

Scaling Relations of Multicomponent Phase Coexistence Boundaries

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Multicomponent phase-separating molecular systems play a key role as membraneless organelles in living cells. Phase diagrams are indispensable for probing the concentration-dependent behavior of these condensates, yet their interpretation has remained largely qualitative due to challenges of modeling complex, multicomponent interactions. Here, we present a generic framework for quantitative analysis and interpretation of phase diagrams. We derive an analytical expression for the phase boundary normal vector, which reveals two distinct scaling regimes: one for associative phase separation, characterized by a power-law scaling analogous to mass-action kinetics that allows for direct extraction of dense phase solute molar ratios, and another for condensate dissolution, where an exponential scaling quantifies three-body repulsion effects triggered by the addition of a new component. We analyze a wide range of experimentally measured phase diagrams, covering proteins NSP5, NSP2, FUS, Whi3, G3BP1, and antimicrobial peptides, and we demonstrate that the additional quantitative insights can aid the mechanistic interpretation of their phase behavior.

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I. INTRODUCTION

Biomolecular condensate formation in cells has emerged as a new paradigm in which nature achieves compartmentalization of macromolecules—including proteins and RNAs—with thermodynamic principles [1,2]. The relevance of these condensates in functions and diseases [3,4] motivates experimental effort in understanding the driving forces [5–7] and molecular grammar of condensate formation [8,9]. A commonly measured descriptor of phase separation is the phase diagram that indicates at which solute concentrations phase separation occurs, and the phase separation propensity is usually deduced by assessing how the phase diagram changes upon a modulation or change in polymer sequence [10,11]. So far, phase diagrams have only been assessed qualitatively, and a general, quantitative framework is lacking that would allow one to systematically derive analytical relations for phase boundary shapes and to extract physical parameters from these measurements.

A major difficulty towards such a framework is the high-dimensional nature of the concentration space and associated phase boundary, which comprises a dense branch and a dilute branch connected by tie lines [Fig. 1(a)]. In *in vitro* and *in cellulo* experiments, many solvent components, such as salt, buffer molecules, crowders, and pH, are present, and the

measured phase diagrams usually represent low-dimensional experimental slices of the full concentration space [Fig. 1(a)]. Mathematically, we denote by c^α the total molar concentration of solute α , where $\alpha = 1, 2, \dots, N$ for N solute species.¹ The dilute and dense phase molar concentrations are denoted as c_-^α and c_+^α , respectively. A typical two-dimensional phase diagram measured along the c^1 and c^2 axes (without loss of generality) can be represented by a plane S_{12} intersecting the dilute phase branch, and the intersection is the measured phase boundary, equivalent to c_-^α 's evaluated on S_{12} [Fig. 1(a)]. A comprehensive theory would need to account for interactions among all N components and then solve the globally constrained optimization problem to give the phase boundary [12,13], before a low-dimensional section of it can be fitted to experimental data. Although detailed theories have been built in the past to account for effects of pH [14,15], crowders [16], and salt [17,18], combining the effect of all possible components is analytically intractable. Model-free measurement approaches are emerging that allow relative energetics of phase-separating systems to be extracted in the presence of an arbitrary number of solutes using dilute phase measurements, termed energy dominance analysis [6,7,19]. Can a similar method be developed to interpret phase diagrams without concentration measurements?

In this work, we derive an expression for the multicomponent phase boundary normal vector, and we evaluate it as a power series by assuming a generic free-energy function

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¹The superscript label follows conventions from differential geometry. For a vector quantity, a superscript α carries units of concentration of α , while a subscript carries the inverse concentration instead.

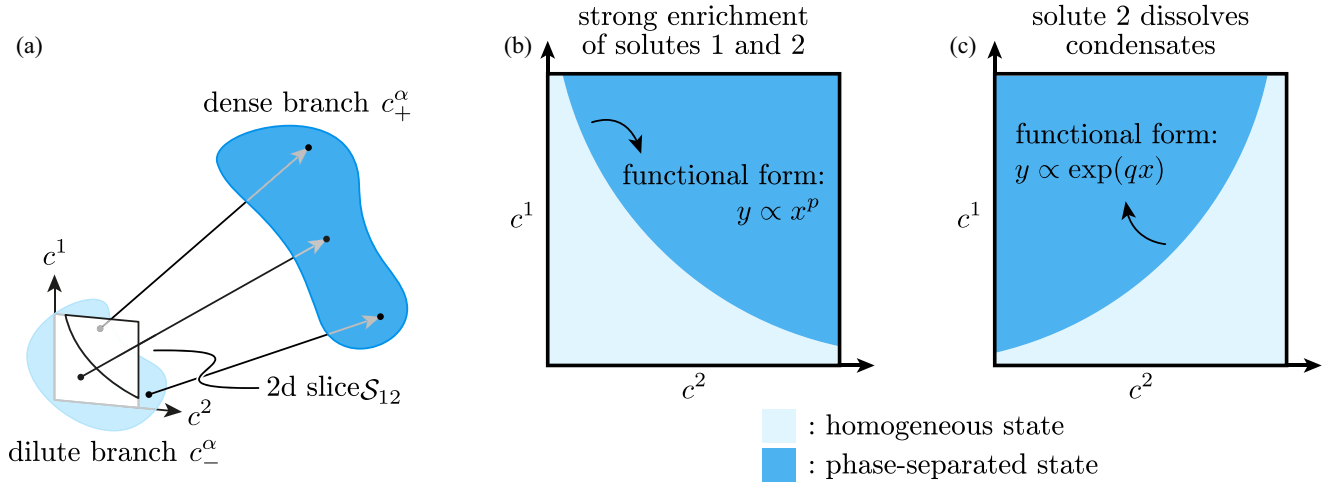


FIG. 1. Phase boundary scaling relations for low-dimensional measurements of multicomponent systems. (a) In the concentration space with many solute components, the dilute (c_-^α) and dense (c_+^α) branches of the phase boundary are high-dimensional. Typical experiments only probe a small discrete set of components and hence measure a low-dimensional slice (S_{12}) of the dilute phase boundary. (b) When both solutes 1 and 2 are strongly enriched in the dense phase, possibly due to heterotypic attraction, a polynomial scaling relation is expected of the phase boundary, with the exponent related to their molar ratio in the dense phase. (c) If instead the solute 2 dissolves condensates comprising solute 1, the phase boundary has a positive gradient and an exponential functional form.

encompassing translational entropy and many-body interactions. We study leading-order contributions of this vector for the experimentally relevant S_{12} slice, which lead to analytical expressions of the phase boundary on S_{12} . These give simple shapes in two scenarios: strong association, where entropic effects dominate for both components [Fig. 1(b)], and dissolution of condensates due to three or more-body interactions [Fig. 1(c)]. These two cases capture the central effects of the two leading-order contributions of the phase boundary normal expression, and higher-order interactions can be systematically incorporated. We make extensive use of the theoretical results to analyze phase boundary data for a wide range of experimental systems, with accompanying analysis scripts, and we draw connections to mass action in chemical kinetics, the energy dominance framework, and solubility product for condensates. Taken together, our work establishes a new quantitative interpretation of low-dimensional phase boundary data.

II. THEORETICAL RESULTS

Associative phase separation is one of the most common motifs of protein condensation, where the heteromolecular attractions between two biomolecular species are essential for condensate formation. In this scenario, their dense phase concentrations are typically much larger than the dilute phase concentrations. Using indices 1 and 2 to label the associative species, we show that the phase boundary on S_{12} satisfies a polynomial scaling relation [Fig. 1(b), Appendix A]

$$\left. \frac{d \ln c_-^1}{d \ln c_-^2} \right|_{S_{12}} \approx -\frac{c_+^2}{c_+^1}, \quad (1)$$

where c_+^α is the dense phase composition connected by a tie line to the point of evaluation on S_{12} . Remarkably, this result is robust in the presence of other hidden components,

and we verify this using numerically generated two- and three-component Flory-Huggins phase separation boundaries (Appendix B). This general scaling behavior has a simple physical interpretation in terms of mass action when the activity of molecules in the dense phase is constant, a point we discuss later. Importantly, in modulation studies where the associative phase boundaries of different systems are compared, Eq. (1) allows quantitative conclusions to be drawn beyond qualitative assessments of expansion or shrinkage of the phase-separated region, which will be a focus in the next section.

Mechanistic insights into the driving forces of phase separation are typically elucidated via a different type of experiment: dissolution of condensate by addition of a phase separation disruptor. Examples of disruptors include salt, RNA, and ATP for electrostatic interactions, and hexanediol for hydrophobic interactions [5,6,10]. Specifically, suppose component 1 is strongly enriched in the dense phase in the absence of solute 2, with condensate formation possibly driven by an associative interaction with another species z ($z = 1$ for homotypic attraction or $z = 3$ for heterotypic attraction with component 3). Furthermore, suppose addition of the solute 2 dissolves condensates, so the phase boundary in S_{12} has a positive gradient. We show that the phase boundary shape in S_{12} becomes an exponential function [Fig. 1(c), Appendix A]

$$\left. \frac{d \ln c_-^1}{d c_-^2} \right|_{S_{12}} \approx \epsilon_{12z}^{(3)} c_+^z, \quad (2)$$

where c_+^z is the dense phase molar concentration of component z , $\epsilon_{12z}^{(3)} > 0$ is a three-body repulsion energy density involving solutes 1, 2, and z , and we work with dimensionless energies by setting the thermal energy to unity $k_B T = 1$. The combined quantity $\epsilon_{12z}^{(3)} c_+^z$ represents a repulsion energy normalised by the concentration of solute 2.

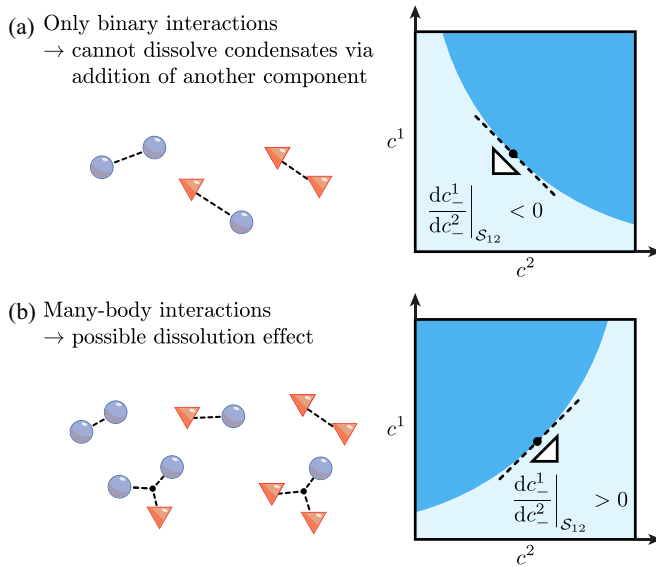


FIG. 2. Order of interactions determines dissolution phenomenology. (a) When only binary interactions are present in the phase separation system, no condensate dissolution upon the addition of another component can be observed in the low-concentration limit where solvent entropy effects are negligible. (b) Dissolution is only possible in the presence of three or more-body interactions, which can be thought of as one molecule disrupting the attraction between two other molecules. Effects from solvent entropy can also lead to condensate dissolution, as is commonly observed in numerical computations of binodal boundaries.

