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Phase stability condition and liquid-liquid phase separation under mesoscale confinement

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ABSTRACT

Here we establish the thermodynamic phase stability condition under mesoscale confinement, which is essential in elucidating how the confinement of solutions inside a droplet, cell or liposome may influence phase separation. To clarify how phase stability is affected by external conditions, a formal analogy between the partially open ensemble and mesoscopic system will be exploited, through which the nonnegligible role of the system boundary will be identified as the crucial difference from the macroscopic stability condition. The thermodynamic stability condition extended for mesoscale is shown to involve several different orders of magnitude that are all considered to be $O(1)$ at a macroscopic limit. Phase instability in mesoscale is shown to ensue when the difference between self-association (relative self-fluctuation of particle number) and mutual association (relative number correlation between different species) reaches the mesoscopic order of magnitude, in contrast to the divergence of particle number fluctuation

(namely, reaching a macroscopic order of magnitude) required in macroscale. Thus, confinement may enhance phase instability.

1. Introduction

Confinement within a small space affects not only biomolecular conformation and association [1–3] but also phase stability. Aqueous biomolecular solutions stable in macroscopic bulk, such as the ones containing DNA, PEG and NaCl [4] or DNA and alginate [5], undergo phase transition when confined in a droplet, and increasingly so as the droplet size decreases [4,5]. Simple solution mixtures [6], even ethanol-water[7–11] mixtures, go through phase separation under confinement. Thus, confinement may affect phase stability. Such an effect is expected to be at work also in droplet protein crystallization [12,13], confined polyelectrolyte solutions [14] and polymer blends [15], and compartmentalization in biological cells [16–18].

Such a universal importance of phase stability under confinement, encountered in diverse disciplines, necessitates the extension of thermodynamic phase stability, developed so far in macroscopic systems [19–23], to solution mixtures confined in small systems. How to deal with small (mesoscopic and nanoscale) systems, for which the effect of system boundary is not negligible, was pioneered by Hill [24–26], yet the formal nature of the theory has seen few applications over the decades [24–26], except, most notably, for the simulation of the Kirkwood-Buff integrals (KBIs) in finite systems [27–29]. The goal of this paper is twofold: (i) to establish the thermodynamic stability condition for a solution mixture under confinement and (ii) to clarify the difference between the stability conditions in the macroscopic and mesoscopic systems.

Clarifying the phase stability condition to mesoscale and nanoscale confinement is timely, considering not only the recent realization on the importance of liquid-liquid phase separation in biology [16–18] but also the recent progress that brought the investigation of biomolecular stability, aggregation, assembly, and binding in aqueous solution mixtures out of phenomenological models onto a statistical thermodynamic foundation [30–33]. It is now possible not only to quantify, from experiments, the competitive (preferential) solvation of water and cosolvents as the driving forces of biological processes [30–33] but also to elucidate the origin of mesoscale fluctuations in solution mixtures [22,23]. These achievements have been made for macroscopic bulk solutions yet can readily be generalized into mesoscale (and nanoscale) confinement to clarify and identify similarity and crucial differences between macroscopic and mesoscopic systems.

2. Mesoscale thermodynamics and local-bulk thermodynamics

Here we present an alternative derivation of Hill’s “nanothermodynamics” or the thermodynamics of small systems [24–26]. To this end, let us consider a binary mixture composed of two components ($i = 1$ and 2), as well as the component m , which constitutes a mesoscopic (or nanoscale) boundary object (such as a cellular membrane, micelle, or chaperone). We shall use “mesoscale” or “mesoscopic” throughout as the general terms for the small system. Following our previous papers [31,32], let us write down the Gibbs-Duhem equation for the mesoscopic subsystem, namely the system in the vicinity of component m :

$$\langle N_m \rangle d\mu_m = -SdT + VdP - \langle N_1 \rangle d\mu_1 - \langle N_2 \rangle d\mu_2 \quad (1)$$

Equating $\langle N_m \rangle d\mu_m$ as the change of thermodynamic function, $d\mathcal{E}$, we obtain

$$d\mathcal{E} = -SdT + VdP - \langle N_1 \rangle d\mu_1 - \langle N_2 \rangle d\mu_2 \quad (2)$$

Eq. (2) is formally equivalent to Hills' nanothermodynamics [24–26]. What is crucial here is that $\langle N_1 \rangle$ and $\langle N_2 \rangle$ are much larger in magnitude than $\langle N_m \rangle$, because of the mesoscopic scale of the boundary object m . Integrating Eq. (2) yields

$$\mathcal{E} = \langle N_m \rangle \mu_m = -TS + PV - \langle N_1 \rangle \mu_1 - \langle N_2 \rangle \mu_2 \quad (3)$$

Hill classified \mathcal{E} as the “intensive” thermodynamic quantity. This classification may be justified when considering that μ_m is an intensive quantity while $\langle N_m \rangle$, representing the number of mesoscopic boundary objects, is far smaller than the macroscopic order of magnitude. Strictly speaking, however, the order of magnitude of \mathcal{E} is either mesoscopic or microscopic, depending on the nature of the boundary object. If the boundary object is mesoscopic in scale, $\langle N_m \rangle$ is smaller than $\langle N_1 \rangle$ and $\langle N_2 \rangle$ by orders of magnitude and μ_m is of mesoscopic scale. A mesoscopic object is different from a molecular species in terms of the magnitudes of $\langle N_m \rangle$ and μ_m within the framework of Eq. (3).

Comparing Eq. (2) to the inhomogeneous solution-based derivation of the preferential solvation theory (i.e., based on a pair of the Gibbs-Duhem equations to link preferential solvation to the KBIs) [30,31,34] is insightful in elucidating the nature of the mesoscopic system. The pair of the Gibbs-Duhem equations consist of the one for the vicinity of a solute and the other for the bulk solution having the same volume as the vicinity [30,31,34]. Here, unlike the case of preferential solvation, the second Gibbs-Duhem equation for the bulk does not exist for the mesoscopic system. This is due to a fundamental difference between a single solute (fixed at the origin) and the mesoscopic boundary object m in terms of the degrees of freedom. When two solvent components

are involved in a single phase, with a solute molecule fixed at the origin as a source of an external field, the Gibbs phase rule shows that there are three degrees of freedom [31,35]. This means that μ_1 and μ_2 are linked via the Gibbs-Duhem equation for the bulk solution, and only one of them can serve as the independent parameter in addition to T and P . In contrast, μ_1 and μ_2 are both independent parameters. This shows that the mesoscopic system forms a separate phase from the reservoir with which species 1 and 2 are exchanged. An additional degree of freedom, specific to a mesoscopic system, has been introduced by the non-zero $\mathcal{E}(T, P, \mu_1, \mu_2; N_m)$, and disappears for a system without a boundary, as can be seen from Eq. (1); the more dilute the component m ($\langle N_m \rangle \rightarrow 0$) the more negligible the influence therefrom, and Eq. (1) reduces to the (macroscopic) Gibbs-Duhem equation for a two-component system. Hence, the effect of the boundary persists throughout a small system.

