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The purification of biomolecular condensates: Bottlenecks and strategies

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Biomolecular condensates are large assemblies of proteins and nucleic acids that form distinct compartments inside the cell without being surrounded by a membrane. They form through multivalent interactions, are not stereospecifically defined, and can scale with component addition. By concentrating specific biomolecules at specific times and cellular locations, condensates play key roles in many processes, such as transcription, RNP assembly, cell cycle, DNA repair, and stress responses. Condensate biology greatly benefited from systematic analyses of their composition. However, condensates often have heterogenous sizes and are built on interaction networks that include stable and labile components. They also have highly variable compositions and dynamics. Their purification thus represents a significant challenge, and it necessitates extensive testing and adaptation of techniques originally designed for other applications. This article aims to synthesize the existing empirical knowledge on the extraction and purification of cellular condensates and analyze the challenges inherent to this field.

What are condensates?

Condensates are large, membraneless cellular assemblies formed by multivalent interactions of varying strength and specificity. They are dynamic structures, lack a defined stereospecificity, and scale with component addition. Their membraneless property and their intermediate size between molecular complexes and membrane-bound compartments give them a unique role within the cell. Condensates have the property to concentrate specific biological molecules at a precise location within the cell. Hence, they have been described as enhancers or regulators of biological processes, as storage sites for certain proteins and RNAs, and as sites where certain phenomena occur, such as ribosomal RNA transcription, splicing, snRNP maturation, DNA repair, and microtubule nucleation (Jain et al., 2016; Cho et al., 2018; Beutel et al., 2019; Woodruff et al., 2017; Boija et al., 2018). Condensates have also become of increasing interest as potential compartments driving neurodegenerative diseases and cancer (Cai et al., 2021; Suzuki and Onimaru, 2022; Spannl et al., 2019; Banani et al., 2022; Boija et al., 2021; Alberti and Hyman, 2021).

While membraneless objects have long been observed (Cajal, 1903), the term condensate has been coined only recently to highlight the ability of P-granule proteins to form liquid-like droplets stemming from diffused components in the cytoplasm (Brangwynne et al., 2009). The term includes but is not limited to the liquid–liquid phase separation (LLPS) model that provides one theoretical basis for condensate formation (Banani et al., 2017). Models of condensate formation have been refined during recent years with the addition of microphases and the occurrence of both high- and low-affinity interactions, which better take into account the molecular heterogeneity within these assemblies (Latham et al., 2024, *Preprint*; Choi et al., 2020; Chattaraj and Shakhnovich, 2025). Stable interactions often originate from folded domains that form specific interactions between protein, RNA, and DNA, while intrinsically disordered regions can provide multivalent, less specific, and weaker interactions (Vernon et al., 2018; Wang et al., 2018). *In vitro* reconstitution of condensates using simplified systems abide with the LLPS model, which states that above a threshold of concentration in biomolecules, the mix of biomolecule–water (solute–solvent) is more stable by demixing into two distinct phases, one dense in biomolecules and the other dilute. This phenomenon can take place because the mean of solute–solute and solvent–solvent interactions is stronger than the solute–solvent interaction (Xu et al., 2023; Flory, 1953). Yet, these *in vitro* reconstitutions often lack the molecular complexity of the cellular milieu and in particular competing nonspecific interactions (Musacchio, 2022). In addition, the LLPS model only partly explain *in vivo* observations (McSwiggen et al., 2019; Riback et al., 2020; Hedtfeld et al., 2024), which, in contrast, can often be understood by considering high-affinity interactions between condensate components (Hedtfeld et al., 2024). Methods have been proposed to

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distinguish condensate assembly models and, in particular, LLPS from condensate assembly driven by high-affinity interactions (Hedtfeld et al., 2024). This issue is directly relevant here as the forces driving condensate formation will be predictive of how condensates behave during purification, as discussed below. Interestingly, several condensates have been found to resist lysis and purification conditions (Bornens et al., 1987; Andersen et al., 2002; Wallace et al., 2015; Hubstenberger et al., 2017; An et al., 2019), suggesting a key contribution of high-affinity, specific interactions for their maintenance.

Condensates generally undergo dynamic formation and dissolution while continuously exchanging with their surrounding environment, as proven by FRAP experiments (Taylor et al., 2019). For a given condensate, the exchange rates of its components can, however, vary strongly, with some molecules exchanging rapidly and others stably associated with the condensates (e.g., DCP1 vs. DCP2 in P bodies, Xing et al., 2020; G3BP1 and mRNAs in stress granules; Moon et al., 2020; Parker et al., 2025). This difference suggests a model where "Scaffold molecules" participate in the condensate architecture while "Client molecules" shuttle in and out of condensates (Banani et al., 2016). The dynamic behavior of condensate is also evident in the cellular response to stress, like DNA damage, heat shock, or oxidative stress, during which the cell is able to rapidly form condensates and dissolve them when the stress is relieved. Dysfunction of condensate dynamics represents an underlying cause of neurodegenerative diseases, where the accumulation of condensates and their transition from a dynamic liquid state to a gel or solid state is a key factor (Alberti and Hyman, 2016; Murakami et al., 2015; Molliex et al., 2015).

In addition to the interplay of passive binding events, it has been demonstrated that active processes requiring ATP are required to maintain condensate exchange with their surrounding environment (Jain et al., 2016; Brangwynne et al., 2011), and several chaperones have been shown to be able to extract proteins from condensates (Buchan, 2024; Bard and Drummond, 2024; Brunello et al., 2025). Enzymes that induce post-translational modifications (PTMs) of condensate proteins can also alter the condensate interaction network. Among these PTMs, SUMOylation (Keiten-Schmitz et al., 2021; Alghoul et al., 2023), phosphorylation (Sridharan et al., 2022; Schisa and Elaswad, 2021), PARylation (Leung, 2020), and methylation (Schisa and Elaswad, 2021; Courchaine et al., 2021; Lee et al., 2021) provide rapid mechanisms to affect condensate formation or dissolution. For example, promyelocytic leukemia protein (PML) mono-SUMOylation maintains the assembly of PML nuclear bodies, but oxidative stress triggers poly-SUMOylation of PML and causes PML nuclear body disassembly (Tatham et al., 2008). In addition to these targeted mechanisms, changes in the level of scaffold condensate biomolecules may play a role in the regulation of condensate formation on longer time scales.