Comparing the left-hand sides of Eqs. (1) and (2), the main difference is changing $d \ln c_-^2$ to dc_-^2 . The logarithmic scaling originates from the entropic penalty of partitioning component 2 in associative phase separation, and three-body disruptive interactions enter as a linear contribution to the phase boundary normal vector (Appendix A), leading to the difference in forms. Interestingly, a corollary of the theoretical results is that under the assumption of weak solvent entropy contributions, condensate dissolution by addition of a component can only arise from three- or more-body interactions, and is not possible with only two-body interactions alone (Fig. 2, Appendix A). Dissolution observed in numerical computations of the two-component Flory-Huggins model [20] arises from solvent entropy effects, and will be absent in the weak solvent limit. Typical molecular species that dissolve condensates are monovalent salts involved in charge screening [5–7,10] and protein or RNA-binding partners that sequester condensate-forming proteins through complex formation [19,21]. Our analysis suggests that these mechanisms should all broadly be considered as many-body interactions. This is sensible since these processes can be treated as one molecule disrupting attractions between two other molecules, and as such at least three molecules are involved in the dissolution mechanism [Fig. 2(b)]. Many-body interactions can be a fruitful avenue for further investigations, as they are found to introduce multiphase behaviors that are richer than binary interactions in numerical computations [22], and robust quantitative interpretation of one-dimensional phase separation data also relies on three-body interactions [23].

Experimental data analysis using Eqs. (1) and (2) involves fitting measured phase boundaries to these functional forms, and the right-hand-side terms in the equations need to be fixed to be constant as a result. This might appear untenable at first, but we observe below that the functional forms of Eqs. (1) and (2) are indeed preserved in many datasets to a large extent. Furthermore, if multiple phase diagrams are measured at varying solution conditions, changes in the fitting parameters can be traced directly (under assumptions used in the analytical derivation) to changes in dense phase concentrations of specific components, and the theoretical expression of the phase boundary can be extended to include these additional variations, too. This turns out to be important in explaining the concave phase boundary shape in protein-RNA systems.

III. APPLICATIONS TO EXPERIMENTAL DATA

A. NSP2/NSP5 viral protein condensate system validates the associative scaling relation

Equation (1) states that the scaling exponent of an associative phase boundary corresponds to the dense phase concentration ratio of the components. We explicitly test this in the viral protein system of NSP2 and NSP5. Condensates formed by these proteins are key sites of viral replication [24]. We take the NSP2/NSP5 phase diagram from [10] and fit a power-law form to the boundary by first plotting the data in double logarithmic scale and then using a support vector machine with linear kernel to estimate the gradient [Figs. 3(a) and 3(b)]. Details of the analysis can be found in the GitHub repository [25]. This fitting gives a dense phase molar stoichiometry of $c_+^{\text{NSP5}}/c_+^{\text{NSP2}} = 2.5 \pm 0.5$. To validate the dense phase molar ratio, we measure this directly using gel electrophoresis (Appendix C). Briefly, NSP2 and NSP5 are mixed at an equimolar concentration of 20 μM each at a final volume of 10 μL , giving a total of 0.2 nmol of each protein in the sample. The sample is centrifuged and the dilute phase is taken out as the supernatant. The amounts of proteins in the supernatant are quantified by comparing the gel stain intensities to samples containing only NSP5 or NSP2 [Fig. 3(c)]. The result showed that (0.1988 ± 0.0003) nmol of NSP5 and (0.073 ± 0.006) nmol of NSP2 entered the dense phase, giving a dense phase molar ratio of $c_+^{\text{NSP5}}/c_+^{\text{NSP2}} = 2.8 \pm 0.2$, consistent with the phase boundary fitting result [Figs. 3(d) and 3(e)].

Using the dense phase molar ratio, we can further give the energy dominance [7] ratio between these proteins. The dominance D^α is the fraction of free-energy decrease due to solute α relative to the total free-energy decrease, and it satisfies $\sum_{\alpha=1}^N D^\alpha = 1$ [7]. We show that for two generic components (Appendix A),

$$\frac{D^1}{D^2} \approx - \left. \frac{dc_-^2}{dc_-^1} \right|_{S_{12}} \frac{c_+^1}{c_+^2}, \quad (3)$$

where $dc_-^2/dc_-^1|_{S_{12}}$ is the inverse of the phase boundary gradient. This implies that the phase boundary alone allows us to deduce the relative energetic contribution of solutes to phase separation, and a dominance ratio of $D^{\text{NSP5}}/D^{\text{NSP2}} \approx 5$ is estimated from data. This means the free-energy decrease associated with NSP5 is about five times that associated with NSP2.

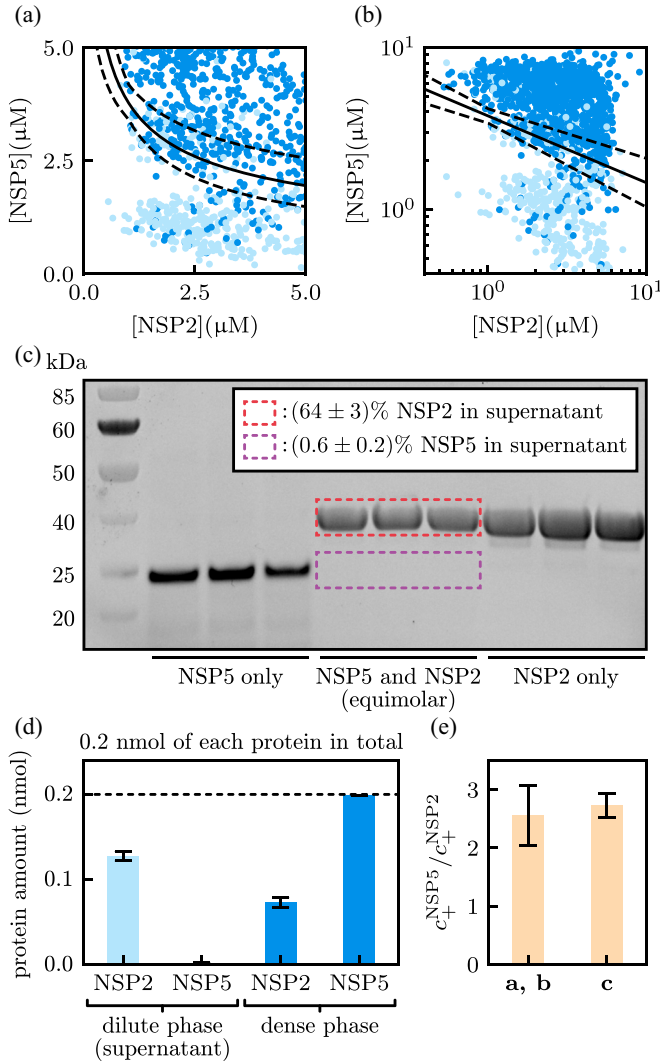


FIG. 3. Phase boundary informs on the stoichiometry of viral protein condensates. Phase boundary data from [10] are reproduced here in (a) linear and (b) double logarithmic scales. Light and dark blue scatter points correspond to homogeneous and phase-separated samples, respectively. We analyze the boundary following the scaling relation Eq. (1). Black solid lines are power-law fits with errors in black dashed lines, giving a dense phase molar ratio of $c_+^{NSP5}/c_+^{NSP2} = 2.5 \pm 0.5$. (c) Gel electrophoresis results for quantifying NSP5 and NSP2 dense phase molar ratio. Equal concentrations of NSP2 and NSP5 are mixed in solution to form condensates, and supernatants of samples with only NSP5 (left), NSP5 and NSP2 (middle), and only NSP2 (right) are measured. (d) Gel electrophoresis gave the amount of proteins in the dilute phase (left, light blue), and the rest are in the dense phase (right, dark blue). This gives $c_+^{NSP5}/c_+^{NSP2} = 2.8 \pm 0.2$. (e) Summary of dense phase molar ratios.

B. Changing FUS/PEG phase boundary shapes reveals detailed mutation and small molecule effects

The NSP5/NSP2 condensate system is relevant *in cellulo* and *in vivo*, while many *in vitro* condensates are constituted using the macromolecule polyethylene glycol (PEG). PEG has been used by the condensate community as an inert crowder in the past but was later shown to have associative interactions in some cases [20], so we revisit the phase separation of the

protein Fused in Sarcoma (FUS) with PEG (molecular weight 6000 dalton, or 6 kDa, in our experiments). We measure the FUS/PEG phase boundary (Appendix D) and found via fitting to Eq. (1) the dense phase molar ratio $c_+^{FUS}/c_+^{PEG} = 0.110 \pm 0.008$ [Fig. 4(a)] [25]. In the past, this molar ratio has been estimated using the dilute phase measurement approach [6,20], which gave 0.0061 ± 0.0005 for PEG (20 kDa) and 0.0039 ± 0.0008 for PEG (10 kDa) in [20], significantly smaller than that measured here. We trace this inconsistency to a correction factor due to the measurement approach developed in [20]. In short, the approach outlined in [20] aims to find two compositions of total FUS and PEG that give the same dilute phase (FUS). These points would have been on the same tie line if the system was entirely two-dimensional, but this breaks down if more “hidden” dimensions are present [6,7]. A brief summary of the results from a more rigorous analysis [7] is given in Appendix E. Denoting by ζ the ratio between the FUS/PEG dense phase stoichiometry measured using the dilute phase approach and that of the true stoichiometry, we have

$$\zeta = \frac{D^2}{1 - D^1} = \frac{1}{1 + \sum_{\beta=3}^N D^\beta / D^2}, \quad (4)$$

where FUS and PEG are solutes 1 and 2, and the sum is over all other solutes. We estimate $\zeta \approx 0.02 \ll 1$ (Appendix E) by comparing tie line gradient results from [20] to the results obtained here, and we deduce that D^2 is small, meaning PEG is only contributing weakly to the system free-energy decrease. This is further corroborated by estimating the FUS/PEG dominance ratio using Eq. (3). The phase boundary gradient is $\frac{dc_+^2}{dc_+^1}|_{S_{12}} \approx -10^3$ since the boundary varies in the μM range for FUS but the mM range for PEG (1% in PEG corresponds to 1.6 mM in molar concentration), leading to $\frac{D^1}{D^2} \approx 10^2$. Using the measured FUS dominance $D^1 \approx 0.6$ in this concentration regime [7], we can estimate $D^2 \approx 0.006$, leading to $\zeta \approx \frac{0.006}{1-0.6} = 0.015$, consistent with the earlier value.

In [10], phase diagrams between the mutant FUS(G156E) and PEG (molecular weight 6 kDa), in the presence or absence of the small molecule bis-ANS, are also measured [Figs. 4(b) and 4(c)]. Compared to the wild-type FUS discussed before, the G156E mutant (where a glycine residue is replaced by a glutamic acid residue at position 156) is more prone to aggregation and implicated in the development of Amyotrophic Lateral Sclerosis (ALS) [26]. The phase-separated regions are markedly larger in the latter cases, and typically this is interpreted as a generic increase in phase separation propensity with the mutation and the small molecule. Equipped with Eq. (1), we further draw quantitative insights from these data. Repeating the log-log scaling analysis, we obtain for the dense phase FUS(G156E)/PEG molar ratio as $c_+^{FUS(G156E)}/c_+^{PEG} = 0.555 \pm 0.028$ [Fig. 4(b)], significantly higher than that obtained for the wild-type FUS [Fig. 4(a)]. Upon addition of bis-ANS, which is documented to be a potent modulator of phase behavior [27], this ratio further increases to $c_+^{FUS(G156E)}/c_+^{PEG} = 1.12 \pm 0.07$ [Fig. 4(c)] [25]. These results are in agreement with the fact that the G156E mutation enhances FUS self-association [26], and the role of bis-ANS as a bivalent linker that can strengthen hydrophobic interactions crucial for FUS condensate formation [5] [Figs. 4(d) and 4(e)].