It is useful to interpret Eq. (2) further from a perspective of a solution confined by the boundary object (i.e., components 1 and 2), namely, as the breakdown of the Gibbs-Duhem equation, because of the presence of $d\mathcal{E}$, as advocated by Hill [26]. In a bulk system consisting only of components 1 and 2, the right-hand side of Eq. (4) is zero. In this sense, the apparent breakdown of the Gibbs-Duhem equation is equivalent to the existence of the following semi-grand ensemble, open to all the microscopic components (components 1 and 2) but not to the mesoscale components (denoted by m), defined as

$$\mathcal{E}(T, P, \mu_1, \mu_2; N_m) = G(T, P, N_1, N_2, N_m) - \sum_{i=1,2} N_i \mu_i \quad (4)$$

where G is the Gibbs free energy of the system comprising not only of components 1 and 2 but also of the boundary object (component m). A shorthand $N_i = \langle N_i \rangle$ may be used for simplicity from now onwards.

Another interpretation of Eq. (4) is possible by comparing with the case when the system does not have the component m and is macroscopic only with regards to components 1 and 2. In this case, the Gibbs free energy is simply $N_1\mu_1 + N_2\mu_2$, and \mathcal{E} is considered to signify the excess of the free energy due to the presence of a boundary. This is consistent with the recent insight that nanothermodynamics is equivalent also to the Gibbs adsorption isotherm [36]. Indeed, Eq. (1) can be shown to be formally equivalent to the Gibbs adsorption isotherm, by averaging out the individual molecular effect $\langle N_m \rangle d\mu_m$ in Eq. (1) to yield $Ad\gamma$ (where A is the surface area and γ is the surface free energy).

Thus, a comparison between a mesoscopic system and the preferential solvation theory has led to a practical insight necessary for constructing a statistical thermodynamic stability theory for mesoscale confinement; mesoscale confinement is formally equivalent to a semi-open ensemble under isobaric condition.

3. Thermodynamic stability condition in mesoscale

3.1. Thermodynamic stability condition for a bulk ternary solution under constant T and P

Before writing down the thermodynamic stability condition for a mesoscopic system, let us revisit the stability condition for a macroscopic system, in order to clarify the concepts and tools essential for derivation presented in Section 3.2 onwards. Consider a three-component solution mixture consisting of species 1, 2 and m under constant pressure P and temperature T . The stability of the

system is governed by the Gibbs free energy, $G(T, P, N_1, N_2, N_m)$. Here we postulate that the same thermodynamic stability condition should hold true for the entire system as well as for its macroscopic subsystems [37]. This means that the stability condition should be written down using intensive variables that represent changes in solution composition [37]. (Note that we are dealing with the case when the confined solution is in a single phase, for which we write down the stability condition. Hence, we are not considering the case when a strong interaction with, or adsorption onto, the boundary makes the confined solution already phase separated.)

When the mixture forms a single phase, the degrees of freedom, according to the Gibbs phase rule, is 4 [19]. This means there are 2 remaining degrees of freedom that governs the stability condition independent of the system size. Such intensive variables can be defined as $c_1 = N_1/N_m$ and $c_2 = N_2/N_m$. Consequently, the thermodynamic stability condition,

$$\delta G = \frac{1}{2} \sum_{i,j=1,2,m} \left(\frac{\partial^2 G}{\partial N_i \partial N_j} \right)_{T,P,N_k} (\delta N_i \delta N_j) > 0 \quad (5a)$$

where the order of magnitude of the terms in Eq. (5a), being a multiplication of $\left(\frac{\partial^2 G}{\partial N_i \partial N_j} \right)_{T,P,N_k} = O\left(\frac{1}{V}\right)$ and $\delta N_i \delta N_j = O(V)$, is indeed $O(1)$. (Throughout this paper, we employ the Landau symbol $O(x)$ for “the same order of magnitude as x ” and $o(x)$ for “infinitesimal in comparison to x ”.) Based on the above postulate, we express Eq. (5a) in a size-independent manner using c_i and $\tilde{G}(T, P, c_1, c_2) = G/N_m$, while keeping the left-hand side of Eq. (5a) the same, as

$$\delta G = \frac{1}{2} \sum_{i,j=1,2} \left(\frac{\partial^2 \tilde{G}}{\partial c_i \partial c_j} \right)_{T,P,c_k} (N_m \delta c_i \delta c_j) > 0 \quad (5b)$$

so that both the stability matrix $\left(\frac{\partial^2 \tilde{G}}{\partial c_i \partial c_j}\right)_{T,P,c_k}$ and fluctuation (i.e., covariance, or the ensemble average of $N_m \delta c_i \delta c_j$) are intensive ($O(1)$) physical quantities. It is convenient to rewrite Eq. (5b) in terms of the following quadratic form

$$\delta G = \frac{1}{2} \boldsymbol{\delta c}^T \mathbf{G} \boldsymbol{\delta c} \quad (6)$$

with

$$\boldsymbol{\delta c} = \sqrt{N_m} \begin{pmatrix} \delta c_1 \\ \delta c_2 \end{pmatrix} \quad (7)$$

$$\mathbf{G} = \left(\left(\frac{\partial^2 \tilde{G}}{\partial c_i \partial c_j} \right)_{T,P,c_k} \right) \quad (8)$$

and with the transposed vector, $\boldsymbol{\delta c}^T$. Note that \mathbf{G} is a Hessian matrix.

Our goal is to write down a relationship between the stability matrix \mathbf{G} and the fluctuation (covariance) matrix involving δc_i s. To do so, diagonalizing the quadratic form makes it easier [38]. Since \mathbf{G} is a symmetry matrix, i.e., $\mathbf{G}^T = \mathbf{G}$, it can be diagonalized by an orthogonal matrix, \mathbf{D} satisfying $\mathbf{D}^T = \mathbf{D}^{-1}$ [39], such that

$$\mathbf{D}^{-1} \mathbf{G} \mathbf{D} = \begin{pmatrix} \alpha_1 & 0 & 0 \\ \dots & \dots & \dots \\ 0 & 0 & \alpha_v \end{pmatrix} \quad (9)$$

Eq. (6) can be rewritten as

$$\delta G = \frac{1}{2} (\boldsymbol{\delta c}^T \mathbf{D}) (\mathbf{D}^{-1} \mathbf{G} \mathbf{D}) (\mathbf{D}^{-1} \boldsymbol{\delta c}) \quad (10)$$

When we define

$$\boldsymbol{\delta c}' = \mathbf{D}^{-1} \boldsymbol{\delta c} = \mathbf{D}^T \boldsymbol{\delta c} \quad (11)$$

due to the orthogonality of \mathbf{D}

$$\boldsymbol{\delta c}'^T = \boldsymbol{\delta c}^T (\mathbf{D}^{-1})^T = \boldsymbol{\delta c}^T \mathbf{D} \quad (12)$$

