

Roadmap for Condensates in Cell Biology

Dilimulati Aierken ^{1,2} Sebastian Aland ^{3,4} Stefano Bo ⁵ Steven Boeynaems ^{6,7,8,9,10} Danfeng Cai ^{11,12,13} Serena Carra ¹⁴ Lindsay B. Case ¹⁵ Hue Sun Chan ¹⁶ Jorge R. Espinosa ^{17,18,19} Trevor K. GrandPre ^{20,21} Alexander Y. Grosberg ²² Ivar S. Haugerud ²³ William M. Jacobs ²⁴ Jerelle A. Joseph ^{1,2,*} Frank Jülicher ^{25,†} Kurt Kremer ²⁶ Guido Kusters ²⁷ Liedewij Laan ²⁸ Keren Lasker ²⁹ Kathrin S. Laxhuber ²⁵ Hyun O. Lee ^{16,‡} Kathy F. Liu ^{30,31,32,33,34} Dimple Notani ³⁵ Yicheng Qiang ²⁷ Paul Robustelli ³⁶ Leonor Saiz ³⁷ Omar A. Saleh ³⁸ Helmut Schiessel ³⁹ Jeremy D. Schmit ⁴⁰ Meng Shen ⁴¹ Krishna Shrinivas ^{42,43} Antonia Statt ⁴⁴ Andres R. Tejedor ^{17,18,19} Tatjana Trcek ⁴⁵ Christoph A. Weber ²³ Stephanie C. Weber ^{46,47,§} Ned S. Wingreen ^{48,49,||} Huaiying Zhang ⁵⁰ Yaojun Zhang ^{51,52} Huan-Xiang Zhou ⁵³ and David Zwicker ^{27,¶}

¹Department of Chemical and Biological Engineering, *Princeton University*, New Jersey 08544, USA

²Omnenn-Darling Bioengineering Institute, *Princeton University*, New Jersey 08544, USA

³Institute of Numerical Mathematics and Optimization, *TU Bergakademie Freiberg*, 09599 Freiberg, Germany

⁴Faculty of Informatics/Mathematics, *HTW Dresden*, 01069 Dresden, Germany

⁵Department of Physics, *King's College London*, London WC2R 2LS, United Kingdom

⁶Department of Molecular and Human Genetics, *Baylor College of Medicine*, Houston, Texas 77030, USA

⁷Jan and Dan Duncan Neurological Research Institute, *Texas Children's Hospital*, Houston, Texas 77030, USA

⁸Therapeutic Innovation Center (THINC), *Baylor College of Medicine*, Houston, Texas 77030, USA

⁹Center for Alzheimer's and Neurodegenerative Diseases (CAND), *Baylor College of Medicine*, Houston, Texas 77030, USA

¹⁰Dan L Duncan Comprehensive Cancer Center (DLCCCC), *Baylor College of Medicine*, Houston, Texas 77030, USA

¹¹Department of Biochemistry and Molecular Biology, *Johns Hopkins Bloomberg School of Public Health*, Baltimore, Maryland 21205, USA

¹²Department of Biophysics and Biophysical Chemistry, *Johns Hopkins School of Medicine*, Baltimore, Maryland 21205, USA

¹³Department of Oncology, *Johns Hopkins School of Medicine*, Baltimore, Maryland 21205, USA

¹⁴Department of Biomedical, Metabolic and Neural Sciences, *University of Modena and Reggio Emilia*, Modena 41125, Italy

¹⁵Department of Biology, *Massachusetts Institute of Technology*, Cambridge, Massachusetts 02139, USA

¹⁶Department of Biochemistry, *University of Toronto*, Toronto, Ontario M5S 1A8, Canada

¹⁷Department of Physical Chemistry, *University Complutense of Madrid*, Madrid 28040, Spain

¹⁸Instituto Pluridisciplinar, *Universidad Complutense de Madrid*, P.º de Juan XXIII, 1, Moncloa - Aravaca, 28040 Madrid, Spain

¹⁹Yusuf Hamied Department of Chemistry, *University of Cambridge*, Lensfield Road, Cambridge CB2 1EW, United Kingdom

²⁰Department of Physics and Center for Biomolecular Condensates, *Washington University in St. Louis*, St. Louis, Missouri 63130, USA

²¹National Institute for Theory and Mathematics in Biology,

Northwestern University and *The University of Chicago*, Chicago, Illinois 60611, USA

²²Department of Physics and Center for Soft Matter Research, *New York University*, 726 Broadway, New York, New York 10003, USA

²³Faculty of Mathematics, Natural Sciences, and Engineering, *Institute of Physics*,

University of Augsburg, Universitätsstrasse 1, 86159 Augsburg, Germany

²⁴Department of Chemistry, *Princeton University*, Princeton, New Jersey 08544, USA

²⁵*Max Planck Institute for the Physics of Complex Systems*, Nöthnitzer Str. 38, 01187 Dresden, Germany

²⁶*Max Planck Institute for Polymer Research*, Ackermannweg 10, 55128 Mainz, Germany

²⁷*Max Planck Institute for Dynamics and Self-Organization*, Am Faßberg 17, 37077 Göttingen, Germany

²⁸Department of Bionanoscience, Kavli Institute of Nanoscience Delft, *Delft University of Technology*, Delft, The Netherlands

²⁹Department of Integrative Structural and Computational Biology, *The Scripps Research Institute*, La Jolla, California 92037, USA

³⁰Department of Biochemistry and Biophysics, *Perelman School of Medicine*, *University of Pennsylvania*, Philadelphia, Pennsylvania 19104, USA

* Contact author: jerellejoseph@princeton.edu

† Contact author: julicher@pks.mpg.de

‡ Contact author: hyunokate.lee@utoronto.ca

§ Contact author: steph.weber@mcgill.ca

|| Contact author: wingreen@princeton.edu

¶ Contact author: david.zwicker@ds.mpg.de

- ³¹Graduate Group in Biochemistry and Molecular Biophysics, Perelman School of Medicine, [University of Pennsylvania](#), Philadelphia, Pennsylvania 19104, USA
- ³²Abramson Family Cancer Research Institute, Perelman School of Medicine, [University of Pennsylvania](#), Philadelphia, Pennsylvania 19104, USA
- ³³Penn Institute for RNA Innovation, [University of Pennsylvania](#), Philadelphia, Pennsylvania 19104, USA
- ³⁴Penn Center for Genome Integrity, [University of Pennsylvania](#), Philadelphia, Pennsylvania 19104, USA
- ³⁵[National Centre for Biological Sciences-TIFR](#), GKVK Campus, Bellary Road, Bangalore 560065, India
- ³⁶Department of Chemistry, [Dartmouth College](#), Hanover, New Hampshire 03755, USA
- ³⁷Department of Biomedical Engineering, [University of California, Davis](#), California 95616, USA
- ³⁸Materials and Physics Departments, [University of California, Santa Barbara](#), California 93110, USA
- ³⁹Cluster of Excellence Physics of Life, [TU Dresden](#), 01062 Dresden, Germany
- ⁴⁰Department of Physics, [Kansas State University](#), Manhattan, Kansas 66506, USA
- ⁴¹Department of Physics, [California State University, Fullerton](#), California 92831, USA
- ⁴²Department of Chemical and Biological Engineering, [Northwestern University](#), Evanston, Illinois 60208, USA
- ⁴³Center for Synthetic Biology, [Northwestern University](#), Evanston, Illinois 60208, USA
- ⁴⁴Department of Materials Science and Engineering, [University of Illinois Urbana-Champaign](#), Urbana, Illinois 61801, USA
- ⁴⁵Department of Biology, [Johns Hopkins University](#), Baltimore, Maryland 21218, USA
- ⁴⁶Department of Biology, [McGill University](#), Montreal, H3A 1B1, Canada
- ⁴⁷Department of Physics, [McGill University](#), Montreal H3A 2T8, Canada
- ⁴⁸Department of Molecular Biology, [Princeton University](#), New Jersey 08544, USA
- ⁴⁹Lewis-Sigler Institute for Integrative Genomics, [Princeton University](#), New Jersey 08544, USA
- ⁵⁰Department of Biological Sciences, [Carnegie Mellon University](#), Pittsburgh, Pennsylvania, 15213, USA
- ⁵¹Department of Physics & Astronomy, [Johns Hopkins University](#), Baltimore, Maryland 21218, USA
- ⁵²Department of Biophysics, [Johns Hopkins University](#), Baltimore, Maryland 21218, USA
- ⁵³Department of Chemistry and Department of Physics, [University of Illinois Chicago](#), Chicago, Illinois 60607, USA



(Received 8 January 2026; accepted 10 July 2026; published 20 August 2026)

Biomolecular condensates govern essential cellular processes yet elude description by traditional equilibrium models. This roadmap, distilled from structured discussions at a workshop and reflecting the consensus of its participants, clarifies key concepts for researchers, funding bodies, and journals. After unifying terminology that often separates disciplines, we outline the core physics of condensate formation, review their biological roles, and identify outstanding challenges in nonequilibrium theory, multiscale simulation, and quantitative in-cell measurements. We close with a forward-looking outlook to guide coordinated efforts toward predictive, experimentally anchored understanding and control of biomolecular condensates.

DOI: [10.1103/3b2k-ftwx](https://doi.org/10.1103/3b2k-ftwx)

I. INTRODUCTION

Biomolecular condensates have emerged as a key organizing principle in cell biology, transforming our understanding of cellular architecture (Fig. 1). Recent studies have highlighted that condensates are involved in a wide range of essential processes, including gene regulation, signal transduction, metabolism, macromolecular processing, signaling, and stress response. Beyond fundamental biology, condensates are linked to human diseases such as neurodegeneration and cancer, as well as to climate-impacting processes such as carbon fixation, underscoring their widespread significance. To fully understand how cells operate, we need to understand condensates, which, in turn, will enable us to target or engineer them for therapeutic and environmental benefit. The concepts of phase separation and condensation offer a powerful framework for understanding these non-membrane-bound organelles, providing insight into how they organize biomolecules in space and time. Understanding biological condensates requires integrating concepts from biology, physics, and chemistry, and collaboration across disciplines is essential to advance the field.

The concept of condensation has deep roots in physics, dating back over a century (Fig. 2). Initially developed to explain phase separation in thermodynamic equilibrium, these concepts have been continuously refined to describe more complex situations in soft-matter physics. Similar principles were invoked in biology long before any molecular details were understood: Early observations of cellular space hinted at numerous liquidlike self-assemblies without membranes. Advances in molecular biology and imaging have since revealed the complex composition and dynamics of these structures, bridging phenomenology with underlying mechanisms in increasing detail (Fig. 2). Today, the combination of soft-matter physics and cell biology forms a truly interdisciplinary field that offers an opportunity for discovery for all. There are a growing number of success stories in this regard; examples include nucleoli that generate protein-synthesizing ribosomes, germ granules that specify developing germ cells, the asymmetric organization of cell-division components in bacteria, and recombination nodules (Box 1).

This interdisciplinarity offers both opportunities for discovery and challenges. Scientists from biology, chemistry, physics, and mathematics often approach condensates with

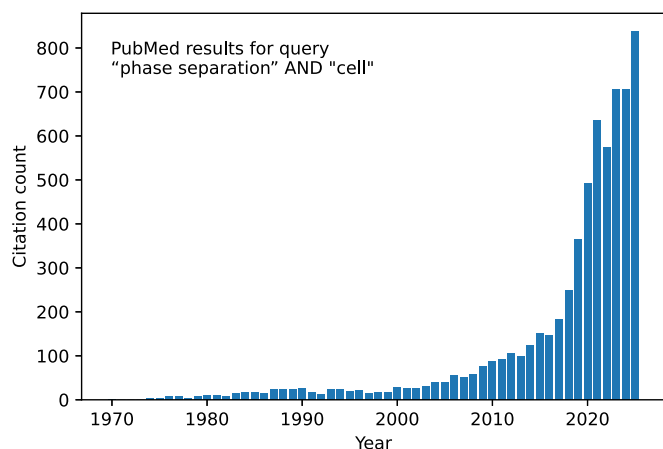


FIG. 1. The strong rise in the number of publications in recent years (obtained from searching PubMed for “phase separation” and “cell”) indicates a growing interest in using the concept of phase separation in cell biology.

different languages and conceptual frameworks, making communication and efficient progress challenging. To address this, researchers convened at the Kavli Institute for Theoretical Physics (KITP) for seven weeks in the summer of 2025. Many of the [scientific talks are available online](#). In addition to these seminars, every Friday, structured 2-hour discussion sessions brought all participants together to share perspectives and address key questions. Ideas were summarized live on the blackboard (Fig. 3) and recorded in detailed notes, which form the foundation of this article.

