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Unlocking the electrochemical functions of biomolecular condensates

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Biomolecular condensation is a key mechanism for organizing cellular processes in a spatiotemporal manner. The phase-transition nature of this process defines a density transition of the whole solution system. However, the physicochemical features and the electrochemical functions brought about by condensate formation are largely unexplored. We here illustrate the fundamental principles of how the formation of condensates generates distinct electrochemical features in the dilute phase, the dense phase and the interfacial region. We discuss the principles by which these distinct chemical and electrochemical environments can modulate biomolecular functions through the effects brought about by water, ions and electric fields. We delineate the potential impacts on cellular behaviors due to the modulation of chemical and electrochemical environments through condensate formation. This Perspective is intended to serve as a general road map to conceptualize condensates as electrochemically active entities and to assess their functions from a physical chemistry aspect.

The chemical composition of the cellular matrix is the most fundamental factor governing the activity of biologics[1]. For example, the chemiosmotic theory[2], which posits a connection between the proton gradient and the change in diverse biochemical activities of living systems, demonstrates a chemical reaction-based mechanism correlating chemical environments and cellular functions. However, in intracellular spaces, our understanding of the rules governing and regulating chemical compositions, which are essential to understand the biochemical functions of living cells, is limited. Recent works in the field of macromolecular condensation, a generic mechanism of living cells to regulate spatiotemporal distribution of macromolecules[3], have gradually shed light on this crucial aspect of the life sciences.

The condensation of biomolecules driven by macromolecular phase transition results in a membraneless structure, named the biomolecular condensate[4–6], which is found to drive diverse cellular processes and disease pathways[7–11]. An emerging feature is the electrochemical functions that are dictated by the chemical environment of condensates. Upon condensate formation, molecules forming the dense phase switch from interactions primarily with solvent (that is, salt, water) to interactions with molecules participating in condensate formation, while molecules remaining in the dilute phase can establish intramolecular interactions[6,12,13]. This feature raises two questions: (1) how does this density-transition process dictate a unique chemical feature of the solvent environment of condensates? And (2) how do differences in the chemical environment between the dilute phase and the dense phase cooperatively generate new electrochemical features in the interfacial region between the two phases? These fundamental questions imply that the biomolecular condensate may not be a chemically inert entity but can control chemical and biological functions directly by the chemical features arising from condensate formation. Also, if the chemical constituents within the condensates are distinct in composition and abundance, then the chemical and electrochemical environment of the cytoplasm might also be affected, suggesting a possible role of biomolecular condensates in regulating global cellular biochemistry.

In this Perspective, we discuss the underlying mechanisms by which condensate formation can create a unique chemical environment. We present some early evidence showing unique chemical features of the microenvironment and the interfacial region of condensates and how such features can serve as the basis to modulate

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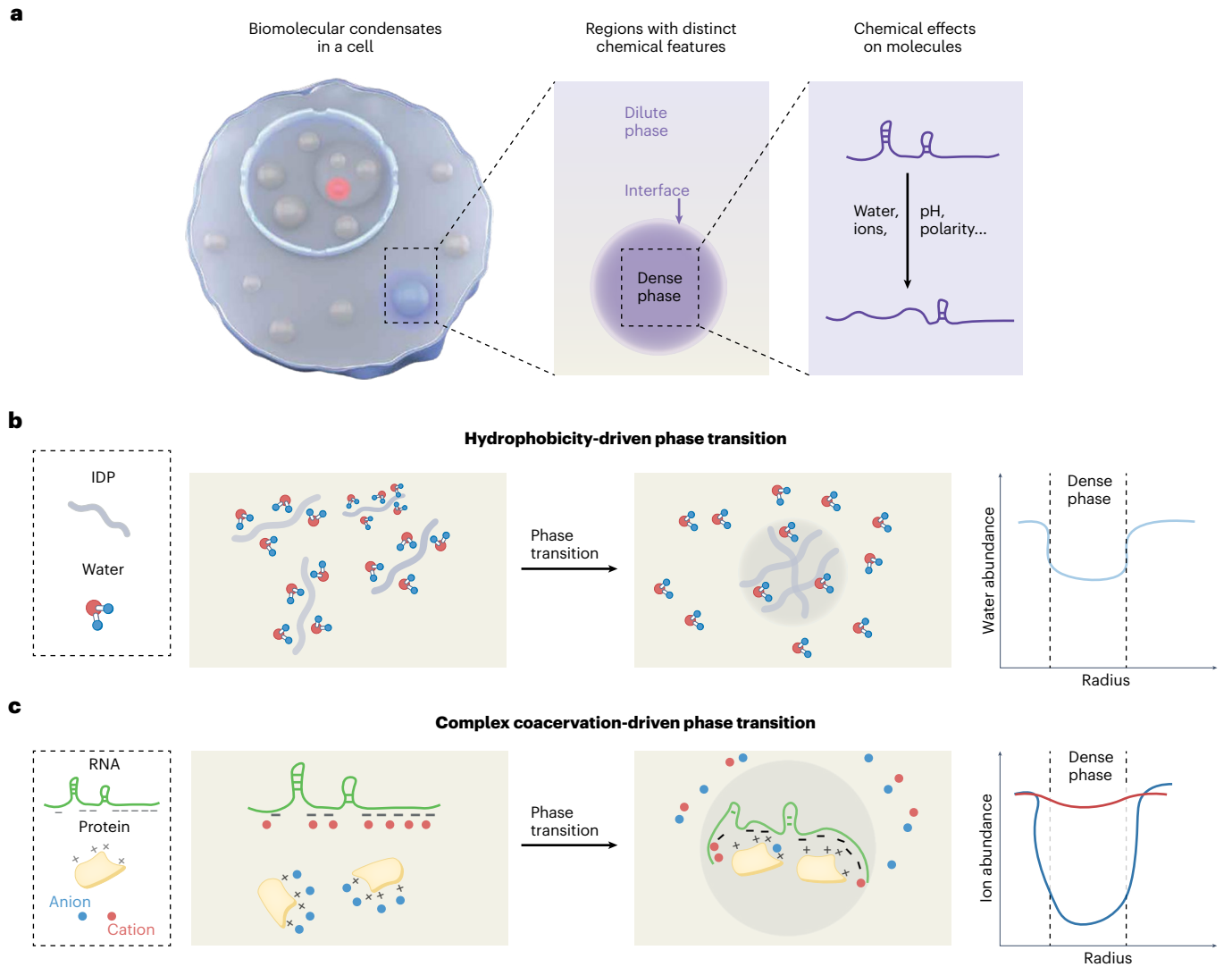