Methods to analyze condensate composition

Given the multitude of distinct biomolecules that comprise cell condensates, an investigation of a given condensate through the lens of a single protein is inherently limited and incomplete. To precisely characterize their composition and interaction network, regulation, dynamics, and response to environmental changes, methods analyzing the condensate as a whole are needed. There are three main methods to screen condensate components: microscopy screens, proximity labeling (PL), and purification.

Microscopy screens

To screen for condensate proteins, microscopy is an interesting tool as the analysis can be straightforward. Usually "scaffold" proteins or RNAs (like DCP1A protein for P bodies; Fillman and Lykke-Andersen, 2005; or Neat1_2 RNA for paraspeckles; Sasaki et al., 2009) can be labeled by IF or FISH or genetically tagged and used as reporters for a given condensate. Other putative biomolecules will then be screened for colocalization with these reporters. The main advantage of microscopy screens is the non-denaturation of cells, although fixation sometimes induces condensation artifacts (Irgen-Gioro et al., 2022). Depending on the microscope, the resolution size may limit observations: smaller condensates, around 100 nm or less, can evade detection, and the presence of weakly enriched client proteins may be also difficult to assess, inducing false negative results. Also, the addition of tags to condensate scaffold proteins could modify their interaction network, their stability, and create artifacts. Furthermore, microscopy screens are labor intensive and require a large amount of antibodies and/or RNA probes that add up to a high final cost.

Proximity Labeling

This technique uses as bait a fusion between a condensate resident protein and one of several dedicated enzymes. These enzymes catalyze the tagging of a diffuse chemical (e.g., biotin for BioID) onto other molecules present in a close radius (~1–10 nm; Qin et al., 2021). Labeled molecules that are in proximity to the condensate bait can then be enriched and identified.

Compared with microscopy, PL labels client proteins and proteins independently of condensate size. It also has the advantage of being a non-denaturating method, as the labeling is done *in vivo*. Yet, some techniques based on the APEX tag need to introduce hydrogen peroxide, leading to a stress in the cell that could modify condensates. Moreover, as for microscopy, adding tags such as APEX (28 kDa) or Turbo-ID (34 kDa) to the N or C terminus of scaffold proteins could modify the structure and dynamics of condensates by itself, although several smaller tags (BioID2, 24 KDa or UltraID, 22 KDa) have been engineered to minimize their impact on protein behavior. It is also important to note that the bait is always present at low concentration throughout the cell in addition to being enriched in the condensate, generating background labeling. Newer versions of PL systems using split and/or opto-manipulable enzymes have been developed to circumvent this issue (Lee et al., 2023).

Purification

Another way to study condensate composition is to purify them and identify their components by mass spectrometry or RNA sequencing. This approach is tempting as isolating a condensate gives access to all its components through a single experiment. It can be conducted by pulling down genetically engineered bait proteins to extract condensates out of the cell lysate (Safieddine et al., 2024; Hubstenberger et al., 2017; Carden et al., 2023; Reddy

et al., 2023; Matheny et al., 2019; Chen et al., 2023, *Preprint*) or by using their different physical properties to separate them from the lysate (Bornens et al., 1987; Moritz et al., 1995; Vogel et al., 1997; Neil et al., 2021; An et al., 2019). The main drawback of purification is the need to open the cell to free the condensates. Such a perilous operation, like disrupting cell barrier with chemical or mechanical means, will also change the condensate environment. The goal of the purification process is to mitigate the disturbance as much as possible to preserve the condensate integrity as discussed below. While often deemed difficult to carry out, purification of biomolecular condensates is the source of many discoveries (Safieddine et al., 2024; Hubstenberger et al., 2017; Carden et al., 2023; Reddy et al., 2023; Sridharan et al., 2022). In the case of condensates that have been implicated in diseases, such as ALS, Alzheimer's, Parkinson's, other tauopathies or cancer, a comparison of the composition of purified condensates could provide crucial information on the rearrangements between native and pathological condensates, thus refining our knowledge of pathological processes (Spannl et al., 2019). The challenges in purifying condensates originate from their defining properties: molecular complexity with stable and labile components, size heterogeneity, and the diversity of the condensates themselves, with each having its own biochemical properties.

Existing experimental techniques for condensate purification

The scientific community has long been engaged in the pursuit of effective condensate purification techniques (Muramatsu et al., 1963). The entire process can be subdivided into two principal steps: the initial extraction by cell lysis and the subsequent enrichment or purification of a condensate.

The challenge of cell lysis

The primary challenge in the purification of condensates stems from their unbound nature, which renders them sensitive to even mild alterations of their surrounding environment. In contrast to protein complexes, which are formed by stable interactions between folded domains, condensate components often include weaker and more labile interactions (Fig. 1 A). Therefore, the cell lysis step must be executed with precision and delicacy to ensure the integrity of the condensates. This can be achieved either through chemical means, by adding salt and detergent to cells (Bornens et al., 1987; Mintz, 1999; Andersen et al., 2003; Saitoh et al., 2004; Schulz et al., 2006; Gogendeau et al., 2015; Hubstenberger et al., 2017; Matheny et al., 2019; An et al., 2019; Neil et al., 2021; Reddy et al., 2023; Carden et al., 2023), or through physical means, by shearing (Reddy et al., 2023; Yang et al., 2022; Jamieson-Lucy and Mullins, 2019), sonication (Lam et al., 2002; Andersen et al., 2002), or grinding (Wallace et al., 2015; Glauninger et al., 2024, *Preprint*; Keyport Kik et al., 2024). The aforementioned methods have each their respective limitations.

Chemical lysis. The composition of the lysis buffer is of paramount importance in the context of chemical lysis. The reagents commonly used are salts and detergents. The concentration of salt can induce cell swelling in hypotonic conditions, which is characterized by a low salt concentration of <150 mM equivalent of Na^+Cl^-. Conversely, hypertonic buffers cause cell shrinkage (Koeppen and Stanton, 2013). Hypotonic buffers on their own can be used to open the plasma membrane by causing excessive cell swelling, which releases the cytoplasm and allows fractionation between the nucleus and cytoplasm. Hence, hypotonic buffer can also be used during the first lysis step for nuclear condensate purification (Gogendeau et al., 2015; Bornens et al., 1987; Bornens and Moudjou, 1998; Andersen et al., 2003).