This roadmap aims to clarify concepts for scientists, funding agencies, and journals working in the area of biomolecular condensate research, reflecting the consensus view of the participants of the KITP workshop. We begin by addressing terminology, which often creates barriers between disciplines (Sec. II). Next we examine the physics behind biological condensates (Sec. III), summarize their biological implications (Sec. IV), and conclude with key challenges (Sec. V) and a future outlook (Sec. VI).

II. WHAT ARE CONDENSATES?

The definition of the term biomolecular condensate (herein called condensate) is controversial [15]. Some might consider

all cellular structures to be condensates since all structures comprise condensed matter, i.e., are made from materials ranging from liquids to solids. Others might claim that nothing is a condensate since they define condensate as emerging through a phase transition, which would require infinitely large systems to unambiguously identify them using tools from statistical mechanics. Neither of these viewpoints is productive for describing and understanding cell biology. In addition, researchers have used a wealth of terms, like aggregates, blobs, bodies, clusters, foci, granules, hubs, macromolecular assemblies, microdomains, nodules, puncta, ribosome exclusion zones, speckles, spots, etc., to describe what might be summarized as biomolecular condensates. Such variety of terminology with subtly distinct meanings hinders coherent communication and suggests that clarifying the concept of condensates might be helpful.

What properties do condensates have in common? Although opinions differ, researchers tend to agree that condensates are compartments with a different composition from their surroundings. The surroundings are separated by a well-defined interface that is not established by a lipid membrane; condensates are non-membrane-bound compartments that can exclude membrane-bound organelles such as the nucleus or mitochondria (sometimes they associate with one another). Researchers also tend to agree that condensates have dynamic, nonstoichiometric composition, so individual complexes, like the ribosome, do not count (clusters of complexes may). Moreover, condensates are typically described as having (at least in part) an amorphous internal structure, in which the relative positions of molecules are not guaranteed, to exclude ordered structures such as amyloid-like aggregates and microtubules. Finally, many people stress that condensates exhibit emergent properties, i.e., that the properties of individual molecules differ from those of the collective. However, it is unclear at what size (or molecule count) such properties emerge (e.g., water exhibits liquid properties for as few as a dozen molecules [16]). Consequently, there is no consensus on the size restrictions of condensates at this time.

There are also more controversial properties that are often discussed in the context of condensates: Some papers equate condensates with liquid-liquid phase separation (LLPS), but it is often neither clear whether the involved objects have liquid-like properties (and solidlike properties have been observed [17]), nor whether they actually form by phase separation.

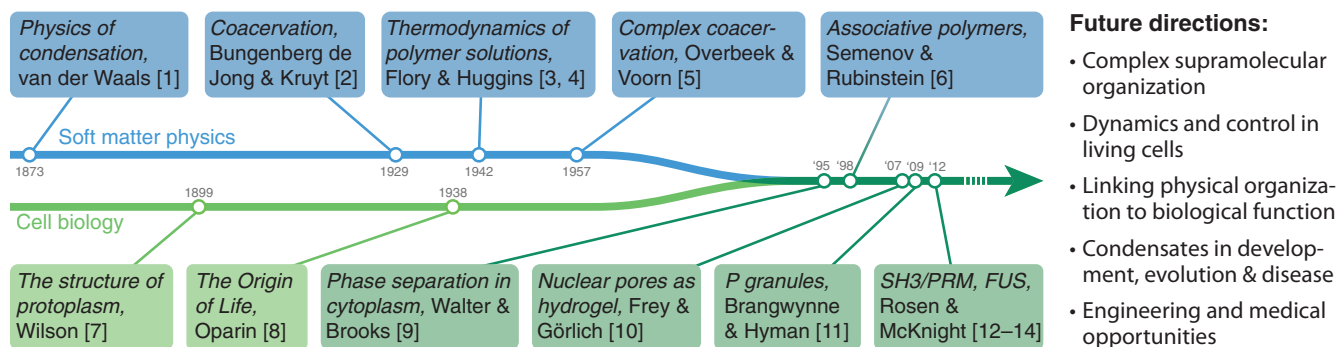


FIG. 2. Historical development of condensates in cell biology [1–14]. The timeline highlights selected landmark papers that influenced biomolecular condensates research substantially. Future directions are discussed in detail in Sec. VI.

BOX 1: Examples of success stories

While many biomolecular condensates have been described [20], the biological consequences of their formation remain unclear in many cases. Here we highlight a few examples for which the concept of condensation has been especially useful for explaining biological phenomena.

- (i) Among the first condensates described as such are *P granules*, which contribute to germ cell specification in the nematode *Caenorhabditis elegans*. *P granules* are initially distributed throughout the one-cell embryo but are ultimately inherited by the posterior daughter cell at division. This asymmetric segregation, first described in 1982 [21], could not be explained by cytoplasmic flows or localized degradation. Instead, Brangwynne *et al.* [11] proposed an alternative mechanism: Before cell division, *P granules* in the anterior half of the embryo shrink and dissolve, while those in the posterior half condense and grow. This novel description explained not only the asymmetric localization of *P granules* in the embryo but also their fusion, dripping, and wetting behavior in the adult gonad [11].
- (ii) The bacterium *Caulobacter crescentus* divides asymmetrically to produce two daughter cells with distinct developmental fates [22,23]. This fate decision is controlled, in part, by a gradient of the active, phosphorylated form of the master transcription factor CtrA [24–26]. How a soluble, freely diffusing regulator is converted into a spatially patterned activity gradient that reliably specifies daughter-cell fate had remained unclear. Lasker *et al.* [27] proposed that this gradient is reinforced by selective sequestration of CtrA, together with proteins in its phosphosignalling cascade within the *polar organizing protein Z (PopZ) condensate*. The physical state of PopZ is itself functionally important: A designed mutation series that either fluidizes or hardens the condensate shows that normal growth is supported only within a narrow range of material properties. Combining an overfluidizing mutant with an oversolidifying mutant restores both wildtype-like material properties and cell growth, demonstrating a direct relationship between condensate properties and organismal fitness [28].
- (iii) In sexually reproducing organisms, genetic diversity is generated through DNA exchange between homologous chromosomes during meiosis [29]. Numerous double-strand breaks (DSBs) are formed along each chromosome, but only a few of these sites become crossovers. Indeed, putative crossover sites “interfere” with one another such that mature crossovers are distantly spaced. First observed in *Drosophila* in 1913 [30], the mechanism underlying crossover interference remained a mystery for more than a century. Recent studies in the plant *Arabidopsis thaliana* [31] and the animal *C. elegans* [32] now suggest that the number and positioning of crossovers are determined by coarsening of *recombination nodules*. Recombination nodules are condensates that form at DSBs. Nodule components, such as the RING finger protein HEI10/ZHP-3, diffuse laterally along the synaptonemal complex that holds homologous chromosomes together, redistributing from many small nodules to a few large nodules, which then promote crossover formation. Since nearby recombination nodules compete for material, the remaining large nodules tend to be distantly spaced.

In addition to these examples, the concept of condensation has significantly advanced our understanding of certain cofactors partitioning at the same sites for transcription [33], organization of heterochromatin [34,35], spatial separation of different steps of ribosome biogenesis [36], and organization of DNA repair [37–40]. Interestingly, although modulation of chemical reaction rates is one of the most commonly speculated function of condensates, these examples instead involve the precise localization of macromolecules on larger length scales [41]. Indeed, biological consequences can occur on multiple length and timescales (i.e., molecular to evolutionary) and investigating condensates at the systems level may be necessary to identify emergent consequences.

Condensation has also brought new insights to our understanding of human disease. One promising example is amyotrophic lateral sclerosis (ALS), a degenerative motor neuron disease. ALS is associated with dozens of different genes [42], which has made it difficult to identify the underlying pathological mechanism(s). However, major breakthroughs since a decade ago pointed to aberrant condensation as a key process in pathogenesis. First, the low complexity domains of a family of ALS-associated RNA-binding proteins (e.g., FUS, TDP-43, and hnRNP1A) drive their phase separation, as well as fibrillization [43,44]. Condensate environment (including surrounding dilute phase and condensate surface) appear to facilitate their fibrillization, which is accelerated by disease-associated mutations on these proteins or stresses. Second, arginine-containing dipeptide repeats, derived from hexanucleotide repeat expansion in *C9orf72*, disrupt the dynamics and function of endogenous condensates, including stress granules, nucleoli, and the nuclear pore complex [45]. These basic discoveries have inspired ongoing development of therapeutics that target aberrant TDP-43 aggregation, such as condensate-modifying drugs [46], protein disaggregases [47], and short RNA chaperones [48].

Many condensate-associated processes highlighted throughout this article are linked to disease processes or aging. For example, condensation has been implicated in the role of BRD4-NUT or EWS-FLI1 in cancer [49,50] and SARS-CoV-2 N-protein replication compartments [51]. Thus, although the role of condensates in disease mechanism is still being evaluated, these examples highlight the potential for targeting condensates for therapeutic benefit.

In any case, the term phase separation denotes the process, whereas the resulting state is better described as phase coexistence. However, phase coexistence is unambiguously defined only in thermodynamically large equilibrium systems, so its validity in small, living cells out of equilibrium is unclear. It is also sometimes said that condensates are transient structures, but long-lived examples, like the Balbiani body in germ cells, also exist [18]. Finally, condensates are often associated with biological function, but condensates can and do form without a clear biological function [19]. Either case, proving biological function requires manipulation that is challenging to achieve in cells in most cases: disrupting condensation but not the normal function or interaction of the molecules involved. We argue that it is still useful to discuss concepts and properties of condensates even when their biological function is yet unclear, for example, to better describe and learn about the structures observed in cells.

These controversies demonstrate that unambiguously defining biomolecular condensates via their properties is virtually impossible and limiting; properties are often difficult to measure or the check-list definitions are not reflective of the wide variety of condensates that exist and thus are highly contested. After all, biological molecules, complexes, and structures exist along a continuum of sizes, shapes, material properties, and dynamics. Likewise, biomolecular condensates span the full range of these parameters. Any sharp boundaries used to define a term are arbitrary and thus semantic. To make headway, we here suggest that one could instead think of condensation as a general concept and ask whether using it to understand a particular biological structure is useful: Can the concept of condensation explain observations (quantitatively)? Can it make testable predictions? In other words, as the initial qualitative question (What are condensates?) easily leads to semantic discussions, we suggest reframing this instead to answer quantitative questions (e.g., How well does condensation explain a phenomenon?), which acknowledges the complex and continuously changing nature of biology.

In following sections, we describe general principles that may be helpful to consider what condensation can achieve. We argue that it will be most productive to use these ideas to understand the process or structure of interest. Indeed, the concept of phase coexistence has been useful to gain new insight into biological phenomena and disease processes (Box 1). Some of the examples remained mysterious for decades, and shifting the perspective to phase coexistence allowed describing them intuitively. In cases where such a description is successful, we might as well call the observed structure a condensate. In contrast, some structures currently called condensates might turn out to be better described by other concepts, in which case we suggest not calling them condensates anymore. Either way, phase coexistence is a generic property of complex mixtures, so this process likely governs some cellular structures. In other words, “condensation exists, so cells need to deal with it” (Alexander “Shura” Grosberg).

III. PHYSICS OF CONDENSATES

Recasting the question from “What is a condensate?” to the quantitative “Can the principles of condensation provide

new tools to understand cellular phenomena?” focuses attention on control parameters and measurable responses that we can use to control, probe, or predict behavior of the structure or process of interest. Practically, we ask which cellular “knobs” control condensation and how they shape the resulting responses. Treating biomolecular condensation as a continuous input-output mapping (from control parameters to responses) enables physically grounded, interpretable models that connect scattered observations and yield testable predictions (Fig. 4). In what follows, we first outline the control parameters that govern condensation in cells and then describe the condensate responses (i.e., measurable properties) that emerge.

