


# Modulating biomolecular condensates: a novel approach to drug discovery

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**Abstract** | In the past decade, membraneless assemblies known as biomolecular condensates have been reported to play key roles in many cellular functions by compartmentalizing specific proteins and nucleic acids in subcellular environments with distinct properties. Furthermore, growing evidence supports the view that biomolecular condensates often form by phase separation, in which a single-phase system demixes into a two-phase system consisting of a condensed phase and a dilute phase of particular biomolecules. Emerging understanding of condensate function in normal and aberrant cellular states, and of the mechanisms of condensate formation, is providing new insights into human disease and revealing novel therapeutic opportunities. In this Perspective, we propose that such insights could enable a previously unexplored drug discovery approach based on identifying condensate-modifying therapeutics (c-mods), and we discuss the strategies, techniques and challenges involved.

For more than a century, scientists have speculated on the structure and organization of the protoplasm<sup>1–4</sup>. In addition to membrane-bound organelles such as the nucleus and mitochondria, microscopists also observed organelles lacking membranes. For instance, the nucleolus was first described in the 1830s<sup>5</sup>. Additional membraneless organelles were identified at the turn of the twentieth century<sup>6–10</sup>, and many others have since been reported. Although the functions of these assemblies — now known as biomolecular condensates — have been described in some cases (TABLE 1), the mechanisms that control their formation, structure, dynamics, composition and activity are only now being studied intensively (FIG. 1).

Experimental evidence to support the hypothesis that biomolecular condensates form by aqueous phase separation was first generated by Cliff Brangwynne, Tony Hyman, Frank Jülicher and colleagues. They demonstrated that P granules — protein–RNA assemblies found in *Caenorhabditis elegans* — exhibit liquid-like behaviour in cells, including dripping, wetting and

fusion, implicating phase separation in their formation<sup>11</sup>. Subsequent work from Brangwynne and Hyman showed that nucleoli in *Xenopus* oocytes also behave as liquids, exhibiting rapid ATP-dependent dynamics<sup>12</sup>.

Publications from other laboratories soon provided additional support for the concept of biological phase separation. The Rosen laboratory was first to recognize the role and importance of weak multivalent interactions in driving phase separation and speculated that cellular organization and regulation across all of biology might be critically dependent upon such phase transitions<sup>13</sup>. Work from the McKnight laboratory showed that proteins containing low-complexity, intrinsically disordered regions (IDRs) phase separate into hydrogels capable of partitioning ribonucleoprotein (RNP) granule components<sup>14,15</sup>. Hanazawa, Yonetani and Sugimoto revealed that just two condensate components can reconstitute P granules in cells, supporting the idea that some proteins are necessary and sufficient to promote condensate assembly<sup>16</sup>. This early work was captured in a series of reviews<sup>17–21</sup>.

The biomolecular condensates field reached an inflexion point in 2015, with multiple publications reporting breakthrough findings (reviewed in REF.<sup>22</sup>); since then, research in the field has grown rapidly.

Several lines of evidence emerging from such research support the relevance of biomolecular condensates for drug discovery. There are a growing number of examples of ‘aberrant behaviours’ of condensates that are associated with disease states, including neurodegeneration<sup>23</sup>, cancer (for example, prostate cancer)<sup>24</sup>, viral infections (for example, respiratory syncytial virus (RSV))<sup>25</sup> and cardiac disease<sup>26,27</sup> (TABLE 2). Proteins of high therapeutic interest in neurodegenerative disease such as TDP43 and FUS have been identified inside condensates<sup>28,29</sup>. The anticancer drugs cisplatin and tamoxifen can partition into transcriptional condensates, altering their composition in cultured cells and in vitro reconstituted model condensates<sup>30</sup>; initial reports show that small molecules can alter condensate behaviours with functional consequences in cell-based studies<sup>31,32</sup>. Finally, the tools to study condensates are rapidly maturing, increasing their applicability to efforts to identify condensate-modifying therapeutics (c-mods).

Several components are crucial to the basis of a c-mod discovery campaign. First, observed associations between condensate characteristics and diseases should be rigorously validated, with the aim of identifying associations that are causal. Furthermore, it should be established that molecular and mechanistic aspects of biomolecular condensates identified through in vitro studies are relevant in vivo. Second, assays that reliably reflect disease-relevant aspects of the biology of condensates need to be developed. In contrast to classical drugs that typically target unique macromolecules, the target for c-mods is a community of molecules engaged in an extended network. Major challenges include identifying the biomolecule(s) required for condensate assembly, as well as understanding the thermodynamics of the extended network and the kinetics of processes that disrupt the equilibrium. As discussed in detail in REFS.<sup>33,34</sup>, caution needs to be exercised to not over-interpret qualitative data and results obtained from simplified

Table 1 | Functional roles of biomolecular condensates

Condensate name	Cellular localization	Roles in cell function	Refs.
A-body	Nucleus	Promote cell dormancy; control of local protein synthesis under stress conditions	198,199
Cajal body	Nucleus	mRNA processing	200
Cleavage body	Nucleus	Protein degradation	201
DNA damage foci	Nucleus	DNA protection and repair	202,203
Gemini of Cajal body (also known as gems)	Nucleus	snRNP maturation	204
Heterochromatin	Nucleus	Gene silencing; chromatin compaction	78,79,205
Histone locus body	Nucleus	Regulation of transcription; processing of histone mRNA	206
Nuclear speckle/ interchromatin granule cluster	Nucleus	Splicing; genome organization	207–209
Nuclear stress body	Nucleus	Transient depots; protection of nuclear proteins	76,210
Nucleolus	Nucleus	Ribosome biogenesis; stress sensing; protein quality control	211
p62 body	Cytoplasm	Autophagy; SUMOylation	212
PML body (also known as Kremer body and nuclear domain 10)	Nucleus	Apoptotic signalling; antiviral response; transcriptional regulation	213,214
Transcriptional condensate/ transcription foci/ super-enhancer	Nucleus	Active transcription	81,215,216
Balbani body	Perinuclear	Storage of organelles and RNA in oocytes	47
Nuclear pore (complex)	Nuclear membrane	Regulation of nucleocytoplasmic traffic by creating a gel-like permeable mesh-like barrier	217
Centrosome	Cytoplasm	Microtubule organization; regulation of mitosis, cell motility, polarity and adhesion	218
Paraspeckles	Nucleus	Gene regulation	219–221
P-body	Cytoplasm	RNA metabolism	222,223
P granule/nuage body/ germ granule	Cytoplasm	Germ cell	11,224
Proteasome storage granule (PSG)	Cytoplasm	Protection of proteasomes from degradation	158
RNA transport granule	Cytoplasm	mRNA transport	225
Stress granule	Cytoplasm	Stress response; protective depot for RNA-binding proteins and translationally arrested mRNA	226,227
U-body	Cytoplasm	Assembly/storage of U snRNPs; associate with P-bodies	228
TIS granule	Endoplasmic reticulum	Translation regulation	121
Signalling cluster	Plasma membrane	Signal transduction	65
Synaptic density	Plasma membrane	Neuronal communication	229,230
Bacterial RNP body (BR body)	Bacterial cytoplasm	Bacterial mRNA decay	231

snRNP, small nuclear ribonucleoprotein.

(for example, in vitro reconstitution) or artificial (for example, overexpression) systems. Nevertheless, these model systems can be leveraged to obtain insights into the structural ensemble and mesoscale organization of a subset of macromolecules inside a condensate-like milieu (reviewed in REF.<sup>35</sup>) and the effects of putative c-mods on the represented interactions, as we discuss later in the Perspective.

Given the infancy of the field, the aspects of the rationale and strategies for pursuing c-mod discovery discussed are built on disparate pieces of evidence from studies that were not necessarily focused on drug discovery, or from drug discovery studies that were not searching for c-mods. However, we believe that there is a substantial amount of data from such studies that support the feasibility of targeting biomolecular condensates and provide a foundation for a guide to future c-mod discovery and assay development when interpreted with a condensate perspective.

With the goal of contributing to such a guide, in this Perspective we first discuss how understanding the properties and functions of condensates may enable a novel approach to drug discovery. After briefly describing the physics and structural basis for the formation of biomolecular condensates, we outline the diverse roles that condensates play in cellular function and some of the evidence for the associations of aberrant condensates with disease. We then describe approaches and technologies for the identification and characterization of drug candidates that can modulate or otherwise exploit disease-relevant condensates and consider the challenges that need to be addressed for them to be effective.

### Overview of condensate biology

Biomolecular condensates have been linked to many cellular processes, including sensing and responding to stress, compartmentalization of biochemical reactions, mechanical regulation and signalling (reviewed in REFS.<sup>34,36</sup>). Their composition is typically complex, consisting of hundreds of different proteins and nucleic acids, which form an extensive intermolecular network spanning length scales of nanometres to micrometres. The underlying mechanisms for biomolecular condensate assembly depend on their composition and architecture (reviewed in REFS.<sup>33,36–38</sup>). As a common denominator, this assembly is mediated by multivalent interactions leading to increased local concentration of a select molecular community, which creates a

microenvironment with unique properties (reviewed in REF.<sup>36</sup>). Here, we focus on the widely used model in which biomolecular condensates assemble through phase separation. We propose that targeting the emergent properties of the molecular community within condensates provides an untapped source of therapeutic agents. Notably, however, the c-mod design and discovery strategies discussed later in this Perspective are agnostic to the mechanisms underlying condensate assembly.

### Principles of condensate assembly

Many biomolecular condensates are thought to assemble in a concentration-dependent manner to form non-stoichiometric macromolecular assemblies, via spontaneous or nucleated phase separation of a select set of proteins and/or nucleic acids. Phase separation occurs when the concentration of biomolecules exceeds the saturation concentration ( $C_{sat}$ ). This threshold defines the phase boundary, above which thermodynamics favours self-solvation of these biomolecules rather than solvation by the surrounding environment, driving formation of mesoscale assemblies rather than discrete biomolecular complexes. Minor changes in biomolecule concentration that cross the phase boundary trigger a sharp, switch-like response, leading to either condensate formation or dissolution, effectively changing the local concentration, sometimes over several orders of magnitude (FIG. 2a). Importantly, this process is fast and reversible, making it an ideal element in sensing stress and other environmental changes.

Assembly of biomolecular condensates is facilitated by multivalency encoded as multiple copies of folded domains or of structural motifs, and/or low-complexity IDRs<sup>13,22,39</sup>. These interactions are typically weak and contribute to fine-tuning the material properties of biomolecular condensates<sup>40,41</sup> (FIG. 2b).

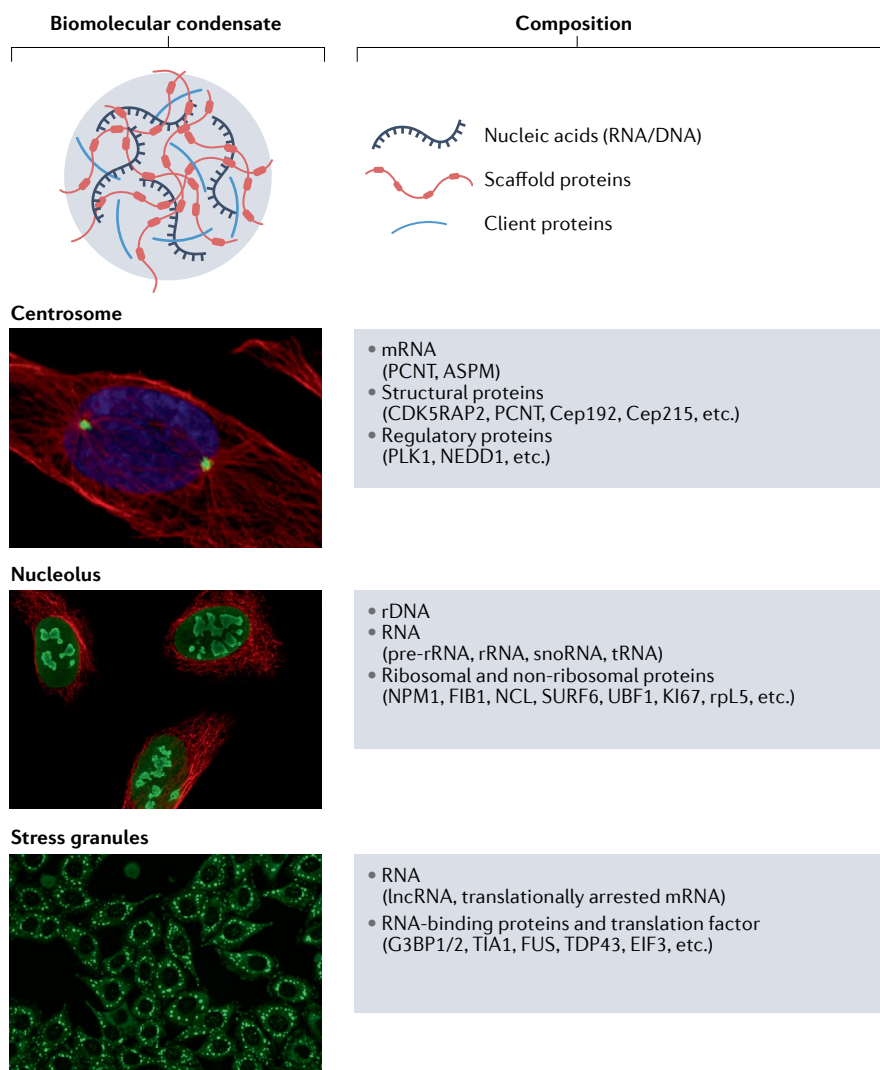
### Physical properties of condensates

Biomolecular condensates exhibit a range of material properties (reviewed in REF.<sup>21</sup>). Stress granules<sup>42,43</sup>, P granules<sup>11</sup> and nucleoli<sup>12</sup> exhibit liquid-like properties. Gel-like assemblies (that is, the centrosome<sup>44</sup>, RNA expansion repeats<sup>45</sup> and the nuclear pore<sup>46</sup>) and solid-like functional amyloids (that is, the Balbiani body<sup>47</sup> and A-bodies<sup>48</sup>) have reduced internal dynamics. These material properties are correlated with the composition and biological functions of the condensates and can be dynamically modulated through changes in the

environment or active biological processes (discussed below). For example, the liquid-like properties allow stress granules to rapidly assemble and disassemble as conditions vary. The gel-like centrosome can withstand the microtubule pulling forces when the mitotic spindle is formed<sup>49</sup>. The Balbiani body is proposed to promote dormancy during oocyte storage by shutting down all biochemical reactions (reviewed in REF.<sup>50</sup>).