From a general standpoint, ions concentration and composition exert a significant influence on the stability and fluidity of condensates (Morishita et al., 2023). Indeed, salts are regulating the charges in the condensate environment and therefore affect the strengths of electrostatic interactions and cation–pi interactions (MacAinsh et al., 2024; Fig. 1 B).

With regard to divalent cations, Ca^{2+} concentrations below 3 mM or above 5 mM result in the disruption of extracted nucleoli (Muramatsu et al., 1963). A similar phenomenon has also been observed in *Drosophila melanogaster*, where the addition of 50 mM $Mg^{2+}Cl^-_2$ reduces the size of Me31B foci extracted from *Drosophila* oocytes (Sankaranarayanan et al., 2021). During the purification of centrosomes from cell lysates, precise tuning of $Mg^{2+}Cl^-_2$ concentration is crucial for the dissociation of centrosomes from nuclei (Gogendeau et al., 2015). The effect of multivalent cations such as Mg^{2+} and Ca^{2+} on the *in vitro* condensation of DEAD box helicase proteins DDX4 (the human ortholog of *Drosophila* Vasa) and DDX3 (the human ortholog of *Drosophila* Belle) has also been the subject of recent investigation, showing that the alteration in condensation is due to the shielding of negatively charged amino acids on the protein by the divalent cations (Nott et al., 2015; Crabtree et al., 2023). The impact of Hoffmeister ion series on condensates has also been investigated utilizing elastin-like polypeptide and resilin-like polypeptides as models (Zhu et al., 2024). This study demonstrated that all added ions influence the microenvironment of the proteins, modifying the polarity, viscosity, mobility, and viscoelasticity of these condensates (Zhu et al., 2024).

Accordingly, empirical evidence suggests that most cytoplasmic condensates are extracted under isotonic or lightly hypotonic conditions, characterized by a concentration of 100–150 mM Na^+Cl^- and 2–5 mM $Mg^{2+}Cl^-_2$ or $Mg^{2+}(OAc)^-_2$ (Table1). One major exception is the centrosome, which was one of the first condensates to be purified (Bornens et al., 1987). In this case, the buffer is hypotonic with almost no monovalent ions but contains mild detergent. One possible explanation for this exception is that the specific structure of centrosomes makes them probably more resistant to harsh lysis conditions.

Detergents like Triton X-100, NP-40, or deoxycholate are the main lysis chemicals employed in the purification of condensates. They facilitate the opening of lipidic cell membranes but are also known to denature proteins at high concentrations (Seddon et al., 2004; Anson, 1939). Their amphiphilic properties can modify the environment of proteins, solvating hydrophobic regions and challenging the equilibrium of condensates (Fig. 1 C). Nevertheless, a precise quantification of the effect of detergents on condensates, both *in vitro* and *in cellulo* is missing. Further research in this area could provide a robust foundation

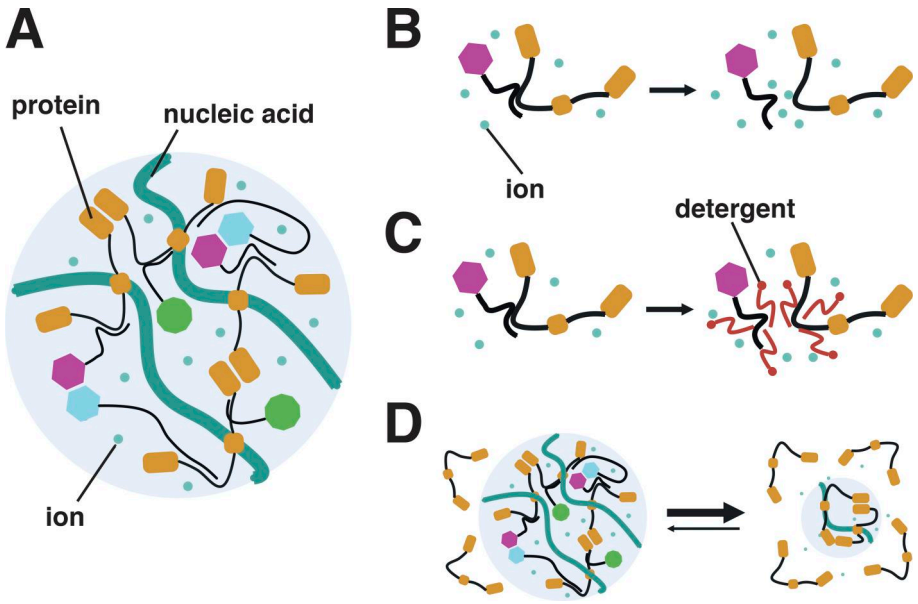

Figure 1. **Effect of lysis on condensate interaction network. (A–D)** Schematics of a condensate. Proteins and nucleic acids scaffold the condensate, creating a microenvironment rich in certain proteins and nucleic acids, with its own pH and ion composition. During chemical lysis, the three major factors that destabilize condensates are (top to bottom): B increasing the overall concentration of salt molecules that can disrupt the structures within biomolecules and the interactions based on hydrogen bindings, electrostatic bindings, and cation–pi bindings; C addition of detergent molecules that can bind to hydrophobic regions of proteins and disrupt hydrophobic pockets within condensates; D the dilution effect that favors a state with more free molecules outside of condensates. Figure created using Biorender (https://biorender.com/).

for determining the optimal detergent choice and establishing a rationale for the quantity of detergent to be used during cell lysis.

In experimental buffers, typical detergents concentrations for plasma membrane lysis are 0.5% NP-40 or 0.2–0.3% Triton X-100 (Table 1). Nuclear condensates require an additional fractionation step to remove the cytoplasm and a more concentrated detergent, up to 1% Triton X-100, or the use of a more potent detergent like deoxycholate. Nuclear membrane opening can also require a high Na^+Cl^- concentration, up to 500 mM. This is a cause for concern since these harsh conditions may disrupt condensate structure, but to date there is no better alternative to perform nuclear lysis using only chemicals. All buffers include antiproteases, with many also including RNase inhibitors, to protect condensates from degradative activities. All steps are conducted at a low temperature to minimize degradation of biological samples and condensate dissolution.