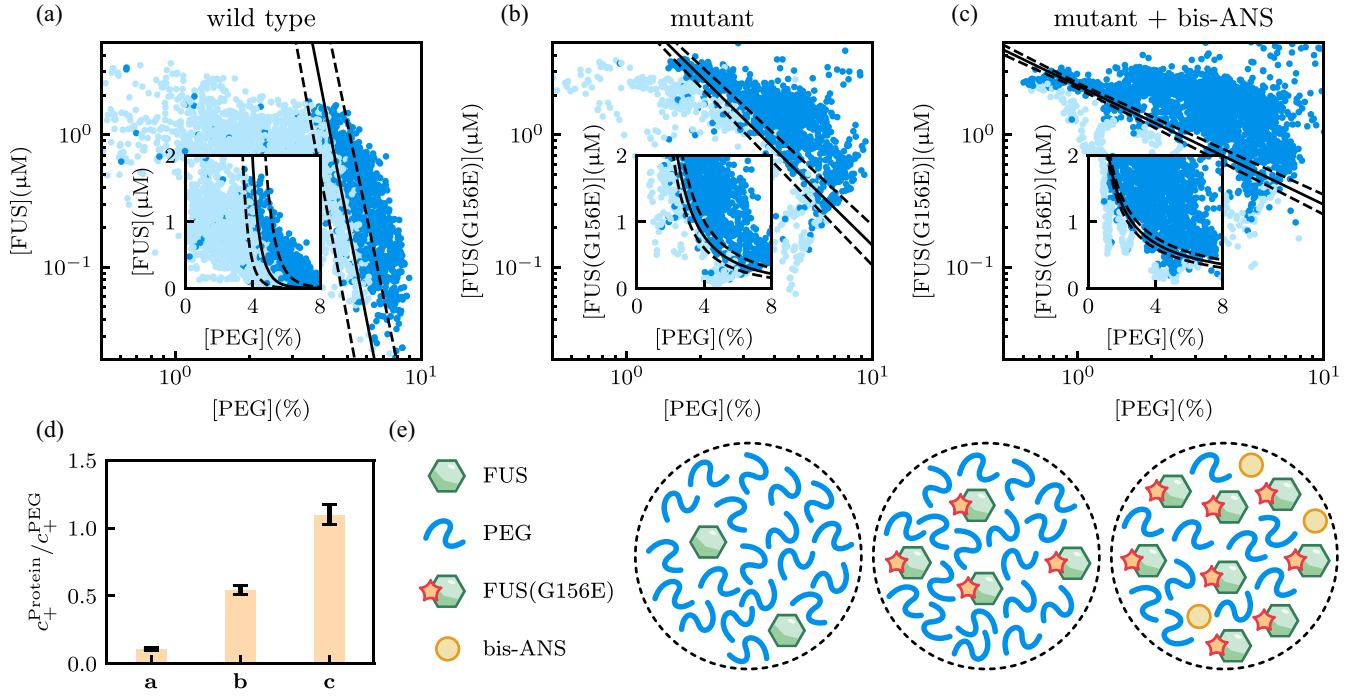


FIG. 4. Phase behavior of FUS with PEG. (a) PhaseScan experiment performed with wild-type FUS is analyzed in double logarithmic scale, giving a dense phase molar ratio $c_{+}^{\text{FUS}}/c_{+}^{\text{PEG}} = 0.110 \pm 0.008$. Inset: same data plotted on a linear scale. (b),(c) We take PhaseScan data of the mutant FUS(G156E) from [10] in the absence and presence of the small-molecule modulator bis-ANS. The mutant FUS is more preferentially enriched than the wild-type FUS, with a dense phase molar ratio $c_{+}^{\text{FUS(G156E)}}/c_{+}^{\text{PEG}} = 0.555 \pm 0.028$. When the small-molecule bis-ANS is added, this further increases to $c_{+}^{\text{FUS(G156E)}}/c_{+}^{\text{PEG}} = 1.12 \pm 0.07$. The phase boundary also shifts towards lower PEG, indicating that bis-ANS enhances the phase separation propensity of FUS(G156E) [10,26]. (d) Summary of protein-to-PEG dense phase molar ratios. (e) Graphical illustration of the estimated condensate stoichiometry values.

C. Quantifying Whi3/RNA binding disruption confirms Whi3's role as a condensate scaffold

As a more biologically relevant example, we analyze data from [28] where the phase behavior of the BNI1 RNA with protein Whi3 was studied. Whi3 is an RNA-binding protein that forms condensates with many different types of RNA, and is thus commonly characterized as a scaffold protein [28]. BNI1 RNA is an mRNA encoding the BNI1 protein that phase-separates with Whi3. BNI1 RNA has five cognate binding sites for Whi3, and in [28] phase diagrams of the wild-type BNI1 RNA as well as a mutant RNA with cognate sites shuffled were measured [Figs. 5(a) and 5(b)]. One complication here is that the RNA can dissolve condensates at high RNA concentrations [Fig. 5(b), left] so we focus on the low-concentration regime for fitting with Eq. (1). We find that the RNA-to-Whi3 molar ratio decreases upon cognate site shuffling, from $c_{+}^{\text{RNA}}/c_{+}^{\text{Whi3}} = (1.7 \pm 0.7)$ to $c_{+}^{\text{RNA}}/c_{+}^{\text{Whi3}} = (0.72 \pm 0.16)$ [Figs. 5(b) and 5(c)] [25]. This suggests the disrupted Whi3/RNA interaction leads to a more Whi3-rich condensate, consistent with the picture that Whi3 acts as a scaffold protein that recruits RNAs via associative interaction [Fig. 5(d)]. This insight would not have been possible to derive by just comparing the phase boundaries, highlighting the value of the quantitative phase boundary analysis framework developed here.

The dissolution trend of Whi3/RNA condensates with increasing RNA concentrations can in principle be analyzed

using Eq. (2), and for completeness we take a small dataset from [29] and demonstrate that Eq. (2) indeed captures the phase boundary shape. In [29], the dissolution effect of CLN3 RNA, an mRNA encoding the protein CLN3, is mapped out against Whi3 (Fig. 6). Following the notation of Eq. (2), since RNA is dissolving condensates, we assign index 1 to Whi3 and index 2 to RNA. The Whi3/RNA phase boundary shape is then [compare to Eq. (2)]

$$c_{-}^{\text{Whi3}} = \bar{c}_{-}^{\text{Whi3}} \exp[\mu c_{-}^{\text{RNA}}], \quad (5)$$

where the constant right-hand side of Eq. (2) is collapsed into a single variable μ since we do not have prior information on which three-body interaction is responsible for the dissolution. μ has the dimension of $k_B T$ (in this work we have set $k_B T = 1$) per concentration unit and represents a concentration-normalized effective repulsion energy for RNA molecules. The inverse of μ then provides an RNA concentration scale over which dissolution occurs. We fit the phase boundary to a straight line in a log-linear plot, and fitting parameters are $\bar{c}_{-}^{\text{Whi3}} = (0.09 \pm 0.04) \mu\text{M}$ and $\mu = 9 \pm 3 k_B T / \mu\text{M}$, so the CLN3 RNA dissolution occurs over a characteristic range of $\sim 0.1 \mu\text{M}$. Detailed analysis scripts can be found in [25]. Equation (5) indeed captures the phase boundary shape to a good extent (Fig. 6), however the sampling in [29] was done in double logarithmic scale, leading to data sparsity in linear scale (Fig. 6, right). To further demonstrate the use of Eq. (2) we turn to publications that performed more extensive experiments on condensate dissolution.

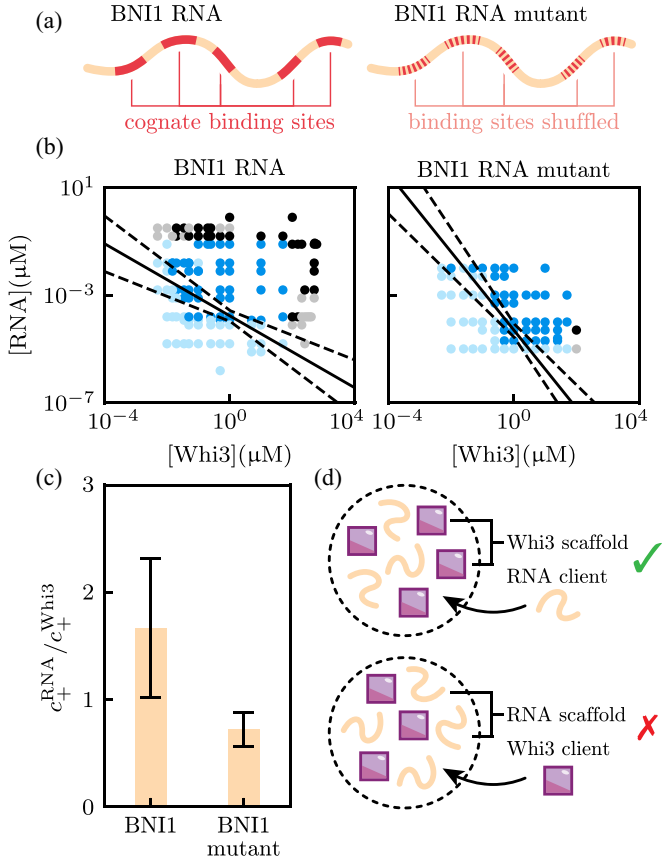


FIG. 5. Whi3/RNA phase behavior changes with RNA cognate binding sites shuffling. (a) Phase behavior of BNI1 RNA and Whi3 were studied in [28]. BNI1 RNA (top) has five cognate binding sites for Whi3, and a mutant with the cognate sites shuffled was also studied (bottom). (b) Phase diagrams of Whi3 against the wild-type (left) and shuffled (right) BNI1 RNA measured in [28]. For phase boundary fitting, we only use points with $[\text{Whi3}] < 100 \mu\text{M}$ and $[\text{RNA}] < 0.1 \mu\text{M}$, as points with higher concentrations might break the strong enrichment assumption and incur dissolution effects [29]. Light and dark blue scatter points are homogeneous and phase-separated data used for the fitting. Gray (homogeneous) and black (phase-separated) points are masked out during fitting. (c) Dense phase molar ratios of the RNAs against Whi3. A decrease in relative RNA content from $c_{+}^{\text{RNA}}/c_{+}^{\text{Whi3}} = (1.7 \pm 0.7)$ to $c_{+}^{\text{RNA}}/c_{+}^{\text{Whi3}} = (0.72 \pm 0.16)$ is observed here after shuffling the cognate sites. This suggests the disruption of Whi3/RNA binding results in RNA leaving the now Whi3-rich condensates, as expected of the common denomination of Whi3 as a scaffold protein. (d) The quantitative analysis and interpretation method allows us to distinguish the scaffold/client behavior of the Whi3/RNA system, which is not possible by qualitative inspection of the phase boundaries.

D. Multicomponent G3BP1 phase boundary reveals nuanced RNA dissolution behaviour

G3BP1 is an RNA-binding protein central to stress granule formation [31], and in [19], phase separation of G3BP1 in the presence of poly(A)-RNA and the small molecule suramin is studied, where detailed phase diagrams are measured [Fig. 7(a)]. Contrary to the convex phase boundary shape suggested by Eq. (2), the G3BP1/RNA boundary is concave [19] [Fig. 7(b)], indicating a breakdown of some

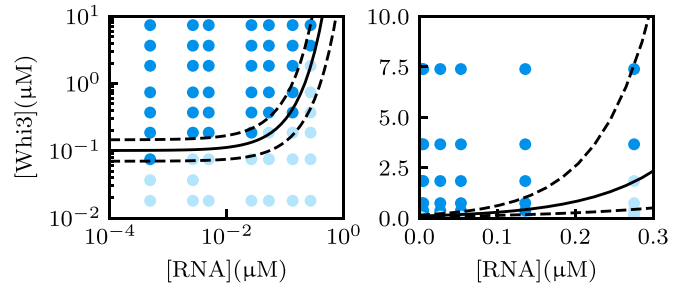


FIG. 6. Exponential function captures CNL3 RNA/Whi3 dissolution boundary. We analyze phase separation data from [29] using Eq. (5), and plot the results in double logarithmic (left) and linear (right) scales. The original sampling was performed in double logarithmic scale, so data appear sparse in the linear scale. Fitting an exponential function leads to a characteristic CNL3 RNA chemical potential of $\mu = 9 \pm 3 k_B T / \mu\text{M}$.

assumptions used in the derivation, such as the constant dense phase composition. However, the G3BP1/suramin boundary conforms well with the exponential shape, and we now show that these additional experimental data allow us to deduce the dense phase RNA concentration change, and consequently explain the concave G3BP1/RNA phase boundary shape.