Eqs. (11) and (12), therefore, leads to the diagonalization of Eq. (10) as

$$\delta G = \frac{1}{2} \boldsymbol{\delta c}^T (\mathbf{D}^{-1} \mathbf{G} \mathbf{D}) \boldsymbol{\delta c}' = \frac{1}{2} \sum_i \alpha_i (\delta c'_i)^2 \quad (13)$$

The diagonalized stability condition, Eq. (13), in conjunction with the quasi-thermodynamic theory of fluctuation [38,40],

$$w = \exp(-\delta G) = \exp\left(-\frac{1}{2} \sum_i \alpha_i (\delta c'_i)^2\right) \quad (14)$$

yields the following relationship between the eigenvalues and fluctuation thanks to the Gaussian distribution

$$\langle \delta c'_i \delta c'_j \rangle = \alpha_i^{-1} \delta_{ij} \quad (15)$$

Noting α_i^{-1} is the diagonal element of $(\mathbf{D}^{-1} \mathbf{G} \mathbf{D})^{-1}$, Eq. (15) can be expressed in terms of the correlation matrix as

$$\langle \boldsymbol{\delta c}' \boldsymbol{\delta c}'^T \rangle = (\mathbf{D}^{-1} \mathbf{G} \mathbf{D})^{-1} = \mathbf{D}^T \mathbf{G}^{-1} \mathbf{D} \quad (16)$$

Converting $\boldsymbol{\delta c}'$ back to $\boldsymbol{\delta c}$ via Eqs. (11) and (12),

$$\langle \mathbf{D}^T \boldsymbol{\delta c} \boldsymbol{\delta c}^T \mathbf{D} \rangle = \mathbf{D}^T \mathbf{G}^{-1} \mathbf{D} \quad (17)$$

which yields

$$\langle \boldsymbol{\delta c} \boldsymbol{\delta c}^T \rangle = \mathbf{G}^{-1} \quad (18)$$

showing an inverse relationship between the correlation matrix and the stability matrix.

Thermodynamic stability condition under isobaric condition is particularly important for mesoscale for biological and soft matter systems. The choice of the isobaric condition is reasonable, considering that the isothermal compressibility of lipids forming lipid bilayer is comparable in magnitude to that of liquid water [41–43]. In addition, the presence of cholesterol does not affect the isothermal compressibility significantly [41–43].

3.2. Thermodynamic stability for a mesoscopic binary solution under constant T and P

In Section 2, the thermodynamic function $\mathcal{E}(T, P, \mu_1, \mu_2; N_m)$ for a mesoscopic two component solution was shown to be analogous formally to the thermodynamic function for a partially open ensemble (open to components 1 and 2 but is closed to m). Here we write down the thermodynamic stability condition for the mesoscopic binary solution under constant temperature and pressure. To do so, its formal analogy with the macroscopic ternary solution (Section 3.1) is helpful. Since the solution composition must change, we carry out the Legendre transform to introduce

$$\mathcal{G}(T, P, N_1, N_2; N_m) = \mathcal{E}(T, P, \mu_1, \mu_2; N_m) + N_1\mu_1 + N_2\mu_2 \quad (19)$$

which governs thermodynamic stability. The degree of freedom for this mesoscopic binary solution is 4, consistent with the corresponding macroscopic ternary solution. Since $\mathcal{G}(T, P, N_1, N_2; N_m)$ is convex with respect to N_1 and N_2 ,

$$\delta\mathcal{G} = \frac{1}{2} \sum_{i,j=1,2} \left(\frac{\partial^2 \mathcal{G}}{\partial N_i \partial N_j} \right)_{T,P,N_k} \delta N_i \delta N_j > 0 \quad (20)$$

is the stability condition for the mesoscale binary solution. See Appendix A for justification.

In the case of the macroscopic ternary solution, the stability condition, postulated to be independent of system size, had to be expressed in terms of the intensive variables, namely concentrations. Consequently, a macroscopic subsystem was introduced with a postulate that the same thermodynamic stability condition holds true regardless of its size [37]. However, a mesoscopic system is fundamentally different from a macroscopic system, because the confined

solution is under the influence of the boundary object, hence a subsystem closer to the boundary would be different from a subsystem away from the boundary.

However, an ensemble consisting of equivalent, non-interacting mesoscopic systems (which, as a whole, obeys the laws of macroscopic thermodynamics) has played a key role in constructing the thermodynamics of small systems [25]. In this context, we postulate that the size invariance of the stability condition refers to the ensemble of mesosystems as a whole, and the stability condition should be size independent in a way that the “subsystem” of the ensemble consists of more than one mesoscopic system(s). Since T and P are kept constant, the remaining number of intensive variables is 2, hence we look for two intensive variables as the descriptors for composition change.

For a macroscopic ensemble consisting of \mathcal{N} mesoscopic systems, the thermodynamic functions scale as

$$\mathcal{N}\mathcal{G}(T, P, N_1, N_2; N_m) = \mathcal{G}(T, P, \mathcal{N}N_1, \mathcal{N}N_2; \mathcal{N}N_m) \quad (21)$$

However, introducing $\tilde{\mathcal{G}}(T, P, c_1, c_2) = \frac{\mathcal{N}\mathcal{G}}{\mathcal{N}N_m} = \frac{\mathcal{G}}{N_m}$, in a manner analogous to our previous discussion for a macroscopic system, Eq. (20) can be rewritten in a form which is invariant under a scaling with respect to size, as

$$\delta\mathcal{G} = \frac{1}{2} \sum_{i,j=1,2} \left(\frac{\partial^2 \tilde{\mathcal{G}}}{\partial c_i \partial c_j} \right)_{T,P,c_k} (N_m \delta c_i \delta c_j) \quad (22)$$

Eq. (22) is formally analogous to Eq. (5b). However, the difference between the two equations is in the order of magnitude. Since, as we emphasised in Section 2, the mesoscopic quantities, N_1 , N_2 and V have a different order of magnitude from N_m , let us introduce the characteristic volume of the mesoscopic system, $v = \frac{V}{N_m}$. Consequently, $O(c_i) = O(v)$ and $O(\tilde{\mathcal{G}}) = O(v)$, which leads

to $O(N_m \delta c_i \delta c_j) = O(v)$ and $O\left(\left(\frac{\partial^2 \tilde{g}}{\partial c_i \partial c_j}\right)_{T,P,c_k}\right) = O\left(\frac{1}{v}\right)$. This order of magnitude analysis reveals a fundamental difference between a mesoscopic system and a macroscopic system. Note that for a macroscopic system, since N_m , N_i and V are all in the same order of magnitude, $O(v)$ reduces to $O(1)$, hence both $N_m \delta c_i \delta c_j$ and $\left(\frac{\partial^2 \tilde{g}}{\partial c_i \partial c_j}\right)_{T,P,c_k}$ are also $O(1)$. The appearance of $O(v)$ is a signature of the mesoscopic thermodynamic stability, which will be clarified further in Section 4.