The complex network of interactions between the various members of a molecular community determines the

material properties of a condensate. The biomolecules within a community share features that contribute to their compatibility and co-localization (reviewed in REF.<sup>51</sup>). These features include similar amino acid bias in IDRs<sup>52</sup>, certain families of folded domains<sup>13,53,54</sup> and similar classes of nucleic acids<sup>28,55,56</sup>. These molecules can be classified as scaffolds or clients (FIG. 2b) based on whether they are essential for the formation of the underlying network of a condensate<sup>53</sup>. Scaffolds are multivalent biomolecules required for condensate formation; they typically



**Fig. 1 | Examples of complex composition of biomolecular condensates.** The molecular community defines the identity of a biomolecular condensate. Examples of three biomolecular condensates and selected components. The centrosome is the central organizer of microtubules and is involved in regulation of mitosis; the image shows a mitotic SiHa cell, with the centrosome structural protein CDK5RAP2 stained green, the nucleus blue and microtubules red. The nucleolus is the site of ribosome biogenesis; the image shows U2OS cells with the nucleolar scaffold protein NPM1 stained green and microtubules red. Stress granules; the image shows stress granules in HeLa cells, visualized via G3BP1 immunofluorescence. lncRNA, long non-coding RNA; rRNA, ribosomal RNA; snoRNA, small nucleolar RNA. The centrosome image is reproduced from <https://www.proteinatlas.org/ENSG00000136861-CDK5RAP2/cell#img>, and the nucleolus image is reproduced from <https://www.proteinatlas.org/ENSG00000181163-NPM1/cell#img>.

exhibit the lowest  $C_{sat}$  among components, initiating the condensation process, and are characterized by a high partition coefficient (the ratio between concentrations inside versus outside the condensate)<sup>13,52,53,57,58</sup>. Clients are molecules that partition into condensates via interactions with the scaffolds; they are typically characterized by lower partition coefficients compared with scaffolds<sup>59</sup>. Typically, multiple macromolecules can function as co-scaffolds (for example, G3BP1/2 in stress granules<sup>60</sup> and PGL1/3 in P granules<sup>16,61,62</sup>). The composition of condensates dynamically adjusts based on changes in bulk concentration of co-scaffolds and clients<sup>53,63,64</sup>, as well as in response to non-equilibrium processes (for example, activity of energy-consuming enzymes).

**Functions of condensates**

Condensates provide a distinct environment optimized for function. The intra-condensate milieu regulates enzymatic reactions by compartmentalizing components involved in related biological processes. This unique environment can modulate one or more of the following

parameters: diffusion of components, enrichment in substrates and/or depletion of inhibitors<sup>65–70</sup>.

Biomolecular condensates can respond rapidly, with a low energy threshold, to sudden environmental changes, such as temperature, stress, starvation, detection of foreign material or other cellular stimuli<sup>71</sup>. Phase separation affords a reversible mechanism for increasing the local concentration of a particular component within the condensate, while reducing it in the outside environment. Such processes are involved in mitigating cellular toxicity, by sequestering excess materials in response to stress. For example, stress signals sensed in the cytoplasm trigger assembly of stress granules, which compartmentalize untranslated RNA and RNA-binding proteins from the cytoplasm and nucleus<sup>28,72</sup> (FIG. 1). Similarly, stress sensed in the nucleus leads to dynamic changes in the nucleolus<sup>73</sup> and formation of nuclear stress condensates<sup>74–76</sup>. Biomolecular condensates also play roles in minimizing cellular noise<sup>77</sup>, control of genome packaging<sup>56,78–80</sup>, transcription<sup>81,82</sup>, cell-cycle control and DNA double-strand breaks<sup>83</sup>, viral assembly<sup>84</sup> and immune responses<sup>66</sup> (TABLES 1, 2).

**Regulation of condensates**

The composition of biomolecular condensates is complex, dynamic and varies with cell type and the type of signal that induces condensate formation. Studies indicate that biological systems are often optimized to reside close to the phase boundary. Thus, small changes in the environment (for example, metabolite or biomolecule concentrations, pH or temperature) tip the equilibrium to either dissolve or assemble the condensate, generating a rapid switch-like signal (reviewed in REF.<sup>71</sup>) (FIG. 2a). Condensates exist in a non-equilibrium state via the action of energy-consuming enzymes<sup>12,85</sup>, competitive interactions with ligands<sup>63,64,86</sup>, hydrotropes<sup>87</sup> and other perturbations, which regulate their function, composition and dynamics. Protein quality control, including chaperones, autophagy and proteasome degradation<sup>85,88–91</sup>, and the post-translational machinery are intimately involved in the regulation of condensates and their emergent properties.

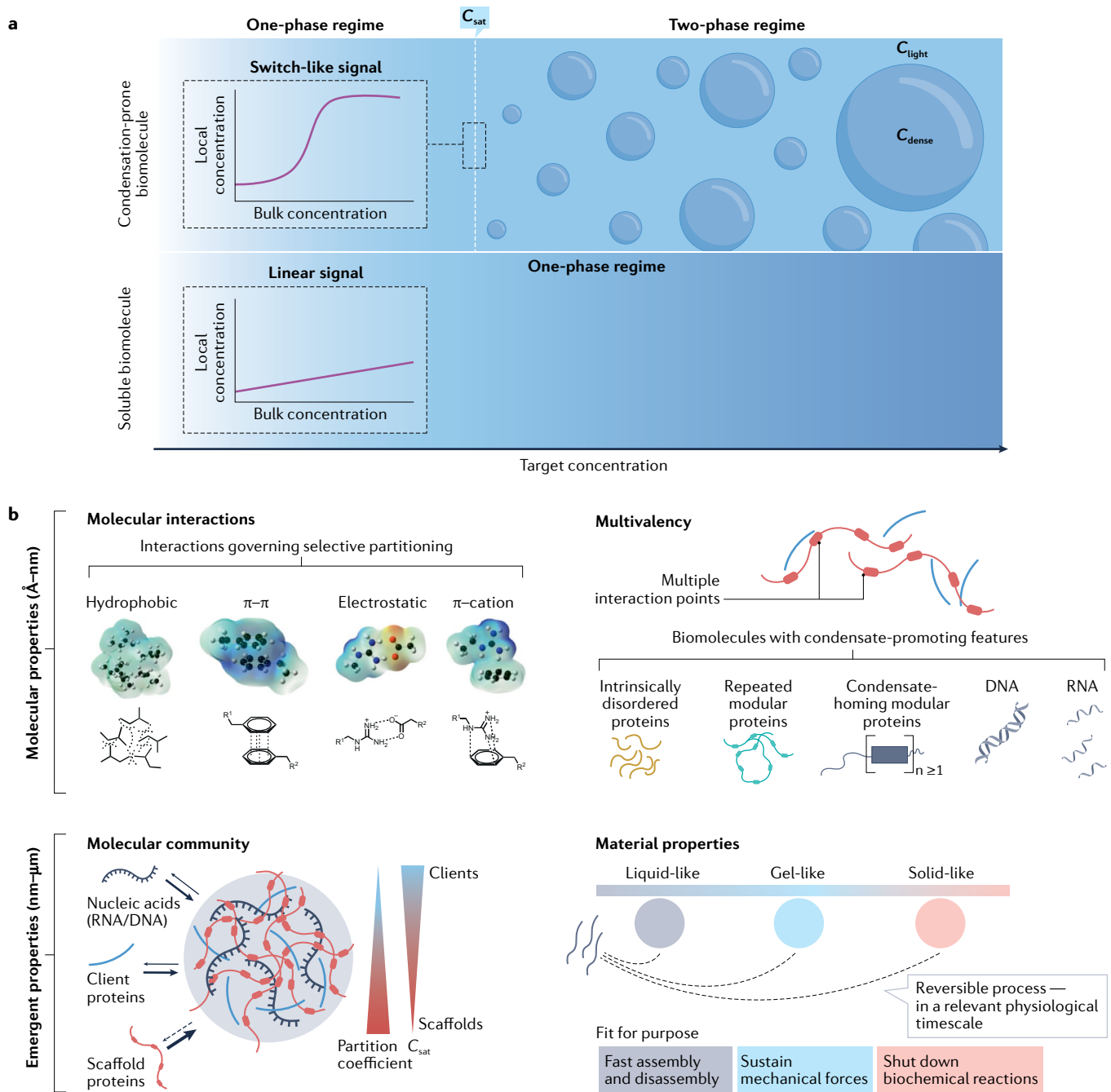
**Post-translational modifications.** Assembly and disassembly of condensates is modulated by covalent post-translational modifications (PTMs) of protein components, such as phosphorylation, acetylation, methylation, SUMOylation, ubiquitination, PARYlation (poly-ADP-ribosylation) and glycosylation (reviewed in REFS.<sup>92,93</sup>). These modifications can have a dramatic effect on the conformational ensemble and dynamics of IDRs involved in condensate scaffolding (reviewed in REFS.<sup>94,95</sup>). For example, epigenetic modifications were shown to induce changes in material properties of chromatin, modulating access of the transcriptional machinery to the genetic information<sup>56</sup>. Additionally, epitranscriptomic and post-transcriptional modifications on RNA contribute similarly to modulating phase behaviour of condensates (reviewed in REF.<sup>39</sup>).

**Competitive interactions.** Proteins that serve as scaffolds for condensates can interact with chaperones (reviewed in REF.<sup>85</sup>) and nucleocytoplasmic transporters (for example, karyopherins)<sup>96</sup>, thereby modulating the  $C_{sat}$ , preventing and reversing aberrant phase transitions. Similarly, helicase action controls the partitioning of RNA within biomolecular condensates<sup>97</sup>. For example, HSP70 is required for the dissolution of aged stress granules containing misfolded SOD1 (REF.<sup>88</sup>). Karyopherins prevent aberrant

Table 2 | Dysregulation of biomolecular condensates in disease

Condensate name	Disease association	Dysregulation type	Refs.
LANA body	Karposi sarcoma herpesvirus	Nuclear bodies formed under latent infection	232
Negri body	Rabies virus	Cytoplasmic neuronal protein and RNA inclusions	10,142
Nucleolus	Solid tumours	Enlarged nucleoli	233
	AML	Aberrant NPM1 cytoplasmic localization, delocalized from nucleoli	234,235
	Neurodegeneration	Insinuation of dipeptide repeats	131
	Viral infections	Hijacking	236
PML body	Viral infections	Hijacking	237
RBM20 condensate	Dilated cardiomyopathy	Aberrant cytoplasmic condensates; RBM20 delocalization from nucleus	26
RNA foci	Neurodegeneration	Aberrant RNA foci recruit RNA-binding proteins	130
Stress granule	Neurodegeneration	Change in persistence, material properties and composition	131,238
	Viral infections	Hijacking	239,240
Transcriptional condensate/transcription foci/super-enhancer	Ewing's sarcoma	EWS1–FLI aberrant transcriptional condensates	138
	AML/AEL	NUP98 fusion aberrant transcriptional condensates	139,140,168
	Various types of cancer	Aberrant super-enhancers and transcriptional condensates	Transcription factor overexpression
			82,122,215,216
Viral factory	Viral infections	Viral mRNP condensates	84,141

AEL, acute erythroid leukaemia; AML, acute myeloid leukaemia; mRNP, messenger ribonucleoprotein.