We first focus on the G3BP1/suramin phase boundaries. Since suramin is found to dissolve condensates by disrupting G3BP1/RNA interactions [19], the three-body interaction of Eq. (2) is a G3BP1/RNA/suramin term. The fitting function is

$$c_{-}^{\text{G3BP1}} = \bar{c}_{-}^{\text{G3BP1}} \exp[(\epsilon c_{+}^{\text{RNA}}) c_{-}^{\text{suramin}}], \quad (6)$$

where $\bar{c}_{-}^{\text{G3BP1}}$ is the saturation concentration of G3BP1 in the absence of suramin, and the G3BP1/RNA/suramin three-body repulsion energy density is written as ϵ . This functional form fits the phase boundary very well [Fig. 7(a)] [25], and the fitting constants at different levels of total [RNA] [Figs. 7(b) and 7(c)] allow us to deduce additional insights to the system. The fitted $\bar{c}_{-}^{\text{G3BP1}}$ values are effectively the G3BP1/RNA phase boundary in the absence of suramin [Fig. 7(b)]. The interesting part is the parameter $\epsilon c_{+}^{\text{RNA}}$ [Fig. 7(c)]. Since ϵ is a constant, changes in $\epsilon c_{+}^{\text{RNA}}$ at different total [RNA] should arise from changes in dense phase [RNA]. In deriving Eq. (2), we assumed c_{+}^{RNA} is constant, while Fig. 7(c) suggests that c_{+}^{RNA} in fact decreases with c_{-}^{RNA} . To analytically incorporate the effect of changes in dense phase concentrations, an expression relating c_{+}^{RNA} to c_{-}^{RNA} is required. First-principles calculation is intractable in this scenario, so we resort to a phenomenological fit of Fig. 7(c) and investigate its effects on the phase boundary. Many fitting functional forms can be used, and we choose the exponential function here to write c_{+}^{RNA} as a function of c_{-}^{RNA} , since it fits to the data well while only introducing two free parameters:

$$c_{+}^{\text{RNA}} = \bar{c}_{+}^{\text{RNA}} e^{-c_{-}^{\text{RNA}} / \Delta c^{\text{RNA}}}. \quad (7)$$

We find the RNA concentration scale $\Delta c^{\text{RNA}} = (33.7 \pm 1.3) \text{ nM}$, or $(70.8 \pm 2.8) \text{ ng}/\mu\text{L}$ [Fig. 7(c)]. This allows us to account for the change in dense phase [RNA] when deriving the phase boundary scaling function of G3BP1 against RNA,

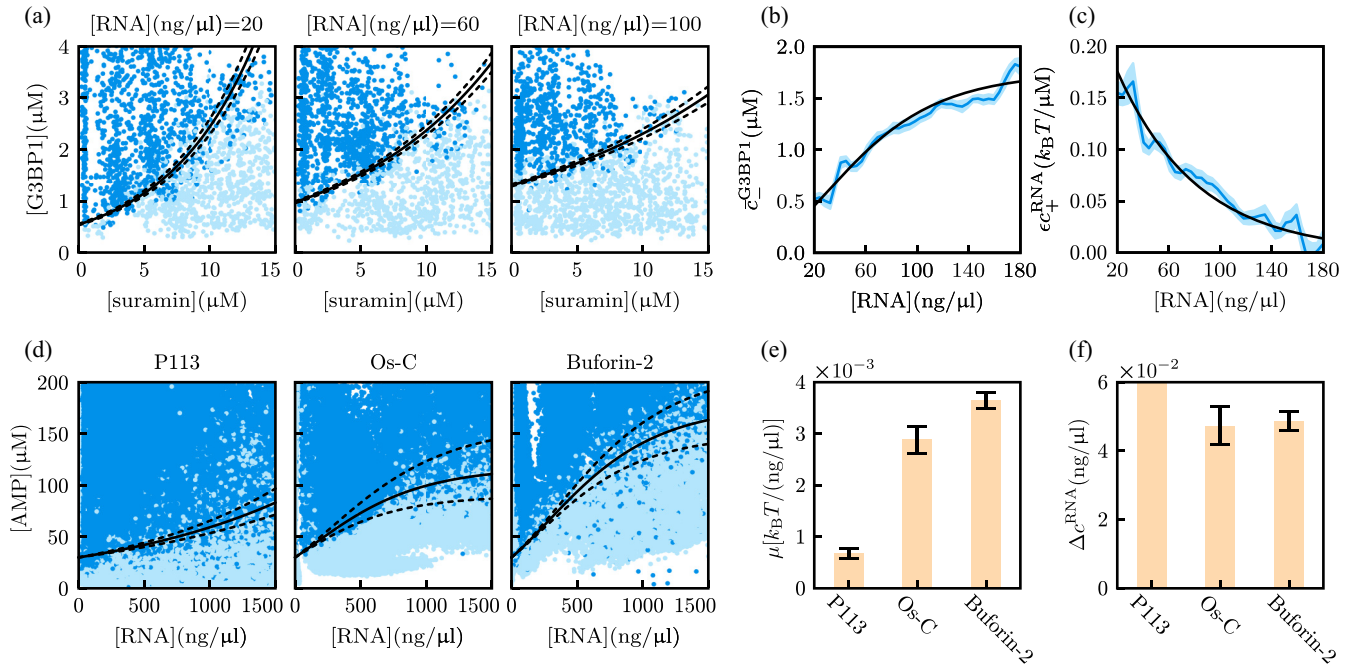


FIG. 7. Condensate dissolution boundary analysis in more complex scenarios. (a) In [19], condensates formed by G3BP1 and poly(A)-RNA are dissolved by the small molecule suramin at varying levels of total [RNA]. An exponential functional form [Eq. (6)] is fitted to the phase boundary (black lines). (b) The G3BP1 saturation concentration in the absence of suramin increases with total [RNA] (blue solid line, and blue shade represents the error estimate). This is effectively the G3BP1/RNA phase boundary, and the simple form of Eq. (2) cannot explain its concave shape. Drawing from the hint that dense phase [RNA] is changing at different total [RNA], we use Eq. (8) for the fitting instead (black solid line). (c) The fitting constant $\epsilon c_+^{\text{RNA}}$ decreases as total [RNA] increases due to the dissolution effect of RNA. This implies the dense phase [RNA] decreases with total [RNA]. Black solid line is a fit using Eq. (7). (d) Phase boundaries of three antimicrobial peptides against poly(A)-RNA from [30]. Black solid and dashed lines are fits using Eq. (9). (e) The effective concentration-normalized repulsion energy μ is lowest for P113, also reflected in the least dissolution effect among the AMPs. Buforin-2 has the highest μ , in line with its strongest dissolution tendency. (f) Characteristic RNA concentration Δc^{RNA} over which dense phase [RNA] decreases. The value obtained for P113 is very large, recovering the functional form of Eq. (2). Δc^{RNA} estimated for Os-C and Buforin-2 are, however, similar, so the difference seen in experiments can be attributed to repulsion energy.

leading to (Appendix F)

$$\bar{c}_{-}^{\text{G3BP1}} = \bar{c}_{-}^{\text{G3BP1}} \exp[-\epsilon' \bar{c}_{+}^{\text{RNA}} \Delta c^{\text{RNA}} e^{-c_{-}^{\text{RNA}}/\Delta c^{\text{RNA}}}], \quad (8)$$

where ϵ' is a protein/RNA/RNA three-body repulsion energy density, $\bar{c}_{-}^{\text{G3BP1}}$ is another fitting parameter that fixes the y-intercept, and we take a previously obtained Δc^{RNA} value. Equation (8) accounts for the data well [Fig. 7(b)], and this example illustrates the flexibility of the approach developed to analyze phase boundary scaling: by writing the phase boundary normal components as sums of terms, increasingly complex scenarios can be included.

E. Quantifying repulsion strength in antimicrobial peptide systems

The concave shape of Fig. 7(c) is rather common for protein/RNA dissolution phase boundaries, and we hypothesize that despite the phenomenological nature of Eq. (7), the derived form [Eq. (8)] can still be used on other protein/RNA systems. We take phase boundary data from [30], where the phase behaviors of three antimicrobial peptides (AMPs), P113, Os-C, and Buforin-2, in the presence of poly(A)-RNA, were mapped out. Equation (8) represents a three-parameter fit if Δc^{RNA} is not estimated. To better constrain the fitting,

we use the following functional form:

$$c_{-}^{\text{AMP}} = \bar{c}_{-}^{\text{AMP}} \exp[\mu \Delta c^{\text{RNA}} (1 - e^{-c_{-}^{\text{RNA}}/\Delta c^{\text{RNA}}})]. \quad (9)$$

Equation (9) is equivalent to Eq. (8) upon a redefinition of fitting parameters, but has the nicer property that the y-intercept is fixed at $\bar{c}_{-}^{\text{AMP}}$ as the exponent vanishes at $c_{-}^{\text{RNA}} = 0$. We fix $\bar{c}_{-}^{\text{AMP}} = 30 \mu\text{M}$ for all three datasets. This constrains the y-intercepts of the fits, and the individual fitting parameters are μ , which has the dimension of repulsion energy per unit RNA concentration, and Δc^{RNA} , a characteristic RNA concentration scale over which dissolution affects dense phase RNA concentration. Equation (9) provides a good fit to the three phase boundaries [Fig. 7(d)]. In the limit of large Δc^{RNA} we recover Eq. (2). Detailed analysis code can be found in [25]. It was originally found in [30] that P113 has the highest phase-separation propensity with RNA, with the largest phase-separated region among the three AMPs [Fig. 7(d)]. Indeed, comparing the fitted μ values shows that P113 has the smallest concentration-normalized repulsion energy, followed by Os-C and Buforin-2 [Fig. 7(e)]. The Δc^{RNA} estimated for P113 is very large, hinting that dense phase RNA concentration is hardly changing [Fig. 7(f)]. Interestingly, Os-C and Buforin-2 have similar Δc^{RNA} , and the difference in measured phase boundary solely originates from μ [Fig. 7(f)].

In addition, since the RNA used is the same across these experiments, the repulsive term has to involve the AMP, further supporting the assumption used to derive Eq. (8) in Appendix F. Taken together, this final example shows Eqs. (2) and (9) can be helpful tools to extract quantitative information from experimentally measured phase boundaries when dissolution is observed.

IV. DISCUSSION

Obtaining high-dimensional phase boundaries in theoretical models is a daunting task, and the framework we developed here represents an alternative route towards quantitative interpretation of experimental data. The two general phase behaviors we explored are observed in many multi-component systems, and connections can be made with other existing theoretical frameworks, too.

It is instructive to compare Eq. (1) with mass action in chemical kinetics. For a process where molecular species A and B combine to yield C



with a, b, c being the stoichiometric coefficients, we have at equilibrium $K^{\text{eq}}[A]^a[B]^b = [C]^c$ for an equilibrium constant K^{eq} . The derivation of this relation is mathematically similar to the binodal equations, where the chemical potential densities of the A and B molecules in the monomeric state and the combined C state are equalized, originating from global free-energy minimization with mass conservation constraints. Taking the logarithm of both sides of Eq. (10) and perturbing the concentrations, while assuming the concentration $[C]$ is approximately a constant (such as C being in a different phase), we obtain

$$d(a \ln[A]) + d(b \ln[B]) \approx 0 \rightarrow \frac{d \ln[A]}{d \ln[B]} \approx -\frac{b}{a}. \quad (11)$$

As such, Eq. (1) has a simple physical interpretation as a solubility product. In [32,33], a similar solubility product idea is proposed, where the dilute phase solute concentrations are multiplied together instead of using the phase boundary values. Using the dilute phase response relations derived in [7], we show that the solubility product of [32,33] is closely related to the phase boundary scaling Eq. (1) via dominance values of the two solutes as well as tie line vector components (Appendix G).

