Depicting a cellular space occupied by condensates

Daniel Liu[a,†], Margot Riggi[b,†], Hyun O. Lee[c,†], Simon L. Currie[d,†], David S. Goodsell[e,f,†], Janet H. Iwasa[b,‡,*], Ofer Rog[g,‡,*]

[a]Historisches Seminar, Abt. Wissenschaftsgeschichte, Ludwig-Maximilians-Universität München, Geschwister-Scholl-Platz 1, 80539 Munich, Germany; [b]Department of Biochemistry, and [g]School of Biological Sciences and Center for Cell and Genome Science, University of Utah, Salt Lake City, UT 84112; [c]Department of Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada; [d]Department of Biophysics, UT Southwestern Medical Center, Dallas, TX 75390 [e]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037; [f]Institute for Quantitative Biomedicine and Research Collaboratory for Structural Bioinformatics Protein Data Bank, Rutgers, The State University of New Jersey, Piscataway, NJ 08854

ABSTRACT **Condensates have emerged as a new way to understand how cells are organized, and have been invoked to play crucial roles in essentially all cellular processes. In this view, the cell is occupied by numerous assemblies, each composed of member proteins and nucleic acids that preferentially interact with each other. However, available visual representations of condensates fail to communicate the growing body of knowledge about how condensates form and function. The resulting focus on only a subset of the potential implications of condensates can skew interpretations of results and hinder the generation of new hypotheses. Here we summarize the discussion from a workshop that brought together cell biologists, visualization and computation specialists, and other experts who specialize in thinking about space and ways to represent it. We place the recent advances in condensate research in a historical perspective that describes evolving views of the cell; highlight different attributes of condensates that are not well-served by current visual conventions; and survey potential approaches to overcome these challenges. An important theme of these discussions is that the new understanding on the roles of condensates exposes broader challenges in visual representations that apply to cell biological research more generally.**

Monitoring Editor
Douglas Kellogg
University of California, Santa Cruz

Received: Mar 22, 2023
Revised: Jun 20, 2023
Accepted: Jul 10, 2023

INTRODUCTION

In the 19th century, cells were seen as being composed of soft fluid or jelly, based largely on physical appearance (Kölliker, 1845; Hertwig, 1895; Reynolds, 2008, 2018; Liu, 2017). By the beginning of the 20th century biologists began to think of the cell as a "colloid," that is, a viscous fluid with small particles dispersed within it (Hardy, 1899; Höber, 1902), and attempted to study its aggregate properties through physical chemistry approaches like precipitation experiments and measurements of gross viscosity (Heilbrunn, 1926). This framework conceptualized the cell as a disordered but dynamic system of semifluid phases. However, these colloid concepts did not provide good mechanistic explanations for cellular functions such as oxidative phosphorylation or secretion, nor did they easily account for the existence of clearly visible, persistent structures such as chromosomes or the Golgi apparatus (Matlin, 2022).

Two kinds of methodological innovations around the 1930s marked a clear break from the past. The first was cell fractionation: breaking the cell apart to study isolated fractions and proteins, and to assemble molecular mechanisms (Bechtel, 2005; Matlin, 2022). The second was the use of imaging tools borrowed from mineralogy and crystallography: polarization microscopy (Schmidt, 1924), x-ray diffraction (Frey-Wyssling, 1937), and later electron microscopy (Geren and Schmitt, 1954). These tools carried the assumption that the structure of cells and macromolecules were rigid and regular, like crystals. These innovations were essential for the development

DOI:10.1091/mbc.E22-11-0519

†co-first authors.

‡co-corresponding authors

*Address correspondence to: Janet H. Iwasa (jiwasa@biochem.utah.edu) and Ofer Rog (ofer.rog@utah.edu).

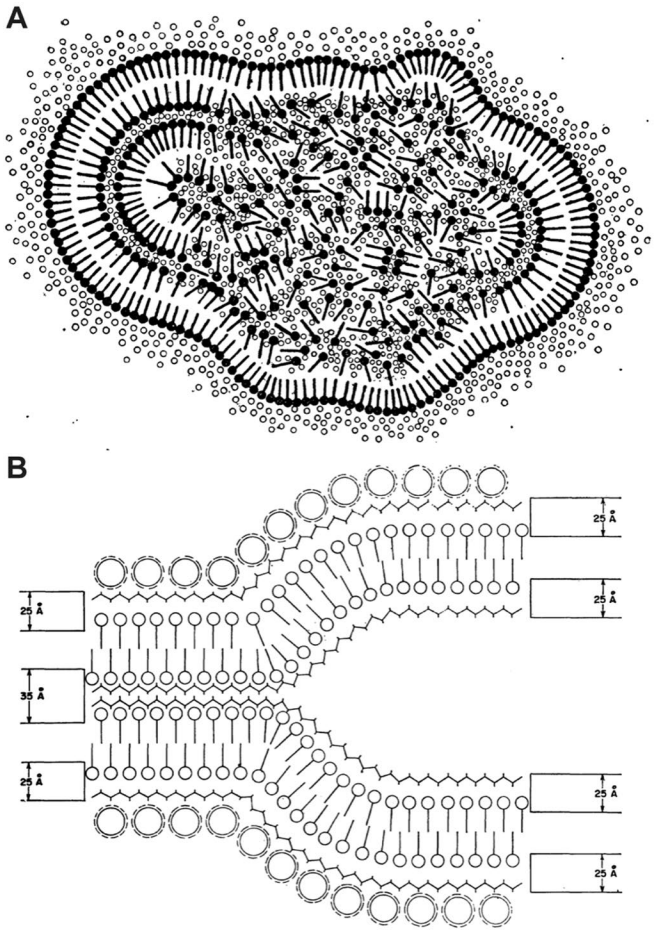

FIGURE 1: Depictions of soft cellular structures "hardened" after 1950. With the advent of x-ray diffraction and electron microscope imaging, illustrations of structures like the cell membrane increasingly emphasized their regularity. Ångström-scale imaging required crystallizing purified biomolecules or plasticizing tissue preparations to achieve such precise dimensional measurements. (A) Highly schematic diagram by polarized light microscopist Wilhelm J. Schmidt (1941) of a lecithin droplet in water (cross section) illustrating how more ordered structures like lipid bilayers could arise from disorder. In the diagram, weighted sticks represent lipids and small circles represent water molecules. (B) Diagram of cell membrane structure by electron microscopist Fritiof S. Sjöstrand (1960), showing a tradeoff between physical measurement and a realistic depiction of soft or fluid texture.

of modern cell and molecular biology, and gave rise to the still-prevailing conception of the cell as being composed of rigid molecular machines.