Mechanical lysis. Mechanical lysis does not modify the chemical environment of condensates; however, it introduces a significant amount of energy into the system, which is sufficient to destroy the plasma membrane.

From a general standpoint, there are three principal methods for achieving mechanical lysis: shearing, grinding, and sonication. Homogenizers operate through the process of shearing, which is generated by the application of a tangential force to the sample. Dounce homogenizer has been employed for the preparation of samples in nuclear condensate purification (Reddy et al., 2023). Grinding relies on the creation of friction through the sandwiching of the sample between two hard surfaces that slide against each other (Burden, 2012) and has been successfully used for the purification of yeast stress granules through cryogenic milling (Wallace et al., 2015; Glauninger et al., 2024, *Preprint*). In the sonication method, sound waves migrate through the medium and induce pressure variations. The generated acoustic cavitations grow and collapse, applying high shear forces that cause disruptions (Zhang et al., 2007). Particularly in the case of nuclear condensates, such as nucleolus and Cajal bodies, sonication has been employed for the simultaneous disruption of the cytoplasmic membrane and the nuclear envelope, as well as chromatin (Lam et al., 2002; Muramatsu et al., 1963).

To mitigate the risk of overheating, the cell suspension is subjected to Dounce homogenization or sonication in ice-chilled conditions or is grinded with a cryogenic apparatus. There is, however, a chance that localized high-pressure/high-temperature points exist, which could compromise the integrity of condensates. However, the extent of this alteration remains to be determined empirically, as there is currently a lack of studies on the impact of mechanical lysis on condensate integrity. With regard to sonication, the collapse of cavitation can result in water thermolysis, leading to the generation of free radicals, including H•, HO•, and HOO•, which can interact with biological molecules and cause a modification of binding strengths within condensates (Riesz et al., 1985; Petrier et al., 1998).

Other factors to consider

The dilution factor. During lysis, cell extracts are diluted in lysis buffer with a wide range of dilution factors. Concerning human cell culture, when the information is available, between 1 and 20 million cells are typically resuspended between 500 µl

Table 1. **Comparison of chemical lysis buffers for condensate extraction**

Publications	Target condensate	Lysed compartment	Lysis buffer	Salts	Detergent[a]	Organism/cell type
Bornens et al. (1987), Bornens and Moudjou (1998), Andersen et al. (2003), Gogendeau et al. (2015)	Centrosome	Cytoplasm	1 mM Tris-HCl, pH 8.0	0.5 mM $MgCl_2$	0.5% IGEPAL CA-630	KE37 cells
Schulz et al. (2006)	Centrosome	Cytoplasm	100 mM Na-PIPES, pH 6.9	2 mM $MgCl_2$	0.3% Triton X-100	*Dictyostelium discoideum*
Carden et al. (2023)	Centrosome	Cytoplasm	50 mM Tris-HCl, pH 8.0	150 mM NaCl	1% (vol/vol) NP-40, 0.5% (wt/vol) Na-deoxycholate, and 0.1% (wt/vol) SDS	HEK293T
Neil et al. (2021)	L-body	Cytoplasm	10 mM HEPES, pH 7.4	100 mM KOAc and 3 mM MgCOAc	0.05% NP-40	*X. leavis* oocyte
Hubstenberger et al. (2017), Safieddine et al. (2024)	P body	Cytoplasm	50 mM Tris, pH 7.4	150 mM NaCl	0.2% Triton X-100	HEK293
Zhou et al. (2024) [b]	DHX9 stress granules	Cytoplasm	50 mM HEPES, pH 7.5	150 mM KCl	1% NP-40	HeLa
Matheny et al. (2019)	Stress granule and P body	Cytoplasm	50 mM Tris HCl, pH 7.4	100 mM KOAc and 2 mM MgOAc	0.5% NP-40	U2OS cells
Mintz (1999), Saitoh et al. (2004)	Nuclear speckle	Nucleus	10 mM Tris-HCl, pH 7.4	500 mM NaCl and 5 mM MgCl2	1% Triton	Swiss Webster female mice liver
Reddy et al. (2023)	Paraspeckle	Nucleus[c]	10 mM Tris-HCl, pH 7.5	150 mM NaCl	1% Triton X-100 and 0.1% deoxycholate	HEK293FRT
An et al. (2019)	Paraspeckle-like	Nucleus	50 mM Tris HCl, pH 7.4	400 mM NaCl, 100 mM KOAc, and 2 mM MgOAc	0.5% NP-40	HEK cells
Jain et al. (2016), Wheeler et al. (2017)	Stress granules	Cytoplasm	50 mM Tris HCl, pH 7.4	100 mM KOAc and 2 mM MgCOAc	0.5% NP-40	Yeast strain BY4741

[a]Tendencies here are that cytoplasmic condensates are extracted under mild conditions with light hypotonicity or isotonicity of the buffer and with mild, low-concentrated detergent. One noticeable exception is the centrosome, for which harsher lysis conditions have been used.
[b]HeLa cells were fixed with paraformaldehyde prior to the lysis process.
[c]Nuclear condensates require a high concentration of salt or a more potent detergent. This has to be taken into consideration while examining the results from these papers, as condensates might have been degraded.

and 1.5 ml (Safieddine et al., 2024; Reddy et al., 2023; Matheny et al., 2019). For yeasts, usually, 50 ml of culture between 0.4 and 0.6 OD_{660}, which represents around 750 million cells, is re-suspended between 100 and 500 µl (Glauninger et al., 2024, *Preprint*; Keyport Kik et al., 2024; Jain et al., 2016; Wallace et al., 2015). This dilution alters the equilibrium between proteins outside and inside condensates and can result in the partial or total dissolution of condensates (Fig. 1 D). To date, there is a lack of information in this area, illustrated by the absence of data on the number of cells pelleted, which could be used to calculate the dilution factor. However, a recent study made use of the substantial cytoplasm content of unfertilized *Xenopus laevis* eggs to work with an undiluted cytoplasm, and a dilution experiment demonstrated that a dilution of as little as 1.2X had an impact on the assembly of several condensate proteins (Keber et al., 2024).