For condensate dissolution, we show that three-body repulsion is necessary in practical situations, and a generic exponential phase boundary shape ensues. The fitting parameter in this case relates to the three-body repulsion energy and dense phase concentrations, so explicit interaction energy values could in principle be obtained if the dense phase concentration can be measured. Furthermore, we find that the concave phase boundary shape typically exhibited by protein/RNA systems can be rationalized by a changing dense phase RNA concentration, which we first deduced from the G3BP1/RNA/suramin dataset of [19] and subsequently deployed to analyze datasets of [30]. Experimental confirmation of Eq. (7) via dense phase quantification in the future will provide further support for the theory. A more interesting direction that this opens up is in linking the

dissolution phenomenon with more detailed mechanisms, such as molecular binding. It is usually surmised that strong protein/RNA binding underlies stable complex formation and subsequent condensate dissolution, and modeling the binding as introducing a new inert molecular species could lead to a binding-associated free-energy contribution as a series expansion at low concentrations, drawing connection between three-body repulsion energy and the binding constant.

A pertinent issue that emerges with the quantitative phase boundary analysis method is the reliability of the data and the fit. Phase diagrams generated using a conventional hand-pipetting method are usually low-resolution, and small changes in the phase boundary upon modulation effects can be hard to discern. The majority of the datasets analyzed here are obtained using the high-throughput PhaseScan platform [10], but some practical problems also exist. For instance, the component concentrations in each sample are tracked using the fluorescent intensities of barcode dyes, and correcting channel cross-talk is paramount to obtaining quantitatively correct phase boundary positions. Furthermore, with the exception of the antimicrobial peptide dataset, the numerical fittings are done using a linear support vector machine, and the fitting quality can deteriorate if the phase diagrams have a disproportionately large number of phase-separated or homogeneous samples. One method to circumvent the issue is by first discretizing the whole concentration space and identifying phase boundary locations using information in each local grid, and then fitting the phase boundary to the identified points, as is done for the antimicrobial peptide dataset. A more fruitful approach will be to augment the screening platform to automatically adjust the concentration ranges over which the phase diagram is measured, and focus on the most information-rich region to scan over. Hopefully, the new possibilities introduced by the quantitative framework can motivate these engineering efforts towards generating more robust, detailed phase diagrams.

In sum, the general mathematical formulation of the phase boundary normal expression allows a quantitative interpretation of phase diagrams without explicitly solving for the phase equilibrium. This work makes extensive use of the theoretical results to demonstrate the applicability of the phase boundary expressions, and the accompanying datasets and analysis scripts can be valuable for future experimental investigations.

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The authors declare no conflicts of interest.

DATA AVAILABILITY

Curated datasets and analysis scripts can be found on [25].

APPENDIX A: MATHEMATICAL DERIVATIONS

We adopt notations from [7]. In an N -component phase separation system, we denote the average volume fraction of component α as ϕ^α , where $\alpha = 1, 2, \dots, N$, and the free-energy density of a homogeneous composition ϕ^α as $f(\phi)$, where the superscript is dropped in function arguments for brevity. In the derivation, the unit thermal energy is set to unity $k_B T = 1$. Given dilute and dense phase component volume fractions $\psi_-^\alpha, \psi_+^\alpha$, as well as the tie line vector connecting the points $k^\alpha \equiv \psi_+^\alpha - \psi_-^\alpha$, the chemical and osmotic equilibrium conditions are [7]

$$(\partial_\alpha f)|_{\psi_-} = (\partial_\alpha f)|_{\psi_+}, \quad (\text{A1a})$$

$$\sum_{\alpha=1}^N \psi_-^\alpha (\partial_\alpha f)|_{\psi_-} - f(\psi_-) = \sum_{\beta=1}^N \psi_+^\beta (\partial_\beta f)|_{\psi_+} - f(\psi_+), \quad (\text{A1b})$$

where $\partial_\alpha \equiv \frac{\partial}{\partial \phi^\alpha}$. Perturbations of Eqs. (A1) lead to an expression of the phase boundary normal vector n_α [7], and we outline the detailed derivation here, which was lacking before. Perturbing the left-hand side of Eq. (A1a) gives

$$\begin{aligned} \delta[(\partial_\alpha f)|_{\psi_-}] &\equiv (\partial_\alpha f)|_{\psi_- + \delta\psi_-} - (\partial_\alpha f)|_{\psi_-} \\ &= (\partial_\alpha f)|_{\psi_-} + \sum_{\beta=1}^N \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-} - (\partial_\alpha f)|_{\psi_-} \\ &= \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-}, \end{aligned} \quad (\text{A2})$$

and repeating it for the right-hand-side dense phase point leads to

$$\sum_{\beta=1}^N \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-} = \sum_{\gamma=1}^N \delta\psi_+^\gamma (\partial_\alpha \partial_\gamma f)|_{\psi_+}. \quad (\text{A3})$$

Similarly, we apply a perturbation to Eq. (A1b). We use the relations

$$\begin{aligned} &\delta \left[\sum_{\alpha=1}^N \psi_-^\alpha (\partial_\alpha f)|_{\psi_-} \right] \\ &= \sum_{\alpha=1}^N \delta\psi_-^\alpha (\partial_\alpha f)|_{\psi_-} + \sum_{\alpha,\beta=1}^N \psi_-^\alpha \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-}, \end{aligned} \quad (\text{A4})$$

as well as

$$\delta[f(\psi_-)] = \sum_{\alpha=1}^N \delta\psi_-^\alpha (\partial_\alpha f)|_{\psi_-}, \quad (\text{A5})$$

so that the first-order perturbation of Eq. (A1b) is

$$\sum_{\alpha,\beta=1}^N \psi_-^\alpha \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-} = \sum_{\gamma,\sigma=1}^N \psi_+^\gamma \delta\psi_+^\sigma (\partial_\gamma \partial_\sigma f)|_{\psi_+}. \quad (\text{A6})$$

Substituting Eq. (A3) into the right-hand side of Eq. (A6) gives

$$\sum_{\alpha,\beta=1}^N \psi_-^\alpha \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-} = \sum_{\gamma,\sigma=1}^N \psi_+^\gamma \delta\psi_+^\sigma (\partial_\gamma \partial_\sigma f)|_{\psi_+}, \quad (\text{A7})$$

and using $k^\alpha \equiv \psi_+^\alpha - \psi_-^\alpha$ this can be simplified into

$$\sum_{\alpha,\beta=1}^N k^\alpha \delta\psi_-^\beta (\partial_\alpha \partial_\beta f)|_{\psi_-} = 0. \quad (\text{A8})$$

Since the phase boundary normal is defined by

$$\sum_{\alpha} n_\alpha \delta\psi_-^\alpha = 0, \quad (\text{A9})$$

in essence any perturbation must lie on this boundary and thus have zero overlap with the normal to the surface, direct inspection gives

$$n_\alpha \propto \sum_{\beta=1}^N k^\beta \partial_\alpha \partial_\beta f|_{\psi_-}. \quad (\text{A10})$$

Equation (A10) can in principle be used as it is, and one could further apply chemical potential density equilibrium to simplify expressions. We now derive another form of n_α by combining Eq. (A10) with Eq. (A1a). Chemical potential densities are defined as $\mu_\alpha(\phi) \equiv (\partial_\alpha f)|_\phi$, and chemical equilibrium requires $0 = \mu_\alpha(\psi_+) - \mu_\alpha(\psi_-)$. In addition, we have $\psi_+^\alpha = \psi_-^\alpha + k^\alpha$ by definition, so that we can write chemical equilibrium in terms of quantities evaluated at the dilute phase $0 = \mu_\alpha(\psi_+) - \mu_\alpha(\psi_-) = \sum_{i=1}^{\infty} \frac{1}{i!} (\sum_{\beta=1}^N k^\beta \partial_\beta)^i \partial_\alpha f|_{\psi_-}$. The $i = 1$ term is proportional to n_α , so

$$n_\alpha \propto - \sum_{i=2}^{\infty} \frac{1}{i!} \left(\sum_{\beta=1}^N k^\beta \partial_\beta \right)^i \partial_\alpha f \Big|_{\psi_-}. \quad (\text{A11})$$

We now take as $f(\phi)$ a sum of components' translational entropy, solvent entropy, and many-body interactions. It is convenient to define the solvent volume fraction as a function $\phi^0(\phi) \equiv 1 - \sum_{\alpha=1}^N \phi^\alpha$, giving

$$\begin{aligned} f(\phi) &= \sum_{\alpha=1}^N \frac{\phi^\alpha}{L^\alpha} \ln \phi^\alpha + \phi^0(\phi) \ln[\phi^0(\phi)] \\ &+ \sum_{i=2}^{\infty} \frac{1}{i!} \sum_{\alpha_1, \dots, \alpha_i=1}^N \chi_{\alpha_1 \dots \alpha_i}^{(i)} \phi^{\alpha_1} \dots \phi^{\alpha_i}, \end{aligned} \quad (\text{A12})$$

where L^α is the size of component α , and $\chi_{\alpha_1 \dots \alpha_i}^{(i)}$ is the i -body contact energy. When only binary interactions $\chi_{\alpha_1 \alpha_2}^{(2)}$ are considered, we recover the Flory-Huggins model [34,35]. Substituting Eq. (A12) into Eq. (A11) term by term leads to an expression for n_α . The contribution to n_α from the first term of Eq. (A12) is

$$\begin{aligned} &- \sum_{i=2}^{\infty} \frac{1}{i!} \left(\sum_{\beta=1}^N k^\beta \partial_\beta \right)^i \left(\sum_{\gamma=1}^N \frac{\phi^\gamma}{L^\gamma} \ln \phi^\gamma \right) \Big|_{\psi_-} \\ &= - \sum_{i=2}^{\infty} \frac{1}{i!} \left(\sum_{\beta=1}^N k^\beta \partial_\beta \right)^i \left(\frac{\phi^\alpha}{L^\alpha} \ln \phi^\alpha \right) \Big|_{\psi_-} \end{aligned}$$

$$\begin{aligned}
 &= -\frac{1}{L^\alpha} \sum_{i=2}^{\infty} \frac{1}{i!} (k^\alpha \partial_\alpha)^i \partial_\alpha (\phi^\alpha \ln \phi^\alpha) \Big|_{\psi_-} \\
 &= -\frac{1}{L^\alpha} \sum_{i=2}^{\infty} \frac{1}{i!} (k^\alpha)^i (\partial_\alpha)^{i+1} (\phi^\alpha \ln \phi^\alpha) \Big|_{\psi_-}. \quad (\text{A13})
 \end{aligned}$$

Note the generic relations

$$\frac{d^i}{dx^i} (x \ln x) = (-1)^i \frac{(i-2)!}{x^{i-1}} \quad \text{for } i \geq 2, \quad (\text{A14a})$$

$$\sum_{i=2}^{\infty} \frac{(-x)^i}{i} = x - \ln(1+x). \quad (\text{A14b})$$

We can further simplify Eq. (A13) to

$$\begin{aligned}
 &-\frac{1}{L^\alpha} \sum_{i=2}^{\infty} \frac{1}{i!} (k^\alpha)^i (\partial_\alpha)^{i+1} (\phi^\alpha \ln \phi^\alpha) \Big|_{\psi_-} \\
 &= -\frac{1}{L^\alpha} \sum_{i=2}^{\infty} (k^\alpha)^i \frac{1}{i!} (-1)^{i+1} \frac{(i-1)!}{(\psi_-^\alpha)^i} \\
 &= \frac{1}{L^\alpha} \sum_{i=2}^{\infty} \frac{1}{i} \left(-\frac{k^\alpha}{\psi_-^\alpha} \right)^i \\
 &= \frac{1}{L^\alpha} \left[\frac{k^\alpha}{\psi_-^\alpha} - \ln \left(1 + \frac{k^\alpha}{\psi_-^\alpha} \right) \right]. \quad (\text{A15})
 \end{aligned}$$