**Fig. 2 | Principles of condensate assembly and their regulation. a** | Phase separation enables a sharp, switch-like response as the concentration of phase-separating biomolecule(s) exceeds the saturation concentration ( $C_{sat}$ ). In this case, a small change in bulk concentration can lead to sudden change in a molecule's local concentration, and may lead to large changes in activity/signalling (top). By contrast, without phase separation, a molecule's local concentration scales linearly with the bulk concentration, resulting in subtle effects (bottom). **b** | At atomic and molecular levels (angstrom to nanometre), various types of interactions (top left) and their valency (top right) define condensates. These interactions, in turn, define condensate composition. Examples of biomolecular topology that promote condensation include intrinsically disordered proteins with multivalency encoded in short linear

motifs, modular proteins with multivalency encoded in repeat folded binding domains, modular proteins with a condensation-prone intrinsically disordered region (IDR) and one or more folded domains that drive specific localization (for example, transcription factors), and nucleic acids (DNA and RNA). Scaffolds (orange) are characterized by higher partition coefficients and lower  $C_{sat}$  values compared with clients (blue). Together, all smaller-scale interactions modulate the biomolecular network of the molecular community (bottom left) and the emergent material properties (bottom right) at the mesoscale (nanometre to micrometre), optimized for condensate function (fit for purpose). Solid-like condensates such as the Balbiani body are reversible under physiological conditions, in contrast to solid-like pathological amyloids. Part **b** is adapted with permission from REF.<sup>124</sup>, Elsevier.

phase transitions of prion-like domains and reverse phase separation of amyotrophic lateral sclerosis (ALS)-associated proteins

(for example, FUS, TDP43 and hnRNP1) by directly binding to cognate nuclear localization signals within the target protein,

aided by weak, non-specific interactions that compete for the condensation-driving interactions<sup>98,99</sup>.

**Compositional change.** As described above, condensate composition, dynamics, material properties and function are interrelated. Partitioning of RNA inside protein condensates tunes the viscosity in vitro and in vivo<sup>100–103</sup>. The level of RNA can either promote or dissolve condensates scaffolded by RNA-binding proteins<sup>104–107</sup>. Such a regulatory mechanism has significant implications in biology; a notable example is regulatory feedback in transcription, where incipient amounts of RNA synthesis promote stabilization of transcriptional condensates, whereas accumulation of transcripts promotes their dissolution<sup>108</sup>. In addition to RNA, other nucleotide polymers can tune the stability of biomolecular condensates, including DNA<sup>56,66,80</sup> and PAR<sup>109,110</sup>. Modulation of the protein composition of a condensate can affect the dynamics of individual components. For example, the centrosome nucleator SPD2 diffuses faster when its binding partners PLK1 and TPXL1 are present in the reconstituted condensates<sup>44</sup>.

In a recent report, An et al.<sup>111</sup> demonstrated that aberrant, persistent pathological stress granules formed by an ALS-associated FUS mutant exhibit different proteomics compared with normal stress granules. These stress granules are characterized by enriched physical interactions between components, consistent with earlier observations that pathological stress granules are less dynamic.

### Conformation-encoded regulation.

Condensate-associated proteins exhibit a modular topology that allows them to function as interaction hubs by engaging with multiple types of macromolecules. They encode structural switches that promote transitions between freely diffusing discreet monomers or oligomers to a ligand-bound scaffold of a large macromolecular network within a condensate, as described for the nucleolar and stress granule scaffolds, NPM1 (REF.<sup>96</sup>) and G3BP1 (REFS.<sup>112,113</sup>), respectively. In these examples, ligand binding alters the conformation of a protein at atomic scale, triggering remodelling of the nanometre-to-micrometre scale molecular network of the condensate.

Within the condensate microenvironment, IDRs can remain disordered, as seen in DDX4 (REF.<sup>114</sup>) and FUS<sup>107</sup>, or can undergo folding upon binding. FUS and TDP43 were shown to form cross- $\beta$  structures within their LCD domains in hydrogels to stabilize intermolecular interactions<sup>115,116</sup>, and the carboxy-terminal LCD of TDP43 stabilizes

a helix structure upon dimerization in liquid-like droplets<sup>117</sup>. Modulation of the helical propensity by mutations, including those associated with ALS, affect not only the  $C_{sat}$  for phase separation but also the material properties of the resulting condensates and the splicing function of TDP43 (REF.<sup>117</sup>).

**Spatial positioning.** Biomolecular condensates provide a means for cells to spatially regulate important processes. For example, at homeostasis, FUS and TDP43 fulfil roles in RNA splicing and metabolism in the nucleus but are sequestered into cytoplasmic stress granules under stress conditions. The function of HSF1, a transcription factor for heat shock chaperones, is modulated under stress conditions via sequestration into nuclear condensates<sup>74</sup>. Organization of the cytoplasm, such as spatial patterning of specific transcripts in polar cells, is achieved by encapsulation of the target RNA molecules in biomolecular condensates<sup>11,101,102</sup>.

Multi-compartment organization of the condensate interior (reviewed in REF.<sup>37</sup>) arises via coexisting, non-miscible phases<sup>40,103,118–120</sup>. The nucleolus exhibits a three-layered architecture, determined by the surface tension with respect to the nucleoplasm<sup>40</sup>. It was proposed that the material properties of the different nucleolar layers are optimized to promote the correct sequence of steps and the vectorial flux in ribosome biogenesis<sup>40,64</sup>.

Cellular surfaces, such as chromatin, membranes and the cytoskeleton, can serve as regulators of spatial positioning of biomolecular condensates. Membranes serve as nucleators to drive phase separation by restricting molecular diffusion and promoting local crowding effects (reviewed in REF.<sup>95</sup>). For example, T cell signalling condensates and TIS granules form at the plasma membrane<sup>65</sup> and on the endoplasmic reticulum membrane<sup>121</sup>, respectively. Su et al. showed that phosphorylation of the T cell receptor triggers clustering of LAT (linker for activation of T cells) into mesoscale condensates at the plasma membrane, and that these condensates recruit components of T cell signalling, which subsequently trigger actin polymerization as a functional output<sup>65</sup>.

Taken together, these mechanisms governing their behaviour, function and regulation play an important role in the normal function of condensates in cells and provide valuable insights for designing strategies to correct their malfunction in disease.

### Condensates in disease

Here, we define a ‘condensatopathy’ as an aberration of a condensate that drives a specific disease phenotype, which has been observed in in vitro model systems and in vivo cellular and animal models of neurodegenerative diseases, dilated cardiomyopathy, certain types of cancer (reviewed in REFS.<sup>23,27,122–124</sup>) and viral infections. Intriguingly, there are multiple examples of genetic mutations showing strong clinical association with diseases that affect proteins that have been identified in biomolecular condensates. This suggests the possibility that these mutations might dysregulate condensate function, and thereby drive disease. We discuss a few better-understood examples below. Although correlations between condensate malfunction and disease in these model systems have been well documented, strong support for causation is still under development.

### Neurodegeneration

ALS and frontotemporal dementia (FTD) pathology have been linked to environmental factors and a diversity of genetic alterations, including numerous point mutations in the low-complexity regions of RNP-granule localized proteins, as well as repeat expansions. Point mutations in proteins such as FUS<sup>42</sup> and hnRNPA1 (REF.<sup>43</sup>) accelerate the kinetics of phase transition and promote amyloid-like fibril formation within the condensate environment in in vitro studies. Altered kinetics of clearance of RNP granules, namely prolonged persistence of condensates, was associated with the ALS-hallmark phenotype of cytoplasmic inclusions in cultured cells and neurons. For example, repetitive cycling of G3BP1-positive condensate formation and increased persistence, modulated via an optogenetics model, evolved towards cytoplasmic proteinaceous inclusions and caused cellular toxicity<sup>125</sup>.

A recurring pathological observation in ALS/FTD is the presence of TDP43-rich cytoplasmic granules, irrespective of whether the *TDP43* gene harbours mutations<sup>126</sup>. In vitro and cellular data suggest that these granules arise as condensates and undergo ageing (reviewed in REF.<sup>23</sup>). This spatial re-localization of nuclear TDP43 into cytoplasmic condensates in cultured neurons was associated with increased condensate viscosity<sup>127</sup> and splicing defects in several motor neuron-specific mRNAs, including that encoding stathmin 2 (STMN2)<sup>128</sup>, a neuron-specific regulator of microtubule stability. The TDP43

condensatopathy causes a loss of function of STMN2 (REF.<sup>128</sup>) and impaired axonal growth and regeneration<sup>127,128</sup>. Furthermore, optogenetic formation of TDP43-positive condensates via blue light illumination was sufficient to recapitulate the progressive motor dysfunction observed in patients with ALS in a *Drosophila* model<sup>129</sup>.

Repeat expansions of a short nucleotide segment are another specific type of genetic alteration associated with diseases such as ALS/FTD, myotonic dystrophy, spinocerebellar ataxias and Huntington disease. The severity of these diseases scales with the length of the repeat (multivalency) of the transcript and/or translated polypeptide. Furthermore, the diagnosed clinical cases exhibit a minimum threshold length of the repeat. The resulting polyvalent RNAs and polypeptides have the hallmarks of biomolecules that will localize to condensates as scaffolds, and result in condensatopathies that sequester other important biomolecules. *C9orf72* G4C2, CAG and CUG repeats are found in ALS/FTD, Huntington disease and myotonic dystrophy, respectively; transcripts containing variable lengths of these repeats form RNA foci in live cells, and dynamically arrested condensates in vitro<sup>45</sup>. polyGA and polyGR peptides, resulting from an ATG-independent translation of the G4C2 repeats, were shown to sequester proteasomal and nucleolar proteins, respectively (reviewed in REF.<sup>130</sup>). Furthermore, overexpressed or exogenously added arginine-rich polypeptides of G4C2 repeats insinuate in pre-existing cellular condensates, such as the nucleolus, RNP granules and the nuclear pore complex. As a result, the condensates change their composition, material properties and function due to competition between the G4C2 peptides with the native interactions<sup>131,132</sup>. Correcting the altered material properties and/or sequestration of biomolecules due to the underlying condensatopathies may prevent or reverse these neurodegenerative diseases.

### Cardiomyopathy

Condensatopathies associated with dysregulation of RNP granules are not limited to neurodegenerative diseases. A mutation in the gene coding for the tissue-specific alternative splicing factor RBM20 that is found in patients with congenital dilated cardiomyopathy is characterized by a RNP condensate defect, coupled with contractile dysfunction and aberrant heart anatomy in a heterozygous pig model<sup>26</sup>. The R636S point mutation

localized in the low-complexity disordered RSRS region causes aberrant sarcoplasmic accumulation of RBM20. At the cellular level, the dominant effect of the mutant leads to RBM20 re-localization from nuclear splicing speckles to cytoplasmic condensates that fuse with other cellular condensates harbouring stress granule markers<sup>26</sup>. This condensatopathy causes sequestration of mRNA, polysomes and cardiac cytoskeleton proteins (for example, ACTC1). Interestingly, mice harbouring the congenital dilated cardiomyopathy mutation exhibited a more severe cardiac dysfunction phenotype than mice lacking *RBM20* (REF.<sup>133</sup>).

Collectively, these observations suggest that the pathological mechanism attributed to the condensate phenotype is complex, involving loss of nuclear splicing function for RBM20, loss of function of proteins that partition into aberrant cytoplasmic RBM20 condensates and a gain of function of these condensates. Therefore, the RBM20 condensatopathy serves as a hub for misregulation of multiple pathways in congenital dilated cardiomyopathy and is an attractive node to target this disease therapeutically.

### Cancer

Recent progress has shown a link between condensatopathies and several types of cancer. These condensatopathies deregulate many processes, including, but not limited to, genomic stability, signalling, protein quality control and transcription (reviewed in REFS.<sup>134,135</sup>). Transcription of key developmental genes is often under the control of super-enhancers. Super-enhancers are classically defined by chromatin immunoprecipitation sequencing as clusters of enhancers bearing large amounts of transcriptional machinery (transcription factors, coactivators and RNA Polymerase II (Pol II)). This high-density assembly of proteins at super-enhancers is now understood to constitute transcriptional condensates that drive gene expression<sup>81,82,136</sup>. These insights challenge the stoichiometric model of transcription, suggesting novel properties and functions of transcription factors and coactivators in a concentrated condensate of protein and DNA. For example, the function of transcription factor activation domains was poorly understood because they contain IDRs not amenable to crystallography. Now it is becoming clear that they may activate genes, in part, by their capacity to condense with coactivators on genomic regulatory elements. Transcription of oncogenes is a general feature of

cancer cells. This often occurs through condensatopathies, such as acquisition of aberrant super-enhancers<sup>137</sup>.

Condensatopathies resulting in aberrant gene expression are also associated with cancers. Several chromosomal translocations have been identified, where a condensation-prone IDR fused to a chromatin-associating folded domain creates aberrant condensates. Two examples are EWS–FLI in Ewing sarcoma<sup>138</sup> and NUP98–KDM5A in leukaemia<sup>139</sup>. NUP98–KDM5A is one of many variations of genetic translocations that fuse the condensation-prone amino-terminal FG-rich IDR of a nucleoporin (for example, NUP98 and NUP124) with a folded domain that anchors it at a specific location on chromatin, such as a DNA-binding domain (for example, HOXA9, HOXA13 and PHF23), a helicase domain (for example, DDX10) or a histone binding domain (for example, KDM5A, NSD1)<sup>140</sup>. These genetic translocations result in condensatopathies that share a common expression reprogramming phenotype, with upregulation of the developmentally silenced *Hox* genes. These cancers with diverse genetic aetiology may be treatable by similar drug strategies aimed at the underlying condensatopathies.

### Viral infections

Biomolecular condensates are also leveraged by pathogens such as viruses to more effectively hijack the host cell and evade the host innate immunity self-defence mechanisms (reviewed in REFS.<sup>10,84,141</sup>). Literature reports link the roles of biomolecular condensates to multiple steps within the viral replication cycle, including viral entry and egress, transcription, protein synthesis, and genome and virion assembly (reviewed in REF.<sup>84</sup>).

Certain viral infections (for example, rabies and mammalian orthoreovirus)<sup>10</sup> induce formation of stress granules. Interestingly, despite the fact that Negri bodies in cells infected with rabies virus share some protein and RNA components with stress granules, the two biomolecular condensates behave similarly to immiscible liquids<sup>142</sup>, highlighting the importance of the whole molecular community in determining the identity, function and material properties of a condensate. This concept of a molecular community-imposed selectivity becomes important when designing compounds that target specific biomolecular condensates.

Viruses have evolved to evade the host's innate immune response via multiple mechanisms. The host senses foreign

cytoplasmic genomic material via pathogen receptors such as RIGI and MDA5 and induces PML body assembly in the nucleus, as a part of the interferon-dependent innate immune response. Partitioning the viral RNA within viral factory condensates provides a shielding mechanism, preventing its detection by the cytoplasmic pathogen-sensing machinery. Additionally, DNA and RNA viruses disrupt PML bodies as part of their nuclear replication (reviewed in REF.<sup>10</sup>).

Viral latency is one of the primary challenges that prevent the development of cures for patients suffering from viral infections such as HIV-1. The histone chaperone CAF1 condenses with the viral HIV-1 LTR to form nuclear bodies that recruit other histone chaperones and epigenetic modifiers, and these condensates maintain the integrated viral genome during latency<sup>143</sup>. These observations could provide a novel intervention point to reactivate latent HIV-1-infected cells, which has been a long-standing focus of efforts to develop a potential cure for HIV-1 infection.

Insights from in vitro and in cell overexpression model systems into the molecular mechanisms of replication and host evasion of SARS-CoV-2 indicate that the dimerization of the nucleocapsid protein<sup>144</sup> promotes phase separation with specific viral RNA elements, primarily located at the 5' and 3' UTRs<sup>145</sup>, as well as with host heterogeneous nuclear RNPs, such as stress granule proteins<sup>146</sup>. Phase separation inhibits PTMs such as Lys63-linked polyubiquitination of a host antiviral signalling protein, MAVS, thereby suppressing activation of the innate immune system<sup>144</sup>.

## Drug discovery strategies

The roles of condensates in normal and aberrant cellular functions are becoming clearer, and a range of tools are now available to study these cellular phenomena, including protein proximity labelling, advanced microscopy techniques and computational methods, as discussed further below. Accordingly, there is a growing opportunity to explore condensate-informed approaches to drug discovery.

We introduce the term condensate-modifying therapeutics (c-mods) to describe drugs that modulate the physical properties, macromolecular network, composition, dynamics and/or function of specific biomolecular condensates to prevent or reverse disease. A c-mod discovery programme may have one of the three following objectives: repairing a

condensatopathy; disrupting the normal functioning of a condensate implicated in disease; or preventing a target from functioning either by disabling it within its native condensate or by de-partitioning the target from its native condensate (FIG. 3a). In each case, the drug discovery strategy will be based on a screening and validation model where a condensate optical phenotype is reliably correlated with one or more functional, disease-relevant outputs.

First, for condensatopathy repair, when an aberrant condensate has clearly been implicated in causing a disease, the objective would be to restore normal condensate behaviour or remove aberrant condensates, either by preventing their formation or eliminating them once formed. This could be considered a phenotypic screening strategy, with condensate behaviour in model systems being assessed in the initial screen, and the hits further validated in a disease-relevant secondary assay, as discussed below. There need not be a specific target or pathway, nor any presumed molecular mechanism by which the effect on the condensate is achieved, although such information may be available at the pathway or target level.

Second, in cases where the normal functioning of a condensate is implicated in a biological process central to a disease, the objective would be to develop a c-mod that interferes with the condensate behaviour, ideally only in the disease-relevant cells. The screening strategy would be similar to that described above.

In the third type of case, the objective would be to render a specific target inactive either by 'disabling' its ability to function within its native condensate environment or by removing it from that environment. This is especially relevant for targets of high therapeutic interest that are often described as 'undruggable' due to selectivity issues or the intrinsic difficulty of finding chemical matter that interferes with their function. If new condensate knowledge indicates that such targets function within a condensate environment, novel strategies could be adopted to disable them. Programmes focused on condensatopathy repair or the disruption of the normal functioning of a condensate implicated in disease may be entirely driven by phenotype. By contrast, the focus of programmes that seek to block target function is to track the behaviour of that specific target. Those targets might be de-partitioned out of the condensate, thereby rendering them inactive. Alternatively, the targets might remain in the condensate but be prevented from engaging in the interactions necessary for function.

## C-mod discovery strategies

A wide variety of strategies may be envisioned to identify c-mods that achieve these three objectives. The preferred strategies in any situation will depend on the desired pharmacological outcomes and the detailed knowledge of components, structure and function of the given condensate.

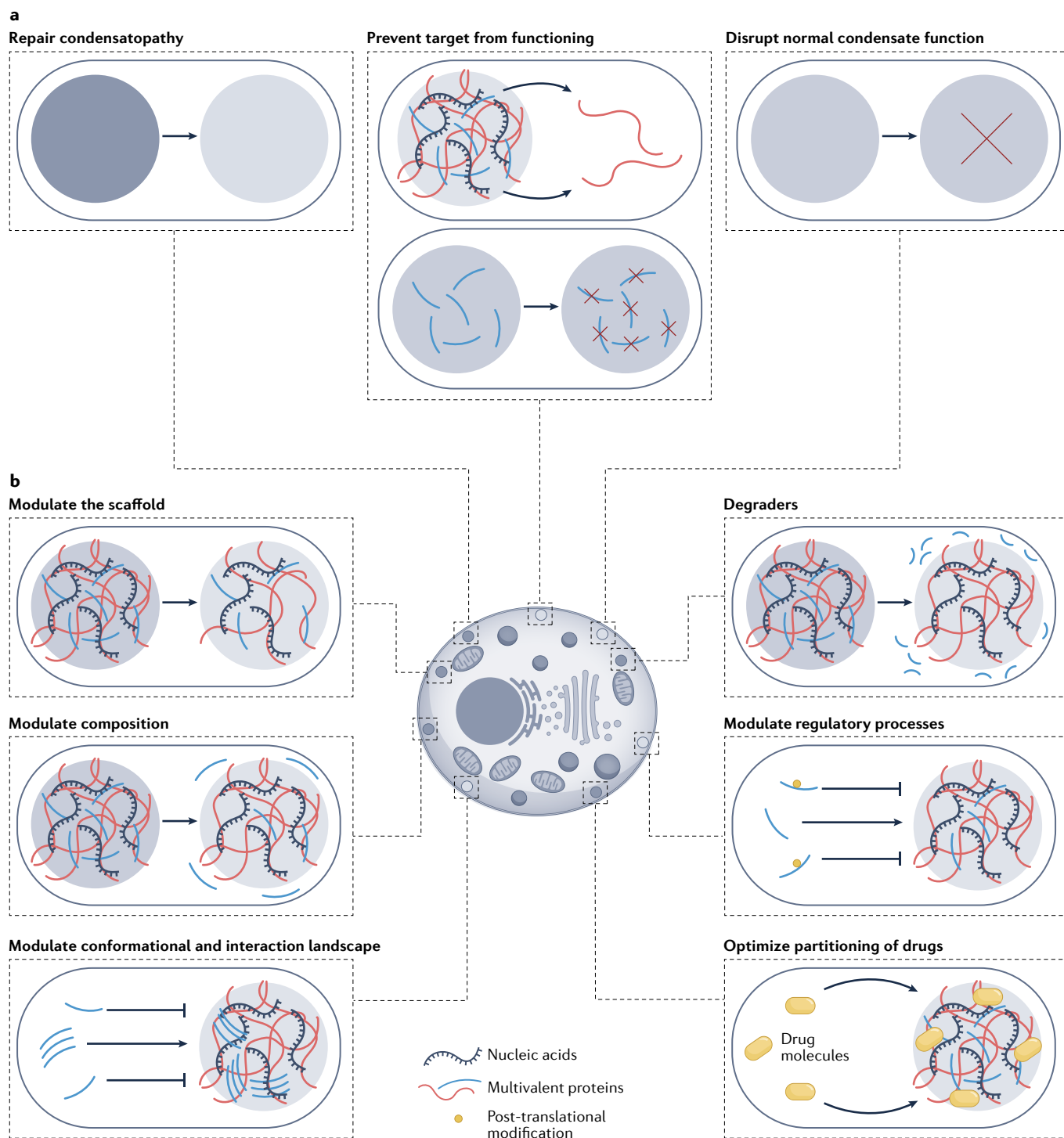
### Modulating the condensate scaffold.

Modulating a condensate scaffold is expected to lead to drastic effects on the stability of condensates, such as persistence,  $C_{\text{sat}}$  (that is, formation or dissolution) (FIG. 3b), material properties and/or composition. If a c-mod intercalates between two or more condensate components, or changes the interaction valency or the interaction strength between (co)-scaffolds (within folded and/or disordered domains), it could also change the material properties. The goal is not full inhibition of a particular protein but, rather, disruption of the composition or stability of a biomolecular condensate, which can be achieved via modest changes in the weak networking interactions.

Scaffold modulation could be achieved in various ways. One approach is tuning valency. For example, a low-valency poly-PR peptide dissolved in vitro heterotypic condensates consisting of NPM1 and a multivalent poly-PR peptide<sup>132</sup>, suggesting that replacing multivalent interactions that mediate network-stabilizing interactions with monovalent, terminal ones is a feasible c-mod mechanism.

A second approach is directly blocking or stabilizing protein–protein<sup>144</sup>, protein–nucleic acid or nucleic acid–nucleic acid interactions that contribute to scaffolding<sup>147</sup>. For example, short bait RNAs prevented formation of TDP43 inclusions in an optogenetic cellular model<sup>148</sup>, probably via a mechanism that outcompetes TDP43–TDP43 self-interaction. A second example is the topoisomerase inhibitor and nucleic acid intercalator mitoxantrone (TABLE 3), which inhibited stress granule formation in a phenotypic high-content screen using two different cell lines and multiple types of stress, and was shown to block the RNA-dependent recruitment of RNA-binding proteins, including TDP43. These compounds reduced persistence of TDP43 puncta in induced pluripotent stem cell-derived motor neurons<sup>31</sup>; the exact mechanism of action and how it relates to the annotated activity of this compound need to be further investigated.





**Fig. 3 | Goals and strategies for developing c-mods.** **a** | Condensate-modifying therapeutics (c-mods) are developed to achieve one or more of the following objectives: to repair or eliminate a condensatopathy (left); to prevent a specific target from functioning by either delocalizing it from its native condensate (centre) or rendering it inactive within the condensate; or to disrupt the function of a normal condensate (right). **b** | Strategies to modulate the emerging properties of condensates with c-mods, described in detail in the text. These strategies can be used individually or in combination, and any one strategy can influence multiple characteristics of a condensate; for example, modulating the scaffold will probably result in changes in composition and material properties.

Several encouraging proofs of concept for condensate-targeted antiviral drug discovery have been reported, although the exact mechanisms of action are not fully elucidated. Small molecules such as

kanamycin (TABLE 3) are able to destabilize nucleocapsid-containing biomolecular condensates, both in vitro and in cultured cells<sup>143</sup>. Additionally, a peptide that inhibits nucleocapsid dimerization prevented

condensation and viral replication, and rescued the innate immune response in live cells and mouse models<sup>142</sup>. Cyclopamine (TABLE 3) analogues modulated the material properties of RSV viral factories in infected

Table 3 | Examples of compounds with evidence of condensate-modulating activity

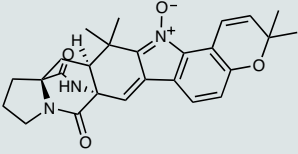
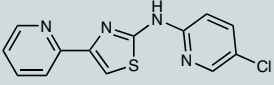
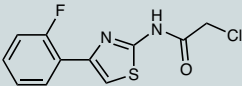
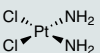
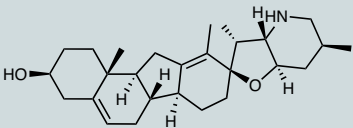
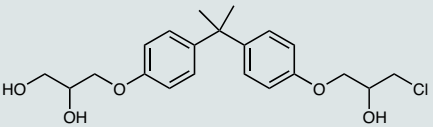
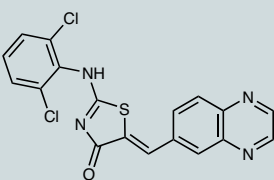
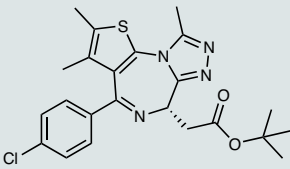
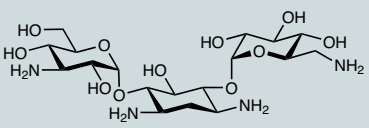
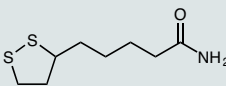
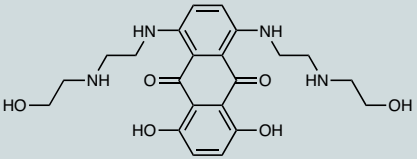
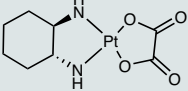
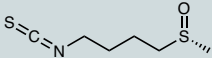
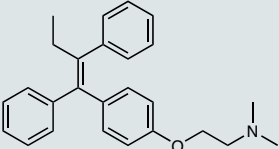
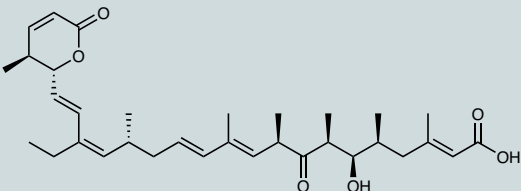
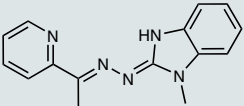
Compound name	Structure	C-mod mechanism evidence	Known targets	Refs.
Avrainvillamide		Restores nucleolar localization of cytoplasmic NPM1 mutant; covalent binder	NPM1	150,241
BAY 249716		Induce condensate formation of DNA-binding defective mutants; dissolve nuclear condensates of structural mutants; covalent binders	p53	242
BAY 1892005				
Cisplatin		Partitions into transcriptional condensates; dissolves MED1 and BRD4 nuclear condensates	DNA	30
Cyclopamine		Hardens RSV viral condensates in infected cells	Sonic hedgehog pathway/smoothed M2-1 viral anti-termination factor	25
EPI-001		Dissolves androgen receptor-rich transcriptional condensates	Androgen receptor	24,152
GSK-626616		Induces condensation of components of the early secretory pathway	DYRK3	243
JQ1		Partitions into transcriptional condensates; dissolves MED1 nuclear condensates	BET family of bromodomain proteins	30
Kanamycin A		Re-localizes SARS-CoV-2 N protein to the nucleus; destabilizes in vitro reconstituted condensates	Bacterial 30S proteasome; nucleic acids	145,244
Lipoamide		Dissolves stress granules	NA	32
Mitoxantrone		Prevents formation of TDP43 cytoplasmic condensates	Topoisomerase II	31

Table 3 (cont.) | Examples of compounds with evidence of condensate-modulating activity

Compound name	Structure	C-mod mechanism evidence	Known targets	Refs.
Oxaliplatin		Alters nucleolar organization	DNA	245
Sulforaphane		Drives formation of nuclear $\beta$ -catenin condensates	Multiple reported targets	149,246
Tamoxifen		Partitions into transcriptional condensates	Oestrogen receptor	30
Leptomycin B		Inhibits formation of aberrant NUP98–HOXA9 transcriptional condensates	CRM1	193
SI-2		Inhibits formation of drug-resistant SRC3/NSD2 condensates	SRC3	197

C-mod, condensate-modifying therapeutic; NA, not available; RSV, respiratory syncytial virus.

cells by reducing the dynamics of the M2-1 protein recovery upon photobleaching, translating into reduced viral replication in the lungs of living mice<sup>25</sup>.

A c-mod could stabilize non-productive conformations (for example, in folded domains or disordered regions), thereby preventing scaffolding contacts; alternatively, it can trap an aberrant or excess protein in inactive condensates (for example, depots). Sulforaphane (TABLE 3) treatment of colorectal cancer cells induces formation of  $\beta$ -catenin nuclear depots that partially co-localize with the transcriptional repressor PRMT5; the appearance of the nuclear depots is associated with a reduction in  $\beta$ -catenin-dependent transcriptional activity<sup>149</sup>.

#### Modulating condensate composition.

C-mods can be envisioned that inhibit or promote the client–scaffold interactions to drive target exclusion or inclusion into the condensate, respectively. For example, an aberrantly de-partitioned protein could be helped to return to its ‘home’ condensate. Nucleolar protein NPM1 is aberrantly localized to the cytoplasm in acute myeloid leukaemia (AML). The natural product avrainvillamide covalently binds mutant

NPM1, returning the protein to the nucleoplasm and nucleolus in cell lines from patients with AML<sup>150</sup>. As discussed in previous sections, changes in condensate composition can affect numerous features, from material properties (for example, viscosity and surface tension), to dynamics and ability to respond to environmental stimuli (for example, persistence and ageing), to enzymatic activity of individual components (for example, cGAS<sup>66</sup>, UBC9 (REF.<sup>68</sup>) and Dcp1/2 (REF.<sup>67</sup>)).

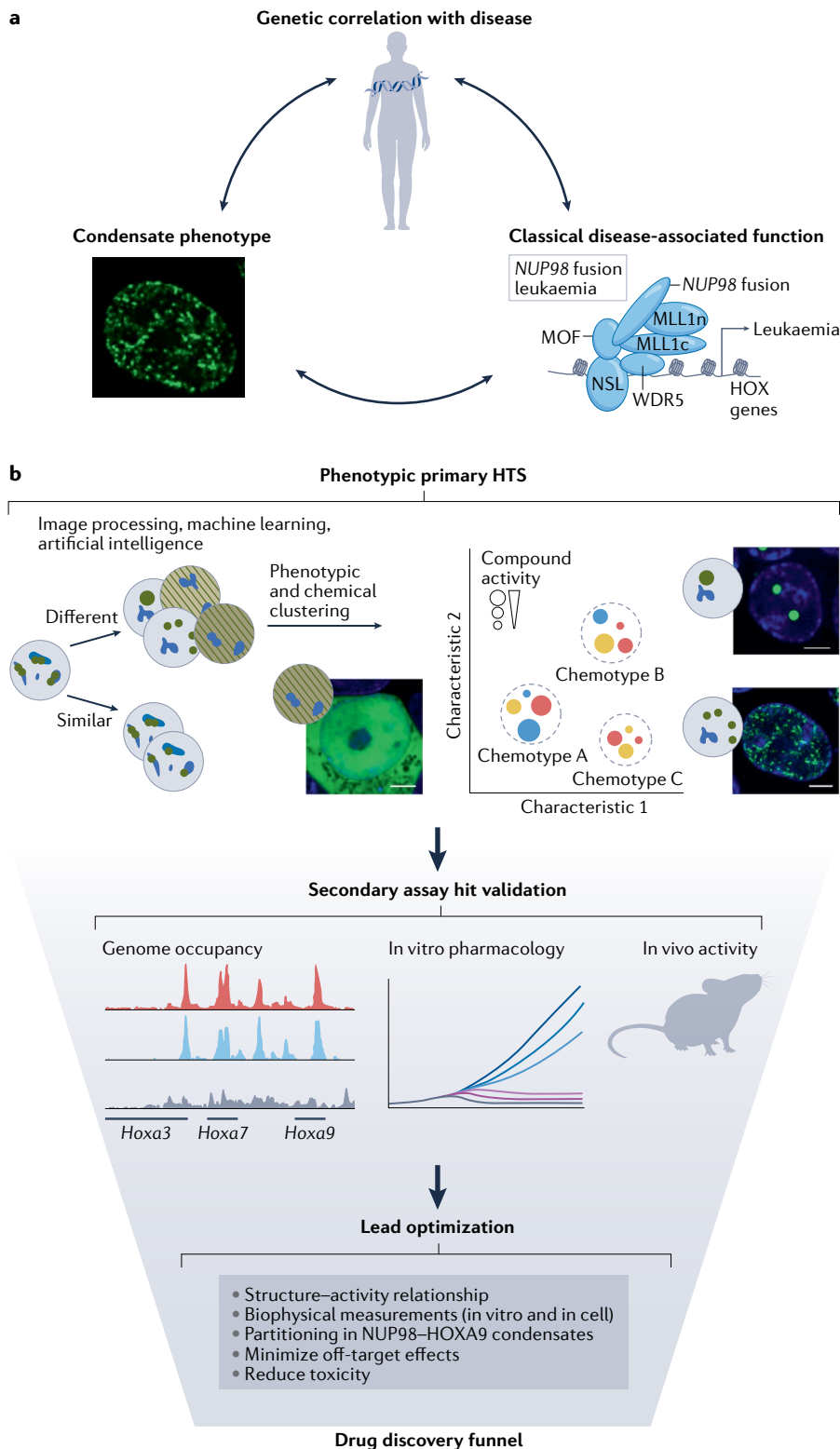
#### Modulating the conformational and interaction landscape.

C-mods that interact with the IDRs of a protein may alter the ability of that protein to partition into a condensate or prevent that protein from forming various intermolecular interactions with other biomolecules within the condensate. Because IDRs are conformationally highly dynamic, it is challenging to use traditional structure-based methods to screen for c-mods that interact with them. However, c-mods could work by engaging with IDRs to either decrease the population of functionally active states or increase the population of inactive or inhibitory states.

Some drug targets, including transcription factors (for example, MYC), hormone receptors (for example, the androgen receptor) and nucleotide-binding proteins (for example, TDP43), contain IDRs and are known to localize to biomolecular condensates. Although small molecules have been identified that bind to these IDRs, they generally do so at micromolar affinities; covalent binders were reported for MYC<sup>151</sup> and the androgen receptor<sup>152</sup> IDRs (TABLE 3), and non-covalent IDR binders were reported for p27<sup>Kip1</sup> (REFS.<sup>153,154</sup>). The ability to produce a high local drug concentration within a condensate might allow for the development of lower affinity, but highly partitioned, drugs that are effective in targeting proteins in these families that have so far been highly challenging.

Differences in protein conformation inside versus outside a condensate might also be leveraged to develop c-mods that are selective for one of the conformations, potentially minimizing off-target effects.

**Degraders.** One approach to effectively remove a specific protein from a condensate is to degrade it using proteolysis-targeting chimera (PROTAC) or molecular glue



**Fig. 4 | Building a c-mod discovery pipeline: NUP98–HOXA9 as a case study.** **a** | The first step is validation of the condensate hypothesis by testing the correlation between the genetic alteration (*NUP98* and *HOXA9* fusion), aberrant condensate phenotype and aberrant transcription of *HOX* genes. **b** | A proof-of-concept drug discovery pipeline for the NUP98–HOXA9 condensatopathy. A primary phenotypic high-throughput screen (HTS) in a cell line expressing NUP98–HOXA9 could identify compounds that change the morphology of aberrant condensates. Hit compounds with various chemotypes could be filtered and prioritized (for example, with the aid of machine learning/artificial intelligence or through traditional methods) based on various characteristics (two are shown in the graph). Selected hits would then move into secondary validation assays, where one or more functional outcomes are monitored, in disease-relevant cell lines (for example, genome occupancy by ChIP-seq, and in vitro pharmacology by proliferation kinetics) and in vivo activity (for example, tumour growth and survival rates in animal models). Lead compound characteristics would then be optimized in a panel of assays, ranging from in vitro binding studies to the target, biophysical characterization of the lead compound effects on composition and material properties of in vitro reconstituted and endogenous condensates, partitioning measurements, toxicity and off-target measurements, in addition to secondary functional assays. Parts **a** and **b** are adapted with permission from REFS.<sup>168,169</sup>, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>), Elsevier.

For example, autophagosome-tethering compounds are molecular glues that selectively target the mutant huntingtin to degradation via autophagy, by selectively binding to the expanded polyQ and LC3 (REF.<sup>163</sup>).

Using similar approaches, one can envision degrading a scaffold to reduce the effective concentration below  $C_{sat}$ , thereby preventing or reversing condensate assembly.

**Modulating condensate regulatory processes.**

Enzymes such as chaperones and helicases can play critical roles in regulating the condensate environment<sup>95</sup>. Such regulation could affect the condensate environment generally, or may more selectively affect the behaviour of particular proteins or nucleic acids, either by preventing them from interacting with their usual partner molecules or by dramatically affecting their properties (for example, conformation, solubility and valency), causing them to de-partition out of the condensate. Affecting turnover kinetics may be another mechanism to regulate condensate composition.

strategies<sup>155</sup>. There are now several reports suggesting that E3 ligases involved in protein degradation function within condensates<sup>156–159</sup>. Several PROTAC design strategies for neurodegeneration targets, including TDP43,  $\alpha$ -synuclein, tau and huntingtin, are discussed in REF.<sup>160</sup>. Similar in concept, RIBOTACs are bifunctional

molecules that target specific RNA molecules for ribonucleolytic degradation<sup>161</sup>.

It has been shown that nuclear p62-containing condensates are essential to efficient proteasomal function, serving as a hub for efficient nuclear protein turnover<sup>162</sup>. Autophagy is also critically dependent upon phase separation.

Another option could be to change the post-translational state of a protein, or the epigenetic or epitranscriptomic state of DNA or RNA, respectively. As discussed earlier, this may either modify the ability of a biomolecule to nucleate the formation of the condensate, change its residence time in the condensate or alter the function of that biomolecule inside the condensate.

Phosphorylation and methylation are some of the most studied PTMs that modulate protein condensation<sup>93</sup>. RNA post-transcriptional modifications are essential regulators of RNA function and may affect the ability of those RNA to phase separate<sup>39,164</sup>. Furthermore, epigenetic regulation via histone methylation and acetylation status tunes the phase separation of chromatin<sup>56</sup>.

**Optimize partitioning of drug into condensates.** Condensates contain key drug targets such as enzymes, transcription factors, DNA and coactivators. This creates a unique local microenvironment that may selectively increase or decrease the concentration of small molecules, thereby having an impact on their target engagement and therapeutic efficacy (FIG. 3b). For example, cisplatin and JQ1 (TABLE 3) are antineoplastic compounds that act by intercalating DNA and inhibiting transcription, respectively. Both have recently been shown to specifically partition in transcriptional condensates<sup>30</sup>. Transformed cells often acquire transcriptional condensates at oncogenes, and high concentrations of intercalating agents or inhibitors at these key genes might account for the heightened sensitivity of cancer cells to agents that target universal cellular processes<sup>92</sup>. This partitioning behaviour might explain their ability to preferentially kill cancer cells, but it is not yet clear whether these compounds function inside the condensates, and a systematic comparison of efficacy versus condensate partitioning within a drug analogue series is yet to be reported.

### Considerations and challenges

The compositional complexity, size and dynamics of biomolecular condensates pose several challenges for drug discovery. First, reliable models reflecting relevant biology and well-defined metrics for characterization of condensates are imperative. Challenges in quantitative characterization of condensates and development of model systems are discussed in REFS.<sup>33,34</sup> Condensates are exquisitely sensitive to variations in expression levels of their

components and regulators, and changes in environment. For example, an overexpressed protein or a protein engineered to undergo phase separation more readily<sup>58,81,136,138,148,165–167</sup> in a model cell line could induce formation of condensates, whereas under endogenous expression levels in the disease-relevant cell line it exists below  $C_{sat}$ , raising the question of relevance of the screening outcome. Such artificial model systems have been used extensively in the field of transcription, where the small size and transient nature of the condensates or hubs make their quantification via conventional microscopy methods challenging.

Second, c-mod discovery will depend on cellular phenotypic assays (as described below), and so the identification of the target(s) driving the observed phenotypes will seldom be straightforward. C-mods may affect condensates via a wide range of mechanisms, from direct interactions with one or more biomolecules within the condensate, to general effects on the emergent properties of the condensate, to altered PTM of proteins, thereby preventing them from entering the condensate. For some c-mod discovery efforts, the target(s) will not be known, and the compound optimization effort will be driven solely by phenotypic cellular assays.

In cases where the targets of interest are known, a different challenge will emerge: correlating often-subtle measures of condensate phenotypic behaviours with more traditional measures used in drug discovery programmes, such as biochemical read-outs, intracellular target engagement, measures of gene expression, disease-relevant functional cellular read-outs or pharmacological effects.

A third challenge is how to optimize selective partitioning of the c-mod into the disease-relevant condensate. The properties of condensates vary widely and are difficult to quantify. To improve the therapeutic index, properties such as polarity, lipophilicity, hydrogen bond donor/acceptor count, charge, overall shape, flexibility, aromaticity and the presence of specific functional groups may influence partitioning and be optimized for a specific condensate environment.

An additional selectivity challenge results from the fact that many biomolecular components are found in multiple condensates. We hypothesize that a c-mod which partitions non-selectively into many condensates may lead to unacceptable off-target effects. However, if a c-mod concentrates in the condensate of interest, as each condensate contains in the order of a hundred other kinds of gene products,

### Box 1 | Computational methods to inform the study of condensates

Algorithms such as CatGranule<sup>247</sup> and P-score<sup>172</sup> have been trained on prior knowledge and are reasonably successful in predicting which protein sequences will phase separate. The PLAAC algorithm<sup>248</sup>, designed to detect prions, performs similarly to algorithms specifically trained to predict phase separation (reviewed in REF.<sup>249</sup>), suggesting that existing predictors might be detecting a particular ‘flavour’ of disorder. However, it is expected that as the volume of training data for such predictors increases, the quality and utility of databases and predictors will also increase.

Of note, the potential for a protein to homotypically phase separate is only one variable controlling the behaviour of condensates. Understanding the mechanism of action of condensate-modifying therapeutics (c-mods) or generating a condensate hypothesis requires more nuance. Such characterization can include an in-depth understanding of protein flexibility and disorder. Many different predictors of protein disorder exist, and aggregators of information on disorder such as D2P2 (REF.<sup>250</sup>) or MobiDB<sup>251</sup> are useful in parsing the results. These databases can inform on disorder and annotated motifs, and hint at hidden structures.

Similarly, when viewed through a lens of disorder or frustrated folding, predictions from AlphaFold<sup>252</sup> can provide information on protein conformational bias. Perhaps the most critical analyses of condensate components investigate how a protein (or nucleic acid) interacts within the condensate network. Tools that assess hidden functions within disordered regions<sup>253,254</sup> or predict short linear motifs in disordered regions<sup>255</sup> provide valuable clues about how flexible regions interact in condensates. This list is far from comprehensive; a wide array of protein property databases and predictors exist. The unifying feature of condensates is the array of distributed interactions with a range of affinities. Any bioinformatic tool that shows how a biomolecule interacts with its environment can aid in understanding condensate structure and the c-mod mechanism of action.

Emerging advances in all-atom simulations can also be used to elucidate the binding mode (as was shown for EPI-002 and EPI-7170 to the intrinsically disordered region (IDR) of the androgen receptor) and infer a structural rationale for their differential potencies<sup>256</sup>. All-atom and coarse-grained molecular simulations can be integrated at several points in the drug discovery pipeline, including but not limited to identification of interesting binding features or interfaces in the target biomolecule, obtaining insights into the mechanism of action of a hit compound<sup>195</sup> or guiding the screening strategies and medicinal chemistry efforts to improve potency.

### Box 2 | Methods for proximity labelling to map condensate composition

Approaches such as affinity purification coupled to mass spectrometry-based proteomics are traditionally used to map stable protein networks<sup>177,257,258</sup>. However, these methods fail to detect weak and transient interactions that are integral to networks within biomolecular condensates because material losses occur during cell lysis and subsequent washing steps. Chemical cross-linking followed by affinity purification overcomes some of these problems at the expense of increasing the rate of false positives<sup>257</sup>.

In proximity labelling techniques, a 'bait' protein is fused to an enzyme that covalently modifies 'prey' proteins or nucleic acids in its vicinity<sup>177</sup>, thus providing a means to preserve sensitivity to labile interactions, while allowing for stringent washes and minimizing carry-over of non-specific binders. Three main strategies have been developed. First, the engineered peroxidase system (APEX<sup>259</sup>, APEX2 (REF.<sup>260</sup>) and HRP<sup>261</sup>) biotinylates tyrosine residues of prey proteins, as well as nucleic acids, within a radius of about 10–20 nm, upon stimulation with peroxide. Second, biotin ligases (BioID<sup>259</sup>, BioID2 (REF.<sup>262</sup>), BASU<sup>263</sup> and TurboID<sup>264</sup>) create activated esters that covalently biotinylate lysine amines of proximal proteins. Last, hybridization-proximity labelling (HyPro) methods use digoxigenin-labelled antisense RNA hybridization probes, which bind to a target RNA in fixed cells, biotinylating proximal proteins and nucleic acids<sup>265</sup>.

APEX labelling occurs on short timescales (<1 min)<sup>257</sup>, and therefore is ideal for probing dynamic and transient cellular processes. BioID labelling operates on longer time frames (~18 h), limiting the application to long-lived structures such as the interaction network of TDP43 aggregates<sup>266</sup>. In contrast to APEX, where long-term exposure to peroxide leads to cell toxicity, BioID is non-toxic, enabling *in vivo* measurements<sup>267</sup>. The HyPro method has the advantage of not requiring expression of an exogenous, modified target protein and, therefore, can be performed on unmodified cells, as close as possible to physiological conditions<sup>265</sup>.

In all approaches, biotinylated biomolecules are enriched via affinity binding to streptavidin beads, purified and analysed by mass spectrometry (for proteins) and/or sequencing (for nucleic acids) to provide a comprehensive proteomic and transcriptomic interactome within the condensate. An in-depth comparison of the various protein–nucleic acid affinity approaches is presented in REFS.<sup>257,268</sup>

Proper controls are essential to map condensate compositions, specifically to decipher whether the detected biomolecules are indeed localized to a specific condensate. Ideally, two independent measurements are performed, one with a condensate present and one without; for example, stress-inducing agents can be used to tune the formation of the stress granules. As a cautionary note, transfection of fusion proteins can lead to overexpression artefacts, mislocalizations and composition alterations; therefore, any engineered system should be validated with functional assays and independent confirmation of the identified components (for example, with immunofluorescence).

the functional selectivity is likely to be very high because many other potential binding partners for that c-mod are not present in the condensate, leading to a high therapeutic index. In addition, if a c-mod can bind, even weakly, to multiple related proteins within the target condensate, this may yield a pharmacologically relevant effect.

#### Building a c-mod discovery platform

Converting our knowledge of biomolecular condensates and their involvement in disease into c-mods requires a novel drug discovery platform, which can be divided into four main parts (FIG. 4): identification of a disease-relevant target condensate, and formulation of a hypothesis on how modification of the target condensate could have desired functional effects in cellular models of disease (the condensate hypothesis); characterization of the target condensate and development of assays to measure such effects that enable validation of the hypothesis; high-throughput screening (HTS) to identify potential c-mods that reverse or

prevent the aberrant condensate phenotype; and hit-to-lead optimization based on the desired, disease-relevant functional outcome. Such a discovery platform will be built on existing (reviewed in REF.<sup>35</sup>) as well as novel interdisciplinary assays.

A foundational piece in the development of a c-mod discovery pipeline is establishing a reliable connection between the target condensate phenotype and a disease-relevant functional read-out. For example, a correlation between the decapping activity of Dcp1/2 (REF.<sup>67</sup>) and condensate formation was determined by tracking the fluorescence of a dual-labelled RNA probe. MED1-IDR-induced condensation was correlated with the transcriptional output measured in an *in vitro* transcription assay<sup>81</sup>. Fluorescence microscopy was used to monitor and quantify actin polymerization in response to signalling cluster formation<sup>65</sup>. Splicing<sup>26</sup> and cardiac defects in transgenic pigs<sup>26</sup> were linked to the phenotypic observation of RBM20 condensatopathy. Behavioural changes (for example, crawling ability) in a *Drosophila* ALS model<sup>129</sup> and

survival curves in a cardiomyopathy pig model<sup>26</sup> have been successfully used to correlate the condensatopathy with clinical presentations of the disease *in vivo*.

These functional assays should also be utilized again later in the pipeline as secondary hit validation assays and to optimize c-mod efficacy. To illustrate how such a c-mod discovery pipeline could look in practice, we use the example of repairing the NUP98–HOXA9 condensatopathy in AML, based on a range of complementary assays described by Chandra et al.<sup>168</sup> and Xu et al.<sup>169</sup> (FIG. 4).

#### Formulate a condensate hypothesis

The initial step of condensate-centric drug discovery is to select a target condensate and formulate a condensate hypothesis. Targets could originate from several sources, including pre-existing data on the disease relevance of a condensate, *de novo* identification of a condensatopathy or *de novo* identification of a condensate–association of a conventional target. Curated databases of genetic variants with strong disease association<sup>170,171</sup>, when combined with predictors of condensation-prone features<sup>172</sup> of the mutated proteins, provide a rich source to draw upon for hypothesis generation and can help prioritize proteins that probably play central roles in condensate assembly<sup>52</sup>.

Additionally, computational methods (BOX 1) have the potential to decode, *a priori*, how biomolecules cooperate to form condensates or how putative c-mods affect condensates, and to aid *de novo* discovery of c-mods. However, the complexity of condensates creates challenges for computational methods. Computational analysis can take the form of data mining the existing knowledge of condensate composition and the properties of simplified systems *in vitro* to generate predictions. In this vein, several databases of phase-separating proteins or condensate components have begun to be curated (reviewed in REF.<sup>173</sup>). Such databases can be an excellent first source when exploring a condensate hypothesis.

A hypothesis that a NUP98–HOXA9 condensatopathy is responsible for cellular transformation in AML cells can be formulated based on the following findings (FIG. 4a). First, human genetics data show a strong correlation between expression of NUP98 fusion oncogenes and AML clinical manifestation<sup>174</sup>. Second, expression of NUP98–HOXA9 in cultured cells induces formation of nuclear puncta, driven by the FG-repeat IDR of NUP98 (REFS.<sup>168,175,176</sup>).

### Condensate characterization

To understand the condensate, one must characterize, to the greatest extent possible, the community of biomolecules which comprise it. This information can be leveraged to characterize the condensatopathy, to select a suitable HTS phenotypic assay to identify *c*-mods and to interrogate the mechanism of action of *c*-mods. The compositional analysis can be performed via subcellular proteomic and transcriptomic analysis<sup>177</sup> (BOX 2) or multiplexed imaging methods<sup>178</sup>.

Due to the compositional complexity of condensates and the various parallel modes of regulation of phase behaviour, disentangling the contributions of specific components to the phenotype and behaviour of a condensate in living cells remains challenging.

Typically, live-cell fluorescence confocal microscopy is used to characterize the localization and emergent properties of condensates (reviewed in REF.<sup>179</sup>). Condensates with sizes below the limit of detection of conventional confocal microscopes<sup>136,180,181</sup> may be visualized, albeit at the expense of speed and throughput, with the advanced techniques<sup>182</sup> described in BOX 3. In-cell phase boundaries of biomolecular condensates can be quantified by correlating the variable levels of expression of a fluorescently tagged marker protein with the formation of condensates<sup>58</sup>. This analysis can measure the effects of disease-associated mutations<sup>183</sup>, or identify co-scaffold interdependencies<sup>64,184</sup>. Furthermore, it can be readily implemented into the HTS pipeline to determine the identity of *c*-mods and obtain mechanistic insights.

Complementary to cellular assays, *in vitro* reconstituted condensates that recapitulate a subset of relevant features of the biological condensate can be used to address more specific questions related to the nature of interactions that drive condensation or are affected by *c*-mods. For example, monitoring the shift in the phase boundary and changes in emergent biophysical properties (such as number, size, morphology, material properties, dynamics and composition) as a function of various parameters (such as ionic strength, pH, ligand concentration and temperature) could identify the most promising points for therapeutic intervention inside the macromolecular network (reviewed in REF.<sup>179</sup>). This informs strategies for *c*-mod design (for example, a protein–ligand interaction, a hydrophobic-driven interaction or an electrostatically driven interaction) and hit optimization.

Methods for probing material properties, such as viscosity, surface tension and component dynamics (for example, diffusion and mobile fraction), can be applied *in vitro* and in live cells<sup>40,44,185</sup> (BOX 4); this information can be leveraged as a read-out to detect a change in condensate milieu, and/or to gain insights into the mechanisms driving a condensatopathy or a *c*-mod.

For example, the condensate hypothesis for the NUP98–HOXA9 condensatopathy is supported by the correlation between the phenotypic observation of aberrant nuclear puncta and transcriptional reprogramming of the *HOX* cluster and *p53* that leads to

leukaemogenesis in primary cells and mouse models<sup>168,169</sup>. The composition of these aberrant condensates has been characterized by proximity labelling proteomic assays<sup>186</sup> (BOX 2), as well as Co-IP and ChIP-seq<sup>169</sup> assays showing an expansive network of interactions with chromatin remodelling factors<sup>168,169</sup>. NUP98–HOXA9 within nuclear condensates recovers from photobleaching in the order of seconds<sup>168</sup>, indicating dynamic on/off binding kinetics and rapid diffusion with and within the extended condensate network. Importantly, these disease-relevant functions depend on the ability of NUP98–HOXA9 to form condensates via FG motif multivalency,

### Box 3 | Advanced microscopy techniques to study condensates

Some condensates, such as transcriptional condensates<sup>181</sup>, are very small and therefore hard to detect with conventional confocal microscopes. In recent years, various advanced imaging technologies have been developed to study condensates across scales<sup>182</sup>. Specifically, super-resolution imaging techniques such as time-correlated photoactivated localization microscopy (tcPALM)<sup>181</sup>, live-cell single particle tracking<sup>138,269</sup> and stimulated emission depletion (STED) can achieve a spatial resolution of tens of nanometres and were successfully used to gain deep understanding into the transcriptional condensate and heterochromatin biophysics<sup>165</sup>. For transcriptional condensate analysis, it might be of great importance to measure whether a transcription factor such as MYC partitions into a MED1 condensate<sup>136</sup>. For such a co-localization analysis, STED is a well-suited tool, because it offers the optimal combination of spatial super-resolution and optimal alignment of the different colour channels.

Structured illumination microscopy bridges the gap between advanced super-resolution techniques such as STED and PALM and conventional confocal microscopy, providing resolutions down to 60 nm (REF.<sup>270</sup>). Moreover, dynamic live-cell imaging can be performed without the need for dedicated sample modifications. By trading resolution for speed and reducing phototoxicity, lattice-light sheet microscopy (LLSM) emerged as a powerful tool for long-term imaging of dynamic objects in living cells<sup>271,272</sup> and even to visualize condensates in *Drosophila* embryos<sup>79</sup>. Although these structured illumination microscopy approaches provided important insights on the dynamics of condensates, automated screening remains challenging because minor alignment errors such as a small tilt of the sample can be detrimental to the image quality. Moreover, the reconstruction algorithms can induce imaging artefacts.

LLSM is a super-resolution method well poised for the study of the dynamic properties of condensates and the early steps of protein aggregation, due to its high spatial and temporal resolution in combination with low phototoxicity<sup>273</sup>. LLSM revealed that even in extended embryonic systems, the HP1A condensate can grow, fuse and dissolve<sup>79</sup>. Recently, light sheet microscopes have been successfully re-engineered into an inverted configuration<sup>274</sup> and LLSM versions are commercially available, making this exciting technology applicable for multiwell plate configurations, which expands the usability for pharma applications. In general, all of these techniques provide limited applicability for large-scale screening purposes. With dedicated optimizations and custom software development, up to 1,000 compounds can be evaluated. Despite their limited throughput, these super-resolution techniques serve as excellent choices for in-depth characterization of the model systems, hit follow-up and investigation of mechanisms of action.

Super-resolution add-ons for confocal systems<sup>275,276</sup> (which we refer to as enhanced resolution) are a very good option to improve the resolution for condensate screening applications, as they are commercially available and can be used in combination with multiwell plate formats. Additionally, no sample modification is required and software integration for automation is provided. These enhanced resolution systems can be reasonably set up to screen up to tens of thousands of compounds.

Phenotypic screening of small condensates, with sizes below the visible light diffraction limit, is challenging due to the requirement for specialized, low(er) throughput instrumentation. Advances in machine learning can be leveraged to compensate for some of these shortcomings<sup>277,278</sup>. For example, machine learning algorithms can be trained on high-quality, low-throughput, super-resolution images (such as STED) to enhance the data quality of conventional microscopy images<sup>279</sup>, enabling high-throughput screening (HTS) of small condensates. Machine learning can also be used to optimize and integrate data analysis from large imaging data sets with data from orthogonal validation assays, in order to expedite the drug discovery process.

## Box 4 | Methods for probing material properties of condensates

Fluorescence recovery after photobleaching (FRAP) measures biomolecular diffusion inside condensates, reporting on a convolution between local viscosity and binding kinetics of the condensate biomarker with the macromolecular network<sup>34,280</sup>. FRAP assays are compatible with most cell lines expressing genetically encoded fluorescent tags; limitations include time-dependent changes in material properties (for example, ageing), long acquisition times and limited throughput. Molecular rotors are fluorescent dyes that are sensitive to environmental changes; they can be conjugated to genetically encoded tags (for example, HaloTag) fused to the condensate biomarker<sup>281</sup> to sense local changes inside specific condensates. Theoretically, this method could be adapted for high-throughput screening (HTS) applications. Time-lapse imaging can track condensate fusion and isotropic growth, which reports on the ratio between viscosity and surface tension<sup>12</sup>. All of these methods are compatible with *in vitro* reconstituted systems and live cells.

Fluorescence lifetime imaging (FLIM) can visualize a change in intracellular material properties<sup>282</sup>. A well automatized FLIM set-up (where the acquisition time for a full field of view is ~5 s) could outcompete FRAP in terms of throughput; the non-destructive nature of FLIM is compatible with imaging in live systems (for example, developing or ageing animals) over a much longer time course.

Stimulated Raman scattering (SRS) microscopy-based methods are a set of powerful label-free techniques, which have been successfully applied to detect peripheral nerve degeneration and tissue degeneration of the spinal cord in mouse models of amyotrophic lateral sclerosis (ALS)<sup>283,284</sup>. They were also used in the quantification and spectral analysis of native polyQ aggregates with subcellular resolution in live cells<sup>285</sup>. SRS microscopy is commercially available and can be coupled to other imaging modalities such as confocal fluorescence microscopy and non-linear imaging, enabling different cellular components such as aggregates and lipid droplets to be separated spectrally.

Active physical perturbations such as defined temperature changes<sup>286,287</sup> or advanced laser-induced hydrodynamic flow changes<sup>49,288</sup> provided detailed insights into the biophysical nature of condensates in living organisms.

Chemical perturbations that globally affect certain classes of interactions, such as 1,6-hexanediol (hydrophobic interactions)<sup>79,289</sup>, salt (electrostatic interactions)<sup>286</sup> and disassembly drugs<sup>290</sup>, can be used in parallel to a screen; these provide insights into the extent to which different types of interactions contribute to condensate stability, and a means of detecting changes in material properties of condensates as a function of drug treatment. As for practical considerations when applying global perturbations, short incubation times are recommended so that instantaneous physicochemical responses can be probed before toxic side effects develop. The latter should be monitored by using cell viability or toxicity assays. Although some of the biophysical perturbations described above might not be suited for large-scale screening campaigns, they can be utilized to distinguish between aggregates (irreversible) and condensates (reversible), which is essential for the characterization of the target before starting a HTS campaign.

and the ability to nucleate condensates by binding DNA through the HOXA9 folded domain<sup>168</sup>.

### Primary screens

At the onset of the HTS campaign for potential c-mods, one should have the following: a validated condensate hypothesis; a robust and scalable set of phenotypic and disease-relevant functional assays; and an assay that enables investigation of structure–activity relationships for hit-to-lead optimization. C-mods are selected based on phenotypic HTS, which monitors a combination of emergent properties (for example, size, number and morphology) and/or co-localization of selected markers. Considerations for the selection of the appropriate HTS set-up that balances speed, throughput and resolution/sensitivity as appropriate for the target condensate are discussed below.

High-content imaging assays can be optimized for screening of large libraries (~10<sup>6</sup> compounds), while monitoring

the optical phenotype of condensates in live or fixed cells<sup>31,32,187</sup>, and *in vitro* reconstituted condensates<sup>188</sup> with sizes above the diffraction limit. This approach has been used to identify hits that inhibit stress-induced aggregation of TDP43 (REF.<sup>187</sup>), stress granule formation<sup>31,32</sup> and p53–Mdm2 interaction<sup>188</sup>.

The imaging technique depends on the size of the condensate. Generally, increasing the optical resolution and signal sensitivity comes at the expense of throughput. Initial target assessment and hit follow-up studies of 1,000 compounds might be achievable with the advanced techniques presented in BOX 3. Alternatively, the model system can be altered to artificially increase the size of the condensate, by using optogenetics<sup>58,189,190</sup> and repeat operon arrays<sup>30,136,138,191,192</sup>. These engineered systems and practical considerations for their selection are reviewed in REF.<sup>179</sup>.

Monitoring the material properties of condensates can also identify c-mods (BOX 4).

These methods are amenable to multiplexing and HTS applications, with libraries of up to 10<sup>4</sup>–10<sup>5</sup> compounds, depending on the technique.

For the example of repairing the NUP98–HOXA9 condensatopathy, a phenotypic primary HTS would identify compounds that change the optical phenotype of the NUP98–HOXA9 nuclear puncta (FIG. 4b). A change from punctate to diffuse staining of NUP98–HOXA9 could indicate c-mods that dissolve the condensates, whereas a change to fewer, larger condensates could indicate c-mods that inhibit binding to chromatin<sup>168</sup>. Similar phenotypic screens have been performed to identify small molecules that prevent oxidative stress-induced TDP43 and G3BP1 cytoplasmic puncta formation in PC12 (REF.<sup>187</sup>), as well as HEK293xT and neural precursor cells<sup>31</sup>, respectively; these serve as model systems for ALS.

### Secondary screens and hit optimization

The primary hits from the HTS are filtered based on cytotoxicity and condensate selectivity (for example, using a phenotypic screen against a panel of unrelated condensates), validated based on disease-relevant functional assays (for example, induced pluripotent stem cell-derived or patient-derived cells) and their mechanism of action characterized via biophysical measurements. Optimization of drug partitioning inside a target condensate (BOX 5) provides the opportunity to improve the therapeutic index by increasing exposure of a drug to its target and minimizing off-target effects.

In our NUP98–HOXA9 condensatopathy example (FIG. 4b), the primary screen hits would be evaluated and further optimized in cell proliferation/transformation (for example, proliferation rates and colony formation) and/or transcriptional reprogramming (for example, qRT-PCR and ChIP-seq) assays, followed by validation in animal models (for example, tumour growth and survival)<sup>169</sup>. Leptomycin B (TABLE 3), a well-characterized inhibitor of the nucleocytoplasmic transporter CRM1, exhibited c-mod properties when it inhibited formation of NUP98–HOXA9 aberrant condensates, and transcriptional reprogramming<sup>193</sup>. We hypothesize that the c-mod acts by inhibiting CRM1-dependent nucleation of NUP98–HOXA9 condensates on chromatin<sup>168,193,194</sup>.

### Outlook

Biomolecular condensates are emerging as attractive novel targets for drug discovery. Many proteins and nucleic acids of high



therapeutic interest, including numerous targets previously considered ‘undruggable’, operate within condensates. Importantly, there is emerging evidence that condensates are ‘druggable’. First, some approved drugs have been shown to partition into condensates<sup>30</sup>. Second, high-content cellular screening has identified drug-like molecules that modulate condensate behaviours in a selective manner<sup>31,148,187</sup>. Third, it is now understood that PTMs can strongly regulate the formation, behaviour and dissolution of condensates. Taken together, it is tempting to speculate that many approved drugs may, in part, be acting as c-mods, for example by exerting a portion of their pharmacological benefit through the modification of disease-relevant condensates.

We hypothesize that condensates could represent nodes of misregulation in polygenic diseases. For example, mutations in binding motifs within IDRs (such as degrons, nuclear export and import signals) can alter regions of transient structure<sup>120</sup> and/or the interactome of the affected protein<sup>126</sup>. Consequently, changes in the IDR interactome can lead to alterations in the condensate scaffolding, composition, dynamics, material properties and functional output. Alternatively, the mutations can lie outside canonical binding regions, where they might affect condensation by changing the physicochemical properties and valency. This paradigm might explain the pathophysiology of certain diseases that exhibit stereotyped phenotypes but complex genetic and environmental causes. Each individual type of ALS/FTD-associated genetic mutation accounts for a relatively small number of patients. However, despite differences in the cause of onset, all ALS subtypes share condensate dysfunction as the common denominator, namely formation and persistence of cytoplasmic TDP43 granules in affected neurons<sup>23</sup>. Targeting the condensate rather than individual mutations within that molecular community might provide an avenue to deliver broader therapies to a larger patient population. It is also possible that the dysfunctional cellular processes observed in some cancers are driven by mutations in diverse genes, all of which form a single condensate. This condensate may integrate oncogenic signals into a single output, such as a high proliferative capacity or sustained signalling.

The high complexity and dynamic nature of condensates raise several unique challenges and opportunities for c-mod discovery. For example, c-mods can exhibit

unusual dose–response behaviour, which can vary depending on the experimental conditions. This behaviour, however, could provide insights into the mechanism of action of the c-mod, such as preferential engagement with one of the phases<sup>195</sup> or engagements of multiple targets<sup>196</sup>. For this reason, a range of biologically relevant assay time frames, windows of drug treatment and phenotypic responses must be measured. In addition, tight control over assay conditions must be maintained to achieve the necessary assay reproducibility required for HTS and medicinal chemistry. Appropriate biochemical and disease-relevant functional read-outs are required to demonstrate clear correlations with the observed condensate phenotypes.

Because of the complexity of condensates, and the nature of the forces that lead to condensate formation, combinations of drugs that engage multiple components of the molecular community may be of particular importance. Furthermore, a c-mod may be envisioned that binds weakly to multiple sites on one protein or to many related proteins; such monovalent compounds, binding in a super-stoichiometric fashion, are expected to reduce the valency on the scaffolds, thereby destabilizing the condensate. To stabilize

a conformational state that promotes interaction, one could adopt a molecular glue strategy to force biomolecules to remain in an associated or proximal state.

Many questions and challenges are topics of active investigation by the community. For example, how do we demonstrate causality between disease and condensatopathies? How do we identify ‘hub’ condensatopathies for polygenic diseases? What are the different signalling and regulatory pathways that are dysregulated via any one target condensate? What are the most informative components for understanding the function of the condensate and the effects of c-mods? To address each of these challenges, the drug-hunter must understand the individual components of a target condensate as well as the collective behaviour of the molecular community. However, this remains challenging, both due to technological limitations of spatial and temporal resolution as well as biological complexity (for example, fluctuations in composition due to stochastic variability in protein expression or differences in cell-cycle state).

The more complete the condensate map, the more opportunities for a successful drug discovery programme. The complexity of the condensate environment requires creative medicinal chemistry approaches to

#### Box 5 | Methods for probing small-molecule partitioning in cells

Detection of small molecules inside condensates remains challenging. Fluorescence confocal microscopy allows for visualization of small molecules that are inherently fluorescent, such as mitoxantrone, or require custom modifications as applied for cisplatin<sup>291</sup> and tamoxifen<sup>292</sup>, but is not generally applicable, as most drug-like molecules are not inherently fluorescent.

In vitro reconstituted systems are amenable to physical separation of the two phases (light and dense) by centrifugation, providing direct access to measuring concentrations of the small molecule using well-established, scalable analytics methods to extract partition coefficients. Reconstitution of condensates from whole cell lysates<sup>293</sup> derived from disease-relevant cell models more closely mimics the complexity of cellular condensates and could be used to directly measure condensate-modifying therapeutic (c-mod) partitioning as described above.

Measurements of compound partitioning into in vitro reconstituted and/or in live-cell condensates can be integrated in an iterative fashion in the medicinal chemistry optimization pipeline, in combination with the functional assays described above.

Stimulated Raman scattering (SRS) microscopy is an imaging method based on contrast generated by Raman-active vibrational frequency of a given chemical bond that allows visualization of label-free small molecules, as well as distinguishing between specific classes of biomolecules and types of protein secondary structure<sup>294</sup>. This imaging technique can be leveraged to quantify drug partitioning in condensates inside living cells. However, the generated scattering signals are inherently weak, and so only a well-tuned system in combination with Raman-active compounds, such as drugs containing an alkyne moiety (for example, ponatinib), might be detected with resolutions and sensitivities<sup>295</sup> that are required to localize the compound within a condensate inside a cell.

Nanoscope subcellular ion beam imaging provides a completely new avenue to visualize the 3D volumetric distributions of genomic regions, RNA transcripts and proteins with 5-nm axial resolution<sup>296</sup>. It was used to monitor the cisplatin distribution with subcellular resolution in cancer cells<sup>297</sup>. The technology requires sample fixation, which locks biological molecules in space; antibody staining is used to visualize co-localizing proteins. However, caution must be exercised as fixation might cause partitioning artefacts by altering the biophysical properties of the condensates. Therefore, either partition experiments should be performed in a live-cell setting or all biological and drug-like molecules need to be (chemically) fixed in space.

## Glossary

## Centrosome

The main microtubule organizing centre of all animal cells. It comprises a pair of centrioles on its core that are surrounded by a mass of proteins called pericentriolar material. Centrosomes nucleate microtubules that form the mitotic spindle upon cell division and are also involved in other functions such as cell polarity and cell signalling.

## Clients

Molecules that partition into condensates, but are not required for the condensate formation.

## C-mods

(Condensate-modifying therapeutics). Compounds that modify the properties of a biomolecular condensate.

## Condensate hypothesis

A testable proposition that links the modification of a particular biomolecular condensate (for example, normal or abnormal) to a desirable functional change in diseased cells.

## Condensatopathy

An aberration of a condensate that drives a specific disease phenotype.

 $C_{\text{sat}}$ 

(Saturation concentration). The threshold concentration above which condensates form.

## Mesoscale assemblies

Biomolecular networks that span the nanometre-to-micrometre length scale.

## Multivalency

The presence of repeated, identical or similar interaction sites or domains on the same molecule.

## Nuclear pore

A nuclear pore is a protein assembly embedded in the nuclear envelope that provides a selectively permeable

barrier via FG-rich intrinsically disordered regions separating the inside of the nucleus from the cytoplasm.

## Nucleolus

A ribonucleoprotein condensate that functions as the site of ribosome biogenesis and in stress sensing. Nucleoli exhibit a three-layer architecture, composed of the fibrillar centre (the site of ribosomal DNA transcription), the dense fibrillar component (the site of pre-ribosomal RNA processing) and the granular component (the site of ribosomal RNA assembly with ribosomal proteins into pre-ribosomal particles).

## Partition coefficient

The ratio between component concentrations inside versus outside the condensate.

## PARYlation

(Poly-ADP-ribosylation). A post-translational modification (PTM) where polymers of ADP-ribose (poly(adenosine diphosphate ribose)) are covalently attached to proteins. This process is mediated by PAR polymerase enzymes.

## P granules

Perinuclear ribonucleoprotein granules found in *Caenorhabditis elegans* germline cells.

## RNA expansion repeats

Repeats of a short nucleotide sequence of variable length.

## Scaffolds

Multivalent biomolecules required for condensate formation.

## Stress granules

Membraneless compartments consisting of RNA and proteins that form in the cell cytoplasm upon stress conditions.

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develop c-mods. For example, knowledge about drugging individual targets that localize to or regulate a condensate can be leveraged to create combination therapies or multifunctional drugs. This information may, in turn, address other challenges, including how to mitigate toxicity by, for example, avoiding inhibition of components that do not exclusively function within the target condensate, or overcoming drug resistance. A promising result in this direction has been reported for multiple myeloma, where patients with high expression of the protein SRC3 experience poor outcomes. Liu et al.<sup>197</sup> discovered that resistance to the proteasome inhibitor bortezomib results from interactions between steroid receptor coactivator SRC3 and the histone methyltransferase NSD2, leading to the stabilization and phase separation of SRC3. A small-molecule compound, SI-2 (TABLE 3), disrupts the interaction between SRC3 and NSD2, eliminating the condensate and restoring the activity of bortezomib<sup>197</sup>.

Incorporating a ‘condensate perspective’ into the drug discovery process holds

significant potential to create medicines that operate through fundamentally different mechanisms. However, to capitalize on the insights that are emerging in the condensate field, it is clear that a novel approach is required. We suggest that successful discovery of c-mods will result from integrating deep understanding of condensate properties and function, pragmatic drug discovery expertise, and robust commitment to the development and application of suitable technologies to measure emergent properties of condensates, to characterize the broad effects of c-mods on condensate behaviour and function, and to further understand the thermodynamics and kinetics of these interactions at a molecular level. Synergy between efforts in the biotechnology and pharmaceutical industries and academia, and expertise from disparate fields, has been and will continue to be the key for success in developing new medicines by targeting biomolecular condensates.

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#### Competing interests

All authors are employees or board members of Dewpoint Therapeutics, a drug discovery company that studies condensates, and have a financial stake in the company.

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