A. Control parameters (“knobs”)

We identified five broad classes of physical control parameters or knobs that affect condensates and could be used to influence their behavior.

(1) *Intermolecular affinities.* These are encoded by the sequences of the biomolecules (proteins, RNA, DNA, etc.) that are found within condensates. For a given set of conditions, associative interactions among biomolecules can drive condensation or their partitioning in condensates by providing favorable binding free energy that compensates for the entropic losses associated with demixing. Chemical modifications (the addition of covalent tags) can reshape this interaction landscape; for example, post-translational modifications can drastically change the phase behavior of proteins and their ability to interact with RNA [52]. The sequence of molecules also shapes their structure, which in turn affects intermolecular affinities. For example, interactions among intrinsically disordered protein sequences, which lack stable tertiary structure and can engage in multivalent, promiscuous associations, facilitate condensation [53]. However, specific interactions are equally important. Repeating RNA motifs and folded protein domains (e.g., RNA-recognition motifs) can form high-affinity contacts that promote protein-RNA condensates [54]; in some assemblies, structured-domain multimerization is the primary driver (e.g., SUMO-SIM). Thus, it is crucial to consider the full spectrum of interaction types—from low-affinity, multivalent contacts to high-specificity binding—that give rise to condensation in cells.

(2) *Molecular concentration.* Another important control parameter of condensation is molecular concentration. This encompasses the cellular concentrations of macromolecules, as well as small molecules and ions. Together, these components determine the effective solvent quality (e.g., pH, ionic strength) and thereby set the accessible phase space—i.e., the number and types of coexisting phases—of the “molecular mixture.” Additionally, molecular concentration directly determines the relative amounts of given macromolecules, thereby influencing their ability to form condensates. For example, at low RNA concentrations, certain proteins undergo condensation, whereas at sufficiently high RNA concentrations, protein condensation can be suppressed [55]. Ion concentration strongly modulates electrostatic screening, thereby altering molecular affinities, especially for highly charged molecules such as RNA [56].

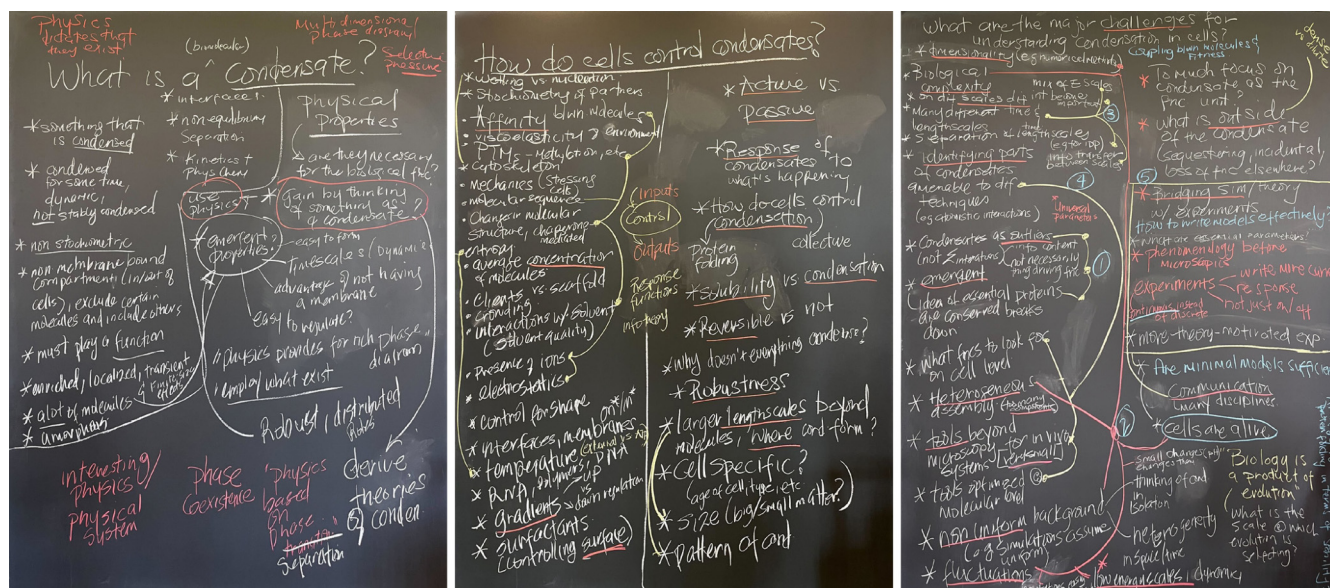


FIG. 3. Pictures of blackboards summarizing weekly discussion sessions at the KITP Workshop “Physical Principles Shaping Biomolecular Condensates” in 2025. The three shown examples are related to Secs. II, III, and V.

As the molecular composition of the cellular milieu changes due to active processes (e.g., transcription, translation, degradation, and transport; discussed below), the accessible phase space changes, and with it the capacity of the multicomponent molecular mixture to condense into distinct phases.

(3) *Cellular structures.* Cellular structures, including membranes, the cytoskeleton, membrane-bound organelles, and other surfaces, also control condensation inside cells [57]. These structures can provide sites for heterogeneous nucleation, promote wetting or prewetting, impose curvature, and exert mechanical stresses, and thereby modulate condensate dynamics and shape. For example, attractive interactions with a surface can create prewetting layers that lower nucleation barriers and control droplet size and location. Conversely, surfaces (or surface-active molecules associated with them) can destabilize condensates by acting as surfactants that alter interfacial properties (e.g., reducing interfacial tension, changing contact angles) or by sequestering key components.

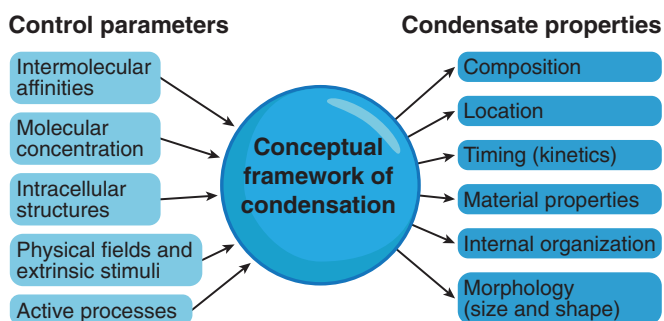


FIG. 4. The physical framework of condensation describes how various control parameters affect condensate properties. Cells can use the control parameters as knobs to tune condensate properties. Alternatively, these properties serve as proxies for environmental parameters to perform sensing tasks.

Cytoskeletal networks can also position condensates, bias coalescence, and influence material exchange through active transport and forces [58].

(4) *Physical fields (including extrinsic stimuli).* Another class of control parameters comprises physical fields—temperature, electric fields and potentials, hydrostatic pressure, pH, salt, cosolvents, and osmotic or mechanical stress—which generally act nonspecifically to shift phase boundaries and modulate condensation. These can be viewed as extrinsic cues that influence the phase behavior of molecules. Temperature, e.g., is a global control parameter: Modest changes can move systems across the binodal. Within single cells, sustained spatial temperature gradients are thought to be minimal, but local energy dissipation and composition changes (e.g., ATP levels, ion fluxes, chaperone activity) can produce temperature-like effects [59]. Electric fields (externally applied or arising from membrane potentials) redistribute ions and reweight electrostatic interactions, altering nucleation, wetting, and condensate dynamics. By contrast, gravity is negligible at most cellular length scales (though sedimentation and centrifugation can matter in large cellular compartments or in larger volume buffer solutions). Importantly, temperature also couples to sequence-dependent changes in molecular structure and solvation, creating a rich interplay between molecular sequence, interaction strengths, physical stimuli, and condensation propensities [60].

(5) *Active processes.* Cells are living entities that metabolize material to avoid reaching thermodynamic equilibrium. For example, cytoskeletal networks and motors create flows and actively move around molecules and organelles, which are continuously generated and degraded in response to certain cues. Such active processes also affect condensates, e.g., by motor-driven active fluxes or active chemical conversions of molecules and their concentration [61]. The latter example allows modifying molecules into an unfavorable state, e.g., turning a molecule inside a condensate into a form that effec-

tively repels the other condensate material, thus biasing this molecule to leave the condensate. Alternatively, molecules dissolved in the cytosol could be actively converted into a form that favors condensation, thereby initiating the formation of a condensate at a controlled location and at a specified time, or enable their partitioning into condensates.

These knobs rarely act in isolation; they are cross coupled, producing rich biomolecular phase behavior. Moreover, some parameters are actively regulated by cells (e.g., post-translational modifications and local concentrations), others vary passively with physiology (e.g., macromolecular crowding and ion balance), and some are largely extrinsic (e.g., temperature, pressure, and applied fields). Cells must sense and respond to fluctuations in these control parameters, and one outcome is the formation or dissolution of condensates or changes in their composition. The distinctions between parameters are also not always clear: e.g., molecular affinities depend on physical fields such as salt. Nevertheless, viewing biology through the lens of condensation organizes diverse mechanisms into a tractable set of control parameters that can be used collectively to modulate the phase behavior of molecules inside, on, and outside cells.

B. Condensate properties (“responses”)

Having considered the set of control parameters, we now discuss the measurable, quantitative responses, i.e., properties of the condensate that vary as these parameters are changed.

(1) *Composition*. One such quantifiable response is the composition of a condensate, which comprises its proteins and nucleic acids, plus small molecules (ions, cofactors, metabolites, water). In cells, condensates are multicomponent and dynamic, typically containing dozens to hundreds of macromolecular species (proteins and RNAs, and in some cases DNA or chromatin). Unlike crystals, they lack fixed stoichiometry; constituent concentrations often vary with environment and time. While stoichiometry can vary over time, the presence of an interface implies a composition that is persistently distinct from the surrounding environment. This nonfixed, exchanging nature—where molecules continuously partition in and out of condensates—complicates quantitative measurement of composition. Proximity-labeling proteomics and RNA profiling, along with curated component databases, have provided useful insights; though further advances are needed to obtain quantitative readouts of condensate-specific composition at high spatiotemporal resolution. Systematically quantifying how molecular composition shifts with control parameters (e.g., concentration, affinities, physical fields) will clarify what determines the makeup of condensates.

(2) *Location*. A second key response pertains to where condensates form in cells. Mapping location was transformative—e.g., P granules in *C. elegans* embryos, whose posterior enrichment arises from spatially regulated dissolution and condensation (Box 1), linked phase separation to development [11]. Site-specific formation also encodes mechanism and function: Condensates on microtubules often reflect scaffolded nucleation or active transport [62]; membrane-proximal condensates (e.g., at junctional domains on cell membranes) can mediate signaling and imply specific

protein-lipid interactions [63]; nuclear-restricted condensates point to local concentration thresholds, binding landscapes, or ionic screening distinct from the cytoplasm. Systematically quantifying the spatial distribution of condensates, therefore, serves as a constraint in our deciphering of their mechanism and function.

(3) *Timing (kinetics)*. Temporal dynamics of condensates, i.e., timing or kinetics, is another measurable response. This concerns how fast condensates nucleate, grow, exchange components, age (waiting-time-dependent hardening), and dissolve, as well as when these processes occur. Because condensation is collective, multiple coupled timescales coexist: molecular binding or unbinding, chemical conversion, molecular diffusion, viscoelastic network relaxation, nucleation-and-growth versus spinodal demixing, growth or coarsening (i.e., coalescence or Ostwald ripening), and possible kinetic arrest or gelation. Disentangling them is difficult, but mapping them is diagnostic, revealing mechanisms: which processes occur, whether active remodeling (e.g., by ATPases, chaperones, helicases) maintains the state, and if coarsening is arrested by gelation. Timing also includes when condensates appear within physiological cycles (cell cycle, development, circadian time, and aging) and how control parameters (e.g., physical stimuli) reshape lifetimes and dissolution rates. By mapping condensate kinetics alongside the timescales of other cellular processes, we can identify plausible links and generate testable hypotheses. Quantifying condensate timescales, therefore, enables predictive, mechanistic models.

