

Liquid-Liquid Phase Separation in Transcription Regulation: A Survey

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Abstract

Liquid-liquid phase separation (LLPS) is a critical mechanism in cellular organization, forming biomolecular condensates that compartmentalize cellular processes without membranes. This survey explores LLPS's role in transcription regulation, emphasizing the formation of transcriptional condensates that enhance gene expression by concentrating transcriptional machinery. The survey details LLPS's molecular mechanisms, highlighting multivalent interactions, intrinsically disordered regions (IDRs), and the stickers and spacers model. It examines the dynamics of phase separation, focusing on electrostatic and hydrophobic interactions, and the regulatory role of RNA. LLPS's implications extend to gene regulation, cellular function, and disease, particularly neurodegenerative disorders and cancer. The survey also discusses LLPS's potential in biotechnology and synthetic biology, offering insights into designing synthetic organelles and biomaterials. Future research should explore predictive models, protein-RNA interactions, and the effects of external noise on LLPS dynamics to enhance our understanding of cellular processes and therapeutic strategies.

1 Introduction

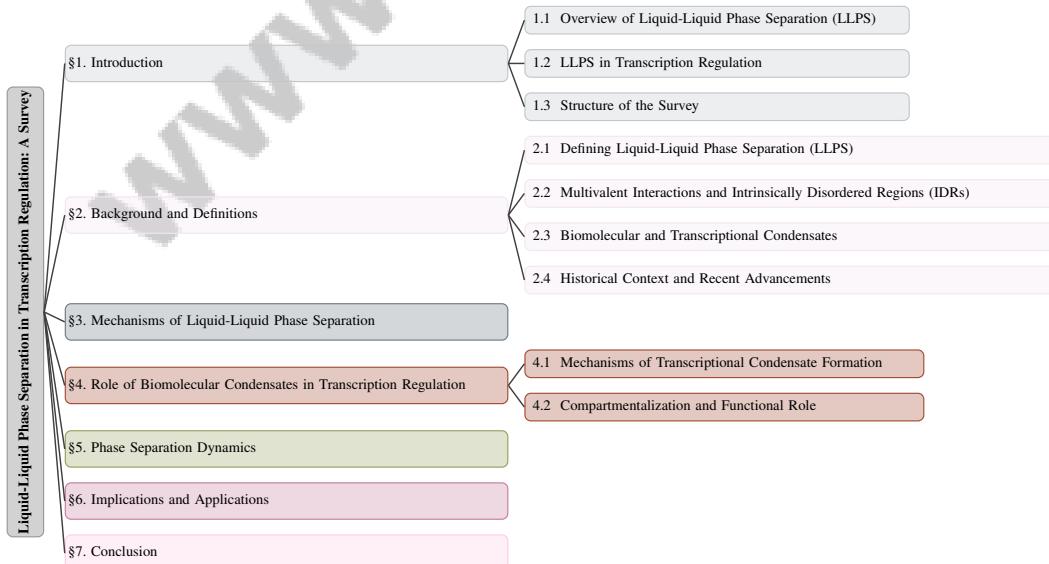


Figure 1: chapter structure

1.1 Overview of Liquid-Liquid Phase Separation (LLPS)

Liquid-liquid phase separation (LLPS) is a fundamental mechanism in cellular organization, facilitating the formation of biomolecular condensates—membraneless organelles (MLOs) that compartmentalize cellular components without membranes [1]. These dynamic structures are crucial for organizing biochemical reactions, impacting various cellular processes [2]. LLPS is primarily driven by multivalent interactions among biomolecules, particularly intrinsically disordered proteins (IDPs), which lack stable three-dimensional structures, enabling versatile interactions [3].

LLPS mediates critical interactions, such as -cation bonds between amino acids like tyrosine and arginine, which are vital for biological functions [3]. Additionally, RNA plays an essential role in the formation and functionality of ribonucleoprotein (RNP) granules, highlighting LLPS's significance in cellular processes and its implications in diseases [4]. The assembly of biomolecular condensates, particularly those involving proteins with multiple modular domains, underscores LLPS's versatility in constructing complex cellular architectures [5].

The potential of LLPS extends to synthetic biomolecular condensates, where IDPs or peptides are utilized to develop innovative biomaterials [6]. Research on synthetic protein condensates reveals unique properties applicable in cellular and metabolic engineering [7]. Furthermore, the study of LLPS in protein partitioning elucidates its role in various cellular functions [8].

External fluctuations significantly influence phase separation processes, as noted by [9], where noise acts as an ordering agent in temporal and spatiotemporal dynamics. The interactions of long biopolymers, such as DNA with protein mixtures, are critical for understanding chromatin organization and gene expression, emphasizing LLPS's relevance in biological systems [10]. Additionally, sequence-dependent interactions in IDPs are crucial for LLPS, underscoring the importance of amino acid residue interactions [11].

As research on LLPS expands, it remains a focal point for understanding cellular organization and its implications across scientific disciplines. Insights into LLPS mechanisms are essential for advancing knowledge in medicine, structural biology, and biotechnology, informing the development of innovative therapeutic strategies and biomaterials. Understanding how LLPS regulates enzyme activity, influences post-translational modifications, and contributes to membrane-less organelle formation can enhance our comprehension of pathological processes, such as protein aggregation in neurodegenerative diseases, guiding targeted interventions that leverage phase separation principles to improve cellular function and resilience under stress [12, 13, 14, 15].

1.2 LLPS in Transcription Regulation

LLPS is integral to transcription regulation by enabling the formation of transcriptional condensates—specialized membraneless organelles that compartmentalize transcriptional machinery and regulatory factors within the nucleus. These condensates arise from the LLPS of proteins with modular domains or intrinsically disordered regions (IDRs), playing a vital role in transcription, chromatin organization, and the DNA damage response. By concentrating key components and modulating the biochemical environment, LLPS enhances transcription efficiency, underscoring its importance in cellular physiology and potential implications in tumorigenesis [12, 14]. These condensates create microenvironments that improve the kinetics of transcriptional processes beyond what is achievable in homogeneous systems. The IDRs of proteins are crucial for this regulatory mechanism, facilitating condensate formation and dynamic remodeling through phase transitions.

Molecular interactions, such as those involving the FUS LC domain, stabilize transcriptional condensates, highlighting LLPS's role in organizing transcriptional components into functional assemblies [16]. RNA significantly influences RNA-protein condensate composition and stability, with factors like RNA length being critical for regulatory roles. Advanced computational strategies are necessary to elucidate the complex interplay of RNA and protein interactions within these condensates [17].

