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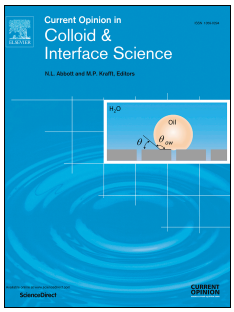

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Complex dynamics of multicomponent biological coacervates

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Abstract

Liquid-liquid phase separation (LLPS) and coacervates play key roles in natural and synthetic soft matter. In particular, the past few years have seen a rapid expansion in studies of these phenomena in the context of dynamic cellular compartmentalization. In this brief review, we mainly focus on a few concepts and selected *in vitro* and cellular examples of recent developments in the areas of dynamics and multicomponent systems. Topics covered include the flexibility and conformational dynamics of polymeric species involved in LLPS, valence and non-monotonic effects, noise modulation and feedback loops, and multicomponent systems and substructure. The fundamental concepts discussed in this review are widely applicable, including in the context of cellular function and development of materials with novel properties.

1 Introduction and motivation

Liquid-liquid phase separation and coacervates play wide and important roles both in the natural world and synthetic materials [1]. Coacervates can be defined as droplets of a dense liquid phase within a dilute liquid phase, formed by a process of liquid-liquid phase separation (LLPS). The term coacervation can be traced back to the early 1900s, in particular with work from Bungenberg-de Jong and Kruyt on colloid systems and with even earlier roots in the work of Tiebackx on gelatin/gum Arabic mixtures. Some years later, Oparin and Haldane also invoked coacervates in the context of protocells and origin of life hypotheses [2, 3]. Coacervates have since been widely studied both experimentally and theoretically, with numerous applications in a range of disciplines. The past decade has seen a large resurgence in interest in this topic in the area of cell biology, beginning with observations reported by Brangwynne et al. of liquid-like coacervate droplets in living cells [4]. Such cellular coacervate phases have since been linked to a number of "membraneless organelles" or condensates in cells, including P-granules, nucleoli and stress granules, with links to diverse biological functions as well as diseases [5]. Additionally, recent thinking in the field has been deeply influenced by previous concepts and theory from polymer chemistry and physics, given that cellular polymers such as proteins and nucleic acids are key components of these cellular coacervates [6, 7].

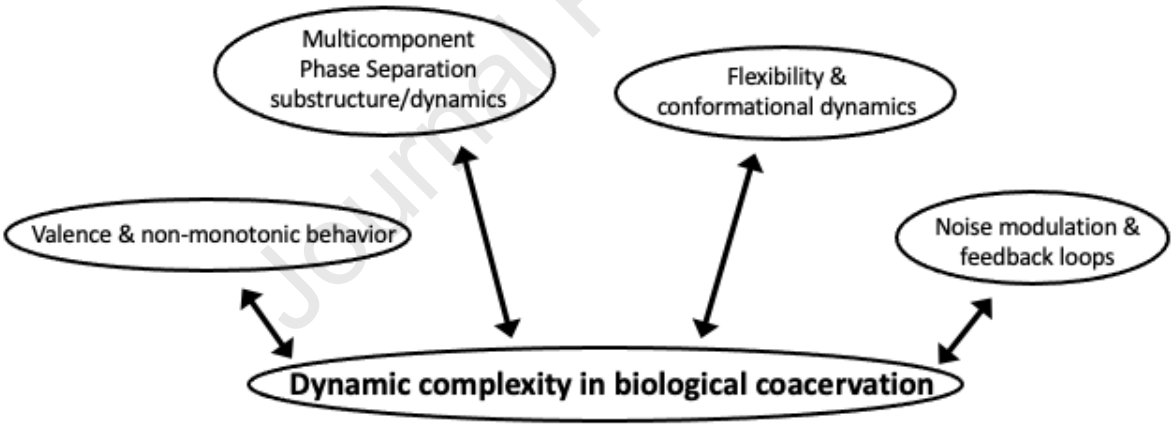

Figure 1. A schematic overview of key topics covered in this review.

A major fraction of research has considered coacervates from the perspective of equilibrium-based models incorporating polymer concepts from single component systems. While such studies have served as a useful framework for conceptualizing phase separation, more complex systems—as are common in multicomponent, biological condensates—may not always behave as expected without additional considerations. For example, Riback et al. observed that in a model stress granule biomimetic, the concentration of the dilute phase was not fixed at a constant C_{sat} as would be the case in a homotypic phase separated system at equilibrium, but rather increased with the concentration of the phase-separating, scaffolding protein [8]. While the principles of simplified thermodynamics based, equilibrium models are still important

and generally underlie more complicated coacervation, multicomponent systems have proven to possess additional complexity, with dependencies on the molecular and stoichiometric characteristics of their constituent parts. This dependence on composition in multicomponent systems—both at a molecular level and from a species perspective—has been shown to drive interesting system dynamics in droplet forming environments. Along these lines, here we place a substantial focus on complex equilibrium and non-equilibrium dynamical behavior of coacervates and the importance of these dynamics especially in the context of multicomponent systems.

The remainder of this review is structured as follows. We first begin a discussion of some basic features of coacervates and their dynamics. We then provide a few interesting and important concepts and examples from the recent literature on coacervates *in vitro* and in cells (Figure 1). In particular, we first discuss the coupling of component dynamics and coacervation. We follow with other sections discussing additional (though related) concepts and topics, where complexity and dynamics again can play important roles, including valence and non-monotonic effects, noise modulation, and substructure in multicomponent systems. While some of the discussion in the following sections is about complexity that can exist even under equilibrium conditions, we note that such phenomena can substantially alter dynamics of the overall system at a variety of length scales, for example the feedback loops in reentrant systems discussed below. Finally, we briefly give our thoughts for emerging and future directions.

2.1 A brief introduction to coacervates and their dynamics

The process of liquid-liquid phase separation gives rise to coacervates, small liquid droplets of a dense phase in a bulk dilute phase. This phase separation occurs due to a balance of enthalpic and entropic driving forces. Flory and Huggins separately developed a widely used mean-field lattice-based formalism to describe the free energy of mixing in terms of enthalpic and entropic terms [6, 9-11]. A key feature of this formalism is the Flory interaction parameter, which incorporates the interplay between polymer-polymer, polymer-solvent and solvent-solvent interactions. The sign and magnitude of this term determines if minimizing the free energy will result in the system undergoing phase separation. Voorn and Overbeek extended this formalism to the phenomenon of complex coacervation which involves phase separation of oppositely charged species, also widely observed in natural and designed systems [6, 12]. They used the Flory-Huggins entropy of mixing, combining it with an electrical free energy of mixing based on Debye-Hückel theory. While these theoretical developments take highly simplified views, and several advancements in theory have been made since, the conceptual underpinnings of this early work are still commonly utilized.

A useful view of cellular LLPS is through the lens of associative polymer theory. In particular, Harmon et al. and Choi et al. have used the sticker-spacer formalism to model this process [13, 14]. Interacting groups or patches on proteins, RNA or DNA can be viewed as stickers, which can interact with complementary or similar patches. Spacers hold the stickers together on a polymer, but can play important roles through

their interactions with other spacers, solvent, and their rigidity. A wide variety of interacting motifs have been found to play roles as stickers, including charge-charge, cation-pi, pi-pi, and hydrophobic motifs. Furthermore, both disordered and folded regions of biomolecules can play key roles in LLPS [6, 15, 16].