(4) *Material properties (mechanics)*. Another key condensate response is material properties—the mechanical behavior of condensates (viscoelastic moduli, viscosity, surface or interfacial tension, gelation, and yield behavior)—which quantify how condensates deform and flow under applied stress or strain. Although sequence-encoded interactions have long been linked to phase behavior, quantitative maps from sequence and environment to mechanical properties are only now emerging, enabled by improved rheology (particle-tracking or active microrheology, optical tweezers, droplet fusion and shape recovery, micropipette aspiration) *in vitro* and, increasingly, in cells [64,65]. These parameters shape aging or maturation, control fusion or coarsening and fission, and determine how condensates respond to mechanical cues and, in turn, remodel their surroundings. Interfacial phenomena can even promote surface-mediated solidification, with disease implications [66,67]. Systematically linking control knobs to material properties will be invaluable for both physiology and engineering.

(5) *Internal organization*. Condensate internal organization also varies with control parameters. Here we consider both the connectivity of molecules—often termed microstructure or substructure, which emerges on molecular length scales—and the formation of emergent condensate architectures, such as multiphasic organization, which occurs on larger length scales. Molecular connectivity governs key properties, including molecular diffusion, local interactions (e.g., enzyme kinetics), and information transfer within condensates (e.g., mechanical stress propagation). For instance, interfacial phenomena, including aging, have motivated comparisons of molecular organization at condensate surfaces versus cores [68]. At larger length scales, interfacial effects can give rise

to multiphasic condensates comprising multiple coexisting liquid phases. A well-studied example is the nucleolus, where distinct subphases within the condensate correlate with function and reflect underlying physical factors such as interfacial free-energy differences [36]. Ultimately, internal organization shapes condensate mechanics and dynamics and provides insight into both function and the physical processes that regulate it.

(6) *Morphology*. Last, control parameters also drive differences in condensate size and shape. Early work emphasized spherical droplets, but morphologies span two-dimensional wetting layers on membranes and anisotropic or multiphase bodies. Crucially, shape only alludes to material state; spherical morphology does not guarantee liquidity. Condensates can be dynamically arrested or gel-like yet remain round, either because interfacial tension dominates or because a spherical liquid droplet has aged into a solidlike state while retaining its original shape. Small condensates also often appear spherical due to optical resolution limits. Quantitative readouts are therefore needed to infer mechanics. Size is likewise informative but biased: Conventional imaging detects larger condensates and can miss nanoscale clusters that precede coarsening (e.g., in stress-granule assembly [69]). Size and shape distributions, aspect ratios, contact angles, and their evolution over time reveal formation pathways (nucleation-and-growth versus spinodal and coalescence versus Ostwald ripening) and active remodeling.

So far, we discussed the control parameters and the condensate responses they elicit at the single-condensate level (Fig. 4). One can also consider collective responses across many condensates. In some systems, condensate number is functionally critical (e.g., centrosomes), whereas in others numbers may be less informative (e.g., nucleoli). Furthermore, condensate count is not an independent response; it emerges from nucleation, growth, coalescence, fission, and dissolution and should be interpreted alongside size and spatial distributions. It is also important to stress that many different sets of control parameters can result in similar condensate responses. Additionally, since control parameters vary in space and time, the resulting response space is inherently high dimensional.

IV. WHAT CONDENSATION CAN ACHIEVE IN, ON, AND OUTSIDE CELLS

Physical principles explain that molecules with sufficiently strong, multivalent interactions will separate into coexisting phases. This occurs with folded proteins (e.g., eye-lens γ -crystallins, lysozyme) as well as with highly disordered conformations (e.g., intrinsically disordered regions) inside cells, on the plasma membrane, and outside cells. Various parameters of this process, such as timing, location, component ratios, internal structures, and material properties can be regulated by cellular mechanisms (Fig. 4). In turn, the emergent properties of resulting condensates greatly influence the cellular space, which can be utilized by cells or researchers for functional benefit. In this section, we discuss (i) how condensation impacts molecules and cells, (ii) how evolution may have harnessed condensation to control cellular processes, and (iii) how researchers can leverage condensation.

A. Effects of condensation on the cellular space

Condensates profoundly affect the cellular organization in at least three different ways:

(1) *Composition and stoichiometry*. Condensates create a unique physicochemical environment that causes certain molecules (including macromolecules, ions, and small molecules) to partition into, be excluded from, or accumulate at the condensate-dilute phase interface to varying extents. This process then modifies the composition or stoichiometry of molecules outside the condensates, potentially generating a physicochemical or compositional gradient across the cellular space.

(2) *Conformation, interaction, and dynamics of molecules*. The condensate composition (point 1) creates unique solvent properties within condensates, at their interface, and in their surrounding environment. This influences the diffusional and conformational dynamics of molecules—such as how they move, “breathe,” or fold—both inside and at the interface of the condensates, which in turn influences their intra- and intermolecular interactions. For instance, proteins that adopt relatively compact conformations in dilute solution due to strong intramolecular interactions can exchange these interactions for more favorable intermolecular interactions within the condensate, causing them to adopt more open conformations individually. The surface of condensates also provides a distinct interface that impacts the dynamics and conformation of molecules. At this interface, proteins can form different intermolecular arrangements; emerging evidence suggests that the condensate interface can promote the formation of amyloid-like interprotein beta-sheet transitions and condensate hardening [67].

(3) *Physical effects*. Depending on their composition, size, location, and material properties, condensates can obstruct, exert forces on, or “glue” nearby structures such as molecules, membrane-bound structures, the cytoskeleton, chromatin, and other condensates. By occupying space as selective compartments, condensates can also function as crowders with varying degrees of physicochemical and material properties, which can alter local viscosity and slow or restrict the diffusion of macromolecules in their vicinity.

These physically intrinsic and thus “unavoidable” effects of condensates may influence cellular processes, or the processes must be resilient against them. We note that what we discussed in this section is not an exhaustive list of what condensation can achieve in biology. Additionally, the examples listed here are not mutually exclusive but closely interconnected, influencing one another and being influenced by other processes (i.e., the control “knobs” mentioned in the previous section and Fig. 4).

B. Ways condensation may be used to control cellular processes

How might cells leverage these effects for their functional benefit? Below, we list examples of roles that condensates might play in cellular processes, based on principles of biomolecular interactions and condensate properties, along with experimental evidence from biology (Box 1).

(1) *Spatial organization*. Condensates concentrate specific molecules in particular locations. The formation of a condensate thus allows cells to regulate their structure and

provide spatiotemporal cues for downstream processes. For example, condensate microenvironments can organize various cellular structures—from cytoskeletal networks (such as the actin cortex [70] and mitotic spindle [71]) to membrane-associated structures (like membrane contact sites [72] and signaling clusters that regulate immune responses [73])—in highly tuned hierarchies that lead to different morphologies. Additionally, condensation can control where signaling occurs (e.g., postsynaptic densities [74] and clustering of polarity factors [27]) or how cells are connected (such as tight junctions [63]), thereby influencing communication between cells and in turn, the organization of cells and tissues.

(2) *Tuning biochemical reactions and pathways.* Condensation can provide spatiotemporal control over biochemical reactions, either to facilitate or inhibit them, as well as influence stochastic processes like nucleation (e.g., actin polymerization [70]). Beyond individual reactions, this control can also tune pathway selection (e.g., branched pathway “channelling” in metabolism [75]) and separate interfering or competing processes. This can be achieved by concentrating or depleting specific molecules at certain locations. Additionally, the local chemistry within condensates can contribute, e.g., by modulating conformations and interactions.

(3) *Buffering.* Beyond a critical concentration, the “extra” molecules can partition into condensates, leaving the effective concentration outside unchanged. Therefore, condensation may help buffer chemical processes and regulate gradients and fluctuations in molecular concentrations [76]. However, the partition coefficient in biomolecular condensates is not always constant, and predicting the saturation concentration within the cellular milieu is not straightforward.

(4) *Force transmission and mechanics.* Condensation can generate and transmit forces that displace chromatin or drive chromosome segregation [77]. Condensates can also serve as glues or plugs to support repair, as seen in DNA end synapsis during double-strand break repair [40,78], and in the repair of ruptured lysosomal membranes [79]. The condensate solvent environment may also influence the structure or arrangement of polymers, e.g., by promoting microtubule aster formation over bundling and vice versa [80].

(5) *Information processing and transduction.* Condensation provides a highly sensitive spatiotemporal response, transferring molecular information (e.g., hydrophobicity, aromaticity, charge, and sequence pattern) to the cellular level across sharp decision boundaries [81]. Therefore, condensates can be used to detect and respond rapidly to changing environmental conditions, such as temperature, light, or chemical signals (e.g., hormones) during plant or animal development and stress responses (e.g., starvation, dormancy, and latency) as observed in bacteria [82], plant seeds [83] or flowering plants [84,85] and tardigrades [86]. Condensation can also transfer information (e.g., domains of epigenetic modifications [87]) or increase variability (e.g., random placement of crossovers during meiosis [31,32]). These responses, processed or translated by condensation, are nonlinear, similar to aggregation processes but different in that they are reversible.

Do cells leverage condensation to enhance their fitness and survival? Condensates are often associated with specific processes based on their composition or localization. It is tempting to speculate that they directly affect these pro-

cesses. If so, then certain “features” of condensates might be conserved or reimaged through evolution to serve similar functions. These questions and possibilities are still actively studied and lack definitive answers. The answers will likely vary by case rather than apply universally. It is also possible that condensation has little to no impact on the process it is associated with or on the fitness of cells or organisms, especially in specific contexts. Regardless of the case, the presence of condensates will influence the cellular environment, which may need to be managed by the cells. It is possible that cellular mechanisms even suppress condensation to avoid such effects.

C. How researchers can apply what we learn about condensates

Understanding the properties and responses of condensates, along with their effects on cellular space and potential functions, offers scientists exciting opportunities to apply these principles to study cellular processes or to design a new generation of chemical factories or therapeutics.

(1) *Probe biological functions.* Using principles of condensation, we can develop methods to control different aspects of condensates, helping us better understand their roles in biological processes and diseases. Specifically, it will be useful to modulate condensation (formation or dissolution of certain molecules), condensate composition (concentration or depletion), or their properties independently, without deleting or altering both copies of endogenous proteins to separate the effects of modifying a condensate component from condensation. This may be accomplished through mutations, engineered peptides, or tags individually or in combination.

(2) *Therapeutics.* As we identify condensates involved in disease states, therapeutics can be designed to target them, e.g., to dissolve them (e.g., to dampen oncogene transcription) or to modify their material properties (e.g., to restore healthy dynamics).

(3) *Designer condensates.* Synthetic condensates can be engineered to have specific properties that control targeted outputs, such as sequestering harmful components, turning signaling or reactions on or off, or generating new materials both inside and outside cells to probe biological functions or serve as therapeutics.

When studying the potential roles of condensates in biology and in disease, it is important to recognize that condensation might not always promote or enhance a process; it can also suppress or inhibit it, even to the point of being toxic to cells. Therefore, it will be crucial to develop ways not only to induce condensation but also to prevent it.

In summary, we suggest considering condensation as a physical tool accessible to both cells and researchers. Does it help to study your process of interest by evaluating and applying the properties of condensates discussed here? The concept of phase coexistence can be a powerful tool for predicting specific behaviors of biomolecules or for explaining biological phenomena through physics. For instance, new sights may arise from considering partitioning rather than stoichiometric binding or unbinding or examining phase boundaries and solvent environments rather than physical obstructions caused by occupancy. Emerging evidence indicates that this approach can be very effective (Box 1). However, predictions and behaviors vary widely across systems, and there is no uni-

versal set of tests to “prove” condensation; close collaboration among biologists, physicists, chemists, mathematicians, and engineers is thus essential.

V. CHALLENGES

While the biomolecular condensates research has made significant progress in advancing the biophysical understanding of condensates and their roles in cellular processes (Box 1), it faces several challenges going forward. Some of these are laid out below under the headings of conceptual, methodological, and social challenges.

