CellPress
OPEN ACCESS

Molecular Cell

Review

Molecular determinants of condensate composition

Alex S. Holehouse[1,2,*] and Simon Alberti[3,*]
[1]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA
[2]Center for Biomolecular Condensates (CBC), Washington University in St. Louis, St. Louis, MO, USA
[3]Center for Molecular and Cellular Bioengineering, Biotechnology Center, Technische Universität Dresden, Dresden, Germany
*Correspondence: alex.holehouse@wustl.edu (A.S.H.), simon.alberti@tu-dresden.de (S.A.)
https://doi.org/10.1016/j.molcel.2024.12.021

SUMMARY

Cells use membraneless compartments to organize their interiors, and recent research has begun to uncover the molecular principles underlying their assembly. Here, we explore how site-specific and chemically specific interactions shape the properties and functions of condensates. Site-specific recruitment involves precise interactions at specific sites driven by partially or fully structured interfaces. In contrast, chemically specific recruitment is driven by complementary chemical interactions without the requirement for a persistent bound-state structure. We propose that site-specific and chemically specific interactions work together to determine the composition of condensates, facilitate biochemical reactions, and regulate enzymatic activities linked to metabolism, signaling, and gene expression. Characterizing the composition of condensates requires novel experimental and computational tools to identify and manipulate the molecular determinants guiding condensate recruitment. Advancing this research will deepen our understanding of how condensates regulate cellular functions, providing valuable insights into cellular physiology and organization.

INTRODUCTION

Cellular organization is hierarchical, regulated, and dynamic. Far from being a homogeneous mixture of biomolecules, the interior of a cell is a highly organized environment with precise spatial and temporal regulation.[1] Illuminated by an ever-expanding toolkit of imaging technologies and spatial proteomics, complex and dynamic localization patterns of biomolecules have been uncovered at unprecedented resolution, revealing the spatial complexity that governs cellular behavior.[2]

Cellular organization is often described in terms of membrane-bound compartments, such as the nucleus, mitochondria, lysosome, and endoplasmic reticulum. Membrane-bound compartments segregate and concentrate macromolecules into specialized microenvironments, often with critical functions for cellular physiology. These compartments are connected through a dynamic network of transport processes and membrane contact sites that shuttle macromolecules.[3] Targeted subcellular localization to membrane-bound organelles can be driven by specific energy-dependent transporters, passive localization following concentration gradients, or by some combination of the two.[4] The cytoskeleton also plays a critical role in the macroscopic organization of the cellular interior and microscopic transport processes.[5] It offers a structural scaffold for the directed movement of vesicles, proteins, and organelles. Through targeted localization of macromolecules among different membrane-bound compartments, cells ensure that the complement of biomolecular processes is appropriately regulated in space and time.

Beyond membrane-bound compartments, an appreciation for the importance of subcellular organization in the cytosol emerged as early as 1899.[6,7] Even as structural biology was in its infancy, it was recognized that labile interactions between proteins in the cellular context may be critical for forming functionally important higher-order assemblies.[8–10] The importance of these assemblies was initially proposed as a conceptual model,[9,11] explored biochemically in the context of various membraneless bodies, and has ultimately re-emerged under the moniker of biomolecular condensates.[12–16]

Biomolecular condensates (condensates) are membraneless, non-stoichiometric cellular assemblies that concentrate and recruit specific biomolecules while excluding others[12,13,17,18] (Figure 1A). Condensates can be small, made up of hundreds of molecules, or large, with diameters of multiple micrometers. From a cellular perspective, they can be constitutive (e.g., nucleoli[19,20]) or inducible (e.g., stress granules[21,22]). They possess emergent properties—properties that appear only as a consequence of the collective effect of the condensate constituents.[23,24] These emergent properties include (but are not limited to) an internal dielectric,[25] interfacial tension,[26] viscosity,[14,27,28] elasticity,[29,30] pH,[31–33] local ion concentration,[34,35] and interfacial electric potentials,[34,36–39] all of which can differ significantly from their surroundings. Moreover, condensates have a boundary that defines the interior and exterior through an interfacial region, which can be just tens of nanometers in width, and that interface can encode properties distinct from both the interior and exterior.[34,40–42] In short, despite lacking a canonical lipid barrier separating their interior and exterior, condensates possess many properties typically ascribed to membrane-bound organelles.[43–45] Importantly, entry and exit into a condensate does not necessarily require an active (energy-dependent) process but can occur via passive diffusion of molecules into and out of the condensate (Figure 1B).

Given that they lack a surrounding membrane, why do condensates form and remain stable? While various physical processes

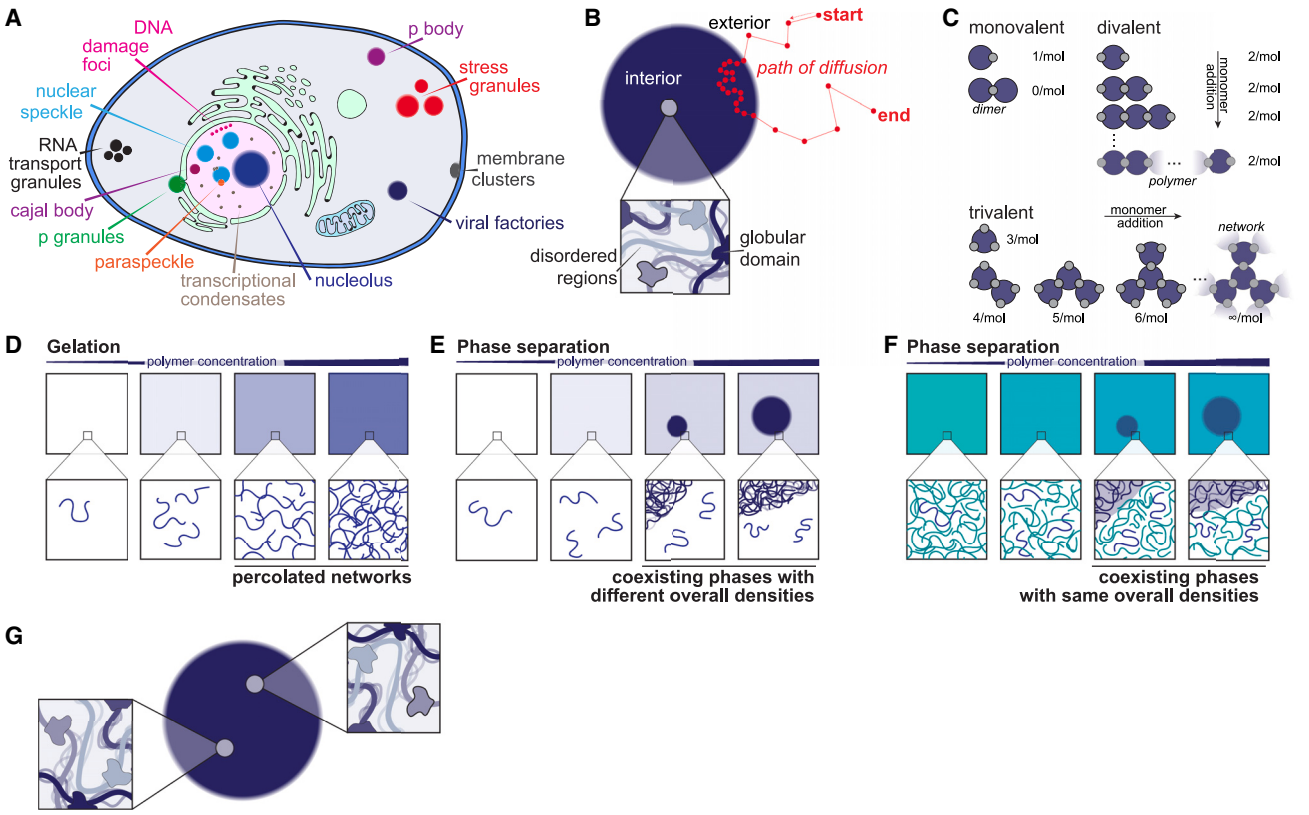

Figure 1. Biomolecular condensates are cellular assemblies that concentrate a specific subset of molecules

(A) A range of different cellular bodies are well described as biomolecular condensates.

(B) Many biomolecular condensates are liquid-like, with constituents showing diffusion into and out of the condensate.

(C) Phase separation depends on multivalent interactions, where the valency of a monomer determines how the number of free sites scales as multimers form (sites per molecule). For trivalent species (or higher), adding additional monomers onto an oligomer generates an ever-increasing valency-per-oligomer, enabling network formation.

(D) Gelation reflects a phase transition that lacks a sharp density transition with respect to one or more components but instead involves a sharp connectivity transition with respect to inter-chain network formation.

(E) Phase separation reflects a phase transition with a sharp density transition leading to specific spatial regions with distinct molecular compositions.

(F) Phase transitions can happen within a dense solution, such that while the absolute density in macromolecules between two phases may be the same, the composition in the two phases can still be very different.

(G) A characteristic of phase separation is that on some length scale, the average properties are homogeneous across distinct regions of the same phase. This does not preclude condensates possessing complex internal substructures on shorter length scales.

could underlie their formation, in many cases, their assembly, maintenance, and dissolution appear to be well described by the physics of phase transitions.[14,16] Direct investigation of this concept through a combination of experimental perturbation and physical modeling has provided evidence that is consistent with phase transitions, offering a cogent framework to describe the behavior of a wide variety of condensates.[14,25,31,44,46–53] Moreover, *in vitro* facsimiles of cellular condensates consisting of one or more different components are quantitatively described by the physics of phase separation.[16,25,27,29,54–57] Thus, applying the physics of phase transitions to describe cellular condensates presents the opportunity to understand and explain their properties and dynamics.

Multivalency, which refers to the ability of a single molecule to interact favorably with multiple partners simultaneously, is often invoked as a key requirement for a macromolecule to be a driver of phase transitions. Monovalency—the ability to interact with one partner only—facilitates the assembly of dimers but no other species (Figure 1C). Divalency—the ability to interact with one or two partners only—facilitates the formation of linear oligomers (of 2, 3, 4, …, *n* molecules long, potentially forming long filaments) but does not allow the formation of a network in 2D or 3D (Figure 1C). However, trivalency—the ability to interact with one, two, or three partners only—and higher-order valencies facilitate percolation in 2D and 3D, allowing the molecules to form fully connected networks (Figure 1C).

The concentration-dependent formation of a connected network is a phase transition.[14,16,23,58] Phase transitions can manifest as gelation, in which a connected network forms without a local region that is enriched for one or more specific molecules (Figure 1D). Phase transitions can also manifest as phase separation, in which one or more specific molecules co-assemble in a local region (Figure 1E). It is worth noting that in phase separation the total concentration of biomolecules may be the same across a phase boundary—that is, the total protein density may be the same inside vs. outside a condensate

(Figure 1F). However, in this case, the compositions of the two phases will be different, such that the different phases have distinct emergent properties. Gelation and phase separation have also been referred to as associative and segregative phase transitions, respectively.[59]

Phase separation has been invoked in many scenarios as the process that underlies the formation of biomolecular condensates.[12,13,23,60] Phase separation enables the formation of two (or more) coexisting phases. The term "phases" here reflects local regions of space with distinct molecular compositions (and hence emergent properties). Importantly, phases are macroscopically homogeneous: a phase is a region of space where the local material/chemical/compositional properties are uniform on a macroscopic scale. This means that two distinct portions of the phase would have identical properties (Figure 1G). While in a strict thermodynamic sense, phases should be in equilibrium with one another, this may not be the case in cells, either due to active processes (e.g., transcription[61] or enzymatic activity[62]) or due to dynamical arrest trapping condensates in metastable states.[15,44,63,64] However, despite this caveat, principles adopted from equilibrium physics have shown remarkable fidelity in at least semi-quantitatively capturing the behavior of condensates in cells, suggesting that many condensates may—at least from a macroscopic phenomenological standpoint—be in approximate equilibrium or steady state.

In the simplest possible system of solvent and polymer (e.g., a single type of protein), phase separation is driven by solubility. Above some solubility limit (saturation concentration, c_{sat}), the solvent can no longer support additional polymer, so any additional polymer "crashes out" into an "insoluble" phase. In the context of many inorganic compounds—and indeed many proteins—that insoluble phase manifests as a (high-concentration) insoluble precipitate that separates from a liquid phase. In the context of liquid-liquid phase separation (LLPS), that insoluble phase is a second, more concentrated liquid. In this situation, we refer to the two coexisting phases as the dilute phase and the dense phase.

Many condensates appear to have liquid-like properties on some length scales and time scales, although we emphasize that liquidity is not a prerequisite for function.[14,23,65] Liquid-like behavior can be qualitatively assessed in terms of dripping, wetting, fusion, and fission and (semi)-quantitatively evaluated in terms of fluorescence recovery after photo bleaching (FRAP), fusion relaxation dynamics, contact angles, and passive/active (micro)-rheology.[14,20,27,56,66,67] However, unambiguously knowing whether a condensate is formally well described as a simple liquid or a viscoelastic material (possessing both liquid and solid properties) is challenging.[59,60] As such, we will use "phase separation" (without the liquid-liquid modifier) to describe phase separation that gives rise to condensates. For convenience, the remainder of this perspective describes condensates under the assumption that they form through phase separation.

The propensity for a molecule to partition into a condensate (or not) can be described by its partition coefficient (K_p) (Figure 2A). This value reflects the ratio of molecular concentrations between the dense phase (i.e., the condensate) and the dilute phase. In practice, partition coefficients are often reported in terms of ratios of fluorescence intensities. While qualitatively this will reflect ratios of concentrations between the two phases, converting fluorescence intensity directly to concentration is extremely challenging.[68] As such, treating K_p obtained from fluorescence-based imaging as semi-quantitative rather than as an absolute concentration measurement is generally safer.

For a simple two-component system (polymer + solvent), the partition coefficient reflects the driving force for phase separation, with a higher partition coefficient commensurate with a stronger drive to separate into two phases. For systems with more than two components, the partition coefficient of any component (for any condensate) is the combined effect of all possible intermolecular interactions dictating the net driving force for recruitment or exclusion into all available phases (Figure 2B). In systems approximating cellular complexity, every possible mobile component—ions, proteins, nucleic acids—has a partition coefficient for each phase. Consequently, the composition of a condensate is dictated by the identity and concentrations of all possible mobile components within a system. Put another way, the overall identity and concentration of every single component determines how all other components are distributed across all phases.

The stickers-and-spacers framework is one convenient conceptual model through which condensate-forming molecules can be described (Figure 2C).[59,69] In this model, sites that play a major role as drivers of attractive interactions (and consequently the specificity of recruitment into condensate) are termed "stickers" while sites that do not are termed "spacers." Stickers can interact with stickers and spacers, and the relative importance of sticker:sticker and sticker:spacer interactions will depend on the molecular details of a system.[54,70] Which regions or residues are stickers and which are spacers can be dependent on context, influenced by the available binding partners and the solution environment. Furthermore, spacers are not inert. They contribute to the specificity of interactions with the solvent, and they influence the cooperativity or lack thereof of sticker:sticker interactions. All together, stickers can be defined phenomenologically as residues, regions, motifs, or domains that, if removed, have a more pronounced effect on the partition coefficient than if an equivalent number/mass of spacers were removed (Figure 2C).[51] The framework also defines condensate interiors as complex networks generated by sticker-driven reversible interactions and by the effects of spacers, which influence the density and lifetimes of those interactions. Finally, we emphasize that the stickers-and-spacers framework is effectively a first-order approximation for describing the phase behaviors of multivalent macromolecules.