In the first line we used Eq. (A14a), and in the last line Eq. (A14b). The contribution from the entropic term of Eq. (A12) can be calculated in a similar fashion, leading to a compact form for n_α :

$$n_\alpha \propto Q_\alpha - Q_0 + X_\alpha, \quad (\text{A16})$$

where

$$\begin{aligned}
 Q_\alpha &\equiv \frac{1}{L^\alpha} \left[\frac{k^\alpha}{\psi_-^\alpha} - \ln \left(1 + \frac{k^\alpha}{\psi_-^\alpha} \right) \right], \\
 Q_0 &\equiv \frac{k^0}{\psi_-^0} - \ln \left(1 + \frac{k^0}{\psi_-^0} \right), \quad (\text{A17})
 \end{aligned}$$

are terms arising from the entropic free energies, with short-hands $\psi_-^0 \equiv \phi^0(\psi_-)$, $\psi_+^0 \equiv \phi^0(\psi_+)$, and $k^0 \equiv \psi_+^0 - \psi_-^0$. X_α contains interaction terms, and only interactions involving three or more bodies enter the X_α expression, with the leading term $X_\alpha^{(3)}$

$$X_\alpha^{(3)} = -\frac{1}{2} \sum_{\beta_1, \beta_2=1}^N \chi_{\alpha\beta_1\beta_2}^{(3)} k^{\beta_1} k^{\beta_2}. \quad (\text{A18})$$

Two-body interactions have disappeared as they are absorbed into Q_α through the chemical equilibrium. Notice all Q_α 's and Q_0 are non-negative since $x - \ln(1+x) \geq 0 \forall x \in \mathbb{R}$. We expect the solvent entropy contribution to be negligible in practical cases, $Q_0 \approx 0$. This is because the volume fraction of water is large in both dilute and dense phases in experiments, far from the logarithmic singularity, and furthermore a large Q_0 would mean any molecule added to *in vitro* experiments can dissolve condensates, which is not the case. As a result, in the absence of three- or more-body interactions ($X_\alpha = 0$), the ratio between any two phase boundary normal components

is always positive, and one cannot dissolve condensates via addition of another molecular species.

Equation (A16) gives the general phase boundary normal direction. To make use of n_α , note that for a vector $\delta\psi_-^\alpha$ lying on the phase boundary, we have by definition

$$\sum_{\alpha=1}^N n_\alpha \delta\psi_-^\alpha = 0. \quad (\text{A19})$$

Since total concentrations of only components 1 and 2 are allowed to vary on S_{12} , $\delta\psi_-^3 = \dots = \delta\psi_-^N = 0$. As a result,

$$\frac{n_2}{n_1} = - \frac{\delta\psi_-^1}{\delta\psi_-^2} \Big|_{S_{12}}. \quad (\text{A20})$$

The right-hand side is closely related to the phase boundary gradient in S_{12} , so an expression for n_2/n_1 allows a link between the experimental observable and the phase space to be established.

Assume solute 1 (without loss of generality) is strongly enriched such that $\frac{1}{L^1} \frac{k^1}{\psi_-^1} \approx \frac{1}{L^1} \frac{\psi_+^1}{\psi_-^1} \gg 1$. Meanwhile, since ψ_-^α and k^α are of $\mathcal{O}(1)$ (volume fractions), and the interaction χ 's are of $\mathcal{O}(1)$ in units of thermal energy $k_B T$, we have $X_\alpha \sim \mathcal{O}(1)$. In the strong enrichment regime, we can approximate

$$n_1 \propto \frac{1}{L^1} \frac{k^1}{\psi_-^1}. \quad (\text{A21})$$

If there is a component 2 that is also strongly enriched, using Eq. (A20) we get

$$-\frac{\delta\psi_-^1}{\delta\psi_-^2} \Big|_{S_{12}} = \frac{k^2 L^1 \psi_-^1}{k^1 L^2 \psi_-^2} \rightarrow -\frac{\delta \ln \psi_-^1}{\delta \ln \psi_-^2} \Big|_{S_{12}} = \frac{k^2/L^2}{k^1/L^1}. \quad (\text{A22})$$

Finally, we convert volume fractions $\psi_-^\alpha, \psi_+^\alpha$ to their counterparts in molar concentration unit c_-^α, c_+^α using

$$\begin{aligned}
 \psi_-^\alpha &= v L^\alpha c_-^\alpha, \\
 \psi_+^\alpha &= v L^\alpha c_+^\alpha, \\
 k^\alpha &= v L^\alpha (c_+^\alpha - c_-^\alpha), \quad (\text{A23})
 \end{aligned}$$

where v is the volume of one mole of lattice sites, to give

$$\frac{d \ln c_-^1}{d \ln c_-^2} \Big|_{S_{12}} = -\frac{c_+^2}{c_+^1}. \quad (\text{A24})$$

Here we also used $c_+^\alpha \gg c_-^\alpha$. This is Eq. (1).

To give the dominance ratio between solutes 1 and 2, recall that [7]

$$D^\alpha = \frac{n_\alpha k^\alpha}{\sum_{\gamma=1}^N n_\gamma k^\gamma}, \quad (\text{A25})$$

so

$$\frac{D^1}{D^2} = \frac{n_1 k^1}{n_2 k^2} = \frac{n_1}{n_2} \frac{n_1 L^1 \psi_-^1}{n_2 L^2 \psi_-^2}. \quad (\text{A26})$$

After algebraic simplifications we obtain

$$\frac{D^1}{D^2} = \frac{dc_-^2}{dc_-^1} \Big|_{S_{12}} \frac{d \ln c_-^2}{d \ln c_-^1} \Big|_{S_{12}} = -\frac{dc_-^2}{dc_-^1} \Big|_{S_{12}} \frac{c_+^1}{c_+^2}. \quad (\text{A27})$$

Next, we derive the phase boundary shape in the case of condensate dissolution. Suppose condensates formed by component 1 are dissolved by the addition of a component 2, through a ternary interaction with component 3 with $\chi_{123}^{(3)} > 0$ (or with component 1, in which case the derivation is similar). We use Eq. (A21) for n_1 and take $n_2 \approx X_2$, assuming component 2 partitioning and solvent entropy are weak factors. This allows us to understand the effect of three-body interactions, and deviations of the phase boundary from the analytical form can be used to detect additional factors, such as a nonzero Q_2 term. Adopting the simplifying assumptions leads to

$$\left. -\frac{\delta \psi_-^1}{\delta \psi_-^2} \right|_{S_{12}} = \frac{n_2}{n_1} \approx \frac{-\frac{1}{2}[\chi_{132}^{(3)} + \chi_{123}^{(3)}]k^1 k^3}{\frac{1}{L^1} \frac{k^1}{\psi_-^1}} = -L^1 \chi_{123}^{(3)} k^3 \psi_-^1, \quad (\text{A28})$$

taking $\chi_{123}^{(3)} = \chi_{132}^{(3)}$. Rearranging, this is

$$\left. \frac{\delta \ln \psi_-^1}{\delta \psi_-^2} \right|_{S_{12}} \approx L^1 \chi_{123}^{(3)} k^3, \quad (\text{A29})$$

which gives an exponential form for the phase boundary in S_{12} . In molar concentration units, this gives

$$c_-^1 = \bar{c}_-^1 \exp[\epsilon_{123}^{(3)} c_+^3 c_-^2], \quad (\text{A30})$$

where $\epsilon_{123}^{(3)} \equiv \chi_{123}^{(3)}(vL^1)(vL^2)(vL^3)/v$ is the molecule-level ternary interaction contact energy density in Eq. (2), and $\bar{c}_-^1$ is the component 1 saturation concentration in the absence of component 2.

APPENDIX B: SCALING IN A SIMPLE COMPUTATIONAL MODEL

We verify the polynomial scaling of the dilute phase boundary by numerically computing the full phase diagrams of two- and three-component Flory-Huggins models. The free energy is taken to be, for $N = 2$ or 3,

$$f = \sum_{\alpha=1}^N \frac{\phi^\alpha}{L^\alpha} \ln \phi^\alpha + \phi^0 \ln \phi^0 + \frac{1}{2} \sum_{\alpha, \beta=1}^N \chi_{\alpha\beta} \phi^\alpha \phi^\beta. \quad (\text{B1})$$

For the two-component case, we set $L^1 = 40$, $L^2 = 70$, $\chi_{11} = -0.8$, $\chi_{12} = \chi_{21} = -3.0$, and $\chi_{22} = -0.2$. This choice of parameters results in strong enrichment of both components in the dense phase, while maintaining the solvent volume fractions to be of order 1 on the phase boundary [Figs. 8(a) and 8(b)]. On a log-log plot, the dilute phase boundary is a straight line in the small- ϕ regime, and we fitted $\frac{d \ln \psi_-^1}{d \ln \psi_-^2} \approx -0.47$. This gives $\frac{k^1}{k^2} = -\frac{L^1}{L^2} \frac{d \ln \psi_-^1}{d \ln \psi_-^2} \approx 1.22$, which is a good approximation since the exact value computed for points in the range $10^{-7} < \psi_-^2 < 10^{-3}$ is $\frac{k^1}{k^2} = 1.21 \pm 0.11$. For the three-component case, we keep the same parameters components 1 and 2, and set $L^3 = 5$, $\chi_{13} = \chi_{31} = 0.1$, and $\chi_{23} = \chi_{32} = 0.1$. The tie lines are three-dimensional vectors [Fig. 8(c)]. The binodal boundary is computed using the convex hull algorithm, and we take cross-sections of it at approximately constant ϕ^3 's, using a window of $\Delta \phi^3 = 0.02$. For each section, we fit the $\phi^1 - \phi^2$ boundary on a double logarithmic plot as before and

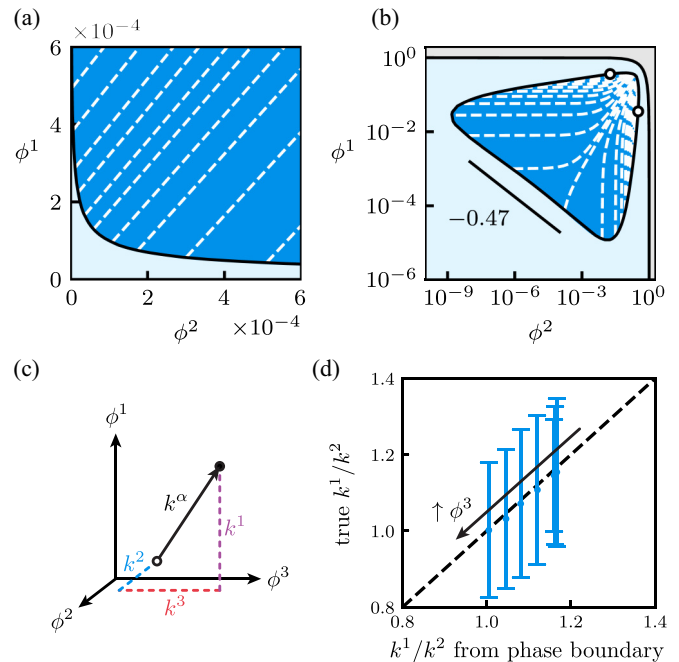


FIG. 8. Numerical investigations using the Flory-Huggins model. (a),(b) Two-component phase diagrams in linear and log scales. Light blue regions are homogeneous, and dark blue regions are phase-separated. White dashed lines are tie lines, and hollow markers are critical points. The gray region is physically forbidden. Polynomial scaling of the phase boundary is observed at small ϕ^1 and ϕ^2 , with the exponent relating to the tie line component ratio. (c) In the three-component system, each tie line is a three-dimensional vector. (d) We take slices of constant ϕ^3 in phase space and compute k^1/k^2 from numerical data as well as the double logarithmic fitting, and they agree well with each other.

estimate the k^1/k^2 ratio from the scaling exponent. Comparing this with k^1/k^2 explicitly computed using numerical data, we observe good agreement between the two methods [Fig. 8(d)].