While the above views of coacervates are from an equilibrium point of view, a wide range of coacervate equilibrium and non-equilibrium dynamics at a range of scales are important for consideration. General concepts are briefly discussed here. The kinetics of coacervate formation and dissolution are considered critically important in many processes. Rapid formation and dissolution can control compartmentalization and further interactions and reactions, a means of regulation in cell biology. A related question is: when coacervate formation is induced, how do they form? One way to think about this process is classical nucleation theory, where opposing bulk (volume) and interfacial (surface-area) forces result in small droplets being unstable, while droplets larger than a certain size become stable. Hence, phase separation has to overcome this nucleation barrier in the free energy surface. Once droplets are formed, they can grow in size by multiple mechanisms. For example, Ostwald ripening occurs through a net molecular diffusion from smaller droplets to larger droplets (driven by the surface area vs. volume considerations discussed above), resulting in growth of larger droplets at the expense of smaller ones. Another growth mechanism is fusion of droplets, again increasing the fraction of larger droplets. It should be noted however that several potential mechanisms (including kinetically trapped [17] or arrested phases and active processes [18, 19]) can alter the above processes, likely contributing to the distributions of smaller droplet species quite commonly observed in a cellular context (Figure 2). We briefly discuss a couple of these mechanisms later in this review. We also note that droplets or condensates can span a wide range of sizes from nm sized clusters to the μm scale (or even larger), and that coarsening mechanisms such as Ostwald ripening and fusion discussed above can also occur at a variety of these size scales. Yet another question is whether coacervate droplets are uniform or can have dynamic substructure, with the latter indeed being the case. In addition, a range of dynamics is also associated with material properties of droplets (ranging from fast to slow dynamics with biological and disease relevance), resulting from and influencing molecular-scale properties, inter- and intra-molecular interactions and diffusion of molecules both within droplets and in exchange with the dilute phase. Finally, it should be noted that non-equilibrium

dynamics are intrinsic to biology. Many of these dynamic non-equilibrium systems can be cast in the framework of active matter, i.e., matter which is actively consuming energy, a process that can lead to seemingly counterintuitive and interesting behavior. Indeed, not only can the dynamics of chemical reactions influence the coacervation process, but coacervation can also influence the course of a chemical reaction [20-24].

2.2 Flexibility and conformational dynamics

Before delving into the dynamics of droplet formation, dissolution, and organization, we first start with the effects of molecular scale dynamics in phase separating species on droplet formation and material properties, where dynamic properties of droplet components often find itself at the center of condensates in multicomponent systems. While it does not have to be the case for all condensates, a significant portion of biomolecules involved in scaffolding phase separation—often proteins with intrinsically disordered regions or single stranded nucleic acid species—are at least somewhat conformationally dynamic. As a result, the role of flexibility and the interplay between structure and disorder based on underlying thermodynamic principles have been useful in understanding how dynamics in biopolymers helps to mediate the formation and stability of networks between multivalent species. While the extent of molecular scale dynamic influence on droplet properties are still being investigated, some examples described here suggest that dynamics could not only play a role in switchable droplet properties, but also in modulating structural and material properties as well.

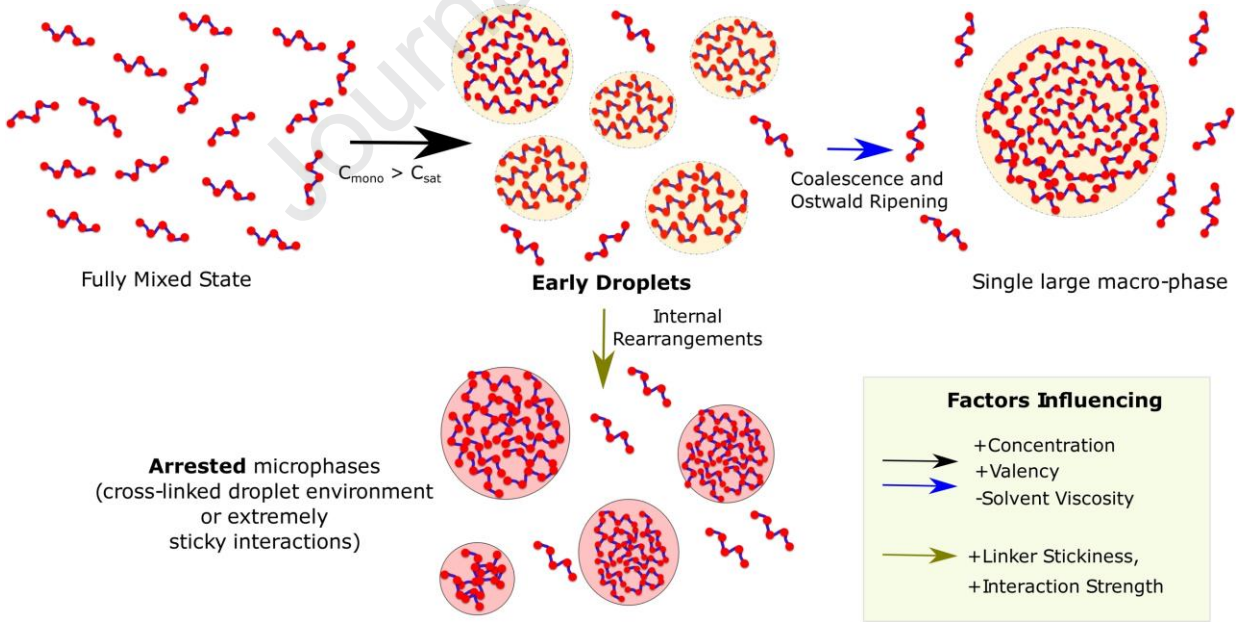

Figure 2. Illustration of droplet growth dynamics during LLPS showing formation of early small clusters that can grow by multiple mechanisms including fusion and Ostwald ripening. Competing kinetics of growth and interaction processes can also result in arrested states of the system. Figure from Ranganathan et al. eLife (2020) 9:e56159; doi: 10.7554/eLife.56159.

In simplified systems designed to probe the extent to which properties of chain or linker dynamics affect phase separation and binding, the role of flexibility often appears to reflect the energetic interplay between enthalpic and entropic contributions. In the context of protein ligand interactions, research into the effects of linkers on multivalent or intramolecular binding interactions suggests that a stiff linker of equal or slightly smaller length relative to the distance between binding pockets can achieve higher binding affinity than its more flexible counterpart [25, 26]. Such models where binding affinity becomes higher with greater rigidity assuming perfect spacing are consistent with a thermodynamic understanding of binding, where given little to no enthalpic cost to fit the binding site, greater rigidity in the interacting species results in a lower entropic penalty upon binding, thereby representing a more favorable binding energy.

While stronger binding affinity coupled with high valency can enable phase separation at lower concentrations [5], multivalent protein-ligand binding models insufficiently describe condensate systems largely due to the spacing and networking aspects of condensates that contribute to overall system behavior. In simulations modeling a single polymeric species interacting with a smaller rigid binding partner (conceptually similar to a system of RNA with an RNA binding protein, Figure 3a), Zumbro and Alexander-Katz showed that for long polymers relative to the multivalent sites, greater flexibility not only affects the minimal binding affinity for phase separation, but also has a stronger effect with increasing valency of the binding partner. This trend can be explained by the addition of an enthalpic cost in rigid structures from loop formation coupled with poorer/fewer contacts at the binding interface relative to a much more favorable enthalpic term in the flexible system [27]. In systems of two chain-like polymeric species, a similar trend of flexibility enabling phase separation propensity and stability also applies. Using a coarse-grained model of asymmetric polyelectrolytes with long flexible polycations and short polyanions, based on a system with a long, positively charged peptide and shorter, single-stranded homopolymeric nucleic acids of varying persistence length (Figure 3b), Shakya et al. observed that with decreasing persistence lengths of the anionic polymer, droplets formed more readily and were more resistant to salt dissolution, consistent with an understanding of improved energetic favorability in droplet formation with more dynamic components [28]. The results not only reconstituted the experimental observations of Shakya and King's previous work, which observed a similar dependence on nucleic acid species flexibility when comparing across dsDNA and single stranded polyA and polyT oligomers with respect to droplet forming and salt resistance properties [29], but also expanded upon it by suggesting the possibility of increased monomer exchange with the dilute phase with increased polyanion flexibility in the presence of an already flexible polycation [28]. In these ways, the systems explored by Zumbro and Alexander and Shakya et al. highlight the role dynamics play in influencing the thermodynamics of droplet formation, driving separation, maintaining droplet stability, and possibly influencing species dynamics.