The spatial organization of macromolecules, including charged entities like DNA and RNA, is essential for forming specific cellular compartments that impact transcription regulation and other cellular processes [18]. LLPS at interfaces, such as evaporating droplets, further illustrates its role in creating distinct cellular patterns and structures [19]. LLPS also influences chromatin organization, as discussed in the context of prewetting phases [10], and may contribute to disease mechanisms,

particularly in neurodegenerative disorders where aberrant phase separation of proteins like tau leads to pathogenic conformations.

Despite its significance, challenges remain in designing on-demand transcriptional condensates due to the incomplete understanding of LLPS molecular mechanisms in transcription regulation [20]. The optogenetic approach to controlling the condensation of TAF-family transcriptional regulators, particularly TAF15, presents a promising avenue for investigating their role in transcriptional output [5]. Additionally, post-translational modifications (PTMs) in IDPs are crucial for LLPS, influencing cellular organization and function [21]. Understanding these processes is vital for elucidating transcriptional regulation mechanisms and their dysregulation in disease states, emphasizing LLPS's critical role in maintaining cellular function and health.

1.3 Structure of the Survey

This survey is meticulously structured to provide a comprehensive exploration of liquid-liquid phase separation (LLPS) and its pivotal role in transcription regulation. The introductory section establishes the foundation by elucidating LLPS concepts and their overarching significance in cellular processes, particularly in transcription regulation. The background section delves into the definitions and essential components of LLPS, clarifying multivalent interactions and intrinsically disordered regions (IDRs), while explaining biomolecular and transcriptional condensate formation and function. This section also reviews the historical context and recent advancements in LLPS research, providing a timeline of key developments.

The survey then transitions to a detailed exploration of the molecular mechanisms driving LLPS, focusing on the critical roles of electrostatic and hydrophobic interactions, and elucidating the "stickers and spacers" model that describes how specific protein motifs contribute to the formation and stability of biomolecular condensates. This analysis integrates insights from recent studies on the structural characteristics of proteins involved in LLPS, the impact of post-translational modifications, and the broader implications for cellular organization and function across various biological processes [22, 23, 15]. This is followed by a focused discussion on how biomolecular condensates influence transcription regulation, highlighting the mechanisms of transcriptional condensate formation and their compartmentalization functions.

Subsequent sections address the dynamics of phase separation, exploring factors influencing condensate formation, maturation, and dissolution. The implications and applications section discusses the broader impact of LLPS on gene regulation, disease associations, and potential therapeutic strategies, along with applications in biotechnology, synthetic biology, material science, and engineering.

The survey concludes by summarizing key insights and emphasizing the importance of understanding LLPS in transcription regulation. Future research directions are suggested to encourage further exploration in this dynamic and evolving field. Through this structured approach, the survey aims to deliver a nuanced exploration of LLPS, elucidating its fundamental principles, practical applications in cellular biology and protocell research, and potential future developments, particularly in regulating enzyme activity and advancing biotechnological innovations [12, 24, 25]. The following sections are organized as shown in Figure 1.

2 Background and Definitions

2.1 Defining Liquid-Liquid Phase Separation (LLPS)

Liquid-liquid phase separation (LLPS) is a fundamental biophysical process where a homogeneous solution of biomolecules demixes into distinct liquid phases, forming biomolecular condensates that are crucial for cellular organization [26]. These condensates enable compartmentalization of biochemical reactions, facilitating dynamic cellular processes without relying on membrane-bound structures [6]. Multivalent interactions among biomolecules, particularly involving intrinsically disordered proteins (IDPs) and their regions (IDRs), drive LLPS, characterized by unique amino acid sequences and charge distributions [27]. Key interactions include electrostatic, hydrophobic, and cation- interactions, each contributing to phase separation [27]. Theoretical frameworks, such as those elucidating poly-tyrosine and poly-arginine interactions, are vital for understanding complex coacervation [11]. While these interactions are pivotal for designing synthetic condensates, the molecular mechanisms governing LLPS remain incompletely understood [28].

RNA's role in LLPS is increasingly recognized, as RNA molecules can form liquid-like droplets through phase separation, influenced by molecular motors under out-of-equilibrium conditions [29]. Inter-domain interactions in dilute solutions provide insights into the condensed phase of biomolecular condensates, questioning if LLPS is solely driven by stoichiometric interactions [10]. Ongoing research reveals LLPS's diverse roles in natural and engineered systems, including its impact on chromatin organization and gene silencing via phase-separated droplets. Understanding environmental factors, such as evaporation at dynamic interfaces, is crucial for comprehending LLPS's implications in cellular and material science contexts [8].

2.2 Multivalent Interactions and Intrinsically Disordered Regions (IDRs)

Multivalent interactions and intrinsically disordered regions (IDRs) are essential to LLPS, driving biomolecular condensate formation critical for cellular organization and function [30]. IDRs, lacking stable three-dimensional structures, enable flexible and transient protein interactions, crucial for the dynamic assembly and disassembly of condensates in response to cellular cues and environmental changes [31]. These interactions regulate processes like gene expression and cellular signaling, highlighting cellular systems' adaptability. The complexity of multivalent interactions is influenced by protein composition and solution conditions, with electrostatic interactions being particularly significant. Charge patterning and electrostatic complementarity drive condensation phenomena, affecting IDPs' phase behavior [11]. Theoretical models emphasize network elasticity's role in LLPS, linking droplet size and dynamics to the surrounding medium's elastic properties [27].

RNA significantly influences LLPS, with intrinsic properties such as structure and sequence affecting its condensate-forming ability [31]. This underscores sequence determinants' importance in IDR behavior, where sequence-specific interactions modulate phase separation [27]. Designing LLPS-promoting peptide building blocks capable of self-coacervating into droplets with tunable properties exemplifies exploiting IDR interactions in synthetic systems [10]. Surface forces and phase separation studies reveal their critical role in altering thermodynamic phase boundaries, influencing droplet behavior [29]. Additionally, post-translational modifications (PTMs) significantly impact IDPs' phase transitions, essential for forming membraneless organelles in cells [21]. Understanding the mechanistic framework integrating spatial gene organization and active transcription with nuclear condensate behavior remains challenging, necessitating advanced theoretical and experimental approaches to elucidate multivalent interactions and IDRs' dynamic and functional implications in cellular processes.