Order, regularity, solidity, and crystallinity were essential for the development of modern molecular biology techniques. x-ray crystallography demands extensive purification of protein and nucleic acid samples, while atomic lattice models and electron micrographs show precise measurements at angstrom scales only if they hold still. Even more importantly, the idea that ligands and binding sites were as regular and rigid as locks and keys was essential for biochemists to usher in the specificity revolution in the 1950s (Olby, 1986; Judson, 1996; Mertens, 2019). Orderly crystalline structures were also easy to illustrate, and our recognizable style of structural biological diagramming echo 1950s trends in modernist architecture, manner, and design (Figure 1).

The growing appreciation of the continuum between order and disorder that underlies cell biology – for example, in the study of lipid bilayers and intrinsically disordered proteins (Liu, 2018) – did not overturn these early 20th century conceptions. While the term "colloid" is rarely invoked nowadays, the cell interior is still mostly conceived as composed of highly structured entities, each diffusing in a crowded aqueous medium. Many biomolecules can be purified and reconstituted into machines that function in isolation. And it is generally assumed that the reverse is also true: that a structural description of cellular components and their interactions will explain their ability to organize the cell and allow it to function.

CONDENSATES: A NEW PARADIGM

Over the last decade, the 19th century interest in the fluid nature of cellular space has been rekindled, challenging the assumed universality of the "molecular machines" conception. Building on a culmination of findings and advances in many areas of cell biology and biophysics, a 2009 paper (Brangwynne *et al.*, 2009) is thought to be an inflection point in this shift. This paper demonstrated that well-known germ cell-specific structures called P-granules behave as liquids, and that physical properties of liquids can account for P-granule dynamics and their asymmetric localization.

More generally, biomolecular condensates (henceforth 'condensates') are cellular assemblies without a membrane enclosure, like P-granules, that selectively concentrate and exclude biomolecules (Banani *et al.*, 2017). Condensates are found throughout the tree of life and play pivotal roles in organizing diverse cellular processes including heterochromatin formation, transcription, DNA repair, cell cycle regulation, meiotic recombination, protection of cellular components under stress, germ cell specification, cell signaling, and RNA transport.

To mediate these processes, condensates are thought to function by locally modulating the concentrations of components that could be included, concentrated, or excluded from them (Lyon *et al.*, 2021). This is in stark contrast to what we typically hold about crystalline structures functioning through the exquisite positioning of biomolecules relative to one another. Condensate formation through multivalent reversible interactions challenges the universality of the lock and key model of molecular interaction and function. This traditional model assumes that biological processes are mediated by highly specific interaction surfaces, such as between enzyme active sites and substrates, transcription factors and promoters, or subunits of macromolecular complexes like the ribosome. Instead of the lock and key positioning, molecules in condensates interact through different interaction surfaces, often exhibit internal dynamics (i.e., rearrangement of molecules and exchange with their surroundings), and collectively exhibit behaviors such as fusion, relaxation, and deformation behaviors that are consistent with the viscosity and surface tension of fluids. These properties vary widely between different condensates – which can take material forms ranging from liquids to gels to solids – and can change over time and in response to cellular signals (Alberti *et al.*, 2019). The dynamic and varied interactions between condensate components has thus changed the way we consider molecular interactions and the functions that arise from them.

The formation of many condensates has been attributed to the process of phase separation, whereby the sum of multivalent reversible interactions drives interacting macromolecules to separate from their surroundings and form discrete phases (Hyman *et al.*, 2014). Phase separation of proteins and nucleic acids can be modeled through a polymer chemistry framework that considers biomolecules as an array of interaction units, or "stickers", connected by

intervening units, or "spacers" (Choi *et al.*, 2020). The number, distribution, and strength of stickers have been shown to contribute to the formation of condensates and their properties. Stickers can be globular interaction motifs such as SH3 and PRM, SUMO and SIM, or RNA binding modules and RNA molecules; or individual residues that interact via hydrophobic interactions, pi–pi interactions between aromatic residues, or interactions between oppositely charged residues (Li *et al.*, 2012; Lin *et al.*, 2015; Nott *et al.*, 2015; Zhang *et al.*, 2015; Wang *et al.*, 2018; Yang *et al.*, 2020).

Most condensates in cells include many different biomolecules, each concentrated to a different degree in the condensate (Currie and Rosen, 2022). These components do not contribute equally to condensate formation and properties but rather their contribution falls within a continuum. At its two extremes are molecules that are required for condensate formation, called scaffolds, and molecules that have a minimal impact on condensate properties but become enriched in the condensate through their interaction with scaffolds, called clients (Banani *et al.*, 2016). In addition to proteins, many condensates include RNAs that have different chemical properties than proteins, further complicating the accurate representation of the complex condensate environment. The heterogeneity of condensate composition and properties that differ between components and condensates as well as change over time and in response to stimuli all contribute to the complexity of studying and conceptualizing cellular condensates.

VISUAL REPRESENTATIONS OF CONDENSATES

The explosive interest and whirlwind progress in researching condensates has unfortunately not been accompanied by similarly rapid development of graphical representations. Cell biologists still often resort to using a graphical language developed in the 1930s to describe the inner workings of the cell (Figure 2, left panel). This language uses colorful, geometric shapes – each indicating a molecule or a domain of specific type and conformation – that specifically interact with one another, and arrows to indicate biochemical or temporal transitions (e.g., a catalytic cycle) or modes of regulation (e.g., activation or repression). These graphical conventions reflected important assumptions of cell biology: that individual components and tight interactions can be studied and conceptualized in isolation, and in turn inform the way these biomolecules function in the complex environment inside the cell.

Applying this established graphical language to condensates fails to capture many of their unique features and limits the full appreciation of their biological functions. The condensate field has also adopted tools from the material sciences, like the phase diagram. However, the rich and dynamic composition of many biomolecular condensates generates complex phase diagrams that are not readily amenable to clear graphical representation (Choi *et al.*, 2020; Riback *et al.*, 2020; Sanders *et al.*, 2020). These challenges highlight the need to develop new visual conventions. As far as we know, there has not been a deliberate effort to evolve the visual language of cell biology to accommodate condensates – for example, finding ways to depict liquid assemblies made of irregular, flexible regions and subunits that rearrange. This process will likely require casting off some of the visual conventions that have served cell biologists well for decades.

