Article:
Alberti, S. orcid.org/0000-0003-4017-6505, Arosio, P. orcid.org/0000-0002-2740-1205, Best, R.B. orcid.org/0000-0002-7893-3543 et al. (31 more authors) (2025) Current practices in the study of biomolecular condensates: a community comment. Nature Communications, 16 (1). 7730. ISSN: 2041-1723

https://doi.org/10.1038/s41467-025-62055-8

Comment

https://doi.org/10.1038/s41467-025-62055-8

Current practices in the study of biomolecular condensates: a community comment

Simon Alberti, Paolo Arosio, Robert B. Best, Steven Boeynaems, Danfeng Cai, Rosana Collepardo-Guevara, Gregory L. Dignon, Rumiana Dimova, Shana Elbaum-Garfinkle, Nicolas L. Fawzi, Monika Fuxreiter, Amy S. Gladfelter, Alf Honigmann, Ankur Jain, Jerelle A. Joseph, Tuomas P. J. Knowles, Keren Lasker, Edward A. Lemke, Kresten Lindorff-Larsen, Reinhard Lipowsky, Jeetain Mittal, Samrat Mukhopadhyay, Sua Myong, Rohit V. Pappu, Karsten Rippe, Tatyana A. Shelkovnikova, Anthony G. Vecchiarelli, Susanne Wegmann, Huaiying Zhang, Mingjie Zhang, Chloe Zubieta, Markus Zweckstetter, Dorothee Dormann & Tanja Mittag

The realization that the cell is abundantly compartmentalized into biomolecular condensates has opened new opportunities for understanding the physics and chemistry underlying many cellular processes[1], fundamentally changing the study of biology[2]. The term biomolecular condensate refers to non-stoichiometric assemblies that are composed of multiple types of macromolecules in cells, occur through phase transitions, and can be investigated by using concepts from soft matter physics[3]. As such, they are intimately related to aqueous two-phase systems[4] and water-in-water emulsions[5]. Condensates possess tunable emergent properties such as interfaces, interfacial tension, viscoelasticity, network structure, dielectric permittivity, and sometimes interphase pH gradients and electric potentials[6–14]. They can form spontaneously in response to specific cellular conditions or to active processes, and cells appear to have mechanisms to control their size and location[15–17]. Importantly, in contrast to membrane-enclosed organelles such as mitochondria or peroxisomes, condensates do not require the presence of a surrounding membrane.

Condensates have been linked to many cellular functions. They can accelerate or suppress biochemical reactions[18–20], aid in the storage or sequestration of molecules[21], and even patch damaged membranes[22] and generate mechanical capillary forces[23–28]. The cell can use phase separation to sense and respond to changes in the environment[29–31] or to buffer against concentration fluctuations in the cytosol or nucleoplasm[32]. Condensation plays a role in genomic activities such as DNA replication, recombination, and repair, as well as in transcription, translation, signaling and stress responses[33]. It has also been linked to various diseases such as neurodegeneration and cancer[34,35]. Condensation may, for instance, lead to a gain of function that drives the specific disease process.

Liquid-liquid phase separation (LLPS) has received attention as a process of condensate assembly, and the analogy to the demixing of oil and water is often used. While useful as a first introduction to the conceptual framework, the term and analogy can be misleading because they imply that both phases are purely viscous liquids and that components segregate nearly completely. In fact, as we will see, condensates enrich molecules to a wide variety of degrees, can possess a wide-range of material states[36,37], and can form through a variety of physical processes[3,38,39]. Many of their constituents are multivalent biopolymers (e.g., proteins and nucleic acids) that form a multiplicity of attractive and repulsive, solvent-mediated, reversible interactions[40–42]. These interactions create internal spatial and dynamic inhomogeneities that generate local or condensate-spanning networks[11,43–47]. Furthermore, macromolecule conformations are environmentally sensitive and heterogeneous, and can display orientational ordering. This means that condensates are viscoelastic, with gel-like or liquid-crystalline or even semi-crystalline organization on different length scales[48,49]. Such systems are referred to in the soft matter literature as complex fluids, because they feature dominantly viscous, a combination of viscous and elastic, or dominantly elastic properties depending on the length- and timescales that are probed[50].

Given these complexities, which have come to light even for simple systems composed of a single macromolecule plus solvent, we will use the inclusive terms “biomolecular condensate” and “phase separation” in this text, as the most detail-agnostic descriptors of the phenomenon. We encourage researchers to do the same, and to use precise terms that correctly identify the types of phase transitions and material states that apply to their system. These aspects of condensates have been reviewed and discussed extensively, but we provide here a practical summary that aims to be accessible to readers across disciplines.

One way to think about the network of interactions among macromolecules that phase separate is in terms of coupled associative and

segregative phase transitions (COAST)[50]. For example, phase separation is a segregative phase transition, giving rise to different coexisting phases. Percolation, on the other hand, is an associative phase transition, akin to self-assembly[3]. Other COAST-like processes exist, and these are "coupled" in the sense that they influence each other. The resulting condensates can be catalogued as macrophases (which grow in size with increasing total concentration of their biomolecular constituents) or microphases (which have defined sizes encoded in the molecular architectures of their constituents via block-copolymeric architectures). Another important concept is that of scaffolds and clients, where scaffold molecules drive condensate formation and recruit and concentrate client molecules, which can in turn influence the phase behavior[40,51–53].

To add further complexity, constituent molecules can often form assemblies even below the saturation concentration or phase boundary (i.e., below the threshold concentration for condensate formation), simply because they are multivalent species[54–57]. These assemblies have been called pre-percolation clusters or higher-order oligomers[54,58]. Lacking a delineating phase boundary due to their small size, they are not easily defined by clear differences in internal microenvironments or network structure, unlike coexisting phases. However, probes that are sensitive to solvent polarity show that these clusters are incipient facsimiles of condensates[58].

The fact that condensates have material properties that go beyond those of simple liquids is now very apparent, and these properties are an area of active research[10,12,59]. Material states are emergent and determined by the network structure of the condensate, the transport properties within it (including diffusion and permeability), and the timescales of making and breaking of molecular contacts[37,60–64]. Of note, classical material properties are only defined for macroscopic systems. Biological systems are frequently small, not exceeding submicron size, and formed from a comparatively small number of molecules. Precise language and physics-based measures that are comparable between different types of condensates are therefore particularly important.

The physical characteristics of condensates can strongly influence biochemical functions. For example, condensates generate a distinct solvation environment, including differences in water concentration, structure and dynamics, pH, biomolecule and ion concentrations, dielectric constant, and partitioning of metabolites and biomacromolecules[6–8,13,65–71]. Solvation properties influence partitioning, reaction rates, and other biochemical processes[31,72–74]. Moreover, in many biological condensates the concentration of components can easily reach what is referred to in polymer science as the semi-dilute regime, in which the macromolecule forming the condensate can become a solvent for itself, dramatically changing the environment that the biomolecule experiences[75,76].

Further, biomolecules at condensate boundaries, or interfaces, exhibit distinct properties compared to those in the bulk dilute or dense phases, primarily because being positioned at the interface is less energetically favorable than in the bulk dense phase. These differences can encompass aspects such as conformation, orientation, extent of networking, and mobility[11,47,77,78]. These specific properties may drive biochemical reactions or aggregation processes[79–81]. Interfaces can also coincide with gradients of pH, ions, metabolites, or other biomolecules[66,68,69,77].

Finally, condensates can have multi-phase architecture, in which multiple mutually immiscible dense phases form layers or subcompartments around each other[82–85]. Such structures result in multiple interfaces and may therefore be particularly suitable for mediating complex biochemical processes.

Many studies begin by abrogating a particular condensate in live cells, which then spurs biochemical or theoretical advances. Other studies start with biophysical characterization or theoretical and computational modeling of a condensate, which then guides molecular biological and cell biological investigations. We believe that only by collaborating across disciplines, working together as a community, will we achieve our ultimate goal: the physics-based understanding of biological phase separation processes and their role in physiological and pathological states.

In an attempt to provide the field with a research framework to foster more of these fruitful exchanges, and while fully aware that each research project is unique, we will attempt to outline here some recommendations for the study of biomolecular condensates and phase separation.

Studying phase separation inside cells

A biologist studying a condensate in a cell may be interested in revealing its function. This may involve a set of experiments to interrogate the properties and behaviors of the condensate followed by genetic, chemical, and physical manipulations to alter these properties, linking such alterations to phenotypes. However, it is important to point out that it is often difficult (if not impossible) to find perturbations that only affect the condensate-forming behavior while sparing all other functions of the macromolecules involved.

While many condensates have clear functions, it is possible that some condensates have no function and are epiphenomena resulting from the complex behavior of many interacting components at high concentration in one location[86]. However, as with any instance of proving a negative, the possibility remains that a condition-specific function has been missed.

There are several ways to characterize the biophysical properties of condensates in cells. These include the mapping of phase diagrams[87]; the measurement of material and transport properties such as molecular transport by fluorescence recovery after photobleaching (FRAP) or single-molecule tracking[88,89], viscosity, and capillary velocity[9,83]; and single-molecule Förster resonance energy transfer (FRET)-based measurements of scaling exponents of disordered protein regions[75], which inform on the apparent solvent quality of the condensates and, if possible, should be compared to different in vitro states. Ideally these physical properties can be experimentally manipulated: if changes to the biophysical state alters functional output, it is a strong indicator that there is biological relevance to the condensate state.

Characterizing condensate assembly in cells. When studying a new type of condensate, it will be useful to determine the conditions under which it forms and disassembles because this could provide first insights into its potential functional role in cells. Many condensates assemble in response to changes in cell cycle, cellular conditions and/or stress[90,91]. Hypotheses regarding driving forces for condensation that emerge from these initial cellular assays can then be tested in more controlled conditions.

If the question is whether a macromolecule of interest forms biomolecular condensates inside the cell, it is important to study the macromolecule at its endogenous expression level, in the cellular or tissue environment where it is relevant. Knocking down/out the endogenous copy and then exogenously expressing the protein at

different levels can be used to dissect concentration-dependence of condensate formation via phase diagram mapping[43,92]. To investigate if a protein is localized in cellular condensates, live–cell imaging approaches avoid potential artifacts from fixing[93] and are recommended whenever possible. To visualize large condensates (>300 nanometers), wide-field or confocal microscopy can be used. For visualizing smaller condensates or clusters (20–300 nanometers), super-resolution imaging (e.g. Airyscan, structured illumination microscopy, photo-activated localization microscopy or stimulated emission depletion microscopy) can be used[54,94]. Single-particle tracking is a powerful technique for the study of protein localization and diffusion within condensates, large or small[88,89,95].

Another goal may be to map the composition of condensates in cells. This could involve crosslinking experiments, immunoprecipitation, or proximity labeling approaches followed by mass spectrometry and/or RNA sequencing with the relevant controls to capture only condensate interactors[96–101]. If the condensate changes composition or physical properties over time, these experiments would ideally be performed at different time points to characterize potential changes in composition. Understanding the drivers of cellular phase separation processes is particularly important, and this can be achieved by genetic screens of all components combined with imaging[43].

Given that nucleic acids are often components and/or drivers of condensate formation[102], it is important to study their contributions. In fixed cells or tissue, target RNA can be probed using in situ hybridization. In live cells, one common scheme for labeling RNA is using the MS2- and PP7-tag systems[103]. Here, the RNA of interest is appended with multiple copies of phage-derived hairpin sequences. RNA is rendered fluorescent by co-expression of the cognate hairpin binding protein labeled with an appropriate fluorophore. These tags require genetic engineering of the target RNA. Another approach is using catalytically inactive RNA-targeting CRISPR systems, such as Cas13, which allow probing endogenous RNA using an appropriate guide[104]. Additionally, hybridization-based approaches with probes, such as peptide nucleic acids that have high affinity for RNA, hold promises for live imaging as well.

To understand the phase behavior of a protein that has been identified as a driver of condensate formation, the characterization of in vivo phase diagrams is informative. This could involve observing a protein over a wide range of concentrations, combined with quantitative imaging[105]. Phase transitions are generally sensitive to environmental cues and physicochemical parameters such as pH[73,74], salt[72], temperature[106–108], and pressure[109], and these parameters can be altered in cells[31]. However, in vivo characterization of the phase behavior of a macromolecule remains a challenge. Many such molecules are not expected to have a fixed saturation concentration in cells because they are part of multicomponent condensates that form through an interplay of homo- and heterotypic interactions. The dilute phase concentrations of the driver components are thus not fixed but depend on the ratio of their total concentrations[110]. A clear cut example of how to proceed with identifying the core scaffolds, the modulators, the ligands, and methods to map phase boundaries emerged in 2020 with three decisive contributions focused on stress granules[43,92,111].

In the early 2000s, hexanediol was used as a chemical that can perturb hydrophobic interactions and thus destroy the permeability barrier of nuclear pore complexes[112]. Later, attempts were made to use hexanediol treatment as a general method to determine whether a given structure is a condensate with liquid-like properties, which it does not report on[113]. Furthermore, hexanediol has been shown to have various detrimental effects in cells[114–116]. In our view, it should thus be avoided and not be used as evidence for the formation of an assembly via phase separation or the fluid-like nature of a condensate in vitro or in vivo.