2.3 Biomolecular and Transcriptional Condensates

Biomolecular condensates formed through LLPS are crucial for cellular organization, creating dynamic, membraneless compartments that facilitate various biochemical processes [32]. These condensates, often involving IDPs, exhibit complex behaviors, including reentrant phase behavior and aggregate formation [1]. Their morphology and stability are intricately regulated by protein and RNA interaction networks, which dictate assembly and function [31]. Transcriptional condensates, a specialized subset, concentrate transcription machinery and regulatory factors, enhancing transcriptional efficiency and specificity. These condensates leverage RNA-mediated interactions to stabilize their structure and function, playing a vital role in modulating gene expression [33]. Competing protein and RNA interactions influence transcriptional condensate formation, altering their morphology and functional dynamics [31].

Biomolecular condensates can sustain significant pH gradients without external energy input, revealing a novel mechanism for intracellular pH regulation [34]. This property underscores condensates' functional versatility in maintaining cellular homeostasis. Algorithms predicting interactions further highlight specific protein interactions' role in phase separation, particularly related to biomaterials and RNA processing [3]. Despite their critical roles, understanding biomolecular condensates' interaction with and remodeling of cellular membranes remains a challenge, essential for proteins involved in condensate formation and cellular organization. Further research into phase separation mechanisms and implications for cellular processes such as signaling, RNA metabolism, and responses to environmental stressors is warranted [35, 22, 3, 36]. Distinguishing between small structures and true condensates, along with formation criteria, requires further exploration to elucidate their complexities and functional roles in natural and engineered systems.

2.4 Historical Context and Recent Advancements

The exploration of LLPS has evolved from focusing on basic phase transition phenomena to emphasizing biomolecular condensates' complex role in cellular environments. Early studies on phase separation dynamics in fluid membranes provided foundational insights into how phase segregation can induce shape transformations [9]. This work laid the groundwork for understanding existing methods' limitations in capturing LLPS's intricate dynamics in more complex biological systems. Recent theoretical advancements have enhanced understanding of LLPS, particularly regarding confinement's role in regulating droplet size and its biological implications. Thermodynamic frameworks, including Gibbs free energy calculations and nucleation activation barriers, have been pivotal in elucidating favorable conditions for phase separation. These frameworks allow nuanced understanding of how network elasticity influences phase separation dynamics by stabilizing supersaturated mixtures and impeding droplet nucleation [21]. Such insights are particularly relevant to biological systems, where mechanical properties of cellular structures critically affect their function.

LLPS dynamics' complexity necessitates novel analytical approaches to predict phase behavior in systems with numerous interacting components. Integrating chemical reaction networks (CRNs) into LLPS models enhances biological system representations' accuracy by accounting for intricate solute interactions. This approach elucidates LLPS's influence on enzyme activity by concentrating reactants, tuning reaction rates, and managing competing metabolic pathways, providing insights into conditions conducive to stable microphase separation and biomolecular condensates' formation crucial for cellular metabolism and stress responses. Incorporating non-ideal solute interactions into CRN frameworks allows deeper understanding of phase separation dynamics, moving beyond traditional ideal-solution assumptions to better reflect biological complexities [12, 37, 38]. Despite advancements, challenges remain in quantitatively linking nanoscale biomolecule dynamics to biomolecular condensates' mesoscale properties, such as viscosity and diffusion characteristics.

In disease contexts, biomolecular condensates have been implicated in various conditions, including cancer and neurodegenerative diseases, underscoring understanding LLPS mechanisms and environmental factors' influence. LLPS research's historical development and recent advancements highlight its intricate nature and pivotal role in natural biological processes and engineered systems. Recent studies demonstrate LLPS regulates enzyme activity by concentrating reactants and modulating reaction rates, influencing metabolic pathways in response to cellular conditions. Innovative techniques like patterned flow manipulation have emerged, allowing precise control over LLPS characteristics, potentially leading to significant technological applications. Machine learning tools for predicting phase separation proteins (PSPs) enhance understanding of LLPS's biological functions and implications in various diseases, emphasizing LLPS's complexity and relevance across multiple domains [12, 25, 39]. Continued integration of theoretical and experimental methodologies is essential for advancing LLPS understanding and its broader implications across various scientific disciplines.

3 Mechanisms of Liquid-Liquid Phase Separation

Category	Feature	Method
Molecular Mechanisms and Influences	Electrostatic Influences	CG-IDP[1]
Role of Electrostatic and Hydrophobic Interactions	Phase Transition Influences	AESM[40], KPFM[26], FSSA[30]
Stickers and Spacers Model	Sequence Influence	SSM[41], CNA[42]

Table 1: This table provides a comprehensive summary of the various methods used to investigate the molecular mechanisms and interactions involved in liquid-liquid phase separation (LLPS). It categorizes these methods into three key areas: molecular mechanisms and influences, the role of electrostatic and hydrophobic interactions, and the stickers and spacers model, highlighting the specific features and techniques employed in each category. The references included offer further insight into the methodologies and their applications in understanding LLPS dynamics.

Understanding the mechanisms of liquid-liquid phase separation (LLPS) involves examining molecular interactions that facilitate this process. As illustrated in ??, the hierarchical categorization of mechanisms involved in LLPS highlights key molecular influences such as intrinsically disordered proteins (IDPs), electrostatic and hydrophobic interactions, and the stickers and spacers model. Table 1 presents a detailed summary of the methods used to explore the molecular mechanisms underlying liquid-liquid phase separation, emphasizing the role of electrostatic and hydrophobic interactions, as

well as the stickers and spacers model. Additionally, Table 2 presents a comprehensive comparison of various methods used to explore these mechanisms, further emphasizing the significance of these interactions and models. The figure further details each category with specific roles and applications, providing a comprehensive overview of LLPS dynamics and their implications for cellular organization and biotechnological applications. The following subsection delves into the molecular mechanisms and influences governing LLPS, thereby providing a foundation for further exploration.

3.1 Molecular Mechanisms and Influences

The molecular underpinnings of LLPS are driven by thermodynamic forces and kinetic processes that enable biomolecular condensate formation. IDPs play a pivotal role due to their flexible structures, which support diverse interactions necessary for the dynamic assembly and disassembly of condensates [1]. The stickers-and-spacers model classifies amino acid residues into adhesive components (stickers) and flexible linkers (spacers), which together dictate protein phase behavior.

