Critical Phenomena of Liquid-Liquid Phase Separation in Biomolecular Condensates

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Biomolecular condensates, an important class of intracellular organelles, is considered to be formed by Liquid-Liquid Phase Separation (LLPS) *in vivo*. Here, in this report, we focus on the critical region of LLPS, where we believe is the state that most cells live for spatiotemporally-resolved physiological activities. First, a brief introduction of LLPS and recent progress is presented. We then discuss critical phenomena of passive LLPS and active LLPS respectively from mean-field perspective with the help of some reported results. Current descriptions of passive LLPS are mainly based on Flory-Huggins model, which is still within the framework of mean-field Landau-Ginzburg model. For active LLPS, the application of external fields do affect the critical behaviour. This not only implies the importance of taking external energy/biochemical reactions into account for more precise description of *in vivo* LLPS, but also could be the origins of such rich intracellular activities.

I. INTRODUCTION

Intracellular compartmentalization enables dynamical and accurate information processes during the growth and development of lives. Compared with well-studied membrane-bound organelles, researches on membrane-less organelles are still in its infancy. It has been well established during the past decade that membrane-less organelles are biomolecular condensates, comprised of proteins and/or RNAs, with significantly higher density compared with their surroundings but also internal mobility and rapid component exchange with surroundings, which highlights its liquid-like properties. Examples include protein condensates and ribonucleoprotein (RNP) condensates such as the nucleolus, Cajal bodies, stress granules, P-bodies, and so on. Therefore, Liquid-Liquid Phase Separation (LLPS) is widely recognized as the mechanism underlying formation and functions of those membrane-less organelles [1]. Controlled by external stimuli or biological signals, proteins and/or RNAs can form into biomolecular condensates or dissolve into solutions so as to fulfill the biological functions of membrane-less organelles in a spatiotemporally resolved manner.Recent researches also suggest that gelation (i.e., liquid-gel transition) could be the intermediate step between the transition from liquid-like biomolecular condensates to solid-like biomolecular condensates, the latter of which are usually observed in diseases, such as Amyotrophic lateral sclerosis (ALS) and Alzheimer's Disease (AD) [2].

All of those exciting experimental discoveries denote the importance of LLPS and related liquid-gel-solid transition on better understanding, modulation of biological processes and disease treatments. Several topics listed as follows are of great interests to the whole community: 1) As a single condensate could contain hundreds of different kinds of proteins, what are mechanisms that guide the formation of multicomponent mixtures of proteins and RNAs? [3] 2) How do external energy/chemicals (such as ATP or post-translational modifications) influence LLPS (i.e., out-of-equilibrium active process)? [4] 3) How does heterogeneous cellular environments interact with condensates? [5]

Although huge amounts of researches have been done, very few of them took a serious investigation on criticality or universality of LLPS/liquid-gel-solid transition. It's highly possible that cells live in the vicinity of the critical point of LLPS such that they can function in a timely manner as responses to any stimuli or biological signals. [6] In this report, we will focus on the critical phenomena of LLPS. Standard and extended Flory-Huggins model will be presented with related analysis of critical points for passive LLPS. A dynamic percolation-like model will be discussed for criticality of out-of-equilibrium active LLPS. Future research directions will be concluded in the final part of this report.

II. PASSIVE LIQUID-LIQUID PHASE SEPARATION

All different kinds of LLPS and liquid-gel-solid transition in biological context can be roughly categorized into two types: passive process and active process [7]. There is no obvious deviation from equilibrium thermodynamics for passive processes, while due to external work or internal energy sources, thermodynamics of active process is fundamentally different (see next section).

One of the simplest models to describe passive LLPS is Flory-Huggins model. Protein chains (i.e., polypeptides) can be considered as a chain of dipoles. Taken solvent molecules into account, there exists three types of interactions: peptide-peptide, solvent-solvent, peptide-solvent. Strong peptide-peptide interactions enable condensation of protein chains and expel solvent molecules into the bulk solution. Through introducing Flory interaction parameter, Flory-Huggins free energy can be

expressed as follows [8, 9]:

$$\beta f = \frac{\phi}{N}\ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi), \quad (1)$$

where ϕ is the volume fraction of peptides, β equals to $\frac{1}{k_{\mathrm{B}}T}$, χ is the Flory interaction parameter, which quantities interactions between peptides and solvent per site and can be expressed as follows:

$$\chi = z\beta\left[u_{\mathrm{ps}} - \frac{1}{2}\left(u_{\mathrm{pp}} + u_{\mathrm{ss}}\right)\right], \quad (2)$$

where u_{pp}, u_{ss} and u_{ps} are interaction energy per site for peptide-peptide, solvent-solvent, peptide-solvent interactions, respectively.