Inducible condensation allows the study of phase separation processes in live cells, with control of nucleation, growth, and size[117]. In addition, it enables the assessment of which functions are specifically gained by inducing phase separation and thus decouples the contributions of condensation from other protein functions. Optogenetic tools have been widely used to study the phase behavior of proteins in cells[38,118–120]. They can be immensely useful for facile manipulation of the state of matter and for mapping phase diagrams. Due diligence is then required to discern the contributions of intrinsic phase behaviors versus the contributions of valence-augmenting domains or cores, and this requires detailed titrations of valence and laser power. If these methods are used, different domain combinations should be carefully considered along with loss-of-function mutants of the protein of interest. While optogenetic induction provides high spatiotemporal resolution and reversibility, chemical induction is ideal for evaluating long-term effects because phototoxicity is reduced[43,117,121]. Chemoptogenetic tools that are based on photosensitive chemical probes can combine the benefits of both approaches[122,123]. For conclusions on function, it is important to ensure that the induced condensates mimic endogenous condensates in terms of location, size, composition, and material properties. This can be achieved by controlling protein expression level, nucleation site, and induction degree (by varying the light intensity/duration or the amount of chemical used). Inducible condensation tools are useful to assess the consequences of condensation processes, particularly in combination with other methods that assess properties of endogenous biomolecules.

Characterizing physical properties of condensates in cells. To study the dynamics of condensate components, they are typically tagged/labeled with a fluorescent dye and then visualized using live-cell imaging. Dyes include fluorescent proteins, self-labeling protein tags, and genetically encoded handles for labeling[75]. Ideally these observations would be performed on the endogenous protein because altered protein levels will very likely influence the dynamics. It is also important that labeling does not interfere with protein function, or with oligomerization properties. A comparison of several labeling strategies is therefore useful[124,125].

Fluorescent components enable the measurement of molecular diffusion and dynamics. Important approaches to investigate these properties are single-particle tracking, FRAP and fluorescence correlation spectroscopy (FCS), which can give insight into the mobility of individual condensate components[9,88,89,126,127]. This can prove valuable for quantifying the strengths of associative interactions within condensates. However, it is worth emphasizing that FRAP readouts need to be interpreted cautiously because they alone are not directly informative regarding material states of condensates and instead report on the mobility of the labeled species[128].

Additional approaches are available to determine the molecular density and organization of condensates, including fluorescence-lifetime imaging microscopy (FLIM), homoFRET, immuno-transmission electron microscopy (TEM), correlative light and electron microscopy (CLEM), optical diffraction tomography (ODT), and hyperspectral imaging combined with phasor plot analysis[13,71,129–134]. To investigate the viscoelasticity of condensates, time-lapse imaging approaches have provided important insight[75], as have new, physics-

based methods of image analysis to extract bending rigidity[135]. These approaches allow capturing condensate fusion events, shape relaxation, or coarsening. Other optical approaches such as Brillouin microscopy can also help determine viscoelastic properties[136]. For condensates that wet surfaces such as membranes or the cytoskeleton, the contact angle can provide important insights into the material properties and underlying forces[13,16,83,134,137,138,139,140].

In recent years, super-resolution microscopy and single-molecule tracking measurements of the diffusive properties of the molecular components of a condensate have become a critical benchmark[89,141,142]. Methods such as photoactivated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM) are powerful because they enable the determination of how proteins behave in time and space by localizing single emitters with 10–30 nanometer localization precision and 10–100 microsecond time resolution[143,144]. Cryogenic electron tomography (cryo-ET) is also emerging as a technique to describe the architecture and morphological properties of condensates[14,145,146].

A common property of condensates is that they have a selective interface. The properties of an interface can be probed by comparing the trajectories of molecules diffusing in the cytoplasm, in the condensate, and those traversing (or being reflected by) the interface using single-molecule tracking approaches[147]. It is worth mentioning that not all trackable molecular species must experience resistance at the interface[3,148].

It should be noted that studying condensates in bacterial cells is particularly difficult because they are typically an order of magnitude smaller than eukaryotic cells. Observations of condensates in bacterial cells are thus almost always diffraction limited. As a result, many criteria listed above are not easily applicable in bacterial cells and distinct approaches with even higher resolutions are actively being developed for these systems[149–153].

Perturbing condensates in cells to assess function. A powerful way to assess the function of a condensate is a perturbation experiment in which the condensate properties are specifically altered, and the cellular phenotype is assessed. This typically requires the identification of component domains and/or residues that are required for phase separation and their subsequent perturbation. The solubility or networking ability of the component, and therefore its saturation concentration, could be altered by introducing specific mutations. Important preconditions include that the molecule retains its biological functions, stability, and biochemical features that are not tied to its condensation activity.

If such a loss-of-function mutant shows a phenotypic defect, it is useful to test whether the wildtype phenotype can be restored by adding back a fusion protein that endows an orthogonally-encoded phase separation activity (e.g., via the PopZ-Tag[152], an intrinsically disordered region such as the FUS N-terminus[154], or via addition of orthogonal oligomerization domains[43]). Restoration of function in such a condensate complementation experiment would be strong evidence for a functional role of condensation, but a negative result is not informative as the lack of complementation could be due to loss of other interactions in the deleted domain.

Importantly, functional defects upon disruption of a condensate are not necessarily sufficient to infer a specific function if interaction patterns with partners in the dilute phase are also disrupted[3]. Identifying variants or mutations that exaggerate the condensation properties can be useful. Sometimes it will also be necessary to change residues or domains involved in client recruitment without affecting condensation itself or substitute residues to drive alternate localization. Such separation-of-function variants may be difficult to achieve but are highly informative[3]. Importantly, in addition to testing the hypothesis that a condensate carries a specific function, alternative hypotheses should be considered, such as that function arises from soluble complexes[155,156].

As already discussed, labeling/tagging of endogenous biomolecules should be attempted whenever possible (e.g., by CRISPR-mediated tagging, inducible titratable promoters, or introducing artificial amino acids and click chemistry). Stable cell lines with low expression of tagged proteins, preferentially with knock-out of the endogenous protein, are the next best solution. Notably, tagging a protein with a fluorescent protein can alter its ability to condense[157], and comparisons of several different fluorescent proteins and/or with immunofluorescence are thus useful[93,124].

While there is an increasing repertoire of in vivo approaches, many questions will require complementary in vitro and in silico approaches to obtain clear answers.

Studying phase separation outside cells

Probing the properties of condensates in vitro provides synergistic information to that obtained from experiments focusing on condensates in living cells, tissues, or animals. For example, studying assembly intermediates on the pathway to condensation is difficult in cells due to limits of optical microscopy and can benefit from controlled in vitro conditions in which concentration, ionic strength, and other parameters can be carefully controlled. Indeed, in vitro systems allow systematic variation of the composition of condensates as well as the chemical and physical conditions under which phase separation takes place. This enables the elucidation of the fundamental interactions that drive biomolecular condensation and of the physicochemical factors that modulate them. Using a simple system of only a few components under controlled and known conditions allows for detailed biophysical, biochemical, biomechanical, and structural interrogation of dilute and condensed phases and their dynamics. The extent to which additional components change the emergent properties of condensates can be systematically addressed in vitro. Characterizing simple condensates in vitro, even those of clients or individual domains, offers important conceptual insights that are not easily inferable from complex intracellular condensates.

The quantitative characterization of in vitro biophysical properties is essential for several reasons. (1) Characterizing driving forces for biomolecular phase separation enables an understanding of molecular grammars underlying the formation of different types of condensates. (2) Understanding interactions driving phase separation or the encoding of emergent properties can generate hypotheses that can be tested in cells or whole organisms. (3) Characterizing the network structure, dynamics of biomolecules within condensates and other emergent properties is critical to understanding the biological activities and pathological properties arising from condensates. (4) Biochemical and biophysical readouts provide information for generating improved theories, computational models and biological hypotheses. In turn, models generate hypotheses that can be tested experimentally and quantified through biophysical measurements.

Methods to characterize condensate properties in vitro. One can take bottom-up or top-down approaches to reconstitute condensates in vitro, i.e., start with simple systems and increase their complexity in

steps, or start investigating the full system and over time isolate the most relevant parts. The existence of two-phase regimes can be qualitatively inferred microscopically[158–160], or by measuring turbidity over a concentration range[127,159–161]. As a cautionary note, turbidity is not a direct measure of the driving force for phase separation, and it is best to progressively reduce the concentration of constituents until background turbidity is reached for inferring saturation concentrations. New microfluidics approaches enable rapid scanning of a large concentration space with minimal sample requirements and may even allow inferring tie lines that provide information about the interplay of homo- and heterotypic interactions mediating phase separation[162–167]. Densities of condensates can be determined by quantitative phase microscopy[129] or FCS[168]. Coexistence lines and tie lines can be characterized explicitly by determining coexisting dilute and dense phase concentrations by separating the coexisting phases and determining the concentrations of the constituents[127,169–171]. In simple systems, this may be accomplished through ultraviolet–visible spectroscopy[172]. More complex systems may require approaches such as analytical high-performance liquid chromatography (HPLC)[169].

To characterize the interactions that mediate associative phase separation, typical biophysical techniques are useful. These include nuclear magnetic resonance (NMR) spectroscopy, which enables mapping of interaction sites with amino acid resolution[173–176]. Mapping by deletion, mutagenesis or through structural approaches are other possibilities[177]. Once the motifs or domains mediating interactions are known, the strengths of these interactions can be determined using biophysical approaches such as isothermal titration calorimetry, surface plasmon resonance, fluorescence anisotropy, NMR and electrophoretic mobility shift assays. NMR has been used to determine the identity of adhesive motifs in intrinsically disordered regions[174,176,178], and pairwise interaction strengths have been extracted by generating mutants with varying valence of these motifs and determining their single-chain dimensions by small-angle X-ray scattering (SAXS)[168,179]. The importance of these interactions for phase separation can then be tested by determining the phase behavior of proteins in which these sites are mutated, which should result in weakening of the driving forces for phase separation[57,87,180]. Once variants with interesting phase behavior have been identified in vitro, their behavior in cells and effects on function can be characterized.

Single-molecule studies enable access to molecular information that is normally averaged in conventional ensemble experiments. For instance, single-molecule FRET has been used to track intermolecular and intramolecular interactions in dilute and dense phases and has shown how intramolecular interactions are exchanged for intermolecular interactions[64,181,182]. It can also directly report on single-chain dimensions in condensates. Nanosecond FCS provides information on segmental and domain motions within single molecules in condensates and how these differ from those in the dilute phase[64]. As with any fluorescence measurement, one should be cautious about the potential artifacts that may arise from the source of fluorescence, such as organic dyes or fluorescent proteins[183]. Types of dyes, size of dyes and dye positions should be varied to test for such effects. In addition, the local environment in the condensate may also affect dye photophysics, such as its quantum yield and brightness, and one must be cautious when interpreting the fluorescence intensities[168]. Fluorescence lifetime measurements can be used to examine the effects of condensate environment on dye properties[134,184]. NMR has also provided complementary information on diffusion and internal motions of disordered domains within condensates with atomic detail[174,175]. Hyperspectral imaging of water-soluble environment-sensitive probes can resolve the dielectric permittivity of both the condensate and the dilute phase and, when combined with contact angle measurements, allows one to correlate permittivity differences with membrane affinity[13].

Biomolecule mobility in biomolecular condensates affects biochemical activities and disease processes. Although FRAP can be applied in many different ways to make various measurements of transport properties, there are two main use-cases: (i) the entire condensate can be photobleached, in which case the recovery of the fluorescence signal depends mostly on the exchange between the inside and outside of the condensate; (ii) a small fraction of a large condensate can be photobleached, in which case the fluorescence recovery mostly reflects the diffusional motion of the phase-separated molecules within the condensate[46,71]. The latter parameter can be directly compared to values obtained from single-particle tracking and FCS[10,64,170,181,185–190].

Uniform diffusion within a condensate may indicate that a condensate is composed of a homogeneous phase at the resolution of the method. Single-fluorophore tracking has revealed that even condensates formed by a single protein species have inhomogeneities which affect their viscoelastic properties[71]. To further enhance resolution and provide additional structural information, single-particle reconstruction algorithms, traditionally used in cryo-EM, can also be implemented into multicolor single-molecule localization microscopy. Internal structure of condensates can be revealed with polarization microscopy[191], solution or solid-state NMR spectroscopy[173–175,192,193], cryo-EM and cryo-ET approaches[14,146,194], or single-fluorophore tracking with environmentally sensitive dyes[71].

As a complement to the resulting static view, a network view of condensates, in which interactions rather than locations are highlighted, is useful for understanding their properties[11,45]. The fusion rate between two neighboring condensates is related to two inherent parameters, interfacial tension and viscosity. The relaxation time of similarly-sized condensates can be plotted as a function of the corresponding condensate diameter, and the positive linear slope reveals the “inverse capillary velocity” (i.e., the ratio of viscosity to surface tension)[87,127]. Using the Stokes-Einstein formula and a measured diffusion constant, one can calculate the viscosity, which in turn reveals interfacial tension. These interpretations assume that the condensates are Newtonian liquids on the relevant timescales, which may not always hold true[10,195].

Particle tracking microrheology of probes embedded in condensates can also be used to infer transport properties, including sub- and super-diffusive processes and dominantly viscous versus elastic regimes[62,170,187,190,196], as well as viscous and elastic moduli[188,190]. Optical tweezers can be used to probe the viscoelasticity of condensates via active[10] or passive measurements[12] or can be repurposed for condensate fusion assays[159]. Users of optical tweezers have to be careful to minimize the exposure of condensates to the focused laser beam, which can locally increase the temperature and thereby change the properties of the condensates, but non-perturbative low power seems to be sufficient for many measurements. The interpretation of the measurements should take into consideration the spatial and time scales of the experiment and the relation between probe and network sizes. Other methods to determine surface tension or viscoelastic material properties include micropipette aspiration[191,197], atomic force microscopy (AFM)[198] and flicker spectroscopy[135].