Because of the formal analogy between Eq. (22) and Eq. (5b), both containing the two concentration variables, c_i and c_j , the discussion in Section 3.1 is applicable to the mesoscale binary solution, to relate the stability matrix

$$\tilde{\mathbf{g}} = \left(\left(\frac{\partial^2 \tilde{g}}{\partial c_i \partial c_j} \right)_{T,P,c_k} \right) \quad (23)$$

and the correlation matrix, $\langle \delta \mathbf{c} \delta \mathbf{c}^T \rangle$, as

$$\langle \delta \mathbf{c} \delta \mathbf{c}^T \rangle = \tilde{\mathbf{g}}^{-1} \quad (24)$$

By virtue of Eq. (24), the stability condition for the mesoscale binary solution,

$$|\tilde{\mathbf{g}}| > 0 \quad (25)$$

can be interpreted in terms of the fluctuation (covariance) matrix as

$$\frac{1}{|\langle \delta \mathbf{c} \delta \mathbf{c}^T \rangle|} > 0 \quad (26)$$

Note that the rank of the mesoscopic stability matrix (Eq. (23)) is two, which is higher than that of the macroscopic binary system by one. Such a difference in rank can be rationalized simply from the existence of the additional component for the mesoscopic system, which is the boundary

object 3 introduced in Section 2. A macroscopic system, on the other hand, is a system devoid of such a boundary object, or whose influence can be neglected. Thus, the fundamental difference between a mesoscopic and macroscopic system can be attributed to the existence of a boundary object as an additional component, and consequently to the number of components and therefore the rank of the stability matrix.

3.3. Thermodynamic stability for a mesoscopic binary solution under constant T , P and μ_1

Here we consider a mesoscale binary solution which is open to component 1 but is closed to component 2. This corresponds, for example, to an aqueous biopolymer solution in confinement where water is in equilibrium with the reservoir and can move in and out of the system. The thermodynamic function, therefore, can be obtained via Legendre transform as

$$\mathcal{J}(T, P, \mu_1, N_2; N_m) = \mathcal{E}(T, P, \mu_1, \mu_2; N_m) + N_2\mu_2 \quad (27)$$

The stability condition can be written as

$$\delta\mathcal{J} = \frac{1}{2} \frac{\partial^2 \mathcal{J}}{\partial N_2^2} (\delta N_2)^2 > 0 \quad (28)$$

See Appendix A for justification. Here we follow the same set of guiding principles as laid out in Section 3.3. Firstly, the thermodynamic stability condition must be independent of system size, which should be the same for the mesoscopic system itself and for the grand systems consisting of independent (uncorrelated) mesoscale systems. Secondly, the number of variables that fluctuate should be subject to the Gibbs phase rule. In this case, the degrees of freedom of a mesoscale binary solution is 4, and three intensive variables are already fixed. Hence, there is only one intensive variable that fluctuates. Therefore, the choice of the “intensive” variable $c_2 = N_2/N_m$

$$\delta\mathcal{J} = \frac{1}{2} \frac{\partial^2 \tilde{\mathcal{J}}}{\partial c_2^2} (N_m \delta c_2)^2 \quad (29)$$

satisfies the above two criteria. (Note that c_2 is an $O(v)$ quantity in mesoscale while being $O(1)$ in macroscale, as has been clarified in Section 3.2). There is no need to diagonalize Eq. (29). Application of the quasi-thermodynamic fluctuation theory yields

$$N_m \langle (\delta c_2)^2 \rangle = \frac{\langle (\delta N_2)^2 \rangle}{N_m} = \left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right)^{-1} \quad (30)$$

Therefore,

$$\frac{1}{\langle (\delta N_2)^2 \rangle} > 0 \quad (31)$$

is the stability condition.

3.4. Thermodynamic stability under constant T, P, μ_1 and μ_2

In this case, all four degrees of freedom for a binary mesoscopic system granted by the Gibbs phase rule are already fixed. This means there is no intensive degrees of freedom left for the system that can lead to the change of solvent composition. Nevertheless, let us write down a thermodynamic stability condition for $\mathcal{E}(T, P, \mu_1, \mu_2; N_m)$. The only “variable” related to composition is N_m , which is the number of molecules that constitute the boundary object. The thermodynamic function \mathcal{E} is a concave function with respect to N_m .

$$\delta \mathcal{E} = \frac{1}{2} \frac{\partial^2 \mathcal{E}}{\partial N_m^2} (\delta N_m)^2 > 0 \quad (32)$$

It is not straightforward to rewrite this stability condition in terms of the intensive, \mathcal{E}/N_m . The closest way that can be

$$\delta \mathcal{E} = \frac{1}{2} \left(N_m \frac{\partial^2 \mathcal{E}}{\partial N_m^2} \right) \frac{(\delta N_m)^2}{N_m} > 0 \quad (33)$$

It is easy to see that the stability condition is linked to $\langle (\delta N_m)^2 \rangle$. If the number of independent mesoscale systems is λ in the grand system, the requirement on the independence of mesosystems

lead to a linear increase of $\langle(\delta N_m)^2\rangle$ with respect to λ . However, this information is hardly useful for our purpose, because our interest here is chiefly on the behaviour of the confined solutions rather than the interactions between boundary objects that have been defined as non-interacting in the first place.

4. How system size affects phase separation

4.1. Linking isobaric fluctuation to isochoric fluctuation and the Kirkwood-Buff integrals

What is the effect of system size on phase separation? To answer this question from a perspective of the phase stability condition, let us start with the case of constant T, P , and μ_2 for its simplicity. The number fluctuation, $\langle(\delta N_2)^2\rangle_{\{T,P,\mu_2\}}$, when converted to grandcanonical ensemble, can be interpreted in terms of the Kirkwood-Buff integrals (KBIs)

$$\begin{aligned}
\left(\frac{\partial^2 \bar{j}}{\partial c_2^2}\right)^{-1} &= \frac{\langle(\delta N_2)^2\rangle_{\{T,P,N_1,\mu_2\}}}{N_m} = \frac{1}{N_m} \langle(\delta N_2 - C\delta N_1)^2\rangle_{\{T,V,\mu_1,\mu_2\}} \\
&= \frac{\langle(\delta N_2)^2\rangle_{\{T,V,\mu_1,\mu_2\}}}{N_m} - 2C \frac{\langle\delta N_1 \delta N_2\rangle_{\{T,V,\mu_1,\mu_2\}}}{N_m} + C^2 \frac{\langle(\delta N_1)^2\rangle_{\{T,V,\mu_1,\mu_2\}}}{N_m} + o(1) \\
&= c_2 \rho_2 (G_{11} + G_{22} - 2G_{12}) + c_2 (1 + C)
\end{aligned} \tag{34}$$

where $C = c_2/c_1$, and the KBI is defined as

$$G_{ij} = V \frac{\langle\delta N_i \delta N_j\rangle - \delta_{ij} \langle N_j \rangle}{\langle N_i \rangle \langle N_j \rangle} \tag{35a}$$