Fig. 1 | The origin of distinct chemical environments in biomolecular condensates. a, Biomolecular condensates can establish distinct chemical environments in different regions. A distinct chemical environment of condensates can modulate the chemical activity of biomolecules. **b**, Hydrophobicity-driven phase transition of IDPs can result in different water contents between the dilute and dense phases. **c**, Complex coacervation-driven phase transition of biomolecules can result in different ion contents between the dilute and dense phases. Enforced charge neutrality within one phase mediates selective partitioning or exclusion of ions into the dense phase.

cellular chemical and electrochemical environments. We also rationalize and propose some speculations on how the environmental effects by condensates can affect biological functions from an electrochemical aspect. This aspect of condensate functions goes beyond the relevant biomolecular functions of biomolecules that drive and participate in condensate formation. This work aims to provide a framework to explore the electrochemical functions of biomolecular condensates from the perspective of physical chemistry.

Chemical environment defined by phase transition

Condensates are formed by the coupling of an associative and segregative phase transition of molecules in the solution system[6]. In a simple in vitro system, transitioning from a homogeneous single phase into two immiscible phases results in a molecular density gradient within the aqueous two-phase system, which suggests that the molecular interactions defining the thermodynamic equilibrium before and after phase transition are different. This switch of intermolecular interactions by phase transition (for example, solvent–protein to protein–protein) is the underlying cause of the simultaneous associative transition of certain biomacromolecules and the segregative transition of others, resulting in a distinct chemical environment in the dense phase (Fig. 1a). This can further affect the chemical and electrochemical features of the interfacial region and the dilute phase. We here illustrate how phase transition can generate a distinct chemical environment using two distinct cases. These cases represent two different thermodynamic driving forces for phase separation: condensate formation driven by (1) hydrophobicity and (2) complex coacervation.

For systems undergoing phase transition through hydrophobicity-dependent driving force, such as an elastin-like polypeptide[14], which is an intrinsically disordered protein (IDP) consists of polar and hydrophobic residues, before phase transition, the IDP is stabilized by water–backbone interactions[15]. With an increase in system temperature surpassing the critical solution temperature of the IDP, phase separation occurs via a lower critical solution temperature transition process[16], in which the increase in entropic cost to hydrate the protein backbone leads to the release of water molecules from the backbone, causing the polypeptides to favor intrachain and interchain interactions and phase separation. Thermodynamically, the release of water to the dilute phase is entropically favorable and can drive phase

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BOX 1

Thermodynamic basis of electrochemical potential equilibrium

We start with the fundamental equation for the Gibbs free energy for a system containing neutral species

$$dG = -SdT + VdP + \sum_i \mu_i dn_i \quad (1)$$

where T is the temperature, S is the entropy, P is the pressure, V is the volume, n_i is mole number of species i, and μ_i is its corresponding chemical potential.

In the presence of charged species, dG needs to account for an additional term due to charging work — that is, electrical work,

$$dW_{el} = \varphi dQ \quad (2)$$

where φ is the electric potential, and dQ is the net change in the charge.

$$dQ = \sum_i z_i F dn_i \quad (3)$$

with z_i being the valency and F = 96,485 C/mol being Faraday's constant. We define the valency z_i such that it is positive for cations, negative for anions, and zero for neutral species.

Adding the charging work term to equation (1), the Gibbs free energy change becomes

$$dG = -SdT + VdP + \sum_i (z_i F\varphi - \mu_i) dn_i \quad (4)$$

Now consider a closed system consisting of two subsystems, 1 and 2 at constant temperature and pressure. The total free energy change due to species exchange between the two subsystems is

$$dG = dG^{(1)} + dG^{(2)} = \sum_i \left(z_i F\varphi^{(1)} - \mu_i^{(1)}\right) dn_i^{(1)} + \sum_i \left(z_i F\varphi^{(2)} - \mu_i^{(2)}\right) dn_i^{(2)} \quad (5)$$

Noting $dn_i^{(2)} = -dn_i^{(1)}$ due to species conservation, equation (5) becomes

$$dG = \sum_i \left(z_i F\Delta\varphi + \mu_i^{(2)} - \mu_i^{(1)}\right) dn_i^{(1)} \quad (6)$$

where $\Delta\varphi = \varphi^{(2)} - \varphi^{(1)}$ is the potential difference between subsystem 2 and subsystem 1 (the Galvani or Donnan potential).