As many condensates in vitro undergo liquid-to-solid phase transitions on the order of minutes or hours[190], timing is critical when examining their properties. When comparing different samples to each other, it is crucial to pay attention to the age of the sample (i.e., the time from first assembly), and to measure time-dependent properties[190,196]. To establish a transition to a solid material, creep tests with optical tweezers can be deployed[190]. Fourier transform infrared spectroscopy and Raman spectroscopy can be used to test whether loss of dynamics is accompanied by solidification[134,190,199].

Typical associative biomolecules that phase separate may form heterogeneous sub-micron sized pre-percolation clusters in subsaturated solutions, which may be a source of biochemical function[58]. They can be detected and characterized by dynamic light scattering (DLS), FCS, or microfluidic confocal spectroscopy. Notably, pre-percolation clusters can also coexist with condensates.

Cell lysates and reconstitution of condensates with purified components. Reconstitution of condensates in vitro from purified biomolecules is a powerful approach that can answer questions about the driving forces for phase separation, and about the formation, functions, and physical properties of condensates. However, it is important to point out that many molecules will form condensates in vitro if extreme solution conditions are chosen, such as high concentrations of crowding agents. Such crowders may not be inert depletants but instead interact with biomolecules. Crowders that are pure depletants can nevertheless be used successfully to determine the intrinsic driving forces for phase separation of biomolecules[200]. In vitro formation of condensates does not imply that the biomolecules in question actually function through condensates. Good judgement should be used regarding the questions that a given in vitro reconstitution can answer.

As a compromise between in vitro and in vivo studies, the use of cell lysates for the reconstitution of more complex condensates with many cellular components represents an attractive intermediate solution[201]. The power of such assays lies in enabling the addition of defined protein/nucleic acid concentrations, the delivery of post-translationally modified proteins or peptides and, more generally, the incorporation of components that cannot be easily produced inside living cells[202,203]. Lysates are also powerful for determining components of specific condensates[201,204].

Studying phase separation using modeling and simulations

Theory and simulation are contributing in meaningful ways to our understanding of the forces driving the formation of biomolecular condensates in single- and multicomponent systems[50,205–209]. Theoretical approaches, including mean-field and more sophisticated field-theoretic approaches[210,211], have yielded key insights, e.g., for charge-rich systems. Meanwhile, computer simulations now play a central role not only in designing and interpreting experiments but also in predicting complex phase behaviors that help guide experimental investigations. The computational studies rely on a range of modeling strategies, each with distinct strengths; understanding the features and limitations of these models is essential for interpreting simulation outcomes effectively.

Even in the simplest systems comprising a single macromolecule, reversible phase transitions involve interactions across multiple length and time scales. To address these distinct scales, researchers use models with varying levels of resolution, guided by a general principle: coarse-grained, lower-resolution models enable access to longer spatiotemporal regimes, while atomistic simulations are constrained to shorter scales. Multi-resolution modeling thus offers the potential to explore phenomena across a broader spectrum of scales. Such multi-resolution approaches deploy models of differing resolutions strategically. Currently, most simulations fix the resolution a priori and aim to predict or interpret experimental outcomes through sequence-specific simulations[212–215]. In parallel phenomenological models provide a valuable complement by seeking to explain observed behaviors or predict experimentally testable outcomes through targeted "what if" scenarios[216,217].

Molecular modeling methods and their resolutions. Given the diversity of models, we adopt a parsimonious classification based on the resolution chosen for the interacting entities. In descending order of molecular detail, the models include: (1) Classical all-atom force fields with explicit representation of solvent and ions[68,69,195,218–222]; (2) Coarse-grained models that preserve sequence-dependent characteristics of biomolecules, including proteins[212–215,223], RNA[224–226], DNA[227–229], and chromatin[229], but omit explicit atomic details of macromolecules or solvent; and (3) Minimal models that represent biomolecules with just a few particles[41,44,53,81,229–231], or use continuum or field-theoretic frameworks, which retain only selected physicochemical details. Multi-resolution models combining two or more levels of representation have also started to emerge in the literature[44,68,69,72,81,229].

With computational resources typically available to the broader community, it is possible to compute (i) bulk thermodynamic properties such as saturation concentrations, critical solution temperatures, inter-residue interactions at the amino acid level, network topology, density of connections[42,44], and material properties[37,42,61] using sequence-dependent coarse-grained models; (ii) atomic-level interaction modes, including protein-solvent interactions and ion partitioning within condensates, using all-atom models[178,232]; and (iii) general physical mechanisms in complex multicomponent systems using minimal on-lattice or off-lattice models and theoretical approaches[45,233,234]. Importantly, results from all-atom and sequence-dependent coarse-grained models can be directly related to experimental observables (e.g., from NMR spectroscopy, SAXS, FRET, absorbance), offering valuable insight into model accuracy and applicability. These computational models are further complemented by physical theories that assist in model development and guide the interpretation of experiments.

Physics-based transferable coarse-grained models. There is a long and rich history of using coarse-grained models to study biomolecular interactions[235–237]. These models often rely on bioinformatically derived interaction scales and incorporate experimental observables to constrain phase space sampling in a manner consistent with experimental results. Efforts have been made to make such approaches transferable, meaning that direct experimental input is not embedded in the model Hamiltonian, as exemplified by the Kim-Hummer model[238], which has been applied to weakly binding multi-protein complexes.

Building upon the successes of these earlier approaches, transferable[212–215] or learned[11,239–243] sequence-dependent coarse-grained modeling have become a popular strategy for probing the role of chemical specificity in biomolecular condensates. The appeal of such models lies in their ability to balance computational efficiency with the retention of essential physicochemical properties (e.g., chemical composition, structure, flexibility), which are critical for

explaining the driving forces underlying phase separation. These approaches share features of simplicity and pairwise interaction potentials. Simplicity is achieved by reducing the number of interaction sites, while specificity is introduced through the nature of the pairwise interactions among coarse-grained interaction units. Although there is often a debate about the relative merits of different modeling approaches, the choice ultimately depends on the questions being addressed and the extent to which a given model enables testable predictions or advances phenomenological understanding.

In transferable models, the resolution defines the nature and types of interaction entities; a potential function is then chosen, and its parameters are chosen to be transferable across systems. These models vary primarily in their treatment of non-bonded interactions between amino acid pairs, which are typically represented by a single interaction site per residue (usually a C_α-based representation), although some models use more or fewer sites[244,245]. Beyond proteins, sequence-dependent transferable models have also been developed for RNA[224,225], DNA[228,229], and chromatin[229]. These systems face the additional challenge of accurately capturing local conformational properties (e.g., torsional and bending rigidity), which affect their phase behavior. A central guiding principle in many transferable, sequence-dependent coarse-grained models is to preserve the simplicity of the energy function while extending applicability to increasingly complex systems, including multidomain proteins with both folded and disordered regions[246], post-translational modifications, and multicomponent mixtures involving proteins, nucleic acids, and chromatin[247].

Important considerations for coarse-grained model selection. Coarse-grained models, and modeling approaches more broadly, are inherently approximate. Rather than being universally applicable, each model's approximations must be evaluated in the context of its intended use. Some models are developed for specific systems and are used to interpret experimental data. Others are designed for broader applicability but may introduce inaccuracies due to their generality. Practitioners are advised to carefully review the range of applicability of the model, particularly how far it can be extended beyond the systems used during parameterization. Ideally, a model should have a demonstrated track record of success in identifying relevant molecular features across diverse systems and matching experimental observations, or at least for the specific class of systems under investigation. Otherwise, their applicability should be carefully evaluated through additional validation studies, preferably in combination with biophysical experiments. When comparing coarse-grained simulation results with experimental data, it is often helpful to consider behavior relative to a well-defined reference, using normalized quantities[248]. This approach is especially valuable for dynamic properties such as diffusion and viscosity, where coarse-grained models typically exhibit accelerated dynamics due to reduced solvent friction and a smoother energy landscape[37]. If needed, mapping schemes can be applied to translate coarse-grained results into experimentally relevant timescales, provided the assumptions underlying the mapping are clearly stated and subjected to further validation[37]. Because multiple approximations are introduced during model development and parameterization, one should not expect absolute agreement between simulation and experiment. Nevertheless, there is growing interest in developing coarse-grained models that achieve quantitative alignment with experimental data on absolute scales across different systems[212–214,249]. The availability of high-quality experimental datasets to refine these models will enhance their predictive capabilities and may yield generalizable insights that extend beyond findings from individual systems.

Choice of a molecular simulation approach. In addition to selecting a transferable model, simulations involve two key decisions: the choice of sampling method (molecular dynamics or Monte Carlo), and whether molecules are represented on or off a lattice. These choices are influenced by factors such as computational efficiency, the complexity of the sampling required, the need to mitigate finite-size artifacts[250], and the nature of the scientific questions that are addressed.

Molecular dynamics sampling has been advanced through the use of slab geometries that reduce system-size effects, particularly those introduced by periodic boundary conditions, compared to droplet geometries containing the same number of protein chains[215,250]. Some practitioners, however, may prefer droplet geometries when system-size effects are negligible, because they offer a more intuitive visual representation. Regardless of geometry, it is important to account for potential finite-size effects[246,250]. Monte Carlo sampling has been advanced through the use of lattice-based simulations[251], which also employ periodic boundary conditions. A key challenge in these approaches lies in constructing a set of Monte Carlo moves that ensure ergodic sampling. The bond-fluctuation model and its generalizations have proven to be quite effective for lattice-based simulations. Both lattice and off-lattice simulations can incorporate more complex representations, either by assigning multiple interaction sites per residue or by grouping multiple residues into larger interaction units. As model resolution changes, careful development and validation become increasingly important.

Many of the approaches mentioned above have been implemented in open-source simulation packages such as LAMMPS[252], GROMACS[253], HOOMD-blue[254], and OpenMM[255]. Depending on the platform, users can take advantage of diverse computational resources, including multi-core CPUs and GPU acceleration, to significantly extend the accessible time and length scales.

Emerging role of all-atom models in dissecting atomic interactions within condensates. Practitioners of atomistic simulations have recently started to push the boundaries of what is feasible, building on the successes of off-lattice coarse-grained models that provide reasonable starting points for condensed phase simulations at atomic resolution[64,68,69]. The development of back-mapping schemes from coarse-grained to atomistic representation has been facilitated by tools such as Modeler[256], Pulchra[257], CAMPARI[258], and Martini[244]. However, the extensive computational resources required for these simulations limit their application primarily to phenomena occurring on shorter timescales. Atomistic simulations are particularly powerful for elucidating the driving forces behind biomolecular phase separation, especially as they relate to solvation thermodynamics, and for characterizing the chemical environment within condensates, including water, ion, and small molecule partitioning[67,259–261]. They are also essential for systems where coarse-grained models fail to capture critical conformational preferences, such as residual secondary structures and their modulation upon condensation (e.g., modulation of helical structures through intermolecular interactions)[57]. Nevertheless, the significant computational overhead associated with fully atomistic simulations has thus far constrained their use for estimating key properties such as phase diagrams and condensate viscosities.

In many cases, valuable insights into the molecular driving forces of biomolecular phase transitions can be obtained by simulating one or two protein molecules, relying on an expected correspondence between molecular interactions in dilute and dense phases[250]. Such studies can be further enhanced by employing advanced sampling techniques to achieve better convergence of thermodynamic averages[180]. Given the inherent limitations of atomistic models, intense activity over the last 15 years has produced modern force fields[262–265] that are sufficiently accurate for simulating biomolecular systems relevant to condensates research[69,180].

Suggestions for the selection of minimal models. Minimal models play a distinct and valuable role in uncovering essential mechanistic details of biomolecular phase behavior and in guiding experimental design[53,216,266]. However, selecting an appropriate low-resolution model can be challenging, as these models are typically developed to address specific questions and are therefore non-transferable in the traditional sense. In some cases, it may be sufficient to represent each protein as a single particle with interaction patches, while in other cases, a polymeric representation may be more appropriate[51,53,230,231,267]. The key to developing effective minimal models lies in clearly defining the scientific questions to be addressed. Although it is not advisable to directly transfer a minimal model from one study to another, prior studies can offer useful guidance and inspiration for creating tailored models suited to new questions[53,231]. Minimal models differ significantly from sequence-dependent coarse-grained and atomistic models in one important respect: predictive capability. While the latter models can often make direct, testable predictions without additional mapping to experimental systems, minimal models typically require additional information to translate their reduced representations into experimentally meaningful variables such as protein sequence. Nevertheless, minimal models remain highly useful for guiding hypotheses and interpreting experimental results.

