,1})\xi_i\xi_j + U_{ij}(r_{ij,2})(1 - \xi_i)(1 - \xi_j) \quad (19)$$

where $r_{ij,1}$ are the distances between particles i and j in phase 1, and $r_{ij,2}$ are the distances between the same particles i and j in phase 2.

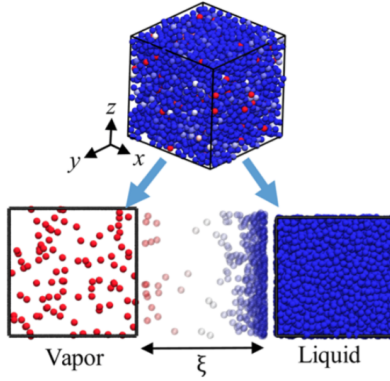


Figure 3: Visual demonstration of GEMD for LJ particles as presented in Gartner e. al. 2016.[31]

The total potential energy of the system is written as

$$U = \sum_{i<j} U_{ij}(r_{ij,1})\xi_i\xi_j + \sum_{i<j} U_{ij}(r_{ij,2})(1 - \xi_i)(1 - \xi_j) + \sum_i g(\xi_i) \quad (20)$$

where $g(\xi)$ is a potential added to the energy landscape to make values of ξ far from 0 or 1 unfavourable, is written as

$$w[\tanh(u\xi_i) + \tanh(u(1 - \xi_i)) - 1] \quad (21)$$

where “steepness” (u) and “height” (w) are constants chosen for the simulation.

The force for ξ on particle i , ξ_i , is

$$\dot{p}_{\xi_i} = m_{\xi}\ddot{\xi}_i = -\frac{dU}{d\xi_i} = -\left[\sum_{j\neq i} U_{ij}(r_{ij,1})\xi_j - \sum_{j\neq i} U_{ij}(r_{ij,2})(1 - \xi_j) + \frac{dg(\xi_i)}{d\xi_i} \right] \quad (22)$$

where $m_{\xi,i}$ is the non-physical mass ξ_i . The bound on ξ values is enforced by making reflective walls at $\xi = 0$ and $\xi = 1$.

As particles join to phase 1 or phase 2, the equality of pressure between phases is maintained by running a barostat for phase 1, set to the instantaneous pressure of phase 2. Likewise, changes to the volume of phase 1 are mirrored in phase 2.

The pressure of each phase is computed by evaluating the virials for each phase as

$$P_1^* = \frac{\sum_i \xi_i k_B T}{V_1} + \frac{\sum_i \vec{r}_{i,1} \cdot \vec{f}_{i,1}}{3V_1} \quad (23)$$

$$P_2^* = \frac{\sum_i (1 - \xi_i) k_B T}{V_2} + \frac{\sum_i \vec{r}_{i,2} \cdot \vec{f}_{i,2}}{3V_2}. \quad (24)$$

where P_2^* is the reference pressure for the barostat acting on phase 1.

The phase volumes are used to scale interatomic distances by effectively changing the location of a particle in phase 1 and a particle in phase 2 by

$$\vec{r}_{i,2} = \left(\frac{V_2}{V_1} \right)^{1/3} \vec{r}_{i,1}. \quad (25)$$

Additionally, the velocities of particle in each phase are related by

$$\vec{v}_{i,2} = \left(\frac{V_2}{V_1} \right)^{1/3} \vec{v}_{i,1} \quad (26)$$

such that the temperature is given as

$$T^* = \frac{1}{3Nk_B} \sum_i \left[m_i v_{i,1}^2 \xi_i + m_i v_{i,2}^2 (1 - \xi_i) \right]. \quad (27)$$

Additionally, Gartner et. al. found it important to add a velocity-rescaling thermostat to the temperature experienced in ξ -space, analogous to real-space temperature,

$$T_\xi = \frac{1}{k_B N} \sum_i m_i \dot{\xi}_i^2, \quad (28)$$

which can be selected to influence the dynamic equilibrium between between the two phases.

T_ξ rescales $\dot{\xi}$ by

$$\dot{\xi}_{new} = \sqrt{\frac{T_\xi^{target}}{T_\xi}} \dot{\xi}. \quad (29)$$

This thermostat seems to make a significant impact on ξ , as evidenced in Fig. 4.

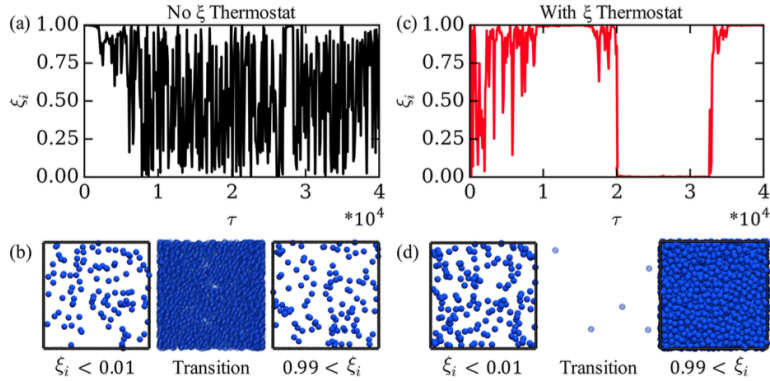


Figure 4: Evolution of a sample ξ_i (a) with and (c) without a ξ thermostat, with corresponding representative system configurations (b) and (d), as presented in Gartner et. al. 2016.[31]

It should be possible to check the chemical potential difference between the two phases by measuring the potential energy difference that happens when the ξ value of a particle is swapped, as in Eq. 8, though Gartner et. al. validated this method by doing Widom insertions, which is certainly tractable given the single-component LJ system used in their work.

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