At equilibrium, we have $dG/dn_i^{(1)} = 0$ resulting in the following general electrochemical equilibrium condition

$$\mu_i^{(2)} = z_i F\Delta\varphi + \mu_i^{(1)} \quad (7)$$

For neutral species, this reduces to the simple equality of chemical potential $\mu_i^{(2)} = \mu_i^{(1)}$. For charged species ($z_i \neq 0$), the potential difference is given by

$$\Delta\varphi = -\frac{1}{z_i F}\left(\mu_i^{(2)} - \mu_i^{(1)}\right) \quad (8)$$

Writing the chemical potential in the standard form

$$\mu_i = \mu_i^* + RT\ln a_i \quad (9)$$

where μ_i^* is a reference chemical potential (taken to be the same for the two subsystems) and a_i is the activity, we then have

$$\Delta\varphi = -\frac{RT}{z_i F}\ln\frac{a_i^{(2)}}{a_i^{(1)}} \quad (10)$$

Equation (10) shows that a difference in the effective concentration (that is, activity) of a charged species between the phases is necessarily associated with an electric potential difference between the phases.

separation along with enthalpically favorable intermolecular interactions. This feature has been experimentally demonstrated for a nuage protein, DDX4, showing positive entropy of transition and negative enthalpy of transition[17]. Similarly, for phase separation of the fused in sarcoma (FUS) protein, the release of entropically unfavorable water molecules into the dilute phase comes along with enthalpically favorable intra-FUS interactions in the dense phase[18]. This process results in the exclusion of solvent from the condensates, reducing the water content in the dense phase compared to the dilute phase (Fig. 1b). This feature has been experimentally tested in the phase separation of the FUS low-complexity sequence[19], in which the water concentration in the condensate is 40% lower than that in the dilute phase.

For systems undergoing phase transition through complex coacervation, such as systems involving proteins and RNA[8], before phase transition, the oppositely charged macromolecules are screened by the corresponding counterions in the solvent. During phase transition, the oppositely charged macromolecules associate together, forming polyion pairs, which leads to the release of the corresponding counterions from the macromolecules[20,21]. The polyion pairs then condense into a dense phase[21]. As such, the macromolecule-rich phase typically contains less ions than the dilute phase[22,23] (Fig. 1c). The ratio of cation and anion in the macromolecule-rich phase then determines the charge condition of the newly formed coacervates. This feature has been studied using solution containing two oppositely charged polypeptides, poly-L-lysine and poly-D,L-glutamic acid, and sodium chloride salt[23]. Measurements on the conductivity in the dilute phase reveal the enrichment of salts in the dilute phase, suggesting that the dense phase and the dilute phase have an ion concentration difference. A similar phenomenon has been demonstrated in the case of complex coacervation of highly charged IDPs, in which counterions are mainly released by the dimerization process[20,24]. As illustrated above, the intermolecular and intramolecular interactions are different before and after phase transition; so the solvent environments from the aspects of water and ion abundance must be different between the homogeneous single phase before phase transition and the dilute phase or the dense phase after phase transition. These features can then mediate environmental chemistry-dependent effects on the structure and functions of biomolecules (Fig. 1a). For example, nucleic acid duplexes can be melted within condensates[25,26]. The detailed mechanisms (that is, water abundance, ions, micropolarity and pH[22,25,27–36]) by which condensate microenvironments can affect the chemistry of biomolecules are illustrated in Supplementary Note 1.

Electrochemical potential equilibrium between phases

What is the consequence of an established ion concentration gradient between phases? The concentration difference of a specific ion between the dilute and dense phases suggests the existence of a cross-phase electric potential gradient[37–39] (Box 1).

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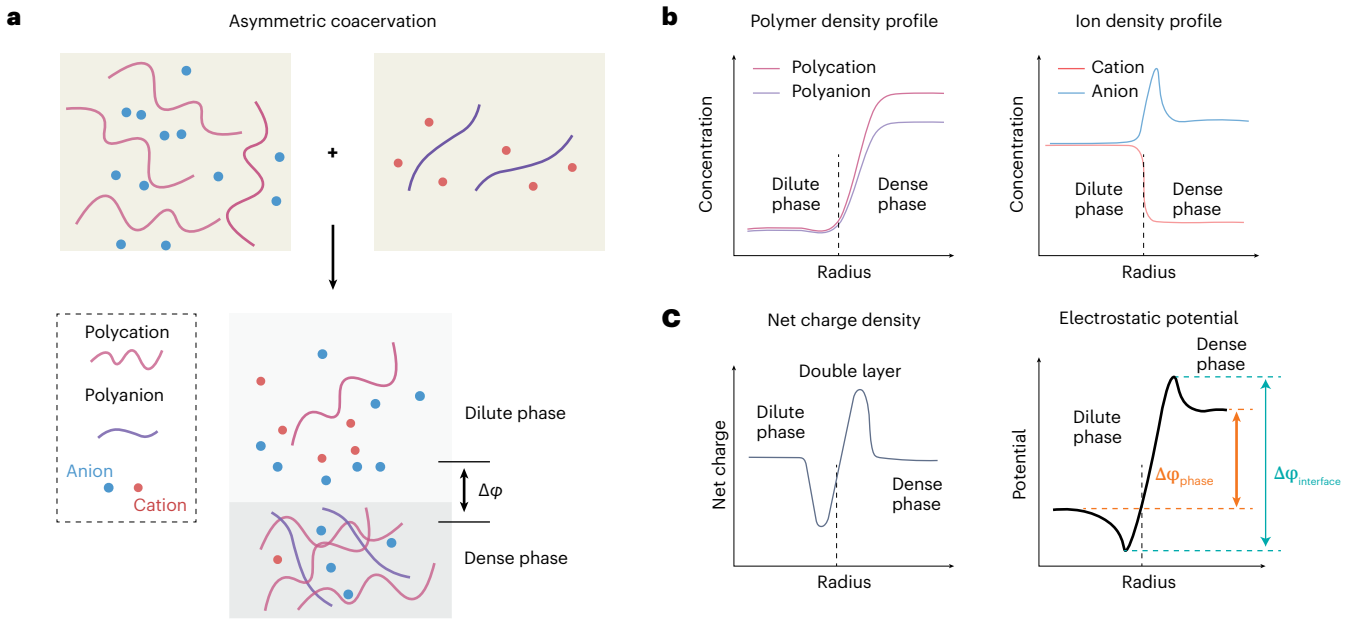