Multivalent biomolecules with many stickers can drive the formation of condensates with liquid-like properties.[25,51,54,71] Multivalency is essential for the formation of a 3D interaction network that sustains the condensate (Figure 1C). The exchange lifetimes of interactions govern the timescales of network re-arrangement, ultimately dictating condensate material properties such as viscoelasticity and interfacial tension.[29,72,73] Moreover, condensates are often viscoelastic materials, meaning their deformation under strain shows both viscous (time-dependent) and elastic (recoverable deformation) behavior. The strengths of intra- and intermolecular interactions will determine the balance

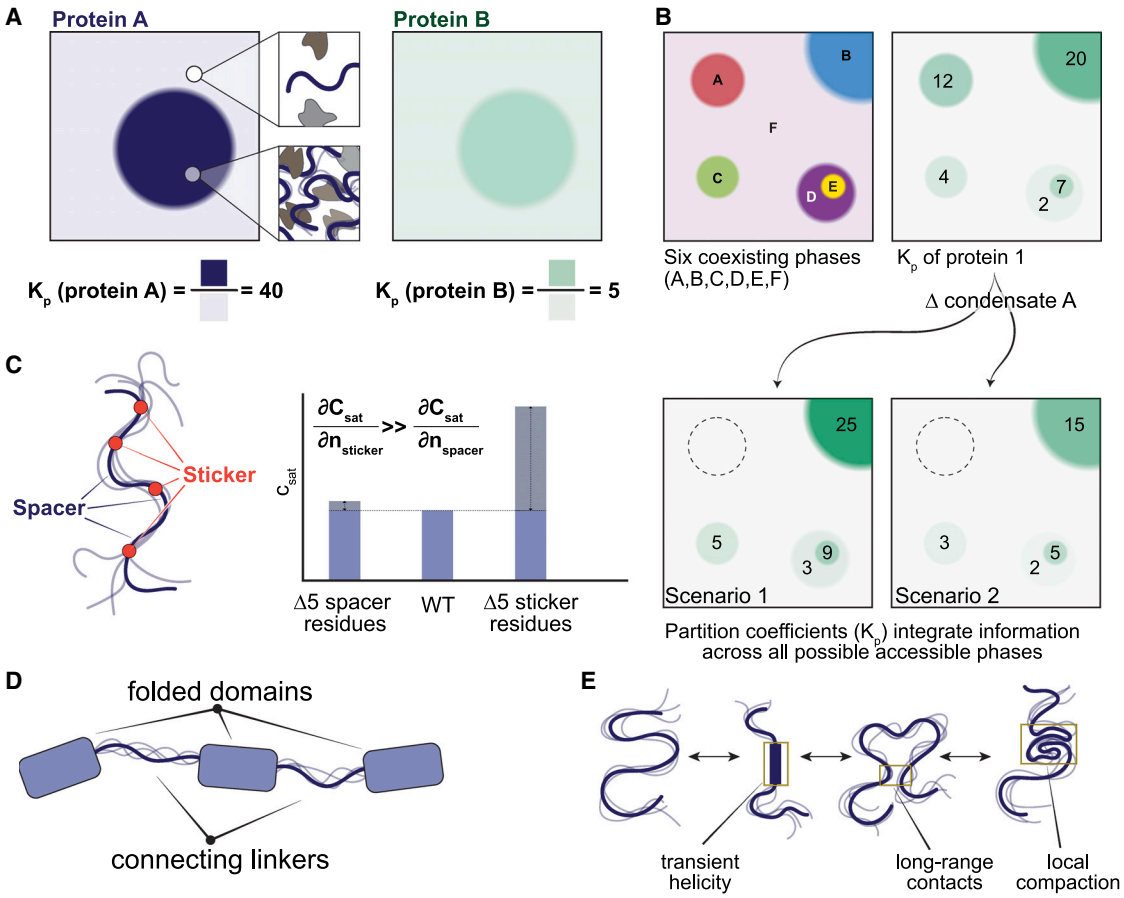

Figure 2. Biomolecular recruitment is quantified by the partition coefficient, which integrates all possible interactions in the system

(A) The partition coefficient quantifies the ratio of a molecule of interest in one phase vs. another. Different proteins within the same condensate may possess very different partition coefficients (protein A vs. B).

(B) For coexisting phases (top left), a single protein will have a partition coefficient for each different phase (top right). Numbers quantify the partition coefficient. If one of those phases is removed (e.g., phase "A"), the protein of interest could redistribute in a wide variety of different ways, in part due to other components previously sequestered within phase A redistributing (bottom). In this way, the partition coefficient of any protein in any phase is an emergent consequence of all possible sets of chemical equilibria in the system.

(C) The stickers and spacers model proposes that distinct protein residues or regions can be designated as stickers and spacers (left). Stickers are defined phenomenologically as those residues or regions where their deletion has a large impact on the saturation concentration compared to an equivalent number/mass of spacer residues (right).

(D) Linear multidomain proteins involve folded domains connected by linkers.

(E) Intrinsically disordered proteins and protein regions (IDRs) lack a stable 3D structure yet still possess local and long-range sequence-encoded biases in their ensemble.

between viscous and elastic behavior. As such, condensate material properties are determined—at least in part—by the same interactions that determine interaction specificity.[28,29,74–77]

Many different types of biomolecules can undergo self-association or association with a partner to form a liquid-like condensate. One example of a molecular architecture that is well described in terms of stickers and spacers is linear multivalent proteins consisting of folded protein-protein interaction domains connected by disordered linkers (Figure 2D). For these molecules, precise site-specific binding interfaces enable highly specific molecular recognition with a corresponding partner protein.[16,58,78] In parallel, intrinsically disordered regions (IDRs) with a variety of sequence features have also been found to form condensates in various contexts.[25,54,71,79–86] Unlike folded domains, IDRs are regions of proteins that lack a stable 3D structure and instead exist in a fluctuating collection of interconverting states known as an ensemble (Figure 2E).[87] IDRs are not necessarily low complexity, although there has been substantial interest by the research community in disordered low-complexity domains in condensates.[88] One common misconception is that IDRs are inherently predisposed to drive phase separation. This is no more the case than the idea that folded domains are inherently predisposed to mediate protein:protein interactions. In both cases, the key feature is the tendency of the proteins to undergo attractive intermolecular interactions, which is governed by the specific amino acid sequence.[84,89]

Given the preceding introduction, we return to how condensates influence cellular organization. For membrane-bound organelles, a critical determinant of their function is determined by what components can enter and exit. The presence of a lipid bilayer generally

necessitates dedicated channels and transporters to enable transport of mobile components into and out of an organelle. This is in stark contrast to condensates, which lack a surrounding membrane. What then determines entry into and exit from a condensate? Phenomenologically, recruitment or exclusion can be quantified in terms of a partition coefficient, but what are the molecular and physical determinants underlying this value?

In this review, we discuss our emerging understanding of the determinants of recruitment and exclusion of proteins into biomolecular condensates. We consider where and how specificity can be encoded, and we delineate two modes of interaction, referred to as site-specific and chemically specific interactions, and how these two interaction modes are regulated and interplay with one another. While our focus here is on proteins, the principles and concepts outlined are directly applicable to other biomolecules. Our protein-centric perspective is driven at least in part by the relative genetic tractability afforded by proteins coupled with the diverse chemical palette offered by amino acids. Finally, we discuss the ways by which site-specific and chemically specific interactions synergize to direct the composition and properties of condensates, facilitate chemical reactions, and regulate cellular processes like metabolism, signaling, and gene expression.

PROTEIN-MEDIATED INTERACTIONS CAN BE SITE SPECIFIC OR CHEMICALLY SPECIFIC

In the following, we will turn to the interactions underlying condensate assembly. Protein-mediated interactions play a major role in organizing the interior of cells.[90] Traditionally, these interactions have been considered from the vantage point of site-specific molecular recognition.[91,92] In site-specific interactions—sometimes referred to as sequence-specific molecular recognition in the context of IDRs[87]—a combination of chemical and shape complementarity between a structured protein interface and a partner that may or may not be structured initially (but becomes structured in the context of the interaction) dictates both specificity and affinity. Structural studies have enabled the detailed elucidation of thousands of distinct protein:protein interfaces. Site-specific molecular recognition events encoded in a relatively small surface area can still have incredible specificity and affinity, enabled by amino acids' broad structural and chemical palette.

Site-specific protein interactions are driven by the precise geometry of residues in the bound state. For folded domains, that geometry is typically predefined (precisely or approximately) by the overall topology and fold of the domain before binding (Figure 3A). Specificity and affinity are then determined by the combined contributions of multiple amino acids across a binding interface interacting simultaneously. As a result, even individual point mutations can entirely abrogate these site-specific interactions, either by disrupting an attractive interaction or by introducing a repulsive interaction (Figure 3B). That said, it is also possible for some interfacial residues to contribute minimally and be relatively permissive to at least a subset of possible mutations. Changes to protein interaction interfaces have emerged as key regulatory mechanisms, for example, by introducing post-translational modifications, and as the underlying cause of various human diseases.

While it might be tempting to assume site-specific interactions are the purview of folded domains, IDRs can also engage in highly specific molecular interactions. For IDRs, site-specific interactions are generally determined by short linear motifs (SLiMs).[93] SLiMs are ~4–~12 amino acid regions with one or more key residues facilitating site-specific molecular recognition with a partner. SLiMs can partially or fully fold upon binding, giving rise to a geometrically defined (albeit potentially short-lived) bound conformation with an interaction partner (Figure 3C). Cases in which bound states remain structurally heterogeneous (despite defined interacting residues) are sometimes referred to as fuzzy binding.[94] Importantly, while some positions in SLiMs are highly permissive to mutations, other positions are tightly constrained in terms of which amino acids are tolerated to preserve an interaction. Consequently, when single-point mutations in IDRs have drastic phenotypic consequences, one molecular mechanism through which this can emerge is by altering SLiMs.[95]

Beyond site-specific molecular recognition, a growing body of work has highlighted the importance of chemically specific molecular recognition.[31,51,96–106] Unlike site-specific interactions, in chemically specific interactions, complementary chemical groups between a protein and a partner facilitate molecular interactions that depend less on the precise order of amino acids than on the local or global presence of specific chemical moieties (Figure 3D). While site-specific interactions give rise to a geometrically defined bound state, chemically specific interactions lack a structurally identifiable bound state.[87,107] Instead, a large number of energetically equivalent bound state configurations are realized.

Because chemically specific interactions do not rely on a precise bound-state geometry, regions that engage in chemically specific interactions can often accommodate mutations if those changes in sequence conserve chemical specificity. This tolerance to mutations emerges from two distinct but related determinants. First, the absence of a defined 3D structure in the bound state (and in the unbound state, in the case of disordered regions) means individual mutations cannot disrupt inter- (or intra-, for disordered regions) molecular native state contacts. Second, the large number of energetically equivalent bound-state configurations introduces substantial redundancy into the bound state, such that disruption of one (or a small number) of those bound states does not necessarily abrogate binding. As an example, chemical specificity underlies the interaction between ProTα and the C-terminal disordered region from histone H1, such that the binding affinities of D- vs. L-enantiomeric versions of the H1 disordered region are indistinguishable from one another (in direct contrast to interactions in which the bound state is highly structured).[108]

In addition to bound-state configurational redundancy, the natural amino acids encode a variety of different intermolecular interactions enabled by different subsets of the amino acids (Figure 3E). This chemical redundancy also encodes a degree of robustness to mutations, although it is worth clarifying that no two amino acids are entirely interchangeable. Moreover, mutations that alter chemical complementarity between two proteins can have a measurable impact on binding affinity, especially if multiple groups of the same chemical type are changed.

Both site-specific and chemically specific molecular recognition can mediate conventional 1:1 stoichiometric interactions.[99,107]

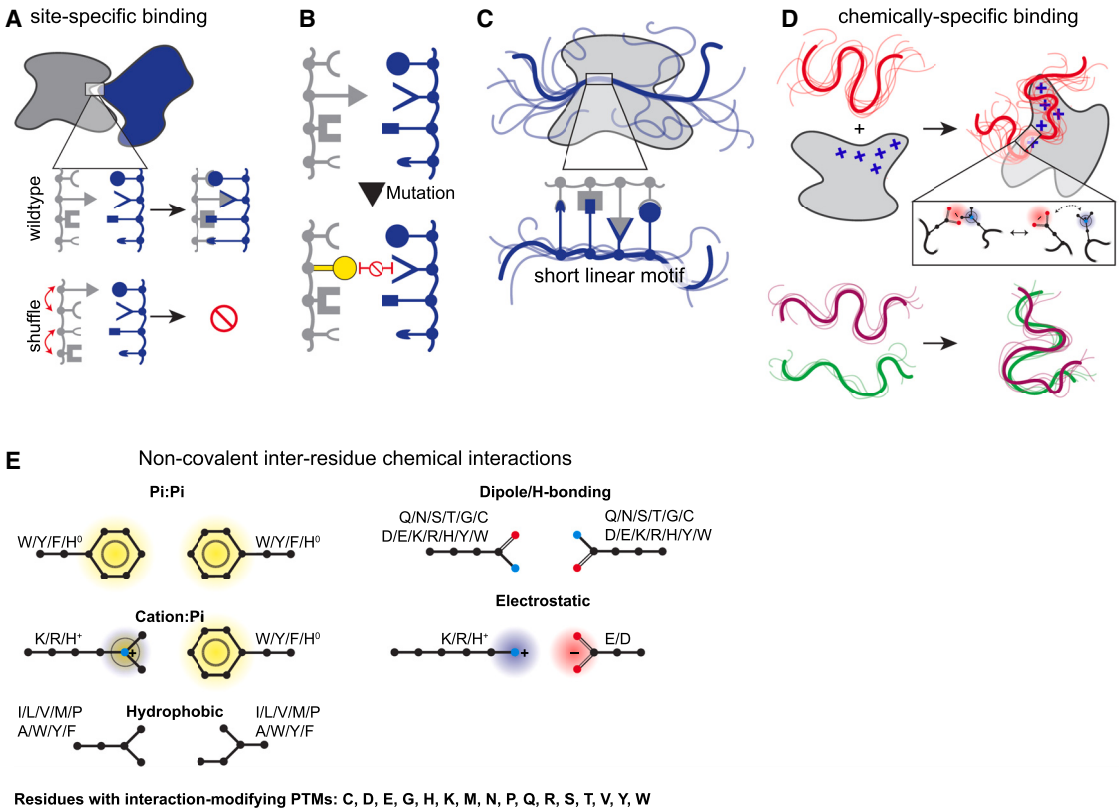

Figure 3. Site-specific and chemically specific binding define two related modes of intermolecular interaction

(A) Site-specific interactions rely on a specific chemical and geometrical bound state and require a specific orientation of amino acids. Changing the order of amino acids, even if the same residues are retained, will generally disrupt site-specific molecular recognition unless an alternative and stable bound-state complex can be accommodated.

(B) Single-point mutations can disrupt site-specific molecular interactions.

(C) Intrinsically disordered regions can engage in site-specific molecular recognition through short linear motifs (SLiMs).

(D) Disordered regions and folded domains can also engage in chemically specific interactions. Here, a disordered region interacts with a folded protein (top) or interacts with another disordered region (bottom). Not shown is a folded region interacting with a folded region.

(E) Distinct non-covalent attractive inter-residue interactions are mediated by different amino acid types. Many amino acids can engage in multiple different types of interactions; for example, salt bridges typically consist of both hydrogen bonding and electrostatic interactions. Different interactions (even among the same amino acids) can vary in strength (e.g., W:W is stronger than F:F). Standard one-letter codes for amino acids are used, with H^0 reflecting neutral histidine and H^+ protonated histidine.

However, they can also enable the multivalency necessary for higher-order assembly into biomolecular condensates. In the context of biomolecular condensates, many principles underlying chemical specificity have been examined and described in terms of the "molecular grammar" that underlies the driving forces for phase separation.[25,28,51,54,55,79–81,83,109–125]

Site-specific and chemically specific interactions exist on a continuum. For example, in IDRs, structured interfaces or SLiMs are often tolerant of specific mutations that preserve local chemistry, indicating that distinct bound states may be sufficiently similar in terms of binding energetics.[126] Emerging work suggests that site-specific molecular recognition is licensed by a chemical context that enables complementary chemical specificity, in effect providing a hierarchical binding model. For example, chemically specific interactions could steer the encounter of two interacting biomolecules to eventually establish productive site-specific interactions.[97,127–130]

For folded domains, favorable surface chemistry may drive certain binding interfaces to be preferred over others, while site-specific cryptic pockets may be accessible only upon local remodeling driven by chemically specific intermolecular interactions. In general, site and chemical specificity unavoidably cooperate to shape the landscape of molecular interactions. In this review, it is important to note that they also determine the attractive and repulsive interactions that recruit or exclude proteins into biomolecular condensates.

SITE-SPECIFIC INTERACTIONS ENABLE PRECISE CONTROL OF CONDENSATE RECRUITMENT

Since site-specific molecular interactions depend on a precise bound-state structure, changes to regions or residues that engage in site-specific interactions offer the most robust means to dramatically influence condensate formation/recruitment with minor changes in sequence. While it is sometimes suggested that phase separation requires "promiscuous" or "non-specific" interactions, this is a misconception. While promiscuous multivalent interactions can drive phase separation, so too can highly

specific multivalent interactions. The attractive interactions underlying phase separation are typically a combination of site-specific and chemically specific interactions.