Electrostatic interactions are key in LLPS, particularly in polyelectrolyte systems, where charge distribution and asymmetry significantly impact condensate morphology and dynamics [11]. Theoretical models, including mean-field approximations and field-theoretic simulations, elucidate how electrostatic forces influence IDPs' phase behavior, showing how charge complementarity can drive phase separation.

RNA acts as a molecular scaffold, influencing nucleation and growth of condensates, thereby affecting their size and composition [31]. The complex interactions between RNA and proteins within these condensates necessitate advanced computational approaches for characterization [2]. Molecular dynamics simulations enhance our understanding of how RNA concentrations modulate interfacial tensions and phase dynamics.

Chemical reactions within phase-separated systems modulate condensate stability and behavior. Integrating LLPS dynamics with chemical reaction networks offers a comprehensive framework to understand the interplay between molecular interactions and reaction kinetics, emphasizing LLPS's role in enzyme regulation and membraneless organelle formation. Recent advancements in manipulating LLPS through techniques like patterned flow enable precise control over droplet formation, paving the way for innovative applications in biotechnology and materials science [24, 12, 25].

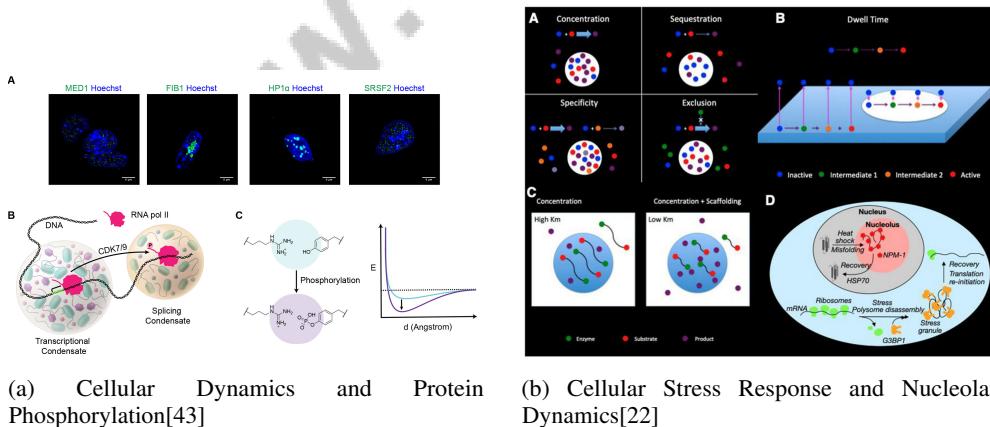


Figure 2: Examples of Molecular Mechanisms and Influences

As shown in Figure 2, LLPS is crucial in cellular biology, organizing compartments without membrane-bound organelles. The first illustration, "Cellular Dynamics and Protein Phosphorylation," combines fluorescence microscopy images and diagrams to depict the localization and interaction of proteins such as MED1, FIB1, HP1, and SRSF2 within the nucleus. The second illustration, "Cellular Stress Response and Nucleolar Dynamics," details protein concentration and distribution in the nucleus during stress responses, offering insights into nucleolar dynamics [43, 22].

3.2 Role of Electrostatic and Hydrophobic Interactions

Electrostatic and hydrophobic interactions are fundamental forces driving LLPS, significantly influencing the formation and stability of biomolecular condensates. In IDP systems, electrostatic interactions arise from charge distributions along protein chains, leading to microphase separation and complex coacervate formation. Recent models highlight the gluonic effect, emphasizing electrostatics' role in inducing reentrant phase transitions in polyelectrolytes, especially with multivalent salts [44].

Theoretical perspectives clarify how electrostatic interactions affect phase behavior, where charge asymmetry in droplets can suppress coarsening dynamics, impacting condensate stability and morphology [45]. The lower effective permittivity within condensed phases enhances electrostatic interactions among IDPs, reinforcing their role in driving LLPS [46]. Finite-size scaling analysis reveals how finite-size effects influence phase transitions, allowing for corrections in transition temperature estimates [30].

Hydrophobic interactions promote the aggregation of nonpolar residues, facilitating phase separation in aqueous environments. These interactions often couple with electrostatic forces to stabilize condensed phases. The statistical mechanics framework, particularly the Random Phase Approximation (RPA), elucidates how charge patterning and hydrophobicity influence polyampholyte phase behavior [47]. The interplay between these interactions is captured in theoretical models integrating thermodynamic and kinetic processes, enabling accurate predictions of phase separation and coarsening behaviors in complex mixtures [26].

Advanced analytical methods, including those utilizing virial coefficients, describe the stability of mixtures against phase separation, emphasizing the balance between electrostatic and hydrophobic interactions [40]. These methods highlight the universal behavior of properties such as osmotic compressibility and relaxation rates under varying solution conditions, underscoring the fundamental nature of these interactions in LLPS [48].

Electrostatic and hydrophobic interactions are thus critical in governing LLPS, with their combined effects dictating the formation, stability, and dynamics of biomolecular condensates. A profound understanding of these interactions is essential for elucidating cellular organization mechanisms, particularly regarding low-complexity protein regions' contributions to compartmentalization in response to environmental stresses and advancing biotechnological applications leveraging phase-separated systems' unique properties in gene regulation, stress response, and signal transduction [49, 36].

3.3 Stickers and Spacers Model

The stickers and spacers model offers a robust framework for understanding LLPS's molecular underpinnings, particularly concerning intrinsically disordered regions (IDRs) and their role in biomolecular condensate formation. This model posits that specific amino acids, termed "stickers," mediate attractive interactions critical for phase separation, while "spacers" are more flexible regions that modulate the physical properties of the resulting condensates [43]. By categorizing amino acids into these functional groups, the model enables a systematic exploration of how sequence properties influence phase behavior [50].

Applying the stickers and spacers framework to IDRs has significantly enhanced our understanding of phase separation dynamics, highlighting sequence-specific interactions' importance in dictating protein phase behavior. This insight allows for the engineering of short peptides for LLPS [51]. The model suggests that entropy minimization leads to attractive forces between stickers, facilitating the dynamic assembly and disassembly of condensates [41]. This perspective is particularly useful for designing synthetic peptides that can undergo phase separation, leveraging intrinsic properties of hydrophobicity and spacer polarity.