Fig. 2 | Electrochemical features of systems undergoing asymmetric complex coacervation. a, A system containing a polycation, a polyanion, a cation and an anion undergoes complex coacervation. With the change in interaction stoichiometry before and after coacervation, a definite Galvani potential (the electric potential difference) is established between the dilute and dense phases. **b**, Spatial density profile of a polycation and a polyanion and the partition function of a cation and an anion from the dilute phase to the dense phase. **c**, Spatial profile of net charge density and electrostatic potential from the dilute phase to the dense phase. This electrostatic potential profile accounts for the thermodynamic electric potential of all the components in the system, which is the Galvani potential at different locations within the solution system. At the interfacial region, where charge separation happens, an interfacial electric potential is set up as an electric double layer. The orange color indicates the interfacial electric potential, and the green color indicates the interphase electric potential.

We illustrate this effect using a simplified scheme, in which a water-based solution contains a pair of oppositely charged polyelectrolytes and a type of monovalent salt, similar to many RNA–protein condensates formed through complex coacervation (Fig. 2). The difference or asymmetry in the initial concentrat−−ion, charge fraction, mixing stoichiometry, chain length and chain flexibility of oppositely charged polyelectrolytes can result in a difference in the concentration of cations, anions, polycations and polyanions in both phases[38]. Such concentration asymmetry then leads to a Galvani potential ($\Delta\varphi$)[40], defined as the electric potential difference between two locations in different phases, which, in our case, are the dense phase and the dilute phase. This feature can be illustrated by the requirement of electrochemical potential equilibrium of all species under phase coexistence[39]: $\mu_i^{\text{dilute phase}} + ez_i\varphi^{\text{dilute phase}} = \mu_i^{\text{dense phase}} + ez_i\varphi^{\text{dense phase}}$, in which μ represents the chemical potential of the species, e represents the elementary charge, z represents the valency of the charge of the species and the difference between $\varphi^{\text{dilute phase}}$ and $\varphi^{\text{dense phase}}$ is $\Delta\varphi$. Therefore, a concentration difference of the charged species in the dilute and dense phases (represented by their differences in chemical potential) corresponds to a definite Galvani potential.

A critical aspect to create such asymmetry is that the stoichiometry between polycations and polyanions in the dense phase does not generate a system with a net charge of zero, due to charge asymmetry, limited polymer chain flexibility and conformational constraints. As demonstrated experimentally[41], when oppositely charged polyelectrolytes are mixed at unequal ratios, the interaction ratio between polycations and polyanions in the dense phase is lower than their mixing ratio at initial loading. Consider a case in which more polycations partition into the dense phase than polyanions after complex coacervation: charge neutrality in the dense phase then requires the partition of anions and the exclusion of cations by the dense phase (Fig. 2a,b). This is an example of an asymmetric coacervate[38].

As studied by an inhomogeneous mean-field theory[42], the net charge density profile shows a positively charged layer forming on the surface of the dense phase and a negatively charged layer forming on the side of the dilute phase (Fig. 2c). This separation of charge at the surface of the condensates corresponds to an electric double-layer structure at the interfacial region[43], which generates an electric field that is strongest at the interface but does extend in either direction beyond the interface[44]. Furthermore, this also leads to a clear difference between interfacial potential (the potential difference at the interfacial region) and phase potential (the potential difference between the two phases) (Fig. 2c). These two separate electrochemical features might be influenced by different solvent properties. For example, compared to the electric potential differences between phases, the interfacial potential can be more markedly affected by the salt concentration in the solution[43], due to the screening effect of salt on the surface electrostatics.

In biological systems, such asymmetry is even more inevitable because of the heterogeneity in sequence, conformation and composition of the components participating in condensate formation. For example, in the case of RNA condensates, such as FUS, WHI3 (an RNA-binding protein in *Saccharomyces cerevisiae*) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) condensates[45–48], the condensate with an asymmetric mixing ratio might possess an overall net negative charge arising from the extensive electronegativity of RNA in the condensates. The stoichiometry between positively charged components and negatively charged components or the charge ratio in a condensate has been found to govern the stability of the condensate, which correlates with the interfacial tension of RNA–protein condensates formed by polyU RNA and RGG disordered proteins[49]. Theoretically, the interfacial tension and structure in an asymmetric system can be determined by the density profiles of components across the interface[42].