Need for models to study non-equilibrium phenomena. Finally, it is important to consider phenomena of interest in three broad categories: equilibrium processes, relaxation toward equilibrium, and steady states under non-equilibrium conditions. Most of the models discussed above are well-suited for studying equilibrium or near-equilibrium behavior. Molecular dynamics simulations have been used together with Metropolis Monte Carlo steps to model non-equilibrium dynamics resulting from phosphorylation[268]. However, many emerging properties of biomolecular condensates, particularly in cellular environments, depend critically on non-equilibrium phenomena. A key focus in the field has been the maturation of initially dominantly viscous condensates into elastic solids and, in some cases, further into amyloid fibrils or other aggregated states[42,81,184,269,270]. As the structural transitions underlying these maturation processes are not captured by most currently available models (coarse-grained models generally lack the necessary structural detail, but some of this can be reintroduced while maintaining a significant level of coarse-graining)[271,272], and atomistic simulations are limited by accessible timescales, one possible path forward is to introduce time-dependent dissipation into the potential energy via specialized algorithms[42,81,184]. Such modifications must be applied carefully, with clearly defined scope and assumptions, as the molecular changes introduced may not faithfully represent the processes occurring in experimental systems.

Establishing the equilibrium behavior of a system in silico can nevertheless serve as an important foundation. Comparisons between simulated behavior and in vitro or in vivo experiments can help clarify the contributions of factors not accounted for in computational models, such as cellular context and associated non-equilibrium dynamics.

Future challenges in the field of phase separation

Fundamentally, the field is and has been aiming to go beyond phenomenology and generate a transferable, physics-based understanding of condensate properties and functions across length and time scales. As we have seen, the barriers separating us from this goal are being tackled on multiple fronts. As a conclusion, we mention some emerging concepts that represent opportunities for future research.

Non-equilibrium phase separation refers to phase separation processes that are linked to active processes, such as biochemical reactions[273]. Enzymes such as ATP-driven chaperones, kinases, and helicases can alter the conformation and concentration of molecules in the dilute and dense phase over time[274]. Non-equilibrium processes can strongly modulate the emergent properties of condensates. For instance, biochemical reactions can modulate material properties and prevent or slow down their time-dependent changes[275]. Furthermore, active processes can maintain spatial concentration gradients over time, as observed in the accumulation of P-granules at the posterior end of *C. elegans* embryos[276,277]. Biochemical reactions can also modulate the size distribution of condensates, and chemically-driven condensates can undergo cycles of growth and division reminiscent of the proliferation of living cells[278].

Time-dependent changes of condensate structure are related to non-equilibrium processes but important enough to be mentioned separately. Many condensates age or mature: they undergo time-dependent changes in material and structural properties. This aspect of phase separation is intricately connected with pathology and disease[1,2,34]. Recent work describes condensates as metastable states that can be precursors or suppressors of different forms of aggregates, including misfolded proteins and amyloid fibers[10,18,61,81,190,269].

Surface-mediated phase separation has been recognized to play an important role in cells. The concentration of components that can be achieved on a surface through adsorption can be orders of magnitude higher than in bulk. Such surfaces include membranes, DNA, and the cytoskeleton, to name just a few[17,138,279–282]. How adsorption on these surfaces alters the rules of phase separation with respect to both kinetics and thermodynamics is an important question[17].

While important insights have been revealed by studying condensates in vitro and inside single cells, the role of condensates when cells go on to build tissues, organs, and ultimately organisms, is less well understood[283]. Whether their components, biophysical properties, and functions remain the same in a multicellular system constitutes an important question. The complex signals that cells experience within tissues (mechanical, hormonal, and metabolic, to name a few) may affect intracellular condensates in interesting ways. While pioneering efforts have been made to visualize condensates in nematode, fly, mouse skin, and in model plants such as *Arabidopsis thaliana*, other models such as organoids and mouse tumor xenografts are actively being developed to study condensates in a three-dimensional tissue environment[284,285].

Finally, we want to mention that phase separation principles have also become powerful tools for bioengineering to control, study and develop new functions in cells[117,286]. Applications range from drug release to generating cells that simultaneously operate multiple

genetic codes[287–290]. All of these efforts will be important as we strive to fully reveal the roles of condensates in biology.

Simon Alberti[1] ✉, Paolo Arosio[2], Robert B. Best[3], Steven Boeynaems[4,5,6,7,8], Danfeng Cai[9], Rosana Collepardo-Guevara[10,11] ✉, Gregory L. Dignon[12], Rumiana Dimova[13], Shana Elbaum-Garfinkle[14,15], Nicolas L. Fawzi[16], Monika Fuxreiter[17,18], Amy S. Gladfelter[19] ✉, Alf Honigmann[20], Ankur Jain[21], Jerelle A. Joseph[22], Tuomas P. J. Knowles[10], Keren Lasker[23], Edward A. Lemke[24,25], Kresten Lindorff-Larsen[26], Reinhard Lipowsky[13], Jeetain Mittal[27] ✉, Samrat Mukhopadhyay[28,29], Sua Myong[30] ✉, Rohit V. Pappu[31], Karsten Rippe[32,33], Tatyana A. Shelkovnikova[34], Anthony G. Vecchiarelli[35], Susanne Wegmann[36], Huaiying Zhang[37], Mingjie Zhang[38], Chloe Zubieta[39,40], Markus Zweckstetter[41,42] ✉, Dorothee Dormann[24,25] ✉ & Tanja Mittag[43] ✉

[1]Biotechnology Center, Technische Universität Dresden, Dresden, Germany. [2]Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland. [3]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA. [4]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. [5]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA. [6]Therapeutic Innovation Center (THINC), Baylor College of Medicine, Houston, TX, USA. [7]Center for Alzheimer's and Neurodegenerative Diseases (CAND), Baylor College of Medicine, Houston, TX, USA. [8]Dan L Duncan Comprehensive Cancer Center (DLDCCC), Baylor College of Medicine, Houston, TX, USA. [9]Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA. [10]Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. [11]Department of Genetics, University of Cambridge, Downing Pl, Cambridge CB2 3EH, UK. [12]Department of Chemical and Biochemical Engineering, Rutgers, State University of New Jersey, Piscataway, USA. [13]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany. [14]Structural Biology Initiative, Advanced Science Research Center, CUNY, New York, NY, USA. [15]Ph.D. Programs in Biology, Biochemistry, and Chemistry, Graduate Center of the City University of New York, New York, NY, USA. [16]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI, USA. [17]Department of Biomedical Sciences, University of Padova, Padova, Italy. [18]Department of Physics and Astronomy, University of Padova, Padova, Italy. [19]Department of Cell Biology, Duke University, Durham, USA. [20]Technische Universität Dresden, Center for Molecular and Cellular Bioengineering (CMCB), Dresden, Germany. [21]Whitehead Institute for Biomedical Research, 455 Main Street, Cambridge, MA, USA. [22]Department of Chemical and Biological Engineering, Omenn-Darling Bioengineering Institute, Princeton University, Princeton, NJ, USA. [23]Department of Integrative Structural & Computational Biology, The Scripps Research Institute, La Jolla, CA, USA. [24]Biocenter, Johannes Gutenberg University Mainz, 55128 Mainz, Germany. [25]Institute of Molecular Biology (IMB gGmbH), 55128 Mainz, Germany. [26]Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Copenhagen, Denmark. [27]Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX, USA. [28]Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Mohali, India. [29]Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER), Mohali, India. [30]Program for Cellular Molecular Medicine (PCMM), Pediatrics Department, Boston Children's Hospital, Boston, USA. [31]Department of Biomedical Engineering and Center for Biomolecular Condensates, James F. McKelvey School of Engineering, Washington University in St. Louis, St. Louis, MO, USA. [32]German Cancer Research Center (DKFZ) Heidelberg, Division of Chromatin Networks, Heidelberg, Germany. [33]Center for Quantitative Analysis of Molecular and Cellular Biosystems (BioQuant), Heidelberg University, Heidelberg, Germany. [34]Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom. [35]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA. [36]German Center for Neurodegenerative Diseases (DZNE), Charitéplatz 1, 10117 Berlin, Germany. [37]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, USA. [38]School of Life Sciences, Southern University of Science and Technology, Shenzhen, Guangdong, China. [39]Laboratoire de Physiologie Cellulaire et Végétale, Commissariat à l'énergie atomique et aux énergies alternatives (CEA), 17 ave. des Martyrs, 38000 Grenoble, France. [40]Centre National de la Recherche Scientifique (CNRS), 38000 Grenoble, France. [41]Department for NMR-based Structural Biology, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany. [42]German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany. [43]Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

✉e-mail: simon.alberti@tu-dresden.de; rc597@cam.ac.uk; amy.gladfelter@duke.edu; jeetain@tamu.edu; sua.myong@childrens.harvard.edu; markus.zweckstetter@dzne.de; ddormann@uni-mainz.de; tanja.mittag@stjude.org

Received: 1 July 2025; Accepted: 7 July 2025;
Published online: 19 August 2025

References

1. Shin, Y. & Brangwynne, C. P. Liquid phase condensation in cell physiology and disease. *Science* **357**, eaaf4382 (2017).
2. Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285–298 (2017).
3. Mittag, T. & Pappu, R. V. A conceptual framework for understanding phase separation and addressing open questions and challenges. *Mol. Cell* **82**, 2201–2214 (2022).
4. Hatti-Kaul, R. Aqueous two-phase systems: a general overview. *Mol. Biotechnol.* **19**, 269–278 (2001).
5. Esquena, J. Water-in-water (W/W) emulsions. *Curr. Opin. Colloid Interface Sci.* **25**, 109–119 (2016).
6. Smokers, I. B. A. et al. Selective Ion Binding and Uptake Shape the Microenvironment of Biomolecular Condensates. *J Am Chem Soc.* **147**, 25692–25704 (2025).
7. Posey, A. E. et al. Biomolecular condensates are characterized by interphase electric potentials. *J. Am. Chem. Soc.* **146**, 28268–28281 (2024).
8. King, M. R. et al. Macromolecular condensation organizes nucleolar sub-phases to set up a pH gradient. *Cell* **187**, 1889–1906.e24 (2024).
9. Brangwynne, C. P. et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* **324**, 1729–1732 (2009).
10. Jawerth, L. et al. Protein condensates as aging Maxwell fluids. *Science* **370**, 1317–1323 (2020).
11. Farag, M. et al. Condensates formed by prion-like low-complexity domains have small-world network structures and interfaces defined by expanded conformations. *Nat. Commun.* **13**, 7722 (2022).
12. Alshareedah, I., Moosa, M. M., Pham, M., Potoyan, D. A. & Banerjee, P. R. Programmable viscoelasticity in protein-RNA condensates with disordered sticker-spacer polypeptides. *Nat. Commun.* **12**, 6620 (2021).
13. Sabri, E., Mangiarotti, A. & Dimova, R. Fluorescence-based mapping of condensate dielectric permittivity uncovers hydrophobicity-driven membrane interactions. *bioRxiv*. https://doi.org/10.1101/2025.03.09.642144 (2025).
14. Zhou, H. et al. Quantitative spatial analysis of chromatin biomolecular condensates using cryoelectron tomography. *Proc. Natl Acad. Sci.* **122** (2025).