Furthermore, the stickers and spacers model has been extended to describe filamentous structures within condensates, providing a novel mechanism for relieving internal stresses during phase separation [42]. This contrasts with the traditional view that phase-separated fluids primarily form spherical droplets, offering new insights into the structural diversity and functional versatility of biomolecular condensates. By integrating these concepts, the stickers and spacers model serves as a powerful tool for elucidating the complex interactions driving LLPS, with implications for both natural and engineered systems.

Feature	Molecular Mechanisms and Influences	Role of Electrostatic and Hydrophobic Interactions	Stickers and Spacers Model
Key Interactions	Thermodynamic Forces	Electrostatic, Hydrophobic	Adhesive Interactions
Core Components	Idps And Rna	Polyelectrolytes	Stickers, Spacers
Application Focus	Biomolecular Condensates	Condensate Stability	Phase Behavior Design

Table 2: This table provides a comparative analysis of three key methodologies for investigating the molecular mechanisms underlying liquid-liquid phase separation (LLPS). It highlights the distinct roles of thermodynamic forces, electrostatic and hydrophobic interactions, and the stickers and spacers model in facilitating biomolecular condensate formation and stability. The table further delineates the core components and application focuses of each method, offering insights into their respective contributions to understanding LLPS dynamics.

4 Role of Biomolecular Condensates in Transcription Regulation

4.1 Mechanisms of Transcriptional Condensate Formation

Transcriptional condensates, formed via liquid-liquid phase separation (LLPS), are pivotal in organizing transcriptional machinery within the nucleus, thereby enhancing transcription regulation. These dynamic structures, devoid of membranes, compartmentalize transcription factors and enzymes, optimizing transcriptional processes [8]. Intrinsically disordered regions (IDRs) of proteins play a crucial role, facilitating multivalent interactions like hydrogen bonding, hydrophobic interactions, and / stacking, particularly involving residues such as glutamine and tyrosine.

RNA is integral in stabilizing and forming transcriptional condensates, influencing their size, number, and internal organization. The interactions between RNA and proteins are vital for the functional dynamics of these condensates, affecting intra-droplet patterning and behavior [31]. The length of RNA significantly impacts the stability of RNA-binding protein (RBP) condensates, emphasizing its role in phase behavior and condensate stability [31].

Electrostatic interactions are fundamental in condensate formation, dictating the phase separation process. The dielectric environment influences the LLPS propensity of polyampholytes, challenging traditional assumptions of uniform dielectric properties in LLPS theories [11]. Interfaces within condensates are critical for efficient biochemical reactions, emphasizing the importance of surface interactions in their functional dynamics [29]. The complete phase diagram for IDP coacervation reveals that charged residue arrangements significantly affect phase behavior, further highlighting electrostatics' role [11].

Quantitative frameworks for nucleation consider the efficacy of nucleation sites modulated by biomolecular features [11]. Additionally, dynamic arrest influences the formation of compositionally distinct condensates, impacting transcription regulation. Controlling wetting transitions in multiphase condensates affects their formation and stability; chemical reactions, particularly enzymatic activity, regulate the size and number of biomolecular condensates [10]. Chemical activity can induce mechanical work against surface tension, leading to droplet division [21].

The mechanisms governing transcriptional condensate formation involve a complex interplay of molecular interactions, including electrostatics, RNA-protein interactions, and sequence-specific effects [27]. Understanding these mechanisms is crucial for elucidating transcriptional condensates' roles in gene regulation and their implications in disease and therapeutic strategies. Integrating novel modeling approaches with experimental data promises to advance our understanding of LLPS dynamics in transcriptional regulation [11]. The role of elasticity in regulating droplet behavior offers insights into cellular organization mechanisms, linking elastic properties to condensate dynamics [34]. Recognizing membraneless organelles (MLOs) as critical for cellular compartmentalization highlights the importance of understanding their material properties for future applications in synthetic biology and therapeutic development [9].

As illustrated in Figure 3, the role of biomolecular condensates in transcription regulation has gained significant attention due to their potential impact on gene expression and cellular function. The study of transcriptional condensate formation mechanisms highlights the intricate processes through which these condensates influence transcriptional regulation. Figure 3 presents three distinct instances elucidating these mechanisms. The first example focuses on the HP1 heterochromatin condensate, illustrating its formation and modification stages, with HP1 proteins depicted in green attached to a gray spherical structure, crucial for gene silencing and chromatin organization. The second example

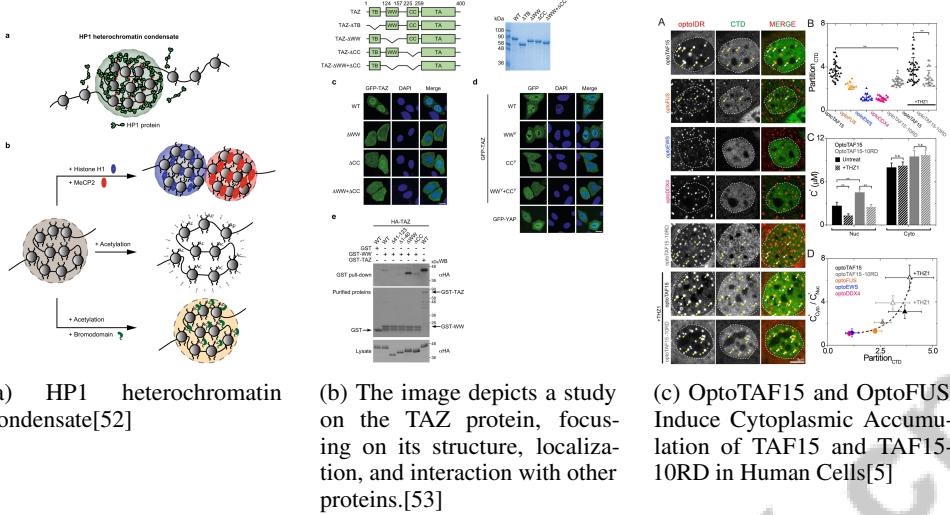


Figure 3: Examples of Mechanisms of Transcriptional Condensate Formation

examines the TAZ protein, emphasizing its structural domains, localization, and interactions with other proteins, as demonstrated through diagrams and Western blot analyses, shedding light on how protein structure and interactions affect transcriptional activity. Lastly, the study of OptoTAF15 and OptoFUS reveals their influence on the cytoplasmic accumulation of TAF15 and TAF15-10RD in human cells, as shown through confocal imaging. These examples collectively enhance our understanding of the diverse mechanisms through which transcriptional condensates form and regulate gene expression, offering insights into their broader biological significance [52, 53, 5].