There is ample evidence that site-specific interactions are critical in condensate assembly. One example is the above-mentioned group of linear multivalent proteins, where folded interaction domains promote interactions with SLiMs. Examples here included (1) SUMO-interacting domains (SIMs) interacting with SUMO moieties,[78] (2) proline-rich SLiMs interacting with SH3 domains (e.g., GRB2),[16,131] (3) PAM2 motifs in Ataxin-2 interacting with PAB1,[132] or (4) a SLiM in Caprin-1 interacting with the G3BP1/2 N-terminal domain.[133] Moreover, canonical protein:protein interfaces are often essential for condensate assembly by promoting protein dimerization or oligomerization. Examples are the (1) N-terminal domains of TDP-43[134] and G3BP1/2[22,135] required for dimerization, (2) the N-terminal domain of NPM1 that promotes pentamer formation,[136] and (3) the obligate oligomeric protein GvpU that assembles into pentamers which drive higher-order assembly in gas vesicle clustering.[137] For G3BP1 and TDP-43, the dimerization domains not only double the valency, but also provide additional interfaces for interactions with other proteins (e.g., Caprin-1 binding to G3BP1/2[133]) or higher order self-assembly (e.g., oligomerization and multimerization of TDP-43[138]). More broadly, work has shown that many proteins are poised to undergo higher-order stoichiometric multimerization, which often leads to condensate formation.[139] However, oligomerization can also have a regulatory role by repressing condensation, especially if oligomerization interfaces are in competition with interactions that otherwise enable multivalency for phase separation.[140,141] In this context the idea of "valence capping"—quenching multivalency through the interaction with monovalent species—offers a way to understand how oligomerization can suppress phase separation.[47]

Beyond oligomers of a fixed stoichiometry, many condensate-forming proteins polymerize into homo- or heteromeric filaments, which carry additional valencies for their assembly into 3D networks to promote condensation.[142] Examples are SAM domain proteins,[143] DIX domain proteins (e.g., Dvl2),[144] and PB1 domain proteins (e.g., ARF19 or p62),[145,146] all of which can polymerize into dynamic homo- or hetero-filaments by a head-to-tail mechanism. The interfaces that underlie filament assembly are expected to be distinct from the interfaces that enable condensate formation because the interfaces required for filament polymerization become capped upon monomer addition. Prior work has suggested that linear polymerization could give rise to "emergent stickers"—novel interfaces that emerge only upon filament formation.[69] In this way, a combination of site-specific (filament formation) and chemically specific (emergent stickers) could encode orthogonal interaction modes to drive condensate formation.

One well-characterized system that combines filament formation and inter-filament interactions is the protein SPOP, which polymerizes through its tandem dimerization domains (BTB and BACK) into linear protein arrays that undergo further assembly.[147–150] The DNA damage sensor PARP1 exhibits various canonical protein:protein interfaces that promote multimerization into protein-DNA co-condensates upon DNA binding.[151] Finally, coiled-coil domains can drive the formation of oligomeric species that undergo phase separation.[152,153] Well-studied examples here include centrosomal proteins (SPD-5),[154] LINE1,[155,156] PopZ,[118] and FLOE1.[157] Moreover, site-specific interactions to facilitate protein multimerization, which in turn enables condensate formation, have been developed as tools for constructing synthetic condensates in various systems.[48,152,158–163] These examples demonstrate the key role of site-specific interactions in condensate recruitment.

Although proteins play a key role in condensate assembly, there is increasing evidence suggesting a central role for nucleic acids[164–168] or nucleic acid-like molecules such as poly(ADP) ribose (PAR) in condensate recruitment.[151,169] In fact, many known condensates contain high concentrations of nucleic acids,[22,170] and changes in RNA concentration have dramatic effects on condensate integrity.[165,171,172] In the case of the nucleolus, ribosomal RNA (rRNA) is produced from clustered rDNA regions that generate a high local RNA concentration.[19] The local production of highly concentrated rRNA is essential for the overall architecture of the nucleolus,[173] presumably because rRNA provides many site-specific binding sites for nucleolar RNA-binding proteins that are essential for maintaining nucleolar integrity. More generally, RNA-binding proteins composed of several structured RNA binding domains, such as RRMs, play crucial roles in the assembly of many condensates, often by binding to specific sequence motifs in RNA molecules locally produced by point sources such as active genes.[174,175] Nucleic acid-like molecules like PAR, which can be linear and/or branched, are produced by PAR polymerases such as PARP1, often upon local cues such as a double-strand break. The three-dimensional PAR network that results from local PAR polymerase activity provides a binding platform for a large set of PAR binding proteins that bind to PAR polymers via site-specific interaction domains such as the BRCA domain.[151,169] Recent work also suggests that RNA molecules can engage in *trans* interactions via Watson-Crick base-pairing.[176,177] However, the contribution of such *trans*-RNA-RNA interactions to condensate recruitment and stability is still unclear.

In summary, site-specific interactions between proteins or proteins and nucleic acids or nucleic acid-like molecules play key roles in condensate assembly. Abrogating these interactions often disrupts condensate assembly altogether, suggesting that these interactions have an essential stabilizing role. Additionally, site-specific interactions appear particularly important for the early stages of condensate assembly, suggesting key roles in initiating condensate assembly as a response to specific local cues in cells.

CHEMICALLY SPECIFIC INTERACTIONS ENABLE RHEOSTATIC CONTROL OF CONDENSATE RECRUITMENT

Thus far, we have considered chemical specificity in the context of two macromolecules interacting with one another. Where condensate recruitment is concerned, chemical specificity depends on the intra-condensate and extra-condensate chemical environments. That intra-condensate environment is a product of both the biomacromolecules within the condensates (proteins, RNA, etc.) and also all metabolites, ions, and small

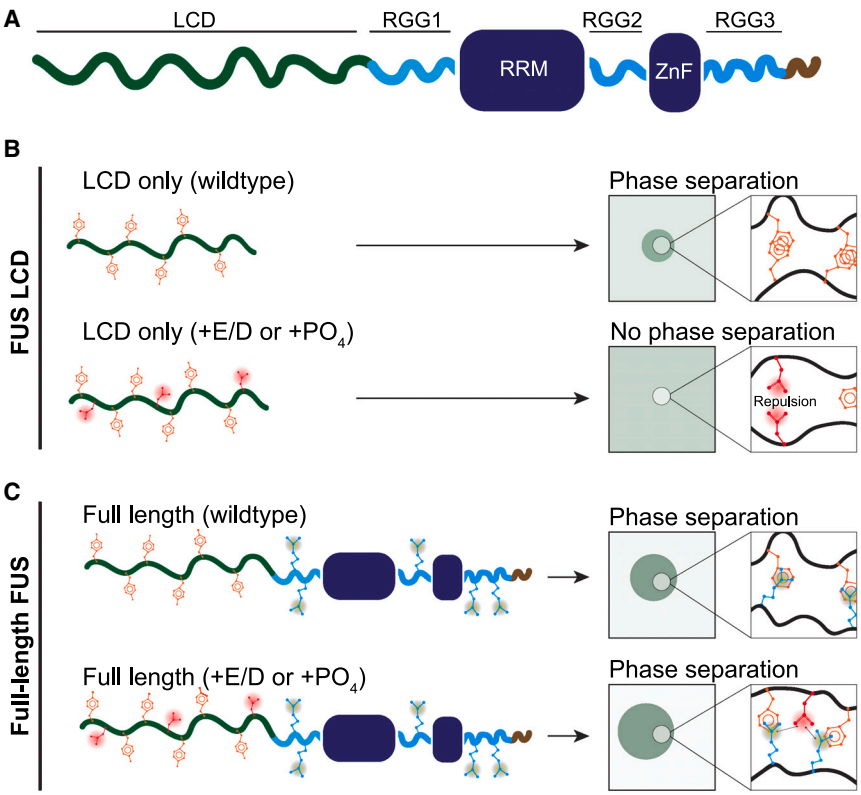

Figure 4. FUS LCD in isolation vs. FUS full-length show opposing responses to phosphorylation

(A) Molecular architecture of the RNA binding protein FUS. An intrinsically disordered N-terminal tyrosine-rich low complex domain (LCD) sits ahead of three arginine-glycine-rich RGG domains (RGG1, RGG2, RGG3), separated by a folded RNA recognition motif (RRM) domain and a folded zinc finger (ZnF).

(B) Schematic of results obtained when the LCD is examined in isolation. Here the addition of negatively charged moieties—either via the introduction of aspartate (D) or glutamate (E) residues or via phosphorylation—suppresses phase separation. This is because for the LCD in isolation, phase separation is primarily driven by tyrosine-mediated interactions, and the introduction of negative charge provides both intermolecular electrostatic repulsion and enhanced solubility due to D/E/PO_4 strongly favorable interaction with solvent.

(C) Schematic of results obtained when examining the full-length FUS. Here, the addition of negative charge into the LCD modestly enhances phase separation. This is because for full-length FUS, the primary interactions that drive phase separation is now tyrosine:arginine interactions (LCD:RGG), with some contribution from other tyrosine-mediated interactions. The addition of negative charge around those tyrosine residues introduces an additional electrostatic contribution to the LCD:RGG interactions.

molecules. This chemical environment can be described in terms of a physicochemical barcode.[178] The extra-condensate environment is similarly determined by the concentration and identities of components outside the condensate. As a result, chemical specificity for condensates is effectively a readout of the integrated chemical environment inside *and* outside a condensate. That environment—in turn—depends on the identity and concentration of all components present in the system. A consequence of this is that while site-specific recruitment can be "digital"—a potential partner possesses or lacks a binding motif necessary to facilitate interaction with condensate components—chemically specific recruitment is "analog" and reflects the partitioning of all components across the system between and among all possible co-existing phases.

Chemically specific intermolecular interactions are most commonly associated with IDRs. For example, mutations that alter the number of aromatic residues (tyrosine, phenylalanine, and tryptophan) have been found to tune phase behavior and—when measured—alter macroscopic binding affinities across a range of systems.[25,54,55,79–81,112,117,179–181] Similar results have been obtained for arginine residues in the context of aromatic-rich IDRs,[25,51,55,182,183] as well as charged residues[83,106,136,184–189] or aliphatic hydrophobic residues.[85,86,190,191] Importantly, in many cases, some combination of these partially or entirely chemically orthogonal interaction modes dictates the overall chemical specificity of an IDR.[51,55,84,109,192]

While loss-of-function mutations can disrupt phase separation if key sticker residues are mutated to spacer residues, gain-of-function mutations can also disrupt phase behavior if spacer (or sticker) residues are replaced by residues that are antagonistic to the attractive interactions that drive phase separation. For example, introducing 12 negatively charged glutamate residues (12E) into the tyrosine-rich low-complexity domain (LCD) of the RNA binding protein FUS suppresses its phase separation (Figures 4A and 4B).[111] However, chemical specificity implies the impact of mutations depends on the chemistry of the partner. In the case of FUS, the 12E variant suppresses phase separation of the LCD-only construct due to electrostatic repulsion impeding attractive tyrosine:tyrosine interactions (Figure 4B). However, introducing 6 or 12 negatively charged residues into the LCD in the context of full-length FUS enhances phase separation,[111] presumably because the glutamate residues enhance interactions between the tyrosine-rich LCD and the arginine-rich C terminus[51,111] (Figure 4C). This example highlights the "specificity" in chemical specificity; chemical complementarity depends on the partner with which a protein interacts.

Beyond amino acid composition, the relative position of different residues (i.e., patterning) can strongly influence affinity and specificity in IDRs. The importance of charge patterning—the relative position of positively and negatively charged residues across an IDR—in dictating IDR conformational behavior was established over a decade ago.[193–195] Charge patterning has subsequently been shown to play a key role in tuning the driving forces for phase separation and also influencing IDR specificity in terms of condensate partitioning.[25,31,83,106,183,185,196] The linear patterning of aromatic residues can also influence condensate formation *in vitro* and likely intermolecular interactions in cells.[54,197–199] The patterning of various other residues has been

shown to influence inter- and intramolecular interactions, with direct implications for phase-separating systems.[121,200,201] In conclusion, the combination of amino acid composition and patterning can encode chemically specific attractive and repulsive intermolecular interactions.

While chemical specificity has been examined most commonly in IDRs, all solvent-exposed biomacromolecule surfaces are subject to the same chemical rules. As such, the surfaces of folded domains can also engage in chemically specific interactions. The fact that folded domains can modulate or even dictate phase behavior is implicitly clear, given the widespread use of solubility tags.[202] Indeed, the highly soluble maltose binding protein (MBP) will often inhibit phase separation of an adjacent domain until cleaved.[112,116,203] A more nuanced appreciation for the tunability with which the chemical specificity of folded domain influences condensate recruitment and exclusion is now beginning to emerge.

Early work on folded domain chemistry in the context of condensates focused on the impact of electrostatics. The surface charge on fluorescent proteins tunes their phase behavior *in vitro* and in cells.[83,204,205] More broadly, surface charge properties of folded domains can influence intra- and inter-molecular interactions in a broad range of systems.[102,184,206–208] For example, condensation of the RNA binding protein Pab1 depends not on its hydrophobic low-complexity IDR (which tunes its temperature dependence) but on the surface chemistry of its RRMs.[85,209] Similarly, the folded helicase domain of Ded1p drives condensate assembly in heat-stressed yeast, while the flanking IDRs have modulatory roles, implying that changes to the solvation state of folded domains could be a driver of condensate assembly.[210,211]

In addition to charge properties, other types of protein chemistry will influence the phase behavior of folded domains. As a prime example, the rates of passage through the nuclear pore complex of GFP molecules with different surface chemistries vary dramatically, reflecting their preferential interaction with the FG-repeat-rich phase inside the nuclear pore.[208] Similarly, just as arginine residues have often been identified as key stickers in IDRs, the gain of arginine residues on folded domains was also shown to drive higher-order assembly.[139,184] More recently, the surface chemistry of fluorescent proteins has been shown to enhance or suppress condensate recruitment or exclusion of tagged proteins in a sequence-dependent manner.[83,204,212,213] Again, fluorescent proteins are not special, and the combined effect of chemical interactions driven by solvent-accessible residues across the surfaces of any globular domain will inevitably influence condensate recruitment and exclusion.[214] The chemical composition of folded domain surfaces is expected to be just as crucial in determining the partition of proteins into condensates as the IDR chemistry.

Beyond the chemical specificity of proteins (disordered or folded), those same chemical rules can impact or determine the partitioning of nucleic acids, small molecules, and even ions. RNA and DNA molecules of different lengths and secondary structures have been shown to display distinct partition coefficients for model condensates lacking canonical nucleic-acid binding domains, implying differences in chemically specific interactions with condensate interiors.[215,216] Although this remains to be shown experimentally, nucleic acid molecules likely display different surface charges and surface hydrophobicity depending on length and conformation. Small molecules can show preferential partitioning into condensates of different chemical compositions, opening the door for subcellular targeting based on complementary chemistry.[43,100,181,217–220] Finally, emerging work has found that different anions and cations (including H^+) have distinct preferences for different condensates, highlighting the potential for condensates to establish electrochemical gradients in the cell.[31,34,37,221]

CONDENSATE RECRUITMENT INVOLVES SITE-SPECIFIC AND CHEMICALLY SPECIFIC INTERACTIONS

Our discussion thus far has segregated two modes of intermolecular interactions into two camps, comparing these two modes. In reality, for endogenous proteins, both modes are expected to dictate condensate recruitment and exclusion, and the resulting hierarchy of interaction strengths, lifetimes, and specificity play key roles in determining condensate form and function.[69,222–226]

In cells, we propose that the assembly of condensates is often initiated by site-specific interactions that locally concentrate specific components. This process frequently involves DNA- or RNA-binding proteins that associate with motifs located in nucleic acid molecules.[22,151] Genetic and biochemical studies have enabled the development of a simplified description for two classes of molecules based on their importance for condensate integrity: scaffolds and clients.[78] Scaffolds typically possess multiple valences, enabling them to undergo extensive interactions that are critical for the initiation and maintenance of condensates. In contrast, clients generally have fewer valences and play a less central role in condensate integrity. Both scaffolds and clients are capable of mediating site-specific and chemically specific interactions. However, given their essential role in condensate formation, scaffolds are likely to rely at least in part on site-specific interactions, while they may be dispensable for the recruitment of clients, where chemically specific interactions may sometimes be sufficient. This does not preclude the possibility that condensates can be initiated solely by chemically specific interactions. However, such a mechanism would necessitate a means to locally enrich these molecules to drive condensate assembly. Once concentrated inside a condensate, components interacting via chemically specific interactions could generate a specific chemical microenvironment that facilitates further site-specific interactions. These considerations suggest that complex feedback loops may exist between chemically and site-specific interactions to aid in the assembly and disassembly of condensates.