Nucleocytoplasmic fractionation. A fractionation step is frequently employed during condensate purification to separate the cytoplasm from the nucleus. This step is particularly susceptible to contamination due to the potential for proteins to leak from one compartment to another during lysis. Free condensate proteins in the cytoplasm and nucleoplasm can thus easily cross loosened membranes. In addition, *in vitro* experiments have shown that condensate proteins form nanometric clusters ranging from a few 10 nm to a few 100 nm (Kar et al., 2022; Gil-Garcia et al., 2024; Tsoi et al., 2024) and that these clusters are precursors of larger scale condensates. These *in vitro* observations of nanoclusters have been confirmed by membrane filtering from cell extracts (Keber et al., 2024). These nanoclusters, like monomeric condensate proteins, may also cross loosened membranes during fractionation and leak from one compartment into the other.

Condensate purification

Differential centrifugation. Differential centrifugation (Fig. 2 A) is one of the earliest tools that was adapted for condensate enrichment. It is straightforward to implement and does not necessitate any further chemical modification of the condensate environment. Differential centrifugation is based on the differences in sedimentation coefficients between the various particles

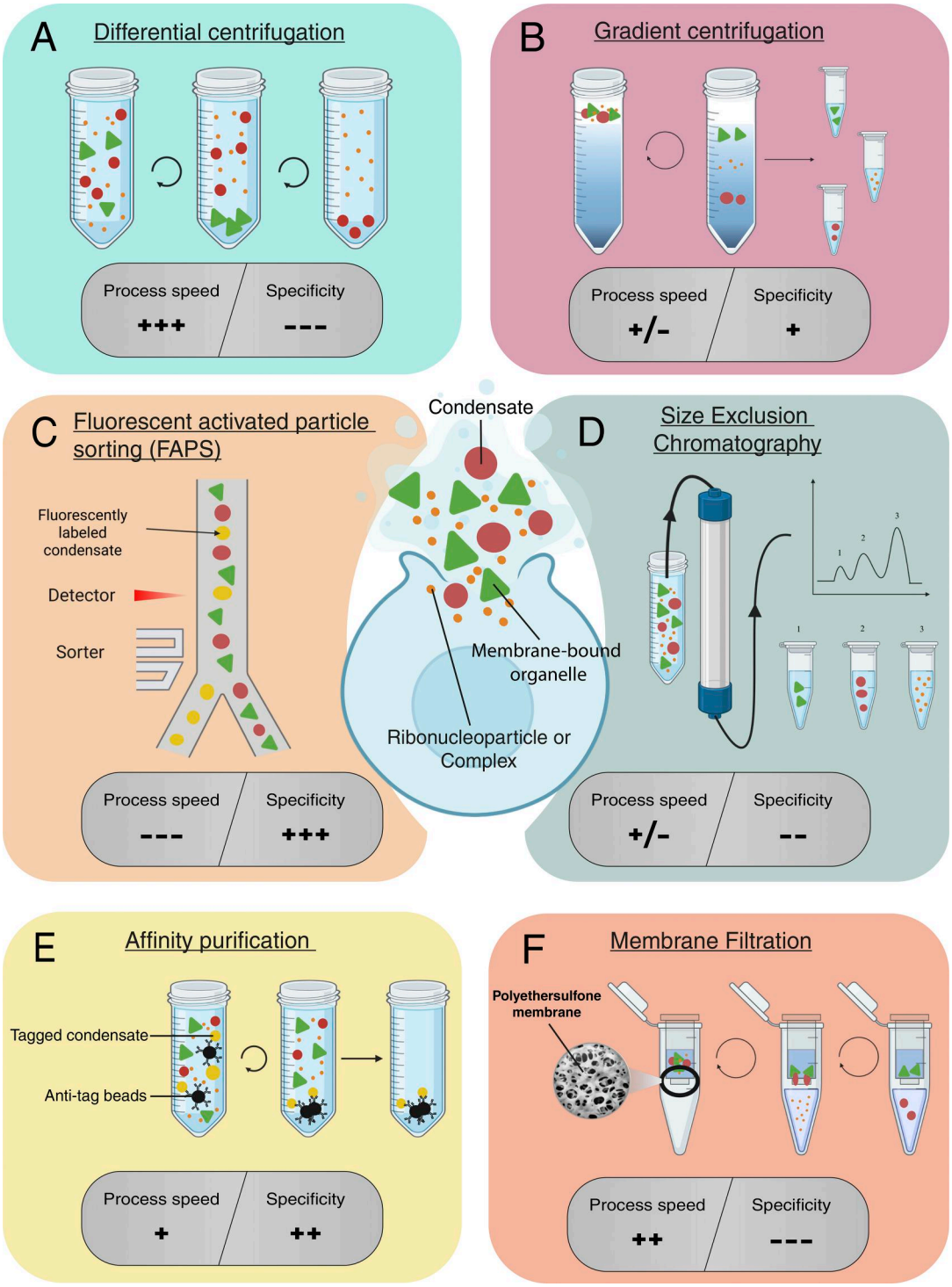

Figure 2. **Condensate purification methods. (A)** The six main ways to purify or enrich condensates: (A) Differential centrifugation postulates that condensates have a specific sedimentation coefficient range that allows their purification through several cycles of centrifugation. **(B)** Gradient centrifugation allows purification of condensates based on their specific densities. **(C)** FAPS takes advantage of flow cytometry to separate fluorescently tagged condensates from the rest of the lysate. **(D)** SEC separates the different particles based on their retention time on porous beads. **(E)** Tagged condensate proteins can be extracted from the rest of the lysate by antibodies grafted on beads. **(F)** Membrane filtration allows for quick sorting between condensate structures and smaller complexes. FAPS, fluorescence-activated particle sorting. Figure created using Biorender (https://biorender.com/).