4.2 Compartmentalization and Functional Role

Biomolecular condensates formed through liquid-liquid phase separation (LLPS) are critical for compartmentalizing transcription machinery, enhancing the efficiency and specificity of transcriptional processes. These condensates create distinct microenvironments within the nucleus that facilitate the localization and concentration of transcription factors and RNA polymerases, effectively increasing reaction rates and buffering cellular noise [22]. Their unique properties allow them to act as dynamic hubs for biochemical reactions, providing essential spatial organization for gene expression regulation.

Condensates enhance reaction rates by concentrating reactants and enzymes in confined spaces, reducing diffusion distances and increasing the likelihood of molecular interactions [22]. This compartmentalization optimizes transcriptional kinetics and allows for the rapid, reversible assembly of transcription complexes in response to cellular signals. The superlinear growth in the number of distinct condensates with increasing molecular diversity further underscores these structures' functional versatility in cellular organization [54].

Network elasticity significantly influences the stability and dynamics of biomolecular condensates, suppressing droplet nucleation within immiscible regions and stabilizing supersaturated mixtures [55]. This property is particularly relevant to transcriptional regulation, as condensate stability can affect the duration and intensity of transcriptional responses. The potential for network elasticity to modulate condensate physical properties presents intriguing avenues for future research, particularly regarding how mechanical properties influence transcriptional regulation [6].

LLPS also contributes to organelle formation in response to stress or neural activity, highlighting its role in adaptive cellular responses [14]. The dynamic nature of condensates enables rapid assembly and disassembly in response to changes in the cellular environment, providing a mechanism for modulating transcriptional activity in response to external stimuli. This adaptability is crucial for maintaining cellular homeostasis and responding to stress conditions, emphasizing LLPS's importance in cellular function and regulation.

Despite their critical roles, challenges remain in visualizing and isolating membrane domains in intact cells, complicating the study of membrane interactions and cytoskeletal influences on membrane organization [35]. These challenges underscore the need for advanced imaging techniques and experimental approaches to elucidate condensate formation and function complexities. Furthermore, the proposal that heterochromatin may represent a distinct prewet phase that excludes transcriptional machinery illustrates LLPS's diverse roles in cellular organization and gene regulation [10].

5 Phase Separation Dynamics

Understanding phase separation dynamics is essential for elucidating the behavior of biomolecular condensates, which are integral to various cellular processes. This section explores the mechanisms underpinning the formation and regulation of these condensates, focusing on the dynamics and regulatory factors influencing their behavior. The characteristics of these condensates are shaped by the interplay of molecular interactions, physical properties, and environmental conditions, which ultimately determine their cellular functionality. The following subsection delves into the dynamics and regulation of condensates, emphasizing intrinsic properties and external factors affecting their formation and stability.

5.1 Dynamics and Regulation of Condensates

The dynamics and regulation of biomolecular condensates formed via liquid-liquid phase separation (LLPS) are pivotal for understanding their roles in cellular processes. Composed primarily of intrinsically disordered proteins (IDPs), these condensates exhibit complex dynamics influenced by their viscoelastic properties and interactions within membraneless organelles. The viscoelasticity significantly affects protein diffusion and overall dynamics within condensates [56].

Theoretical frameworks highlight the role of elasticity in determining droplet size, migration dynamics, and spatial localization within heterogeneous materials. Models incorporating capillary forces, network heterogeneities, and nonlinear mechanical properties underscore elasticity's importance [57]. Chemical kinetics also play a crucial role in regulating condensates. Integrating phase separation dynamics with chemical reaction networks offers insights into how external factors influence phase-separated systems, providing a thermodynamically consistent framework for controlling biomolecular condensates [38]. Linear stability analyses of continuum equations describing chemically active particles further elucidate regulatory mechanisms [58].

RNA is a significant modulator of condensate dynamics, with RNA gradients driving motion and offering insights into phase separation dynamics [59]. RNA-mediated interactions can lead to spatial patterning within droplets, emphasizing RNA's regulatory role through competition among distinct RNA-protein complexes for shared binding partners.

Advanced methodologies, including Monte Carlo simulations, analyze configurational characteristics of mixtures, focusing on local order parameters and phase diagram analysis [28]. High-speed ellipsometry measurements reveal changes in precursor films prior to nucleation in main droplets, providing insights into initial phase separation stages [29]. Charge neutrality among polypeptide chains in condensates is proposed to drive pH gradients, illustrating these systems' complexity [34].

The dynamics and regulation of biomolecular condensates are governed by molecular interactions, electrostatic forces, and chemical kinetics, influencing their formation, stability, and functional roles in cellular processes. These condensates, formed through phase separation, are regulated by post-translational modifications affecting interaction dynamics. The coupling of diffusion and reaction kinetics within condensates optimizes chemical processes, highlighting their significance in cellular organization and biochemical regulation across scales [60, 61, 22]. Understanding these mechanisms is vital for elucidating cellular organization principles and developing applications exploiting phase-separated systems' unique properties. Future research should integrate sophisticated models to capture polyelectrolyte interactions' complexity and electrostatic screening's role in concentrated solutions.

5.2 Diffusive Dynamics and Viscoelastic Properties

The diffusive dynamics and viscoelastic properties of biomolecular condensates formed through LLPS are critical for understanding their functional roles in cellular processes. Composed of IDPs and RNA, these condensates exhibit complex behaviors influenced by their structural and mechanical properties [56]. The viscoelastic nature is pivotal in modulating protein diffusion and overall dynamics, affecting biochemical reactions and cellular functions.

Studies indicate that condensates' viscoelastic properties are determined by network elasticity and molecular interactions, dictating mechanical stability and response to external forces [62]. Network elasticity influences droplet size, migration dynamics, and spatial localization within heterogeneous environments [63]. Numerical simulations elucidate how varying stiffness conditions regulate droplet size and coarsening dynamics, emphasizing elasticity's significance [57].