Eq. (1) is a lattice model based on mean-field assumption. The first and second terms of are mixing entropy per lattice site and third term is mixing energy per lattice site (similar to question 1 of chapter 1 [10]). According to

$$\frac{\partial^2(\beta f)}{\partial\phi^2} = \frac{1}{N\phi} + \frac{1}{1-\phi} - 2\chi = 0, \quad (3)$$

$$\frac{\partial^3(\beta f)}{\partial\phi^3} = -\frac{1}{N\phi^2} + \frac{1}{(1-\phi)^2} = 0, \quad (4)$$

it's easy to find the Flory interaction parameter at critical point approximates 0.5 (the exact solution is $\chi_c = \frac{N+2\sqrt{N}+1}{2N}$), which means phase separation will be facilitated for Flory interaction parameter larger than 0.5. Expanding at the vicinity of critical points, we can have

$$|\phi - \phi_c| \propto |\chi - \chi_c|^{\frac{1}{2}}, \quad (5)$$

which denotes that the scaling exponent β equals to $\frac{1}{2}$. Although its simplicity, it has been adopted as a framework to understand LLPS [11].

Besides short-range dipole-dipole interactions, long-range electrostatic interactions between charged peptides also play an important role in LLPS [12]. Taken that into consideration, Overbeek and Voorn extended Flory-Huggins free energy equation as follows:

$$\beta f = \frac{\phi}{r}\ln\frac{\phi}{2} + (1-\phi)\ln(1-\phi) - \alpha(\sigma\phi)^{3/2}, \quad (6)$$

where r is molecular weight of peptides, σ is charge density, which is deduced according to Debye–Hückel theory and based on the assumption that the influence of polyelectrolytes is similar to that of monovalent ions with equivalent charges [13]. α is a solvent constant determined by thermal energy $k_{\mathrm{B}}T$ and molar volume of solvent, which equals to 3.5 for water under room temperature.

We can find that firstly, the Flory interaction term is neglected here compared with long-range electrostatic interactions. Secondly, the mixing entropy of peptides (i.e., $\frac{\phi}{r}\ln\frac{\phi}{2}$) is reduced due to the strong electrostatic interactions. Following the steps as Eq. (3-4) and expanding near the critical point, we can obtain the critical point $\sigma^3 r \approx 0.5$ of extended Flory-Huggins free energy equation and the same scaling exponent $\beta = \frac{1}{2}$ (i.e., $|\phi - \phi_c| \propto |\sigma - \sigma_c|^{\frac{1}{2}}$) as the standard Flory-Huggins theory. Therefore, although taking electrostatic interactions into consideration makes Flory-Huggins model more realistic and useful, such modification does not substantially change the description of critical behavior. Those two equations are in same universal class as mean-field Landau-Ginzburg model.

III. ACTIVE LIQUID-LIQUID PHASE SEPARATION

Most of the LLPS phenomena observed in biological context are influenced or driven by external energy (e.g., mechanical forces by cytoskeleton) or internal energy sources (e.g., ATP), the active (i.e., out-of-equilibrium) essence of which inevitably alters boundaries and kinetics of phase separation [7]. Here, we will use directed percolation model, one of the simplest possible models exhibiting the out-of-equilibrium phase separation, to describe the dynamic interactions between different protein/RNA regions. For simplicity, directed percolation model can be considered as a network with N nodes, in which each node can be in either active or inactive state. Any active nodes can be inactivated stochastically at the rate of μ, while only inactive nodes at the nearest sites of active nodes can be activated randomly at the rate of λ. There exists sustained activity and stable formation of biomolecular condensates in the 'active phase' above the critical point. To the contrary, below the critical point is the 'absorbing phase', where activity will decay into quiescence and no stable biomolecular condensates can be observed. Thus, in this region, stochastic fluctuations will greatly influence dynamics and is non-negligible.