Chemically specific intermolecular interactions are not something proteins can "switch off." The chemical moieties accessible on the surface of proteins (either on folded domain surfaces or across IDRs) will unavoidably have preferences in their attractive and repulsive interactions. As a result, even for proteins where condensate recruitment depends on site-specific molecular interactions, chemically specific interactions unavoidably contribute to the overall recruitment and exclusion. This offers the potential for exquisite and multi-modal control of condensate

specificity; recruitment can be tuned by altering site-specific binding and/or chemically specific interactions.[96,227,228]

Post-translational modifications offer one route to tune chemically specificity and site-specific interactions, even within the same protein. For example, the tight junction proteins ZO1 and ZO2 engage in site-specific interactions facilitated by canonical protein-protein interaction domains alongside chemically specific IDR interactions, licensed through phosphorylation to enable condensate formation.[229] Similarly, TDP-43 phosphorylation of its N-terminal dimerization domain provides an apparent switch,[230] while post-translational modification of its C-terminal disordered region offers finer-tuned rheostatic control of condensate formation.[113] In summary, identical post-translational modifications can have radically different impacts depending on what types of interactions they modify.

Chemically specific interactions mediated by IDRs can occur intramolecularly as well as intermolecularly. While it is often implied that condensate formation requires IDRs, in several examples for full-length proteins that form condensates *in vitro*, the folded domains examined in isolation undergo aggregation.[64,231] These observations imply that these folded domains possess an intrinsic ability to self-assemble, albeit in a manner that appears biologically maladaptive. The presence of an IDR here functions as a tethered lubricant, disrupting strong chemically specific folded domain interactions and likely contributing to attractive intermolecular interactions.[231,232] Again, the synergy between IDRs and folded domains here implies that IDRs may play a role in tuning the dynamics of intermolecular interactions instead of dictating the energetics of phase separation.

One condition where site-specific and chemically specific interactions synergize in condensate assembly is when cells are exposed to changing environmental conditions.[233,234] Cellular stress is often associated with changes in key chemical and/or physical parameters such as temperature, pH, ionic strength, or water availability.[25,85,231,233,235–238] This can alter protein surface charges and conformations, revealing new interfaces for protein-protein interactions. For instance, the partial unfolding of proteins under stress conditions can trigger the emergence of hydrophobic surfaces and/or desolvation of proteins to promote the assembly into condensates.[85,191,210,239] Likewise, changes in ionic strength or pH alter protein surface charges and protein solvation,[35,231] thus providing opportunities for new site-specific or chemically specific interactions. Indeed, condensates are particularly abundant under stress conditions, and many of these condensates are linked to the control of gene expression, affecting the flow of genetic information from the nucleus to the cytosol.[233,234,240] This includes various RNA-containing condensates associated with transcription, RNA processing, splicing, nuclear export, or translation. Widespread regulation of site-specific and chemically specific interactions to achieve differential condensate recruitment appears to be an adaptive strategy used across the evolutionary tree to adjust gene expression programs to new environmental conditions.

As a final note, given that proteins cannot choose to turn on or off their surface-exposed chemical moieties, it should be clear that almost any protein will—under an "appropriate" set of solution conditions—be able to undergo self-assembly. Studies examining phase separation of a single protein reconstituted *in vitro* (i.e., homotypic phase separation) have been instrumental in elucidating our modern understanding of chemical specificity. However, it bears noting that whether the homotypic phase is a biologically relevant phenomenon, in general, is unclear. More broadly, the partitioning of a biomolecule into an *in vitro* condensate in isolation does not imply that this partitioning is biologically meaningful.[92,241] Instead, it simply reflects the fact that, given the dense and dilute phase chemical environments within an *in vitro* system, the biomolecule in question engages more favorably with the condensate interior than the exterior (formally speaking, equalizing the chemical and osmotic potentials between the dense and dilute phase).[23,59,242] Finally, the fact that partition coefficients are a consequence of the collective interactions among all components in all phases means that a biomacromolecule's partition coefficient will, in reality, be dependent on concentration, reflecting many competing interactions in multiple phases.[49,243] All told, this means that to ascertain biological relevance, site-specific and chemically specific interactions underlying condensate recruitment *in vitro* must be perturbed and tested in living cells, ideally quantitatively.

PRACTICAL RECOMMENDATIONS FOR INVESTIGATING CONDENSATE SPECIFICITY

Given the complexity of the molecular rules underlying condensate assembly, there is a need for guidelines and novel methodology to dissect the mechanisms underlying condensate specificity. The following provides a brief practical guide for investigating the molecular rules underlying condensate assembly, although we note others have written on this topic from the perspective of experimental practicalities.[17,60,203,241]

Hypotheses surrounding the importance of site-specific molecular recognition can be examined by disrupting the precise order of amino acids that determine a binding interface. In the context of folded domains, care should be taken that mutations here do not disrupt or destabilize the native structure. However, single-point mutations within binding interfaces are often well tolerated and provide a way to investigate the molecular determinants of site-specific interactions.[244] Moreover, advances in modern protein design enable the accurate re-design of interfaces in a structure-preserving way with relative ease.[245] In the context of disordered regions, locally shuffling motifs (ideally several times to generate several different shuffle constructs) offer a route to abrogate site-specific intermolecular interactions with minimal impact on chemical specificity.[96,246] Alternatively, targeted point mutations that remove key interface residues in SLiMs can be useful, although such mutations may unavoidably alter chemically specific and site-specific interactions simultaneously.

Hypotheses surrounding the importance of chemically specific molecular recognition can be examined by making mutations to surface-accessible residues to rewire overall chemical interactions. For folded domains, one must ensure these larger-scale mutations of surface residues do not alter protein structure or oligomerization state. In this context, fluorescent proteins are a convenient model system in that they at least provide an intrinsic control against native-state destabilization; mutations that disrupt folding will be fluorescently dark. Nevertheless, mutations that change surface chemistry run the risk of

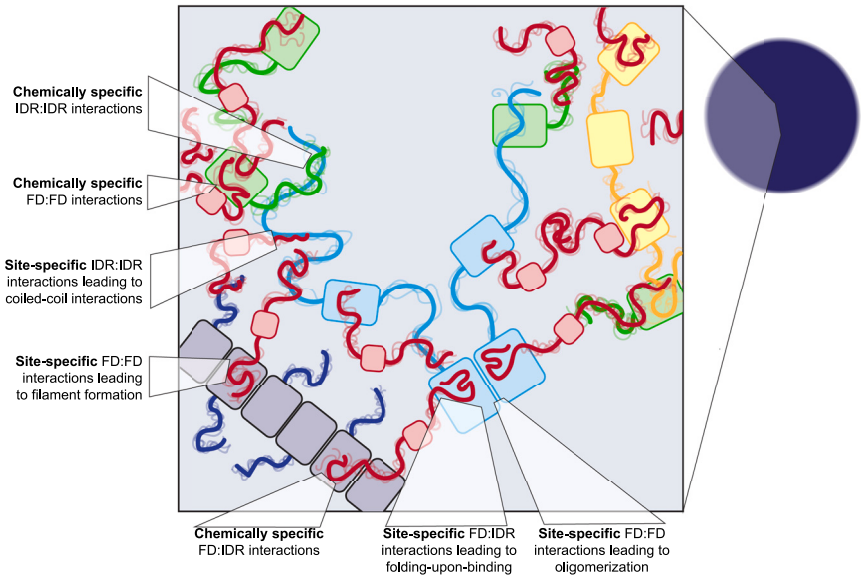

Figure 5. Chemically specific and site-specific interactions occur within biomolecular condensates to underlie condensate formation

These interactions cooperate together to determine specificity and material properties of condensates.

driving oligomerization, which can confound interpretations.[208] For IDRs, changes in overall sequence chemistry can be achieved through the mutation of specific amino acids. As with folded domains, care should be taken that these changes do not lead to substantial but unintended changes in interactions with other components. As a concrete example, adding aromatic residues with the objective of investigating homotypic phase separation in cells will—unavoidably—also lead to unintended heterotypic interaction with other cellular components.

Recent advances in computational biophysics offer convenient tools for the rational design of folded domains and disordered regions. For folded domains, deep learning approaches enable the construction of similar protein scaffolds with distinct surface residues.[245] It is worth noting that it may be tempting to make a small number of point mutations and investigate the predicted 3D structure of the resulting sequence using AlphaFold2 or AlphaFold3.[247] However, this will likely give a high-confidence prediction that appears native, even if those point mutations substantially destabilize the protein.[248] As such, we recommend specific approaches developed for protein design instead of general approaches developed for the *de novo* structure prediction of naturally occurring proteins.[245] For disordered regions, emerging tools to facilitate the design of IDRs with specific biophysical and chemical properties have recently emerged. The design package GOOSE enables the rational design of large libraries of IDR sequences that systematically tune chemical composition in minutes.[246] Finally, recent work has enabled the direct prediction of chemically specific IDR-mediated interactions from sequence, opening the door to an interpretable understanding of how IDR sequence chemistry may tune chemical specificity.[102]

CONCLUSION

While the mechanisms behind membrane-dependent cellular organization have been studied extensively over the past decades, the molecular principles guiding the assembly of condensates have only recently come to light. Here, we focused on the role of site-specific and chemically specific interactions in condensate assembly. Site-specific condensate recruitment involves defined interaction sites and interfaces that allow selective interaction with components. In contrast, chemically specific recruitment reflects the interaction of a protein with a unique intra-condensate chemical environment that reflects the properties of all components across a condensate.

Site-specific and chemically specific interactions must work together for proper condensate assembly (Figure 5). Condensate formation often begins with site-specific interactions that concentrate particular components locally, frequently involving the binding of proteins to specific sequence motifs in DNA or RNA.[22,151] Additionally, localized enzymatic processes, such as PARylation, SUMOylation, or ubiquitylation, can trigger condensate assembly by prompting the confined assembly of condensates via site-specific protein-protein interactions.[86,151,169,249–251] Membranes can also serve as critical sites for condensate assembly via site-specific interactions, as observed for membrane-bound receptor multimers or the assembly of the nuclear pore selectivity filter.[79,208,252] The resulting high local concentration of specific biomolecules with specific chemistries creates a distinct chemical environment that facilitates the recruitment or exclusion of further components through site or chemically specific interactions. Conversely, the assembly of some condensate may initially be driven by chemically specific interactions leading to local concentration of specific components, which can then engage in site-specific interactions.

Many questions regarding the disparate effects of chemically specific and site-specific interactions remain to be addressed: What is the hierarchical relationship of chemically specific versus site-specific interactions? Is the proportion of chemically versus site-specific interactions dependent on the foldedness of a protein or RNA molecule? Are there differences in regulatory precision? How can one define the valency of chemically specific interactions, which lack clear boundaries and show context dependence? What are the chemically specific and site-specific interactions of other interacting biomolecules, such as RNAs, and how do they contribute to condensate assembly? What is the dominating mode of interaction in the cytosol, nucleoplasm, or various locales embedded in or surrounded by membranes? Are there physiological processes that rely more on chemically specific versus site-specific interactions?

Evidence suggests that condensates generate specific microenvironments that exert chemical specificity by attracting or repelling macromolecules, metabolites, and ions.[25,31,37,43,181,217–220] An additional layer of complexity is added by the fact that the local condensate environment impacts which interactions may be enhanced or suppressed. For example, changes in local dielectric or redox state may enhance or suppress the relative importance of electrostatic or dipole interactions, such that new modes of interactions emerge only in the context of the condensate interior. These changes could influence both chemically specific and site-specific interactions, tuning the identity and valence of sticker regions. Moreover, condensate microenvironments can facilitate specific chemical reactions, such as ATP hydrolysis or redox processes,[34,36,253–255] while condensates may generate chemical environments tailored to regulate specific enzymatic reactions or biophysical processes.[31,253,256–258] This model could explain why, for instance, some enzymes exhibit extreme pH optima, with optimal enzyme activation requiring recruitment to specific condensates. Moreover, if enzymes alter the condensate environment, then catalysis could enable changes in condensate composition and properties in a manner that depends on the extent of a reaction.[62,256,259]

The emerging viewpoint of condensates as microenvironments with specific chemistries and solvent conditions provides rich opportunities for understanding cellular enzymology and the regulation of metabolism and signaling. It also implies that synthetic condensates could, in principle, enable the construction of genetically programmable microreactors with bespoke chemical interiors for complex synthetic chemistry.[159,160] While much work is required in this space—including new tools for synthetic condensate control, assembly, and targeting—the opportunities for genetically programmable intracellular chemical biology are already beginning to be realized.[118,125,163,260]

In conclusion, condensates play crucial roles in cellular organization, affecting the distribution of a diverse array of macromolecules, small molecules, and ions. The functional implications of condensate-driven cellular organization are just beginning to be uncovered. Dissecting the roles of site-specific and chemically specific interactions and mapping the chemical environments of condensates will require the development of new methodological approaches, particularly in experimental and computational biophysics. Organizing the cellular interior through condensates involves a complex interplay between site-specific and chemically specific interactions. Our growing understanding of this interplay and our increasing ability to manipulate interactions now positions us to unveil the roles of condensate-dependent cellular organization in a vast array of cellular processes such as metabolism, signaling, gene expression, and stress responses.

ACKNOWLEDGMENTS

We thank Titus Franzmann, Jackie Pelham, Steven Boeynaems, Bede Portz, and Rohit Pappu for their helpful comments. A.S.H. thanks the members of The Water and Life Interface Institute (WALII), supported by NSF DBI grant #2213983, for helpful discussion. A.S.H. is funded by the NIH (DP2-CA290639). S.A. thanks the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for funding under Germany's Excellence Strategy (EXC-2068 – 390729961) and for funding of additional projects (DFG project numbers 369799452, 471025906, and 419138288) as well as support by the European Research Council (grant agreement number 725836).

DECLARATION OF INTERESTS

S.A. is an advisor on the scientific advisory board of Dewpoint Therapeutics. A.S.H. is an advisor on the scientific advisory board of Prose Foods.

REFERENCES

1. McCafferty, C.L., Klumpe, S., Amaro, R.E., Kukulski, W., Collinson, L., and Engel, B.D. (2024). Integrating cellular electron microscopy with multimodal data to explore biology across space and time. Cell *187*, 563–584.
2. Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Cuellar, L.K., Förster, F., Hyman, A.A., Plitzko, J.M., and Baumeister, W. (2016). Visualizing the molecular sociology at the HeLa cell nuclear periphery. Science *351*, 969–972.
3. Cohen, S., Valm, A.M., and Lippincott-Schwartz, J. (2018). Interacting organelles. Curr. Opin. Cell Biol. *53*, 84–91.
4. Rapoport, T.A., Li, L., and Park, E. (2017). Structural and mechanistic insights into protein translocation. Annu. Rev. Cell Dev. Biol. *33*, 369–390.
5. Barlan, K., and Gelfand, V.I. (2017). Microtubule-based transport and the distribution, tethering, and organization of organelles. Cold Spring Harb. Perspect. Biol. *9*, a025817.
6. Cohen, R.D., and Pielak, G.J. (2017). A cell is more than the sum of its (dilute) parts: A brief history of quinary structure. Protein Sci. *26*, 403–413.
7. Wilson, E.B. (1899). The structure of protoplasm. Science *10*, 33–45.
8. Edelstein, S.J. (1980). Patterns in the quinary structures of proteins. Plasticity and inequivalence of individual molecules in helical arrays of sickle cell hemoglobin and tubulin. Biophys. J. *32*, 347–360.
9. McConkey, E.H. (1982). Molecular evolution, intracellular organization, and the quinary structure of proteins. Proc. Natl. Acad. Sci. USA *79*, 3236–3240.
10. Mathews, C.K., Sjöberg, B.M., and Reichard, P. (1987). Ribonucleotide reductase of Escherichia coli. Cross-linking agents as probes of quaternary and quinary structure. Eur. J. Biochem. *166*, 279–285.
11. Mathews, C.K. (1993). The cell-bag of enzymes or network of channels? J. Bacteriol. *175*, 6377–6381.
12. Banani, S.F., Lee, H.O., Hyman, A.A., and Rosen, M.K. (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. *18*, 285–298.
13. Shin, Y., and Brangwynne, C.P. (2017). Liquid phase condensation in cell physiology and disease. Science *357*, eaaf4382.
14. Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science *324*, 1729–1732.
15. Wang, J.T., Smith, J., Chen, B.-C., Schmidt, H., Rasoloson, D., Paix, A., Lambrus, B.G., Calidas, D., Betzig, E., and Seydoux, G. (2014). Regulation of RNA granule dynamics by phosphorylation of serine-rich, intrinsically disordered proteins in C. elegans. Elife *3*, e04591.
16. Li, P., Banjade, S., Cheng, H.-C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. Nature *483*, 336–340.
17. Alberti, S., Gladfelter, A., and Mittag, T. (2019). Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. Cell *176*, 419–434.
18. Lyon, A.S., Peeples, W.B., and Rosen, M.K. (2021). A framework for understanding the functions of biomolecular condensates across scales. Nat. Rev. Mol. Cell Biol. *22*, 215–235.