The diffusive dynamics are characterized by constituent molecules' mobility, influenced by condensate matrix viscoelastic properties. Factors such as molecular crowding, interactions, and surrounding environment properties affect protein and RNA diffusion within condensates [59]. Advanced methodologies, including Monte Carlo simulations and high-speed ellipsometry, analyze configurational characteristics, providing insights into local order parameters and phase diagram analysis.

RNA plays a crucial role in modulating diffusive dynamics, with RNA gradients driving condensate motion and providing insights into phase separation dynamics [59]. The complex interactions between RNA and proteins necessitate advanced computational approaches for thorough characterization. Competition among distinct RNA-protein complexes for binding partners emphasizes RNA's regulatory role.

Understanding diffusive dynamics and viscoelastic properties is vital for elucidating cellular organization principles and developing applications leveraging phase-separated systems' unique properties. Future research should prioritize advanced models incorporating polyelectrolyte interactions' intricate dynamics, focusing on electrostatic screening's influence on phase behavior in concentrated solutions. This includes exploring charge regulation mechanisms, multivalent salts' impact on phase transitions, and sequence-specific properties' role in LLPS, essential for understanding condensate formation in cellular environments. Integrating these aspects will provide deeper insights into polyelectrolyte systems' thermodynamics and biological implications [49, 64, 65, 44, 36].

5.3 Impact of Charge Patterns and Sequence-Dependent Interactions

Charge patterns and sequence-dependent interactions significantly influence biomolecular condensates' dynamics formed through LLPS. The arrangement of charged residues within IDPs and other biomolecules critically affects phase behavior and stability. Theoretical and computational studies demonstrate that charge asymmetry within droplets can create energy barriers impeding coalescence, impacting coarsening dynamics and stability [45].

Interactions based on specific amino acid sequences, such as distinct motifs and charge distributions, are vital in determining proteins' propensity to undergo LLPS. Research indicates that electrostatic, hydrophobic, and cation- interactions, along with low-complexity regions' arrangement, significantly contribute to IDPs' phase behavior. These interactions are essential for understanding how variations in amino acid composition and sequence modulate condensate formation, particularly under changing environmental conditions like temperature and pH [66, 67, 36]. Charge patterns regulate interaction strength, affecting condensates' dynamics and morphology. Theoretical models capture these effects, providing insights into sequence-specific interactions' influence on complex mixtures' phase behavior.

Moreover, charge patterns impact condensate dynamics regulation through electrostatic screening and intermolecular forces modulation. The interplay between charged residues and the surrounding dielectric environment affects condensates' internal organization and functional properties, influencing their ability to compartmentalize biochemical reactions. Understanding charge patterns and sequence-dependent interactions is crucial for deciphering LLPS mechanisms in biomolecules. This knowledge informs fundamental biology regarding biomolecular condensates, significantly influenced by IDPs' interactions, and facilitates designing engineered biomolecules with specific phase separation properties. Such advancements have promising implications for synthetic biology and biotechnology applications, where tailored phase separation behaviors can enhance biomolecular systems' functionality and efficacy [66, 67].

6 Implications and Applications

The study of liquid-liquid phase separation (LLPS) has profound implications for both biological and material sciences, offering insights into gene regulation and cellular function. This foundational understanding is crucial for exploring the complex interactions between phase separation phenomena and biological processes. The following subsection delves into LLPS's impact on gene regulation and cellular function, highlighting its significance in biomolecular condensate formation and transcription dynamics.

6.1 Implications for Gene Regulation and Cellular Function

LLPS significantly affects gene regulation and cellular function by facilitating the formation of biomolecular condensates, which serve as dynamic compartments for transcriptional machinery and regulatory factors. These condensates enhance transcriptional efficiency and specificity by concentrating reactants and enzymes, thus optimizing reaction kinetics and influencing gene expression patterns. The ability of LLPS to create distinct microenvironments is crucial for understanding chromatin organization and cellular structure self-organization [10].

RNA plays an essential role in regulating protein interactions and cellular processes, affecting the stability and dynamics of biomolecular condensates like stress granules and nucleoli. This modulation impacts their biophysical properties, including size, viscosity, and composition, emphasizing RNA's vital role in cellular organization and homeostasis, supporting transcriptional regulation, and protecting RNA from degradation, which affects gene expression and cellular responses to environmental changes [33, 68, 69]. Insights from studies on phase-separated states' interconversion are crucial for understanding nonequilibrium phase separation in biological systems and the nature of nuclear foci.

Biomolecular condensates' ability to maintain significant pH gradients is vital for cellular organization and function, influencing various biochemical pathways [34]. The interaction of sequence-dependent interactions and external noise driving phase separation provides deeper insights into dynamic systems, essential for elucidating mechanisms underlying cellular function.

Post-translational modifications (PTMs) critically regulate the phase behavior of intrinsically disordered proteins (IDPs), essential for the formation and dynamics of membraneless organelles [21]. Understanding these modifications can lead to innovative insights into biomaterials and therapeutic systems, revealing the molecular interactions driving LLPS [27].

Insights into LLPS can inform the development of synthetic organelles and advanced biotechnological applications, as protein phase separation can guide the design of novel cellular components and enhance our understanding of cellular organization. LLPS's influence on droplet spreading dynamics highlights its role in cellular processes and potential applications in manipulating biological systems. LLPS offers profound insights into gene regulation and cellular function, with extensive implications for biotechnology and medicine [11].

6.2 Disease Associations and Therapeutic Strategies

LLPS is a pivotal factor in the pathogenesis of various diseases, particularly neurodegenerative disorders and cancer, where aberrant phase separation of IDPs leads to pathogenic aggregate formation. These aggregates often transition from liquid-like to solid-like states, disrupting cellular functions and contributing to disease progression [34]. Understanding electrostatic interactions and specific protein modifications, such as those in tau, is crucial for elucidating these transitions and their neurotoxic implications [3].

In cancer, LLPS influences oncogenic pathways by regulating biomolecular condensates (BCs) that modulate cellular processes, presenting potential therapeutic targets. Modulating condensate dynamics through RNA modifications further implicates LLPS in disease states, particularly neurological disorders where RNA aggregation is critical [31]. Charge-specific transport studies highlight the potential for coacervates to act as regulators of molecular transport, akin to membranes, which could inform therapeutic interventions [11].

Despite advancements, challenges remain in thoroughly elucidating LLPS influences on enzyme kinetics and the specific roles of various biomolecular condensates in disease contexts. Current studies often lack comprehensive understanding due to biological system complexities and *in vitro*

model limitations [30]. Future research should focus on elucidating regulatory networks governing LLPS, exploring its implications in disease contexts, and developing novel experimental approaches to study these dynamic systems.