Other models for non-uniform homopolymers have also been developed as condensates can also form from biomolecules with more obviously defined sticker-spacer organizations (Figure 3c), and have found that properties of dynamic linkers influence the formation and gel transitions of droplets. Notably, Harmon et al. [14]

observed that at an intermediate linker length, negative or near-zero effective solvation of linkers enable networking transitions, while positive effective solvation of linkers can suppress phase transitions and droplet formation through positive and negative global cooperativity modulation respectively. Building from this thermodynamically based model to examine droplet growth, Ranganathan and Shaknovich [17] found that linker stiffness not only modulated the droplet density—forming more open, less dense networks—but also had the potential to alter the size of droplets by modulating the time it takes polymer species to exhaust valences within a cluster before a new chain joins (Figure 2). In this sense, from both thermodynamic and non-equilibrium perspectives, molecular scale dynamic properties of participating polymers can influence evolution of droplet physical and material properties (Figure 3d).

While in vivo biological condensates typically involve more complexity—both in the number of component species and in the heterogeneity of dynamic properties within a particular polymer sequence, the effect of molecular scale dynamics observed in model systems can still be realized. Most dramatically, structurally dynamic biomolecules can regulate multivalency via conformational change and thus, modulate the network forming properties of scaffolding proteins. An example of this can be found in recent work by Guillen-Boixet et al. which examines the role of G3BP conformational changes in inducing the formation of stress granules—condensates which form in response to cellular stress to stabilize mRNA and/or inhibit translation—in vivo and in a simplified system in vitro [30]. The authors used a combination of simulations and experimental data to demonstrate that when under stress, released unstructured mRNAs can act as a scaffold for G3BP and induce a conformational change, freeing the protein's RG-rich region to induce protein-protein or protein-RNA interactions and act as a crosslinker [30]. In this case, the number of available interacting domains is controlled by the conformation of the scaffolding and crosslinking proteins, presenting a potential regulatory mechanism for cellular condensate formation.

Similarly, structural elements and conformational dynamics may also influence the assembly and networking properties and shapes of droplets. Boeynaems et al. observed that droplets formed between RNA with the ability to form stable structures (polyG homopolymers or polyA:polyU) and PR peptides formed fractal-like networks in kinetically arrested phase separated states [31]. In a more recent work, Ma et al. [32] examined 3' UTRs and mRNAs in the TIS granule network and have found that RNA's containing more unstructured regions and less rigid secondary structural elements in bulk solution have been associated with the formation of more mesh-like droplets. Here, unstructured RNA is thought to interact to form a pervasive, largely immobile, molecular network within non-spherical droplets with dynamic, liquid-like properties relative to the protein species within. While not similarly rigidifying the overall diffusive properties of the proteins localized to the TIS droplets, the different mesh-like form of the droplet inhibited complete droplet fusion of the skeleton, providing an example of species with potentially interesting viscoelastic properties [32]. The irregular structure results in high surface area condensates, which is particularly interesting as the granules are observed to be highly intertwined with the ER. Previous research has suggested that interface between TIS granules and the ER plays a role in protein-recruitment, binding, and

localization [33]. Thus, overall flexibility at a molecular level can not only influence the droplet forming thresholds, but also play a role in defining the material properties through scaffolding and network formation with potential physiological implications. Efforts have been made to begin quantifying relative conformational properties of flexible chains in coacervates [34-38]. Continued advancement in both techniques and potentially interesting cellular systems, coupled with theoretical, computational, and in vitro model systems, leading to detailed probing of molecular scale, conformational dynamics within droplets of these *in vivo* systems could yield unique insight into biological processes.

2.3 Valence and non-monotonic effects

An interesting phenomenon arises for certain multicomponent systems, where monotonic increase in the concentration of a particular component results in non-monotonic changes in phase behavior. For example, Banerjee, Milin et al. demonstrated this type of behavior in a simple model RNA-peptide phase separating system that can serve as a model system to study the influence of primarily charge interactions in LLPS [39, 40]. In this work, a titration of poly-U RNA into a sample of peptide gave rise to phase separation at balanced RNA/peptide ratios, but resulted in loss of these droplets at higher ratios. This complex non-monotonic behavior, termed a reentrant phase transition, tested a prediction based on previous work on other systems. It can also be understood simply in terms of relative stoichiometries of complementary valences, in this case oppositely charge motifs on the peptide and RNA. When there is an approximate balance of the two types of valences in multivalent species, the network of interactions can propagate in an intermolecular fashion allowing for phase separation. However, when there is an overrepresentation of either of the valences, phase separation is suppressed since the network is terminated on a short length scale. Thus, in the case of the peptide/RNA system, at low ratio, positively charged peptide molecules with repulsive charge interactions dominate the solution, while at high ratios, overscreening by negatively charged RNA would result in small negatively charged complexes. Between these extremes lies the window of concentration ratios that promotes phase separation (Figure 4a).

It is noteworthy that several other systems have shown such reentrant behavior consistent with general applicability of such valence balancing considerations [40]. Examples have included a number of subsequent reports, as well as previous observations in the literature that can now be simply explained by such a reentrant phenomenon. This general window-like behavior could have several important biological implications. For example, a common mechanism of regulation in cells is post-translational modifications including phosphorylation, which have been shown to alter LLPS. The reentrant phase transition concept provides for an alternative mechanism, where increase of relative concentration of one of the interacting components could both form and dissolve droplets depending on the conditions. Interestingly, the Banerjee et al. study also showed that the reentrant behavior could give rise to dynamic formation and loss of vacuole-like substructure within droplets. Furthermore, as discussed in the

next section, the non-monotonic response of the system can also give rise to a feedback loop that modulates RNA concentration.