APPENDIX C: EXPERIMENTAL DETAILS FOR NSP5/NSP2 DENSE PHASE QUANTIFICATION

Expression of NSP5 nStrep (strain RF) and purification/solubilization of inclusion bodies was conducted as described in [36,37]. Solubilized inclusion bodies were dialyzed [37], and the cleared supernatant was further purified using a Strep-tactin superflow column (iba lifescience). The column was washed with 1 M NaCl in 20 mM MOPS buffer at pH 7.1, and NSP5 was eluted using a 20 mM MOPS buffer containing 1 M NaCl and 2.5 mM Desthiobiotin (Sigma Aldrich) at pH 7.1. Protein-containing fractions were concentrated using an Amicon Spin column (Millipore) with a molecular weight cutoff of 10 kDa, and a final NaCl concentration of 150 mM was adjusted by addition of an appropriate volume of 50 mM MOPS at pH 7.1. Recombinant NSP2 cHis (strain RF) was expressed and purified as described in [24,36]. Protein concentration and purity were monitored using a Spectrophotometer (Extinction coefficient NSP5: 10555 and NSP2: 41620) and SDS-PAGE, respectively.

To perform the condensate partitioning assay, NSP2 and NSP5 were mixed at an equimolar concentration to form a final sample of 10 μl in volume, with 20 μM of each protein. The sample is centrifuged for 30 minutes at 16000 rcf, alongside controls containing only NSP5 or NSP2. The supernatant was mixed with 4 $\times$ mPAGE LDS Sample Buffer (Merck) and boiled at 70 $^{\circ}\text{C}$ for 10 min. A 12% mPAGE Bis-Tris Polyacrylamide Precast Gels (Merck) was run in MES buffer for sample visualization, and the BenchMark Prestained Protein Ladder (Thermo Fisher Scientific) was used as a protein standard. The gel was stained in Bio-Safe Coomassie Stain (Bio-Rad Laboratories) and imaged using a ChemiDoc imaging system (Bio-Rad Laboratories). The intensities and volumes of individual protein bands were quantified using the Image Lab software v.6.1.0 (Bio-Rad Laboratories). The intensity values of the NSP5 and NSP2-only samples were used as a standard to estimate the remaining protein concentration in the supernatant of the mixed NSP5/NSP2 sample.

APPENDIX D: EXPERIMENTAL CONDITIONS FOR FUS/PEG PHASESCAN

For the PhaseScan [10] experiment, we purify FUS protein using a previously established protocol [38]. PEG with molecular weight 6000 dalton is purchased from Sigma, and 5 μM of Alexa647 dye is added to the PEG inlet to track its concentration in droplets. Both FUS and PEG are dissolved in 50 mM Tris buffer with 150 mM KCl at pH 7.4.

APPENDIX E: ESTIMATING DENSE PHASE MOLAR RATIO USING DILUTE PHASE RESPONSE

Using notations from Appendix A, the change in the dilute phase of a sample $\delta\psi_{-}^{\alpha}$ in response to a change in total composition $\delta\phi^{\alpha}$ (not constrained on S_{12}) in the vicinity of the phase boundary is given by

$$\delta\psi_{-}^{\alpha} = \sum_{\beta=1}^N \left(\delta_{\beta}^{\alpha} - \frac{n_{\beta}}{n_{\alpha}} D^{\alpha} \right) \delta\phi^{\beta}, \quad (\text{E1})$$

so that when $\delta\phi^{\alpha}$ is constrained on S_{12} we have an expression for the dilute phase component 1 concentration

$$\delta\psi_{-}^1 = (1 - D^1) \delta\phi^1 - \frac{n_2}{n_1} D^1 \delta\phi^2. \quad (\text{E2})$$

Setting $\delta\psi_{-}^1 = 0$ then gives a set of total compositions on S_{12} with a fixed dilute phase component 1 concentration, and this is

$$\begin{aligned} K_2^1 &\equiv \left(\frac{\delta\phi^1}{\delta\phi^2} \right)_{\psi_{-}^1} = \frac{n_2}{n_1} \frac{D^1}{1 - D^1} = \frac{n_2}{n_1} \frac{D^1}{D^2} \frac{D^2}{1 - D^1} \\ &= \frac{k^1}{k^2} \frac{D^2}{1 - D^1} = \frac{k^1}{k^2} \frac{1}{1 + \sum_{\beta=3}^N D^{\beta}/D^2}, \end{aligned} \quad (\text{E3})$$

so the correction factor ζ is

$$\zeta \equiv \frac{K_2^1}{k^1/k^2} = \frac{D^2}{1 - D^1} = \frac{1}{1 + \sum_{\beta=3}^N D^{\beta}/D^2}. \quad (\text{E4})$$

To estimate ζ using the molar ratio obtained from the scaling and from the dilute phase measurement approach, we have

to take into account the different molecular weights of PEG used in these experiments. The K_2^1 obtained in molar units are 0.0061 ± 0.0005 for PEG (20 kDa) and 0.0039 ± 0.0008 for PEG (10 kDa) [20], which appeared to be proportional to the molecular weight of PEG used (very crudely). For PEG of 6 kDa, we thus estimate a K_2^1 of 0.002 (in molar units), which is combined with the measured $c_{+}^{\text{FUS}}/c_{+}^{\text{PEG}} = 0.110 \pm 0.008$ to give $\zeta \approx 0.02$.

APPENDIX F: ANALYZING THE G3BP1/RNA PHASE BOUNDARY

The dissolution branch of the G3BP1/RNA phase boundary Fig. 7(b) is concave, so Eq. (2) cannot be directly applied. Using indices 1 and 2 to denote G3BP1 and RNA, the problem lies in the assumption of a constant k^2 . The three-dimensional PhaseScan data suggest k^2 is in fact changing as a function of total RNA concentration [Fig. 7(c)], so the derivation leading to Eq. (A30) needs to be modified. From the data, we postulate that k^2 changes with ψ_{-}^2 according to the functional form

$$k^2(\psi_{-}^2) \approx \bar{k}^2 e^{-\psi_{-}^2/s}, \quad (\text{F1})$$

with constants $\bar{k}^2$ and s . The task now is to rederive Eq. (A28). Since the phase boundary [Fig. 7(c)] corresponds to a system without suramin, we assume the dominant n_2 contribution to be of the form

$$n_2 \approx X_2 \approx -\chi_{122}^{(3)} k^1 k^2. \quad (\text{F2})$$

It is possible to add three-body interaction terms corresponding to disruption of G3BP1/G3BP1 interaction by RNA [$\chi_{112}^{(3)}$] or disruption of RNA/RNA interaction [$\chi_{222}^{(3)}$]. However, from the dilute phase G3BP1 measurement, it was postulated that RNA acts on interactions involving RNA [7], so we drop the $\chi_{112}^{(3)}$ term. Furthermore, since we are assuming strong G3BP1 enrichment, we expect a large k^1 and $\chi_{112}^{(3)}$ should dominate the n_2 expression. We can now calculate

$$\begin{aligned} -\frac{\delta\psi_{-}^1}{\delta\psi_{-}^2} \Big|_{S_{12}} &= \frac{n_2}{n_1} \approx \frac{-\chi_{122}^{(3)} k^1 k^2}{\frac{1}{L^1} \frac{k^1}{\psi_{-}^1}} = -L^1 \chi_{122}^{(3)} k^2 \psi_{-}^1, \\ \frac{\ln \delta\psi_{-}^1}{\delta\psi_{-}^2} \Big|_{S_{12}} &= L^1 \chi_{122}^{(3)} \bar{k}^2 e^{-\psi_{-}^2/s}. \end{aligned} \quad (\text{F3})$$

Integrating, this leads to

$$\psi_{-}^1 = \bar{\psi}_{-}^1 \exp \left[-\chi_{122}^{(3)} L^1 \bar{k}^2 s e^{-\psi_{-}^2/s} \right], \quad (\text{F4})$$

or, in molar units,

$$c_{-}^1 = \bar{c}_{-}^1 \exp \left[-\epsilon_{122}^{(3)} \bar{c}_{+}^2 \Delta c^2 e^{-c_{-}^2/\Delta c^2} \right], \quad (\text{F5})$$

where $\epsilon_{122}^{(3)} \equiv \chi_{122}^{(3)} (vL^1)(vL^2)/v$ and $\Delta c^2 \equiv vL^2 s$. To perform the fitting, first note that 1 ng/ μl of the RNA corresponds to approximately 0.47 nM. Fitting Fig. 7(c) data to an exponential form yields $\Delta c^2 \approx (33.7 \pm 1.3)$ nM, or (70.8 ± 2.8) ng/ μl . Using this value of Δc^2 in Eq. (F3) we find $\bar{c}_{-}^1 = (1.90 \pm 0.03)$ μM , and $\epsilon_{122}^{(3)} \bar{c}_{+}^2 \Delta c^2 = (1.60 \pm 0.07) k_B T$.

APPENDIX G: SOLUBILITY PRODUCT IN THE PROXIMITY OF THE PHASE BOUNDARY

On every point on the two-dimensional slice of the phase boundary, we have complete information on the dilute composition since it is exactly the total composition in the limit of zero dense phase volume. However, for samples within the phase-separated regime, the dilute phase composition differs quickly from the total composition as the dilute phase point moves away from the two-dimensional slice and into the N -dimensional space [Fig. 1(a)]. As a result, multiplying the dilute phase concentrations within the phase-separated regime, as outlined in [32,33], requires a separate treatment. We first take a point on the phase boundary with composition ψ_-^α and bring it into the phase-separated regime by addition of some amounts of solutes 1 and 2, with this displacement denoted as $\delta\phi^\alpha$. The dilute phase composition of this new sample is $\psi_-^\alpha + \delta\psi_-^\alpha$, where $\delta\psi_-^\alpha = \sum_{\beta=1}^N R_\beta^\alpha \delta\phi^\beta$ and $R_\beta^\alpha = \delta_{\beta\alpha} - \frac{n_\beta}{n_\alpha} D^\alpha$ near the phase boundary, with $\delta_{\beta\alpha}^\alpha$ the Kronecker delta [7]. Direct substitution gives dilute compositions of components 1 and 2 as

$$\begin{aligned}\psi_-^1 + \delta\psi^1 &= \psi_-^1 + \delta\phi^1 - D^1\delta\phi^1 - \frac{n_2}{n_1}D^1\delta\phi^2, \\ \psi_-^2 + \delta\psi^2 &= \psi_-^2 + \delta\phi^2 - D^2\delta\phi^2 - \frac{n_1}{n_2}D^2\delta\phi^1,\end{aligned}\quad (G1)$$

and $(\psi_-^1 + \delta\psi^1)(\psi_-^2 + \delta\psi^2)$ is effectively the solubility product referred to in [32,33,39]. The present framework, however, implies the appropriate product combination on the phase boundary should have the concentrations raised to the power of their dense phase molar compositions. We thus define the product P as

$$\begin{aligned}\ln P &\equiv \frac{k^1}{L^1} \ln(\psi_-^1) + \frac{k^2}{L^2} \ln(\psi_-^2), \\ \ln P + \delta \ln P &\equiv \frac{k^1}{L^1} \ln(\psi_-^1 + \delta\psi^1) + \frac{k^2}{L^2} \ln(\psi_-^2 + \delta\psi^2),\end{aligned}\quad (G2)$$

so that P is a constant on the phase boundary in S_{12} . Treating $\delta\phi^{1,2}$ as small perturbations, direct substitution leads to

$$\delta \ln P \approx (1 - D^1 - D^2) \left(\frac{k^1 \delta\phi^1}{L^1 \phi_{\text{dil}}^1} + \frac{k^2 \delta\phi^2}{L^2 \phi_{\text{dil}}^2} \right). \quad (G3)$$

In the case where $D^1 + D^2 = 1$, we have $\delta \ln P = 0$, and the system is effectively two-dimensional. On the other hand, if $D^1 = D^2 = 0$, $\ln P + \delta \ln P$ is equal to the product formed using the total concentrations of 1 and 2 instead of the dilute concentrations, which is the product in the absence of phase separation.