Exploring LLPS as a therapeutic target is promising, especially in neurodegenerative diseases, where modulating phase separation dynamics could alter disease progression. Integrating environmental factors into predictive models of LLPS and employing advanced computational techniques, including machine learning and analytical formulations, can enhance our understanding of phase-separated systems. This approach not only improves the prediction of phase separation proteins (PSPs) but also clarifies how LLPS regulates cellular processes, including enzyme activity and metabolic pathways. Such insights are vital for uncovering LLPS dysregulation implications in diseases, contributing to targeted therapeutic strategies [12, 66, 39]. The potential to engineer size-controlled microdroplets through elastic limitations presents innovative therapeutic strategies for manipulating phase-separated structures in disease contexts.

Understanding molecular interactions and environmental factors influencing synthetic condensates' behavior and stability remains critical for future research, with significant implications for therapeutic development [3]. The potential applications in studying diseases associated with protein misfolding and aggregation underscore LLPS's importance in developing novel therapeutic strategies.

6.3 Applications in Biotechnology and Synthetic Biology

LLPS offers transformative potential in biotechnology and synthetic biology, where its principles can be harnessed to engineer novel biomaterials and cellular systems with enhanced functionalities. The ability of LLPS to form dynamic, membraneless compartments provides a versatile framework for designing synthetic organelles that mimic natural cellular processes, enabling precise control over biochemical reactions and cellular functions [70]. By leveraging LLPS's intrinsic properties, researchers can develop smart materials responsive to environmental cues, offering innovative solutions for drug delivery, biosensing, and tissue engineering.

The conceptual model of phase separation, particularly regarding intrinsically disordered protein regions (IDRs), facilitates predicting phase behavior based on sequence alterations. This understanding allows for the rational design of synthetic biomolecules and materials with tunable properties, providing insights into biological processes and disease mechanisms [50]. Predicting and manipulating phase separation dynamics is particularly valuable in synthetic biology, where creating modular, self-assembling systems can lead to new therapeutic strategies and biomaterials with specific functional attributes.

Recent advancements in understanding the wetting and spreading dynamics of LLPS systems further highlight their potential in biotechnology. Designing synthetic biomaterials with tunable wetting properties opens avenues for creating surfaces and interfaces that dynamically interact with biological systems, enhancing the integration of synthetic and natural components in biomedical applications [71]. These innovations are poised to revolutionize synthetic biology, providing tools for constructing complex biological systems and materials that adapt to changing environments.

Integrating LLPS principles into biotechnology and synthetic biology presents substantial opportunities for enhancing our capacity to design and manipulate intricate biological systems and materials. By leveraging LLPS mechanisms that govern enzyme activity and membraneless organelle formation, researchers can engineer synthetic biomolecular condensates that regulate cellular functions and enable precise control over biochemical reactions. This approach facilitates the concentration of reactants, tuning of reaction rates, and rerouting of metabolic pathways, thus fostering innovative applications in cellular engineering and the development of novel therapeutic strategies [72, 12, 39]. Harnessing the unique properties of phase-separated systems allows for innovative solutions addressing current challenges in medicine, materials science, and environmental sustainability.

6.4 Material Science and Engineering Applications

LLPS presents significant opportunities in material science and engineering, particularly for developing advanced materials with tunable properties. Harnessing LLPS principles enables the creation of materials exhibiting dynamic phase behavior, allowing for systems with responsive characteristics suitable for various applications. Controlling phase separation processes at the molecular level

facilitates engineering materials with specific mechanical, optical, and thermal properties essential for advanced technological applications [26].

Integrating LLPS into material design allows for creating composite materials with enhanced functionalities. By manipulating interactions within phase-separated systems, it is possible to engineer materials that adapt to environmental changes, offering potential applications in smart coatings, sensors, and actuators. Studying phase separation dynamics provides insights into the self-assembly processes driving complex material structures' formation, informing new materials with hierarchical organization and multifunctional capabilities [70].

Furthermore, LLPS's application in designing biomimetic materials highlights its potential to bridge the gap between biological systems and engineered materials. Mimicking natural phase separation processes enables creating materials that interact seamlessly with biological environments, paving the way for innovations in biomedical engineering and tissue regeneration. Exploring LLPS in material science and engineering underscores its transformative potential, offering new avenues for developing materials that meet modern technology and industry demands [50].

The versatility of LLPS in material science is further exemplified by its role in designing materials with controlled wetting and spreading properties. By understanding the thermodynamic principles governing phase separation, researchers can develop surfaces with tailored interactions, enhancing material performance in applications ranging from fluid dynamics to surface coatings. LLPS's potential to inform the design of materials with unique properties underscores its significance in advancing material science and engineering, providing a foundation for future innovations [71].

7 Conclusion

7.1 Conclusion

The exploration of liquid-liquid phase separation (LLPS) reveals its critical role in cellular organization and transcription regulation, highlighting its significance in forming membraneless organelles and biomolecular condensates. LLPS facilitates the compartmentalization of cellular components, enhancing biochemical processes through multivalent interactions and the dynamic behavior of intrinsically disordered proteins (IDPs). These mechanisms underscore the versatility of LLPS in constructing complex cellular architectures and its potential in synthetic biomolecular applications.

Advancements in understanding LLPS have profound implications for medicine, biotechnology, and structural biology. Insights into the molecular interactions driving LLPS, such as -cation bonds and the influence of RNA, provide a foundation for developing innovative therapeutic strategies and biomaterials. Furthermore, the study of LLPS in transcription regulation elucidates the formation of transcriptional condensates, which concentrate transcriptional machinery and regulatory factors, thereby enhancing transcription efficiency and impacting chromatin organization.

Future research should focus on elucidating the intricate dynamics of LLPS, particularly the role of protein-RNA interactions and post-translational modifications in disease mechanisms. Investigating the influence of external fluctuations and sequence-dependent interactions in IDPs will further enhance our understanding of LLPS's adaptability and robustness in various biological contexts. Additionally, the design of synthetic protein condensates presents opportunities for innovative applications in cellular and metabolic engineering.

By addressing these research directions, we can deepen our comprehension of LLPS's pivotal roles in cellular function and disease, paving the way for novel therapeutic strategies and biotechnological innovations.

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