In the large N limit (i.e., continuum limit) and taken stochastic noise into account, directed percolation model can be described by the following Langevin equation [14]:

$$\dot{\rho}(\mathbf{r},t) = a\rho(\mathbf{r},t) - b\rho^2(\mathbf{r},t) + D\nabla^2\rho(\mathbf{r},t) + \sqrt{\rho(\mathbf{r},t)}\eta(\mathbf{r},t), \quad (7)$$

where $\rho(\mathbf{r},t)$ is the density of activity at $\mathbf{r}$ and time t, a is the distance of order parameter, active rate, towards the critical point, b, D are constant and $\eta(\mathbf{r},t)$ is Gaussian noise with mean zero and variance σ^2. Such probabilistic description of noise distinguishes directed percolation model from other deterministic model, such as Ising model.

Fluctuations mentioned above normally lead to the so-called 'avalanching behavior', in which absorbing phase will be perturbed by an activation seed and a series of events will be triggered before the whole system returning

back to absorbing state again. The PDF of avalanche-size S and avalanche-time T in the vicinity of critical point are scale-invariant and expressed as follows [15]:

$$P(S) \sim S^{-\tau}, \tag{8}$$
$$F(T) \sim T^{-\alpha}. \tag{9}$$

Similarly, the averaged avalanche-size scales with avalanche-time in the following manner [16]:

$$\langle S \rangle \sim T^{\gamma}, \tag{10}$$
$$\gamma = \frac{\alpha - 1}{\tau - 1}. \tag{11}$$

A more general expression of Langevin equation can be deduced from Eq. (7) as follows:

$$\dot{\rho} = \sqrt{\rho}\xi(t), \tag{12}$$

where $\xi(t)$ is the Gaussian noise with mean zero and variance $2\sigma^2$, $D\nabla^2\rho(\mathbf{r},t)$ becomes zero in the mean-field limit, $a\rho(\mathbf{r},t)$ is neglected near the critical point, $b\rho^2(\mathbf{r},t)$ vanishes through re-scaling. Eq. (12) can be further rewritten to an equivalent expression [17] as follows in order to guarantee $\rho = 0$ is the absorbing state:

$$\dot{\rho} = -\frac{\sigma^2}{2} + \sqrt{\rho}\eta(t), \tag{13}$$

where $\eta(t)$ is the Gaussian noise with mean zero and variance $\frac{\sigma^2}{2}$. Through applying $x = \sqrt{\rho}$ and $U(x) = \lambda \log x$,

$$\frac{dx}{dt} = -\frac{\sigma^2}{4x} + \eta(t) \tag{14}$$
$$= -\frac{dU(x)}{dx} + \eta(t) \tag{15}$$
$$= -\frac{\lambda}{x} + \eta(t). \tag{16}$$

In the case that we denote variance of the Gaussian noise as 2μ, it is easy to find that $\mu = \lambda = \frac{\sigma^2}{4}$. Thus, $\beta \equiv \lambda/\mu = 1$, which quantities the relationship between the logarithmic potential and the noise variance and it is well-balanced here.

In order to further mimic dynamic interactions between different protein/RNA regions, we can introduce an external field allowing the spontaneous activation of any inactive sites at a fixed rate h [18]. In this case, Eq. (14) becomes

$$\frac{dx}{dt} = -\frac{\sigma^2 - 4h}{4x} + \eta(t), \tag{17}$$

the balance between the logarithmic potential and the noise variance is broken down as β becomes $1 - \frac{h}{\mu}$.

According to Ref. [19], we have

$$F(T) = \frac{4\mu\epsilon^{2v}}{\Gamma(v-1)}(1+\beta)(4\mu T)^{-\nu-1}e^{-\frac{x^2}{4\mu T}} \tag{18}$$
$$\sim T^{-v-1} = T^{-\frac{3+\beta}{2}}, \tag{19}$$

which indicates that α equals to $2 - \frac{h}{2\mu}$. The avalanche-size can be obtained through the following integral [18]:

$$S = \int_0^T v(t)dt \sim T^2 \int_0^1 \tilde{v}(\frac{t}{T})d(\frac{t}{T}) \tag{20}$$

where homogeneity assumption is applied and $v(t)$ is percolation displacement at time t. Therefore, $\gamma = 2$ and $\tau = \frac{3}{2} - \frac{h}{4\mu}$ according to Eq. (11).

Based on the review and discussion above, we have two interesting findings as follows. Firstly, if external fields do not exist, we will have $\alpha = 2$, $\tau = \frac{3}{2}$ and $\gamma = 2$, which coincide with results of standard random walk as reported by Ref. [20]. External fields decrease the scaling exponent α and τ, which means due to the random activation of inactive nodes, it takes longer time for systems in absorbing phase return back to quiescence after perturbation. Secondly, as external fields vary from each other, there is no universal class for such out-of-equilibrium behavior.