To address the condensate representation problem, a group of cell biologists, technology developers, and other visualization experts met in Fall 2022 at the University of Utah to participate in a workshop entitled "Re-imagining a cellular space occupied by condensates" (https://theroglab.org/condensates). We discussed the challenges of visualizing condensates and started to chart potential solutions to address them. Below, we summarize some of the discussions from this unique forum.

HETEROGENEITY AND DYNAMICS

The term "biomolecular condensate" describes an increasingly diverse group of compartments that vary in their composition, dynamics, and association with other cellular features. Beyond the obvious heterogeneity between different types of condensates and compositional stochasticity inherent to biological systems, we are learning that remarkable compositional heterogeneity exists even within and

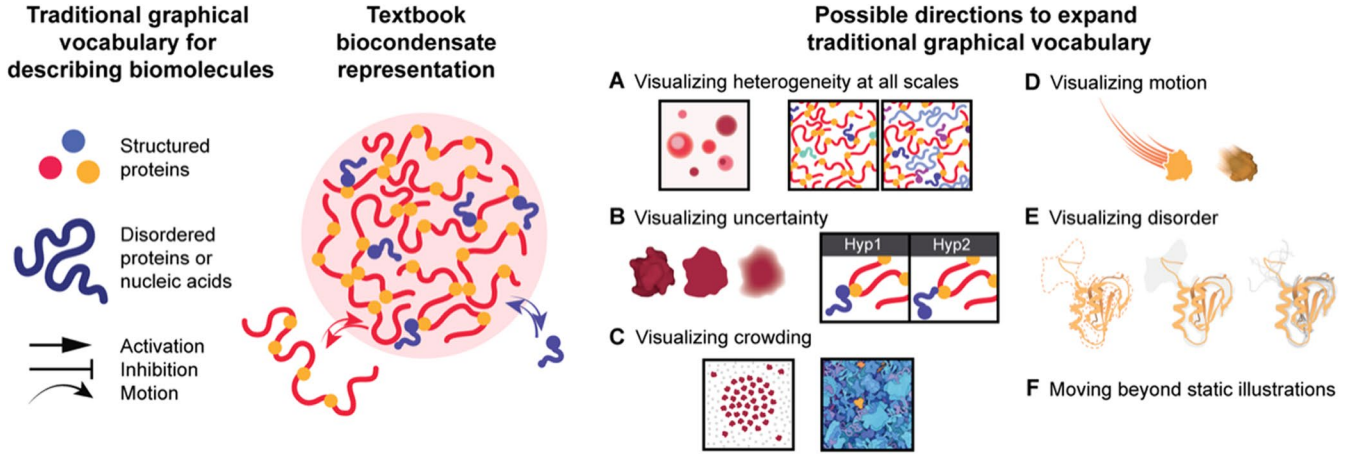

FIGURE 2: Commonly used graphical conventions. Left: Biologists commonly depict structured proteins as simple colorful shapes, nucleic acid or disordered proteins as "spaghetti," and arrows to depict movement or functional relationship (e.g. activation). These conventions are often combined in order to describe condensates. Expanding upon these conventions will allow biologists to better describe molecular features. (A) For example, use of zoom boxes can describe characteristics, such as heterogeneity, across different scales. (B) Uncertainty can be represented by the use of blurring or by showing alternative hypotheses. (C) Crowding may be depicted using simplified or space-filling forms. (D) Motion may be indicated using comic conventions, such as movement lines and motion blurs. (E) Disorder can be described by overlaying multiple structures, or indicating conformations that specific domains may sample. (F) Other solutions cannot fit into a standard printed figure, and include animations and interactive technologies such as AR and VR.

between instances of the same condensate, as shown for cytoplasmic processing bodies and stress granules (Xing *et al.*, 2020; Yang *et al.*, 2020; Currie and Rosen, 2022). Furthermore, condensates like RNA transport granules, PML bodies, and Balbiani bodies change their biophysical properties based on cellular location, cell-cycle stage, and developmental stage, respectively (Dellaire *et al.*, 2006; Grousl *et al.*, 2009; Rai *et al.*, 2018). In addition, condensate components are not positionally stable. Unlike ordered supramolecular structures – for example, centrioles or nuclear pore complexes – subunits in most condensates are assumed to not keep a constant position or orientation relative to one another. How do we depict the dynamics of the multivalent reversible interactions driving condensate assembly or the manner in which components rearrange, transit through, or concentrate in the condensates? The multiple levels of heterogeneity and complexity in condensate composition, dynamics, and biophysical properties highlight the need to move beyond static representations to include spatial and temporal information.

One method to convey motion is to take advantage of motion blurs and digital presentations (Figure 2D). Comic artists have extensively used motion blurs – faded images of past and future locations – to communicate movement within 2D static images. These comic conventions can easily be adopted to convey the dynamics of condensates on paper, while moving images and interactive tools can be used in digital presentations. In addition, blurring could represent random motion (Figure 2E) or experimental uncertainty in structural conformation (Figure 2B), and may also be used to reflect highly regulated compositional transitions. Another way to represent levels of uncertainty or changes in concentration is to use gradients of colors as is often done for electron cloud models.

In addition to temporal information, representing condensate heterogeneity or complexity in composition can be achieved using different combinations of shapes and colors (Figure 2, A and C) that shift in subsequent panels representing the passage of time. Depicting the dynamics of condensates in the context of a cell, as opposed to presenting them in isolation, can help communicate these concepts to broader audiences (Figure 2A). However, trying to pack too much information in a visualization of condensates in a crowded cellular space can be confusing. How do we effectively balance the information presented? In printed materials, insets can be used to zoom in from the cellular scale down to the molecular condensate scale, focusing on certain molecules by blurring and fading the color of surrounding components (Figure 2C). In digital representations, built-in layers of fading and blurring can highlight specific scales or objects.

BRIDGING SPATIAL AND TEMPORAL SCALES

To accurately describe biological processes, it is often necessary to bridge large spans of space and time, linking mesoscale events with molecular events. For example, the switching between structural conformations or binding partners of a multivalent interaction – events that occur over timescales of microseconds and spatial scales of angstroms – will cumulate and could have radical implications for cellular processes that occur over seconds and minutes and affect cells many micrometers in diameter. How do we visually represent such large spatial and temporal scales?