A. Conceptual challenges

1. Sensitivity to conditions and perturbations

A central conceptual challenge has to do with the energy scale associated with condensation. By the very nature of condensates as structures that can form dynamically according to cellular needs, condensation must occur on the border of stability. Thus, energy scales for condensation are modest, typically on the order of a few $k_B T$ per molecule, to allow for regulation via changes in conditions such as pH or temperature or via phosphorylation, etc. This, in turn, means that condensates are highly sensitive to both external conditions and to changes of condensate components, such as modifications or mutations. Many of the known challenges to understanding condensate biophysics and biology arise directly from this sensitivity:

(1) The observation that condensates often behave differently *in vitro* from *in vivo* reflects sensitivity to the complex environment of the cell, including ions, and other small molecules, crowding by macromolecules, changing composition (see point b), the presence of active, energy-consuming processes, and more generally the fact that cells are not in equilibrium.

(2) The subtle dependence of condensate properties on polymer sequence likely also reflects this sensitivity. Notably, evolution has fine-tuned the relevant protein and RNA sequences to condense and function in the exact conditions of the cell. Moreover, sequence features interplay with the conformational degrees of freedom of the unstructured regions in condensate components. As such, the internal organization of condensates can be quite complex and heterogeneous. Indeed, in view of the selective pressure of evolution on sequence details, nothing about natural condensates can be assumed to reflect “typical” homopolymer behavior.

(3) The small energy scales associated with condensation present a challenge to theory: The inevitable small inaccuracies in modeling can drastically change predictions. As such, theory remains very limited in its ability to predict condensate phase diagrams, physical and chemical properties, and functional behavior.

(4) In cells, condensates can participate in positive or negative feedback loops, which act back on specific components, making their behavior additionally complex. For example, enrichment of kinases that phosphorylate certain components can modify condensate properties or even lead to condensate dissolution.

2. Multicomponent nature of condensates

Another conceptual challenge, of particular relevance to condensates in cells, is that the exact components of condensates are often not known, and even the known components may be chemically modified in unknown ways. In addition to the dominant phase-separating components, often called “scaffolds,” *in vivo* condensates may contain hundreds of low abundance components, also known as “clients.” Rather than being passive hitchhikers, clients can affect the stability and dynamics of condensates, again reflecting the overall small energy scales involved in condensation.

3. Linking condensate properties to function and dysfunction

The field of condensate research is presented with both opportunities and challenges in translating fundamental biophysical insights into clinically relevant health applications. A significant hurdle lies in establishing clear causal relationships between condensate dysfunction and disease pathogenesis, as many condensate-related disorders may involve subtle alterations in phase behavior that are difficult to detect and quantify in living systems. Additionally, developing therapeutic strategies that can precisely modulate condensate properties without disrupting essential cellular functions remains challenging. The complexity of condensate composition noted above—often involving hundreds of different proteins and nucleic acids with varying interaction strengths—makes it challenging to predict how genetic mutations or environmental factors will affect condensate behavior and subsequently contribute to pathological conditions such as neurodegenerative diseases or cancer. Of particular relevance to condensate-mediated disease, many condensates are observed to age, i.e., to become more viscous and solidlike with time. It remains a significant challenge to predict which condensates are subject to aging and on what timescales. While beta-sheet formation is implicated in some aging processes [88], other mechanisms may also be involved, e.g., gradual rearrangements into more favorable bonding configurations. As such, aging or its lack thereof may depend on the recruitment into condensates with interacting molecules that help prevent beta-sheet stacking or other stable arrangements such as chaperones or post-translational modification enzymes. Insofar as aging may depend on the nucleation of rare structures, rates of aging are likely to be extremely sensitive to heterogeneity and fluctuations within condensates.

4. De novo functional condensates

Finally, a major conceptual challenge is the design of novel functional condensates. Since condensates are ubiquitous in nature, it seems evident that creating condensates *de novo* will be a powerful tool for cellular engineering. Particular applications include redirecting metabolism toward desired products (biofuels, fine chemicals) via coclustering of enzymes within a desired pathway and condensates that act as biosensors to report on cellular states. However, just getting desired molecules to condense inside a cell is not enough to guarantee proper function. A worthy goal will be the design of condensates with the right components (both who is in, and who is out), size, localization, dynamics (forming when and where needed), physical properties (e.g., exchange rates,

viscosity, and surface tension), and possibly interactions with other organelles and cellular structures.

B. Methodological challenges

1. Probing microstructure of condensates

One of the main technical challenges in the study of condensates is the difficulty of probing structures of ~ 100 -nm scale. Atomic-scale structures can be probed by x-ray crystallography, NMR, and electron microscopy, while macroscopic structures can be probed by light microscopy. Natural condensates often occupy the approximately hundreds-of-nanometers “no man’s land” in between. Moreover, because condensates are often amorphous, tools such as cryoelectron microscopy and tomography typically only yield very rough pictures of condensates with little information about detailed structure (albeit with some exceptions [89,90]). To learn more about internal condensate structure, alternative approaches include single-particle tracking and reporters of local organizations such as FRET, FLIM, and EPR. Since many probes of condensates require labeling, e.g., with fluorescent proteins or other fluorophores, it is important to keep in mind that these tags can change phase behavior in unpredictable ways [91].

2. Composition and molecular states in condensates

Another technical challenge, touched on above, is the difficulty of knowing precisely what is inside a condensate. This is particularly true in cells, where the primary scaffolds may exist in a variety of modification states, and there are likely to be multiple clients and miscellaneous interlopers. Biochemical isolation of condensates faces the challenge of preventing their full or partial dissolution, e.g., during standard fractionation procedures. Nonetheless, it is notable that condensate isolation followed by mass spectrometry analysis has yielded insights in some cases, as have proximity-labeling approaches [92,93]. For *in vitro* studies, it is still challenging to know the exact densities and ratios of condensate components, or which molecules interact with which others and how; even for single-component condensates, the range of polymer conformations is generally unknown and may vary, e.g., between the surface and bulk, both *in vivo* and *in vitro*.

3. Condensate physical properties

Measuring the physical properties of condensates, like surface tension, viscosity, or electrical charge, presents its own challenges. Direct measurement of these properties *in vivo* is generally out of reach. FRAP has been used productively both in cells and *in vitro* to measure exchange rates of condensate material and diffusion coefficients within condensates [94], although one must keep its limitations in mind as well [94,95]. Chemical treatments that dissolve condensates (like 1,6-hexanediol) are often nonspecific and may have off-target effects. Genetic manipulations can change *in vivo* condensate properties, but these take time to manifest and may trigger compensatory responses. Optogenetic techniques hold promise but will require additional development to yield quantitative measures of *in vivo* condensate properties. *In vitro* studies allow more direct probing of condensate properties but are typically limited to a small number of components and

do not necessarily reflect *in vivo* composition or conditions. (A perhaps obvious point is that many proteins or RNAs will phase separate *in vitro* at high enough concentrations, but that is not enough to establish meaningful phase separation *in vivo*.) Growing interest in reproducing condensate dynamics—such as nucleation, coarsening, size control, dissolution, etc.—*in vitro* also faces the need for high spatial and temporal resolution measurements and additional components, such as energy sources, when active processes are at play. The latter include post-translational modifications that may regulate condensate molecular rearrangements or dissolution *in vivo* and are difficult to reproduce *in vitro*.

4. Sequence-resolved modeling of condensates

As remarked above, condensates’ intrinsically dynamical character and their fine-tuning by evolution present substantial challenges to theory and modeling. As noted, the proteins and RNAs that form condensates cannot be expected to behave like homopolymers or even random heteropolymers, limiting the ability of generic polymer models, such as Flory-Huggins theory, to address quantitative questions. In some cases, sequence-dependent analytical theories such as random-phase approximation and renormalized Kuhn length can quantitatively rationalize experimental data and even make high-throughput predictions for sequence-specific partitioning into condensates [96], but these theories are still limited in their ability to capture structural and energetic details [97]. While molecular and field-theoretic simulations have proven valuable in studying condensates, the wide range of relevant timescales presents significant difficulties. In particular, all-atom simulations, which capture the nuances of specific sequences of residues, are typically limited to timescales of microseconds for small condensates. Abstract coarse-grained models are useful to test conceptual points but cannot be mapped one-to-one on specific proteins or RNAs. Coarse-graining at the residue level is an attractive compromise for modeling the condensation of specific sequences [98], but it only approximately captures detailed interactions and dynamics [99] and does not yet reach the long timescales associated with aging, without *ad hoc* modifications [100].

5. Deciphering the biological function of condensates

Finally, an elephant in the condensate room is the question of what exactly does condensation do for function? The challenge here is to separate condensation per se from other processes. Mutations or other perturbations that abrogate condensation may affect function in multiple ways: For example, if condensation follows from phosphorylation of a particular protein, then a mutant that lacks phosphorylation sites will not condense—but the loss of function might instead be due to the lack of phosphorylation. Moreover, assays for biological function are often quite indirect, occurring downstream of condensate-mediated activities, adding uncertainty to inferring the role of condensation. A related challenge is that the function of condensation in cells may itself be indirect. For example, condensation could be employed to localize factors within a cell or to keep out unwanted factors from a particular location. These are important functions but have little to do

with the direct ability of condensates to create high densities of particular factors.

Going forward, to address the function of condensation the framework of control parameters and condensate responses provides a practical experimental strategy: Perturb one physical knob as selectively as possible, measure many condensate responses, and ask which response best predicts biological function. Several strategies could help make these challenges tractable. First, the field would benefit from perturbations that are acute, tunable, and reversible, such as optogenetic clustering, chemically induced dimerization, inducible degradation, anchor-away approaches, or acute recruitment of modifying enzymes, chaperones, and RNA helicases. Such perturbations can change condensate formation, dissolution, or material state on timescales short enough to eliminate or at least reduce compensatory effects. Second, separation-of-function allelic series can be designed to tune valence, charge patterning, aromaticity, sticker spacing, or interaction strength while preserving catalytic domains, localization signals, post-translational modification sites, and overall protein abundance. Third, endogenous perturbations can be complemented by orthogonal synthetic condensates that recruit the same client molecules to unrelated scaffolds, helping distinguish effects of condensation or localization *per se* from scaffold-specific interactions. Finally, whenever possible all these perturbative approaches should be coupled to multi-dimensional quantitative readouts such as condensate size, number, composition, partition coefficients, exchange rates, material properties, and biological outputs. In this way, function can be related continuously to measured condensate properties.

C. Social challenges

1. Condensate studies straddle physics and biology

A major “social” challenge to the study of condensates is the need to communicate and collaborate across disciplinary barriers. The research area draws from cell biology, biophysics, biochemistry, structural biology, polymer physics, and materials science, each with distinct experimental approaches, theoretical frameworks, and preferred conferences and journals. For example, biophysicists may focus on phase diagrams and scaling relations while cell biologists emphasize mutant studies and functional outcomes. Such differences make it difficult to establish close collaborations with shared research questions. There are many illustrative examples in biology of successes of integrating experiment and theory, *e.g.*, going back to Luria and Delbrück [101], but it is still the exception for experimental labs to involve theory prior to doing experiments. This is equally the case in studying condensates, where theorists are typically faced with analyzing preexisting data. A particular challenge will be to convince experimentalists to involve theorists at earlier stages, *e.g.*, in choice of model system and experimental approach, and with an eye toward addressing conceptual questions and interpreting the underlying biophysics. On the flip side, it will be important for theorists to engage with the details of the experiment and the underlying biology of specific systems. There are only a limited number of universal conceptual questions in biomolecular condensates—likely not enough to keep

all the interested theorists occupied—but there is a wealth of questions regarding how specific condensates exploit physics to robustly perform their unique functions. Thus the future of biophysical theory in the study of condensates is likely to be increasingly “biological.” To this end, bringing together experimentalists and theorists in conferences or workshops with specific opportunities to develop collaborations will be productive. Since ease of communication is one key to making such collaborations happen, it should be a priority to educate members of each group in the language of the opposite side.