15. Folkmann, A. W., Putnam, A., Lee, C. F. & Seydoux, G. Regulation of biomolecular condensates by interfacial protein clusters. *Science* **373**, 1218–1224 (2021).
16. Mangiarotti, A. & Dimova, R. Biomolecular condensates in contact with membranes. *Annu. Rev. Biophys.* **53**, 319–341 (2024).
17. Snead, W. T. et al. Membrane surfaces regulate assembly of ribonucleoprotein condensates. *Nat. Cell Biol.* **24**, 461–470 (2022).
18. Das, T. et al. Tunable metastability of condensates reconciles their dual roles in amyloid fibril formation. *Molecular Cell*, **85**, 2230–2245 (2025).
19. Smokers, I. B. A., Visser, B. S., Slootbeek, A. D., Huck, W. T. S. & Spruijt, E. How droplets can accelerate reactions—coacervate protocells as catalytic microcompartments. *Acc. Chem. Res.* **57**, 1885–1895 (2024).
20. Peeples, W. & Rosen, M. K. Mechanistic dissection of increased enzymatic rate in a phase-separated compartment. *Nat. Chem. Biol.* **17**, 693–702 (2021).
21. Küffner, A. M. et al. Sequestration within biomolecular condensates inhibits Aβ–42 amyloid formation. *Chem. Sci.* **12**, 4373–4382 (2021).
22. Bussi, C. et al. Stress granules plug and stabilize damaged endolysosomal membranes. *Nature* **623**, 1062–1069 (2023).
23. Jülicher, F. & Weber, C. A. Droplet physics and intracellular phase separation. *Annu. Rev. Condens Matter Phys.* **15**, 237–261 (2024).
24. Wang, Y. et al. Biomolecular condensates mediate bending and scission of endosome membranes. *Nature* **634**, 1204–1210 (2024).
25. Strom, A. R. et al. Condensate interfacial forces reposition DNA loci and probe chromatin viscoelasticity. *Cell* **187**, 5282–5297.e20 (2024).
26. Gouveia, B. et al. Capillary forces generated by biomolecular condensates. *Nature* **609**, 255–264 (2022).
27. Bergeron-Sandoval, L.-P. et al. Endocytic proteins with prion-like domains form viscoelastic condensates that enable membrane remodeling. *Proc. Natl Acad. Sci. USA* **118** (2021).
28. Lipowsky, R. Response of membranes and vesicles to capillary forces arising from aqueous two-phase systems and water-in-water droplets. *J. Phys. Chem. B.* **122**, 3572–3586 (2018).
29. Palumbo, R. J. et al. Coevolution of the Ess1-CTD axis in polar fungi suggests a role for phase separation in cold tolerance. *Sci. Adv.* **8**, eabq3235 (2022).
30. Riback, J. A. et al. Stress-triggered phase separation is an adaptive, evolutionarily tuned response. *Cell* **168**, 1028–1040.e19 (2017).
31. Iserman, C. et al. Condensation of Ded1p promotes a translational switch from housekeeping to stress protein production. *Cell* **181**, 818–831.e19 (2020).
32. Klosin, A. et al. Phase separation provides a mechanism to reduce noise in cells. *Science* **367**, 464–468 (2020).
33. Hirose, T., Ninomiya, K., Nakagawa, S. & Yamazaki, T. A guide to membraneless organelles and their various roles in gene regulation. *Nat. Rev. Mol. Cell Biol.* **24**, 288–304 (2023).
34. Alberti, S. & Dormann, D. Liquid-liquid phase separation in disease. *Annu. Rev. Genet.* **53**, 171–194 (2019).
35. Cai, D., Liu, Z. & Lippincott-Schwartz, J. Biomolecular condensates and their links to cancer progression. *Trends Biochem Sci.* **46**, 535–549 (2021).
36. Wu, H. & Fuxreiter, M. The structure and dynamics of higher-order assemblies: amyloids, signalosomes, and granules. *Cell* **165**, 1055–1066 (2016).
37. Sundaravadivelu Devarajan, D. et al. Sequence-dependent material properties of biomolecular condensates and their relation to dilute phase conformations. *Nat. Commun.* **15**, 1912 (2024).
38. Shimobayashi, S. F., Ronceray, P., Sanders, D. W., Haataja, M. P. & Brangwynne, C. P. Nucleation landscape of biomolecular condensates. *Nature* **599**, 503–506 (2021).
39. Lee, D. S. W. et al. Size distributions of intracellular condensates reflect competition between coalescence and nucleation. *Nat. Phys.* **19**, 586–596 (2023).
40. Banani, S. F. et al. Compositional control of phase-separated cellular bodies. *Cell* **166**, 651–663 (2016).
41. Ruff, K. M., Dar, F. & Pappu, R. V. Ligand effects on phase separation of multivalent macromolecules. *Proc. Natl Acad. Sci.* **118** (2021).
42. Tejedor, A. R. et al. Protein structural transitions critically transform the network connectivity and viscoelasticity of RNA-binding protein condensates but RNA can prevent it. *Nat. Commun.* **13**, 5717 (2022).
43. Yang, P. et al. G3BP1 is a tunable switch that triggers phase separation to assemble stress granules. *Cell* **181**, 325–345.e28 (2020).
44. Espinosa, J. R. et al. Liquid network connectivity regulates the stability and composition of biomolecular condensates with many components. *Proc. Natl Acad. Sci.* **117**, 13238–13247 (2020).
45. Dar, F. et al. Biomolecular condensates form spatially inhomogeneous network fluids. *Nat. Commun.* **15**, 3413 (2024).
46. Li, P. et al. Phase transitions in the assembly of multivalent signalling proteins. *Nature* **483**, 336–340 (2012).
47. Wang, J., Devarajan, D. S., Nikoubashman, A. & Mittal, J. Conformational properties of polymers at droplet interfaces as model systems for disordered proteins. *ACS Macro Lett.* **12**, 1472–1478 (2023).
48. Azzari, P., Bagnani, M. & Mezzenga, R. Liquid-liquid crystalline phase separation in biological filamentous colloids: nucleation, growth and order-order transitions of cholesteric tactoids. *Soft Matter* **17**, 6627–6636 (2021).
49. Chatterjee, S. et al. Reversible kinetic trapping of FUS biomolecular condensates. *Adv. Sci.* **9** (2022).
50. Pappu, R. V., Cohen, S. R., Dar, F., Farag, M. & Kar, M. Phase transitions of associative biomacromolecules. *Chem. Rev.* **123**, 8945–8987 (2023).
51. Rana, U. et al. Asymmetric oligomerization state and sequence patterning can tune multiphase condensate miscibility. *Nat. Chem.* **16**, 1073–1082 (2024).
52. Farag, M., Borcherds, W. M., Bremer, A., Mittag, T. & Pappu, R. V. Phase separation of protein mixtures is driven by the interplay of homotypic and heterotypic interactions. *Nat. Commun.* **14**, 5527 (2023).
53. Welles, R. M. et al. Determinants that enable disordered protein assembly into discrete condensed phases. *Nat. Chem.* **16**, 1062–1072 (2024).
54. Lan, C. et al. Quantitative real-time in-cell imaging reveals heterogeneous clusters of proteins prior to condensation. *Nat. Commun.* **14**, 4831 (2023).
55. Ray, S. et al. Mass photometric detection and quantification of nanoscale α-synuclein phase separation. *Nat. Chem.* **15**, 1306–1316 (2023).
56. Thomson, J. A., Schurtenberger, P., Thurston, G. M. & Benedek, G. B. Binary liquid phase separation and critical phenomena in a protein/water solution. *Proc. Natl Acad. Sci.* **84**, 7079–7083 (1987).
57. Conicella, A. E. et al. TDP-43 α-helical structure tunes liquid-liquid phase separation and function. *Proc. Natl Acad. Sci. USA* **117**, 5883–5894 (2020).
58. Kar, M. et al. Phase-separating RNA-binding proteins form heterogeneous distributions of clusters in subsaturated solutions. *Proc. Natl Acad. Sci. USA* **119**, e2202222119 (2022).
59. Ghosh, A., Kota, D. & Zhou, H.-X. Shear relaxation governs fusion dynamics of biomolecular condensates. *Nat. Commun.* **12**, 5995 (2021).
60. Tanaka, H. Viscoelastic phase separation in biological cells. *Commun. Phys.* **5**, 167 (2022).
61. Tejedor, A. R., Collepardo-Guevara, R., Ramírez, J. & Espinosa, J. R. Time-dependent material properties of aging biomolecular condensates from different viscoelasticity measurements in molecular dynamics simulations. *J. Phys. Chem. B.* **127**, 4441–4459 (2023).
62. Sundaravadivelu Devarajan, D. & Mittal, J. Sequence-encoded spatiotemporal dependence of viscoelasticity of protein condensates using computational microrheology. *JACS Au.* **4**, 4394–4405 (2024).
63. Biswas, S. & Potoyan, D. A. Molecular drivers of aging in biomolecular condensates: desolvation, rigidification, and sticker lifetimes. *PRX Life* **2**, 023011 (2024).
64. Galvanetto, N. et al. Extreme dynamics in a biomolecular condensate. *Nature* **619**, 876–883 (2023).
65. Nott, T. J., Craggs, T. D. & Baldwin, A. J. Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. *Nat. Chem.* **8**, 569–575 (2016).
66. Dai, Y. et al. Interface of biomolecular condensates modulates redox reactions. *Chem* **9**, 1594–1609 (2023).
67. Krevert, C. S. et al. Liquid–liquid phase separation of the intrinsically disordered domain of the fused in sarcoma protein results in substantial slowing of hydration dynamics. *J. Phys. Chem. Lett.* **14**, 11224–11234 (2023).
68. Welsh, T. J. et al. Surface electrostatics govern the emulsion stability of biomolecular condensates. *Nano Lett.* **22**, 612–621 (2022).
69. Zheng, W. et al. Molecular details of protein condensates probed by microsecond long atomistic simulations. *J. Phys. Chem. B.* **124**, 11671–11679 (2020).
70. Ambadi Thody, S. et al. Small-molecule properties define partitioning into biomolecular condensates. *Nat. Chem.* **16**, 1794–1802 (2024).
71. Wu, T. et al. Single-fluorogen imaging reveals distinct environmental and structural features of biomolecular condensates. *Nat. Phys.* **21**, 778–786 (2025).
72. Krainer, G. et al. Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions. *Nat. Commun.* **12**, 1085 (2021).
73. Adame-Arana, O., Weber, C. A., Zaburdaev, V., Prost, J. & Jülicher, F. Liquid phase separation controlled by pH. *Biophys. J.* **119**, 1590–1605 (2020).
74. Munder, M. C. et al. A pH-driven transition of the cytoplasm from a fluid- to a solid-like state promotes entry into dormancy. *Elife* **5** (2016).
75. Yu, M. et al. Visualizing the disordered nuclear transport machinery in situ. *Nature* **617**, 162–169 (2023).
76. De Gennes, P. *Scaling Concepts in Polymer Physics* (Cornell University Press, 1979).
77. Sanchez-Burgos, I., Espinosa, J. R., Joseph, J. A. & Collepardo-Guevara, R. Valency and binding affinity variations can regulate the multilayered organization of protein condensates with many components. *Biomolecules* **11**, 278 (2021).
78. Wang, J. et al. Sequence-dependent conformational transitions of disordered proteins during condensation. *Chem. Sci.* **15**, 20056–20063 (2024).
79. Linsenmeier, M. et al. The interface of condensates of the hnRNPA1 low-complexity domain promotes formation of amyloid fibrils. *Nat. Chem.* **15**, 1340–1349 (2023).
80. Sanchez-Burgos, I., Joseph, J. A., Collepardo-Guevara, R. & Espinosa, J. R. Size conservation emerges spontaneously in biomolecular condensates formed by scaffolds and surfactant clients. *Sci. Rep.* **11**, 15241 (2021).
81. Garaizar, A. et al. Aging can transform single-component protein condensates into multiphase architectures. *Proc. Natl Acad. Sci.* **119** (2022).
82. Fare, C. M., Villani, A., Drake, L. E. & Shorter, J. Higher-order organization of biomolecular condensates. *Open Biol.* **11**, 210137 (2021).
83. Feric, M. et al. Coexisting liquid phases underlie nucleolar subcompartments. *Cell* **165**, 1686–1697 (2016).
84. Regy, R. M., Dignon, G. L., Zheng, W., Kim, Y. C. & Mittal, J. Sequence dependent phase separation of protein-polynucleotide mixtures elucidated using molecular simulations. *Nucleic Acids Res.* **48**, 12593–12603 (2020).