See Appendix B for the derivation of the first line of Eq. (34) (where N_1 was written explicitly for clarity), using our novel algebraic approach to ensemble transformation [37]. Rewriting the fluctuations in Eq. (35a) in a system-size independent manner, we obtain

$$G_{ij} = \frac{V}{N_m} \frac{\frac{\langle \delta N_i \delta N_j \rangle}{N_m} - \delta_{ij} \frac{\langle N_j \rangle}{N_m}}{\frac{\langle N_i \rangle \langle N_j \rangle}{N_m N_m}} = v \frac{\frac{\langle \delta N_i \delta N_j \rangle}{N_m} - \delta_{ij} \frac{\langle N_j \rangle}{N_m}}{\frac{\langle N_i \rangle \langle N_j \rangle}{N_m N_m}} \quad (35b)$$

where $v = V/N_m$, as has been introduced in Section 3. Eq. (34) can therefore be rewritten as

$$\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right) = \frac{1}{c_2 \rho_2 (G_{11} + G_{22} - 2G_{12}) + c_2 (1+C)} \quad (36)$$

Let us carry out an order of magnitude analysis of Eq. (34). When the phase stability condition is satisfied, the right-most side, $\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right)$, is $O\left(\frac{1}{v}\right)$ and $\frac{\langle (\delta N_2)^2 \rangle}{N_m}$ is $O(v)$. Rewriting this quantity in terms of the KBIs (i.e., the last line of Eq. (34)) is helpful in understanding how the stability condition is broken in mesoscale. Eq. (35b) shows that the KBI in the mesoscopic system is still $O(1)$ when species i and j are well mixed within the mesoscale object.

It is useful here to consider a limiting case of Eq. (35b) for microscopic solutes for comparison. In this case, both c_2 and ρ_2 are $O(1)$, hence $\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right)$ and $\frac{\langle \delta N_i \delta N_j \rangle}{N_m}$ both go back to $O(1)$ when the mixture is stable. Because c_2 and ρ_2 in Eq. (36) are $O(1)$, in order to break the phase stability condition, KBI should become $O(V)$ so as to make $O\left(\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2}\right)\right) = O\left(\frac{1}{v}\right) = o(1)$,

For a mesoscopic system, in contrast, the phase stability condition requires $\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right) = O\left(\frac{1}{v}\right)$ and $\frac{\langle (\delta N_2)^2 \rangle}{N_m} = O(v)$. Since KBIs are $O(1)$ for a stable mesoscopic mixture, the last line of Eq. (34) shows that the $O(v)$ magnitude of $\frac{\langle (\delta N_2)^2 \rangle}{N_m}$ for stability comes entirely from c_2 . However, when the KBIs reach $O(v)$, $\frac{\langle (\delta N_2)^2 \rangle}{N_m} = O(v^2)$ and $\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2} \right)$ is $O\left(\frac{1}{v^2}\right)$.

Note that for a macroscopic system it is simply that $O(v) = O(v^2) = O(1)$, because $v = O(1)$. Only for mesoscopic systems that a distinction between the orders of magnitude, $O(1)$, $O(v)$ and $O(v^2)$ does emerge. Indeed, whether $O\left(\frac{1}{v^2}\right)$ for $\left(\frac{\partial^2 \tilde{J}}{\partial c_2^2}\right)$ can indeed be considered as $o\left(\frac{1}{v}\right)$, thereby breaking the phase stability condition, depends on the interplay between the limitation in precision of observation and the magnitude of v ; the phase stability condition is broken at a large but finite v with which $O\left(\frac{1}{v^2}\right)$ is below the detection limit. Thus, unlike the case of bulk solutions, KBIs do not need to reach the macroscopic scale $O(V)$ to break the phase stability. This is how breaking the phase stability condition may be easier under mesoscopic confinement.

Here, a comparison to mesoscale fluctuations within a macroscopic system [22,23] is in order, for which the left-hand side of Eq. (36) reduces from $O(1)$ to $O\left(\frac{1}{v}\right)$, where v refers to mesoscopic scale. This is parallel to the breaking of phase stability condition in a mesoscopic system, in which the left-hand side of Eq. (36) reduces from $O\left(\frac{1}{v}\right)$ to $O\left(\frac{1}{v^2}\right)$. Both reductions are due to the growths of KBIs from $O(1)$ to $O(v)$, yet the former does not lead to phase separation. In a macroscopic system, phase separation means inhomogeneity of concentration (or density) over $O(V)$ scale, and an $O(v)$ inhomogeneity is too small in scale to be regarded as the emergence of distinct phases. In a mesoscopic system, on the other hand, an $O(v)$ fluctuation already covers the whole system and distinct regions are observed within the boundary.

Under constant T and P (Section 3.2), rewriting isobaric fluctuations in terms of the KBIs leads to a complex mathematical relationship, as shown in Appendix C. The most useful insight,

however, is that the isobaric fluctuation for a dilute component can be approximated by the isochoric fluctuation, thereby expressed easily in terms of the KBIs. In addition, thermodynamic stability under isochoric conditions have been derived in Appendix D, in which case there is no need for an isobaric to isochoric conversion as presented above.

4.2. Phase separation via preferential adsorption and depletion interaction

Preferential adsorption onto the boundary of one species over another has been observed universally, both for alcohol-water mixtures [7–11] and macromolecules [4,5] from experiments and theory, and has been recognized as the key towards phase separation. Based on the mesoscale stability theory developed above, we aim to clarify (a) the relationship between adsorption and phase separation and (b) what constitutes a full set of evidence for the adsorption-induced phase separation as a suggestion for experiments and simulation.

To achieve these aims, the stability theory is indispensable, because the study of preferential adsorption on its own cannot give a complete insight into phase separation for the following reasons. Here, adsorption refers to $\langle N_m N_1 \rangle$ and $\langle N_m N_2 \rangle$, whereas, from Eq. (26), phase separation takes place when

$$|\langle \delta \mathbf{c} \delta \mathbf{c}^T \rangle| = c_1 c_2 \left[\frac{\langle N_2^2 \rangle - \langle N_2 \rangle^2}{\langle N_2 \rangle} \frac{\langle N_1^2 \rangle - \langle N_1 \rangle^2}{\langle N_1 \rangle} - \frac{(\langle N_1 N_2 \rangle - \langle N_1 \rangle \langle N_2 \rangle)^2}{\langle N_1 \rangle \langle N_2 \rangle} \right] = O(1) \quad (37)$$

is broken. Indeed, a direct evaluation of the self- and mutual-correlation terms, $\langle N_i^2 \rangle - \langle N_i \rangle^2$ and $(\langle N_1 N_2 \rangle - \langle N_1 \rangle \langle N_2 \rangle)^2$, requires either a composition dependence of $\langle N_1 \rangle$ and $\langle N_2 \rangle$, rather than $\langle N_1 \rangle$ and $\langle N_2 \rangle$ themselves, or a direct evaluation of number correlations. Unfortunately, at the

present stage, there is no experimental data available in the literature that enables us to evaluate all the terms of Eq. (37).