2. More rigor and precise language

While the concept of condensates is successfully rewriting cell biology textbooks, there is some danger of overhype and backlash. The excitement around condensates has led to claims that phase separation explains many previously mysterious cellular phenomena. The pressure to publish or rapidly commercialize is particularly high in cases of potential therapeutic relevance of condensation, *e.g.*, in neurodegeneration and cancer. However, this enthusiasm around condensates may sometimes outpace rigorous experimental validation, potentially creating skepticism. It is thus important for high standards to be maintained in the face of all these pressures. More concretely, claims regarding the roles of condensates should (when possible) include (i) distinguishing condensate-like appearance from phase-separation mechanism and from biological function; (ii) avoiding reliance on any single diagnostic assay; (iii) quantifying partitioning, exchange, concentration dependence, and material properties; (iv) controlling for effects of tags, overexpression, mutations, and chemical perturbations; and (v) testing prospective predictions from physical models. Such practices would help the field move from generically assigning the label “condensate” toward establishing when condensation provides a necessary and predictive explanation for cellular behavior.

3. Future job and funding opportunities

For the field to flourish, it is also essential that researchers focused on condensates be able to find jobs and get funded. Universities do not have condensate departments, so academic researchers need to find jobs in departments such as molecular biology, biochemistry, chemistry, or biophysics. Will the more biological departments be willing to hire scientists interested in biophysical or theoretical aspects of condensates? Will funding agencies be willing to support condensate research that cuts across traditional disciplines and model systems? Will biotech and pharma companies step up to pursue condensate research? Only time will tell.

VI. OUTLOOK

Biomolecular condensation has emerged as a fundamental principle of cellular organization, transforming how we think about the spatial and temporal organization of molecules inside cells. The preceding sections have laid out how condensates can be understood in terms of control parameters, measurable responses, and their diverse roles in cell physiology, as well as the challenges faced in this endeavor. The field started from simple premises and has matured to provide deep

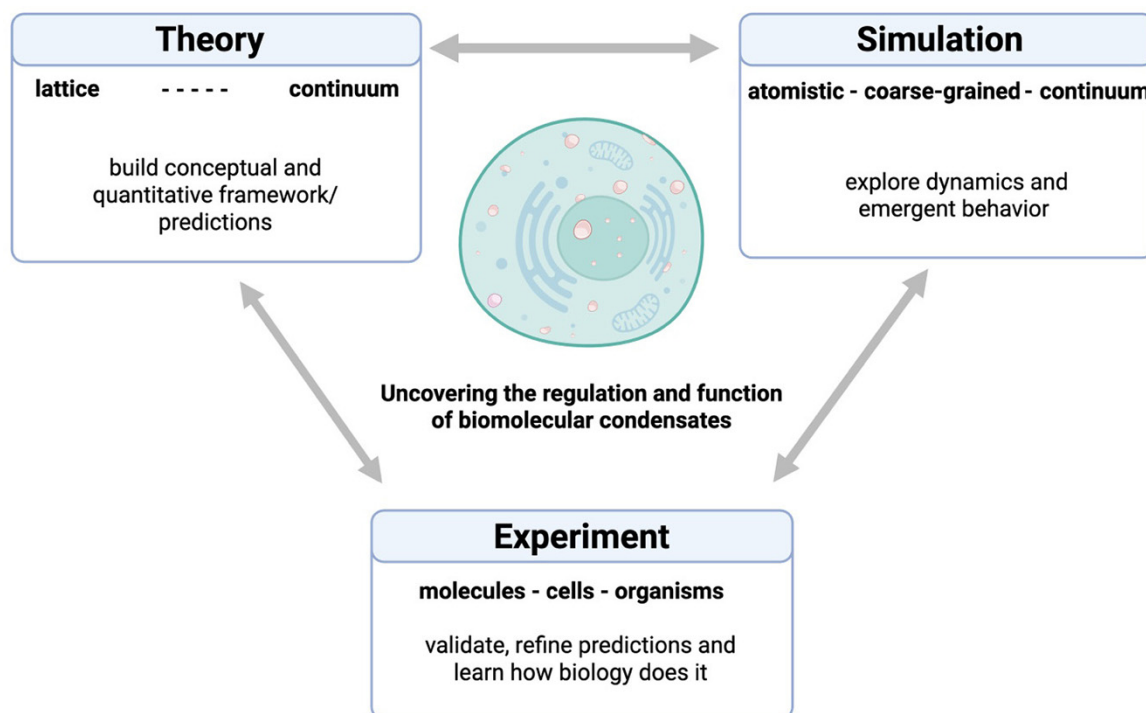


FIG. 5. Understanding biomolecular condensates and their biological consequences will require integrating theoretical, numerical, and experimental approaches. Each approach offers extensive tools, such as chemically nonspecific, composition-dependent, and sequence-specific theories, as well as exploring similarities and variability across different size and timescales (e.g., evolution) in experiments. Figure generated using BioRender.

new insights into cell biology. However, to embrace the full biological complexity, we must tackle conceptual, technical, and social challenges that span scales and disciplines.

A. From phase separation to complex supramolecular organization

Phase separation provides a powerful framework to understand the segregation of molecules in distinct phases separated by an interface. Cellular organization is typically more complex. In the out-of-equilibrium environment of cells, structures and phenomena emerge that go well beyond segregated phases. Phase separation is a generic physical concept, like diffusion, fluid dynamics, and mechanics, that is key to identifying the principles of cellular organization. However, many challenges lie ahead in obtaining a full understanding of the physics of structures such as the nuclear envelope, the spindle, and chromatin organization.

B. Dynamics and control in living cells

Condensates are active, nonequilibrium structures shaped by chemical reactions, mechanical forces, and energy fluxes. Future work will focus on how cells regulate nucleation, growth, coarsening, and dissolution of condensates on physiological timescales, and how feedback from metabolic activity, cytoskeletal organization, or signaling pathways tunes these dynamics. A particular goal is to understand how robustness and adaptability could emerge from the interplay of active processes with phase separation.

C. Linking physical organization to biological function

Condensates influence gene regulation, signaling, metabolism, and mechanical properties of cells and tissues. Determining when condensation is causal for these functions—rather than merely correlated—remains a major frontier. New perturbative approaches that can specifically modulate condensate formation or material state, combined with quantitative readouts of biochemical and mechanical outcomes, will be key to establishing causal links.

D. Condensates in development, evolution, and disease

The role of condensates across scales—from subcellular patterning to tissue morphogenesis and organismal development—presents rich opportunities. Exploring how condensate properties are selected and maintained by evolution, and how their dysregulation could contribute to aging, neurodegeneration, and cancer, will deepen both basic and biomedical insights. Identifying conserved physical design principles may also clarify how different organisms exploit condensation for robustness and adaptability.

E. Engineering and medical opportunities

A deeper physical understanding of condensate formation in the multicomponent environment of the cell could enable the rational design of synthetic condensates with specified composition, dynamics, and material properties. Such designer condensates could rewire metabolism, organize artificial organelles, or serve as drug-delivery systems. Conversely, precise interventions to dissolve or remodel pathological condensates offer new therapeutic strategies.

F. Convergence of disciplines

Progress will depend on close interaction between physics, chemistry, and biology. Developing multiscale theory, high-resolution and high-throughput experimental tools, and data-driven analysis methods will require sustained collaboration and a converging and shared language. The study of biomolecular condensates in cells and organisms could bring about a new research field as a convergence of existing disciplines.

By integrating theory, simulation, and experiment, and by embracing the complexity of living systems (Fig. 5), condensate research could play a key role in opening new avenues in cell biology and also bring new approaches to control and engineer the molecular organization of life.

ACKNOWLEDGMENTS

Many colleagues have contributed to stimulating discussions on these topics over the years. This work was supported in part by Grant No. NSF PHY-2309135 and the Gordon and Betty Moore Foundation Grant No. 2919.02 to the Kavli Institute for Theoretical Physics (KITP).

DATA AVAILABILITY

There are no publicly available research data or software supporting this manuscript. Requests for further information or data should be sent to the authors.

-
- [1] J. D. Van der Waals, *Over de Continuïteit van den Gas-en Vloeistoftoestand* (A. W. Sijthoff, Leiden, 1873), Vol. 1.
 - [2] H. G. B. de Jong and H. R. Kruyt, Coacervation (partial miscibility in colloid systems), *Proc. Koninkl. Nederland. Akad. Wetenschap.* **32**, 849 (1929).
 - [3] P. I. Flory, Thermodynamics of high polymer solutions, *J. Chem. Phys.* **10**, 51 (1942).
 - [4] M. L. Huggins, Solutions of long chain compounds, *J. Chem. Phys.* **9**, 440 (1941).
 - [5] J. T. Overbeek and M. J. Voorn, Phase separation in polyelectrolyte solutions. Theory of complex coacervation, *J. Cell. Comp. Physiol.* **49**, 7 (1957).
 - [6] A. N. Semenov and M. Rubinstein, Thermoreversible gelation in solutions of associative polymers. 1. Statics, *Macromolecules* **31**, 1373 (1998).
 - [7] E. B. Wilson, The structure of protoplasm, *Science* **10**, 33 (1899).
 - [8] A. I. Oparin, *The Origin of Life* (MacMillan, New York, 1938).
 - [9] H. Walter and D. E. Brooks, Phase separation in cytoplasm, due to macromolecular crowding, is the basis for microcompartmentation, *FEBS Lett.* **361**, 135 (1995).
 - [10] S. Frey and D. Görlich, A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes, *Cell* **130**, 512 (2007).
 - [11] C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, and A. A. Hyman, Germline P granules are liquid droplets that localize by controlled dissolution/condensation, *Science* **324**, 1729 (2009).
 - [12] P. Li, S. Banjade, H.-C. Cheng, S. Kim, B. Chen, L. Guo, W. Il, J. V. Hollingsworth, D. S. King, S. F. Banani, P. S. Russo, Q.-X. Jiang, B. T. Nixon, and M. K. Rosen, Phase transitions in the assembly of multivalent signalling proteins., *Nature (Lond.)* **483**, 336 (2012).
 - [13] M. Kato, T. W. Han, S. Xie, K. Shi, X. Du, L. C. Wu, H. Mirzaei, E. J. Goldsmith, J. Longgood, J. Pei, N. V. Grishin, D. E. Frantz, J. W. Schneider, S. Chen, L. Li, M. R. Sawaya, D. Eisenberg, R. Tycko, and S. L. McKnight, Cell-free formation of RNA granules: Low complexity sequence domains form dynamic fibers within hydrogels, *Cell* **149**, 753 (2012).
 - [14] T. W. Han, M. Kato, S. Xie, L. C. Wu, H. Mirzaei, J. Pei, M. Chen, Y. Xie, J. Allen, G. Xiao, and S. L. McKnight, Cell-free formation of RNA granules: Bound RNAs identify features and components of cellular assemblies, *Cell* **149**, 768 (2012).
 - [15] A. Musacchio, On the role of phase separation in the biogenesis of membraneless compartments, *EMBO J.* **41**, EMBJ2021109952 (2022).
 - [16] A. Rognoni, R. Conte, and M. Ceotto, How many water molecules are needed to solvate one? *Chem. Sci.* **12**, 2060 (2021).
 - [17] L. Jawerth, E. Fischer-Friedrich, S. Saha, J. Wang, T. Franzmann, X. Zhang, J. Sachweh, M. Ruer, M. Ijavi, S. Saha, J. Mahamid, A. A. Hyman, and F. Jülicher, Protein condensates as aging Maxwell fluids., *Science* **370**, 1317 (2020).
 - [18] J. B. Woodruff, A. A. Hyman, and E. Boke, Organization and function of non-dynamic biomolecular condensates, *Trends Biochem. Sci.* **43**, 81 (2018).
 - [19] A. Putnam, L. Thomas, and G. Seydoux, RNA granules: Functional compartments or incidental condensates? *Genes & Dev.* **37**, 354 (2023).
 - [20] K. Kuznetsova, M. Scheremetjew, J. Yin, H. Moon, D. A. Vargas, A. Hadarovich, N. Lewis, C. Hoege, C. F. W. Chow, D. Kuster, J. Nijssen, A. Hernandez-Armendariz, J. C. Savage, Y. Wei, S. Zedlitz, H. R. Singh, S. Ghosh, A. P. Kemraj, L. Hersemann, A. A. Hyman, *et al.*, CD-CODE 2.0: An enhanced condensate knowledgebase integrating pathobiology, condensate modulating drugs, and host–pathogen interactions, *Nucleic Acids Res.* **54**, D375 (2026).
 - [21] S. Strome and W. B. Wood, Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*., *Proc. Natl. Acad. Sci. USA* **79**, 1558 (1982).
 - [22] K. Lasker, T. H. Mann, and L. Shapiro, An intracellular compass spatially coordinates cell cycle modules in *Caulobacter crescentus*, *Curr. Opin. Microbiol.* **33**, 131 (2016).
 - [23] S. K. Govers and C. Jacobs-Wagner, *Caulobacter crescentus*: Model system extraordinaire, *Curr. Biol.* **30**, R1151 (2020).
 - [24] Y. E. Chen, C. Tropini, K. Jonas, C. G. Tsokos, K. C. Huang, and M. T. Laub, Spatial gradient of protein phosphorylation underlies replicative asymmetry in a bacterium, *Proc. Natl. Acad. Sci. USA* **108**, 1052 (2011).
 - [25] I. J. Domian, K. C. Quon, and L. Shapiro, Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle, *Cell* **90**, 415 (1997).