19. Lafontaine, D.L.J., Riback, J.A., Bascetin, R., and Brangwynne, C.P. (2021). The nucleolus as a multiphase liquid condensate. Nat. Rev. Mol. Cell Biol. *22*, 165–182. https://doi.org/10.1038/s41580-020-0272-6.

20. Feric, M., Vaidya, N., Harmon, T.S., Mitrea, D.M., Zhu, L., Richardson, T.M., Kriwacki, R.W., Pappu, R.V., and Brangwynne, C.P. (2016). Coexisting Liquid Phases Underlie Nucleolar Subcompartments. Cell *165*, 1686–1697.

21. Protter, D.S.W., and Parker, R. (2016). Principles and Properties of Stress Granules. Trends Cell Biol. *26*, 668–679.

22. Guillén-Boixet, J., Kopach, A., Holehouse, A.S., Wittmann, S., Jahnel, M., Schlüßler, R., Kim, K., Trussina, I.R.E.A., Wang, J., Mateju, D., et al. (2020). RNA-Induced Conformational Switching and Clustering of G3BP Drive Stress Granule Assembly by Condensation. Cell *181*, 346–361.e17.

23. Hyman, A.A., Weber, C.A., and Jülicher, F. (2014). Liquid-Liquid Phase Separation in Biology. Annu. Rev. Cell Dev. Biol. *30*, 39–58.

24. Jülicher, F., and Weber, C.A. (2024). Droplet Physics and Intracellular Phase Separation. Annu. Rev. Condens. Matter Phys. *15*, 237–261.

25. Nott, T.J., Petsalaki, E., Farber, P., Jervis, D., Fussner, E., Plochowietz, A., Craggs, T.D., Bazett-Jones, D.P., Pawson, T., Forman-Kay, J.D., and Baldwin, A.J. (2015). Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. Mol. Cell *57*, 936–947.

26. Jawerth, L.M., Ijavi, M., Ruer, M., Saha, S., Jahnel, M., Hyman, A.A., Jülicher, F., and Fischer-Friedrich, E. (2018). Salt-Dependent Rheology and Surface Tension of Protein Condensates Using Optical Traps. Phys. Rev. Lett. *121*, 258101.

27. Elbaum-Garfinkle, S., Kim, Y., Szczepaniak, K., Chen, C.C.-H., Eckmann, C.R., Myong, S., and Brangwynne, C.P. (2015). 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.

28. Galvanetto, N., Ivanović, M.T., Chowdhury, A., Sottini, A., Nüesch, M.F., Nettels, D., Best, R.B., and Schuler, B. (2023). Extreme dynamics in a biomolecular condensate. Nature *619*, 876–883.

29. Alshareedah, I., Borcherds, W.M., Cohen, S.R., Singh, A., Posey, A.E., Farag, M., Bremer, A., Strout, G.W., Tomares, D.T., Pappu, R.V., et al. (2024). Sequence-specific interactions determine viscoelasticity and aging dynamics of protein condensates. Nat. Phys. *20*, 1482–1491.

30. Alshareedah, I., Singh, A., Yang, S., Ramachandran, V., Quinn, A., Potoyan, D.A., and Banerjee, P.R. (2024). Determinants of viscoelasticity and flow activation energy in biomolecular condensates. Sci. Adv. *10*, eadi6539.

31. King, M.R., Ruff, K.M., Lin, A.Z., Pant, A., Farag, M., Lalmansingh, J.M., Wu, T., Fossat, M.J., Ouyang, W., Lew, M.D., et al. (2024). Macromolecular condensation organizes nucleolar sub-phases to set up a pH gradient. Cell *187*, 1889–1906.e24.

32. Jambon-Puillet, E., Testa, A., Lorenz, C., Style, R.W., Rebane, A.A., and Dufresne, E.R. (2024). Phase-separated droplets swim to their dissolution. Nat. Commun. *15*, 3919.

33. Ausserwöger, H., Scrutton, R., Sneideris, T., Fischer, C.M., Qian, D., de Csilléry, E., Saar, K.L., Białek, A.Z., Oeller, M., Krainer, G., et al. (2024). Biomolecular condensates sustain pH gradients at equilibrium driven by charge neutralisation. Preprint at bioRxiv. https://doi.org/10.1101/2024.05.23.595321.

34. Posey, A.E., Bremer, A., Erkamp, N.A., Pant, A., Knowles, T.P.J., Dai, Y., Mittag, T., and Pappu, R.V. (2024). Biomolecular condensates are characterized by interphase electric potentials. J. Am. Chem. Soc. https://doi.org/10.1021/jacs.4c08946.

35. Crabtree, M.D., Holland, J., Pillai, A.S., Kompella, P.S., Babl, L., Turner, N.N., Eaton, J.T., Hochberg, G.K.A., Aarts, D.G.A.L., Redfield, C., et al. (2023). Ion binding with charge inversion combined with screening modulates DEAD box helicase phase transitions. Cell Rep. *42*, 113375.

36. Guo, X., Farag, M., Qian, N., Yu, X., Ni, A., Ma, Y., Yu, W., King, M.R., Liu, V., Lee, J., et al. (2024). Biomolecular condensates can function as inherent catalysts. Preprint at bioRxiv, 2024.07.06.602359. https://doi.org/10.1101/2024.07.06.602359.

37. Dai, Y., Zhou, Z., Yu, W., Ma, Y., Kim, K., Rivera, N., Mohammed, J., Lantelme, E., Hsu-Kim, H., Chilkoti, A., and You, L. (2024). Biomolecular condensates regulate cellular electrochemical equilibria. Cell *187*, 5951–5966.e18. https://doi.org/10.1016/j.cell.2024.08.018.

38. Hoffmann, C., Murastov, G., Tromm, J.V., Moog, J.-B., Aslam, M.A., Matkovic, A., and Milovanovic, D. (2023). Electric potential at the interface of membraneless organelles gauged by graphene. Nano Lett. *23*, 10796–10801.

39. van Haren, M.H.I., Visser, B.S., and Spruijt, E. (2024). Probing the surface charge of condensates using microelectrophoresis. Nat. Commun. *15*, 3564.

40. Linsenmeier, M., Faltova, L., Morelli, C., Capasso Palmiero, U., Seiffert, C., Küffner, A.M., Pinotsi, D., Zhou, J., Mezzenga, R., and Arosio, P. (2023). The interface of condensates of the hnRNPA1 low-complexity domain promotes formation of amyloid fibrils. Nat. Chem. *15*, 1340–1349.

41. Choi, C.-H., Lee, D.S.W., Sanders, D.W., and Brangwynne, C.P. (2024). Condensate interfaces can accelerate protein aggregation. Biophys. J. *123*, 1404–1413.

42. Farag, M., Cohen, S.R., Borcherds, W.M., Bremer, A., Mittag, T., and Pappu, R.V. (2022). Condensates formed by prion-like low-complexity domains have small-world network structures and interfaces defined by expanded conformations. Nat. Commun. *13*, 7722.

43. Gouveia, B., Kim, Y., Shaevitz, J.W., Petry, S., Stone, H.A., and Brangwynne, C.P. (2022). Capillary forces generated by biomolecular condensates. Nature *609*, 255–264.

44. Folkmann, A.W., Putnam, A., Lee, C.F., and Seydoux, G. (2021). Regulation of biomolecular condensates by interfacial protein clusters. Science *373*, 1218–1224.

45. Welsh, T.J., Krainer, G., Espinosa, J.R., Joseph, J.A., Sridhar, A., Jahnel, M., Arter, W.E., Saar, K.L., Alberti, S., Collepardo-Guevara, R., and Knowles, T.P.J. (2022). Surface Electrostatics Govern the Emulsion Stability of Biomolecular Condensates. Nano Lett. *22*, 612–621.

46. Shimobayashi, S.F., Ronceray, P., Sanders, D.W., Haataja, M.P., and Brangwynne, C.P. (2021). Nucleation landscape of biomolecular condensates. Nature *599*, 503–506.

47. Sanders, D.W., Kedersha, N., Lee, D.S.W., Strom, A.R., Drake, V., Riback, J.A., Bracha, D., Eeftens, J.M., Iwanicki, A., Wang, A., et al. (2020). Competing Protein-RNA Interaction Networks Control Multiphase Intracellular Organization. Cell *181*, 306–324.e28.

48. Bracha, D., Walls, M.T., Wei, M.-T., Zhu, L., Kurian, M., Avalos, J.L., Toettcher, J.E., and Brangwynne, C.P. (2018). Mapping Local and Global Liquid Phase Behavior in Living Cells Using Photo-Oligomerizable Seeds. Cell *175*, 1467–1480.e13.

49. Riback, J.A., Zhu, L., Ferrolino, M.C., Tolbert, M., Mitrea, D.M., Sanders, D.W., Wei, M.-T., Kriwacki, R.W., and Brangwynne, C.P. (2020). Composition-dependent thermodynamics of intracellular phase separation. Nature *581*, 209–214.

50. Berry, J., Weber, S.C., Vaidya, N., Haataja, M., and Brangwynne, C.P. (2015). RNA transcription modulates phase transition-driven nuclear body assembly. Proc. Natl. Acad. Sci. USA *112*, E5237–E5245.

51. Wang, J., Choi, J.-M., Holehouse, A.S., Lee, H.O., Zhang, X., Jahnel, M., Maharana, S., Lemaitre, R., Pozniakovsky, A., Drechsel, D., et al. (2018). A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. Cell *174*, 688–699.e16.

52. Fritsch, A.W., Diaz-Delgadillo, A.F., Adame-Arana, O., Hoege, C., Mittasch, M., Kreysing, M., Leaver, M., Hyman, A.A., Jülicher, F., and Weber, C.A. (2021). Local thermodynamics govern formation and dissolution of Caenorhabditis elegans P granule condensates. Proc. Natl. Acad. Sci. USA *118*, e2102772118.

53. Cho, W.-K., Spille, J.-H., Hecht, M., Lee, C., Li, C., Grube, V., and Cisse, I.I. (2018). Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science *361*, 412–415.

54. Martin, E.W., Holehouse, A.S., Peran, I., Farag, M., Incicco, J.J., Bremer, A., Grace, C.R., Soranno, A., Pappu, R.V., and Mittag, T. (2020). Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. Science *367*, 694–699.

55. Bremer, A., Farag, M., Borcherds, W.M., Peran, I., Martin, E.W., Pappu, R.V., and Mittag, T. (2022). Deciphering how naturally occurring sequence features impact the phase behaviours of disordered prion-like domains. Nat. Chem. *14*, 196–207.

56. Jawerth, L., Fischer-Friedrich, E., Saha, S., Wang, J., Franzmann, T., Zhang, X., Sachweh, J., Ruer, M., Ijavi, M., Saha, S., et al. (2020). Protein condensates as aging Maxwell fluids. Science *370*, 1317–1323.

57. Martin, E.W., Harmon, T.S., Hopkins, J.B., Chakravarthy, S., Incicco, J.J., Schuck, P., Soranno, A., and Mittag, T. (2021). A multi-step nucleation process determines the kinetics of prion-like domain phase separation. Nat. Commun. *12*, 4513.

58. Harmon, T.S., Holehouse, A.S., Rosen, M.K., and Pappu, R.V. (2017). Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. Elife *6*, e30294. https://doi.org/10.7554/eLife.30294.

59. Pappu, R.V., Cohen, S.R., Dar, F., Farag, M., and Kar, M. (2023). Phase Transitions of Associative Biomacromolecules. Chem. Rev. *123*, 8945–8987.

60. Mittag, T., and Pappu, R.V. (2022). A conceptual framework for understanding phase separation and addressing open questions and challenges. Mol. Cell *82*, 2201–2214.

61. Henninger, J.E., Oksuz, O., Shrinivas, K., Sagi, I., LeRoy, G., Zheng, M.M., Andrews, J.O., Zamudio, A.V., Lazaris, C., Hannett, N.M., et al. (2021). RNA-Mediated Feedback Control of Transcriptional Condensates. Cell *184*, 207–225.e24.

62. Harmon, T.S., and Jülicher, F. (2022). Molecular assembly lines in active droplets. Phys. Rev. Lett. *128*, 108102.

63. Lin, A.Z., Ruff, K.M., Dar, F., Jalihal, A., King, M.R., Lalmansingh, J.M., Posey, A.E., Erkamp, N.A., Seim, I., Gladfelter, A.S., and Pappu, R.V. (2023). Dynamical control enables the formation of demixed biomolecular condensates. Nat. Commun. *14*, 7678.

64. Sankaranarayanan, M., Emenecker, R.J., Wilby, E.L., Jahnel, M., Trussina, I.R.E.A., Wayland, M., Alberti, S., Holehouse, A.S., and Weil, T.T. (2021). Adaptable P body physical states differentially regulate bicoid mRNA storage during early Drosophila development. Dev. Cell *56*, 2886–2901.e6.

65. Woodruff, J.B., Hyman, A.A., and Boke, E. (2018). Organization and Function of Non-dynamic Biomolecular Condensates. Trends Biochem. Sci. *43*, 81–94.

66. Alshareedah, I., Kaur, T., and Banerjee, P.R. (2021). Methods for characterizing the material properties of biomolecular condensates. Methods Enzymol. *646*, 143–183.

67. Taylor, N.O., Wei, M.-T., Stone, H.A., and Brangwynne, C.P. (2019). Quantifying Dynamics in Phase-Separated Condensates Using Fluorescence Recovery after Photobleaching. Biophys. J. *117*, 1285–1300.

68. McCall, P.M., Kim, K., Ruer-Gruß, M., Peychl, J., Guck, J., Hyman, A.A., and Brugués, J. (2023). Label-free composition determination for biomolecular condensates with an arbitrarily large number of components. Preprint at bioRxiv. https://doi.org/10.1101/2020.10.25.352823.

69. Choi, J.-M., Holehouse, A.S., and Pappu, R.V. (2020). Physical Principles Underlying the Complex Biology of Intracellular Phase Transitions. Annu. Rev. Biophys. *49*, 107–133. https://doi.org/10.1146/annurev-biophys-121219-081629.

70. Choi, J.-M., Hyman, A.A., and Pappu, R.V. (2020). Generalized models for bond percolation transitions of associative polymers. Phys. Rev. E *102*, 042403.

71. Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M., et al. (2015). A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell *162*, 1066–1077.

72. Cohen, S.R., Banerjee, P.R., and Pappu, R.V. (2024). Direct computations of viscoelastic moduli of biomolecular condensates. Preprint at bioRxiv, 2024.06.11.598543. https://doi.org/10.1101/2024.06.11.598543.

73. Galvanetto, N., Ivanović, M.T., Del Grosso, S.A., Chowdhury, A., Sottini, A., Nettels, D., Best, R.B., and Schuler, B. (2024). Mesoscale properties of biomolecular condensates emerging from protein chain dynamics. Preprint at arXiv. https://doi.org/10.48550/arXiv.2407.19202.

74. Gibson, B.A., Doolittle, L.K., Schneider, M.W.G., Jensen, L.E., Gamarra, N., Henry, L., Gerlich, D.W., Redding, S., and Rosen, M.K. (2019). Organization of Chromatin by Intrinsic and Regulated Phase Separation. Cell *179*, 470–484.e21.

75. Kroschwald, S., Maharana, S., Mateju, D., Malinovska, L., Nüske, E., Poser, I., Richter, D., and Alberti, S. (2015). Promiscuous interactions and protein disaggregases determine the material state of stress-inducible RNP granules. Elife *4*, e06807.

76. Kroschwald, S., Munder, M.C., Maharana, S., Franzmann, T.M., Richter, D., Ruer, M., Hyman, A.A., and Alberti, S. (2018). Different Material States of Pub1 Condensates Define Distinct Modes of Stress Adaptation and Recovery. Cell Rep. *23*, 3327–3339.

77. Weber, S.C. (2017). Sequence-encoded material properties dictate the structure and function of nuclear bodies. Curr. Opin. Cell Biol. *46*, 62–71.

78. Banani, S.F., Rice, A.M., Peeples, W.B., Lin, Y., Jain, S., Parker, R., and Rosen, M.K. (2016). Compositional Control of Phase-Separated Cellular Bodies. Cell *166*, 651–663. https://doi.org/10.1016/j.cell.2016.06.010.