85. Kelley, F. M., Favetta, B., Regy, R. M., Mittal, J. & Schuster, B. S. Amphiphilic proteins coassemble into multiphasic condensates and act as biomolecular surfactants. *Proc. Natl Acad. Sci. USA* **118** (2021).
86. Putnam, A., Thomas, L. & Seydoux, G. RNA granules: functional compartments or incidental condensates?. *Genes Dev.* **37**, 354–376 (2023).
87. Schuster, B. S. et al. Identifying sequence perturbations to an intrinsically disordered protein that determine its phase-separation behavior. *Proc. Natl Acad. Sci.* **117**, 11421–11431 (2020).
88. Chong, S. et al. Tuning levels of low-complexity domain interactions to modulate endogenous oncogenic transcription. *Mol. Cell* **82**, 2084–2097.e5 (2022).
89. Du, M. et al. Direct observation of a condensate effect on super-enhancer controlled gene bursting. *Cell* **187**, 331–344.e17 (2024).
90. Gaglia, G. et al. HSF1 phase transition mediates stress adaptation and cell fate decisions. *Nat. Cell Biol.* **22**, 151–158 (2020).
91. Boyd-Shiwarski, C. R. et al. WNK kinases sense molecular crowding and rescue cell volume via phase separation. *Cell* **185**, 4488–4506.e20 (2022).
92. Sanders, D. W. et al. Competing protein-RNA interaction networks control multiphase intracellular organization. *Cell* **181**, 306–324.e28 (2020).
93. Irgen-Gioro, S., Yoshida, S., Walling, V. & Chong, S. Fixation can change the appearance of phase separation in living cells. *Elife* **11** (2022).
94. Mitrea, D. M. et al. Methods for physical characterization of phase-separated bodies and membrane-less organelles. *J. Mol. Biol.* **430**, 4773–4805 (2018).
95. Hao, S. et al. YAP condensates are highly organized hubs. *iScience* **27**, 109927 (2024).
96. Matheny, T., Rao, B. S. & Parker, R. Transcriptome-wide comparison of stress granules and P-bodies reveals that translation plays a major role in RNA partitioning. *Mol. Cell Biol.* **39** (2019).
97. Markmiller, S. et al. Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell* **172**, 590–604.e13 (2018).
98. Padrón, A., Iwasaki, S. & Ingolia, N. T. Proximity RNA labeling by APEX-seq reveals the organization of translation initiation complexes and repressive RNA granules. *Mol. Cell* **75**, 875–887.e5 (2019).
99. Elmsaouri, S., Markmiller, S. & Yeo, G. W. APEX proximity labeling of stress granule proteins. *Methods Mol. Biol.* **2428**, 381–399 (2022).
100. Youn, J.-Y. et al. High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and Bodies. *Mol. Cell* **69**, 517–532.e11 (2018).
101. Jain, S. et al. ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* **164**, 487–498 (2016).
102. Decker, C. J., Burke, J. M., Mulvaney, P. K. & Parker, R. RNA is required for the integrity of multiple nuclear and cytoplasmic membrane-less RNP granules. *EMBO J.* **41**, e110137 (2022).
103. Tutucci, E. et al. An improved MS2 system for accurate reporting of the mRNA life cycle. *Nat. Methods* **15**, 81–89 (2018).
104. Lenstra, T. L. & Larson, D. R. Single-molecule mRNA detection in live yeast. *Curr. Protoc. Mol. Biol.* **113**, 14.24.1–14.24.15 (2016).
105. Yan, X. et al. Intra-condensate demixing of TDP-43 inside stress granules generates pathological aggregates. *Cell* **188**, 4123-4140.e18 (2025).
106. Dignon, G. L., Zheng, W., Kim, Y. C. & Mittal, J. Temperature-controlled liquid–liquid phase separation of disordered proteins. *ACS Cent. Sci.* **5**, 821–830 (2019).
107. Wadsworth, G. M. et al. RNAs undergo phase transitions with lower critical solution temperatures. *Nat. Chem.* **15**, 1693–1704 (2023).
108. Wuttke, R. et al. Temperature-dependent solvation modulates the dimensions of disordered proteins. *Proc. Natl Acad. Sci.* **111**, 5213–5218 (2014).
109. Cinar, S., Cinar, H., Chan, H. S. & Winter, R. Pressure-sensitive and osmolyte-modulated liquid–liquid phase separation of eye-lens γ-crystallins. *J. Am. Chem. Soc.* **141**, 7347–7354 (2019).
110. Riback, J. A. et al. Composition-dependent thermodynamics of intracellular phase separation. *Nature* **581**, 209–214 (2020).
111. Guillén-Boixet, J. et al. RNA-induced conformational switching and clustering of G3BP drive stress granule assembly by condensation. *Cell* **181**, 346–361.e17 (2020).
112. Ribbeck, K. & Gorlich, D. The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. *EMBO J.* **21**, 2664–2671 (2002).
113. Zheng, T. et al. Molecular insights into the effect of 1,6-hexanediol on FUS phase separation. *EMBO J.* **44**, 2725–2740 (2025).
114. Düster, R., Kaltheuner, I. H., Schmitz, M. & Geyer, M. 1,6-Hexanediol, commonly used to dissolve liquid–liquid phase separated condensates, directly impairs kinase and phosphatase activities. *J. Biol. Chem.* **296**, 100260 (2021).
115. Itoh, Y. et al. 1,6-hexanediol rapidly immobilizes and condenses chromatin in living human cells. *Life Sci. Alliance* **4**, e202001005 (2021).
116. Barrientos, E. C. R., Otto, T. A., Mouton, S. N., Steen, A. & Veenhoff, L. M. A survey of the specificity and mechanism of 1,6 hexanediol-induced disruption of nuclear transport. *Nucleus* **14**, 2240139 (2023).
117. Qian, Z.-G., Huang, S.-C. & Xia, X.-X. Synthetic protein condensates for cellular and metabolic engineering. *Nat. Chem. Biol.* **18**, 1330–1340 (2022).
118. Li, M., Huang, W., Duan, L. & Sun, F. Control intracellular protein condensates with light. *ACS Synth. Biol.* **13**, 3799–3811 (2024).
119. Bracha, D. et al. Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. *Cell* **175**, 1467–1480.e13 (2018).
120. Shin, Y. et al. Spatiotemporal control of intracellular phase transitions using light-activated optodroplets. *Cell* **168**, 159–171.e14 (2017).
121. Yang, J. et al. MYC phase separation selectively modulates the transcriptome. *Nat. Struct. Mol. Biol.* **31**, 1567–1579 (2024).
122. Hernandez-Candia, C. N., Brady, B. R., Harrison, E. & Tucker, C. L. A platform to induce and mature biomolecular condensates using chemicals and light. *Nat. Chem. Biol.* **20**, 452–462 (2024).
123. Hernández-Candia, C. N., Pearce, S. & Tucker, C. L. A modular tool to query and inducibly disrupt biomolecular condensates. *Nat. Commun.* **12**, 1809 (2021).
124. Dörner, K. et al. Tag with Caution — How Protein Tagging Influences the Formation of Condensates. *BioRxiv*. https://doi.org/10.1101/2024.10.04.616694 (2024).
125. Fatti, E., Khawaja, S. & Weis, K. The dark side of fluorescent protein tagging—the impact of protein tags on biomolecular condensation. *Mol. Biol. Cell.* **36** https://doi.org/10.1091/mbc.E24-11-0521 (2025).
126. Beutel, O., Maraspini, R., Pombo-García, K., Martin-Lemaitre, C. & Honigmann, A. Phase separation of zonula occludens proteins drives formation of tight junctions. *Cell* **179**, 923–936.e11 (2019).
127. Chen, N., Zhao, Z., Wang, Y. & Dimova, R. Resolving the Mechanisms of Soy Glycinin Self-Coacervation and Hollow-Condensate Formation. *ACS Macro Lett.* **9**, 1844–1852 (2020).
128. Taylor, N. O., Wei, M.-T., Stone, H. A. & Brangwynne, C. P. Quantifying dynamics in phase-separated condensates using fluorescence recovery after photobleaching. *Biophys. J.* **117**, 1285–1300 (2019).
129. McCall, P. M. et al. Label-free composition determination for biomolecular condensates with an arbitrarily large number of components. *bioRxiv* 2020.10.25.352823. https://doi.org/10.1101/2020.10.25.352823 (2023).
130. Fahim, L. E. et al. Fluorescence lifetime sorting reveals tunable enzyme interactions within cytoplasmic condensates. *J. Cell Biol.* **224** (2025).
131. Rey, T. et al. Mitochondrial RNA granules are fluid condensates positioned by membrane dynamics. *Nat. Cell Biol.* **22**, 1180–1186 (2020).
132. Joshi, A. et al. Intermolecular energy migration via homoFRET captures the modulation in the material property of phase-separated biomolecular condensates. *Nat. Commun.* **15**, 9215 (2024).
133. Ambroggio, E. E., Costa Navarro, G. S., Pérez Socas, L. B., Bagatolli, L. A. & Gamarnik, A. V. Dengue and Zika virus capsid proteins bind to membranes and self-assemble into liquid droplets with nucleic acids. *J. Biol. Chem.* **297**, 101059 (2021).
134. Mangiarotti, A. et al. Biomolecular condensates modulate membrane lipid packing and hydration. *Nat. Commun.* **14**, 6081 (2023).
135. Law, J. O. et al. A bending rigidity parameter for stress granule condensates. *Sci. Adv.* **9** (2023).
136. Beck, T. et al. Optical characterization of molecular interaction strength in protein condensates. *Mol. Biol. Cell* **35**, ar154 (2024).
137. Kusumaatmaja, H., Li, Y., Dimova, R. & Lipowsky, R. Intrinsic contact angle of aqueous phases at membranes and vesicles. *Phys. Rev. Lett.* **103**, 238103 (2009).
138. Mangiarotti, A., Chen, N., Zhao, Z., Lipowsky, R. & Dimova, R. Wetting and complex remodeling of membranes by biomolecular condensates. *Nat. Commun.* **14**, 2809 (2023).
139. Mangiarotti, A. et al. Lipid packing and cholesterol content regulate membrane wetting and remodeling by biomolecular condensates. *Nat. Commun.* **16**, 2756 (2025).
140. Lipowsky, R. Complex remodeling of biomembranes and vesicles by condensate droplets Soft Matter. https://doi.org/10.1039/D5SM00585J.
141. Ramat, A., Haidar, A., Garret, C. & Simonelig, M. Spatial organization of translation and translational repression in two phases of germ granules. *Nat. Commun.* **15**, 8020 (2024).
142. Guo, Y. & Zhang, X. Unveiling intracellular phase separation: advances in optical imaging of biomolecular condensates. *Trends Biochem. Sci.* **49**, 901–915 (2024).
143. Scalisi, S., Ahmad, A., D'Annunzio, S., Rousseau, D. & Zippo, A. Quantitative analysis of PcG-associated condensates by stochastic optical reconstruction microscopy (STORM). *Methods Mol. Biol.* **2655**, 183–200 (2023).
144. Schubert, A. et al. Superresolution microscopy localizes endogenous Dvl2 to Wnt signaling-responsive biomolecular condensates. *Proc. Natl Acad. Sci. USA* **119**, e2122476119 (2022).
145. Wilfling, F. et al. A selective autophagy pathway for phase-separated endocytic protein deposits. *Mol. Cell* **80**, 764–778.e7 (2020).
146. Tollervey, F. et al. Cryo-electron tomography of reconstituted biomolecular condensates. *Methods Mol. Biol.* **2563**, 297–324 (2023).
147. Zhang, Y. et al. The exchange dynamics of biomolecular condensates. *Elife* **12** (2024).
148. Kelley, F. M. et al. Controlled and orthogonal partitioning of large particles into biomolecular condensates. *Nat Commun* **16**, 3521 (2025).
149. Hoang, Y. et al. An experimental framework to assess biomolecular condensates in bacteria. *Nat. Commun.* **15**, 3222 (2024).
150. Parmar, B. S. & Weber, S. C. Single-molecule tracking of RNA polymerase in and out of condensates in live bacterial cells. *Methods Mol. Biol.* **2563**, 371–381 (2023).
151. Saurabh, S. et al. ATP-responsive biomolecular condensates tune bacterial kinase signaling. *Sci. Adv.* **8** (2022).
152. Lasker, K. et al. The material properties of a bacterial-derived biomolecular condensate tune biological function in natural and synthetic systems. *Nat. Commun.* **13**, 5643 (2022).
153. Xu, P. et al. In situ architecture of a nucleoid-associated biomolecular co-condensate that regulates bacterial cell division. *Proc. Natl Acad. Sci. USA* **122**, e2419610121 (2025).
154. Sabari, B. R., Hyman, A. A. & Hnisz, D. Functional specificity in biomolecular condensates revealed by genetic complementation. *Nat. Rev. Genet.* **26**, 279–290 (2024).