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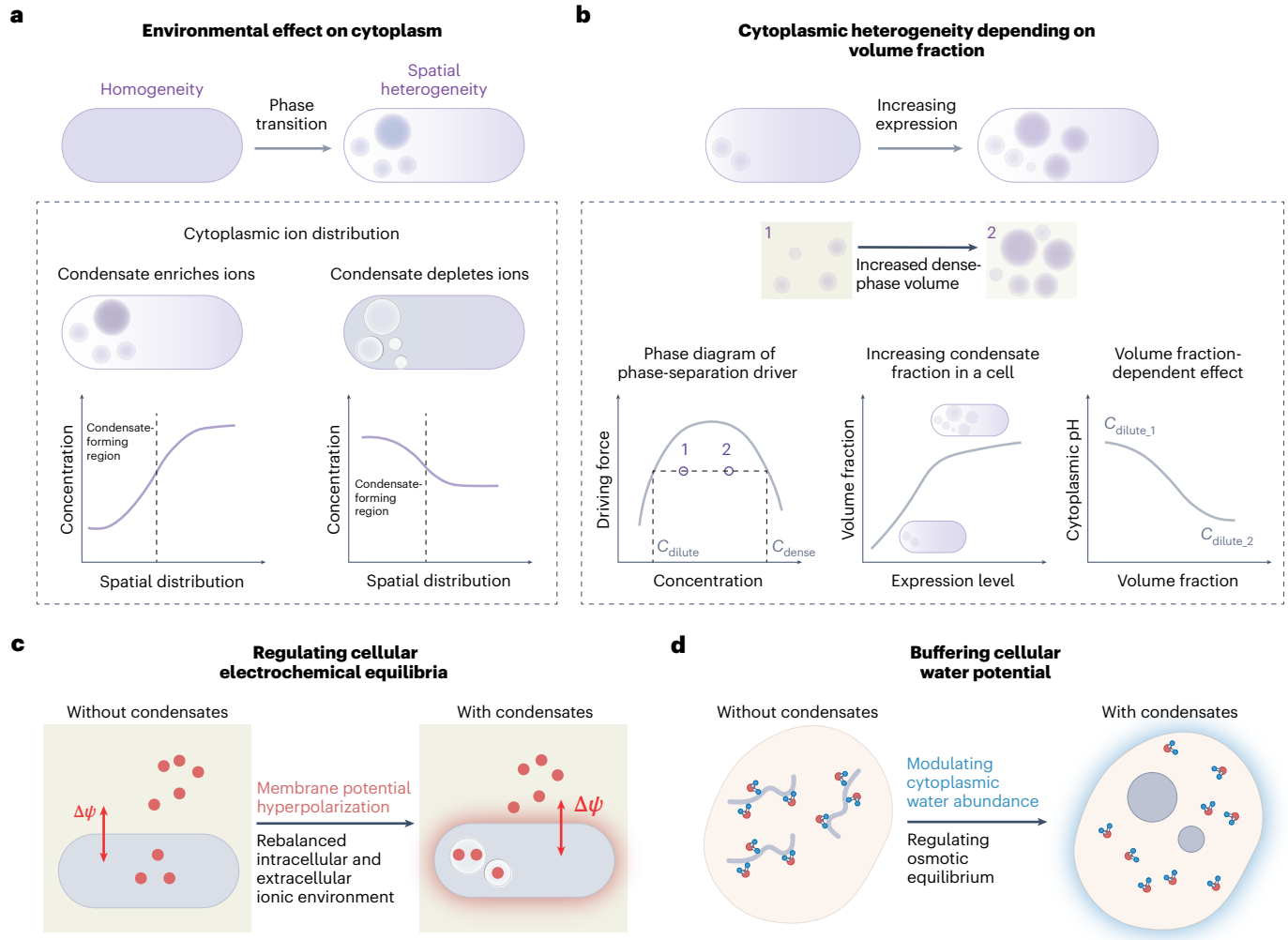

Fig. 3 | Passive environmental effect by condensates increases cellular heterogeneity. a, Condensates can enrich or deplete ions. This passive effect can affect spatial ion concentration differences in the cytoplasm. **b**, The spatial distribution of ions is affected by the volume fraction of condensates occupying a cell, enlarging cellular heterogeneity by condensate formation. **c**, Condensate formation can modulate cytoplasmic ion concentration, thus changing the electrochemical equilibrium of the cell. A phenotypic behavior is the change in membrane potential upon condensation[56]. **d**, Condensate formation releases the water molecules bound to proteins, thus changing the water abundance in the cytoplasm. This feature was observed to regulate osmotic stress in both hypo-osmotic and hyperosmotic conditions[59].

Furthermore, a definitive zeta potential of condensates was measured to be at the level of 10 mV[50]. Zeta potential is the electric potential at the interface separating the dilute phase from the solutes attached to the condensate surface[51]; so it does not directly represent the surface charge of the condensate but is an indication of the net charge of ions contained by the functional group of the condensate surface relative to the dilute phase. Therefore, the existence of a charged surface also suggests the formation of an oppositely charged layer of ions as required by charge neutrality, which establishes an electric double layer. The same conclusion can be reached from observations of cellular condensates. Many condensates, rather than undergo fusion, stay as distinct puncta[52], and the size distribution of these condensates is mostly uniform. This behavior can be explained by the surface electrostatics of condensates[50], suggesting that the capability of condensates to modulate ion distributions is an important mechanism for condensates to regulate their size distribution[53]. Variations in condensate size have been associated with condensate dysfunction, such as the nucleolus[54,55], suggesting a critical role of surface charge on condensate functions. As discussed above, the distribution of charged biomacromolecules and ions between phases collectively determines the interfacial characteristics of condensates.