- [26] K. C. Quon, B. Yang, I. J. Domian, L. Shapiro, and G. T. Marczynski, Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin, *Proc. Natl. Acad. Sci. USA* **95**, 120 (1998).
- [27] K. Lasker, L. von Diezmann, X. Zhou, D. G. Ahrens, T. H. Mann, W. E. Moerner, and L. Shapiro, Selective sequestration of signalling proteins in a membraneless organelle reinforces the spatial regulation of asymmetry in *Caulobacter crescentus*, *Nat. Microbiol.* **5**, 418 (2020).
- [28] K. Lasker, S. Boeynaems, V. Lam, D. Scholl, E. Stainton, A. Briner, M. Jacquemyn, D. Daelemans, A. Deniz, E. Villa, A. S. Holehouse, A. D. Gitler, and L. Shapiro, The material properties of a bacterial-derived biomolecular condensate tune biological function in natural and synthetic systems, *Nat. Commun.* **13**, 5643 (2022).
- [29] C. Girard, D. Zwicker, and R. Mercier, The regulation of meiotic crossover distribution: A coarse solution to a century-old mystery? *Biochem. Soc. Trans.* **51**, 1179 (2023).
- [30] A. H. Sturtevant, The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association, *J. Exp. Zool.* **14**, 43 (1913).
- [31] C. Morgan, J. A. Fozard, M. Hartley, I. R. Henderson, K. Bomblies, and M. Howard, Diffusion-mediated HEI10 coarsening can explain meiotic crossover positioning in *Arabidopsis*, *Nat. Commun.* **12**, 4674 (2021).
- [32] L. Zhang, W. Stauffer, C. Liu, H. Shao, N. Abuzahriyeh, R. Jiang, D. Zwicker, X. Liu, X. Yao, and A. F. Dernburg, Crossover patterning through condensation and coarsening of pro-crossover factors, *Nat. Cell Biol.* **27**, 1161 (2025).
- [33] B. R. Sabari, A. Dall'Agnese, A. Boija, I. A. Klein, E. L. Coffey, K. Shrinivas, B. J. Abraham, N. M. Hannett, A. V. Zamudio, J. C. Manteiga, C. H. Li, Y. E. Guo, D. S. Day, J. Schuijers, E. Vasile, S. Malik, D. Hnisz, T. I. Lee, I. I. Cisse, R. G. Roeder, *et al.*, Coactivator condensation at super-enhancers links phase separation and gene control, *Science* **361**, eaar3958 (2018).
- [34] A. G. Larson, D. Elnatan, M. M. Keenen, M. J. Trnka, J. B. Johnston, A. L. Burlingame, D. A. Agard, S. Redding, and G. J. Narlikar, Liquid droplet formation by HP1 α suggests a role for phase separation in heterochromatin, *Nature (Lond.)* **547**, 236 (2017).
- [35] A. R. Strom, A. V. Emelyanov, M. Mir, D. V. Fyodorov, X. Darzacq, and G. H. Karpen, Phase separation drives heterochromatin domain formation, *Nature (Lond.)* **547**, 241 (2017).
- [36] M. Feric, N. Vaidya, T. S. Harmon, D. M. Mitrea, L. Zhu, T. M. Richardson, R. W. Kriwacki, R. V. Pappu, and C. P. Brangwynne, Coexisting liquid phases underlie nucleolar sub-compartments, *Cell* **165**, 1686 (2016).
- [37] S. Kilic, A. Lezaja, M. Gatti, E. Bianco, J. Michelena, R. Imhof, and M. Altmeyer, Phase separation of 53bp1 determines liquid-like behavior of dna repair compartments, *EMBO J.* **38**, EMBJ2018101379 (2019).
- [38] R. Oshidari, R. Huang, M. Medghalchi, E. Y. W. Tse, N. Ashgriz, H. O. Lee, H. Wyatt, and K. Mekhail, DNA repair by Rad52 liquid droplets, *Nat. Commun.* **11**, 695 (2020).
- [39] V. Spegg, A. Panagopoulos, M. Stout, A. Krishnan, G. Reginato, R. Imhof, B. Roschitzki, P. Cejka, and M. Altmeyer, Phase separation properties of RPA combine high-affinity ssDNA binding with dynamic condensate functions at telomeres, *Nat. Struct. Mol. Biol.* **30**, 451 (2023).
- [40] C. Chin Sang, G. Moore, M. Tereshchenko, H. Zhang, M. L. Nosella, M. Dasovich, T. R. Alderson, A. K. L. Leung, I. J. Finkelstein, J. D. Forman-Kay, and H. O. Lee, Parp1 condensates differentially partition DNA repair proteins and enhance DNA ligation, *EMBO Rep.* **25**, 5635 (2024).
- [41] A. S. Lyon, W. B. Peeples, and M. K. Rosen, A framework for understanding the functions of biomolecular condensates across scales., *Nat. Rev. Mol. Cell Biol.* **22**, 215 (2021).
- [42] J. P. Taylor, R. H. Brown Jr, and D. W. Cleveland, Decoding ALS: From genes to mechanism, *Nature (Lond.)* **539**, 197 (2016).
- [43] A. Patel, H. O. Lee, L. Jawerth, S. Maharana, M. Jahnel, M. Y. Hein, S. Stojnov, J. Mahamid, S. Saha, T. M. Franzmann, A. Pozniakovski, I. Poser, N. Maghelli, L. A. Royer, M. Weigert, E. W. Myers, S. Grill, D. Dreschel, A. A. Hyman, and S. Alberti, A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation, *Cell* **162**, 1066 (2015).
- [44] A. Molliex, J. Temirov, J. Lee, M. Coughlin, A. P. Kanagaraj, H. J. Kim, T. Mittag, and J. P. Taylor, Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization, *Cell* **163**, 123 (2015), number: 1.
- [45] K.-H. Lee, P. Zhang, H. J. Kim, D. M. Mitrea, M. Sarkar, B. D. Freibaum, J. Cika, M. Coughlin, J. Messing, A. Molliex, B. A. Maxwell, N. C. Kim, J. Temirov, J. Moore, R.-M. Kolaitis, T. I. Shaw, B. Bai, J. Peng, R. W. Kriwacki, and J. P. Taylor, C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles., *Cell* **167**, 774 (2016).
- [46] G. Welch, M. Fernandez, J. Lai, M. Cho, S. Wang, E. Riguat, M. Wagner, I. A. Klein, and V. Yu, Small molecule modulators of TDP-43 condensates rescue TDP-43 loss of function and neurodegeneration in ALS patient-derived motor neurons and in mouse models of TDP-43 proteinopathy, <https://dewpointx.com/small-molecule-modulators-of-tdp-43-condensates-rescue-tdp-43-loss-of-function-and-neurodegeneration-in-als-patient-derived-motor-neurons-and-in-mouse-models-of-tdp-43-proteinopathy/> (2025).
- [47] J. J. Ryan, K. R. Miller, A. Puri, M. L. Sprunger, M. M. Bochantine, A. Lopez, S. Chen, A. Bao, C. Ling, A. Xu, B. Bell, B. S. Sohn, L. Li, C. Chang, K. Mohammed, M. V. Staller, and M. E. Jackrel, High-throughput screening approach identifies substrate-selective Hsp104 variants that counter amyloid seeding with diminished off-target effects, *Mol. Cell* **86**, 1012 (2026).
- [48] K. E. Copley, J. C. Mauna, H. L. Danielson, Q. Chen, B. Ozguney, M. Ngo, L. Xie, A. Smirnov, M. Davis, L. Mayne, M. Linsenmeier, J. D. Rubien, C. A. Bergmann, B. Portz, B. L. Lee, H. M. Odeh, L. Lai, Y.-W. Chang, M. Hallegger, J. Ule, *et al.*, Short RNA chaperones promote aggregation-resistant TDP-43 conformers to mitigate neurodegeneration, *Science* **392**, eadv3301 (2026).
- [49] M. Kosno, S. L. Currie, A. Kumar, C. Xing, and M. K. Rosen, Molecular features driving condensate formation and gene expression by the BRD4-NUT fusion oncoprotein are overlapping but distinct, *Sci. Rep.* **13**, 11907 (2023).
- [50] E. E. Selig, E. J. Sohn, A. Stoja, A. K. Moreno-Romero, S. Akula, X. Xu, A. J. R. Bishop, and D. S. Libich, Phase