155. Sabri, N. et al. Reduction of oligomer size modulates the competition between cluster formation and phase separation of the tumor suppressor SPOP. *J. Biol. Chem.* **299**, 105427 (2023).
156. Bremer, A. et al. Reconciling competing models on the roles of condensates and soluble complexes in transcription factor function. *Mol Cell.* **85**, 2718-2732.e7 (2025).
157. Zhou, Z. K., Hong, K., Huang, B. & Narlikar, G. J. Understanding how genetically encoded tags and crowding agents affect phase separation by heterochromatin protein HP1α. *Cell Rep. Methods.* **5**, 101029 (2025).
158. Wang, J. et al. A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. *Cell* **174**, 688–699.e16 (2018).
159. Ceballos, A. V., McDonald, C. J. & Elbaum-Garfinkle, S. Methods and Strategies to Quantify Phase Separation of Disordered Proteins. *Methods Enzymol.* **611**, 31–50 (2018).
160. Alberti, S., Gladfelter, A. & Mittag, T. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. *Cell* **176**, 419–434 (2019).
161. Alberti, S. et al. A user's guide for phase separation assays with purified proteins. *J. Mol. Biol.* **430**, 4806–4820 (2018).
162. Villois, A. et al. Droplet microfluidics for the label-free extraction of complete phase diagrams and kinetics of liquid–liquid phase separation in finite volumes. *Small* **18**, 2202606 (2022).
163. Arter, W. E. et al. Biomolecular condensate phase diagrams with a combinatorial microdroplet platform. *Nat. Commun.* **13**, 7845 (2022).
164. Bremer, A., Mittag, T. & Heymann, M. Microfluidic characterization of macromolecular liquid–liquid phase separation. *Lab Chip* **20**, 4225–4234 (2020).
165. Kopp, M. R. G. et al. Microfluidic shrinking droplet concentrator for analyte detection and phase separation of protein solutions. *Anal. Chem.* **92**, 5803–5812 (2020).
166. Erkamp, N. A., Qi, R., Welsh, T. J. & Knowles, T. P. J. Microfluidics for multiscale studies of biomolecular condensates. *Lab Chip* **23**, 9–24 (2023).
167. Celetti, G. et al. The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *J. Cell Biol.* **219** (2020).
168. Martin, E. W. et al. Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. *Science* **367**, 694–699 (2020).
169. Bremer, A. et al. Quantifying coexistence concentrations in multi-component phase-separating systems using analytical HPLC. *Biomolecules* **12** (2022).
170. Elbaum-Garfinkle, S. et al. The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. *Proc. Natl Acad. Sci. USA* **112**, 7189–7194 (2015).
171. Nott, T. J. et al. Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol. Cell* **57**, 936–947 (2015).
172. Milkovic, N. M. & Mittag, T. Determination of protein phase diagrams by centrifugation. *Methods Mol. Biol.* **2141**, 685–702 (2020).
173. Sang, D. et al. Condensed-phase signaling can expand kinase specificity and respond to macromolecular crowding. *Mol. Cell* **82**, 3693–3711.e10 (2022).
174. Brady, J. P. et al. Structural and hydrodynamic properties of an intrinsically disordered region of a germ cell-specific protein on phase separation. *Proc. Natl Acad. Sci.* **114** (2017).
175. Burke, K. A., Janke, A. M., Rhine, C. L. & Fawzi, N. L. Residue-by-residue view of in vitro FUS granules that bind the C-terminal domain of RNA polymerase II. *Mol. Cell* **60**, 231–241 (2015).
176. Wake, N. et al. Expanding the molecular grammar of polar residues and arginine in FUS phase separation. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-024-01828-6 (2025).
177. Conicella, A. E., Zerze, G. H., Mittal, J. & Fawzi, N. L. ALS Mutations disrupt phase separation mediated by α-helical structure in the TDP-43 low-complexity C-terminal domain. *Structure* **24**, 1537–1549 (2016).
178. Murthy, A. C. et al. Molecular interactions underlying liquid–liquid phase separation of the FUS low-complexity domain. *Nat. Struct. Mol. Biol.* **26**, 637–648 (2019).
179. Bremer, A. et al. Deciphering how naturally occurring sequence features impact the phase behaviours of disordered prion-like domains. *Nat. Chem.* **14**, 196–207 (2022).
180. Mohanty, P. et al. A synergy between site-specific and transient interactions drives the phase separation of a disordered, low-complexity domain. *Proc. Natl Acad. Sci.* **120** (2023).
181. Joshi, A. et al. Single-molecule FRET unmasks structural subpopulations and crucial molecular events during FUS low-complexity domain phase separation. *Nat. Commun.* **14**, 7331 (2023).
182. Mitrea, D. M. et al. Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA. *Elife* **5** (2016).
183. Holla, A. et al. Identifying sequence effects on chain dimensions of disordered proteins by integrating experiments and simulations. *JACS Au* **4**, 4729–4743 (2024).
184. Shen, Y. et al. The liquid-to-solid transition of FUS is promoted by the condensate surface. *Proc. Natl Acad. Sci.* **120** (2023).
185. Pandey, G., Budhathoki, A. & Spille, J.-H. Characterizing properties of biomolecular condensates below the diffraction limit in vivo. *Methods Mol. Biol.* **2563**, 425–445 (2023).
186. Franzmann, T. M. et al. Phase separation of a yeast prion protein promotes cellular fitness. *Science* **359** (2018).
187. Fisher, R. S. & Elbaum-Garfinkle, S. Tunable multiphase dynamics of arginine and lysine liquid condensates. *Nat. Commun.* **11**, 4628 (2020).
188. Mason, T. G. Estimating the viscoelastic moduli of complex fluids using the generalized Stokes-Einstein equation. *Rheol. Acta* **39**, 371–378 (2000).
189. Agarwal, A., Arora, L., Rai, S. K., Avni, A. & Mukhopadhyay, S. Spatiotemporal modulations in heterotypic condensates of prion and α-synuclein control phase transitions and amyloid conversion. *Nat. Commun.* **13**, 1154 (2022).
190. Alshareedah, I. et al. Sequence-specific interactions determine viscoelasticity and aging dynamics of protein condensates. *Nat. Phys.* **20**, 1482–1491 (2024).
191. Wang, H., Kelley, F. M., Milovanovic, D., Schuster, B. S. & Shi, Z. Surface tension and viscosity of protein condensates quantified by micropipette aspiration. *Biophys. Rep.* **1** (2021).
192. Najbauer, E. E., Ng, S. C., Griesinger, C., Görlich, D. & Andreas, L. B. Atomic resolution dynamics of cohesive interactions in phase-separated Nup98 FG domains. *Nat. Commun.* **13**, 1494 (2022).
193. Damman, R. et al. Atomic-level insight into mRNA processing bodies by combining solid and solution-state NMR spectroscopy. *Nat. Commun.* **10**, 4536 (2019).
194. Hutin, S. et al. Phase separation and molecular ordering of the prion-like domain of the Arabidopsis thermosensory protein EARLY FLOWERING 3. *Proc. Natl Acad. Sci.* **120** (2023).
195. Rekhi, S. et al. Expanding the molecular language of protein liquid-liquid phase separation. *Nat. Chem.* **16**, 1113–1124 (2024).
196. Vidal Ceballos, A. et al. Liquid to solid transition of elastin condensates. *Proc. Natl Acad. Sci. USA* **119**, e2202240119 (2022).
197. Roggeveen, J. V., Wang, H., Shi, Z. & Stone, H. A. A calibration-free model of micropipette aspiration for measuring properties of protein condensates. *Biophys. J.* **123**, 1393–1403 (2024).
198. Costa, L., Li-Destri, G., Pontoni, D., Konovalov, O. & Thomson, N. H. Liquid–liquid interfacial imaging using atomic force microscopy. *Adv. Mater. Interfaces* **4** (2017).
199. Avni, A., Joshi, A., Walimbe, A., Pattanashetty, S. G. & Mukhopadhyay, S. Single-droplet surface-enhanced Raman scattering decodes the molecular determinants of liquid-liquid phase separation. *Nat. Commun.* **13**, 4378 (2022).
200. Chauhan, G., Bremer, A., Dar, F., Mittag, T. & Pappu, R. V. Crowder titrations enable the quantification of driving forces for macromolecular phase separation. *Biophys. J.* **123**, 1376–1392 (2024).
201. Freibaum, B. D., Messing, J., Yang, P., Kim, H. J. & Taylor, J. P. High-fidelity reconstitution of stress granules and nucleoli in mammalian cellular lysate. *J. Cell Biol.* **220** (2021).
202. Ryan, V. H. et al. Tyrosine phosphorylation regulates hnRNPA2 granule protein partitioning and reduces neurodegeneration. *EMBO J.* **40** (2021).
203. Bock, A. S. et al. N-terminal acetylation modestly enhances phase separation and reduces aggregation of the low-complexity domain of RNA-binding protein fused in sarcoma. *Protein Sci.* **30**, 1337–1349 (2021).
204. Lyons, H. et al. Functional partitioning of transcriptional regulators by patterned charge blocks. *Cell* **186**, 327–345.e28 (2023).
205. Saar, K. L. et al. Theoretical and data-driven approaches for biomolecular condensates. *Chem. Rev.* **123**, 8988–9009 (2023).
206. Lin, Y.-H., Forman-Kay, J. D. & Chan, H. S. Theories for sequence-dependent phase behaviors of biomolecular condensates. *Biochemistry* **57**, 2499–2508 (2018).
207. von Bülow, S., Tesei, G. & Lindorff-Larsen, K. Machine learning methods to study sequence-ensemble-function relationships in disordered proteins. *Curr. Opin. Struct. Biol.* **92**, 103028 (2025).
208. Zeng, X. & Pappu, R. V. Developments in describing equilibrium phase transitions of multivalent associative macromolecules. *Curr. Opin. Struct. Biol.* **79**, 102540 (2023).
209. Dignon, G. L., Zheng, W. & Mittal, J. Simulation methods for liquid-liquid phase separation of disordered proteins. *Curr. Opin. Chem. Eng.* **23**, 92–98 (2019).
210. McCarty, J., Delaney, K. T., Danielsen, S. P. O., Fredrickson, G. H. & Shea, J.-E. Complete phase diagram for liquid–liquid phase separation of intrinsically disordered proteins. *J. Phys. Chem. Lett.* **10**, 1644–1652 (2019).
211. Wessén, J., Das, S., Pal, T. & Chan, H. S. Analytical formulation and field-theoretic simulation of sequence-specific phase separation of protein-like heteropolymers with short- and long-spatial-range interactions. *J. Phys. Chem. B.* **126**, 9222–9245 (2022).
212. Tesei, G., Schulze, T. K., Crehuet, R. & Lindorff-Larsen, K. Accurate model of liquid–liquid phase behavior of intrinsically disordered proteins from optimization of single-chain properties. *Proc. Natl Acad. Sci.* **118** (2021).
213. Joseph, J. A. et al. Physics-driven coarse-grained model for biomolecular phase separation with near-quantitative accuracy. *Nat. Comput. Sci.* **1**, 732–743 (2021).
214. Regy, R. M., Thompson, J., Kim, Y. C. & Mittal, J. Improved coarse-grained model for studying sequence dependent phase separation of disordered proteins. *Protein Sci.* **30**, 1371–1379 (2021).
215. Dignon, G. L., Zheng, W., Kim, Y. C., Best, R. B. & Mittal, J. Sequence determinants of protein phase behavior from a coarse-grained model. *PLoS Comput. Biol.* **14**, e1005941 (2018).
216. Lin, A. Z. et al. Dynamical control enables the formation of demixed biomolecular condensates. *Nat. Commun.* **14**, 7678 (2023).
217. Fei, J. et al. Quantitative analysis of multilayer organization of proteins and RNA in nuclear speckles at super resolution. *J. Cell Sci.* **130**, 4180–4192 (2017).
218. De Sancho, D. Phase separation in amino acid mixtures is governed by composition. *Biophys. J.* **121**, 4119–4127 (2022).
219. Paloni, M., Bussi, G. & Barducci, A. Arginine multivalency stabilizes protein/RNA condensates. *Protein Sci.* **30**, 1418–1426 (2021).
220. Paloni, M., Bailly, R., Ciandrini, L. & Barducci, A. Unraveling molecular interactions in liquid–liquid phase separation of disordered proteins by atomistic simulations. *J. Phys. Chem. B* **124**, 9009–9016 (2020).
221. Rauscher, S. & Pomès, R. The liquid structure of elastin. *Elife* **6** (2017).