Notably, this issue predates the consideration of condensates. A common strategy in molecular biology has been to focus on events that occur at the single-molecule level and then to extrapolate these events to the cellular level. This strategy has been further reified by popular research techniques – fluorescence microscopy, western blots, and biochemical reconstitutions – all of which yield data relating to a limited set of components. However, the nontrivial effects of events at the molecular scale on the mesoscale in condensates – for example, generation of highly persistent cellular condensates from very transiently interacting components – makes the application of this reductionist approach more challenging.

Visualizing different spatial scales is a challenge that has been tackled in various ways. Illustrators will often utilize a magnified inset to highlight a region of an illustration and show it in a more zoomed-in view. Space constraints in manuscripts typically limit how many levels of magnification can be effectively presented: visualizations thus need to be chosen carefully to highlight relevant events and scales, with the caveat that not all scales can be represented. In animation, it is possible to offer a "continuous zoom" effect that encompasses a large continuum of scale (Eames and Eames, 1977). For examples of using these methods – and others discussed below – in animation, please see Phase Separation 101: https://animationlab.utah.edu/phase-separation.

Representing processes that occur over different time scales also pose significant challenges to which few solutions exist. In scientific animations, it is common to exaggerate or disregard time scales, for example, by showing events that occur over seconds and over hours at the same pace. Such simplifications can give meaningful approximations in some cases – for example, the effect of changing concentrations on enzymatic activities and the resulting cellular metabolism. However, many other cases, especially those involving condensates, involve nonlinear processes – such as nucleation events or liquid to solid transitions – which will be poorly served by them. If an exaggerated time scale is required to represent the condensate phenomenon in question, then it may also be heuristically useful to animate condensate dynamics in "real time" as a means of comparing relevant time scales.

CROWDING

The intracellular environment is a highly packed space, where molecules relentlessly wade through a tight crowd. While cellular concentrations of specific macromolecules are relatively low, the total concentration of all biomolecules (up to 400 g/L; Zimmerman and Trach, 1991) means that they fill a significant fraction of the cell volume (Ellis and Minton, 2003), leaving essentially no unoccupied space.

The effects of this extreme crowding on both the rates and the equilibria of a wide range of processes have been increasingly appreciated (Ellis, 2001; Kuznetsova *et al.*, 2014). Condensates are also heavily influenced by crowding. In in vitro experiments, addition of chemicals that mimic crowding can dramatically modulate the critical protein concentration required for phase separation. For example, crowding agents decrease the critical protein concentration required for the stress-granule components hnRNP1 and FUS to phase separate with RNA, but increase the critical protein concentrations required for these proteins to phase separate in the absence of RNA (Lin *et al.*, 2015; Protter *et al.*, 2018). In some cases, like FCA (Fang *et al.*, 2019) and NPM1 (Ferrolino *et al.*, 2018), crowding agents are essential to observe phase separation. Crowders can also change the biophysical properties of condensates, either acting as ideal inert species or through specific interactions with condensate components. For example, crowding promotes the irreversible liquid to gel transition of FUS prion-like domain condensates (Kaur *et al.*, 2019). Despite the experimental evidence that crowding impacts condensate formation and properties, it is still rarely depicted in visualizations, making it all too easy not to take it into account when building models, designing experiments, and interpreting data.