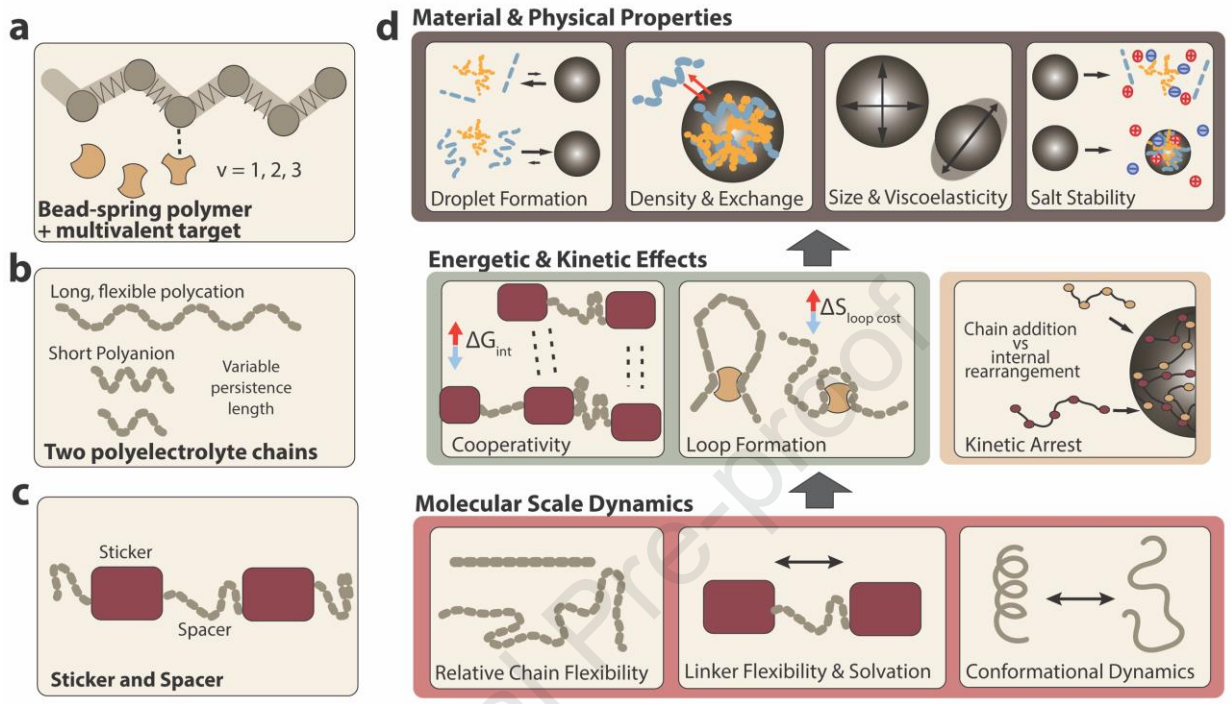

Figure 3. Component dynamic properties modulate material and physical properties. Different model systems can be used to represent biopolymer dynamics, including (a) bead-spring polymer with a multivalent target models where polymers are represented by spherical beads connected by harmonic springs of varying stiffness (v=valency; figure based on model from reference [27], (b) asymmetric polymer chain models of polyelectrolytes with varying persistence lengths (reference [28]), and (c) models based on the sticker-spacer formalism. (d) Biopolymer dynamics (red) can alter the thermodynamics (green) and kinetics (orange) of network formation, resulting in changes to droplet material and physical properties (brown). Different ways of thinking about molecular scale dynamics of polymers include the total chain flexibility, linker properties, and conformational heterogeneity. These can affect the strength of interactions between sticker regions or change the relative change in entropy of network formation, or also alter the rate of chain addition (ΔG_{int} = free energy change of interactions, $\Delta S_{loop\ cost}$ = entropic penalty of loop formation). Changes to thermodynamics or the kinetics of formation can alter droplet properties including phase separation propensity, network density, monomer exchange with dilute phase, droplet size and stability, and viscosity/viscoelasticity.

A different type of non-monotonic reentrant behavior has also been shown for larger proteins with multiple types of interactions. Krainer et al. [41] showed that several proteins such as the ALS linked proteins FUS and TDP43 that undergo phase separation at low salt concentrations dissolve as the salt concentration is increased, consistent with that phase being stabilized primarily by electrostatic interactions. Interestingly however, higher salt concentrations cause the system to reenter a dense

phase. Using a combination of experiments and simulations, the authors provide evidence that the latter dense phase is stabilized by hydrophobic and non-ionic interactions. This work shows how the effects of a single variable (here salt concentration) with opposite effects on different interaction components of phase separation can give rise to non-monotonic behavior. Indeed, a related effect had been previously reported in a different type of system by Onuchic et al. [42]. Here, based on previous reports and the opposing effects of divalent cations on the stability of complex coacervates and RNA structure and collapse, the authors predicted that divalent cations could produce a switching of phase behavior between RNA-protein/peptide (stabilized largely by charge interactions) condensates and RNA condensates (stabilized by RNA-RNA and RNA-cation interactions). This non-monotonic behavior was indeed proven to be the case, with the resulting two types of condensates having substantially different physical and biochemical properties consistent with the opposing stability considerations noted above.

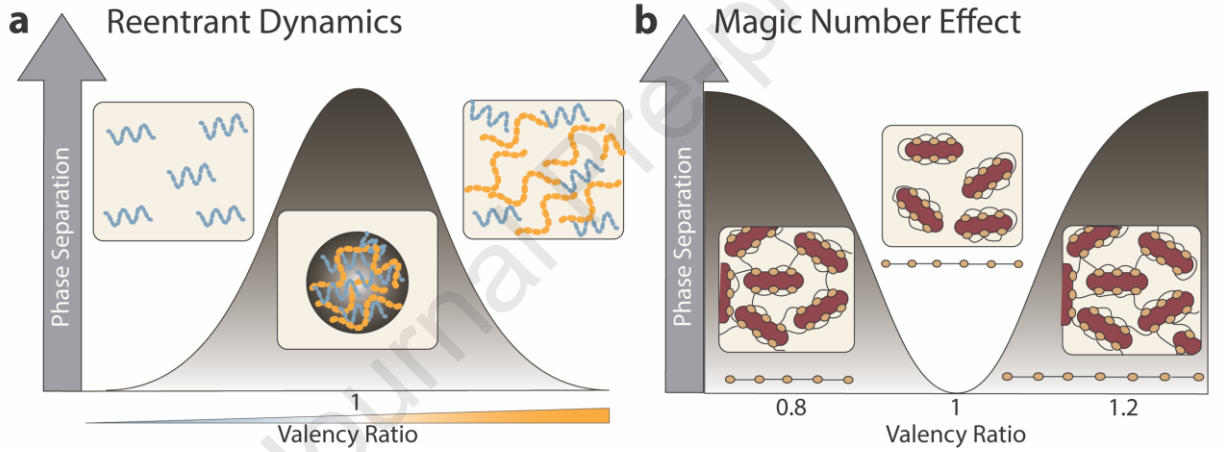

Figure 4. Reentrant dynamics and the magic number effect modulate phase behavior. (a) Biocondensates can undergo reentrant phase transitions based on valency. In charge based, polyelectrolytic systems, charge overscreening can cause the dissolution of droplets (reference [40]). (b) The magic number effect can also modulate phase behavior based on the valency ratios of interacting species (reference [18]).

We also note that valence balance can have other effects. For example, Rosenzweig et al. proposed potential magic number effects for the eukaryotic pyrenoid [43]. Magic number effects have been invoked in several areas including in nuclear structure (of atoms), condensation of colloids and quantum interference effects that control electron transport in single molecules [44, 45]. In the pyrenoid work, based on simulations of two key interacting proteins (EPYC1 and Rubisco) in this system, the authors proposed an effect where integral ratios of complementary valences on these two proteins would result in formation of small oligomers where all valences are satisfied. By the same considerations as discussed above, this terminates propagation of the network and therefore would result in loss of phase separation. Subsequent simulation work by Xu et al. has built on this idea, noting that rigidity in one of the interacting components and strengths of the interactions would enhance this effect [46, 47] (Figure 4b).

Valence effects have also been studied in the context of the dynamics of growth and maturation of droplets. For example, recent computational work by Ranganathan and Shakhnovich indicates that the relative timescales of interaction formation and molecular diffusion can influence occurrence of this growth mechanism [17]. The results revealed the importance of competition between the interaction formation timescale within clusters and a diffusion-limited encounter between clusters. A particularly interesting situation can arise when the rearrangement of initially formed smaller clusters to maximize intra-cluster interactions (which in turn is dependent on other parameters including flexibility of monomer chains) occurs much faster than further encounters between clusters. This can result in small clusters quickly exhausting available valences and therefore not coalescing readily with others, i.e., growth arrest.

The relatively simple concept of valence (and its connections to graph theory) can therefore be used to understand and predict a number of complex features of LLPS. Furthermore, multiple valence-related effects could be superimposed or convoluted to produce even more complex outputs in phase separating systems. Additional examples of this valence concept also appear later in this article.

2.4 Noise modulation and feedback loops

Fluctuations in concentrations RNA, proteins and other molecules are inherent to cells. While these fluctuations can be essential in many cases, suppression of this noise would be useful in many other circumstances. Here, LLPS provides a potential feedback mechanism for suppression of such concentration-related noise in cellular circuitry.