222. Johnson, C. N. et al. Insights into molecular diversity within the FUS/EWS/TAF15 protein family: unraveling phase separation of the N-terminal low-complexity domain from RNA-binding protein EWS. *J. Am. Chem. Soc.* **146**, 8071–8085 (2024).
223. Latham, A. P. & Zhang, B. Consistent force field captures homologue-resolved HP1 phase separation. *J. Chem. Theory Comput* **17**, 3134–3144 (2021).
224. Aierken, D., Joseph, J. A. Accelerated Simulations Reveal Physicochemical Factors Governing Stability and Composition of RNA Clusters. *J Chem Theory Comput.* **20**, 10209–10222 (2024).
225. Nguyen, H. T., Hori, N. & Thirumalai, D. Condensates in RNA repeat sequences are heterogeneously organized and exhibit reptation dynamics. *Nat. Chem.* **14**, 775–785 (2022).
226. Yasuda, I. et al. Coarse-grained model of disordered RNA for simulations of biomolecular condensates. *J. Chem. Theory Comput.* **21**, 2766–2779 (2025).
227. Kapoor, U., Kim, Y. C. & Mittal, J. Coarse-grained models to study protein–DNA interactions and liquid–liquid phase separation. *J. Chem. Theory Comput.* **20**, 1717–1731 (2024).
228. Latham, A. P. & Zhang, B. On the stability and layered organization of protein-DNA condensates. *Biophys. J.* **121**, 1727–1737 (2022).
229. Farr, S. E., Woods, E. J., Joseph, J. A., Garaizar, A. & Collepardo-Guevara, R. Nucleosome plasticity is a critical element of chromatin liquid–liquid phase separation and multivalent nucleosome interactions. *Nat. Commun.* **12**, 2883 (2021).
230. Statt, A., Casademunt, H., Brangwynne, C. P. & Panagiotopoulos, A. Z. Model for disordered proteins with strongly sequence-dependent liquid phase behavior. *J. Chem. Phys.* **152** (2020).
231. Rekhi, S. et al. Role of strong localized vs weak distributed interactions in disordered protein phase separation. *J. Phys. Chem. B.* **127**, 3829–3838 (2023).
232. Murthy, A. C. et al. Molecular interactions contributing to FUS SYGQ LC-RGG phase separation and co-partitioning with RNA polymerase II heptads. *Nat. Struct. Mol. Biol.* **28**, 923–935 (2021).
233. Feric, M. et al. Mesoscale structure–function relationships in mitochondrial transcriptional condensates. *Proc. Natl Acad. Sci.* **119** (2022).
234. Michels, J. J., Brzezinski, M., Scheidt, T., Lemke, E. A. & Parekh, S. H. Role of solvent compatibility in the phase behavior of binary solutions of weakly associating multivalent polymers. *Biomacromolecules* **23**, 349–364 (2022).
235. Betancourt, M. R. & Thirumalai, D. Pair potentials for protein folding: choice of reference states and sensitivity of predicted native states to variations in the interaction schemes. *Protein Sci.* **8**, 361–369 (1999).
236. Miyazawa, S. & Jernigan, R. L. Residue–residue potentials with a favorable contact pair term and an unfavorable high packing density term, for simulation and threading. *J. Mol. Biol.* **256**, 623–644 (1996).
237. Tanaka, S. & Scheraga, H. A. Medium- and long-range interaction parameters between amino acids for predicting three-dimensional structures of proteins. *Macromolecules* **9**, 945–950 (1976).
238. Kim, Y. C. & Hummer, G. Coarse-grained models for simulations of multiprotein complexes: application to ubiquitin binding. *J. Mol. Biol.* **375**, 1416–1433 (2008).
239. Izvekov, S. & Voth, G. A. A multiscale coarse-graining method for biomolecular systems. *J. Phys. Chem. B.* **109**, 2469–2473 (2005).
240. Lu, L., Dama, J. F. & Voth, G. A. Fitting coarse-grained distribution functions through an iterative force-matching method. *J. Chem. Phys.* **139** (2013).
241. Ercolessi, F. & Adams, J. B. Interatomic potentials from first-principles calculations: the force-matching method. *Europhys. Lett. (EPL)* **26**, 583–588 (1994).
242. van Hoof, B., Markvoort, A. J., van Santen, R. A. & Hilbers, P. A. J. A novel method for coarse graining of atomistic simulations using Boltzmann inversion. *Biophys. J.* **100**, 309a (2011).
243. Reith, D., Pütz, M. & Müller-Plathe, F. Deriving effective mesoscale potentials from atomistic simulations. *J. Comput. Chem.* **24**, 1624–1636 (2003).
244. Souza, P. C. T. et al. Martini 3: a general purpose force field for coarse-grained molecular dynamics. *Nat. Methods* **18**, 382–388 (2021).
245. Baul, U., Chakraborty, D., Mugnai, M. L., Straub, J. E. & Thirumalai, D. Sequence effects on size, shape, and structural heterogeneity in intrinsically disordered proteins. *J. Phys. Chem. B.* **123**, 3462–3474 (2019).
246. Cao, F., von Bülow, S., Tesei, G. & Lindorff-Larsen, K. A coarse-grained model for disordered and multi-domain proteins. *Protein Sci.* **33** (2024).
247. Perdikari, T. M. et al. A predictive coarse-grained model for position-specific effects of post-translational modifications. *Biophys. J.* **120**, 1187–1197 (2021).
248. Her, C. et al. Molecular interactions underlying the phase separation of HP1α: role of phosphorylation, ligand and nucleic acid binding. *Nucleic Acids Res.* **50**, 12702–12722 (2022).
249. von Bülow, S., Tesei, G., Zaidi, F. K., Mittag, T. & Lindorff-Larsen, K. Prediction of phase-separation propensities of disordered proteins from sequence. *Proc. Natl Acad. Sci.* **122** (2025).
250. Dignon, G. L., Zheng, W., Best, R. B., Kim, Y. C. & Mittal, J. Relation between single-molecule properties and phase behavior of intrinsically disordered proteins. *Proc. Natl Acad. Sci. USA* **115**, 9929–9934 (2018).
251. Choi, J.-M., Dar, F. & Pappu, R. V. LASSI: A lattice model for simulating phase transitions of multivalent proteins. *PLoS Comput. Biol.* **15**, e1007028 (2019).
252. Thompson, A. P. et al. LAMMPS - a flexible simulation tool for particle-based materials modeling at the atomic, meso, and continuum scales. *Comput. Phys. Commun.* **271**, 108171 (2022).
253. Abraham, M. J. et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **1–2**, 19–25 (2015).
254. Anderson, J. A., Glaser, J. & Glotzer, S. C. HOOMD-blue: A Python package for high-performance molecular dynamics and hard particle Monte Carlo simulations. *Comput. Mater. Sci.* **173**, 109363 (2020).
255. Eastman, P. et al. OpenMM 7: Rapid development of high performance algorithms for molecular dynamics. *PLoS Comput. Biol.* **13**, e1005659 (2017).
256. Webb, B. & Sali, A. Comparative Protein Structure Modeling Using MODELLER. *Curr. Protoc. Bioinformatics* **54** (2016).
257. Rotkiewicz, P. & Skolnick, J. Fast procedure for reconstruction of full-atom protein models from reduced representations. *J. Comput. Chem.* **29**, 1460–1465 (2008).
258. Vitalis, A. & Pappu, R. V. Methods for Monte Carlo simulations of biomacromolecules. *Annu. Rep. Comput. Chem.* **5**, 49–76 (2009).
259. Mukherjee, S. & Schäfer, L. V. Thermodynamic forces from protein and water govern condensate formation of an intrinsically disordered protein domain. *Nat. Commun.* **14**, 5892 (2023).
260. Latham, A. P. et al. Microphase separation produces interfacial environment within diblock biomolecular condensates. *Elife* **12** (2025).
261. Rekhi, S. & Mittal, J. Amino acid transfer free energies reveal thermodynamic driving forces in biomolecular condensate formation. *Proc. Natl. Acad. Sci. USA.* **122**, e2425422122 (2025).
262. Tang, W. S., Fawzi, N. L. & Mittal, J. Refining all-atom protein force fields for polar-rich, prion-like, low-complexity intrinsically disordered proteins. *J. Phys. Chem. B.* **124**, 9505–9512 (2020).
263. Best, R. B., Zheng, W. & Mittal, J. Balanced protein–water interactions improve properties of disordered proteins and non-specific protein association. *J. Chem. Theory Comput.* **10**, 5113–5124 (2014).
264. Robustelli, P., Piana, S. & Shaw, D. E. Developing a molecular dynamics force field for both folded and disordered protein states. *Proc. Natl Acad. Sci.* **115** (2018).
265. Lindorff-Larsen, K. et al. Improved side-chain torsion potentials for the Amber ff99SB protein force field. *Proteins: Struct. Funct. Bioinform.* **78**, 1950–1958 (2010).
266. Ronceray, P., Zhang, Y., Liu, X. & Wingreen, N. S. Stoichiometry controls the dynamics of liquid condensates of associative proteins. *Phys. Rev. Lett.* **128**, 038102 (2022).
267. Rana, U., Brangwynne, C. P. & Panagiotopoulos, A. Z. Phase separation vs aggregation behavior for model disordered proteins. *J. Chem. Phys.* **155** (2021).
268. Zippo, E., Dormann, D., Speck, T. & Stelzl, L. S. Molecular simulations of enzymatic phosphorylation of disordered proteins and their condensates. *Nat Commun.* **16**, 4649 (2025).
269. Garaizar, A., Espinosa, J. R., Joseph, J. A. & Collepardo-Guevara, R. Kinetic interplay between droplet maturation and coalescence modulates shape of aged protein condensates. *Sci. Rep.* **12**, 4390 (2022).
270. Dekker, M., van der Klok, M. L., Van der Giessen, E. & Onck, P. R. A Coarse-Grained MD Model for Disorder-To-Order Transitions in PolyQ Aggregation. *J. Chem. Theory Comput.* https://doi.org/10.1021/acs.jctc.5c00384 (2025).
271. Rizuan, A., Jovic, N., Phan, T. M., Kim, Y. C. & Mittal, J. Developing bonded potentials for a coarse-grained model of intrinsically disordered proteins. *J. Chem. Inf. Model* **62**, 4474–4485 (2022).
272. Zhang, Y., Li, S., Gong, X. & Chen, J. Toward accurate simulation of coupling between protein secondary structure and phase separation. *J. Am. Chem. Soc.* **146**, 342–357 (2024).
273. Weber, C. A., Zwicker, D., Jülicher, F. & Lee, C. F. Physics of active emulsions. *Rep. Prog. Phys.* **82**, 064601 (2019).
274. Hondele, M., Heinrich, S., De Los Rios, P. & Weis, K. Membraneless organelles: phasing out of equilibrium. *Emerg. Top. Life Sci.* **4**, 331–342 (2020).
275. Hondele, M. et al. DEAD-box ATPases are global regulators of phase-separated organelles. *Nature* **573**, 144–148 (2019).
276. Smith, J. et al. Spatial patterning of P granules by RNA-induced phase separation of the intrinsically-disordered protein MEG-3. *Elife* **5** (2016).
277. Saha, S. et al. Polar positioning of phase-separated liquid compartments in cells regulated by an mRNA competition mechanism. *Cell* **166**, 1572–1584.e16 (2016).
278. Zwicker, D., Seyboldt, R., Weber, C. A., Hyman, A. A. & Jülicher, F. Growth and division of active droplets provides a model for protocells. *Nat. Phys.* **13**, 408–413 (2017).
279. Hsu, C.-P., Aretz, J., Hordeichyk, A., Fässler, R. & Bausch, A. R. Surface-induced phase separation of reconstituted nascent integrin clusters on lipid membranes. *Proc. Natl Acad. Sci. USA* **120**, e2301881120 (2023).
280. Hernández-Vega, A. et al. Local nucleation of microtubule bundles through tubulin concentration into a condensed tau phase. *Cell Rep.* **20**, 2304–2312 (2017).
281. Morin, J. A. et al. Sequence-dependent surface condensation of a pioneer transcription factor on DNA. *Nat. Phys.* **18**, 271–276 (2022).
282. Ghosh, R., Satarifard, V. & Lipowsky, R. Different pathways for engulfment and endocytosis of liquid droplets by nanovesicles. *Nat. Commun.* **14**, 615 (2023).
283. Quiroz, F. G. et al. Liquid-liquid phase separation drives skin barrier formation. *Science* **367** (2020).
284. Tang, Y. et al. A cofactor-induced repressive type of transcription factor condensation can be induced by synthetic peptides to suppress tumorigenesis. *EMBO J.* **43**, 5586–5612 (2024).
285. Liu, X. et al. Small-molecule-induced epigenetic rejuvenation promotes SREBP condensation and overcomes barriers to CNS myelin regeneration. *Cell* **187**, 2465–2484.e22 (2024).
286. Song, S. et al. Synthetic Biomolecular Condensates: Phase-Separation Control, Cytomimetic Modelling and Emerging Biomedical Potential. *Angew. Chem. Int. Edn.* **64** (2025).

287. Dai, Y., You, L. & Chilkoti, A. Engineering synthetic biomolecular condensates. *Nat. Rev. Bioeng.* **1**, 466–480 (2023).
288. Bracha, D., Walls, M. T. & Brangwynne, C. P. Probing and engineering liquid-phase organelles. *Nat. Biotechnol.* **37**, 1435–1445 (2019).
289. Reinkemeier, C. D., Girona, G. E. & Lemke, E. A. Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes. *Science* **363** (2019).
290. Reinkemeier, C. D. & Lemke, E. A. Dual film-like organelles enable spatial separation of orthogonal eukaryotic translation. *Cell* **184**, 4886–4903.e21 (2021).

Acknowledgements
P.A. kindly acknowledges the European Research Council through the Horizon 2020 research and innovation programme (ERC grant agreement No. 101002094) for financial support. R.B.B. was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, NIH. S.B. is a CPRIT Scholar in Cancer Research. Work in the S.B. lab is funded by NIH (1DP2NS142714, 1R01NS138605), NSF (DBI #2213983, WALII), CPRIT (RR220094) and the Welch Foundation (Q-2180-20240404). D.C. acknowledges funding: NIH R35GM142837, and Department of Defense Kidney Cancer Idea Development Award (W81XWH2210900). R.C.-G. is supported by UK Research and Innovation (UKRI) through the Engineering and Physical Sciences Research Council (EPSRC) under the UK Government's Horizon Europe guarantee scheme (Grant: EP/Z002028/1), following the award of an European Research Council (ERC) Consolidator Grant ('ChromatinDroplets') under the European Union's Horizon Europe research and innovation programme. G.L.D. acknowledges support from Rutgers University Start-up funds, and NIH award R35GM150589. R.D. acknowledges the ComeIn-Cell network funded by the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101168939. N.L.F. acknowledges grants R01GM147677 and R01NS116176. M.F. acknowledges Fondazione AIRC per la ricerca sul cancro IG 26229 and PRIN 2022EMZJL4. A.S.G. acknowledges funding: NIH R35-GM156800. A.H. acknowledges SPP 2191 "Molecular Mechanisms of Functional Phase Separation" of the German Science Foundation for financial support (DFG project number: 419138182). J.A.J. acknowledges: Chan Zuckerberg Initiative DAF (an advised fund of Silicon Valley Community Foundation; grant 2023-332391), National Science Foundation (NSF) through the Princeton University (PCCM) Materials Research Science and Engineering Center DMR-2011750, National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM155259. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health and other sponsors. The research of T.P.J.K. is funded in part by the European Research Council through the ERC Grant DiProPhys (Agreement 101001615). K.L. acknowledges the Gordon & Betty Moore Foundation—Moore Inventor Fellowship #579361. E.A.L. and D.D. acknowledge CRC1551 'Polymer concepts in cellular function' of the Deutsche Forschungsgemeinschaft (DFG project number 464588647). K.L.-L. is supported by the PRISM (Protein Interactions and Stability in Medicine and Genomics) centre funded by the Novo Nordisk Foundation (NNF18OC0033950). R.L. acknowledges support by the Max Planck School Matter to Life supported by the German Federal Ministry of Education and Research (BMBF) in collaboration with the Max Planck Society and the Max Planck Institute of Colloids and Interfaces. J.M. acknowledges the following NIH grants: R35GM153388, R01NS116176, R01GM136917. S. Mukhopadhyay acknowledges Anusandhan National Research Foundation (formerly SERB) for research grants (SPR/2020/000333 and CRG/2021/002314) and the JC Bose Fellowship (JCB/2023/000016). S. Myong acknowledges funding: 1 RF1AG071326; 1 RF1 NS113636-01. K.R. acknowledges grant RI1283/16-2 in the Priority Program 2191 "Molecular Mechanisms of Functional Phase Separation" of the Deutsche Forschungsgemeinschaft (DFG). T.A.S. acknowledges a UKRI Future Leaders Fellowship (MR/W004615/1). This work was supported by the National Science Foundation to A.G.V. (NSF CAREER Award No. 1941966). S.W. acknowledges the German Research Foundation (DFG), program SPP2191, grant number 506373047. H.Z. acknowledges support from The National Science Foundation (NSF MCB-2145083). Work in M. Zhang's lab is supported by a grant (82188101) from the National Natural Science Foundation of China. C.Z. received funding from the Agence National de la Recherche (ANR-23-CE20-0019 and ANR-21-CE11-0037). M. Zweckstetter was supported by the European Research Council (ERC) under the EU Horizon 2020 research and innovation programme (grant agreement No. 787679). D.D. acknowledges funding from the Heisenberg Programme (project number 442698351). T.M. acknowledges funding by NIH grants R01GM154414 and R01NS121114, by the St. Jude Children's Research Hospital Research Collaborative on the Biology and Biophysics of RNP Granules, and by the American Lebanese Syrian Associated Charities.

Author contributions
Every author contributed to the writing of this Comment.

Competing interests
S.A. is a scientific advisor and shareholder of Dewpoint Therapeutics. T.P.J.K. Is a co-founder of Transition Bio. K.L. is co-inventor on U.S. Patent Application US 2023/0044825 A1, which covers the use of protein condensates for synthetic applications. K.L.-L. holds stock options in and is a consultant for Peptone Ltd. R.V.P. is a member of the Scientific Advisory Board and shareholder in Dewpoint Therapeutics Inc. The remaining authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Simon Alberti, Rosana Collepardo-Guevara, Amy S. Gladfelter, Jeetain Mittal, Sua Myong, Markus Zweckstetter, Dorothee Dormann or Tanja Mittag.