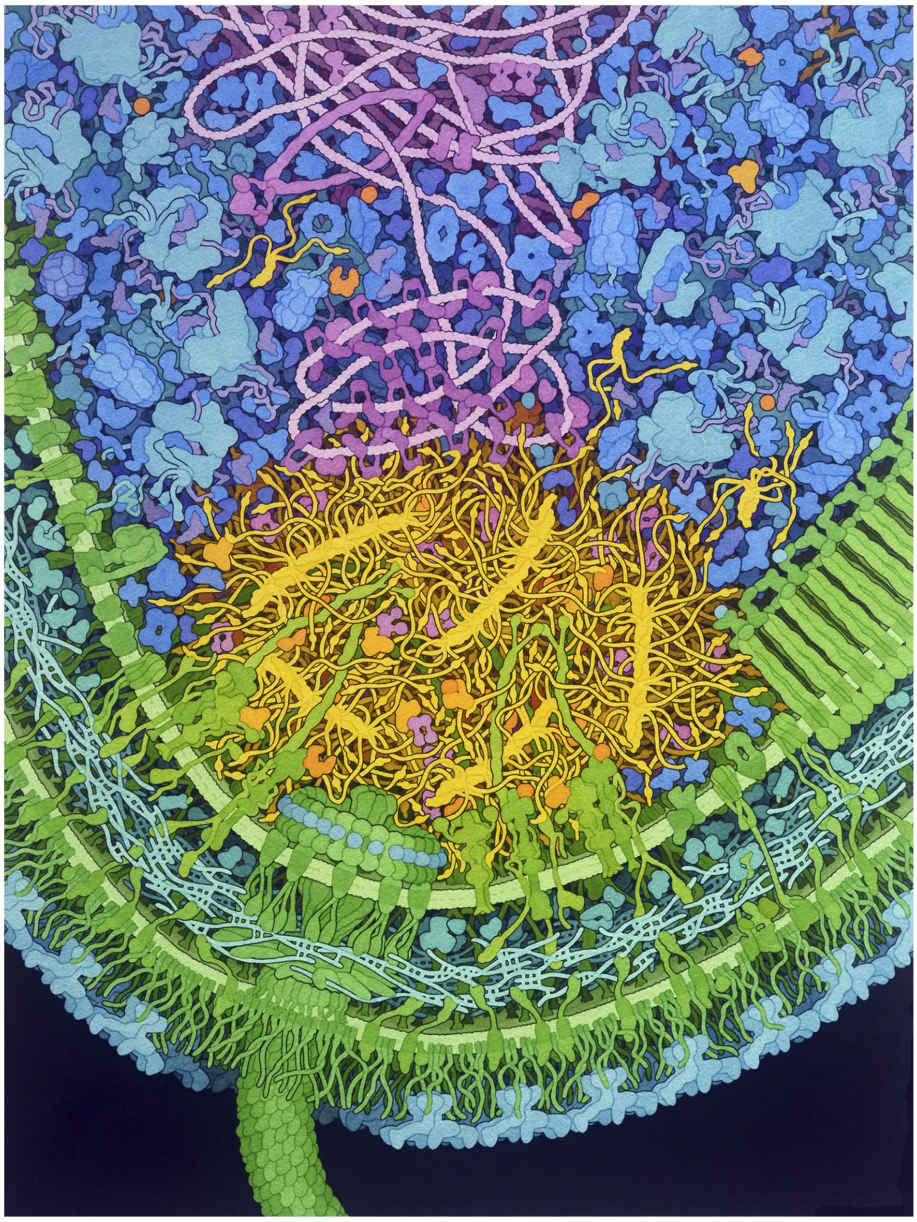

FIGURE 3: Illustration of the *Caulobacter crescentus* polar microdomain (https://pdb101.rcsb.org/sci-art/goodsell-gallery/caulobacter-polar-microdomain). Some of the strategies utilized to represent the crowded scene include: (1) The cartoony, flat color approach avoids visual distractions that could come from more detailed shading, and thereby makes it easier to comprehend the whole scene. In particular, the color scheme is carefully chosen to highlight physical compartmentation of the molecules: all the molecules present in the condensate are depicted in shades of yellow, clearly standing out from the surrounding crowd, depicted in shades of blue. (2) The choice of a cross-sectional view in orthographic projection (instead of an immersive view) allows the display of large fields of molecules with the main players occupying prominent positions in the scene, and allowing easy comparison of molecular sizes and shapes across the scene. Several depth-cued layers of molecules are added behind the foreground to improve the perception of depth. (3) Small molecules and water are omitted for clarity. (4) While the molecular distribution is based on accurate concentrations for species that are present in high copy numbers, artistic license is used to include a selection of molecules that are found in lower concentrations and would normally only be seen by chance, in order to highlight cellular functions of interest.

One of the biggest challenges for visualizing crowding is to find a sweet spot that balances scientific accuracy and clarity: how can we focus the attention of the viewer on the process of interest within a dense cytoplasm? How would a viewer visually distinguish a subset of interacting molecules in the middle of a crowded condensate? Different tools and strategies enable us to move toward clearly representing specific processes or molecules in complex and crowded environments. In figures, one can represent the complexity while maintaining focus on the key events or molecules by using different color schemes or using fading and blurring methods discussed previously. The diffusive random motion of molecules further complicates the design of dynamic animations to describe a process, as efficient storytelling often requires one to follow the process in a coherent step by step series of events. In molecular animations, an effective strategy to alleviate this problem is to layer stochastic motion on top of the keyframed steps used to script the main story.

To represent the state of crowding in figures, scientists can create 2D representations of molecules and arrange them in close proximity (Figure 2C). For a more detailed and accurate representation of the cellular space with condensates, scientist/artist collaborations have proven extremely beneficial. Figure 3, created in collaboration with Keren Lasker at Scripps Research, combines multiple strategies to capture salient features of a PopZ condensate at the pole of a *Caulobacter crescentus* cell (Goodsell and Lasker, 2023). While the PopZ condensate is particularly well studied, many decisions, and appreciable artistic license, were necessary to resolve missing data. For example, structures of many of the membrane-bound components were available only as AlphaFold2-predicted structures, the PopZ filament is based largely on current hypotheses from the Lasker laboratory, and interactions between PopZ with itself and with clients are not fully characterized. This semiquantitative 2D painting approach is intended as a prelude to full 3D modeling, providing an opportunity to gather and curate the necessary biochemical, structural, and micrographic data in collaboration with experimentalists. These data then feed into a computational, automated workflow to generate 3D models of mesoscale environments (Johnson *et al.*, 2015), and even of entire cells (Maritan *et al.*, 2022), generating models that can be interactively explored, analyzed, and easily updated to describe specific studies. Additional suggestions on how to depict biological information within a crowded space are included in the Figure 3 legend.

DISORDER

Structural biology has focused on atomic-level elucidation of discrete conformations adopted by macromolecules and by the multicomponent complexes that they form. This could be a single

conformation, or a handful of conformations, for example, reflecting catalytic steps or alternative binding partners. Discrete conformations are thought to represent functional states – one of few low-energy folding configurations that uniquely create an active site or a protein–protein interacting interface. The adoption of ordered secondary and tertiary structures entails both fixed stoichiometries in macromolecular complexes (e.g., the ribosome) and mesoscale symmetrical or stereotypical organization (e.g., clathrin-coated vesicles or a polarized spindle). Two of the major techniques that probe such structures – crystallography and, more recently, single-particle cryo-electron microscopy – rely on these assumptions in sample preparation and data analysis.

Condensates place renewed emphasis on biological scenarios that do not easily fit into this mold, either at the intra- or intermolecular levels. Intramolecularly, condensates reinvigorated an interest in so-called intrinsically disordered regions and proteins, many of which can also form condensates. Despite their names, these proteins do not completely lack internal order, but rather adopt an ensemble of possible conformations that are influenced by the solvent environment or presence of binding partners (Ruff *et al.*, 2019). In addition to disordered protein regions, conformational diversity of nucleic acids contribute to condensate formation and identity (Zhang *et al.*, 2015; Langdon *et al.*, 2018). On the intermolecular level, disorder exists as a result of molecules interacting in a nonordered or nonstoichiometric manner. This can occur due to relatively weak interactions between macromolecules with concatenated interacting surfaces. While each interaction domain may be traditionally folded, a cluster of macromolecules does not adopt a single conformation or have a fixed stoichiometry. Disorder at this level is further amplified by non–fixed ratios of scaffold and client proteins.

While a certain degree of disorder is an important distinguishing feature of condensates, many condensates likely exist on a continuum of order–disorder. An example of a condensate with order–disorder characteristics is the meiotic synaptonemal complex, which behaves as a fluid condensate but also contains ordered structures that have been resolved by electron microscopy (Moses, 1956; Rog *et al.*, 2017). Many condensates are also anchored to, or are associated with, structured entities, such as the diffusion barrier at the center of the nuclear-pore complex (Schmidt and Görlich, 2015) or pericentriolar material around centrioles (Woodruff *et al.*, 2017). In these cases, significant order is exhibited at least on some dimensions.