79. Frey, S., Richter, R.P., and Görlich, D. (2006). FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. Science *314*, 815–817.

80. Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., et al. (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell *149*, 753–767.

81. Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G., and McKnight, S.L. (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell *149*, 768–779.

82. Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A.P., Kim, H.J., Mittag, T., and Taylor, J.P. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Cell *163*, 123–133.

83. Pak, C.W., Kosno, M., Holehouse, A.S., Padrick, S.B., Mittal, A., Ali, R., Yunus, A.A., Liu, D.R., Pappu, R.V., and Rosen, M.K. (2016). Sequence Determinants of Intracellular Phase Separation by Complex Coacervation of a Disordered Protein. Mol. Cell *63*, 72–85.

84. Martin, E.W., and Holehouse, A.S. (2020). Intrinsically disordered protein regions and phase separation: sequence determinants of assembly or lack thereof. Emerg. Top. Life Sci. *4*, 307–329.

85. Riback, J.A., Katanski, C.D., Kear-Scott, J.L., Pilipenko, E.V., Rojek, A.E., Sosnick, T.R., and Drummond, D.A. (2017). Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell *168*, 1028–1040.e19.

86. Dao, T.P., Kolaitis, R.-M., Kim, H.J., O'Donovan, K., Martyniak, B., Colicino, E., Hehnly, H., Taylor, J.P., and Castañeda, C.A. (2018). Ubiquitin Modulates Liquid-Liquid Phase Separation of UBQLN2 via Disruption of Multivalent Interactions. Mol. Cell *69*, 965–978.e6.

87. Holehouse, A.S., and Kragelund, B.B. (2024). The molecular basis for cellular function of intrinsically disordered protein regions. Nat. Rev. Mol. Cell Biol. *25*, 187–211.

88. Martin, E.W., and Mittag, T. (2018). Relationship of Sequence and Phase Separation in Protein Low-Complexity Regions. Biochemistry *57*, 2478–2487.

89. Chong, S., and Mir, M. (2021). Towards Decoding the Sequence-Based Grammar Governing the Functions of Intrinsically Disordered Protein Regions. J. Mol. Biol. *433*, 166724.

90. Oughtred, R., Rust, J., Chang, C., Breitkreutz, B.-J., Stark, C., Willems, A., Boucher, L., Leung, G., Kolas, N., Zhang, F., et al. (2021). The BioGRID database: A comprehensive biomedical resource of curated protein, genetic, and chemical interactions. Protein Sci. *30*, 187–200.

91. Nooren, I.M.A., and Thornton, J.M. (2003). Diversity of protein–protein interactions. EMBO J. *22*, 3486–3492. https://doi.org/10.1093/emboj/cdg359.

92. Musacchio, A. (2022). On the role of phase separation in the biogenesis of membraneless compartments. EMBO J. *41*, e109952.

93. Davey, N.E., Simonetti, L., and Ivarsson, Y. (2023). The next wave of interactomics: Mapping the SLiM-based interactions of the intrinsically disordered proteome. Curr. Opin. Struct. Biol. *80*, 102593.

94. Tompa, P., and Fuxreiter, M. (2008). Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions. Trends Biochem. Sci. *33*, 2–8.

95. Kliche, J., Simonetti, L., Krystkowiak, I., Kuss, H., Diallo, M., Rask, E., Nilsson, J., Davey, N.E., and Ivarsson, Y. (2024). Proteome-scale characterisation of motif-based interactome rewiring by disease mutations. Mol. Syst. Biol. *20*, 1025–1048.

96. Langstein-Skora, I., Schmid, A., Huth, F., Shabani, D., Spechtenhauser, L., Likhodeeva, M., Kunert, F., Metzner, F.J., Emenecker, R.J., Richardson, M.O.G., et al. (2024). Sequence- and chemical specificity define the functional landscape of intrinsically disordered regions. Preprint at bioRxiv. https://doi.org/10.1101/2022.02.10.480018.

97. Friis Theisen, F., Salladini, E., Davidsen, R., Jo Rasmussen, C., Staby, L., Kragelund, B.B., and Skriver, K. (2022). $\alpha\alpha$-hub coregulator structure and flexibility determine transcription factor binding and selection in regulatory interactomes. J. Biol. Chem. *298*, 101963.

98. Teilum, K., Olsen, J.G., and Kragelund, B.B. (2021). On the specificity of protein-protein interactions in the context of disorder. Biochem. J. *478*, 2035–2050.

99. Borgia, A., Borgia, M.B., Bugge, K., Kissling, V.M., Heidarsson, P.O., Fernandes, C.B., Sottini, A., Soranno, A., Buholzer, K.J., Nettels, D., et al. (2018). Extreme disorder in an ultrahigh-affinity protein complex. Nature *555*, 61–66.

100. Kilgore, H.R., and Young, R.A. (2022). Learning the chemical grammar of biomolecular condensates. Nat. Chem. Biol. *18*, 1298–1306.

101. Jonas, F., Carmi, M., Krupkin, B., Steinberger, J., Brodsky, S., Jana, T., and Barkai, N. (2023). The molecular grammar of protein disorder guiding genome-binding locations. Nucleic Acids Res. *51*, 4831–4844.

102. Ginell, G., Emenecker, R.J., Lotthammer, J.M., Usher, E.T., and Holehouse, A. (2024). Direct prediction of intermolecular interactions driven by disordered regions. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.03.597104.

103. Chen, F., and Jacobs, W.M. (2024). Emergence of multiphase condensates from a limited set of chemical building blocks. J. Chem. Theory Comput. *20*, 6881–6889.

104. Welles, R.M., Sojitra, K.A., Garabedian, M.V., Xia, B., Wang, W., Guan, M., Regy, R.M., Gallagher, E.R., Hammer, D.A., Mittal, J., and Good, M.C. (2024). Determinants that enable disordered protein assembly into discrete condensed phases. Nat. Chem. *16*, 1062–1072.

105. De La Cruz, N., Pradhan, P., Veettil, R.T., Conti, B.A., Oppikofer, M., and Sabari, B.R. (2024). Disorder-mediated interactions target proteins to specific condensates. Mol. Cell *84*, 3497–3512.e9.

106. Lyons, H., Veettil, R.T., Pradhan, P., Fornero, C., De La Cruz, N., Ito, K., Eppert, M., Roeder, R.G., and Sabari, B.R. (2023). Functional partitioning of transcriptional regulators by patterned charge blocks. Cell *186*, 327–345.e28.

107. Schuler, B., Borgia, A., Borgia, M.B., Heidarsson, P.O., Holmstrom, E.D., Nettels, D., and Sottini, A. (2020). Binding without folding - the biomolecular function of disordered polyelectrolyte complexes. Curr. Opin. Struct. Biol. *60*, 66–76.

108. Newcombe, E.A., Due, A.D., Sottini, A., Elkjær, S., Theisen, F.F., Fernandes, C.B., Staby, L., Delaforge, E., Bartling, C.R.O., Brakti, I., et al. (2024). Stereochemistry in the disorder-order continuum of protein interactions. Nature *636*, 762–768.

109. Rekhi, S., Garcia, C.G., Barai, M., Rizuan, A., Schuster, B.S., Kiick, K.L., and Mittal, J. (2024). Expanding the molecular language of protein liquid–liquid phase separation. Nat. Chem. *16*, 1113–1124.

110. Julia Maristany, M., Gonzalez, A.A., Collepardo-Guevara, R., and Joseph, J.A. (2023). Universal predictive scaling laws of phase separation of prion-like low complexity domains. Preprint at bioRxiv. https://doi.org/10.1101/2023.06.14.543914.

111. Monahan, Z., Ryan, V.H., Janke, A.M., Burke, K.A., Rhoads, S.N., Zerze, G.H., O'Meally, R., Dignon, G.L., Conicella, A.E., Zheng, W., et al. (2017). Phosphorylation of the FUS low-complexity domain disrupts phase separation, aggregation, and toxicity. EMBO J. *36*, 2951–2967.

112. Burke, K.A., Janke, A.M., Rhine, C.L., and Fawzi, N.L. (2015). Residue-by-residue view of in vitro FUS granules that bind the C-terminal domain of RNA polymerase II. Mol. Cell *60*, 231–241.

113. Gruijs da Silva, L.A., Simonetti, F., Hutten, S., Riemenschneider, H., Sternburg, E.L., Pietrek, L.M., Gebel, J., Dötsch, V., Edbauer, D., Hummer, G., et al. (2022). Disease-linked TDP-43 hyperphosphorylation suppresses TDP-43 condensation and aggregation. EMBO J. *41*, e108443.

114. Hofweber, M., Hutten, S., Bourgeois, B., Spreitzer, E., Niedner-Boblenz, A., Schifferer, M., Ruepp, M.-D., Simons, M., Niessing, D., Madl, T., and Dormann, D. (2018). Phase Separation of FUS Is Suppressed by Its Nuclear Import Receptor and Arginine Methylation. Cell *173*, 706–719.e13.

115. Yoshizawa, T., Ali, R., Jiou, J., Fung, H.Y.J., Burke, K.A., Kim, S.J., Lin, Y., Peeples, W.B., Saltzberg, D., Soniat, M., et al. (2018). Nuclear import receptor inhibits phase separation of FUS through binding to multiple sites. Cell *173*, 693–705.e22.

116. Lin, Y., Protter, D.S.W., Rosen, M.K., and Parker, R. (2015). Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. Mol. Cell *60*, 208–219.

117. Lin, Y., Currie, S.L., and Rosen, M.K. (2017). Intrinsically disordered sequences enable modulation of protein phase separation through distributed tyrosine motifs. J. Biol. Chem. *292*, 19110–19120.

118. Lasker, K., Boeynaems, S., Lam, V., Scholl, D., Stainton, E., Briner, A., Jacquemyn, M., Daelemans, D., Deniz, A., Villa, E., et al. (2022). The material properties of a bacterial-derived biomolecular condensate tune biological function in natural and synthetic systems. Nat. Commun. *13*, 5643.

119. Boeynaems, S., Bogaert, E., Kovacs, D., Konijnenberg, A., Timmerman, E., Volkov, A., Guharoy, M., De Decker, M., Jaspers, T., Ryan, V.H., et al. (2017). Phase Separation of C9orf72 Dipeptide Repeats Perturbs Stress Granule Dynamics. Mol. Cell *65*, 1044–1055.e5.

120. Murthy, A.C., Tang, W.S., Jovic, N., Janke, A.M., Seo, D.H., Perdikari, T.M., Mittal, J., and Fawzi, N.L. (2021). 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.

121. Schmidt, H.B., Barreau, A., and Rohatgi, R. (2019). Phase separation-deficient TDP43 remains functional in splicing. Nat. Commun. *10*, 4890.

122. Murthy, A.C., Dignon, G.L., Kan, Y., Zerze, G.H., Parekh, S.H., Mittal, J., and Fawzi, N.L. (2019). Molecular interactions underlying liquid-liquid phase separation of the FUS low-complexity domain. Nat. Struct. Mol. Biol. *26*, 637–648.

123. Conicella, A.E., Zerze, G.H., Mittal, J., and Fawzi, N.L. (2016). ALS Mutations Disrupt Phase Separation Mediated by α-Helical Structure in the TDP-43 Low-Complexity C-Terminal Domain. Structure *24*, 1537–1549. https://doi.org/10.1016/j.str.2016.07.007.

124. Simon, J.R., Carroll, N.J., Rubinstein, M., Chilkoti, A., and López, G.P. (2017). Programming molecular self-assembly of intrinsically disordered

proteins containing sequences of low complexity. Nat. Chem. *9*, 509–515.

125. Dzuricky, M., Rogers, B.A., Shahid, A., Cremer, P.S., and Chilkoti, A. (2020). De novo engineering of intracellular condensates using artificial disordered proteins. Nat. Chem. *12*, 814–825.

126. Wiggers, F., Wohl, S., Dubovetskyi, A., Rosenblum, G., Zheng, W., and Hofmann, H. (2021). Diffusion of a disordered protein on its folded ligand. Proc. Natl. Acad. Sci. USA *118*, e2106690118. https://doi.org/10.1073/pnas.2106690118.

127. Ubbink, M. (2009). The courtship of proteins: understanding the encounter complex. FEBS Lett. *583*, 1060–1066.

128. DelRosso, N., Suzuki, P.H., Griffith, D., Lotthammer, J.M., Novak, B., Kocalar, S., Sheth, M.U., Holehouse, A.S., Bintu, L., and Fordyce, P. (2024). High-throughput affinity measurements of direct interactions between activation domains and co-activators. Preprint at bioRxiv, 2024.08.19.608698. https://doi.org/10.1101/2024.08.19.608698.

129. Prestel, A., Wichmann, N., Martins, J.M., Marabini, R., Kassem, N., Broendum, S.S., Otterlei, M., Nielsen, O., Willemoës, M., Ploug, M., et al. (2019). The PCNA interaction motifs revisited: thinking outside the PIP-box. Cell. Mol. Life Sci. *76*, 4923–4943.

130. Bugge, K., Brakti, I., Fernandes, C.B., Dreier, J.E., Lundsgaard, J.E., Olsen, J.G., Skriver, K., and Kragelund, B.B. (2020). Interactions by Disorder - A Matter of Context. Front. Mol. Biosci. *7*, 110.

131. Wang, H.-Y., Chan, S.H., Dey, S., Castello-Serrano, I., Rosen, M.K., Ditlev, J.A., Levental, K.R., and Levental, I. (2023). Coupling of protein condensates to ordered lipid domains determines functional membrane organization. Sci. Adv. *9*, eadf6205.

132. Boeynaems, S., Dorone, Y., Zhuang, Y., Shabardina, V., Huang, G., Marian, A., Kim, G., Sanyal, A., Şen, N.-E., Griffith, D., et al. (2023). Poly(A)-binding protein is an ataxin-2 chaperone that regulates biomolecular condensates. Mol. Cell *83*, 2020–2034.e6.

133. Liboy-Lugo, J.M., Espinoza, C.A., Sheu-Gruttadauria, J., Park, J.E., Xu, A., Jowhar, Z., Gao, A.L., Carmona-Negrón, J.A., Wittmann, T., Jura, N., and Floor, S.N. (2024). Protein-protein interactions with G3BPs drive stress granule condensation and gene expression changes under cellular stress. Preprint at bioRxiv, 2024.02.06.579149. https://doi.org/10.1101/2024.02.06.579149.

134. Carter, G.C., Hsiung, C.-H., Simpson, L., Yang, H., and Zhang, X. (2021). N-terminal domain of TDP43 enhances liquid-liquid phase separation of globular proteins. J. Mol. Biol. *433*, 166948.

135. Yang, P., Mathieu, C., Kolaitis, R.-M., Zhang, P., Messing, J., Yurtsever, U., Yang, Z., Wu, J., Li, Y., Pan, Q., et al. (2020). G3BP1 Is a Tunable Switch that Triggers Phase Separation to Assemble Stress Granules. Cell *181*, 325–345.e28.

136. Mitrea, D.M., Cika, J.A., Stanley, C.B., Nourse, A., Onuchic, P.L., Banerjee, P.R., Phillips, A.H., Park, C.-G., Deniz, A.A., and Kriwacki, R.W. (2018). Self-interaction of NPM1 modulates multiple mechanisms of liquid–liquid phase separation. Nat. Commun. *9*, 842.

137. Li, Z., Shen, Q., Usher, E.T., Anderson, A.P., Iburg, M., Lin, R., Zimmer, B., Meyer, M.D., Holehouse, A.S., You, L., et al. (2024). Phase transition of GvpU regulates gas vesicle clustering in bacteria. Nat. Microbiol. *9*, 1021–1035.

138. Afroz, T., Hock, E.-M., Ernst, P., Foglieni, C., Jambeau, M., Gilhespy, L.A.B., Laferriere, F., Maniecka, Z., Plückthun, A., Mittl, P., et al. (2017). Functional and dynamic polymerization of the ALS-linked protein TDP-43 antagonizes its pathologic aggregation. Nat. Commun. *8*, 45.

139. Garcia-Seisdedos, H., Empereur-Mot, C., Elad, N., and Levy, E.D. (2017). Proteins evolve on the edge of supramolecular self-assembly. Nature *548*, 244–247.

140. Seim, I., Posey, A.E., Snead, W.T., Stormo, B.M., Klotsa, D., Pappu, R.V., and Gladfelter, A.S. (2022). Dilute phase oligomerization can oppose phase separation and modulate material properties of a ribonucleoprotein condensate. Proc. Natl. Acad. Sci. USA *119*, e2120799119.