In one example, Klosin et al. tested the potential for noise suppression via a fundamental basis of LLPS [48]. The argument goes as follows. The thermodynamics of phase separation of a single component solution is such that above the critical concentration, changes in concentration of the component just results in changes in volume fractions of the dense and dilute phases at equilibrium. Thus, noise in the total concentration, for example due to stochastic gene expression, would be buffered in terms of the concentrations in the dilute and dense phases. Klosin et al. tested this idea in cells using engineered variants of the DDX4 phase separating protein and did find evidence for the proposed noise suppression in cells. However, it should be noted that the proposed mechanism is based on ideas for a simplified system. In contrast, the concentration characteristics are more complex (no longer constant) for multicomponent mixtures [8], which would be represented by many or most cellular condensates as also discussed in previous sections. Thus, while some type of buffering or modulation could still occur, the characteristics would be more complex.

Another example is a mechanism we proposed based on our work on reentrant phase transitions [40]. Here, the non-monotonic window-like concentration dependence (discussed above) on the RNA/protein ratio, if coupled with accelerated transcription within a dense phase, could result in a feedback loop that modulates local RNA concentration within the window (Figure 5). Work over the past few years has provided evidence for transcriptional condensates, in effect the accelerated transcription component of the above proposed mechanism. A recent study by Henninger et al. provides experimental support for such a mechanism within cells [49]. Their results provide evidence that transcription initiation enhances LLPS, but transcription elongation then results in dissolution.

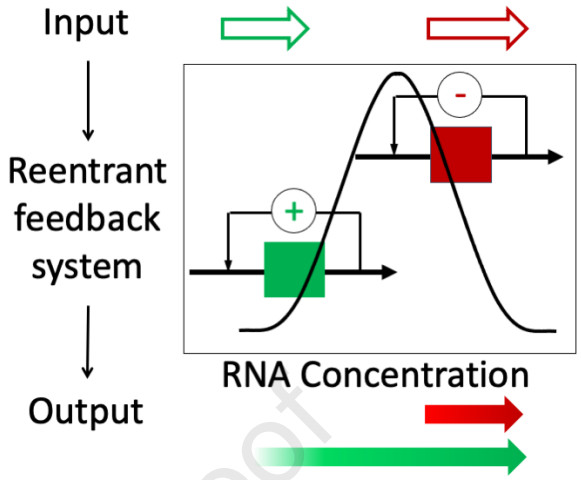

Figure 5. Reentrant phase transitions can potentially encode positive or negative feedback loops (see text for discussion).

We note that LLPS might also be able to modulate noise in other ways. For example, the nature of a phase transition itself can increase fluctuations in the system, and thus amplify noise in the system. In another example, if the noise amplitude lies in the left half of the reentrant phase diagram, the effect of LLPS would be to increase the noise amplitude acting as a positive feedback loop (Figure 5). This is because a noise fluctuation that brings the system stochastically into left part of the phase separation regime would pull the system further into the LLPS window due to the considerations discussed above. Thus, the system could act as a noise amplifier. However, as already discussed above, this amplifier could also act as a limiter, i.e., fluctuations would be amplified until the amplitude began to exit the right side of the LLPS window, where the negative feedback would kick in. These types of nonlinear behaviors could have significant implications in various cellular and biological phenomena, including in the context of transcriptional bursting.

2.5 Multicomponent phase separation substructure/dynamics

Complexity in multicomponent condensates also show up in droplet substructure and organization. In living systems, hierarchical organization and dynamic partitioning in response to cellular conditions can be observed within nuclei [50], nuclear speckles [51], stress granules [52], paraspeckles [53], and germ granules [54], where clustering of interacting biomolecules within droplets creates distinct sub-compartments or core/shell architectures of different compositions. These additional species in biological

systems add dynamic complexity to the models through inherent competing attractive and repulsive interactions, driving droplet organization.

Initial observations of hierarchies and core/shell architectures in biological condensates prompted questions as to what drove such partitioning in multicomponent systems. Efforts by Feric et al. laid the groundwork for the understanding that the relative magnitudes of interfacial tensions between phases largely govern the organization of multiphase behavior. Using a minimalist model composed of two protein species and rRNA, the authors observed that by parameterizing pairwise affinities in the presence of a competing solvent, they could recapitulate the core/shell organization observed in their experiments that modeled organization in part of the nucleolus. The resultant Flory parameters from these interactions could be related to surface tension, from which the relative rank orders of the phases could be determined [50]. Thus, the authors proposed that the immiscibility observed was the product of differences in interfacial tensions of separating phases and hypothesized that these differences were due to differences in intermolecular interactions.

The idea of immiscibility as a product of molecular interactions has been expanded upon through model systems. Polyelectrolyte-based models have shown that charge density and valency can significantly contribute to the degree of mixing and the relative interfacial tensions of phases. Lu et al. initially highlighted an observed relationship between differential critical salt concentration—a property determined by a combination of charge density, ion type, polymer backbone flexibility, and accessibility of the charged groups—with the ability to generate multiple phases, which they related to differences in interfacial energy using the Flory-Huggins formalism [55]. This framework, which could be used to predict multiphase droplet formation, was expanded upon by Mountain and Keating, who observed that when mixing two polyanions and two polycations, where either polycation can separate with either polyanion, the inner core was composed of the two more strongly interacting electrolytes when considering charge density and charge per molecule, with the other species separating into an outer shell [56]. When only one of the two polycations is shared amongst the two anionic species, competition between the polyelectrolytes can lead to more mixing across the two phases. When using polyU/Glu100 and protamine/2xRRASL, the stronger interacting species preferentially excluded lower valency/charge species, with both polycations separating into the inner phase with the shared, interacting polyanion polyU, and the outer shell consisting of a lower density of the shared polycation and the remaining anionic species [56]. Fisher and Elbaum-Garfinkle [57] observed a similar trend in polyK and polyR droplets forming core-shell architectures in the presence of UTPs and polyU. Although the compositions and relative partitioning of the systems was dependent upon the species of the polyelectrolytes added, the systems demonstrated by Mountain and Keating [56] as well as Fisher and Elbaum-Garfinkle [57] suggest that partitioning can be heavily influenced by a combination of valency and interaction strengths, where the more strongly interacting species outcompete and exclude weaker interacting species to form hierarchical levels of separation with distinct compositions.

Beyond charge, interaction strength can also be modulated by other types of intermolecular interactions. Boeynaems et al. observed multiphase separation in mixtures containing the polycation $(PR)_{30}$ and two sets of equivalent length and total charge homopolymers, polyrA and polyrC, where organization was dependent upon the stoichiometries of polyA:polyC [31]. Despite a common polycation, the separation of a polyA and polyC suggested that stronger intermolecular interactions between polyA-polyA relative to polyA-polyC, possibly as a result of preferable base stacking interactions, could also be an additional factor for organizing species within droplets.