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- [1] F. W. van Tartwijk and C. F. Kaminski, Protein condensation, cellular organization, and spatiotemporal regulation of cytoplasmic properties, *Adv. Biol.* **6**, e2101328 (2022).
 - [2] S. F. Banani, H. O. Lee, A. A. Hyman, and M. K. Rosen, Biomolecular condensates: Organizers of cellular biochemistry, *Nat. Rev. Mol. Cell Biol.* **18**, 285 (2017).
 - [3] S. Alberti and A. A. Hyman, Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing, *Nat. Rev. Mol. Cell Biol.* **22**, 196 (2021).
 - [4] Y. Shin and C. P. Brangwynne, Liquid phase condensation in cell physiology and disease, *Science* **357**, eaaf4382 (2017).
 - [5] G. Krainer, T. J. Welsh, J. A. Joseph, J. R. Espinosa, S. Wittmann, E. De Csilléry, A. Sridhar, Z. Toprakcioglu, G. Gudīskytė, M. A. Czekalska, W. E. Arter, J. Guillén-Boixet, T. M. Franzmann, S. Qamar, P. S. George-Hyslop, A. A. Hyman, R. Collepardo-Guevara, S. Alberti, and T. P. J. Knowles, Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions, *Nat. Commun.* **12**, 1085 (2021).
 - [6] H. Ausserwöger, E. De Csilléry, D. Qian, G. Krainer, T. J. Welsh, T. Sneideris, T. M. Franzmann, S. Qamar, N. A. Erkamp, J. Nixon-Abell, M. Kar, P. St. George-Hyslop, A. A. Hyman, S. Alberti, R. V. Pappu, and T. P. J. Knowles, Quantifying collective interactions in biomolecular phase separation, *Nat. Commun.* **16**, 7724 (2025).
 - [7] D. Qian, H. Ausserwöger, T. Sneideris, M. Farag, R. V. Pappu, and T. P. J. Knowles, Dominance analysis to assess solute contributions to multicomponent phase equilibria, *Proc. Natl. Acad. Sci. USA* **121**, e2407453121 (2024).
 - [8] A. Bremer, M. Farag, W. M. Borchers, I. Peran, E. W. Martin, R. V. Pappu, and T. Mittag, Deciphering how naturally occurring sequence features impact the phase behaviours of disordered prion-like domains, *Nat. Chem.* **14**, 196 (2022).
 - [9] Y. An, M. A. Webb, and W. M. Jacobs, Active learning of the thermodynamics-dynamics trade-off in protein condensates, *Sci. Adv.* **10**, eadj2448 (2024).
 - [10] W. E. Arter, R. Qi, N. A. Erkamp, G. Krainer, K. Didi, T. J. Welsh, J. Acker, J. Nixon-Abell, S. Qamar, J. Guillén-Boixet, T. M. Franzmann, D. Kuster, A. A. Hyman, A. Borodavka, P. S. George-Hyslop, S. Alberti, and T. P. J. Knowles, Biomolecular condensate phase diagrams with a combinatorial microdroplet platform, *Nat. Commun.* **13**, 7845 (2022).
 - [11] I. R. Trussina, A. Hartmann, C. Desroches Altamirano, J. Natarajan, C. M. Fischer, M. Aleksejczuk, H. Ausserwöger, T. P. Knowles, M. Schlierf, T. M. Franzmann, and S. Alberti, G3BP-driven RNP granules promote inhibitory RNA-RNA interactions resolved by DDX3X to regulate mRNA translatability, *Mol. Cell* **85**, 585 (2025).
 - [12] Y.-H. Lin, J. Wessén, T. Pal, S. Das, and H. S. Chan, Numerical techniques for applications of analytical theories to sequence-dependent phase separations of intrinsically disordered proteins, *Methods Mol. Biol.* **2563**, 51 (2023).
 - [13] D. Zwicker, O. W. Paulin, and C. ter Burg, Physics of droplet regulation in biological cells, *arXiv:2501.13639*.
 - [14] O. Adame-Arana, C. A. Weber, V. Ziburdaev, J. Prost, and F. Jülicher, Liquid phase separation controlled by pH, *Biophys. J.* **119**, 1590 (2020).
 - [15] H. Ausserwöger, R. Scrutton, T. Sneideris, C. M. Fischer, D. Qian, E. De Csilléry, K. L. Saar, A. Z. Bialek, M. Oeller, G. Krainer, T. M. Franzmann, S. Wittmann, J. M. Iglesias-Artola, G. Invernizzi, A. A. Hyman, S. Alberti, N. Lorenzen, and

- T. P. J. Knowles, Biomolecular condensates sustain pH gradients at equilibrium driven by charge neutralisation, *bioRxiv* 2024.05.23.595321.
- [16] G. Chauhan, A. Bremer, F. Dar, T. Mittag, and R. V. Pappu, Crowder titrations enable the quantification of driving forces for macromolecular phase separation, *Biophys. J.* **123**, 1376 (2024).
- [17] A. Kudlay and M. O. De la Cruz, Precipitation of oppositely charged polyelectrolytes in salt solutions, *J. Chem. Phys.* **120**, 404 (2004).
- [18] J. T. G. Overbeek and M. J. Voorn, Phase separation in polyelectrolyte solutions. Theory of complex coacervation, *J. Cell. Comp. Physiol.* **49**, 7 (1957).
- [19] D. Qian, H. Ausserwöger, W. E. Arter, R. M. Scrutton, T. J. Welsh, T. Kartanas, N. Ermann, S. Qamar, C. M. Fischer, T. Sneideris, P. St. George-Hyslop, R. V. Pappu, and T. P. J. Knowles, Molecular mechanisms of condensate modulation from energy-dominance analysis, *Phys. Rev. Appl.* **23**, 064017 (2025).
- [20] D. Qian, T. J. Welsh, N. A. Erkamp, S. Qamar, J. Nixon-Abell, G. Krainer, P. St. George-Hyslop, T. C. T. Michaels, and T. P. J. Knowles, Tie-Line analysis reveals interactions driving heteromolecular condensate formation, *Phys. Rev. X* **12**, 041038 (2022).
- [21] I. Alshareedah, T. Kaur, J. Ngo, H. Seppala, L. A. D. Kounatse, W. Wang, M. M. Moosa, and P. R. Banerjee, Interplay between short-range attraction and long-range repulsion controls reentrant liquid condensation of ribonucleoprotein-rna complexes, *J. Am. Chem. Soc.* **141**, 14593 (2019).
- [22] C. Luo, Y. Qiang, and D. Zwicker, Beyond pairwise: Higher-order physical interactions affect phase separation in multicomponent liquids, *Phys. Rev. Res.* **6**, 033002 (2024).
- [23] M. Farag, A. S. Holehouse, X. Zeng, and R. V. Pappu, FIREBALL: A tool to fit protein phase diagrams based on mean-field theories for polymer solutions, *Biophys. J.* **122**, 2396 (2023).
- [24] F. Geiger, J. Acker, G. Papa, X. Wang, W. E. Arter, K. L. Saar, N. A. Erkamp, R. Qi, J. P. Bravo, S. Strauss, G. Krainer, O. R. Burrone, R. Jungmann, T. P. Knowles, H. Engelke, and A. Borodavka, Liquid-liquid phase separation underpins the formation of replication factories in rotaviruses, *EMBO J.* **40**, e107711 (2021).
- [25] dq219 <https://github.com/dq219/phase-boundary-scaling> (2025).
- [26] T. Nomura, S. Watanabe, K. Kaneko, K. Yamanaka, N. Nukina, and Y. Furukawa, Intranuclear aggregation of mutant FUS/TLS as a molecular pathomechanism of amyotrophic lateral sclerosis, *J. Biol. Chem.* **289**, 1192 (2014).
- [27] W. M. Babinchak, B. K. Dumm, S. Venus, S. Boyko, A. A. Putnam, E. Jankowsky, and W. K. Surewicz, Small molecules as potent biphasic modulators of protein liquid-liquid phase separation, *Nat. Commun.* **11**, 5574 (2020).
- [28] A. Z. Lin, K. M. Ruff, F. Dar, A. Jaliha, M. R. King, J. M. Lalmansingh, A. E. Posey, N. A. Erkamp, I. Seim, A. S. Gladfelter, and R. V. Pappu, Dynamical control enables the formation of demixed biomolecular condensates, *Nat. Commun.* **14**, 7678 (2023).
- [29] H. Zhang, S. Elbaum-Garfinkle, E. M. Langdon, N. Taylor, P. Occhipinti, A. A. Bridges, C. P. Brangwynne, and A. S. Gladfelter, RNA Controls polyq protein phase transitions, *Mol. Cell* **60**, 220 (2015).
- [30] T. Sneideris, N. A. Erkamp, H. Ausserwöger, K. L. Saar, T. J. Welsh, D. Qian, K. Katsuya-Gaviria, M. L. L. Y. Johncock, G. Krainer, A. Borodavka, and T. P. J. Knowles, Targeting nucleic acid phase transitions as a mechanism of action for antimicrobial peptides, *Nat. Commun.* **14**, 7170 (2023).
- [31] P. Yang, C. Mathieu, R. M. Kolaitis, P. Zhang, J. Messing, U. Yurtsever, Z. Yang, J. Wu, Y. Li, Q. Pan, J. Yu, E. W. Martin, T. Mittag, H. J. Kim, and J. P. Taylor, G3BP1 Is a tunable switch that triggers phase separation to assemble stress granules, *Cell* **181**, 325 (2020).
- [32] A. Chattaraj, M. L. Blinov, and L. M. Loew, The solubility product extends the buffering concept to heterotypic biomolecular condensates, *eLife* **10**, e67176 (2021).
- [33] A. Chattaraj and L. M. Loew, The maximum solubility product marks the threshold for condensation of multivalent biomolecules, *Biophys. J.* **122**, 1678 (2023).
- [34] M. L. Huggins, Solutions of long chain compounds, *J. Chem. Phys.* **9**, 440 (1941).
- [35] P. J. Flory, Thermodynamics of high polymer solutions, *J. Chem. Phys.* **10**, 51 (1942).
- [36] A. Borodavka, E. C. Dykeman, W. Schrimpf, and D. C. Lamb, Protein-mediated RNA folding governs sequence-specific interactions between rotavirus genome segments, *eLife* **6**, e27453 (2017).
- [37] D. Martin, M. Ouldali, J. Ménétrey, and D. Poncet, Structural organisation of the rotavirus nonstructural protein NSP5, *J. Mol. Biol.* **413**, 209 (2011).
- [38] S. Qamar, G. Z. Wang, S. J. Randle, F. S. Ruggeri, J. A. Varela, J. Q. Lin, E. C. Phillips, A. Miyashita, D. Williams, F. Ströhl, W. Meadows, R. Ferry, V. J. Dardov, G. G. Tartaglia, L. A. Farrer, G. S. Kaminski Schierle, C. F. Kaminski, C. E. Holt, P. E. Fraser, G. Schmitt-Ulms *et al.*, FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation- π interactions, *Cell* **173**, 720 (2018).
- [39] A. Chattaraj, Z. Baltaci, S. Chung, B. J. Mayer, L. M. Loew, and J. A. Ditlev, Measurement of solubility product reveals the interplay of oligomerization and self-association for defining condensate formation, *Mol. Biol. Cell* **35**, ar122 (2024).