Effect of condensation on the cytoplasmic environment

With the understanding of the mechanisms by which condensates possess diverse physicochemical properties, we next rationalize and propose how these features caused by phase transition can affect the cytoplasmic environment (Fig. 3a). In other words, when condensates form and grow, how does the electrochemical environment characterized by the concentration of ions and water in the dilute phase (cytoplasm) change? Upon phase separation, the condensate possesses an ion concentration dictated by the concentration of phase-separation-driving macromolecules; however, the ion concentration in the cytoplasm would be affected by the volume fraction of condensates in a cell[56] (Fig. 3a). Assuming a cell with negligible change in its volume, such as a cell in stationary phase, if the condensate can partition certain ions, then an increased volume fraction of the condensates corresponds to a lower concentration of that type of ion in the cytoplasm. This aspect suggests that, for cells with condensates, the continuous expression of condensate-forming proteins would continuously modulate the cellular environment (Fig. 3b), possibly contributing to the maintenance of ion homeostasis under stressed conditions[57].

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BOX 2

Ion concentrations modulate buffering capacity

Variations in the buffering capacity of the cytoplasm are a fundamental driving force for generating a distinct ionic environment. Cells can use the protonation of amino acids to buffer pH, such as the proton equilibrium of hemoglobin for buffering hydronium ions in blood[133]. However, such buffering capacity is insufficient to mediate pH homeostasis. Therefore, considering the ubiquity of condensation and the capability of condensates to modulate ions, the ion-dependent buffering mechanism of condensation can be another important mechanism to modulate cellular pH.

To illustrate how ions can affect pH, considering a solution containing only HCl and NaOH, the pH of the solution needs to satisfy the following conditions:

a. Equilibrium of water dissociation:

$$K_W = [H_3O^+][OH^-] \quad (11)$$

where K_W is the dissociation constant of the water molecule in the case that one water molecule can donate a proton to another water molecule, generating a pair of conjugate acid ($[H_3O^+]$) and conjugate base ($[OH^-]$). The concentration difference between $[H_3O^+]$ and $[OH^-]$ dictates the acidity of the environment.

b. Charge neutrality:

$$[Na^+] + [H_3O^+] = [Cl^-] + [OH^-] \quad (12)$$

where sodium and chloride ions are strong ions (SIs) and completely dissociated from the salt form. The concentration difference ([DSI]) between sodium and chloride ions ($[Na^+]-[Cl^-]=[DSI]$) can be used to represent the difference between hydronium ions and hydroxide ions, which is the basis of pH.

Next, the combination of equations (11) and (12) becomes:

$$[H_3O^+] - Kw/[H_3O^+] + [DSI] = 0 \quad (13)$$

Equation (13) can be solved as a quadratic equation with solutions as follows:

$$[H_3O^+] = 0.5(-[DSI] + \sqrt{[DSI]^2 + 4K_W}) \quad (14)$$

Equation (14) suggests that the concentration of H_3O^+ is not solely determined by the dissociation constant but is also determined by the availability of unpaired SIs. The above analysis shows the foundation of how the concentration of salt species can affect the pH of the environment. In the case of condensates, if associative transition of biomacromolecules can lead to a different abundance of a specific type of ion (for example, the sodium ion[35]) between the dilute and dense phases, then there must be a pH difference between the two phases.

A recent study showed that the formation of biomolecular condensates in bacteria substantially changes the ionic environment of the cytoplasm, thereby regulating the global electrochemical equilibrium of the cell[56]. Phase separation was found to induce a shift in cytoplasmic pH. This intracellular ion imbalance further induces a change in membrane potential. This capability suggests a new role of condensates in modulating intracellular electrochemistry (Fig. 3c), a role previously attributed primarily to membrane ion channels[58]. Similarly, from the perspective of water molecules, a recent study showed that the formation and dissolution of condensates liberate and capture water molecules, establishing a new water potential equilibrium with the cytoplasm[59] (Fig. 3d). Both these works imply that condensate formation can dynamically regulate cellular environments, and such effects are likely influenced by both the degree of ion–water modulation by condensates and the volume fraction of condensates within a cell. Thus, studies on condensate function need to go beyond the condensate itself and consider the overall changes in cellular environments caused by condensate formation.