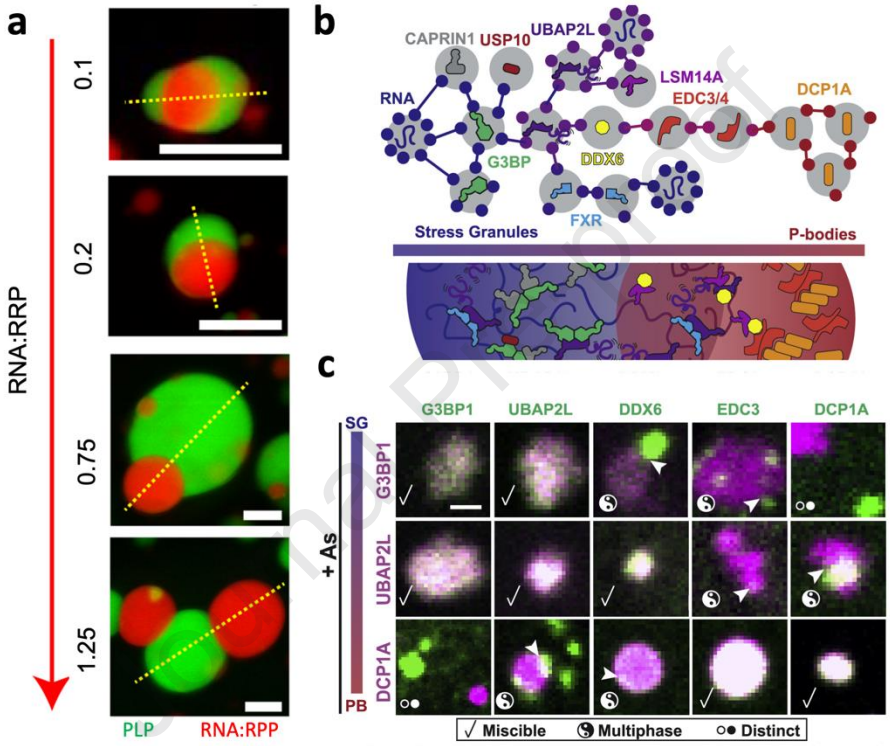

Figure 6. Composition of droplets influences interfacial properties and organization. (a) A ternary mixture of prion-like protein (green), arginine-rich peptide (red) and RNA show a transition from engulfed to separated droplets as a function of RNA:peptide ratio. Figure adapted from Kaur et al. (2021): *Nat. Commun.,* 12, 872, doi:10.1038/s41467-021-21089-4 [58] (b) and (c) Network interaction model and in-cell images for fluorescently labeled protein pairs showing droplet organization of stress granules and P-bodies. (b) and (c) adapted with permission from Sanders et al. (2020): Cell*,* 18, doi: 10.1016/j.cell.2020.03.050 [59]

Competing interactions influence not only the existence of multiple phases, but also their organization. In a study looking at a ternary system of PLP (prion-like polypeptide, pi electron rich and polar amino acids), RRP (Arg rich polypeptides), and RNA, Kaur et al. observed that the organization of the core/shell architecture could largely be driven by RNA relative concentrations [58]. Because RRP-RNA interactions are much more favorable than RPP-PLP interactions, RNA could outcompete PLP and effectively served as a sequestering agent for removing RRP from PLP droplets by triggering a de-mixing transition. In doing so, the concentration of RPP on RPP-RNA droplet surfaces

could be modulated, with decreasing RPP on the surface in response to increasing RNA also corresponding to an increase in interfacial tension between the phases. These differences could macroscopically be observed in the organization of the droplets, where lower differences in interfacial tensions result in engulfed droplets and high differences result in separate droplets existing within a bulk solvent with little to no shared interface (Figure 6a) [58]. In this way, the reentrant properties governing heterotypic droplets of RPP and RNA, in a ternary system manifested in complex mesoscale organizational properties.

In more complex systems involving more molecular species, organization can also respond to changes to the valency of the participating species. In an investigation into the organization of stress granules and P-bodies, Sanders et al. observed the effects of increasing multivalent nodes (v>=3), bridges (v=2), and caps (removal of a valence site) in the networking properties not only within a particular phase, but in creating and modulating interfacial connectivity between different phases (Figure 6b). They not only observed that the N-terminal region of G3BP could interact with other proteins that amplify its valency or with another that limited the total valency by binding and "capping" it, but also observed that the network of SG, PB and their interactions with on another could be modulated based on protein expression and concentration. In a minimal model inspired by their experimental observations using a reduced set of protein complexes with monovalent binding sites and prescribed pairwise interactions, the authors recapitulated their experimental results with respect to the tunability in the networks formed. The introduction of a saturating cap was able to increase interfacial tensions between different networks disrupting interactions on the interfaces of capped phases, while increasing valency, particularly through bridges or nodes capable of interacting with proteins sequestered in another phase, resulted in networks that interacted more despite being compositionally distinct [59]. This dependence on valence and affinity was observed in Sanchez-Burgos and Espinoza et al.'s coarse-grained, patchy particle model in which the authors noticed a tunable difference in the organizational hierarchy and molecular exchange in and out of the droplets when altering valence and the strength of interactions [60]. The recapitulation of multiphase droplet structure with larger systems, and in the case of Sanders et al., in cellular environments, can provide insight into more complex systems.

3. Concluding remarks and emerging/future directions

In this brief review, we have highlighted a few interesting directions in the biological LLPS field, with a particular emphasis on dynamics and multicomponent systems that are widely representative of the cellular context. We anticipate continued rapid growth in several directions.

A particularly interesting direction is the development of a set of foundational principles by which to understand and predict the dynamic behavior of such complex LLPS systems [7, 16, 61]. Here, both *in vitro* and in-cell work are anticipated to make a substantial impact. As discussed above, several well-established concepts from the polymer physics and materials science fields have been adapted in this context. This

effort is expected to continue to include more complex dynamical and multicomponent effects, and there is also the potential for observations in the field to encourage the development of new theory in this area, especially in the complex biological contexts.

Additionally, the change in dynamic properties of biomolecules within droplets are another avenue of investigation. From the perspective of the scaffolding molecules, there remains question about the extent to which their conformational dynamics change in droplets and under what conditions they do so. The work highlighted by Ma et al. [32] with the formation of RNA networks from unstructured chains represents one type of dynamic rearrangement. However, simulations have suggested that molecules may exhibit different dynamic properties in droplets than in bulk solution in different contexts, with Joseph et al. [62] observing chain compaction under some conditions, and Ngyuen et al., bioRxiv, doi: 10.1101/2021.02.20.432119, observing chain expansion as well as reptation-like polymer dynamic motions in other contexts. Experimentally, the questions of compaction, expansion, and rigidification seems equally diverse; Spruijt et al. [35] observe neither chain stiffening nor globulation in their polyelectrolyte coacervate system using neutron scattering, while more recently, Emmanouilidis et al. [37] observed FUS compaction in FUS droplets. Similarly, model systems have suggested that the diverse environments within droplets and their substructures can also influence client recruitment [31, 56], and may have the potential to alter the behavior of recruited species [63]. While there have been studies probing the conformational properties of species within droplets [34, 35, 37, 38, 63-65], the effects of distinct droplet microenvironments on molecular dynamics remains an area to be investigated. In this sense, the use and development of more direct methods (FRET, NMR, FCS, fluorescence anisotropy, EPR, neutron scattering or other experimental techniques) can be coupled with the continued development of computational models to answer questions about the effect droplets have on the structure, function, and dynamics of scaffold or client biomolecules.

As discussed throughout this review, multicomponent LLPS systems encode complexity of various kinds. Studies will continue to probe in more detail the mesoscale and nanoscale structural dynamics of these systems. In particular, these systems can give rise to complex and changing compartments and interfaces, which can tune molecular partitioning, localization, interactions and biochemistry, which will be further explored. Topological effects are also particularly interesting, for example in the context of polymer movement by reptation, entanglement of chains, or the influence of loops or knots. How mechanical constraints, crowding and confinement in cells affects the properties of droplet species, and conversely whether droplets can exert dynamic forces on cellular surfaces and other compartments, also warrant further exploration [66, 67]. Given the networked characteristics of the types of droplets, it will also be of interest to understand how and with what dynamical characteristics information input (change in characteristics) in one location of the droplet might be transmitted to other locations in the droplet, adjoining droplets or other surrounding structures.

Finally, another exciting area of development can be found in synthetic biology and biomaterials that comes from improved understanding of the physical and chemical principles involved in tuning droplet dynamics and material properties. For example, in

the development of microparticles, the use of phase separation and multiphase complexes, Roberts et al. have demonstrated the ability to form unique and stable geometries with IDP's through the tuning of component sequences and temperature, with the addition of photo-crosslinking [68]. Similar developments in DNA nanotechnology have enabled the formation of both classically driven DNA coacervates with controllable multiphase properties [69, 70] and active process based ones [71, 72]. While the synthetic developments in biological coacervates are beyond the scope of this review, the creation of biological mimics, materials, and other bioengineering applications of multicomponent coacervates continue to be an area looked towards for advancement.