IV. CONCLUSION

In conclusion, we have discussed two simplest possible models from mean-field perspective for passive and active LLPS, respectively and particularly focused on critical phenomena. Flory-Huggins model is widely used to explore passive LLPS. Although extended Flory-Huggins model may give out a better description through considering electrostatic interactions, there still exist several important factors needed to be taken into account, such as charge pattern of protein/RNA chains, hydrophobic interactions, rigidity of existed condensates. Besides, there is still the lack of experimental exploration of these critical phenomena. For active LLPS, we find that different external fields may lead to totally different critical behaviours, which highlights the importance of taking external fields (e.g., posttranslational modifications, ATP, mechanical forces, etc.) into consideration due to the complexity and heterogeneity of intracellular environments. In the future, more experimental researches can be conducted at the vicinity of critical point for the better understanding of *in vivo* LLPS, while in the theory side, more universal descriptions of this intricate process is highly demanded.

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[1] C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, and A. A. Hyman, Germline p granules are liquid droplets that localize by controlled dissolution/condensation, Science **324**, 1729 (2009).

[2] Y. Shin and C. P. Brangwynne, Liquid phase condensation in cell physiology and disease, Science **357** (2017).

[3] J. A. Riback, L. Zhu, M. C. Ferrolino, M. Tolbert, D. M. Mitrea, D. W. Sanders, M.-T. Wei, R. W. Kriwacki, and C. P. Brangwynne, Composition-dependent thermodynamics of intracellular phase separation, Nature **581**, 209 (2020).

[4] J. D. Wurtz and C. F. Lee, Chemical-reaction-controlled phase separated drops: formation, size selection, and coarsening, Physical review letters **120**, 078102 (2018).

[5] T. Wiegand and A. A. Hyman, Drops and fibers—how biomolecular condensates and cytoskeletal filaments influence each other, Emerging Topics in Life Sciences **4**, 247 (2020).

[6] M. A. Munoz, Colloquium: Criticality and dynamical scaling in living systems, Reviews of Modern Physics **90**, 031001 (2018).

[7] J. Berry, C. P. Brangwynne, and M. Haataja, Physical principles of intracellular organization via active and passive phase transitions, Reports on Progress in Physics **81**, 046601 (2018).

[8] M. L. Huggins, Some properties of solutions of long-chain compounds., The Journal of Physical Chemistry **46**, 151 (1942).

[9] P. J. Flory, Thermodynamics of high polymer solutions, The Journal of chemical physics **10**, 51 (1942).

[10] M. Kardar, *Statistical physics of fields* (Cambridge University Press, 2007).

[11] C. F. Lee, C. P. Brangwynne, J. Gharakhani, A. A. Hyman, and F. Jülicher, Spatial organization of the cell cytoplasm by position-dependent phase separation, Physical review letters **111**, 088101 (2013).

[12] G. L. Dignon, R. B. Best, and J. Mittal, Biomolecular phase separation: From molecular driving forces to macroscopic properties, Annual review of physical chemistry **71**, 53 (2020).

[13] J. T. G. Overbeek and M. Voorn, Phase separation in polyelectrolyte solutions. theory of complex coacervation, Journal of Cellular and Comparative Physiology **49**, 7 (1957).

[14] M. Henkel, H. Hinrichsen, and S. Lübeck, *Non-equilibrium phase transitions: volume 1: absorbing phase transitions* (Springer Science & Business Media, 2008).

[15] L. P. Kadanoff, S. R. Nagel, L. Wu, and S.-m. Zhou, Scaling and universality in avalanches, Physical Review A **39**, 6524 (1989).

[16] J. P. Sethna, K. A. Dahmen, and C. R. Myers, Crackling noise, Nature **410**, 242 (2001).

[17] N. G. Van Kampen, *Stochastic processes in physics and chemistry*, Vol. 1 (Elsevier, 1992).

[18] S. di Santo, P. Villegas, R. Burioni, and M. A. Muñoz, Simple unified view of branching process statistics: Random walks in balanced logarithmic potentials, Physical Review E **95**, 032115 (2017).

[19] A. Bray, Random walks in logarithmic and power-law potentials, nonuniversal persistence, and vortex dynamics in the two-dimensional xy model, Physical Review E **62**, 103 (2000).

[20] S. Zapperi, K. B. Lauritsen, and H. E. Stanley, Self-organized branching processes: mean-field theory for avalanches, Physical review letters **75**, 4071 (1995).