How is the functioning mechanism of the passive effect distinct from that of the active modulation process executed by components of condensates? Upon condensate formation, cytoplasmic protein concentration homeostasis was demonstrated in the case of phase transition driven by a single component[60], in which the noise originating from stochastic gene expression of the phase-separation-driving protein in the cytoplasm is reduced. This phenomenon can be understood through the phase equilibrium of a single-component system[4,61], in which the phase-separation driver has a fixed dilute-phase concentration (cytoplasmic concentration) upon crossing the coexisting concentration. However, gene expression is always stochastic; then where is the noise reflected? The stochastic features will be reflected by the noise of the volume fractions within a cell population. Considering a population of cells, in which gene expression is typically noisy[62], the variability in phase-separating protein concentrations can lead to variability in volume fraction of condensates in identical cells. This variability in volume fraction is reflected by differences in cellular phenotypes created by the passive effects of condensates within the cell population. Most importantly, this feature highlights that, when condensate function correlates with its change in volume fraction, the downstream function should be more heterogeneously dependent on the gene expression level. A recent work demonstrated that the cellular noise of the passive environmental effect (that is, cytoplasmic pH) brought about by condensation is enlarged after condensate formation, with around 15–20% of cytoplasmic volume occupied by the dense phase in a bacterium[56]. This behavior is conceptually different from the ability of condensates to modulate the concentration fluctuations of phase-separating proteins, which calls attention to the heterogeneity aspect of condensate functions. Further understanding how condensate size is regulated would expand our knowledge of the dynamic regulations of cellular environments by phase transition.

Environmental effects on biological functions

The effects arising from the change in ion and water molecules are cooperative. With the change in one aspect, there must be a corresponding change in the other aspect. For example, water abundance determines the solvation of ions, while ion abundance influences the accessibility and structure of water molecules around biomolecules[63]. Their quantitative relationship and the effects on water activity (for example, auto-ionization of water) are essentially a question of solvent buffering capacity at different ion concentrations (Box 2). We next discuss the biochemical mechanisms by which distinct environmental features can potentially affect biological functions by modulating the activity and accessibility of biomolecules.

Ions contribute to various aspects of cellular functions, from cellular physiology to osmotic homeostasis[64,65]. Condensate formation can deplete certain types of monovalent ions from the dense phase due to local hydrophobicity[35]. As described in the examples in the

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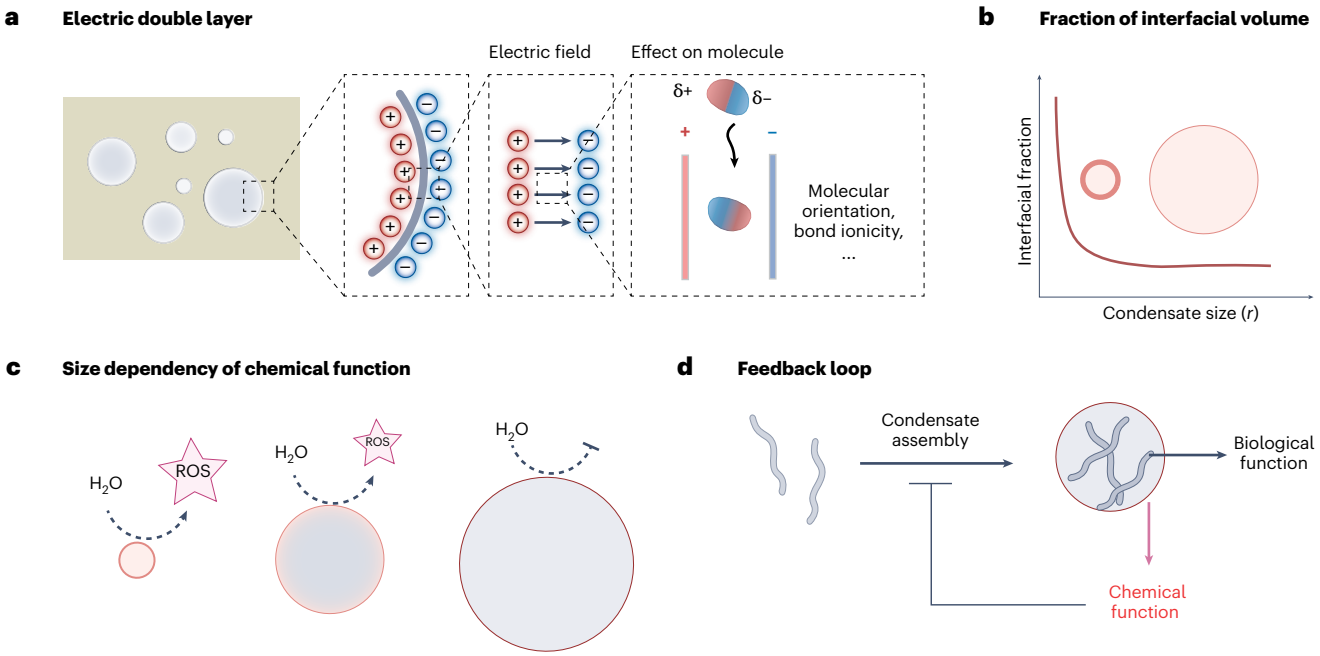

Fig. 4 | The role of the electric double layer in the electrochemical activity of condensates. a, An electric double layer forms at the surface of biomolecular condensates, establishing an interfacial electric field. The electric field can dictate diverse effects on molecules, including molecular alignment and change in bond ionicity. **b**, The ratio of interfacial volume to total volume increases with decreasing size of the condensates. **c**, Chemical activity depending on the interfacial electric field drops drastically with increasing size of condensates, as exemplified by the loss of an electron of the water dimer to generate ROS in the form of hydrated hydroxyl radicals. **d**, Sequestration and enrichment of biomolecules within condensates dictate biological functions, and the interface of condensates modulates chemical functions. These two distinct functioning pathways can possibly form a negative feedback loop to mediate the homeostasis of cellular functions.

previous section, the depletion of ions in condensates corresponds to an enrichment in the same type of ions in the cytoplasm (Fig. 3a). Increasing concentration of ions can screen effective electrostatic interactions between interaction pairs[66]. Meanwhile, multivalent ions can also mediate interbiomolecular and intrabiomolecular interactions by bridging charged residues on biomolecules, resulting in phase transition or aggregation[67,68]. From the aspect of functional dynamics, a specific ionic environment shapes individual Coulomb interactions among molecules that might modulate the dynamic structures of biomolecules[24,69], thereby regulating their biomolecular functions. Ions can also compete with biomolecules to interact with water, as shown by the ion type-dependent salting-out effects of proteins[70,71]. Similar to ions, water can alter biomolecular interactions, such as bridging protein–protein interactions[72]. For example, an ordered water structure at a protein surface plays a key role in driving aggregation and polymorphism[73].