Overall, we anticipate a host of rapid advances in theory and conceptual understanding, adaptation and development of tools for studies of dynamic complexity [73, 74], a close interplay of developments from in-cell and *in vitro* systems, and material and bioengineering/biomedical applications [75, 76] of these types of systems.

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References

[1] Astoricchio E, Alfano C, Rajendran L et al. The Wide World of Coacervates: From the Sea to Neurodegeneration. Trends Biochem Sci 2020; 45:706-717.
[2] Oparin AI. Proiskhozhdenie Zhizny. 1924.
[3] Haldane JBS. The Origin of Life. The Rationalist Annual 1929; 148:3.
[4] Brangwynne CP, Eckmann CR, Courson DS et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 2009; 324:1729-1732.
[5] Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 2017; 18:285-298.
[6] Brangwynne CP, Tompa P, Pappu RV. Polymer physics of intracellular phase transitions. Nat Phys 2015; 11:899-904.
**[7] Choi JM, Holehouse AS, Pappu RV. Physical Principles Underlying the Complex Biology of Intracellular Phase Transitions. Annu Rev Biophys 2020; 49:107-133.
This review article summarizes a few recent conceptual advances from the Pappu lab, especially related to the stickers and spacers model of associative polymers that is being used very successfully to understand and predict biological LLPS behavior.
[8] Riback JA, Brangwynne CP. Can phase separation buffer cellular noise? Science 2020; 367:364-365.
[9] Huggins ML. Solutions of long chain compounds. J Chem Phys 1941; 9:440-440.

[10] Flory P. Principles of Polymer Chemistry. 1953.
[11] Flory PJ. Thermodynamics of high polymer solutions. J Chem Phys 1941; 9:660-661.
[12] Overbeek JT, Voorn MJ. Phase separation in polyelectrolyte solutions; theory of complex coacervation. J Cell Physiol Suppl 1957; 49:7-22; discussion, 22-26.
[13] Choi JM, Hyman AA, Pappu RV. Generalized models for bond percolation transitions of associative polymers. Phys Rev E 2020; 102:042403.
[14] Harmon TS, Holehouse AS, Rosen MK, Pappu RV. Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. Elife 2017; 6.
*[15] Martin EW, Holehouse AS, Peran I et al. Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. Science 2020; 367:694-699.
This work used an innovative combination of experiment and simulation to systematically uncover and define aromatic sticker motifs in a phase separating prion like domain
[16] Das S, Lin YH, Vernon RM et al. Comparative roles of charge, pi, and hydrophobic interactions in sequence-dependent phase separation of intrinsically disordered proteins. Proc Natl Acad Sci U S A 2020; 117:28795-28805.
**[17] Ranganathan S, Shakhnovich EI. Dynamic metastable long-living droplets formed by sticker-spacer proteins. Elife 2020; 9.
This article uses Langevin dynamics and coarse-grained MD simulations to study droplet growth and make the interesting finding of arrested smaller droplets due to a competition between rates of interaction formation within droplets and diffusion limited encounters between droplets.
[18] Bentley EP, Frey BB, Deniz AA. Physical Chemistry of Cellular Liquid-Phase Separation. Chemistry 2019; 25:5600-5610.
*[19] Narayanan A, Meriin A, Andrews JO et al. A first order phase transition mechanism underlies protein aggregation in mammalian cells. Elife 2019; 8.
This interesting paper uses a combination of high-resolution fluorescence imaging and comparison to theory to provide evidence for a first order transition giving rise to a distribution of protein cluster sizes in live cells, along with a potential active mechanism for suppression on larger clusters.
[20] Poudyal RR, Guth-Metzler RM, Veenis AJ et al. Template-directed RNA polymerization and enhanced ribozyme catalysis inside membraneless compartments formed by coacervates. Nat Commun 2019; 10:490.
[21] Sokolova E, Spruijt E, Hansen MM et al. Enhanced transcription rates in membrane-free protocells formed by coacervation of cell lysate. Proc Natl Acad Sci U S A 2013; 110:11692-11697.
[22] O'Flynn BG, Mittag T. The role of liquid-liquid phase separation in regulating enzyme activity. Curr Opin Cell Biol 2021; 69:70-79.
[23] Tibble RW, Depaix A, Kowalska J et al. Biomolecular condensates amplify mRNA decapping by biasing enzyme conformation. Nat Chem Biol 2021; 17:615-623.
[24] Saleh OA, Jeon BJ, Liedl T. Enzymatic degradation of liquid droplets of DNA is modulated near the phase boundary. Proc Natl Acad Sci U S A 2020; 117:16160-16166.

[25] Liese S, Netz RR. Influence of length and flexibility of spacers on the binding affinity of divalent ligands. Beilstein Journal of Organic Chemistry 2015; 11:804-816.
[26] Krishnamurthy VMS, Vincent; Bracher, Paul J.; Shen, Nan; Whitesides, George M. Dependence of effective molarity on linker length for an intramolecular protein-ligand system. Journal of the American Chemical Society 2007; 129:1312-1320.
[27] Zumbro E, Alexander-Katz A. Polymer Stiffness Regulates Multivalent Binding and Liquid-Liquid Phase Separation. Biophys J 2020; 119:1849-1864.
[28] Shakya A, Girard M, King JT, Olvera de la Cruz M. Role of Chain Flexibility in Asymmetric Polyelectrolyte Complexation in Salt Solutions. Macromolecules 2020; 53:1258-1269.
[29] Shakya A, King JT. DNA Local-Flexibility-Dependent Assembly of Phase-Separated Liquid Droplets. Biophys J 2018; 115:1840-1847.
[30] Guillen-Boixet J, Kopach A, Holehouse AS et al. RNA-Induced Conformational Switching and Clustering of G3BP Drive Stress Granule Assembly by Condensation. Cell 2020; 181:346-361 e317.
**[31] Boeynaems S, Holehouse AS, Weinhardt V et al. Spontaneous driving forces give rise to protein-RNA condensates with coexisting phases and complex material properties. Proc Natl Acad Sci U S A 2019; 116:7889-7898.
In a systematic investigation into the effects of anion sequence, structure, and composition on droplet material and organizational properties, the authors not only observe the effects of the anion identity on droplet viscosity and multiphase hierarchies, but also may be the first to characterize kinetically arrested phase separation for condensates of biological molecules.
*[32] Ma W, Zheng G, Xie W, Mayr C. In vivo reconstitution finds multivalent RNA-RNA interactions as drivers of mesh-like condensates. Elife 2021; 10.
This study follows up on prior work in which the authors observed in vivo formation of non-spherical coacervates intertwined with the ER and containing dynamic protein components. This work suggests that higher order RNA structure (or the absence of it) may play a role in the structural morphology of condensates, with long unstructured regions in RNA correlating to the formation of in vitro mesh-like granules.
[33] Ma W, Mayr C. A Membraneless Organelle Associated with the Endoplasmic Reticulum Enables 3'UTR-Mediated Protein-Protein Interactions. Cell 2018; 175:1492-1506 e1419.
[34] Ortony JH, Hwang DS, Franck JM et al. Asymmetric collapse in biomimetic complex coacervates revealed by local polymer and water dynamics. Biomacromolecules 2013; 14:1395-1402.
[35] Spruijt E, Leermakers FAM, Fokkink R et al. Structure and Dynamics of Polyelectrolyte Complex Coacervates Studied by Scattering of Neutrons, X-rays, and Light. Macromolecules 2013; 46:4596-4605.
[36] Ortony JH, Choi SH, Spruell JM et al. Fluidity and water in nanoscale domains define coacervate hydrogels. Chemical Science 2014; 5:58-67.
[37] Emmanouilidis L, Esteban-Hofer L, Damberger FF et al. NMR and EPR reveal a compaction of the RNA-binding protein FUS upon droplet formation. Nat Chem Biol 2021; 17:608-614.
[38] Fares HM, Ghoussoub YE, Delgado JD et al. Scattering Neutrons along the Polyelectrolyte Complex/Coacervate Continuum. Macromolecules 2018; 51:4945-4955.