Nevertheless, Eq. (37) can be used to clarify the mechanism of macromolecular phase separation in mesoscale. The depletion of macromolecules (such as polyethylene glycol, PEG [4], polyelectrolytes [5] and actin filaments [44]) has been proposed, based on the Flory-Huggins model, to be a driving force for the segregation of DNA-macromolecule mixtures under confinement. The depletion of the macromolecules from the surface contribute to their self-association as well as to DNA-DNA association on the surface. If the self-association exceeds mutual association and reaches the mesoscopic order, $O(v)$, the phase stability condition (Eq. (37)) is broken. There was no explicit consideration on the mutual association, yet it is natural to expect it to decrease through segregation in the scale of $O(v)$.

The recent investigations [45,46] on the hydrogen bonding structure of ethanol-water mixtures in confinement examine not only self-association but also mutual association. That water-water and ethanol-ethanol H-bonding were enhanced from bulk upon confinement while ethanol-water H-bonding was reduced [45] is consistent with the increase of self-correlation $\langle N_1^2 \rangle - \langle N_1 \rangle^2$ and $\langle N_2^2 \rangle - \langle N_2 \rangle^2$ and the decrease of $(\langle N_1 N_2 \rangle - \langle N_1 \rangle \langle N_2 \rangle)^2$ which is the direction for phase separation. When the difference between self- and mutual- association reaches the mesoscopic order of magnitude, phase separation takes place. This insight is underscored further by a systematic analysis of ethanol-water mixtures near different surfaces, which has shown that the stronger the water-ethanol hydrogen bond the less separated the two species [46], which is in line with the mesoscale stability condition (Eq. (37)).

5. Conclusion

Confinement of solutions in mesoscale, such as droplet, cell, liposome or nanofluids, has been known to influence phase separation, as has been evidenced in wide-ranging fields. This paper has established the stability condition for a solution under mesoscale confinement, facilitated by the formal analogy between Hill's small system thermodynamics [25] and a partially open ensemble.

The thermodynamic stability condition extended to mesoscale is shown to involve several different orders of magnitude that are all considered to be $O(1)$ at a macroscopic limit. Reaching phase instability is much easier in the mesoscale compared to the macroscale, which is caused by net self-fluctuation (compared to mutual-fluctuation) reaching the mesoscopic order of magnitude $O(v)$, rather than the macroscopic order $O(V)$ (which, due to the macroscopic ($V \rightarrow \infty$) system size, is commonly referred to as divergence). Hence, a solution mixture in which the individual components tend to self-aggregate under confinement can easily break the stability condition, and the interaction with the surface, when it enhances self-association, may help satisfy phase instability. It should be noted that our arguments were concerned only with the extent of fluctuation and the system size. Accordingly, the analyses are valid in general when the system is not too small and the specific interactions with the boundary objects play a minor role.

Appendix A

Here we prove that the convexity of a function is preserved under certain Legendre transformation.

$G(T, P, N_1, N_2; N_m)$ is a special case of a three-component macroscopic mixture, $G(T, P, N_1, N_2, N_3)$, where m was rewritten as 3 for convenience, hence we focus on it throughout this Appendix. Since G is a convex (and at least twice differentiable) function with respect to N_1 , N_2 , and N_3 , under constant T and P [47–49],

$$\delta G = \frac{1}{2} \sum_{i,j=1}^3 g_{ij} \delta N_i \delta N_j > 0 \quad (\text{A1})$$

where

$$g_{ij} = \left(\frac{\partial^2 G}{\partial N_i \partial N_j} \right)_{T,P,N_k} \quad (\text{A2})$$

is positive definite.

Firstly, we prove that if a function is convex function with respect to N_1 , N_2 and N_3 , then the same function is (i) a convex function with respect to N_1 and N_2 under constant N_3 , and also (ii) a convex function with respect to N_1 under constant N_1 and N_3 . Our basis is the condition for the positive definiteness of Eq. (A1), which is a simultaneous fulfilment of Eq. (A3)-(A5) [38,39]:

$$g_{11} > 0 \quad (\text{A3})$$

$$\begin{vmatrix} g_{11} & g_{12} \\ g_{21} & g_{22} \end{vmatrix} > 0 \quad (\text{A4})$$

$$\begin{vmatrix} g_{11} & g_{12} & g_{13} \\ g_{21} & g_{22} & g_{23} \\ g_{31} & g_{32} & g_{33} \end{vmatrix} > 0 \quad (\text{A5})$$

The proof for (i) comes from Eqs. (A3) and (A4) that serve as the condition for the positive definiteness of the following quadratic term when N_3 is kept constant:

$$\delta G = \frac{1}{2} \sum_{i,j=1}^2 g_{ij} \delta N_i \delta N_j \quad (\text{A6})$$

The proof for (ii) follows Eq. (A3) which, on its own, can serve as the condition for the positive definiteness, under constant N_1 and N_2 , of the following:

$$\delta G = \frac{1}{2} g_{11} \delta N_1^2 \quad (\text{A7})$$

We have thus proven that G is a convex function with respect to N_1 and N_2 under constant N_3 , and a convex function with respect to N_1 under constant N_2 and N_3 . These results can be applied straightforwardly to $\mathcal{G}(T, P, N_1, N_2; N_m)$ because it is a special case of $G(T, P, N_1, N_2, N_3)$.

An alternative proof, which is applicable even for undifferentiable functions, will be presented here, in order to facilitate the discussion on convexity under Legendre transformation. This proof comes directly from the definition of a convex function, namely for any two vectors inside the space where the function G is defined, namely $\mathbf{N}_a = (N_{1a}, N_{2a}, N_{3a})$ and $\mathbf{N}_b = (N_{1b}, N_{2b}, N_{3b})$, for which the following holds true [47–49]:

$$G(\lambda \mathbf{N}_a + (1 - \lambda) \mathbf{N}_b) \leq \lambda G(\mathbf{N}_a) + (1 - \lambda) G(\mathbf{N}_b) \quad (\text{A8})$$

Rewriting Eq. (A8) for the special case, $\mathbf{N}'_a = (N_{1a}, N_{2a}, N_3)$ and $\mathbf{N}'_b = (N_{1b}, N_{2b}, N_3)$, leads to the convexity with respect to (N_1, N_2) when N_3 is kept constant. $\mathbf{N}''_a = (N_{1a}, N_2, N_3)$ and $\mathbf{N}''_b = (N_{1b}, N_2, N_3)$, leads to the convexity with respect to N_1 when N_2 and N_3 is kept constant. For differentiable G , Eq. (1) can be derived straightforwardly by considering $\mathbf{N} + \epsilon$ and $\mathbf{N} - \epsilon$ in place of \mathbf{N}_a and \mathbf{N}_b at $\lambda = \frac{1}{2}$.