Table 2. **Centrifugation settings for condensate purification or enrichment**

Publications	Condensate	Compartment	Organism/ cell type	Last debris pelleting settings	Condensate pelleting settings	Sedimentation coefficient lower bound (S)[a]	Sedimentation coefficient high bound (S)
Mintz (1999)	Nuclear speckle	Nucleus	Swiss Webster female mice liver	20,800 g × 2 min	157,000 g × 1 h	3.61E+01	8.18E+03
Hubstenberger et al. (2017)	P body	Cytoplasm	HEK293	200 g × 5 min	10,000 g × 7 min	4.86E+03	3.40E+05
Teixeira et al. (2005)	P body	Cytoplasm	Yeast	2,000 g × 2 min	10,000 g × 10 min	3.40E+03	8.50E+04
An et al. (2019)	Paraspeckle-like	Nucleus	HEK	1,000 g × 5 min	17,000 g × 20 min	1.00E+03	6.80E+04
Jain et al. (2016), Wheeler et al. (2017)	Stress granule	Cytoplasm	Yeast strain BY4741	1,000 g × 5 min	18,000 g × 15 min	1.26E+03	6.80E+04
Wallace et al. (2015)	Stress granule	Cytoplasm	Yeast strain BY4741	3,000 g × 30 s	100,000 g × 20 min	1.70E+02	2.27E+05
Glauninger et al. (2024, *Preprint*)	Stress granule	Cytoplasm	Yeast strain BY4741	3,000 g × 30 s	20,000 g × 10 min	1.70E+03	2.27E+05
Matheny et al. (2019)	Stress granule and P body	Cytoplasm	U2OS cells	1,000 g × 5 min	16,000 g × 20 min	1.06E+03	6.80E+04
Namkoong et al. (2018)	Stress granule and P body	Cytoplasm	HEK293/ NIH3T3	2,000 g × 2 min	10,000 g × 10 min	3.40E+03	8.50E+04

[a]The sedimentation coefficient is defined as $s = v/a$, where a is the acceleration of the particle given by the centrifugation settings and v is the linear speed. The unit of s is the Svedberg (S): 1 S = 1.10^{-13} s. There is a strong assumption here that the distance traveled by the particle is in the range of 2.10^{-2} m, which corresponds to the height of regular Eppendorf tubes of 1.5 and 2 ml. Yet, the order of magnitude remains valid.

present in the solution. If the sedimentation coefficients of membranes, membrane-bound organelles, soluble proteins, and condensates differ, they can be separated by successive centrifugation steps. Yet, the properties of condensates are largely unknown, and their sedimentation coefficients and variability still have to be determined. However, centrifugation has been employed to purify or enrich condensates with known acceleration, time, and tube dimensions, allowing us to approximate the sedimentation coefficient range for each experiment.

Experimental data indicate that condensates are pelleted in the range of 10^2 to 5.10^3 S (where S is the Svedberg unit, 1 S = 10^{-13} s) and that debris and contaminants are removed with centrifugation steps that pellet particles around 10^5S (Table 2). Comparatively, mitochondria have a sedimentation coefficient between 5.10^3 and 10^5 S (Mertens-Strijthagen and De Schryver, 1989; Slinde et al., 1976), and polysomes are in the range of 80 to 500 S (Cross, 1970; Morton, 1974). Therefore, there is a range of possible centrifugation speed for purifying condensates.

However, the size and sedimentation characteristics of a given condensate can be highly variable and may exhibit considerable overlap between different condensate types or cellular debris. Consequently, empirical trials are necessary to refine the experimental procedure and identify the optimal centrifugation settings for each type of condensate.

Gradient centrifugation. Gradient ultracentrifugation (Fig. 2 B) is a related technique that enables the separation of cell components based on their densities or on their size and mass. Gradients are highly concentrated in sucrose or glycerol, and this change in the chemical environment may affect the integrity of the condensates, although it should remain mild compared with the lysis process. While this method is more time consuming than differential centrifugation and requires ultracentrifuge equipment, it is a powerful tool for the precise isolation of an object from a lysate. In particular, this method has been used to purify centrosomes from a wide range of organisms (Bornens et al., 1987; Moritz et al., 1995; Vogel et al., 1997; Gräf et al., 1998; Carden et al., 2023).

Fluorescence-activated particle sorting. This method has been developed for the purification of P bodies using a flow particle sorting machine (Fig. 2 C). By fluorescently labeling LSM14A, a core protein of the P body, and preclearing the cell lysate by centrifugation, P bodies were sorted and analyzed by proteomics and RNA-seq (Hubstenberger et al., 2017; Safieddine et al., 2024). However, the requirement for a large number of cells to provide sufficient material for analysis, coupled with the lengthy process, represents a significant challenge, while the advantage is that it provides condensates of very good purity. This method has also been used to purify GFP-FUS droplets in HEK293T cells (Reber et al., 2021), nucleoli and Cajal bodies in *Arabidopsis thaliana* (Pontvianne et al., 2016; Zhou et al., 2025, *Preprint*), and DHX9 stress granules from HeLa cells (Zhou et al., 2024), demonstrating its versatility.

Size exclusion. An alternative purification method for condensates is size-exclusion chromatography (SEC), which

exploits the distinctive size profile of these structures (Fig. 2 D). In this method, the cell lysate is introduced into a column containing a porous bead matrix. If the particles present in the solution are of a smaller diameter than that of the pores, they will elute at a later point. This technique also yields several fractions that require further analysis to determine which condensates have eluted. SEC should be employed with caution, as condensates possess liquid-like properties and may deform on the column, potentially leading to prolonged retention times. Additionally, the buffer must be adapted to maintain condensate integrity. This method has been successfully employed to purify L-bodies from *X. laevis* oocytes (Neil et al., 2021).

Affinity purification. To obtain a specific condensate from a condensate-enriched fraction, a labeled protein can be employed as a marker of this condensate. This protein can be immunoprecipitated, resulting in the isolation of the condensate (Fig. 2 E). Yet, to achieve an optimal purification, the condensate sample must be free of labeled molecules in the dilute phase. Paraspeckles (Reddy et al., 2023), centrosomes (Carden et al., 2023), P bodies (Matheny et al., 2019), and stress granules (Matheny et al., 2019) have been purified in this manner, typically following an enrichment process involving centrifugation steps to remove proteins of the dilute phase. A recent study took advantage of improvements in image treatment to identify condensates *in cellulo* in fixed cells and photolabel them with biotin, allowing further affinity purification of their components (Chen et al., 2023, *Preprint*). In this manner, proteins in condensates are precisely targeted, avoiding contaminants from the dilute phase.

Membrane filtration. A recent experiment using filtration on polyethersulfone membranes demonstrated that the passage time through the membrane was significantly higher for condensed proteins, which need to deform to go through membrane pores (Keber et al., 2024) (Fig. 2 F). This method represents a promising tool with the potential to be adapted as a new technique for condensate enrichment.