- separation of the oncogenic fusion protein EWS::FLI1 is modulated by its DNA-binding domain, *Proc. Natl. Acad. Sci. USA* **122**, e2221823122 (2025).
- [51] A. Savastano, A. Ibáñez de Opakua, M. Rankovic, and M. Zweckstetter, Nucleocapsid protein of SARS-CoV-2 phase separates into RNA-rich polymerase-containing condensates, *Nat. Commun.* **11**, 6041 (2020).
- [52] Y.-Y. Luo, J.-J. Wu, and Y.-M. Li, Regulation of liquid–liquid phase separation with focus on post-translational modifications, *Chem. Commun.* **57**, 13275 (2021).
- [53] A. S. Holehouse and B. B. Kragelund, The molecular basis for cellular function of intrinsically disordered protein regions, *Nat. Rev. Mol. Cell Biol.* **25**, 187 (2024).
- [54] N. Hess and J. A. Joseph, Structured protein domains enter the spotlight: Modulators of biomolecular condensate form and function, *Trends Biochem. Sci.* **50**, 206 (2025).
- [55] P. R. Banerjee, A. N. Milin, M. M. Moosa, P. L. Onuchic, and A. A. Deniz, Reentrant phase transition drives dynamic substructure formation in ribonucleoprotein droplets, *Angew. Chem. Int. Ed.* **56**, 11354 (2017).
- [56] G. Krainer, T. J. Welsh, J. A. Joseph, J. R. Espinosa, S. Wittmann, E. de Csilléry, A. Sridhar, Z. Toprakcioglu, G. Gudískyte, M. A. Czekalska, W. E. Arter, J. Guillén-Boixet, T. M. Franzmann, S. Qamar, P. S. George-Hyslop, A. A. Hyman, R. Collepardo-Guevara, S. Alberti, and T. P. J. Knowles, Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions, *Nat. Commun.* **12**, 1085 (2021).
- [57] A. Mangiarotti and R. Dimova, Biomolecular condensates in contact with membranes, *Annu. Rev. Biophys.* **53**, 319 (2024).
- [58] T. Wiegand and A. A. Hyman, Drops and fibers—How biomolecular condensates and cytoskeletal filaments influence each other, *Emerg. Top. Life Sci.* **4**, 247 (2020).
- [59] J. Mabillard, C. A. Weber, and F. Jülicher, Heat fluctuations in chemically active systems, *Phys. Rev. E* **107**, 014118 (2023).
- [60] K. M. Ruff, S. Roberts, A. Chilkoti, and R. V. Pappu, Advances in understanding stimulus-responsive phase behavior of intrinsically disordered protein polymers, *J. Mol. Biol.* **430**, 4619 (2018).
- [61] C. A. Weber, D. Zwicker, F. Jülicher, and C. F. Lee, Physics of active emulsions, *Rep. Prog. Phys.* **82**, 064601 (2019).
- [62] M. R. King and S. Petry, Phase separation of TPX2 enhances and spatially coordinates microtubule nucleation, *Nat. Commun.* **11**, 270 (2020).
- [63] K. Pombo-García, O. Adame-Arana, C. Martin-Lemaitre, F. Jülicher, and A. Honigsmann, Membrane prewetting by condensates promotes tight-junction belt formation, *Nature (Lond.)* **632**, 647 (2024).
- [64] I. Alshareedah, W. M. Borchers, S. R. Cohen, A. Singh, A. E. Posey, M. Farag, A. Bremer, G. W. Strout, D. T. Tomares, R. V. Pappu, *et al.*, Sequence-specific interactions determine viscoelasticity and ageing dynamics of protein condensates, *Nat. Phys.* **20**, 1482 (2024).
- [65] H. H. Cheng, J. V. Roggeveen, H. Wang, H. A. Stone, Z. Shi, and C. P. Brangwynne, Micropipette aspiration reveals differential RNA-dependent viscoelasticity of nucleolar subcompartments, *Proc. Natl. Acad. Sci.* **122**, e2407423122 (2025).
- [66] M. Linsenmeier, M. Hondele, F. Grigolato, E. Secchi, K. Weis, and P. Arosio, Dynamic arrest and aging of biomolecular condensates are modulated by low-complexity domains, RNA and biochemical activity, *Nat. Commun.* **13**, 3030 (2022).
- [67] Y. Shen, A. Chen, W. Wang, Y. Shen, F. S. Ruggeri, S. Aime, Z. Wang, S. Qamar, J. R. Espinosa, A. Garaizar, P. S. George-Hyslop, R. Collepardo-Guevara, D. A. Weitz, D. Vigolo, and T. P. J. Knowles, The liquid-to-solid transition of FUS is promoted by the condensate surface, *Proc. Natl. Acad. Sci. USA* **120**, e2301366120 (2023).
- [68] D. Tan, D. Aierken, P. L. Garcia, and J. A. Joseph, Biomolecular condensate microstructure is invariant to sequence-encoded molecular and macroscopic properties, *Soft Matter* **21**, 8635 (2025).
- [69] Y. Ge, T. Paul, M. Gordiychuk, N. Das, Y. Zhang, and S. Myong, FUS nanoclusters are a distinct state within the dilute phase, *Nat. Commun.* **16**, 9956 (2025).
- [70] V. T. Yan, A. Narayanan, T. Wiegand, F. Jülicher, and S. W. Grill, A condensate dynamic instability orchestrates actomyosin cortex activation, *Nature (London)* **609**, 597 (2022).
- [71] D. Oriola, F. Jülicher, and J. Brugués, Active forces shape the metaphase spindle through a mechanical instability, *Proc. Natl. Acad. Sci. USA* **117**, 16154 (2020).
- [72] N. Kim, H. Yun, H. Lee, and J.-Y. Yoo, Interplay between membranes and biomolecular condensates in the regulation of membrane-associated cellular processes, *Exp. Mol. Med.* **56**, 2357 (2024).
- [73] L. B. Case, J. A. Ditlev, and M. K. Rosen, Regulation of transmembrane signaling by phase separation, *Annu. Rev. Biophys.* **48**, 465 (2019).
- [74] X. Chen, X. Wu, H. Wu, and M. Zhang, Phase separation at the synapse, *Nat. Neurosci.* **23**, 301 (2020).
- [75] A. Martínez-Calvo, J. Zhou, Y. Zhang, and N. S. Wingreen, Sticky enzymes: Increased metabolic efficiency via substrate-dependent enzyme clustering, *PRX Life* **3**, 033011 (2025).
- [76] A. Klosin, F. Oltsch, T. Harmon, A. Honigsmann, F. Jülicher, A. A. Hyman, and C. Zechner, Phase separation provides a mechanism to reduce noise in cells, *Science* **367**, 464 (2020).
- [77] B. Gouveia, Y. Kim, J. W. Shaevitz, S. Petry, H. A. Stone, and C. P. Brangwynne, Capillary forces generated by biomolecular condensates, *Nature (London)* **609**, 255 (2022).
- [78] N. Chappidi, T. Quail, S. Doll, L. T. Vogel, R. Aleksandrov, S. Felekyan, R. Kühnemuth, S. Stoyanov, C. A. M. Seidel, J. Brugués, M. Jahnel, T. M. Franzmann, and S. Alberti, Parp1-DNA co-condensation drives DNA repair site assembly to prevent disjunction of broken DNA ends, *Cell* **187**, 945 (2024).
- [79] C. Bussi, A. Mangiarotti, C. Vanhille-Campos, B. Aylan, E. Pellegrino, N. Athanasiadi, A. Fearn, A. Rodgers, T. M. Franzmann, A. Šarić, R. Dimova, and M. G. Gutierrez, Stress granules plug and stabilize damaged endolysosomal membranes, *Nature (Lond.)* **623**, 1062 (2023).
- [80] S. Montenegro Gouveia, S. Zitouni, D. Kong, P. Duarte, B. Ferreira Gomes, A. L. Sousa, E. M. Tranfield, A. Hyman, J. Loncarek, and M. Bettencourt-Dias, Plk4 is a microtubule-associated protein that self-assembles promoting de novo MTOC formation, *J. Cell Sci.* **132**, jcs219501 (2019).
- [81] A. Murugan, D. Zwicker, C. Lorenz, and E. R. Dufresne, Could living cells use phase transitions to process information? *Phys. Rev. X* **16**, 020501 (2026).

- [82] M. Sasazawa, D. T. Tomares, W. S. Childers, and S. Saurabh, Biomolecular condensates as stress sensors and modulators of bacterial signaling, *PLoS Pathog.* **20**, e1012413 (2024).
- [83] Y. Dorone, S. Boeynaems, E. Flores, B. Jin, S. Hateley, F. Bossi, E. Lazarus, J. G. Pennington, E. Michiels, M. De Decker, K. Vints, P. Baatsen, G. W. Bassel, M. S. Otegui, A. S. Holehouse, M. Exposito-Alonso, S. Sukenik, A. D. Gitler, and S. Y. Rhee, A prion-like protein regulator of seed germination undergoes hydration-dependent phase separation, *Cell* **184**, 4284 (2021).
- [84] Y. Lyu, K. Wu, Y. Zhou, J. Du, C. Liu, Y. Wang, Q. Yuan, X. Dong, Z. Hong, M. Fahad, Y. Shen, F. Rong, X. Fu, P. Hu, and L. Wu, Fkf1 nuclear condensates control anti-florigen turnover and flowering onset in response to nitrogen availability in monocots, *Dev. Cell* **61**, 518 (2026).
- [85] X. Huang, Z. Ma, D. He, X. Han, X. Liu, Q. Dong, C. Tan, B. Yu, T. Sun, L. Nordenskiöld, L. Lu, Y. Miao, and X. Hou, Molecular condensation of the CO/NF-YB/NF-YC/FT complex gates floral transition in Arabidopsis, *EMBO J.* **44**, 225 (2025).
- [86] S. Sanchez-Martinez, K. Nguyen, S. Biswas, V. Nicholson, A. V. Romanyuk, J. Ramirez, S. Kc, A. Akter, C. Childs, E. K. Meese, E. T. Usher, G. M. Ginell, F. Yu, E. Gollub, M. Malferrari, F. Francia, G. Venturoli, E. W. Martin, F. Caporaletti, G. Giubertoni, *et al.*, Labile assembly of a tardigrade protein induces biostasis, *Protein Science* **33**, e4941 (2024).
- [87] S. Mukherjee, E. Skoruppa, H. Merlitz, J.-U. Sommer, and H. Schiessel, A self-organised liquid reaction container for cellular memory, *Adv. Sci.* **13**, e12500 (2026).
- [88] L. Emmanouilidis, E. Bartalucci, Y. Kan, M. Ijavi, M. E. Pérez, P. Afanasyev, D. Boehringer, J. Zehnder, S. H. Parekh, M. Bonn, T. C. T. Michaels, T. Wiegand, and F. H. T. Allain, A solid beta-sheet structure is formed at the surface of FUS droplets during aging, *Nat. Chem. Biol.* **20**, 1044 (2024).
- [89] S. He, H.-T. Chou, D. Matthies, T. Wunder, M. T. Meyer, N. Atkinson, A. Martinez-Sanchez, P. D. Jeffrey, S. A. Port, W. Patena, G. He, V. K. Chen, F. M. Hughson, A. J. McCormick, O. Mueller-Cajar, B. D. Engel, Z. Yu, and M. C. Jonikas, The structural basis of Rubisco phase separation in the pyrenoid, *Nature Plants* **6**, 1480 (2020).
- [90] O. Rog, S. Köhler, and A. F. Dernburg, The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors, *eLife* **6**, e21455 (2017).
- [91] K. Dörner, M. J. Gut, D. Overwijn, F. Cao, M. Siketanc, S. Heinrich, N. Beuret, J. Meyer, T. Sharpe, K. Lindorff-Larsen, and M. Hondele, Fluorescent protein and peptide tags alter condensate formation and dynamics in vivo and in vitro, *EMBO Rep.* **27**, 89 (2026).
- [92] J.-Y. Youn, W. H. Dunham, S. J. Hong, J. D. R. Knight, M. Bashkurov, G. I. Chen, H. Bagci, B. Rathod, G. MacLeod, S. W. M. Eng, S. Angers, Q. Morris, M. Fabian, J.-F. Côté, and A.-C. Gingras, High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and bodies, *Mol. Cell* **69**, 517 (2018).
- [93] S. Markmiller, S. Soltanieh, K. L. Server, R. Mak, W. Jin, M. Y. Fang, E.-C. Luo, F. Krach, D. Yang, A. Sen, A. Fulzele, J. M. Wozniak, D. J. Gonzalez, M. W. Kankel, F.-B. Gao, E. J. Bennett, E. Lécuyer, and G. W. Yeo, Context-dependent and disease-specific diversity in protein interactions within stress granules, *Cell* **172**, 590 (2018).
- [94] N. O. Taylor, M.-T. Wei, H. A. Stone, and C. P. Brangwynne, Quantifying dynamics in phase-separated condensates using fluorescence recovery after photobleaching, *Biophys. J.* **117**, 1285 (2019).
- [95] D. T. McSwiggen, M. Mir, X. Darzacq, and R. Tjian, Evaluating phase separation in live cells: Diagnosis, caveats, and functional consequences, *Genes & Dev.* **33**, 1619 (2019).
- [96] J. Wessén, N. De La Cruz, H. Lyons, H. S. Chan, and B. R. Sabari, Sequence-based prediction of condensate composition reveals that specificity can emerge from multivalent interactions among disordered regions, *Commun. Chem.* **8**, 304 (2025).
- [97] Y.-H. Lin, J. Wessén, T. Pal, S. Das, and H. S. Chan, Numerical techniques for applications of analytical theories to sequence-dependent phase separations of intrinsically disordered proteins, in *Methods in Molecular Biology*, edited by H.-X. Zhou, J.-H. Spille, and P. R. Banerjee (Springer US, New York, 2023), Vol. 2563, pp. 51–94.
- [98] J.-M. Choi, A. S. Holehouse, and R. V. Pappu, Physical principles underlying the complex biology of intracellular phase transitions, *Annu. Rev. Biophys.* **49**, 107 (2020).
- [99] A. Feito, I. Sanchez-Burgos, I. Tejero, E. Sanz, A. Rey, R. Collepardo-Guevara, A. R. Tejedor, and J. R. Espinosa, Benchmarking residue-resolution protein coarse-grained models for simulations of biomolecular condensates, *PLoS Comput. Biol.* **21**, e1012737 (2025).
- [100] A. R. Tejedor, R. Collepardo-Guevara, J. Ramírez, and J. R. Espinosa, Time-dependent material properties of aging biomolecular condensates from different viscoelasticity measurements in molecular dynamics simulations, *J. Phys. Chem. B* **127**, 4441 (2023).
- [101] S. E. Luria and M. Delbrück, Mutations of bacteria from virus sensitivity to virus resistance, *Genetics* **28**, 491 (1943).