Representations of disorder have been misleading or overly simplistic, and the condensate field has yet to coalesce on a successful convention. A molecular-level representation that quickly rose to prominence is the yarn-like threads produced by structure-prediction softwares like AlphaFold to designate the absence of a high-confidence fold (Figure 2). These threads, which loop around alpha helices and beta sheets in structured parts of proteins, capture the lack of fixed structure but fail to communicate that the actual structure is not completely random but rather falls within a statistical ensemble of conformations (Ruff and Pappu, 2021). Other potential directions are representations of ensembles, such as those produced by nuclear magnetic resonance (NMR) studies and resembling a jumbled ball of spaghetti noodles. However, these representations are not intuitive to interpret and, due to technical constraints, can only be experimentally derived for relatively short peptides. One way to get around these limitations and represent ensembles of disordered regions is to show different conformations of flexible regions that are faded or blurred to communicate that one peptide chain assumes varied structures or draw a "cloud" that represents a range of space that the region can occupy at different conformations (Figure 2E). Building on this, one could depict mesoscale order–disorder by including detailed structures alongside more faded ensembles of nonstructured interactions.

UNCERTAINTY

Depictions of condensates would ideally indicate size, composition, and structure, as well as the concentrations, internal distribution, interactions, and dynamics of each of its components. Some of these factors may be grounded in experimental evidence and associated with error statistics, while others may be largely speculative. For example, fluorescence imaging of yeast P-bodies was used to generate a quantitative inventory of the major components and their cytoplasmic exchange rates – properties that could be assigned quantitative uncertainty (Xing *et al.*, 2020). These experiments investigated population averages, however, so that the distribution and dynamics of all molecules within a single condensate remain unknown.

Uncertainty can and should strongly impact our interpretation of data, and therefore needs to be accessible in visualizations. Indeed, the issue of how to represent uncertainty is considered a key problem in visualization research (Johnson, 2004). Graphs in peer-reviewed articles indicate the uncertainty that comes with quantitative measurements using precise and space-efficient approaches, such as error bars and confidence bands. However, it is more challenging to depict uncertainty in other kinds of visualizations, including structural models of both condensates and noncondensates.

Possible solutions include combining various rendering styles, depicting multiple possible scenarios or using visual cues such as different color schemes (such as color gradients), transparency, or blurriness to indicate high uncertainty (Figure 2). Because these visualizations can quickly become confusing, information could be organized in interactive layers, as discussed below.

INTERACTIVE TECHNOLOGIES

Interactive technologies can alleviate some of the challenges of accurate and effective condensate visualization. Perhaps the most basic are physical models that can be viewed, touched, and manipulated. Historically, important scientific insights have been gained through such models, including the discovery of the protein alpha helix by Linus Pauling (1994) and, legendarily, the benzene ring by August Kekulé (Rocke, 2010).

3D printing, which has recently become widely accessible, can be used to create complex models that can be made of a variety of materials with properties that are more flexible than typical wood or plastic modeling kits. Such 3D-printed models have proved instrumental in generating testable hypotheses about unusual chemical structures called quasi-crystals (Ajlouni, 2019). A disordered protein can be printed as a flexible or floppy chain, with embedded magnets providing a means to demonstrate the impact of molecular attraction or repulsion. Such models of viral capsids (Chemical & Engineering News, 2011) were instrumental in conveying self-assembly capabilities of large and complex macromolecular structures from individual components. Similar demonstrations for condensates may likewise prove influential.

Virtual reality (VR), augmented reality (AR), and game engines now place the user in the "driver's seat," enabling them to explore complex models in biology (Pettersen *et al.*, 2021). These tools have already proven effective in designing small molecules (Walters *et al.*, 2022) and can likewise provide important insight into biological problems including those involving condensates. For

instance, users may toggle the visibility of groups of molecules to reduce the complexity of a model, or play a 3D simulation of molecular interactions in real time or slowed down – all while being able to rotate the model or walk around it. By transforming the observers from passive viewers to active users, interactive technologies may allow condensate researchers to appreciate and gain insights into complex and dynamic biological processes that may be difficult to achieve by other means of visualization. Despite the strong potential benefit of VR and AR technologies in biological research, support for technical development and access to such tools for researchers has so far been limited. With increased funding support, rapidly evolving technology can more readily spread beyond early adopters, allowing them to realize their full potential as hypothesis generation and modeling tools, while also making them more accessible in scientific communication and publishing.

EDUCATION

Our ability to imagine cellular space, and to share this understanding with trainees and other researchers, can be greatly facilitated by the development of analogies. Some of the most historically successful analogies related biological processes to the motion of bodies and objects in our daily lives. Such analogies allow us to imagine the "walking" of motor proteins on cytoskeletal elements and to predict how such directed motions will result in diffusion regimes different from those of Brownian motion, likened to aimless (or random) walks.

While analogies require careful and specific use (Reynolds 2018), they are likely to be particularly useful for developing intuitions in the classroom. However, adequate analogies for condensates have been elusive. The analogy of water and oil separating in a vinaigrette has often been used to explain the process of phase separation. While this analogy captures some aspects of the phenomenon, it fails to convey many of the complexities of cellular condensates described above. For example, water/oil represents single-component phase separation – where a single "scaffold" dictates the formation of a condensate – which is in poor agreement with data for cellular condensates (McSwiggen *et al.*, 2019; Riback *et al.*, 2020). It is important to discuss these limitations with students when using this analogy.

New technologies provide exciting potential for condensate-biology education. The move away from paper-based teaching materials and toward digital media paves the way for the creation of tools that can easily move between scales. As described above, such digital tools obviate the need to focus on a single level of interpretation – intramolecular dynamics, intermolecular dynamics, or mesoscale – and can also allow to modulate how much complexity is visible (see Phase Separation 101 for many useful animations: https://animationlab.utah.edu/phase-separation).

Finally, the importance of the local environment and 3D space for condensate biology should prompt cell biologists to be inspired by other fields, such as architecture. In these disciplines, both traditional (drawing) and modern (computer-aided design) tools are used throughout training. Moving towards more active engagement with these approaches will allow students and practitioners to gain spatial intuition, hone visualization skills to communicate findings effectively, and to become adroit in moving between a 4D world and reductive 2D representations.

OUTLOOK

Traditional depictions do not suffice to describe the dynamic, disordered, complex, and crowded nature of biomolecular condensates. However, tools and common practices to visualize condensates are rapidly evolving in parallel to the research on condensates. In the workshop and in this perspective, we have explored existing conventions for diagramming and representing condensates and reimagined how these conventions may be expanded and improved to grow with the rapidly progressing research. Accurately conveying dynamics, complexity, and heterogeneity within a visualization is difficult, and practical solutions will require collaboration with visualization experts. Conventions and methods may be borrowed from adjacent fields of inquiry – for example, many of the visualizations presented here build on methods perfected over decades of work in molecular graphics. But perhaps the greatest insight that we gained from the workshop was that successful visualization of condensates is not a trivial task, and provides an exciting challenge for future work. A key step will be the development of new visualization tools that enable researchers to readily visualize and share dynamic, complex, and multidimensional data (Lyons *et al.*, 2022). With better tools, training, collaboration with visualization experts, and increased support for visualization careers, we expect that representations of biological space can more accurately reflect our hypotheses.