Empirical data lead to a paradox that needs to be (dis)solved

The question is what enables condensates to resist the purification process. Initial size could play a role in their resistance to environment modification. Small condensates detected in *X. laevis* egg lysates dissolve promptly upon dilution (Keber et al., 2024), whereas micrometer size condensates may be more resistant to dilution. It could also be a difference in nature, as only a handful of known condensates have been successfully purified so far (stress granules, centrosomes, P bodies, paraspeckles, nuclear speckles, nucleolus, and Cajal bodies; Hubstenberger et al., 2017; Bornens et al., 1987; Saitoh et al., 2004; Lam et al., 2002; Jain et al., 2016; Reddy et al., 2023; Muramatsu et al., 1963), and maybe these are more stable than others. Because these experiments yielded purified condensates from biological samples, we must acknowledge the resistance of some condensates to mild to harsh chemical or physical conditions, as well as to high dilution factors. In stark contrast to the studies of reconstituted condensates *in vitro*, which have shown that condensates are extremely sensitive to the chemistry of their environment (Zhu et al., 2024; Crabtree et al., 2023; Grese et al., 2021; Qamar et al., 2018).

In vitro condensates are frequently composed of a few selected components, including one or a few proteins often containing intrinsically disordered regions occasionally combined with a nucleic acid. In this experimental setup, the modification of a few factors, like salt level, pH, or concentration of biomolecules, may have a profound impact. By contrast, *in cellulo* condensates, due to their intricate and more complex network of interactions, may tolerate these modifications more readily. Moreover, *in vitro* condensates are often created in a simple chemical environment that may not fully capture the equilibrium between the condensate and its environment in the cell. One solution to create *in vitro* condensates more accurately could be the use of more complex setups that better recapitulate the physical and chemical properties inside the cell (Hedtfeld et al., 2024).

So where is the truth? It is challenging to ascertain to which extent purified condensates are altered throughout the purification process, and it may thus be wise to use multiple purification methods in parallel. This also highlights the necessity for rigorous monitoring of condensate integrity during purification and conducting validation experiments as discussed below. Nearly all the experiments conducted so far have incorporated one or more controls to assess condensate integrity, including microscopy imaging, comparison of western blots or proteomics results with preexisting databases (from mass spectrometry or microscopy), or size-exclusion assays.

How to control condensate integrity during purification

A significant challenge in working with cell condensates is the difficulty in establishing robust controls. Often, the lack of data on the composition of condensates and on the effect of lysis conditions makes it difficult to control condensate integrity with one method. Consequently, the most effective approach at present appears to be the combination of several orthogonal methods to validate the purification process.

Microscopy

Microscopy represents the most straightforward approach to visualize condensates during the lysis process (Fig. 3 A). The most common way is expressing a fluorescent protein fused to a condensate protein. This allows for the observation of condensates in cells and throughout the purification process, enabling the assessment of their size and shape. As always, the utmost caution is required, as many studies demonstrate that increasing the protein concentration results in more robust and artificial condensation. It is therefore recommended that transient transfection or stably overexpressed proteins be avoided. To prevent overexpression, insertion of modified proteins within the genome with CRISPR-Cas9 engineering allows expression of fluorescent proteins at their endogenous level. Additionally, special care should be taken when choosing the fused tag, as some are capable of oligomerizing, which can induce a bias in condensate formation (Jain et al., 2001). Furthermore, it has recently been demonstrated that the choice of fluorescent protein tag and its position in the fused protein can influence the size, shape, and number of condensates even for monomeric fluorescent tags (Uebel and Phillips, 2019; Zhou and Narlikar,