141. Roden, C.A., Dai, Y., Giannetti, C.A., Seim, I., Lee, M., Sealfon, R., McLaughlin, G.A., Boerneke, M.A., Iserman, C., Wey, S.A., et al. (2022). Double-stranded RNA drives SARS-CoV-2 nucleocapsid protein to undergo phase separation at specific temperatures. Nucleic Acids Res. *50*, 8168–8192.

142. Bienz, M. (2020). Head-to-tail polymerization in the assembly of biomolecular condensates. Cell *182*, 799–811.

143. Seif, E., Kang, J.J., Sasseville, C., Senkovich, O., Kaltashov, A., Boulier, E.L., Kapur, I., Kim, C.A., and Francis, N.J. (2020). Phase separation by the polyhomeotic sterile alpha motif compartmentalizes Polycomb Group proteins and enhances their activity. Nat. Commun. *11*, 5609.

144. Kang, K., Shi, Q., Wang, X., and Chen, Y.-G. (2022). Dishevelled phase separation promotes Wnt signalosome assembly and destruction complex disassembly. J. Cell Biol. *221*, e202205069. https://doi.org/10.1083/jcb.202205069.

145. Powers, S.K., Holehouse, A.S., Korasick, D.A., Schreiber, K.H., Clark, N.M., Jing, H., Emenecker, R., Han, S., Tycksen, E., Hwang, I., et al. (2019). Nucleo-cytoplasmic partitioning of ARF proteins controls auxin responses in Arabidopsis thaliana. Mol. Cell *76*, 177–190.e5.

146. Gallagher, E.R., and Holzbaur, E.L.F. (2023). The selective autophagy adaptor p62/SQSTM1 forms phase condensates regulated by HSP27 that facilitate the clearance of damaged lysosomes via lysophagy. Cell Rep. *42*, 112037.

147. Marzahn, M.R., Marada, S., Lee, J., Nourse, A., Kenrick, S., Zhao, H., Ben-Nissan, G., Kolaitis, R., Peters, J.L., Pounds, S., et al. (2016). Higher-order oligomerization promotes localization of SPOP to liquid nuclear speckles. EMBO J. *35*, 1254–1275.

148. Sabri, N., Cuneo, M.J., Marzahn, M.R., Lee, J., Bouchard, J.J., Güllülü, Ö., Vaithiyalingam, S., Borgia, M.B., Schmit, J., and Mittag, T. (2023). Reduction of oligomer size modulates the competition between cluster formation and phase separation of the tumor suppressor SPOP. J. Biol. Chem. *299*, 105427.

149. Cuneo, M.J., O'Flynn, B.G., Lo, Y.-H., Sabri, N., and Mittag, T. (2023). Higher-order SPOP assembly reveals a basis for cancer mutant dysregulation. Mol. Cell *83*, 731–745.e4.

150. Bouchard, J.J., Otero, J.H., Scott, D.C., Szulc, E., Martin, E.W., Sabri, N., Granata, D., Marzahn, M.R., Lindorff-Larsen, K., Salvatella, X., et al. (2018). Cancer Mutations of the Tumor Suppressor SPOP Disrupt the Formation of Active, Phase-Separated Compartments. Mol. Cell *72*, 19–36.e8.

151. Chappidi, N., Quail, T., Doll, S., Vogel, L.T., Aleksandrov, R., Felekyan, S., Kühnemuth, R., Stoynov, S., Seidel, C.A.M., Brugués, J., et al. (2024). PARP1-DNA co-condensation drives DNA repair site assembly to prevent disjunction of broken DNA ends. Cell *187*, 945–961.e18.

152. Ramšak, M., Ramirez, D.A., Hough, L.E., Shirts, M.R., Vidmar, S., Eleršič Filipič, K., Anderluh, G., and Jerala, R. (2023). Programmable de novo designed coiled coil-mediated phase separation in mammalian cells. Nat. Commun. *14*, 7973.

153. Ramirez, D.A., Hough, L.E., and Shirts, M.R. (2024). Coiled-coil domains are sufficient to drive liquid-liquid phase separation in protein models. Biophys. J. *123*, 703–717.

154. Woodruff, J.B., Ferreira Gomes, B., Widlund, P.O., Mahamid, J., Honigmann, A., and Hyman, A.A. (2017). The Centrosome Is a Selective Condensate that Nucleates Microtubules by Concentrating Tubulin. Cell *169*, 1066–1077.e10.

155. Sil, S., Keegan, S., Ettefa, F., Denes, L.T., Boeke, J.D., and Holt, L.J. (2023). Condensation of LINE-1 is critical for retrotransposition. Elife *12*, e82991. https://doi.org/10.7554/eLife.82991.

156. Newton, J.C., Naik, M.T., Li, G.Y., Murphy, E.L., Fawzi, N.L., Sedivy, J.M., and Jogl, G. (2021). Phase separation of the LINE-1 ORF1 protein is mediated by the N-terminus and coiled-coil domain. Biophys. J. *120*, 2181–2191.

157. Dorone, Y., Boeynaems, S., Flores, E., Jin, B., Hateley, S., Bossi, F., Lazarus, E., Pennington, J.G., Michiels, E., De Decker, M., et al. (2021).

A prion-like protein regulator of seed germination undergoes hydration-dependent phase separation. Cell *184*, 4284–4298.e27.

158. Shin, Y., Berry, J., Pannucci, N., Haataja, M.P., Toettcher, J.E., and Brangwynne, C.P. (2017). Spatiotemporal Control of Intracellular Phase Transitions Using Light-Activated optoDroplets. Cell *168*, 159–171.e14.

159. Hastings, R.L., and Boeynaems, S. (2021). Designer Condensates: A Toolkit for the Biomolecular Architect. J. Mol. Biol. *433*, 166837.

160. Dai, Y., You, L., and Chilkoti, A. (2023). Engineering synthetic biomolecular condensates. Nat. Rev. Bioeng. *1*, 466–480.

161. Dine, E., Gil, A.A., Uribe, G., Brangwynne, C.P., and Toettcher, J.E. (2018). Protein Phase Separation Provides Long-Term Memory of Transient Spatial Stimuli. Cell Syst. *6*, 655–663.e5.

162. Hernández-Candia, C.N., Pearce, S., and Tucker, C.L. (2021). A modular tool to query and inducibly disrupt biomolecular condensates. Nat. Commun. *12*, 1809.

163. Heidenreich, M., Georgeson, J.M., Locatelli, E., Rovigatti, L., Nandi, S.K., Steinberg, A., Nadav, Y., Shimoni, E., Safran, S.A., Doye, J.P.K., and Levy, E.D. (2020). Designer protein assemblies with tunable phase diagrams in living cells. Nat. Chem. Biol. *16*, 939–945.

164. Roden, C., and Gladfelter, A.S. (2021). RNA contributions to the form and function of biomolecular condensates. Nat. Rev. Mol. Cell Biol. *22*, 183–195. https://doi.org/10.1038/s41580-020-0264-6.

165. Maharana, S., Wang, J., Papadopoulos, D.K., Richter, D., Pozniakovsky, A., Poser, I., Bickle, M., Rizk, S., Guillén-Boixet, J., Franzmann, T.M., et al. (2018). RNA buffers the phase separation behavior of prion-like RNA binding proteins. Science *360*, 918–921. https://doi.org/10.1126/science.aar7366.

166. Morin, J.A., Wittmann, S., Choubey, S., Klosin, A., Golfier, S., Hyman, A.A., Jülicher, F., and Grill, S.W. (2022). Sequence-dependent surface condensation of a pioneer transcription factor on DNA. Nat. Phys. *18*, 271–276.

167. Wadsworth, G.M., Srinivasan, S., Lai, L.B., Datta, M., Gopalan, V., and Banerjee, P.R. (2024). RNA-driven phase transitions in biomolecular condensates. Mol. Cell *84*, 3692–3705.

168. Boeynaems, S., Holehouse, A.S., Weinhardt, V., Kovacs, D., Van Lindt, J., Larabell, C., Van Den Bosch, L., Das, R., Tompa, P.S., Pappu, R.V., and Gitler, A.D. (2019). Spontaneous driving forces give rise to protein-RNA condensates with coexisting phases and complex material properties. Proc. Natl. Acad. Sci. USA *116*, 7889–7898.

169. Leung, A.K.L. (2020). Poly(ADP-ribose): A Dynamic Trigger for Biomolecular Condensate Formation. Trends Cell Biol. *30*, 370–383.

170. McCall, P.M., Kim, K., Fritsch, A.W., Iglesias-Artola, J.M., Jawerth, L.M., Wang, J., Ruer, M., Peychl, J., Poznyakovskiy, A., Guck, J., et al. (2020). Quantitative phase microscopy enables precise and efficient determination of biomolecular condensate composition. Preprint at bioRxiv. https://doi.org/10.1101/2020.10.25.352823.

171. Milin, A.N., and Deniz, A.A. (2018). Reentrant Phase Transitions and Non-Equilibrium Dynamics in Membraneless Organelles. Biochemistry *57*, 2470–2477. https://doi.org/10.1021/acs.biochem.8b00001.

172. Banerjee, P.R., Milin, A.N., Moosa, M.M., Onuchic, P.L., and Deniz, A.A. (2017). Reentrant Phase Transition Drives Dynamic Substructure Formation in Ribonucleoprotein Droplets. Angew. Chem. Int. Ed. Engl. *56*, 11354–11359.

173. Riback, J.A., Eeftens, J.M., Lee, D.S.W., Quinodoz, S.A., Donlic, A., Orlovsky, N., Wiesner, L., Beckers, L., Becker, L.A., Strom, A.R., et al. (2023). Viscoelasticity and advective flow of RNA underlies nucleolar form and function. Mol. Cell *83*, 3095–3107.e9.

174. McCluggage, F., and Fox, A.H. (2021). Paraspeckle nuclear condensates: Global sensors of cell stress? Bioessays *43*, e2000245.

175. Elguindy, M.M., and Mendell, J.T. (2021). NORAD-induced Pumilio phase separation is required for genome stability. Nature *595*, 303–308.

176. Van Treeck, B., and Parker, R. (2018). Emerging Roles for Intermolecular RNA-RNA Interactions in RNP Assemblies. Cell *174*, 791–802.

177. Majumder, S., Coupe, S., Fakhri, N., and Jain, A. (2024). Sequence programmable nucleic acid coacervates. Preprint at bioRxiv. https://doi.org/10.1101/2024.07.22.604687.

178. King, M.R., Ruff, K.M., and Pappu, R.V. (2024). Emergent microenvironments of nucleoli. Nucleus *15*, 2319957.

179. Boke, E., Ruer, M., Wühr, M., Coughlin, M., Lemaitre, R., Gygi, S.P., Alberti, S., Drechsel, D., Hyman, A.A., and Mitchison, T.J. (2016). Amyloid-like Self-Assembly of a Cellular Compartment. Cell *166*, 637–650.

180. Vernon, R.M., Chong, P.A., Tsang, B., Kim, T.H., Bah, A., Farber, P., Lin, H., and Forman-Kay, J.D. (2018). Pi-Pi contacts are an overlooked protein feature relevant to phase separation. Elife *7*, e31486. https://doi.org/10.7554/eLife.31486.

181. Basu, S., Martínez-Cristóbal, P., Frigolé-Vivas, M., Pesarrodona, M., Lewis, M., Szulc, E., Bañuelos, C.A., Sánchez-Zarzalejo, C., Bielskutė, S., Zhu, J., et al. (2023). Rational optimization of a transcription factor activation domain inhibitor. Nat. Struct. Mol. Biol. *30*, 1958–1969.

182. Brady, J.P., Farber, P.J., Sekhar, A., Lin, Y.-H., Huang, R., Bah, A., Nott, T.J., Chan, H.S., Baldwin, A.J., Forman-Kay, J.D., and Kay, L.E. (2017). Structural and hydrodynamic properties of an intrinsically disordered region of a germ cell-specific protein on phase separation. Proc. Natl. Acad. Sci. USA *114*, E8194–E8203.

183. Schuster, B.S., Dignon, G.L., Tang, W.S., Kelley, F.M., Ranganath, A.K., Jahnke, C.N., Simpkins, A.G., Regy, R.M., Hammer, D.A., Good, M.C., and Mittal, J. (2020). Identifying sequence perturbations to an intrinsically disordered protein that determine its phase-separation behavior. Proc. Natl. Acad. Sci. USA *117*, 11421–11431.

184. Boeynaems, S., Ma, X.R., Yeong, V., Ginell, G.M., Chen, J.-H., Blum, J.A., Nakayama, L., Sanyal, A., Briner, A., Van Haver, D., et al. (2023). Aberrant phase separation is a common killing strategy of positively charged peptides in biology and human disease. Preprint at bioRxiv. https://doi.org/10.1101/2023.03.09.531820.

185. Yamazaki, H., Takagi, M., Kosako, H., Hirano, T., and Yoshimura, S.H. (2022). Cell cycle-specific phase separation regulated by protein charge blockiness. Nat. Cell Biol. *24*, 625–632.

186. Mitrea, D.M., Cika, J.A., Guy, C.S., Ban, D., Banerjee, P.R., Stanley, C.B., Nourse, A., Deniz, A.A., and Kriwacki, R.W. (2016). Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA. Elife *5*, e13571. https://doi.org/10.7554/eLife.13571.

187. Ferrolino, M.C., Mitrea, D.M., Michael, J.R., and Kriwacki, R.W. (2018). Compositional adaptability in NPM1-SURF6 scaffolding networks enabled by dynamic switching of phase separation mechanisms. Nat. Commun. *9*, 5064.

188. Kim, T.H., Payliss, B.J., Nosella, M.L., Lee, I.T.W., Toyama, Y., Forman-Kay, J.D., and Kay, L.E. (2021). Interaction hot spots for phase separation revealed by NMR studies of a CAPRIN1 condensed phase. Proc. Natl. Acad. Sci. USA *118*, e2104897118.

189. Kim, T.H., Tsang, B., Vernon, R.M., Sonenberg, N., Kay, L.E., and Forman-Kay, J.D. (2019). Phospho-dependent phase separation of FMRP and CAPRIN1 recapitulates regulation of translation and deadenylation. Science *365*, 825–829.

190. Gibbs, E., Miao, Q., Ferrolino, M., Bajpai, R., Hassan, A., Phillips, A.H., Pitre, A., Kümmerle, R., Miller, S., Heller, W., et al. (2023). p14ARF forms meso-scale assemblies upon phase separation with NPM1. Res. Sq. https://doi.org/10.21203/rs.3.rs-3592059/v1.

191. Ruff, K.M., Choi, Y.H., Cox, D., Ormsby, A.R., Myung, Y., Ascher, D.B., Radford, S.E., Pappu, R.V., and Hatters, D.M. (2022). Sequence grammar underlying the unfolding and phase separation of globular proteins. Mol. Cell *82*, 3193–3208.e8. https://doi.org/10.1016/j.molcel.2022.06.024.

192. Das, S., Lin, Y.-H., Vernon, R.M., Forman-Kay, J.D., and Chan, H.S. (2020). Comparative roles of charge, π, and hydrophobic interactions

in sequence-dependent phase separation of intrinsically disordered proteins. Proc. Natl. Acad. Sci. USA *117*, 28795–28805.

193. Das, R.K., and Pappu, R.V. (2013). Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. Proc. Natl. Acad. Sci. USA *110*, 13392–13397.

194. Sawle, L., and Ghosh, K. (2015). A theoretical method to compute sequence dependent configurational properties in charged polymers and proteins. J. Chem. Phys. *143*, 085101.

195. Tesei, G., Schulze, T.K., Crehuet, R., and Lindorff-Larsen, K. (2021). Accurate model of liquid-liquid phase behavior of intrinsically disordered proteins from optimization of single-chain properties. Proc. Natl. Acad. Sci. USA *118*, e2111696118.

196. Lin, Y.-H., and Chan, H.S. (2017). Phase Separation and Single-Chain Compactness of Charged Disordered Proteins Are Strongly Correlated. Biophys. J. *112*, 2043–2046.

197. Naderi, J., Magalhaes, A.P., Kibar, G., Stik, G., Zhang, Y., Mackowiak, S.D., Wieler, H.M., Rossi, F., Buschow, R., Christou-Kent, M., et al. (2024). An activity-specificity trade-off encoded in human transcription factors. Nat. Cell Biol. *26*, 1309–1321.

198. Holehouse, A.S., Ginell, G.M., Griffith, D., and Böke, E. (2021). Clustering of Aromatic Residues in Prion-like Domains Can Tune the Formation, State, and Organization of Biomolecular Condensates. Biochemistry *60*, 3566–3581.