The limitations of traditional 2D schematic illustrations and the need for complementary visualization techniques that more accurately reflect the processes they depict were highlighted. Interestingly, many of the promising solutions came from individuals who are not biologists, highlighting the importance of cross-disciplinary forums like this workshop. Unfortunately, hyperspecialization makes such forums rare, with most communication about condensates occurring exclusively amongst cell biologists. Related to this challenge is the lack of sustainable funding structures (e.g., academic positions or grants) that allow long-term investment in individuals and communities that will actively evolve a graphical language and visualization technologies. Another challenge is how we integrate new immersive technologies, such as animation, AR, and VR, within our current communication methods, which are largely limited to 2D representations (such as in publications and posters). With findings from our workshop, we hope that more opportunities will arise for cross-disciplinary discussions and funding mechanisms to support them, as well as for supporting individuals that specialize in visualizing dynamic processes in the complex, crowded environment of a cell.

ACKNOWLEDGMENTS

We would like to thank all participants of the workshop for lively discussions and for contributing ideas covered in this perspective: Amy Strom, Martina Maritan, Alex Holehouse, Rima Ajlouni, Abby Dernburg, Blair Lyons, Steven Boeynaems, and Tamara Bidone. We would like to thank Sara Nakielny for comments and editorial work. The workshop was supported by National Science Foundation (NSF) grant (2213354), and by the Center for Cell and Genome Science and the Vice President for Research at the University of Utah. M.R. was supported by an European Molecular Biology Organization Postdoctoral Fellowship (ALTF 26-2019). D.L. is supported by a grant from the German Research Foundation (DFG), project number (463389772). H.O.L.'s work on biomolecular condensates is supported by the Canada Research Chairs program. O.R. is supported by NSF grant (2219605), and would like to thank the Taft-Nicholson Center at the University of Utah, where early work on this proposal was performed.

REFERENCES

Ajlouni R (2019). A surface-stacking structural model for icosahedral quasicrystals. Struct Chem 30.

Alberti S, Gladfelter A, Mittag T (2019). Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. Cell 176, 419–434.

Banani SF, Lee HO, Hyman AA, Rosen MK (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 18, 285–298.

Banani SF, Rice AM, Peeples WB, Lin Y, Jain S, Parker R, Rosen MK (2016). Compositional control of phase-separated cellular bodies. Cell 166, 651–663.

Bechtel W (2005). Discovering Cell Mechanisms: The Creation of Modern Cell Biology, Cambridge, UK: Cambridge University Press.

Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, Jülicher F, Hyman AA (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324, 1729–1732.

Chemical & Engineering News (2011). 3D printed model of a virus self assembles when shaken. Available at: https://www.youtube.com/watch?v=br-YxeXWx6s (accessed February 23, 2023).

Choi J-M, Holehouse AS, Pappu RV (2020). Physical principles underlying the complex biology of intracellular phase transitions. Annu Rev Biophys 49, 107–133.

Currie SL, Rosen MK (2022). Using quantitative reconstitution to investigate multicomponent condensates. RNA 28, 27–35.

Dellaire G, Ching RW, Dehghani H, Ren Y, Bazett-Jones DP (2006). The number of PML nuclear bodies increases in early S phase by a fission mechanism. J Cell Sci 119, 1026–1033.

Eames C, Eames R (1977). Powers of ten and the relative size of things in the universe. https://www.youtube.com/watch?v=0fKBhvDjuy0 (accessed 20 July 2023).

Ellis RJ (2001). Macromolecular crowding: obvious but underappreciated. Trends Biochem Sci 26, 597–604.

Ellis RJ, Minton AP (2003). Cell biology: join the crowd. Nature 425, 27–28.

Fang X, *et al*. (2019). Arabidopsis FLL2 promotes liquid-liquid phase separation of polyadenylation complexes. Nature 569, 265–269.

Ferrolino MC, Mitrea DM, Michael JR, Kriwacki RW (2018). Compositional adaptability in NPM1-SURF6 scaffolding networks enabled by dynamic switching of phase separation mechanisms. Nat Commun 9, 5064.

Frey-Wyssling A (1937). Röntgenometrische vermessung der submikroskopischen räume in gerüstsubstanzen. Protoplasma 27, 372–411.

Geren BB, Schmitt FO (1954). The Structure of the Schwann cell and its relation to the axon in certain invertebrate nerve fibers. Proc Natl Acad Sci USA 40, 863–870.

Goodsell DS, Lasker K (2023). Integrative visualization of the molecular structure of a cellular microdomain. Protein Sci 32, e4577.

Grousl T, *et al*. (2009). Robust heat shock induces eIF2alpha-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast, Saccharomyces cerevisiae. J Cell Sci 122, 2078–2088.

Hardy WB (1899). On the structure of cell protoplasm: part I. The structure produced in a cell by fixative and post-mortem change. The structure of colloidal matter and the mechanism of setting and of coagulation. J Physiol 24, 158-210.1.

Heilbrunn LV (1926). The absolute viscosity of protoplasm. J Exp Zool 44, 255–278.

Hertwig O (1993). The chemico-physical and morphological Properties of the cell. ed. HJ Campbell, London, UK: Swan Sonnenschein & Co.

Höber R (1902). Physikalische Chemie der Zelle und der Gewebe, Leipzig: Wilhelm Engelmann.

Hyman AA, Weber CA, Jülicher F (2014). Liquid-liquid phase separation in biology. Annu Rev Cell Dev Biol 30, 39–58.

Johnson C (2004). Top scientific visualization research problems. IEEE Comput Graph Appl 24, 13–17.

Johnson GT, Autin L, Al-Alusi M, Goodsell DS, Sanner MF, Olson AJ (2015). cellPACK: a virtual mesoscope to model and visualize structural systems biology. Nat Methods 12, 85–91.

Judson HF (1996). The Eighth Day of Creation: Makers of the Revolution in Biology, Long Island, NY: CSHL Press.

Kaur T, Alshareedah I, Wang W, Ngo J, Moosa MM, Banerjee PR (2019). Molecular crowding tunes material states of ribonucleoprotein condensates. Biomolecules 9, 71.