**[39] Banerjee PR, Milin AN, Moosa MM et al. Reentrant Phase Transition Drives Dynamic Substructure Formation in Ribonucleoprotein Droplets. Angew Chem Int Ed Engl 2017; 56:11354-11359.
This work demonstrated reentrant phase transitions in peptide/RNA systems, and their ability to encode complex and dynamic substructure.
[40] Milin AN, Deniz AA. Reentrant Phase Transitions and Non-Equilibrium Dynamics in Membraneless Organelles. Biochemistry 2018; 57:2470-2477.
[41] Krainer G, Welsh TJ, Joseph JA et al. Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions. Nat Commun 2021; 12:1085.
[42] Onuchic PL, Milin AN, Alshareedah I et al. Divalent cations can control a switch-like behavior in heterotypic and homotypic RNA coacervates. Sci Rep 2019; 9:12161.
[43] Freeman Rosenzweig ES, Xu B, Kuhn Cuellar L et al. The Eukaryotic CO2-Concentrating Organelle Is Liquid-like and Exhibits Dynamic Reorganization. Cell 2017; 171:148-162 e119.
[44] Wang J, Mbah CF, Przybilla T et al. Magic number colloidal clusters as minimum free energy structures. Nat Commun 2018; 9:5259.
[45] Ulcakar L, Rejec T, Kokalj J et al. On the resilience of magic number theory for conductance ratios of aromatic molecules. Sci Rep 2019; 9:3478.
[46] Xu B, He G, Weiner BG et al. Rigidity enhances a magic-number effect in polymer phase separation. Nat Commun 2020; 11:1561.
[47] Zhang Y, Xu B, Weiner BG et al. Decoding the physical principles of two-component biomolecular phase separation. Elife 2021; 10.
[48] Klosin A, Oltsch F, Harmon T et al. Phase separation provides a mechanism to reduce noise in cells. Science 2020; 367:464-468.
[49] Henninger JE, Oksuz O, Shrinivas K et al. RNA-Mediated Feedback Control of Transcriptional Condensates. Cell 2021; 184:207-225 e224.
[50] Feric M, Vaidya N, Harmon TS et al. Coexisting Liquid Phases Underlie Nucleolar Subcompartments. Cell 2016; 165:1686-1697.
[51] Fei J, Jadaliha M, Harmon TS et al. Quantitative analysis of multilayer organization of proteins and RNA in nuclear speckles at super resolution. J Cell Sci 2017; 130:4180-4192.
[52] Jain S, Wheeler JR, Walters RW et al. ATPase-Modulated Stress Granules Contain a Diverse Proteome and Substructure. Cell 2016; 164:487-498.
[53] West JA, Mito M, Kurosaka S et al. Structural, super-resolution microscopy analysis of paraspeckle nuclear body organization. J Cell Biol 2016; 214:817-830.
[54] Trcek T, Grosch M, York A et al. Drosophila germ granules are structured and contain homotypic mRNA clusters. Nat Commun 2015; 6:7962.
[55] Lu T, Spruijt E. Multiphase Complex Coacervate Droplets. J Am Chem Soc 2020; 142:2905-2914.
**[56] Mountain GA, Keating CD. Formation of Multiphase Complex Coacervates and Partitioning of Biomolecules within them. Biomacromolecules 2020; 21:630-640.
This work systematically studied LLPS for a series of multiple polycations and polyanions, finding that partitioning and layering of droplets can be heavily influenced by a combination of valency and interaction strengths.

[57] Fisher RS, Elbaum-Garfinkle S. Tunable multiphase dynamics of arginine and lysine liquid condensates. Nat Commun 2020; 11:4628.
**[58] Kaur T, Raju M, Alshareedah I et al. Sequence-encoded and composition-dependent protein-RNA interactions control multiphasic condensate morphologies. Nat Commun 2021; 12:872.
Using a triad of biologically related model polymers, the authors observe the mechanistic effects of competing intermolecular interactions on regulating droplet morphology and organization.
**[59] Sanders DW, Kedersha N, Lee DSW et al. Competing Protein-RNA Interaction Networks Control Multiphase Intracellular Organization. Cell 2020; 181:306-324 e328.
In the context of complex stress granule formation, the authors systematically probe the role of valence on protein-protein network formation and subsequent hierarchical droplet organization.
[60] Sanchez-Burgos I, Espinosa JR, Joseph JA, Collepardo-Guevara R. Valency and Binding Affinity Variations Can Regulate the Multilayered Organization of Protein Condensates with Many Components. Biomolecules 2021; 11.
[61] Sing CE, Perry SL. Recent progress in the science of complex coacervation. Soft Matter 2020; 16:2885-2914.
[62] Joseph JA, Espinosa JR, Sanchez-Burgos I et al. Thermodynamics and kinetics of phase separation of protein-RNA mixtures by a minimal model. Biophys J 2021; 120:1219-1230.
[63] Nott TJ, Craggs TD, Baldwin AJ. Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. Nat Chem 2016; 8:569-575.
[64] Majumdar A, Dogra P, Maity S, Mukhopadhyay S. Liquid-Liquid Phase Separation Is Driven by Large-Scale Conformational Unwinding and Fluctuations of Intrinsically Disordered Protein Molecules. J Phys Chem Lett 2019; 10:3929-3936.
[65] Tange H, Ishibashi D, Nakagaki T et al. Liquid-liquid phase separation of full-length prion protein initiates conformational conversion in vitro. J Biol Chem 2021:100367.
[66] Jawerth L, Fischer-Friedrich E, Saha S et al. Protein condensates as aging Maxwell fluids. Science 2020; 370:1317-1323.
[67] Andre AAM, Spruijt E. Liquid-Liquid Phase Separation in Crowded Environments. Int J Mol Sci 2020; 21.
[68] Roberts S, Miao V, Costa S et al. Complex microparticle architectures from stimuli-responsive intrinsically disordered proteins. Nat Commun 2020; 11:1342.
[69] Jeon BJ, Nguyen DT, Saleh OA. Sequence-Controlled Adhesion and Microemulsification in a Two-Phase System of DNA Liquid Droplets. J Phys Chem B 2020; 124:8888-8895.
[70] Sato Y, Sakamoto T, Takinoue M. Sequence-based engineering of dynamic functions of micrometer-sized DNA droplets. Science Advances 2020; 6:eaba3471.
[71] Karoui H, Seck MJ, Martin N. Self-programmed enzyme phase separation and multiphase coacervate droplet organization. Chemical Science 2021; 12:2794-2802.
[72] Deng J, Walther A. Programmable ATP-Fueled DNA Coacervates by Transient Liquid-Liquid Phase Separation. Chem 2020; 6:3329-3343.
[73] Van Lindt J, Bratek-Skicki A, Nguyen PN et al. A generic approach to study the kinetics of liquid-liquid phase separation under near-native conditions. Commun Biol 2021; 4:77.

[74] Le Ferrand H, Duchamp M, Gabryelczyk B et al. Time-Resolved Observations of Liquid-Liquid Phase Separation at the Nanoscale Using in Situ Liquid Transmission Electron Microscopy. J Am Chem Soc 2019; 141:7202-7210.
[75] Klein IA, Boija A, Afeyan LK et al. Partitioning of cancer therapeutics in nuclear condensates. Science 2020; 368:1386-1392.
[76] Reinkemeier CD, Girona GE, Lemke EA. Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes. Science 2019; 363.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: