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REVIEW

Small molecules modulate the biomolecular condensates and liquid−liquid phase separation

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Abstract Phase separation of biological macromolecules is a ubiquitous cellular mechanism for concentrating and compartmentalizing biochemical reactions. Emerging evidence reveals that biomolecular condensates formed *via* liquid−liquid phase separation (LLPS) play integral roles in diverse physiological processes, including gene expression and intracellular signaling, enabling rapid and delicate cellular responses. Dysregulation of phase separation is increasingly implicated in the pathogenesis of major diseases, ranging from neurodegenerative disorders and cancers to viral infections and aging. Consequently, deciphering the molecular mechanisms governing LLPS and developing strategies for its pharmacological modulation, particularly *via* small molecules, represent promising therapeutic targets offering novel approaches for disease intervention. In this review, we provide a concise overview of biomolecular condensates formation and their functions in diseases regulation. Mainly, we catalog commonly employed tool compounds, reported small-molecule modulators of LLPS, and related clinical progress within this rapidly evolving research area. Furthermore, we integrate recent technological breakthroughs in LLPS to envision the future trajectory and therapeutic potential of this field.

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1. Introduction

Phase separation represents a distinct aggregation state of biological macromolecules within cellular contexts, standing as one of the cardinal mechanisms for cellular regulation. Biomolecular condensates, in essence, are membraneless organelles spontaneously formed from homogeneous solutions through liquid−liquid phase separation (LLPS) or solid-liquid phase separation. This formation relies on proteins, nucleic acids, and other biopolymers *via* multivalent interactions under specific physicochemical conditions (*e.g.*, concentration, temperature, pH, and ionic strength). Biomolecular condensates provide a spatially confined chemical reaction platform for key life processes such as signal transduction, gene transcription, and metabolic regulation[1−5]. Major biomolecular condensates include the nucleolus, stress granules (SGs), processing bodies (PBs), chromatin condensates, and postsynaptic densities. As a condensate within the cell nucleus, the nucleolus is the core of ribosome biogenesis and has substructures such as the fibrillar center (FC), dense fibrillar component (DFC), peripheral dense fibrillar component (PDFC), and granular component (GC); in these regions, ribosomal RNA (rRNA) genes are transcribed, processed, and assembled with proteins into ribosomal subunits. Cytoplasmic SGs form under stress conditions (*e.g.*, heat shock) and protect untranslated messenger RNA (mRNA) by storing it, while PBs degrade excess or defective mRNA and participate in mRNA quality control. Chromatin condensates regulate gene expression through chromatin compaction, facilitating the formation of cell-type-specific gene expression patterns. The postsynaptic density of neurons enables efficient neurotransmitter signal transduction and plays an important role in synaptic plasticity and memory formation[6−10].

The primary mechanism driving phase separation is the formation of a dynamic network among biological macromolecules through the synergistic effect of multivalent weak interactions, resulting in an increase in local concentration and spontaneous separation into high-density liquid phases and low-density liquid phases[11]. The fundamental driving forces can be categorized as follows (Fig. 1): (i) Multivalency: A single molecule engages with other molecules or itself *via* multiple weak binding sites, creating a dynamic cross-linking network, such as protein−protein interactions and protein−RNA interactions. This multivalent nature enables the establishment of a complex web of associations that is crucial for phase separation[12,13]. (ii) Intrinsic disordered regions (IDRs) and low complexity sequences (LCRs): IDRs lack a fixed three-dimensional structure. Instead, they are abundant in polar or charged amino acids (*e.g.*, serine, tyrosine, and glutamine), enhancing the diversity of interactions through dynamic conformation. LCRs are repetitive simple sequences (*e.g.*, glycine−serine and arginine−glycine), they facilitate phase separation primarily through hydrophobic or electrostatic interactions. Their repetitive nature provides a platform for these interactions to occur in a coordinated manner. (iii) Electrostatic interactions: Electrostatic plays a vital role. Cationic−π interactions occur between positively charged arginine (R) and aromatic amino acids, such as tyrosine (Y). Additionally, salt bridge interactions form between oppositely charged amino acids or phosphorylation sites. These electrostatic interactions contribute to the stability and specificity of the molecular assemblies during phase separation (iv) Hydrophobic effect: Non-polar amino acids (*e.g.*, leucine, isoleucine, and phenylalanine). minimize their free energy by reducing hydrophobic surface exposure in aqueous environment, promoting droplet formation. This natural tendency promotes the formation of droplets, which is a key aspect of phase separation. (v) Aromatic interactions: The benzene rings of aromatic residues, namely tyrosine (Y), phenylalanine (F) and tryptophan (W), enhance multivalent cross-linking through $\pi-\pi$ stacking or cationic−π interactions. These aromatic interactions further reinforce the network structure formed during phase separation, stabilizing the resulting LLPS states. (vi) Regulation by external environmental factors: External factors such as temperature, ionic strength and molecular concentration also significantly impact phase separation. For instance, changes in temperature can alter the kinetic and thermodynamic properties of molecular interactions, while variations in ionic strength can modulate electrostatic interactions. Molecular concentration directly influences the likelihood of intermolecular encounters, thereby affecting the formation of the dynamic network. The formation of SGs is intricately linked to the aforementioned driving forces. These forces work in concert, creating a complex and finely-tuned mechanism that governs the formation and function of membraneless organelles, including SGs, which are essential for cellular homeostasis and stress response[11,14−16].

The core function of phase separation is realized through dynamic liquid-phase assembly, which plays a dual role in pathological process (Fig. 2). On one hand, it activates specific

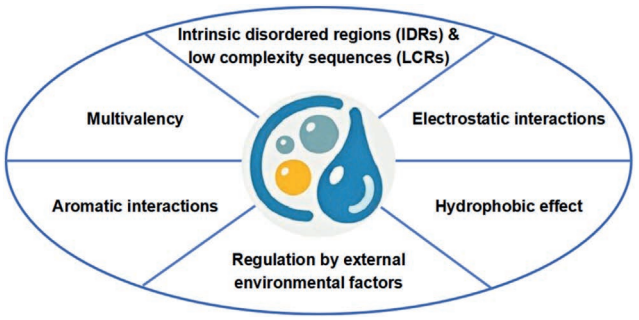

Figure 1 Fundamental driving forces of phase separation.

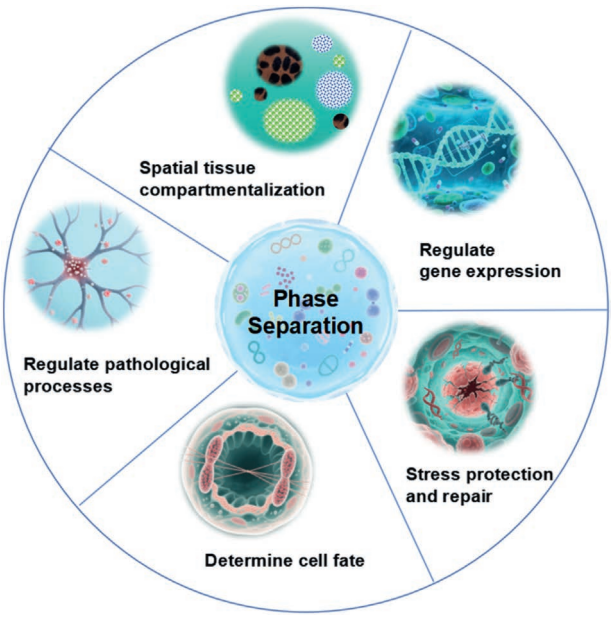

Figure 2 Functions of phase separation.

signaling pathways, such as kinase cascade reactions, through an enrichment effect. By concentrating key molecules in a specific region, it facilitates the sequential activation of signaling components, thereby promoting the transmission of biological signals[2,17]. On the other hand, it inhibits non-essential molecular interactions, like the aggregation of misfolded proteins, through a physical isolation effect. This isolation mechanism helps maintain the normal conformation and function of proteins, preventing the formation of harmful aggregates that can disrupt cellular processes[18−20]. Functionally, phase separation can be systematically categorized into: (i) Spatial tissue compartmentalization: Phase separation acts as a natural "compartmentalizer" within cells. It creates distinct liquid-phase regions that separate different cellular components, much like the rooms in a building. This compartmentalization allows for the spatial organization of various biochemical reactions, ensuring that they occur in an orderly and efficient manner without interference from other cellular processes[21,22]. (ii) Precise regulation of gene expression: It is intricately involved in the regulation of gene expression at multiple levels, including transcription, translation, and RNA metabolism. During transcription, phase-separated condensates can recruit transcription factors (TFs) and other regulatory proteins to specific genomic loci, either enhancing or repressing gene transcription[23,24]. In translation, it can control the assembly of ribosomes and the availability of mRNA, modulating the rate of protein synthesis[25]. Additionally, phase separation also participates in RNA processing, such as splicing and degradation, to ensure the proper maturation and function of RNA molecules[26,27]. (iii) Stress protection and repair: When cells encounter stress, such as oxidative stress or heat shock, phase separation comes into play as a protective mechanism. It enables the formation of stress-induced condensates, like SGs, which sequester translationally inactive mRNAs and associated proteins. This helps cells conserve energy and resources during stress and provides a platform for the repair and restoration of normal cellular functions once the stress is relieved[28,29]. (iv) Determination of cell fate: Phase separation is crucial for determining cell fate decisions, including cell differentiation, polarization, and the cell cycle. During differentiation, it can regulate the expression of lineage-specific genes by organizing TFs networks into phase-separated compartments[30]. In cell polarization, it helps establish and maintain the asymmetric distribution of proteins and organelles, which is essential for cell motility and function. Moreover, it also participates in the regulation of the cell cycle by controlling the activation and inactivation of key cell-cycle regulators[31]. (v) Regulation of pathological processes: Given its far-reaching effects on cellular functions, phase separation also has a significant impact on pathological processes. Dysregulation of phase separation has been associated with various diseases, including neurodegenerative diseases, cancer, and metabolic disorders[32,33]. For example, in neurodegenerative diseases, abnormal phase separation and aggregation of proteins can lead to the formation of toxic protein aggregates, which are characteristic of these disorders. Analyzing the mechanism of phase separation not only uncovers the fundamental laws governing the self-organization of biological macromolecules, but also provides a novel perspective for understanding the environmental dependence of protein functions. It also provides valuable insights into the imbalance of phase transitions associated with diseases, opening up new avenues for the development of targeted therapeutic strategies.

2. Phase separation in diseases

Since the concept of phase separation was first introduced, substantial evidence has demonstrated that abnormal phase separation is closely related to the onset and progression of various diseases.

2.1. Neurodegenerative diseases

An ever-expanding series of studies have firmly established the association between LLPS and neurodegenerative disorders. Tau, a microtubule-associated protein, is crucial for microtubule assembly. It has been closely implicated in the development of multiple neurodegenerative diseases, including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasement membrane degeneration (CBD), silver granulosa disease, Pick's disease, certain forms of frontotemporal dementia (FTD), and chronic traumatic encephalopathy (CTE). Tau filaments exhibit the characteristics of amyloid fibers, and the tau protein has a strong propensity for LLPS, either alone or in the presence of RNA, forming liquid-like droplets that can transition into amyloid fibrils[34]. This aberrant phase separation may initiate tau aggregation, accelerate the deposition of amyloid-β protein (Aβ), and drive the progression of devastating neurodegenerative diseases such as AD and FTD[35,36]. α-Synuclein (α-syn, SNCA), an intrinsically disordered protein (IDP), is highly expressed in the synaptic precursors of nerve endings and plays a pivotal role in the nervous system and neurodegenerative pathologies, particularly in Parkinson's disease (PD)[37]. α-Syn undergoes dynamic LLPS to regulate synaptic vesicle dynamics, highlighting its complex phase behavior in both synaptic homeostasis and pathological aggragation[20,38]. Furthermore, abnormal phase separation of proteins like fused in sarcoma/translocated in liposarcoma (FUS) and TAR DNA-binding protein 43 (TDP-43) results in the formation of fibrous solids. This phenomenon leads to neuronal dysfunction and ultimately cell death, and is closely associated with diseases such as amyotrophic lateral sclerosis (ALS) and FTD[39−42].

2.2. Cancers

As our understanding of LLPS continues to deepen, many oncogenic processes are being re-evaluated as manifestations of LLPS-driven mechanisms. LLPS is closely associated with epigenetic dysregulation, a factor that significantly promotes tumor initiation and progression[43,44]. Additionally, phase separation condensates have the potential to modulate drug distribution and concentration within tumor cells, thereby influencing drug efficacy and contributing to drug resistance[45]. For instance, prostate cancer-associated SPOP (speckle-type POZ protein) mutations enhance autophagy and activate the NFE2L2/NRF2 (NFE2 like BZIP transcription factor 2/nuclearrespiratoty factor 2) pathway by directly regulating the LLPS and ubiquitination of sequestosome 1 (SQSTM1)[46]. This discovery suggests that targeting this oncogenic pathway could potentially be a viable strategy for treating SPOP-mutated cancers. In cellular models of prostate cancer, the androgen receptor (AR) forms dynamic, AR-rich, liquid-like foci with the co-activator mediator complex subunit 1 (MED1) super-enhancers (SEs). These foci play a role in promoting oncogenic transcriptional programs, diving the development of diseases[47]. Regarding osteosarcoma, previous studies have highlighted theoretical role of the MYC-driven SEs signaling pathway in osteosarcoma tumorigenesis[48]. Homeobox B8 (HOXB8) and fos-like

antigen 1 (FOSL1), key regulatory circuit components located near the SEs in osteosarcoma, can form dense and dynamically phase-separated droplets *in vitro*. Moreover, these components can form fluid-like spots within the nucleus, further emphasizing the role of LLPS in osteosarcoma development[49]. LLPS also contributes to drug resistance in multiple myeloma (MM)[50]. In this cancer, LLPS of Src-3 (steroid receptor coactivator 3) enhances the recruitment of histone methyltransferase NSD2 (nuclear receptor binding SET domain protein 2). This, in turn, affects H3K36me (histone lysine 36 site H3) and apoptosis, ultimately promoting drug resistance in MM. EML4/ALK (echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase) variations undergo phase separation in tumors, enhancing STAT3 (signal transducer and activator of transcription 3) phosphorylation, facilitating tumor transformation and highlighting yet another way in which LLPS is involved in the oncogenic process[51]. Furthermore, the long non-coding RNA (lncRNA) MNX1-AS1 demonstrated a potent anti-tumor effect in xenotransplantation models by amplifying c-Myc expression and stabilizing TF1 mRNA *via* interaction with IGF2BP1 (insulin-like growth factor 2 mRNA binding protein 1), positioning it as a promising biomarker and a therapeutic target[52]. In breast cancer, mutations in the tumor suppressor gene SPOP can induce the disease[53]. These mutations disrupt phase separation, leading to mislocalization of key cellular components. Additionally, adenosine triphosphate (ATP), acting as an estrogen cofactor, participates in the dynamic phase separation process, thereby influencing the pathophysiology of breast cancer[54,55]. The cell polarity protein Par3 promotes LLPS of the adhesion molecule a-catenin during metastasis, altering osmotic pressure and mechanical properties. Inhibiting aPKC (atypical protein kinase C) reduces Par3-mediated mechanical signaling, impairing invasion and migration of breast cancer, while potentially improving patient survival[56]. Pancreatic cancer progression is similarly driven by LLPS-mediated mechanisms. SRPK2 (serine/arginine-rich protein kinase 2) emerges as a key regulator of SGs formation in obesity-associated pancreatic ductal adenocarcinoma, governed by hyperactivation of the IGF1 (insulin-like growth factor 1)/PI3K (phosphatidylinositol-3-kinase)/mTOR1 (mammalian target of rapamycin 1)/S6K1 (ribosomal protein S6 kinase beta-1) pathway. Moreover, the low-complexity sequence domain of histone lysine (K)-specific methyltransferase 2D (KMT2D) fosters an LLPS-favorable environment. This environment aids in regulating histone monomethylation and transcription, thereby facilitating the progression of pancreatic cancer[57]. LLPS also underpins oncogenic processes in Ewing's sarcoma[53,58], leukemia[59,60], liposarcoma[61], glioblastoma[62,63], ovarian cancer[64], esophageal cancer[65], colorectal cancer (CRC)[66,67], nephroblastoma[68] and liver cancer[69,70]. This growing body of evidence underscores the broad relevance in tumorigenesis and disease progression across diverse cancer types.

2.3. Aging

Biological aging is characterized by nine distinct hallmarks: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulation of nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication[71]. Dysregulated phase separation disrupts cellular homeostasis and impairs tissue function by promoting the formation of pathological biomolecular aggregates, interfering with transcriptional regulation and chromatin architecture, compromising stem cell function, and activating pro-aging signaling pathways. In senescent cells, the SGF29 (SAGA complex associated factor 29) protein undergoes LLPS, form nuclear condensates that recruit transcriptional regulators to the promoter region of the cell cycle inhibitor p21, leading to cell cycle arrest and cellular senescence[72]. In aged hematopoietic stem cells (HSCs), the RNA-binding protein FUS undergoes abnormal phase separation *via* its RGG domain, inhibiting protein binding to chromatin, disrupting the three-dimensional genomic organization, perturbing gene expression, and ultimately causing the hematopoietic system decline[73]. In intestinal stem cells (ISCs), the BuGZ (Bub3 interacting and GLEBS motif containing ZNF207) protein undergoes phase separation in the nucleus, regulating the downstream MAPK (mitogen-activated protein kinase) signaling pathway to regulate ISCs proliferation. Imbalances in BuGZ phase separation compromise intestinal regenerative capacity and accelerate organismal aging[74]. Additionally, numerous other pathological mechanisms highlight how aberrant phase separation contributes to cellular dysfunction and aging. For a more comprehensive overview, we direct readers to read recent reviews[75].

2.4. Infectious diseases

Phase separation exhibits a "double-edged sword" characteristic in infectious diseases, predominantly influencing in viral replication, immune escape, and the host's defensive mechanisms[33]. Viral proteins, such as the 52K protein of adenovirus, exploit phase separation to form membraneless condensates. These condensates function as viral replication compartments, providing an optimal environment for the replication and assembly of the viral genome. By concentrating viral nucleic acids, enzymes, and capsid proteins, they significantly boost the replication efficiency[76]. Herpeviruses further manipulate host immunity through phase separation. Their interstitial proteins form condensates *via* multivalent interactions, competitively binding host cytoplasmic DNA (*e.g.*, pathogenic DNA) and disrupting the phase-separated assembly of cGAS (cyclized GMP−AMP synthase). This interference prevents cGAS from recognizing DNA signals, suppressing interferon pathway activation and enabling immune evasion[77]. Additionally, poliovirus hijacks or disassembles the host's SGs, suppressing the expression of interferon-stimulated genes (ISGs), and blunting the innate immune response. Conversely, the host can leverage phase separation for antiviral defense. Phase separation condensates like cGAS and SGs are crucial components of the innate immunity, any dysregulation in them can directly lead to immune failure. Upon recognition of pathogenic DNA, host cGAS undergoes pre-phase separation, forming a droplet-like structure that accumulates cGAS. This amplification of the downstream STING (stimulator of interferon genes) pathway signal promotes the production of interferons. SGs, on the other hand, impede viral replication by sequestering viral RNA and proteins[78].

2.5. Other diseases

Research has further revealed that phase separation is integral to various physiological processes, including gene expression regulation[79,80], cell division[81−83], and circadian rhythm regulation[84]. Abnormalities in phase separation during these processes may precipitate a wide range of pathological consequences, highlighting its significance in maintaining physiological homeostasis and the potential consequences of its malfunction.

3. Biomolecular phase behavior tools

3.1. LLPS toolbox: synthetic modulators, labeling tools, and auxiliary reagents

Commonly utilized tool compounds can be primarily categorized as follows (Table 1), each serving the purpose of regulating the phase separation process, labeling relevant molecules, or facilitating mechanistic studies.

3.2. Naturally occurring biological modulators of LLPS/condensates

Beyond synthetic tools, cells employ endogenous molecules to dynamically regulate LLPS, ensuring phase behavior is tightly coupled to physiological states. For instance, ATP plays a dual role in the formation and dissociation of cellular biomolecular condensates through energy-dependent mechanisms involving phosphorylation[92,93], chaperone activity[94,95] and ATP hydrolysis-driven structural rearrangement[96]. Its hydrolysis product, ADP, and related metabolites—including ADP-β-D-manno-heptose (ADP-Hep), polyADP-ribose (PAR) chains, and NAD^+—orchestrate phase separation events such as TIFA (TRAF-interacting protein with fork head-associated domain)-mediated innate immune activation, transcriptional pausing, and inhibition of programmed necrosis[97−99]. The second messenger cyclic adenosine monophosphate (cAMP) further modulates condensate dynamics by allosterically regulating protein interactions, driving PKA (protein kinase A) condensate assembly, controlling SUMOylation, and contributing to pathological aggregation and nuclear gene regulation[100−106]. Additionally, metabolic cues induce phase separation: glucose triggers aggregation of calmodulin and ubiquitin-related proteins[107], while cardiolipin and palmitate promote NLRP3 (NOD-like receptor thermal protein domain associated protein 3) inflammasome activation *via* conformational changes[85,108]. Endogenous polyphosphate (PolyP) facilitates electrostatic-driven LLPS of cationic proteins in a chain-length-dependent manner[109]. Dextran forms an aqueous two-phase system with PEG for biomolecule separation and phase behavior research[110]. Arginine promotes the formation of RNA−protein condensates through charge-driven interactions[111]. Heparin interferes with protein−protein or protein−RNA interactions to inhibit condensates formation[112].

3.3. Common techniques and model systems for studying LLPS

Research on protein phase separation generally relies on the combination of *in vitro* reconstitution, live-cell dynamic imaging, functional validation, and multi-dimensional analytical techniques.

3.3.1. Prediction of phase separation and bioinformatics tools

The prediction of protein phase separation propensity primarily relies on bioinformatics tools. Sequence-based prediction algorithms (*e.g.*, PSPHunter[113,114]) employ machine learning to predict phase-separating capability and identify key IDRs. Charge/hydrophobicity analysis tools (*e.g.*, FuzDrop[115]) compute net charge distribution, hydrophobic residue ratios, and $\pi-\pi$ interactions to forecast LLPS conditions.

3.3.2. In vitro phase separation validation techniques

In vitro reconstitution assays purify target proteins (*e.g.*, His/fluorescent-tagged) *via* prokaryotic expression systems (*e.g.*, *E. coli*), inducing LLPS by modulating pH, ionic strength, or temperature. Cloud-point extraction (CPE) isolates hydrophobic proteins (*e.g.*, membrane proteins). Dynamic behavior characterization employs Fluorescence Recovery After Photobleaching (FRAP) to quantify molecular mobility and time-lapse microscopy to record droplet fusion/fission kinetics, thereby verifying liquid-like properties. Structural resolution techniques further utilize cryo-electron microscopy (Cryo-EM) to capture submicron

Table 1 LLPS toolbox.

Category	Specific substance/tool	Functional description	Ref.
Synthetic modulators	1,6-Hexanediol (1,6-HEX)	Promotes or inhibits LLPS in different conditions	23,85−87
	Polyethylene glycols (PEGs)	Water-soluble, low-immunogenic, biocompatible polymer (ethylene glycol repeating units); induce biomolecule (protein/nucleic acid) phase separation *via* concentration adjustment	88
	BI-3802	Targets the BTB domain of Bcl6, inducing its aggregation and subsequent formation of phase separation droplets	89
	BI-3812	Prevents the binding of BI-3802 and Bcl6, enabling reversible regulation of the phase separation process	89
Labeling tools	Fluorescent proteins (*e.g.*, GFP, mCherry)	Fused with the target protein, enabling the observation of phase separation dynamics *via* a fluorescence microscope	88
	Organic fluorescent dyes	Used to label nucleic acids or proteins for imaging in *in vitro* phase separation systems	90
	Biotin-labeled compounds	Used in pull-down experiments or phase separation studies on solid-phase supports when combined with streptavidin	90
Auxiliary reagents	Salts (*e.g.*, NaCl and KCl)	Influence charge interactions and regulate phase separation behavior by modulating ionic strength	90,91
	Surfactants	Disrupt membrane structures or interfere with hydrophobic interactions, useful for studying membrane-dependent phase separation processes	90

condensate architectures, and nuclear magnetic resonance (NMR) reveals residue-specific interaction analysis[116,117].

3.3.3. Intracellular phase separation research techniques

Live-cell imaging with fluorescent tags (*e.g.*, EGFP/mCherry) enables real-time observation of phase separation dynamics *via* confocal microscopy and 3D reconstruction. Functional perturbation experiments introduce key mutations (*e.g.*, aromatic residue substitutions) *via* CRISPR/Cas9 or small-molecule inhibitors to dissolve condensates. Disease modeling uses neurodegenerative organoids to confirm solid-like aggregation (*e.g.*, tau fibrillization), while tumor models to analyze dysregulated condensates[116,118].

3.3.4. Functional validation and interaction studies of phase separation

In vitro co-condensation assays quantify synergistic phase separation *via* sedimentation analysis. Proximity labeling (*e.g.*, TurboID[119,120]) marks droplet-associated proteins in live cells, mapping dynamic interaction networks through mass spectrometry.

3.3.5. Emerging technologies and integrated strategies

High-throughput screening platforms (*e.g.*, microfluidic chips[121]) automate environmental parameter control (temperature, ionic strength) and optimize conditions *via* fluorescence detection. Multi-omics integration strategies combine TurboID interactomes, transcriptomics, and proteomics data to map RNA-protein co-condensation in membraneless organelles. Computational/AI tools leverage coarse-grained models (*e.g.*, Mpipi[122]) for phase diagrams and AI platforms to predict mutation effects.

4. Phase separation modulators

In recent years, with the continuous accumulation of extensive research efforts, a diverse array of small molecules with regulatory functions in phase separation have been reported. Some of these molecules have been proven to have positive effects by inducing, promoting, or stabilizing phase separation condensates, while others have been shown to have negative effects by disrupting, inhibiting, or dissolving phase separation condensates. Moreover, some modulators have been demonstrated to exhibit dual effects under different conditions. Here, we will classify them according to their respective disease fields.

4.1. Nucleoli/RNA-related modulators

The nucleolus, serving as the assembly hub for ribonucleoprotein particles, holds significant biological importance, primarily in the biogenesis of ribosomes. The LLPS offers a unified quantitative framework for nucleolus assembly, structural maintenance and function, and is of great significance for gene regulation within the entire nucleus and the assembly of ribonucleoprotein particles. A number of small molecule modulators targeting nucleoli and RNA-related condensates have been identified in studies (Table 2, Fig. 3). The conventional mechanisms of anti-cancer natural products include directly damaging cancer cell DNA, interfering with the cancer cell proliferation cycle, and inducing apoptosis; these enable anti-cancer drugs and natural products to modulate biomolecular condensates/LLPS either directly or indirectly. Ribosomal inhibitors (anisomycin **1**, neomycin **2**, cycloheximide **3**, emetine **4**) reduce the number of SGs per cell; cytoskeleton-targeting compounds (vincristine **5**, vinblastine **6**) and actin filament stabilizer prieurianin (**7**) alter the average size and/or quantity of SGs; Na^+/K^+-ATPase inhibitors (digitoxin **8**, proastragaloside a **9**) and tyrosine kinase receptor inhibitor tyrphostin A9 (**10**) act on the cell surface to regulate SGs; phospholipase A2 inhibitor quinacrine (**11**) and cyclooxygenase inhibitor oxyphenbutazone (**12**) target intracellular processes to modulate SGs; planar aromatic modulators mitoxantrone (**13**), acamycin (**14**), and daunorubicin (**15**) reduce TDP-43 accumulation in human induced pluripotent stem cell-derived motor neurons (iPS-MNs)[40]. Actinomycin D (**16**) inhibits nucleolar RNA synthesis, disrupts nucleolar structure, promotes LLPS within the nucleolus, and induces the formation of splicing factor proline and glutamine rich protein (SFPQ) condensates[123,124]. Camptothecin (CPT, **17**),

Table 2 Nucleoli/RNA-related modulators.

Molecule	Target condensate	Mechanism	Ref.
1−**15**	SGs	Alter SGs size and/or quantity.	40
16 (Actinomycin D)	Nucleolar condensates, SFPQ condensates	Inhibits nucleolar RNA synthesis/structure, forms stable protein aggregates, promotes nucleolar LLPS; inhibits RNA polymerase and induces SFPQ condensates.	123,124
17 (CPT)	CITIs	Blocks RNAPII elongation; induces CITIs, alters genome structure, promotes NHEJ-mediated chromosome fusion; activates SLX4 phase separation *via* TOP1−DNA complexes (TOP1-DPCs); regulates SLX4 dissolution *via* RNF4.	125,126
18, **19**	CITIs	Block RNAPII elongation; induces CITIs, promote NHEJ-mediated chromosome fusion.	123,126
20 (THZ1)	SFPQ/NONO condensates; Pol II CTD transcriptional condensates	Relocates/Forms SFPQ/NONO large nuclear condensates. Inhibits Pol II CTD condensates: Blocks CDK7 phosphorylation, reduces Pol II nuclear aggregation and phase separation.	123,127
21 (Oxaliplatin)	Nucleolus condensates	Targets NPM1, induces nucleolar LLPS; arrests cell cycle and triggers cell death; clickable derivatives enable dynamic phase separation analysis.	128,129
22 (PD98059)	SGs	Reduces SGs formation.	130

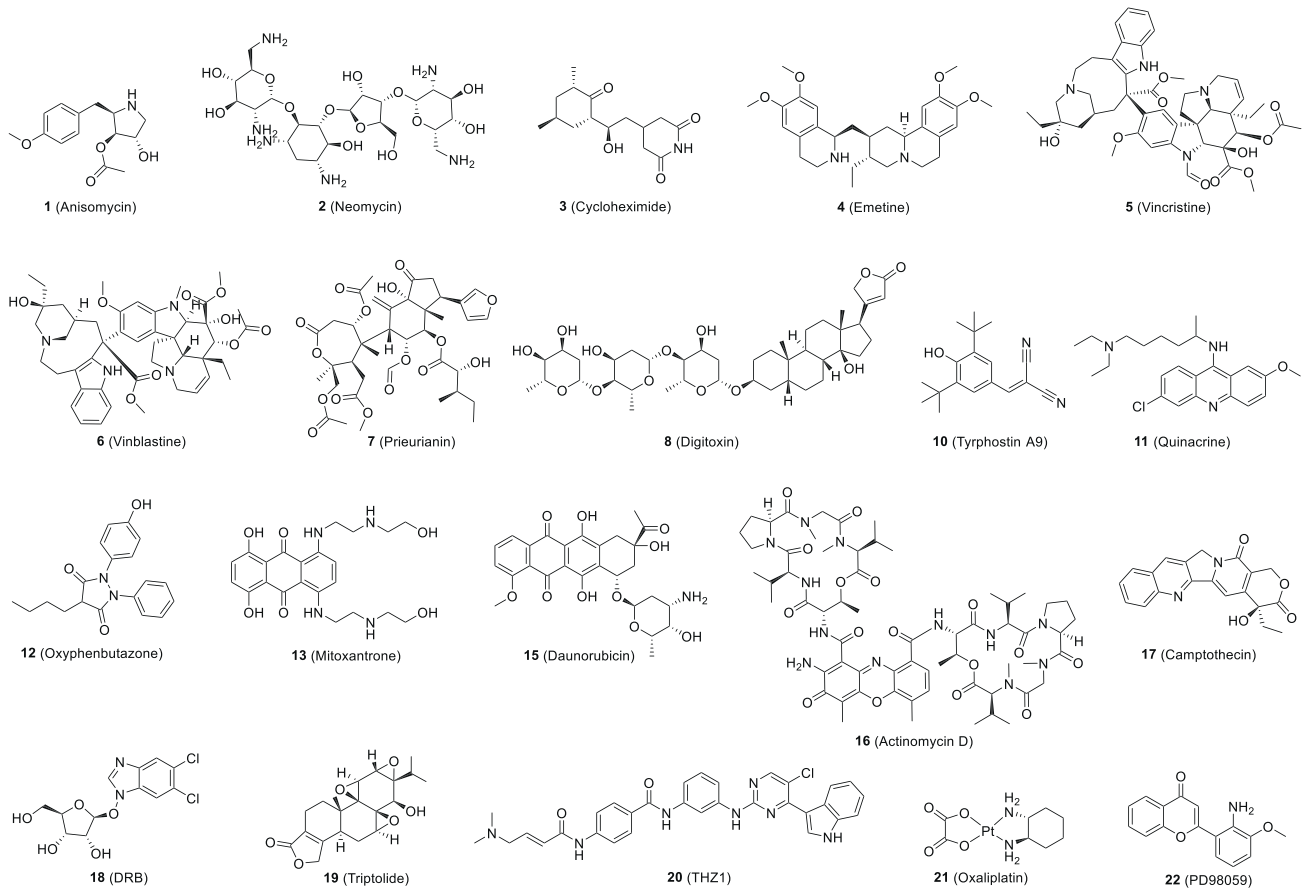

Figure 3 Representative structures of nucleoli/RNA-related modulators.

5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB, **18**), and triptolide (**19**) induce the formation of transcription inhibition-induced condensates (CITIs)[125,126]. THZ1 (**20**), a covalent inhibitor of CDK7, relocalizes the nucleolar peripheral speckle-associated proteins, thereby promoting the formation of large nuclear condensates; it also prevents Pol II phosphorylation by inhibiting CDK7 to block transcription initiation and elongation, and, reduces Pol II aggregation, and decreases the LLPS capacity of its carboxy-terminal domain (CTD), thereby inhibiting target gene expression[123,127]. Oxaliplatin (**21**) disrupts nucleolar function by targeting the nucleolar protein nucleophosmin 1 (NPM1), induces nucleolar LLPS, and causes cell cycle arrest[128,129]. PD98059 (**22**) can affect SGs formation under certain conditions[130].

4.2. *Immunity and autophagy-related modulators*

Mitochondrial autophagy represents a highly selective autophagic program for eliminating damaged, senescent, or dysfunctional mitochondria, crucial for intracellular homeostasis and mitochondrial quality control. Small molecules can modulate LLPS in immunity and autophagy (Table 3, Fig. 4). Celastrol (tripterine, **23**) regulates selective mitochondrial autophagy by inducing LLPS *via* binding to the nuclear receptor Nur77[131]. XS561 (**24**), a novel Nur77 ligand, binds the lateral organ boundaries domain of Nur77, triggering its nuclear-to-mitochondria translocation and inducing co-condensate formation with B-cell lymphoma-2 (Bcl-2), causing mitochondrial apoptosis[132]. Autophagy related 4B cysteine peptidase (ATG4B), an autophagy-related protein, functions *via*

Table 3 Immunity and autophagy-related modulators.

Molecule	Target condensate	Mechanism	Ref.
23 (Celastrol)	Mitochondrial condensates	Binds Nur77, translocates it to mitochondria, forms dynamic condensates with p62.	131
24 (XS561)	Nur77/Bcl-2 mitochondrial condensates	Binds Nur77 LBD, induces Nur77/Bcl-2 LLPS; forms pro-apoptotic condensates altering Bcl-2, triggering mitochondrial apoptosis; activates the apoptotic pathway of tumor cells.	132
25 (Lopinavir)	ATG4B condensates	Enhances circSPECC1 to promote ATG4B degradation *via* proteasome.	133
26 (EGCG)	α-syn condensates, Aβ	Inhibits α-syn and Aβ fibrinogenesis; remodels large, mature fibrils into smaller, amorphous aggregates.	134−137

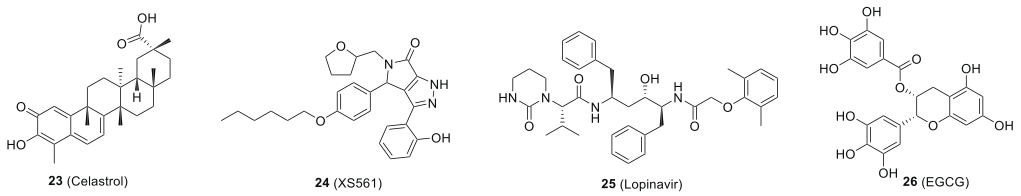

Figure 4 Representative structures of immunity and autophagy-related modulators.

condensates formed by LLPS. Lopinavir (**25**) activates the ELAVL1 (ELAV like RNA binding protein 1)-circSPECC1-ATG4B pathway, enhancing apoptosis and suppressing metastasis[133]. Epigallocatechin gallate (EGCG, **26**) inhibits interferon production by disrupting the interaction between G3BP1 (GTPase-activating protein SH3 domain-binding protein 1) and cGAS[134].

4.3. Cancers-related modulators

Targeted therapy for cancer is a research hotspot in LLPS studies. With the in-depth understanding of cancer-related phase separation mechanisms, more small molecules have been proven to modulate phase separation (Table 4, Fig. 5). Splicing variants of AF-1 and AR-V7 form molecular condensates *via* LLPS with dynamic characteristics, and selective AR irreversible covalent antagonists represented by UT-143 (**27**) abrogate LLPS-driven condensate formation, inhibit ARAF-1 LLPS and dissolve AR-V7 condensates. Purified AF-1 protein forms LLPS condensates in the absence of UT-143, whereas AF-1 folded in the presence of UT-143 remains monomeric and fails to undergo phase separation (Fig. 6A)[138]. JQ1 (**28**) decreases MED1 condensate stability and MYC (myelocytomatosis oncogene)-region platinum-DNA binding in HCT116 cells[45,139]; GSK-J4 (**29**) targets HOXB8 to reduce CRC cell condensates[49]; SI-2 (**30**) inhibits Src-3 LLPS in breast cancer and lung cancer[50]; Arsenic trioxide (ATO, **31**) promots L3MBTL2 (lethal malignant brain tumor-like protein 2) condensate formation and suppresses osteosarcoma growth[140]; β-Alanine (**32**) enhances chemotherapy by inhibiting p53 lactylation and promoting LLPS[141]; and icFSP1 (**33**) induces FSP1 (ferroptosis suppressor protein 1) phase separation, synergizing with GPX4 (glutathione peroxidase 4) inhibition to suppress tumors without off-target effects[142]. Metformin (Met, **34**), a first-line type 2 diabetes mellitus (T2DM) drug, is confirmed as an antitumor agent[143]. It disrupts TWIST1 (twist family bHLH transcription factor 1)-YY1-P300 phase separation and inhibits hepatocellular carcinoma (HCC) epithelial−mesenchymal transition[144]. Other relevant small molecules include: LY2835219 (**35**) etc. can dissolve PS-DBD fusion condensates in Ewing's sarcoma[145]; PD98059 (**22**) target SGs in pancreatic ductal adenocarcinoma (PDAC)[130,146]; Elvitegravir (EVG, **36**) disrupts Src-1 LLPS in

Table 4 Cancers-related modulators.

Molecule	Target condensate	Mechanism	Ref.
27 (UT-143)	AF-1/AR-V7 condensates	Binds regulatory domain, altering conformation, physicochemical properties and chromatin landscape.	138
28 (JQ1)	MED1 condensates	Targets BRD4, dissolves MED1 condensates; reduces condensates stability and oncogene effects.	45,139
29 (GSK-J4)	HOXB8 condensates	Binds HOXB8 IDRs, modulates folding to disrupt regulatory condensates.	49
30 (SI-2)	SRC-3-NSD2 condensates	Binds SRC-3 to disrupt SRC-3−NSD2 interaction and promotes degradation.	50
31 (ATO)	L3MBTL2-induced condensates	Inhibits UBE2O, stabilizes L3MBTL2 to enhance LLPS-driven condensates formation.	140
32 (β-Alanine)	p53 condensates	Blocks AARS1-lactic acid binding; inhibits p53 lactoylation, restores LLPS-driven tumor suppression.	141
33 (icFSP1)	FSP1 cytoplasmic condensates	Translocates FSP1, triggers phase separation; induces ferroptosis synergizes with GPX4 inhibition.	142
34 (Met)	TWIST1-YY1-P300 condensates	Dissolves TWIST1−YY1−P300 condensates by disrupting charge/hydrophobic interactions.	144
35 (LY2835219)	FET-ETS fusion condensates	Dissolves FET−ETS condensates; rescues the abnormal expression of target genes.	145
36 (EVG)	Src-1/YAP/TEAD condensates	Binds Src-1 to disrupt its phase separation ability.	147
37, **38**	Mutant SHP2 aggregates	Lock SHP2 in closed conformation to disrupt mutant LLPS.	148
39 (ET516)	AR condensates	Destroys AR condensates; inhibits AR transcriptional activity; suppresses the proliferation and tumor growth.	149
40 (FIP4)	FOXM1 condensates	Targets FOXM1 UDR1; induces electrostatic repulsion; disrupts intermolecular interactions; inhibits LLPS.	150
41 (ReACp53)	Mutant p53 aggregates	Masks p53 adhesion fragments (252−258); inhibits further aggregation.	151
42, 43	Mutant p53 condensates	Dissolve p53 mutant condensates; cause nuclear p53 aggregation.	152

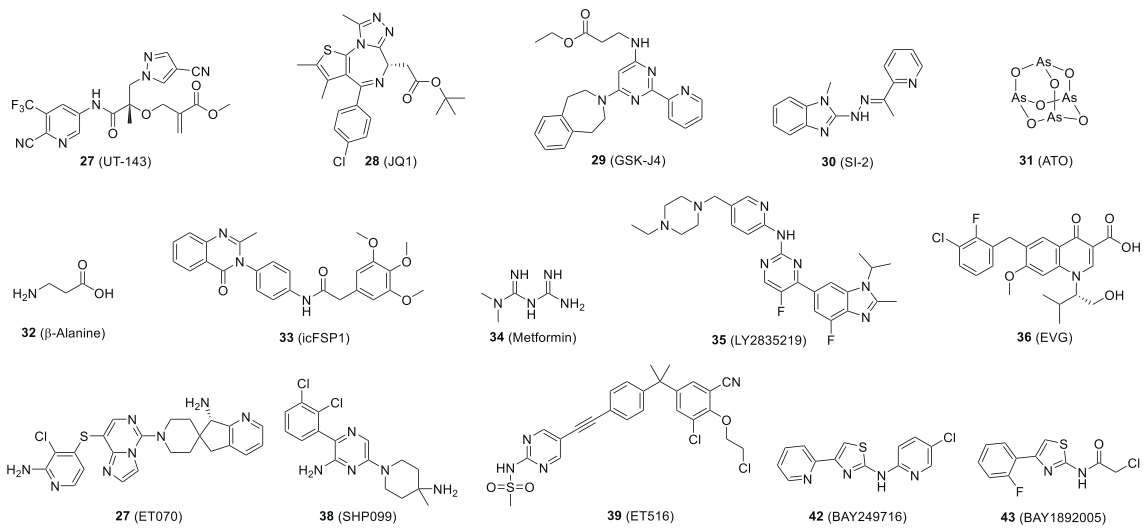

Figure 5 Representative structures of cancers-related modulators.

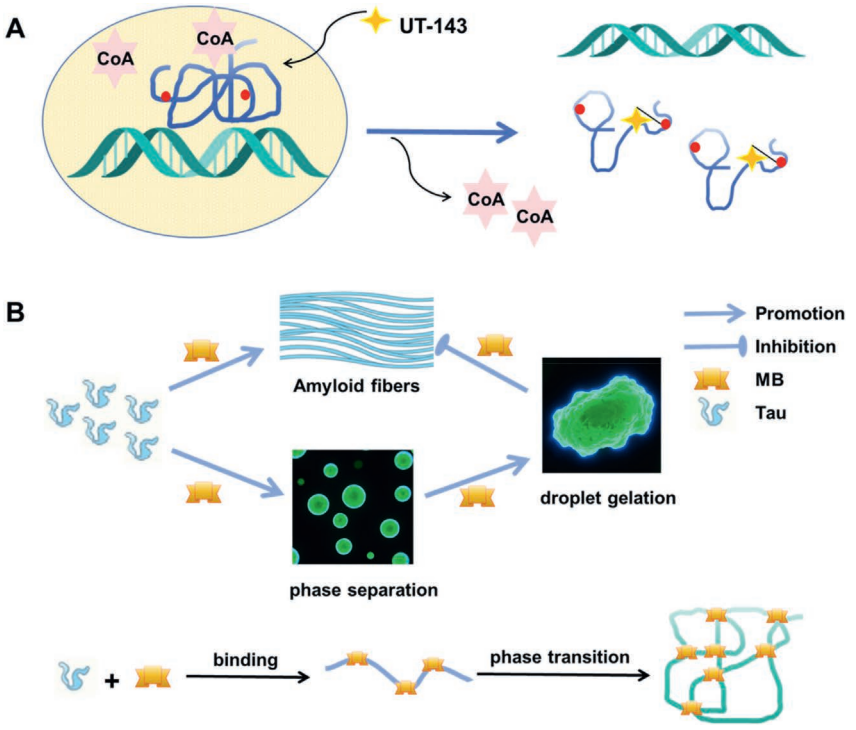

Figure 6 Mechanisms of condensates/LLPS modulated by molecules. (A) shows UT-143 (**27**) inhibits condensate formation; (B) shows the mechanism of MB (**64**)-induced phase separation.

Hippo/YAP signaling[147]; ET070 (**37**) and SHP099 (**38**) block SHP2 (SH2 domain-containing protein-tyrosine phosphatase-2) LLPS in Noonan syndrome[148]; ET516 (**39**) disrupts AR aggregation and suppresses prostate cancer cells proliferation[149]; D-amino acid-based interference peptide FIP4 (**40**) reduces FOXM1 (forkhead box M1) condensates and inhibits FOXM1 LLPS[150]; Cell-penetrating peptide ReACp53 (**41**) prevents p53 aggregation in high-grade serous ovarian cancer (HGSOC)[151]; BAY249716 (**42**) and BAY1892005 (**43**) dissolve condensates of fluorescently labeled structural mutant p53 in cells expressing fluorescent DNA contact mutant p53 but induce p53 nuclear aggregation[152].

4.4. Inflammatory responses-related modulators

The pathological progression of diverse diseases is linked to inflammatory responses, and phase separation modulators can play a role in these fields (Table 5, Fig. 7). Curcumin C1 (**44**) regulates phase separation-mediated gene transcription microenvironment, disrupts P300-BRD4 (bromodomain protein 4)-mediated inflammatory gene transcriptional condensates, and regulates RNA N^6-methyladenosine (m^6A)-modified RNA binding protein (RBP) phase separation[153−155]. Doxorubicin (**45**) reduces cytoplasmic TDP-43 accumulation in ALS patient cells; paclitaxel (**46**) alters the

Table 5 Inflammatory responses-related modulators.

Molecule	Target condensate	Mechanism	Ref.
44 (Curcumin C1)	Tau/P300-BRD4/m^{6}A condensates	Inhibits tau LLPS, oligomerization, filament formation and toxicity; inhibits P300; regulates m^{6}A-dependent RBPs.	153−155
45 (Doxorubicin)	TDP-43/SGs/NLRP3 condensates	Reduces TDP-43 accumulation; induces NLRP3 LLPS *via* reduced solubility.	40,85
46 (Paclitaxel)	SGs/NLRP3 condensates	Alters SGs size and number per cell; induces NLRP3 phase separation.	85,85
47, **48**	NLRP3 inflammasome condensates	Bind NLRP3, induce conformational changes; drive LLPS *via* FISAN domain, lowering phase separation threshold for inflammasome activation.	85,108
49 (MCC950)	NLRP3 inflammasomes	Suppresses NLRP3-3 K/A and NLRP3 aggregation and LLPS by inhibiting ATPase activity and binding NACHT/LRR domains; blocks low K^+-induced LLPS.	85,156
50, **51**	NLRP3 inflammasomes	Inhibit NLRP3 LLPS *via* cysteine modification and a fixed NLRP3 conformation.	85
52 (Compound **22**)	TIFA-TRAF6 condensates	Enhances ADP-Hep-induced TIFA LLPS, activates ALPK1−TIFA−TRAF6 inflammatory.	98

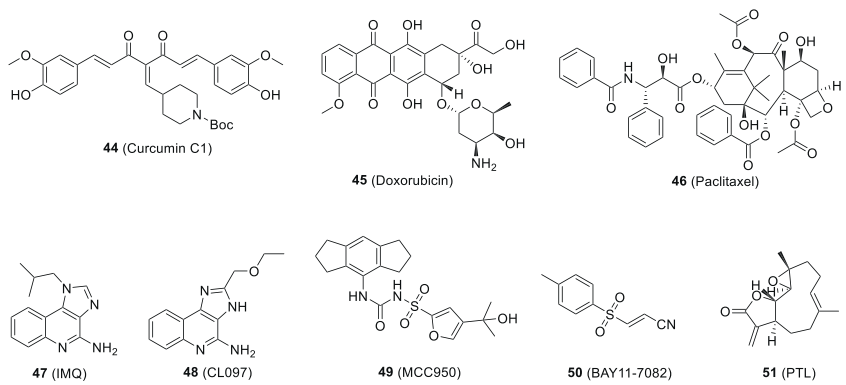

Figure 7 Representative structures of inflammatory responses-related modulators.

average SGs size and/or quantity per cell, and both of them directly induce NLRP3 phase separation and activation by reducing its solubility, independent of ZDHHC7 (zinc finger DHHC domain-containing protein 7)-mediated palmitoylation[40,85]. Imiquimod (IMQ, **47**) and CL097 (**48**) bind NLRP3 to lower its phase separation threshold, promoting LLPS[85,108]. MCC950 (**49**) inhibits NLRP3-3 K/A aggregation, thereby inhibiting NLRP3 LLPS and activation[85,156]. BAY11-7082 (**50**) and PTL (**51**) covalently inhibit NLRP3 LLPS through irreversible cysteine modification and stabilization of NLRP3 in a fixed conformation[85]. The ALPK1 (alpha kinase 1)-TIFA-TRAF6 (tumor necrosis factor receptor-associated factor 6) signaling cascade serving as a pivotal regulator and its activation by small molecules relies on TIFA LLPS. Adenosine monophosphate compound **22** (**52**) enhances ADP-Hep-induced TIFA phase separation, promoting downstream inflammatory signaling activation[98].

4.5. Viral infections-related modulators

In viral infections (Table 6, Fig. 8), (−)-Gallocatechin gallate (GCG, **53**) binds the SARS-CoV-2 nucleocapsid protein (N protein), disrupts N protein LLPS and inhibits SARS-CoV-2 replication. EGCG (**26**) weakly inhibits N protein LLPS, modulates viral-induced inflammation[136]. CVL218 (**54**) and PJ34 (**55**) target N protein to reduce the density and fluidity of the N protein-RNA-nsp12 complex droplets, inducing a more "loose" morphology that enhances their vulnerability to other drugs (*e.g.*, remdesivir)[157,158]. Cyclopamine (**56**) and A3E (**57**) solidify respiratory syncytial virus (RSV) inclusion bodies, blocking viral replication, and bind M2-1 to dismantle the replication microenvironment[159]. Several compounds in the FDA-approved drug library (*e.g.*, LDK378 (**58**), nelfinavir (**59**), nilotinib (**60**), crystal violet (**61**), tocapone (**62**) and chlorhexidine (**63**)) can influence SARS-CoV-2 N protein condensates quantity, size and morphology[160].

4.6. Neurodegenerative diseases-related modulators

The application of phase separation modulation in neurodegenerative diseases is highly concerned, mainly involving targeting FUS, TDP-43, tau, α-syn, prion proteins (PrPSc) and SGs to develop small molecule modulators (Table 7, Fig. 9). Methylene blue (MB,

Table 6 Viral infections-related modulators.

Molecule	Target condensate	Mechanism	Ref.
26, **53**	SARS-CoV-2 N-protein condensates	Disrupt SARS-CoV-2 N-protein LLPS.	136
54, **55**	SARS-CoV-2 N Protein-RNA-nsp12 condensates	Bind N-NTD, disrupt RNA binding, reduce condensates density/fluidity.	157,158
56, **57**	RSV inclusion bodies	Bind RSV M2-1, solidifiy liquid inclusion bodies; inhibit viral transcription and assembly.	159
58, **59**	SARS-CoV-2 N-protein condensates	Induce larger and fewer aggregates.	160
60 (Nilotinib)	SARS-CoV-2 N-protein condensates	Increases volume, viscosity and alters morphology.	160
61−**63**	SARS-CoV-2 N-protein condensates	Enhance phase separation by increasing both the number and size of aggregates.	160

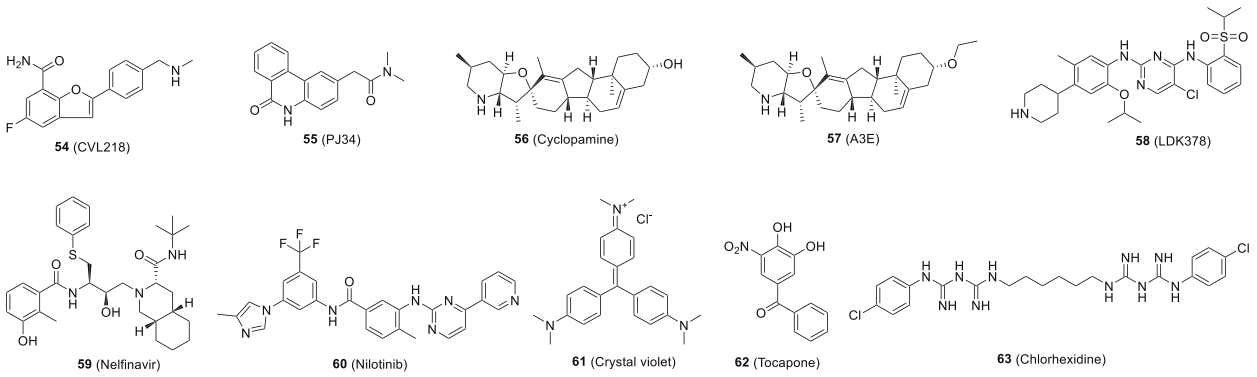

Figure 8 Representative structures of viral infectious-related modulators.

Table 7 Neurodegenerative diseases-related modulators.

Molecule	Target condensate	Mechanism	Ref.
64, **65**	Tau protein condensates	Promote tau LLPS and gelation; reduce cytotoxicity without affecting microtubule, delay amyloid fiber formation.	161
66, **67**	FUS condensates	Specifically modulate FUS LLPS.	41,162
68 (AIM4)	TDP-43^{2C}-A315T condensates	Binds TDP-43^{2C} C-terminal to inhibit LLPS.	163,164
69 (Anle138b)	PrPSc and α-syn condensates	Blocks pathological aggregation of PrPSc and α-syn; inhibits oligomer accumulation, neuronal degeneration and disease progression *in vivo*.	165,166
70 (SMM 1c)	Zn-mediated tau condensates	Inhibits and dissolves Zn^{2+}-induced tau LLPS.	167
71, **72**	Tau/FUS/TDP-43/Ded1p condensates	Bifunctional concentration-dependent: Promote LLPS (low conc.) *via* hydrophobic/$\pi-\pi$ stacking; Destroy (high conc.). Non-specific. Salt insensitive.	168

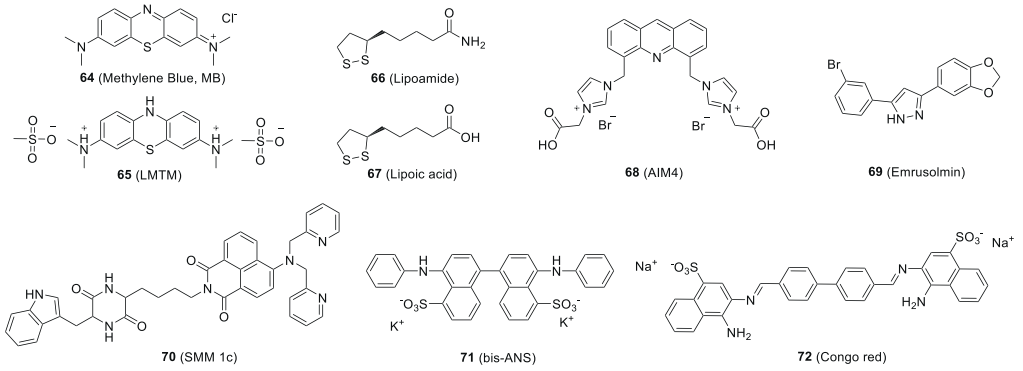

Figure 9 Representative structures of neurodegenerative diseases-related modulators.

64) and hydromethylthionine mesylate (HMTM, TRx0237, LMTM or LMTX, **65**) inhibit tau protein amyloid aggregation, recent studies revealed they also promote tau phase separation and droplet gelation. Through extensive interactions with multiple domains of tau protein, these compounds promote the conformational extension of tau protein and form an intermolecular electrostatic/hydrophobic interaction network. MB-induced phase separation gel state of tau protein reduces cytotoxicity without impairing tau's microtubule assembly function, delaying cytotoxic amyloid fibers formation (Fig. 6B)[161]. Lipoamide (**66**) and lipoic acid (**67**) specifically modulate FUS LLPS without disrupting other biomolecular condensates[41,162]. AIM4 (**68**) interferes with TDP-43 phase separation, suppresses its abnormal aggregation, and inhibits *in vitro* LLPS of TDP-43^{2C} with A315T mutation[163,164]. Curcumin C1 (**44**) blocks tau oligomers toxicity, inhibits all steps of tau protein aggregation, and prevents tau oligomers transformation[154]. Emrusolmin (anle138b, **69**) inhibits PrPSc and α-syn aggregation, oligomer accumulation, neuronal degeneration and disease progression *in vivo*, with low toxicity, high oral bioavailability and good blood−brain barrier permeability[165,166]. SMM 1c (**70**) blocks Zn^{2+}-induced tau LLPS and reduces droplet number and size[167]. EGCG (**26**) inhibits α-syn and Aβ fibrinogenesis and remodels large, mature α-syn and Aβ fibrils into smaller, amorphous protein aggregates[135,137]. 4,4′-diphenylaniline-1,1′-dinaphthyl-5,5′-disulfonic acid (bis-ANS, **71**) is a concentration-dependent LLPS modulator (inducing at low concentrations, inhibiting at high concentrations), with naphthalene groups likely mediating effects *via* hydrophobic interactions and/or $\pi-\pi$ stacking[168]. Congo red (**72**) oligomerizes them molecules into spherical oligomers *via* binding hydrophobic surfaces and electrostatic interactions; further studies confirmed it regulates phase separation concentration-dependently like bis-ANS[168].

5. Clinical progress of phase separation drugs

As aforementioned, HMTM (**65**, Table 7, Fig. 9), a second-generation tau protein aggregation inhibitor, targets the pathological mechanisms of multiple neurodegenerative diseases. TauRx Pharmaceuticals has completed several clinical trials (NCT03539380, NCT03446001, NCT02245568, NCT01689246, NCT01689233, NCT01626391, and NCT01626378). The data show that HMTM significantly reduces key serum biomarkers of neurodegenerative diseases (*e.g.*, serum neurofilament light chain, NFL), can reduce the incidence of dementia, and alleviate cognitive impairment. TauRx Pharmaceuticals submitted a marketing authorization application to the UK MHRA on July 1, 2024, positioning HMTM as a pioneering disease-modifying therapy for early-stage AD.

SHP2, a non-receptor tyrosine phosphatase, undergoes dynamic regulation of its activity through its intrinsic conformational changes. Studies have revealed that mutations in SHP2 can lead to its abnormal LLPS[148]. This phase separation depends on the multivalent interaction between IDRs of SHP2 and other proteins. ET0038 (aka, ETS-001), an allosteric inhibitor of SHP2 developed by ETERN Therapeutics Co., Ltd., acts by binding to non-catalytic sites of SHP2. This binding stabilizes its self-inhibitied conformation and blocks the phosphatase activity[169], which may indirectly affect SHP2 phase separation. In May 2021, ET0038 received approval from the US Food and Drug Administration (FDA) for new drug clinical trial (IND) (NCT05525559). Concurrently, China's National Medical Products Administration (NMPA) accepted its clinical trial application and designated it for the "Breakthrough Therapy Drugs" channel (NCT05354843). In July 2021, the NMPA officially granted the implied approval for two Phase I clinical trials, targeting advanced solid tumors with abnormal MAPK signaling pathways. As of May 2025, ET0038 remains in the Ib/II phase clinical trial stage, with no Phase II data published to date.

Yes-associated protein (YAP) is the core transcriptional co-activator of the Hippo signaling pathway. The YAP/TEAD (TEA domain transcription factor) complex can regulate the expression of genes related to cell proliferation, survival and immune escape during tumorigenesis. Upon activation, YAP undergoes LLPS to form liquid condensates, recruiting TFs to create transcriptionally active sites within the nucleus, thereby promoting oncogene transcription. ETS-006 is an oral highly selective YAP/TEAD protein−protein interaction inhibitor developed by ETERN Therapeutics Co., Ltd. based on its LLPS technology platform. It targets the binding interface between YAP and TEAD to disrupt both their interaction and YAP-driven LLPS, thereby blocking the spatial microenvironment essential for oncogene transcription and inhibiting tumor growth[170]. The US FDA approved ETS-006 for IND and granted it orphan drug designation for the treatment of malignant pleural mesothelioma — marking it as the world's first small-molecule drug entering clinical trials based on a phase separation mechanism. China's NMPA has approved its IND application for multiple advanced solid tumors (CXHL2500014, CXHL2500015). As of August 2025, ETS-006 remains in the early clinical trial stage, with no efficacy data yet disclosed.

6. New technologies for phase separation regulation

Nap-o-Nap is an artificial synthetic molecule inspired by IDPs. It comprises two naphthalene moieties linked by ethylene glycol chains, enabling reversible cross-linking through $\pi-\pi$ interactions. The secondary amine groups undergo pH-dependent protonation (pH 7.5−8.0), triggering LLPS. By introducing cucurbituron and amantadine hydrochloride, its hydrophilic and hydrophobic balance is precisely tuned, allowing reversible LLPS control[171].

Proteolysis targeting chimeras (PROTAC) technology is an emerging protein degradation strategy that has witnessed remarkable advancements in recent years. The basic principle of PROTAC relies on bifunctional small molecules to induce the ubiquitination of target proteins through the ubiquitin-proteasome system, thereby enabling their degradation. Rao's team[172] leveraged PROTAC technology to degrade BRD4 and study its role in biomolecular condensate dynamics. Using bifunctional molecules, PROTAC induces ubiquitin-mediated degradation *via* the proteasome. Immunofluorescence staining and high-throughput sequencing revealed that BRD4 degradation alters condensate morphology and disrupts functional interactions with co-condensates.

Phase-separated tumor killer (psTK) fusion protein is an innovative tumor therapy tool developed by Li's team[173]. psTK is a fusion protein combining a phase-separation module (P2S2), tumor-targeting nanobody (*e.g.*, CXCR4-binding CNB), and apoptosis-inducing nanobody (*e.g.*, DR5-activating DNB) and flexible linkers. P2S2 is composed of bivalent proline-rich domain (PRM) and Src homologous domain 3 (SH3), which triggers LLPS to cluster receptors on cancer cells, while DNB triggers ligand-independent apoptosis. Flexible linkers ensure spatial precision, enabling tumor-specific killing with minimal off-target effects *in vivo*.

The Killswitch system uses stress-responsive low-complexity domains (LCDs) to form condensates under specific triggers (*e.g.*, high ROS in tumors). These condensates recruit CRISPR-Cas9 or apoptosis inducers, enabling spatially controlled gene editing or cell death. In glioblastoma models, Killswitch reduced off-target effects by $> 90\%$ compared to conventional therapies[174].

PSD percolation network regulation technology, developed by Zhang's team[175], targets the mesoscale percolation molecular network within the postsynaptic density (PSD). This technology utilizes two key tools: the DLS-PBM inhibitory peptide and the SAP90/PSD-95-associated protein (SAPAP)-95FingR chimeric protein. The former disrupts PSD-95/SAPAP interactions, splitting the PSD network into core/surface sub-networks to reduce complexity and accelerate AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor) diffusion. The latter enhances network stability by reinforcing PSD-95/SAPAP nodes. It has demonstrated efficacy in correcting memory deficits in mouse models and elucidating *trans*-synaptic anteroposterior membrane cooperative assembly mechanisms.

Additionally, other emerging advancements warrant attention, such as the fluorescence optical tweezers-microfluidics integrated platform[176] and innovative cancer therapeutic platforms (*e.g.*, RIOK1-stress granule targeting therapy[177] and KAT8-IRF1 phase separation inhibitors[178]), though these will not be detailed here.

7. Conclusions and prospects

In recent years, phase separation research has transcended physical chemistry to address core issues in life sciences, revealing how biological macromolecules dynamically regulate cellular compartmentalization, gene expression and stress response through LLPS. Emerging evidence demonstrate that phase separation serves not only as the physical foundation for membraneless organelles (*e.g.*, nucleoli and SGs) but also acts as a molecular bridge linking macromolecular interactions to complex biological processes such as RNA modification, chromatin remodeling and protein homeostasis. For instance, the aggregation of TFs driven by phase separation can enhance gene expression efficiency, while abnormal phase separation is closely related to pathological states such as neurodegenerative diseases and tumor drug resistance. Breakthroughs in phase separation research, including novel technologies, new platforms, mechanistic insights, and therapeutic molecules, have established robust theoretical frameworks and empirical foundations for targeted LLPS interventions in disease therapy. This is particularly significant for "undruggable targets" such as IDPs and protein−protein interactions, which have long been challenging for traditional drug development approaches. These advances provide a fundamental soft matter physics perspective to decode biological complexity, reshaping our understanding of life phenomena at the chemistry-physics-biology interface. By integrating principles from multiple disciplines, this field enables unprecedented exploration of dynamic macromolecular condensates—key regulators of both normal cellular functions and pathological processes.

Despite its broad prospects, phase separation research still faces significant challenges, including the difficulty in defining the functional heterogeneity of dynamic condensates, the insufficient spatiotemporal specificity of regulatory methods, and the risk that excessive intervention could disrupt cellular homeostasis. To address these issues and promote the transition from basic discoveries to clinical applications, it will be essential in the future to establish interdisciplinary collaboration networks that integrate expertise from fields such as chemistry, physics, biology, and medicine, while simultaneously developing ethical assessment frameworks to ensure the responsible and safe translation of research findings. These combined efforts will be critical for overcoming current limitations and unlocking the full therapeutic potential of phase separation principles in diverse medical contexts.

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Author contributions

Kaiyue Lian conceived the manuscript and outlined it, conducted the investigation and then wrote the original draft. Fajun Nan and Kaiyue Lian reviewed and edited the draft. All authors passed the final review and submission.

Conflicts of interest

The authors declare no conflicts of interest.

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