Kölliker A (1845). Die Lehre von der thierischen Zelle. In: Zeitschrift Für Wissenschaftliche Botanik 1, 46–102.

Kuznetsova IM, Turoverov KK, Uversky VN (2014). What macromolecular crowding can do to a protein. Int J Mol Sci 15, 23090–23140.

Langdon EM, *et al*. (2018). mRNA structure determines specificity of a polyQ-driven phase separation. Science 360, 922–927.

Li P, *et al*. (2012). Phase transitions in the assembly of multivalent signalling proteins. Nature 483, 336–340.

Lin Y, Protter DSW, Rosen MK, Parker R (2015). Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. Mol Cell 60, 208–219.

Liu D (2017). The cell and protoplasm as container, object, and substance, 1835–1861. J Hist Biol 50, 889–925.

Liu D (2018). Heads and tails: molecular imagination and the lipid bilayer, 1917-1941. In: Visions of Cell Biology: Reflections Inspired by Cowdry's General Cytology, ed. K Matlin, J Maienschein, M Laubichler, Chicago, IL: University of Chicago Press, 209–245.

Lyon AS, Peeples WB, Rosen MK (2021). A framework for understanding the functions of biomolecular condensates across scales. Nat Rev Mol Cell Biol 22, 215–235.

Lyons B, *et al*. (2022). The Simularium Viewer: an interactive online tool for sharing spatiotemporal biological models. Nat Methods 19, 513–515.

Maritan M, Autin L, Karr J, Covert MW, Olson AJ, Goodsell DS (2022). Building structural models of a whole mycoplasma cell. J Mol Biol 434, 167351.

Matlin KS (2022). Crossing the Boundaries of Life: Günter Blobel and the Origins of Molecular Cell Biology, Chicago, IL: University of Chicago Press.

McSwiggen DT, Hansen AS, Teves SS, Marie-Nelly H, Hao Y, Heckert AB, Umemoto KK, Dugast-Darzacq C, Tjian R, Darzacq X (2019). Evidence for DNA-mediated nuclear compartmentalization distinct from phase separation. eLife 8, e47098.

Mertens R (2019). The Construction of Analogy-Based Research Programs: The Lock-and-Key Analogy in 20th Century Biochemistry, Bielefeld, Germany: Transcript Publishing.

Moses MJ (1956). Chromosomal structures in crayfish spermatocytes. J Biophys Biochem Cytol 2, 215–218.

Nott TJ, *et al*. (2015). Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. Mol Cell 57, 936–947.

Olby RC (1986). Structural and Dynamical Explanations in the World of Neglected Dimensions. In: History of Embryology, Cambridge, UK: Cambridge University Press, 275–308.

Perutz MF (1994). Linus Pauling. 1901-1994. Nat Struct Biol 1, 667–671.

Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Morris JH, Ferrin TE (2021). UCSF ChimeraX: structure visualization for researchers, educators, and developers. Protein Sci 30, 70–82.

Protter DSW, Rao BS, Van Treeck B, Lin Y, Mizoue L, Rosen MK, Parker R (2018). Intrinsically disordered regions can contribute promiscuous interactions to RNP granule assembly. Cell Rep 22, 1401–1412.

Rai AK, Chen J-X, Selbach M, Pelkmans L (2018). Kinase-controlled phase transition of membraneless organelles in mitosis. Nature 559, 211–216.

Reynolds A (2008). Amoebae as exemplary cells: the protean nature of an elementary organism. J Hist Biol 41, 307–337.

Reynolds AS (2018). The Third Lens: Metaphor and the Creation of Modern Cell Biology, Chicago, IL: University of Chicago Press.

Riback JA, Zhu L, Ferrolino MC, Tolbert M, Mitrea DM, Sanders DW, Wei M-T, Kriwacki RW, Brangwynne CP (2020). Composition-dependent thermodynamics of intracellular phase separation. Nature 581, 209–214.

Rocke AJ (2010). Image and Reality: Kekulé, Kopp, and the Scientific Imagination, Chicago, IL: University of Chicago Press.

Rog O, Köhler S, Dernburg AF (2017). The synaptonemal complex has liquid crystalline properties and spatially regulates meiotic recombination factors. eLife 6, e21455.

Ruff KM, Pappu RV (2021). AlphaFold and implications for intrinsically disordered proteins. J Mol Biol 433, 167208.

Ruff KM, Pappu RV, Holehouse AS (2019). Conformational preferences and phase behavior of intrinsically disordered low complexity sequences: insights from multiscale simulations. Curr Opin Struct Biol 56, 1–10.

Sanders DW, *et al*. (2020). Competing protein-RNA interaction networks control multiphase intracellular organization. Cell 181, 306–324.e28.

Schmidt HB, Görlich D (2015). Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permselectivity. eLife 4, e04251.

Schmidt WJ (1924). Die Bausteine des Tierkörpers in polarisiertem Lichte, Bonn: Friedrich Cohen.

Schmidt WJ (1941). Die Doppelbrechung des Protoplasmas und ihre Bedeutung für die Erforschung seines submikroskopischen Baues. Ergeb Physiol 44, 27–95.
Sjöstrand FS (1960). Morphology of ordered biological structures. Radiat Res 2, 349–386.
Walters RK, Gale EM, Barnoud J, Glowacki DR, Mulholland AJ (2022). The emerging potential of interactive virtual reality in drug discovery. Expert Opin Drug Discov 17, 685–698.
Wang J, *et al*. (2018). A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. Cell 174, 688–699.e16.
Woodruff JB, Ferreira Gomes B, Widlund PO, Mahamid J, Honigmann A, Hyman AA (2017). The centrosome is a selective condensate that nucleates microtubules by concentrating tubulin. Cell 169, 1066–1077.e10.
Xing W, Muhlrad D, Parker R, Rosen MK (2020). A quantitative inventory of yeast P body proteins reveals principles of composition and specificity. eLife 9, e56525.
Yang P, *et al*. (2020). G3BP1 is a tunable switch that triggers phase separation to assemble stress granules. Cell 181, 325–345.e28.
Zhang H, Elbaum-Garfinkle S, Langdon EM, Taylor N, Occhipinti P, Bridges AA, Brangwynne CP, Gladfelter AS (2015). RNA controls PolyQ protein phase transitions. Mol Cell 60, 220–230.
Zimmerman SB, Trach SO (1991). Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of Escherichia coli. J Mol Biol 222, 599–620.