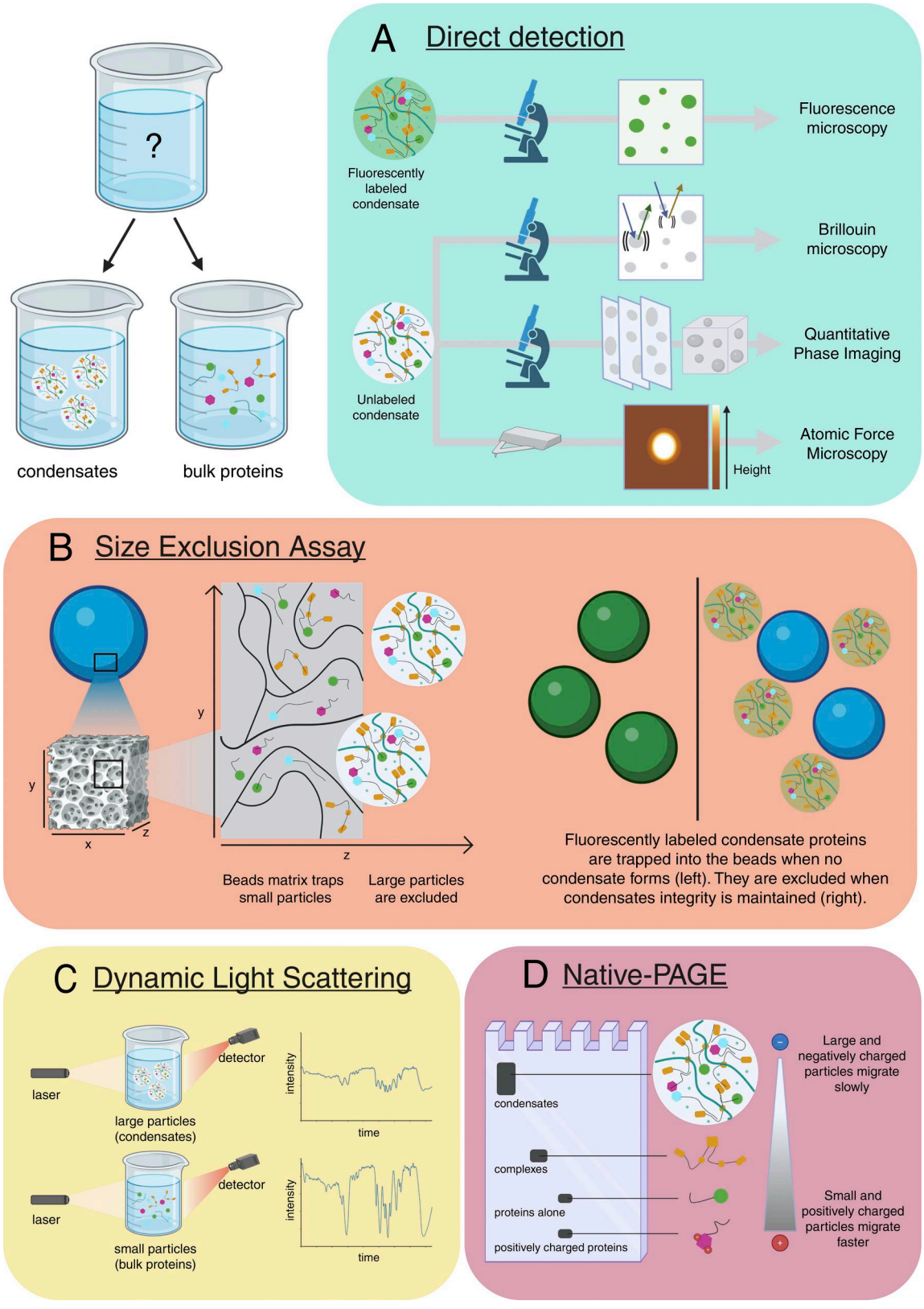

Figure 3. **Condensates detection methods. (A)** Microscopy methods used to observe condensates can be divided into two groups: Fluorescently labeled condensates can be directly observed by fluorescence microscopy. Other methods use physical properties of condensates to detect them without labeling. **(B–D)** Size-exclusion beads packed in a column or dispersed in the sample discriminate condensates based on their size. (C) Dynamic light scattering takes advantage of Rayleigh diffusion from particles in the medium to assess the particles size. (D) Proteins and particles migration speed in native PAGE vary accordingly with their size and charge. Figure created using Biorender (https://biorender.com/).

2023, *Preprint*; Pandey et al., 2024; Barkley et al., 2024; Fatti et al., 2025).

Additionally, purification has also been monitored by transmission electron microscopy in the case of nucleoli (Andersen et al., 2002), nuclear speckles (Mintz, 1999), and centrosomes (Bornens et al., 1987). With its nanometric resolution, it offers a distinctive insight into the structural characteristics of the purified entity and thus a better quality assessment. Other less common microscopy methods, like Brillouin microscopy (Santis et al., 2019; Schlüßler et al., 2022; Antonacci et al., 2018), quantitative phase imaging (Hong et al., 2021), or atomic force microscopy (Yamasaki et al., 2020; Singatulina et al., 2019), have been used to detect condensates based on their physical properties. For more insight on condensate microscopy, see this review by Ibrahim et al. (2024) (Ibrahim et al., 2024).

All of these methods have different advantages and drawbacks concerning image resolution, sample preparation, ability to do time-lapse, or availability of the machines.

Size-exclusion assays

Size-exclusion techniques are employed to ascertain whether the size of the condensate remains constant throughout the purification process (Fig. 3 B). This approach, though, does require prior knowledge of the size range of the target condensate. Fluorescent proteins can be combined with dispersed size-exclusion beads to observe the penetration of the proteins into the polymer matrix and to quantify the partition of fluorescent particles between bulk and beads (Keber et al., 2024). On another hand, SEC can be combined with immunoblotting to evaluate in which fraction, i.e., around what size, the condensate proteins elute from the column.

Dynamic light scattering

Another powerful tool is dynamic light scattering (Fig. 3 C). This method provides an indication of the size distribution of particles in suspension, ranging from 1 nm to 10 µm in diameter. It is a valuable technique for verifying that purified condensates in solution are of the expected size. Notably, this approach can surpass the detection threshold of a widefield microscope, offering detailed insights into sizes below 100 nm, where smaller assemblies have been observed (Gil-Garcia et al., 2024; Tsoi et al., 2024; Hochmair et al., 2022). Although powerful, this technique requires quite pure samples with small size dispersion and therefore has only been used to measure *in vitro* condensates so far.

Native PAGE

The native PAGE technique enables the separation of proteins based on their size and charge in a non-denaturing gel (Fig. 3 D). This method provides information on the partitioning between the oligomerized state and the free proteins. Native PAGE has been primarily employed to examine the oligomerized state of *in vitro* condensate proteins and the impact of diverse conditions, including pH, salt concentration, and protein concentration (Bullier-Marchandin et al., 2023; Dizani et al., 2024, *Preprint*; Hochmair et al., 2022; Li et al., 2024; Stewart et al., 2024; Harmon et al., 2017). The effects of lysis buffers can then be evaluated by conducting assays with a range of salts and detergents and investigating the oligomerized versus free state by native PAGE. However, only a few proteins can be detected by western blotting, which makes the work on an entire condensate time consuming and resource intensive.

Concluding remarks

The purification of biomolecular condensates is a powerful approach for understanding the subtle mechanisms that govern these fascinating biological objects. However, there are many obstacles to the development of robust purification methods, and each may thus be tailored to a specific need. Condensates form and dissolve depending on the metabolism of the cell; they have variable stability, can be heterogenous in size and molecular composition, include stable and labile components, and are found in both the cytoplasm and the nucleus. They are not protected from changes in their chemical environment, making any lysis process risky.

Nevertheless, many teams have managed to extract condensates from biological samples. A number of condensates have been purified to study their composition, PTM, and morphology. A range of techniques were used, including differential centrifugation, gradient centrifugation, SEC, fluorescence-activated particle sorting, and membrane filtration. Each method has its strengths and weaknesses, enforcing the need for thorough controls of condensate integrity during the purification process, as well as subsequent validation experiments by smFISH or IF, using antibodies or tagged proteins that can be exogenously expressed or modified genomically.

In most cases, purified condensates were obtained despite quite stringent lysis conditions, whether chemical or physical. These results are in striking contrast to *in vitro*–reconstituted condensates that are often fragile and can react strongly to minor environmental changes. This suggests that important factors, such as specific RNA or DNA or PTMs or the overall molecular complexity, may have been absent in simplified reconstitution approaches as well as in cytomimetic medium. Future work will be needed to reconcile these observations with models and will certainly lead to new and exciting discoveries.

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