199. Patil, A., Strom, A.R., Paulo, J.A., Collings, C.K., Ruff, K.M., Shinn, M.K., Sankar, A., Cervantes, K.S., Wauer, T., St Laurent, J.D., et al. (2023). A disordered region controls cBAF activity via condensation and partner recruitment. Cell *186*, 4936–4955.e26.

200. Bowman, M.A., Riback, J.A., Rodriguez, A., Guo, H., Li, J., Sosnick, T.R., and Clark, P.L. (2020). Properties of protein unfolded states suggest broad selection for expanded conformational ensembles. Proc. Natl. Acad. Sci. USA *117*, 23356–23364.

201. Zheng, W., Dignon, G., Brown, M., Kim, Y.C., and Mittal, J. (2020). Hydropathy Patterning Complements Charge Patterning to Describe Conformational Preferences of Disordered Proteins. J. Phys. Chem. Lett. *11*, 3408–3415.

202. Esposito, D., and Chatterjee, D.K. (2006). Enhancement of soluble protein expression through the use of fusion tags. Curr. Opin. Biotechnol. *17*, 353–358.

203. Alberti, S., Saha, S., Woodruff, J.B., Franzmann, T.M., Wang, J., and Hyman, A.A. (2018). A User's Guide for Phase Separation Assays with Purified Proteins. J. Mol. Biol. *430*, 4806–4820. https://doi.org/10.1016/j.jmb.2018.06.038.

204. Obermeyer, A.C., Mills, C.E., Dong, X.-H., Flores, R.J., and Olsen, B.D. (2016). Complex coacervation of supercharged proteins with polyelectrolytes. Soft Matter *12*, 3570–3581.

205. Yeong, V., Werth, E.G., Brown, L.M., and Obermeyer, A.C. (2020). Formation of biomolecular condensates in bacteria by tuning protein electrostatics. ACS Cent. Sci. *6*, 2301–2310.

206. Kim, J., Qin, S., Zhou, H.-X., and Rosen, M.K. (2024). Surface charge can modulate phase separation of multidomain proteins. J. Am. Chem. Soc. *146*, 3383–3395.

207. Zhou, H.-X., and Pang, X. (2018). Electrostatic interactions in protein structure, folding, binding, and condensation. Chem. Rev. *118*, 1691–1741.

208. Frey, S., Rees, R., Schünemann, J., Ng, S.C., Fünfgeld, K., Huyton, T., and Görlich, D. (2018). Surface properties determining passage rates of proteins through nuclear pores. Cell *174*, 202–217.e9.

209. Chen, R., Glauninger, H., Kahan, D.N., Shangguan, J., Sachleben, J.R., Riback, J.A., Drummond, D.A., and Sosnick, T.R. (2024). HDX-MS finds that partial unfolding with sequential domain activation controls condensation of a cellular stress marker. Proc. Natl. Acad. Sci. USA *121*, e2321606121.

210. Iserman, C., Altamirano, C.D., Jegers, C., Friedrich, U., Zarin, T., Fritsch, A.W., Mittasch, M., Domingues, A., Hersemann, L., Jahnel, M., et al. (2020). Condensation of Ded1p Promotes a Translational Switch from Housekeeping to Stress Protein Production. Cell *181*, 818–831.e19. https://doi.org/10.1016/j.cell.2020.04.009.

211. Jegers, C., Franzmann, T.M., Hübner, J., Schneider, J., Landerer, C., Wittmann, S., Toth-Petroczy, A., Sprangers, R., Hyman, A.A., and Alberti, S. (2022). A conserved and tunable mechanism for the temperature-controlled condensation of the translation factor Ded1p. Preprint at bioRxiv. https://doi.org/10.1101/2022.10.11.511767.

212. Fatti, E., Khawaja, S., and Weis, K. (2024). The dark side of fluorescent protein tagging: the impact of protein tags on biomolecular condensation. Preprint at bioRxiv. https://doi.org/10.1101/2024.11.23.624970.

213. D/"orner, K., Gut, M., Overwijn, D., Cao, F., Siketanc, M., Heinrich, S., Beuret, N., Sharpe, T., Lindorff-Larsen, K., and Hondele, M. (2024). Tag with Caution - How protein tagging influences the formation of condensates. Preprint at bioRxiv. https://doi.org/10.1101/2024.10.04.616694.

214. Ausserwöger, H., Krainer, G., Welsh, T.J., Thorsteinson, N., de Csilléry, E., Sneideris, T., Schneider, M.M., Egebjerg, T., Invernizzi, G., Herling, T.W., et al. (2023). Surface patches induce nonspecific binding and phase separation of antibodies. Proc. Natl. Acad. Sci. USA *120*, e2210332120.

215. Nott, T.J., Craggs, T.D., and Baldwin, A.J. (2016). Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. Nat. Chem. *8*, 569–575.

216. Poudyal, R.R., Sieg, J.P., Portz, B., Keating, C.D., and Bevilacqua, P.C. (2021). RNA sequence and structure control assembly and function of RNA condensates. RNA *27*, 1589–1601.

217. Klein, I.A., Boija, A., Afeyan, L.K., Hawken, S.W., Fan, M., Dall'Agnese, A., Oksuz, O., Henninger, J.E., Shrinivas, K., Sabari, B.R., et al. (2020). Partitioning of cancer therapeutics in nuclear condensates. Science *368*, 1386–1392.

218. Kilgore, H., Chinn, I., Mikhael, P., Mitnikov, I., Van Dongen, C., Zylberberg, G., Afeyan, L., Banani, S., Wilson-Hawken, S., Lee, T., et al. (2024). Chemical codes promote selective compartmentalization of proteins. Preprint at bioRxiv. https://doi.org/10.1101/2024.04.15.589616.

219. Schmidt, H.B., Jaafar, Z.A., Wulff, B.E., Rodencal, J.J., Hong, K., Aziz-Zanjani, M.O., Jackson, P.K., Leonetti, M.D., Dixon, S.J., Rohatgi, R., and Brandman, O. (2022). Oxaliplatin disrupts nucleolar function through biophysical disintegration. Cell Rep. *41*, 111629.

220. Ambadi Thody, S., Clements, H.D., Baniasadi, H., Lyon, A.S., Sigman, M.S., and Rosen, M.K. (2024). Small-molecule properties define partitioning into biomolecular condensates. Nat. Chem. *16*, 1794–1802.

221. Dai, Y., Wang, Z.-G., and Zare, R.N. (2024). Unlocking the electrochemical functions of biomolecular condensates. Nat. Chem. Biol. *20*, 1420–1433. https://doi.org/10.1038/s41589-024-01717-y.

222. Schmit, J.D., Feric, M., and Dundr, M. (2021). How Hierarchical Interactions Make Membraneless Organelles Tick Like Clockwork. Trends Biochem. Sci. *46*, 525–534.

223. Dignon, G.L., Best, R.B., and Mittal, J. (2020). Biomolecular Phase Separation: From Molecular Driving Forces to Macroscopic Properties. Annu. Rev. Phys. Chem. *71*, 53–75.

224. Currie, S.L., Xing, W., Muhlrad, D., Decker, C.J., Parker, R., and Rosen, M.K. (2023). Quantitative reconstitution of yeast RNA processing bodies. Proc. Natl. Acad. Sci. USA *120*, e2214064120.

225. Xing, W., Muhlrad, D., Parker, R., and Rosen, M.K. (2020). A quantitative inventory of yeast P body proteins reveals principles of composition and specificity. Elife *9*, e56525. https://doi.org/10.7554/eLife.56525.

226. Case, L.B., De Pasquale, M., Henry, L., and Rosen, M.K. (2022). Synergistic phase separation of two pathways promotes integrin clustering and nascent adhesion formation. Elife *11*, e72588.

227. Protter, D.S.W., Rao, B.S., Van Treeck, B., Lin, Y., Mizoue, L., Rosen, M.K., and Parker, R. (2018). Intrinsically Disordered Regions Can

Contribute Promiscuous Interactions to RNP Granule Assembly. Cell Rep. *22*, 1401–1412.

228. Mohanty, P., Shenoy, J., Rizuan, A., Mercado-Ortiz, J.F., Fawzi, N.L., and Mittal, J. (2023). A synergy between site-specific and transient interactions drives the phase separation of a disordered, low-complexity domain. Proc. Natl. Acad. Sci. USA *120*, e2305625120.

229. Beutel, O., Maraspini, R., Pombo-García, K., Martin-Lemaitre, C., and Honigmann, A. (2019). Phase separation of zonula occludens proteins drives formation of tight junctions. Cell *179*, 923–936.e11.

230. Wang, A., Conicella, A.E., Schmidt, H.B., Martin, E.W., Rhoads, S.N., Reeb, A.N., Nourse, A., Ramirez Montero, D., Ryan, V.H., Rohatgi, R., et al. (2018). A single N-terminal phosphomimic disrupts TDP-43 polymerization, phase separation, and RNA splicing. EMBO J. *37*, e97452.

231. Franzmann, T.M., Jahnel, M., Pozniakovsky, A., Mahamid, J., Holehouse, A.S., Nüske, E., Richter, D., Baumeister, W., Grill, S.W., Pappu, R.V., et al. (2018). Phase separation of a yeast prion protein promotes cellular fitness. Science *359*, eaao5654. https://doi.org/10.1126/science.aao5654.

232. Franzmann, T.M., and Alberti, S. (2019). Prion-like low-complexity sequences: Key regulators of protein solubility and phase behavior. J. Biol. Chem. *294*, 7128–7136.

233. Franzmann, T.M., and Alberti, S. (2019). Protein Phase Separation as a Stress Survival Strategy. Cold Spring Harb. Perspect. Biol. *11*, a034058. https://doi.org/10.1101/cshperspect.a034058.

234. Alberti, S., and Hyman, A.A. (2021). Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat. Rev. Mol. Cell Biol. *22*, 196–213.

235. Romero-Perez, P.S., Dorone, Y., Flores, E., Sukenik, S., and Boeynaems, S. (2023). When Phased without Water: Biophysics of Cellular Desiccation, from Biomolecules to Condensates. Chem. Rev. *123*, 9010–9035. https://doi.org/10.1021/acs.chemrev.2c00659.

236. Watanabe, K., Morishita, K., Zhou, X., Shiizaki, S., Uchiyama, Y., Koike, M., Naguro, I., and Ichijo, H. (2021). Cells recognize osmotic stress through liquid–liquid phase separation lubricated with poly (ADP-ribose). Nat. Commun. *12*, 1353.

237. Jalihal, A.P., Pitchiaya, S., Xiao, L., Bawa, P., Jiang, X., Bedi, K., Parolia, A., Cieslik, M., Ljungman, M., Chinnaiyan, A.M., and Walter, N.G. (2020). Multivalent Proteins Rapidly and Reversibly Phase-Separate upon Osmotic Cell Volume Change. Mol. Cell *79*, 978–990.e5.

238. Villegas, J.A., Heidenreich, M., and Levy, E.D. (2022). Molecular and environmental determinants of biomolecular condensate formation. Nat. Chem. Biol. *18*, 1319–1329.

239. Desroches Altamirano, C., Kang, M.-K., Jordan, M.A., Borianne, T., Dilmen, I., Gnädig, M., von Appen, A., Honigmann, A., Franzmann, T.M., and Alberti, S. (2024). eIF4F is a thermo-sensing regulatory node in the translational heat shock response. Mol. Cell *84*, 1727–1741.e12. https://doi.org/10.1016/j.molcel.2024.02.038.

240. Desroches Altamirano, C., and Alberti, S. (2024). Surviving the heat: the role of macromolecular assemblies in promoting cellular shutdown. Trends Biochem. Sci. https://doi.org/10.1016/j.tibs.2024.09.008.

241. Hedtfeld, M., Dammers, A., Koerner, C., and Musacchio, A. (2024). A validation strategy to assess the role of phase separation as a determinant of macromolecular localization. Mol. Cell *84*, 1783–1801.e7.

242. Chew, P.Y., and Reinhardt, A. (2023). Phase diagrams—Why they matter and how to predict them. J. Chem. Phys. *158*, 030902.

243. Ruff, K.M., Dar, F., and Pappu, R.V. (2021). Polyphasic linkage and the impact of ligand binding on the regulation of biomolecular condensates. Biophys. Rev. *2*, 021302.

244. Pancotti, C., Benevenuta, S., Birolo, G., Alberini, V., Repetto, V., Sanavia, T., Capriotti, E., and Fariselli, P. (2022). Predicting protein stability changes upon single-point mutation: a thorough comparison of the available tools on a new dataset. Brief. Bioinform. *23*, bbab555.

245. Khakzad, H., Igashov, I., Schneuing, A., Goverde, C., Bronstein, M., and Correia, B. (2023). A new age in protein design empowered by deep learning. Cell Syst. *14*, 925–939.

246. Emenecker, R.J., Guadalupe, K., Shamoon, N.M., Sukenik, S., and Holehouse, A.S. (2023). Sequence-ensemble-function relationships for disordered proteins in live cells. Preprint at bioRxiv. https://doi.org/10.1101/2023.10.29.564547.

247. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature *596*, 583–589.

248. Pak, M.A., Markhieva, K.A., Novikova, M.S., Petrov, D.S., Vorobyev, I.S., Maksimova, E.S., Kondrashov, F.A., and Ivankov, D.N. (2023). Using AlphaFold to predict the impact of single mutations on protein stability and function. PLoS One *18*, e0282689.

249. Nosella, M.L., Kim, T.H., Huang, S.K., Harkness, R.W., Goncalves, M., Pan, A., Tereshchenko, M., Vahidi, S., Rubinstein, J.L., Lee, H.O., et al. (2024). Poly(ADP-ribosyl)ation enhances nucleosome dynamics and organizes DNA damage repair components within biomolecular condensates. Mol. Cell *84*, 429–446.e17.

250. Yang, W., Robichaux, W.G., 3rd, Mei, F.C., Lin, W., Li, L., Pan, S., White, M.A., Chen, Y., and Cheng, X. (2022). Epac1 activation by cAMP regulates cellular SUMOylation and promotes the formation of biomolecular condensates. Sci. Adv. *8*, eabm2960.

251. Dao, T.P., Martyniak, B., Canning, A.J., Lei, Y., Colicino, E.G., Cosgrove, M.S., Hehnly, H., and Castañeda, C.A. (2019). ALS-Linked Mutations Affect UBQLN2 Oligomerization and Phase Separation in a Position- and Amino Acid-Dependent Manner. Structure *27*, 937–951.e5.

252. Case, L.B., Ditlev, J.A., and Rosen, M.K. (2019). Regulation of transmembrane signaling by phase separation. Annu. Rev. Biophys. *48*, 465–494.

253. Dai, Y., Chamberlayne, C.F., Messina, M.S., Chang, C.J., Zare, R.N., You, L., and Chilkoti, A. (2023). Interface of biomolecular condensates modulates redox reactions. Chem *9*, 1594–1609.

254. Gil-Garcia, M., Benítez-Mateos, A.I., Papp, M., Stoffel, F., Morelli, C., Normak, K., Makasewicz, K., Faltova, L., Paradisi, F., and Arosio, P. (2024). Local environment in biomolecular condensates modulates enzymatic activity across length scales. Nat. Commun. *15*, 3322.

255. Tibble, R.W., Depaix, A., Kowalska, J., Jemielity, J., and Gross, J.D. (2021). Biomolecular condensates amplify mRNA decapping by biasing enzyme conformation. Nat. Chem. Biol. *17*, 615–623.

256. Sang, D., Shu, T., Pantoja, C.F., Ibáñez de Opakua, A., Zweckstetter, M., and Holt, L.J. (2022). Condensed-phase signaling can expand kinase specificity and respond to macromolecular crowding. Mol. Cell *82*, 3693–3711.e10.

257. Smokers, I., Visser, B., Lipiński, W., Nakashima, K., and Spruijt, E. (2024). Phase-separated droplets can direct the kinetics of chemical reactions including polymerization, self-replication and oscillating networks. Preprint at ChemRxiv. https://doi.org/10.26434/chemrxiv-2024-xzl0t.

258. Peeples, W., and Rosen, M.K. (2021). Mechanistic dissection of increased enzymatic rate in a phase-separated compartment. Nat. Chem. Biol. *17*, 693–702.

259. Chen, X., Seyboldt, R., Sommer, J.-U., Jülicher, F., and Harmon, T. (2024). Droplet differentiation by a chemical switch. Phys. Rev. Lett. *133*, 028402.

260. Dai, Y., Farag, M., Lee, D., Zeng, X., Kim, K., Son, H.-I., Guo, X., Su, J., Peterson, N., Mohammed, J., et al. (2023). Programmable synthetic biomolecular condensates for cellular control. Nat. Chem. Biol. *19*, 518–528.