Secondly, we shall prove that $J(T, P, \mu_1, N_2, N_3)$ is a convex function with respect to N_2 when all other parameters are kept constant. Our starting point is the result above that $G(T, P, N_1, N_2, N_3)$ is a convex function with respect to N_2 when other parameters are all kept constant. We therefore

need to show that the convexity of N_2 is not affected by the Legendre transform of N_1 to μ_1 . To facilitate the proof, we omit all the constants to denote $J(\mu_1, N_2)$ and $G(N_1, N_2)$, where

$$J(\mu_1, N_2) = G(N_1, N_2) - \mu_1 N_1 \text{ under the condition that } \mu_1 = \left(\frac{\partial G}{\partial N_1} \right)_{N_2} \quad (\text{A9})$$

The convexity of G with respect to N_2 is defined as the following in terms of the two points, $N_2 = N_{2a}$ and N_{2b} as

$$G(N_1, \lambda N_{2a} + (1 - \lambda) N_{2b}) \leq \lambda G(N_1, N_{2a}) + (1 - \lambda) G(N_1, N_{2b}) \quad (\text{A10})$$

Subtracting both sides by $\mu_1 N_1$, we obtain

$$\begin{aligned} & G(N_1, \lambda N_{2a} + (1 - \lambda) N_{2b}) - \mu_1 N_1 \\ & \leq \lambda [G(N_1, N_{2a}) - \mu_1 N_1] + (1 - \lambda) [G(N_1, N_{2b}) - \mu_1 N_1] \end{aligned} \quad (\text{A11})$$

Even though $\mu_1 = \left(\frac{\partial G}{\partial N_1} \right)_{N_2}$ in all the terms in Eq. (A11) were taken at different N_2 , μ_1 s nevertheless have the same constant value, because we are considering the convexity of $J(\mu_1, N_2)$ under constant μ_1 . Consequently, Eq. (A11) leads to

$$J(\mu_1, \lambda N_{2a} + (1 - \lambda) N_{2b}) \leq \lambda J(\mu_1, N_{2a}) + (1 - \lambda) J(\mu_1, N_{2b}) \quad (\text{A12})$$

which is indeed the convexity of $J(\mu_1, N_2)$ with respect to N_2 under constant μ_1 . Using the similar argument as before, we can obtain $\left(\frac{\partial^2 J}{\partial N_2^2} \right) > 0$ when J is differentiable.

Appendix B

Here we convert $\langle (\delta N_2)^2 \rangle_{\{T, P, \mu_2\}}$ to the corresponding expression for fluctuation in the grandcanonical ensemble. To do so, we employ our recent algebraic method for ensemble conversion based on (i) system size-invariance of concentration (in this case C) and its fluctuation and (ii) invariance of C and its fluctuation under ensemble transformation [37]. In Ref [37], we

employed isobaric and isochoric subsystems for the re-derivation of the KB theory for macroscopic solution mixtures. In contrast, our goal here is to convert $\langle(\delta N_2)^2\rangle_{\{T,P,\mu_1,\mu_2\}}$ (with full information on ensemble) to $\langle(\delta N_2)^2\rangle_{\{T,V,\mu_1,\mu_2\}}$; unlike Ref [37], we need to employ an ensemble of independent, non-interacting mesoscopic systems.

The isobaric ensemble can therefore be written as $\langle(\delta \mathcal{N}_2)^2\rangle_{\{T,P,\mathcal{N}_1,\mu_2\}}$, with the specified number of total \mathcal{N}_1 , while the isochoric ensemble becomes $\langle(\delta \mathcal{N}_2)^2\rangle_{\{T,V,\mu_1,\mu_2\}}$. Note that $\frac{\mathcal{N}_i}{V}$ is identical to the mean concentration for the small system. With this setup, the change of mole ratio, $C = \mathcal{N}_2/\mathcal{N}_1$, can be expressed under isobaric and isochoric conditions as

$$\frac{\mathcal{N}_2 + (\delta \mathcal{N}_2)_P}{\mathcal{N}_1} = \frac{\mathcal{N}_2 + (\delta \mathcal{N}_2)_V}{\mathcal{N}_1 + (\delta \mathcal{N}_1)_V} \quad (\text{B1})$$

The Maclaurin expansion of the right-hand side of Eq. (B1) yields

$$\frac{\mathcal{N}_2 + (\delta \mathcal{N}_2)_P}{\mathcal{N}_1} = \frac{(\mathcal{N}_2 + (\delta \mathcal{N}_2)_V) \left(1 - \frac{(\delta \mathcal{N}_1)_V}{\mathcal{N}_1} + O\left(\frac{1}{V}\right) \right)}{\mathcal{N}_1} \quad (\text{B2})$$

Comparing both sides of Eq. (B2) yields

$$(\delta \mathcal{N}_2)_P = (\delta \mathcal{N}_2)_V - \frac{\mathcal{N}_2}{\mathcal{N}_1} (\delta \mathcal{N}_1)_V + O(1) \quad (\text{B3})$$

An expression analogous to Eq. (34) can be derived from Eq. (B3) as

$$\frac{\langle(\delta \mathcal{N}_2)^2\rangle_{\{T,P,\mathcal{N}_1,\mu_2\}}}{\mathcal{N}_m} = \frac{1}{\mathcal{N}_m} \langle(\delta \mathcal{N}_2 - C \delta \mathcal{N}_1)^2\rangle_{\{T,V,\mu_1,\mu_2\}} + o(1) \quad (\text{B4})$$

The next step is to convert Eq. (B4) to an expression in a small system. To do so, let us first note that the small systems, that comprise the ensemble thereof, are all independent. Under this condition, the denominator and numerator in the both side of Eq. (B4) scale with the number of subsystems in an ensemble. Hence, we recover $\langle(\delta N_2)^2\rangle_{\{T,P,N_1,\mu_2\}}$. Noting that the size information

is implicit in the small system in terms of N_m , which is the hidden extensive quantity; instead of the mean of N_1/N_m , being an intensive quantity, μ_1 can be adopted. Thus, using the constancy of mean concentrations, we obtain the first step in Eq. (34). This constitute the generalization of the “intensive nature of relative fluctuation” [37] to small systems.

Appendix C

Here we convert the fluctuation in number of species i , $(\delta N_i)_P$ in a T, P, μ_1, μ_2, N_m ensemble to that in a T, V, μ_1, μ_2, N_m ensemble, $(\delta N_i)_V$. Note that only pressure and volume have been denoted in the variances for simplicity. Here we extend our recently proposed method without thermodynamic variable conversion [37]. Let us consider an ensemble of independent, non-interacting mesoscopic systems with volume \mathcal{V} , which contains \mathcal{N}_i molecules of species i . The change of number density, $\mathcal{N}_i/\mathcal{V}$, can be expressed under isobaric and isochoric conditions as

$$\frac{\mathcal{N}_i + (\delta \mathcal{N}_i)_V}{\mathcal{V}} = \frac{\mathcal{N}_i + (\delta \mathcal{N}_i)_P}{\mathcal{V} + (\delta \mathcal{V})_P} \quad (\text{C1})$$

The Maclaurin expansion of the right-hand side of Eq. (C1) yields

$$\frac{\mathcal{N}_i + (\delta \mathcal{N}_i)_V}{\mathcal{V}} = \frac{(\mathcal{N}_i + (\delta \mathcal{N}_i)_P) \left(1 - \frac{(\delta \mathcal{V})_P}{\mathcal{V}} + O\left(\frac{1}{\mathcal{V}}\right) \right)}{\mathcal{V}} \quad (\text{C2})$$

Comparing both sides of Eq. (C2) yields

$$(\delta \mathcal{N}_i)_V = (\delta \mathcal{N}_i)_P - \frac{\mathcal{N}_i}{\mathcal{V}} (\delta \mathcal{V})_P + O(1) \quad (\text{C3})$$

Therefore, the elements of the correlation matrix, because they are system size independent, can be transformed as

$$\frac{\langle \delta \mathcal{N}_i \delta \mathcal{N}_j \rangle_V}{\mathcal{N}_m} = \frac{1}{\mathcal{N}_m} \left[\langle \delta \mathcal{N}_i \delta \mathcal{N}_j \rangle_P - \frac{\mathcal{N}_i}{\mathcal{V}} \langle \delta \mathcal{V} \delta \mathcal{N}_j \rangle_P - \frac{\mathcal{N}_j}{\mathcal{V}} \langle \delta \mathcal{V} \delta \mathcal{N}_i \rangle_P + \frac{\mathcal{N}_i \mathcal{N}_j}{\mathcal{V}^2} \langle \delta \mathcal{V} \delta \mathcal{V} \rangle_P \right] \quad (\text{C4})$$

Since Eq. (C4) is independent of system size, it can be rewritten for a mesoscopic system, as

$$\frac{\langle \delta N_i \delta N_j \rangle_V}{N_m} = \frac{1}{N_m} \left[\langle \delta N_i \delta N_j \rangle_P - \frac{N_i}{V} \langle \delta V \delta N_j \rangle_P - \frac{N_j}{V} \langle \delta V \delta N_i \rangle_P + \frac{N_i N_j}{V^2} \langle \delta V \delta V \rangle_P \right] \quad (C5)$$

It is well-known that the last term of Eq. (C5) is related to the isothermal compressibility, κ_T , via $RT\kappa_T = \frac{\langle \delta V \delta V \rangle_P}{V}$. Isothermal compressibility makes a negligibly small contribution compared to number fluctuation, hence is neglected. $\langle \delta V \delta N_i \rangle_P$ in the second and third terms can be linked to

$$\frac{1}{\langle V \rangle_P} \left(\frac{\partial \langle V \rangle_P}{\partial \mu_i} \right)_{T, \mu_j, N_m} = \frac{1}{RT} \frac{\langle \delta V \delta N_i \rangle_P}{\langle V \rangle_P} \quad (C6)$$

When one of the components (say, i) is dilute, Eq. (C5) can be simplified further, by neglecting

$$\frac{1}{N_m} \left[-\frac{N_i}{V} \langle \delta V \delta N_j \rangle_P - \frac{N_j}{V} \langle \delta V \delta N_i \rangle_P \right] = -\frac{N_i}{V} \frac{N_j}{N_m} \frac{\langle \delta V \delta N_j \rangle_P}{N_j} - \frac{N_i}{V} \frac{N_j}{N_m} \frac{\langle \delta V \delta N_i \rangle_P}{N_i} \quad (C7)$$

because $\frac{N_i}{V}$ is small. Eq. (C5) can then be simplified as

$$\frac{\langle \delta N_i \delta N_j \rangle_P}{N_m} = \frac{\langle \delta N_i \delta N_j \rangle_V}{N_m} \quad (C8)$$

Appendix D

Here we summarise briefly the two most interesting cases regarding thermodynamic stability under an isochoric condition for a mesoscopic binary solution. In the following, we use the result from Appendix A, namely, the convexity of thermodynamic functions with respect to unconstrained variables. For understanding how the stability condition is broken in mesoscale, the same order-of-magnitude consideration with respect to the system size scale (as in Section 4.1) is valid for these cases.

(i) The $(T, V, N_1, N_2; N_m)$ ensemble. Starting from Eq. (19), we carry out the Legendre transform to introduce

$$\mathcal{G}'(T, V, N_1, N_2; N_m) = \mathcal{G}(T, P, N_1, N_2; N_m) - PV \quad (\text{D1})$$

which governs thermodynamic stability. When we constraint the system volume as constant.

$\mathcal{G}'(T, V, N_1, N_2; N_m)$ is still convex with respect to N_1 and N_2 , hence

$$\delta\mathcal{G}' = \frac{1}{2} \sum_{i,j=1,2} \left(\frac{\partial^2 \mathcal{G}'}{\partial N_i \partial N_j} \right)_{T,V,N_k} \delta N_i \delta N_j > 0 \quad (\text{D2})$$

is the stability condition for the mesoscale binary solution. Parallel to Section 3.2, the size-invariance of the stability through the use of an ensemble consisting of equivalent, non-interacting mesoscopic systems transforms Eq. (D2) into the following form:

$$\delta\mathcal{G}' = \frac{1}{2} \sum_{i,j=1,2} \left(\frac{\partial^2 \tilde{\mathcal{G}}'}{\partial c_i \partial c_j} \right)_{T,V,c_k} (N_m \delta c_i \delta c_j) \quad (\text{D3})$$

where $\tilde{\mathcal{G}}'(T, V, c_1, c_2) = \frac{\mathcal{N}\mathcal{G}'}{\mathcal{N}N_m} = \frac{\mathcal{G}'}{N_m}$. An argument parallel to Section 3.2 leads to the following expression of the stability condition in terms of the covariance matrix:

$$\frac{1}{|\langle \delta c \delta c^T \rangle|} > 0 \quad (\text{D4})$$

Note that the covariance in Eq. (D4) was taken under an isochoric condition rather than the isobaric condition in Eq. (26). This quantity is analogous to the KB theory for macroscopic solutions except for the presence of the boundary object.

(ii) The $(T, V, \mu_1, N_2; N_m)$ ensemble. Starting from Eq. (27), a Legendre transformation yields an isochoric thermodynamic function

$$\mathcal{J}'(T, V, \mu_1, N_2; N_m) = \mathcal{J}(T, P, \mu_1, N_2; N_m) - PV \quad (\text{D5})$$

The stability condition can be written as

$$\delta \mathcal{J}' = \frac{1}{2} \frac{\partial^2 \mathcal{J}'}{\partial N_2^2} (\delta N_2)^2 > 0 \quad (\text{D4})$$

Following an argument parallel to Section 3.3, the stability condition is expressed in terms of $\widetilde{\mathcal{J}}' =$

$\frac{\mathcal{J}'}{N_m}$ and the “intensive” variable $c_2 = N_2/N_m$,

$$\delta \mathcal{J}' = \frac{1}{2} \frac{\partial^2 \widetilde{\mathcal{J}}'}{\partial c_2^2} (N_m \delta c_2)^2 \quad (\text{D5})$$

from which the isochoric stability condition,

$$\frac{1}{\langle (\delta N_2)^2 \rangle} > 0 \quad (\text{D6})$$

analogous to the isobaric counterpart (Eq. (31)) can be derived.

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