Aside from the role of ions in modulating biomolecular interactions, similar to the role of water in hydrolysis reactions, ions can contribute directly to cellular functions. For example, magnesium ions are critical to ribosome structure[74]. The increased intracellular abundance of magnesium ions can direct ribosome assembly to promote translation[75] and correlate with cellular resistance to ribosome-targeting antibiotics[76]. Another divalent cation, the calcium ion, contributes to various cellular signaling processes and enzymatic reactions[77]. These functions are largely decided by the spatiotemporal abundance of the calcium ion[78]. For example, callose synthesis, a polysaccharide critical to cell wall synthesis and cell survival, is directed by the gradient of calcium ion concentration[79]. These facts imply that, if condensation can generate an intracellular ion gradient, such a gradient can possibly orchestrate cellular functions, with a directional preference dictated by the spatial arrangement of condensates (Fig. 3b). Other than a specific molecular interaction that can be determined by a specific ion, considering that such an environmental effect actually acts on the entire cellular environment[76], a recent study found that the change in cytoplasmic environments by condensates results in a globally differentially expressed transcriptome[56]. Many of the differentially expressed gene clusters are governed by transcriptional factors that are sensitive to ion abundance.

Another key feature driving biological functions that can be affected by ion and water concentration is the osmotic pressure of cells[80]. Osmotic pressure represents the minimum amount of pressure applied to the cellular membrane to balance the influx and the efflux of molecules[81]. For example, the equilibrium between intracellular and extracellular ion concentrations dictates the difference in the chemical potential of water across the cellular membrane, which determines the thermodynamic tendency of water to diffuse across the membrane[82]. Therefore, changes in cytoplasmic ion concentration from condensation would affect the equilibrium of water potential, possibly triggering a desiccation response of cells[65]. A recent work demonstrated that the ability of condensate formation to liberate water molecules into the cytoplasm can counteract extracellular osmotic perturbations[59]. The above discussion strongly suggests that the environmental effect due to macromolecular condensation can lead to a global modulation of biological functions.

Electrochemical functions of interfacial electric fields

In addition to the direct influence of cellular processes by solvent environment-dependent effects, in a previous section, we introduced the consequence of an electric potential gradient between the dilute and dense phases and the formation of an electric double layer at the interface. We next discuss a potential functioning mechanism of the interfacial electric field and introduce some early evidence on the electrochemical functions.

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Electric field is a vector field established by oppositely charged particles, and the field is defined as the region between the charged structures[82] (Fig. 4a). Consider a point charge positioned inside the field. This positive charge would migrate in the direction of the field vector. In the case of a molecule inside the field, the same effect can modify the structure of the molecule by influencing the electron density between bonds and causing polarization and alignment of the molecule[83], thereby affecting the reactivity of the molecule[84]. Such an electric field-mediated polarization effect has been implemented to catalyze chemical reactions and affect endoselectivity[85]. In the biological realm, the same kind of electrostatic effect generated by the amino acid residues within an enzyme is the driving force for many enzymatic reactions by shifting the binding energy between the enzyme and the substrate from the reactant state to the transition state[86]. Biomolecular condensates have been demonstrated to accelerate chemical reactions, and the rate enhancement could not be solely explained by the increase in local concentrations of the reactants[87]. As rationalized above, we suspect that this phenomenon might be further understood by decoding the interfacial electric field of condensates.

The interfacial electrostatic effect has been studied in the case of a micrometer-sized water droplet, in which an electric field is established at the interface of the water microdroplet[88–90]. This interfacial electric field has been found to drive spontaneous redox reactions and enhance enzymatic reactions[89,91,92]. One of the major driving forces for diverse chemical reactivity is the production of reactive oxygen species (ROS) through the interfacial electric field[90,93]. The interfacial electric field can transform the water dimer cation $(H_4O_2)^+$ in the form of hydrated hydroxyl radicals $\bullet OH{-}H_3O^+$ (a hydroxyl radical combined with a hydronium cation through a hydrogen bond) into $\bullet OH{-}H_2O$ by transferring a hydrogen bond[94,95]. Subsequently, $\bullet OH{-}H_3O^+$ and $\bullet OH{-}H_2O$, and $\bullet OH{-}H_2O$ and $\bullet OH{-}H_2O$ can further combine spontaneously to produce H_2O_2. It is also possible that •OH arises from the loss of an electron from the hydroxide anion OH^- based on the same electric field-dependent effect[89,90].

Similar to water microdroplets, the interface of biomolecular condensates has also been found to possess an electric field[37,96] and is able to modulate spontaneous redox reactions[37]. The underlying mechanism for the formation of such an electric field has been discussed in previous sections, highlighting the functional consequences of an electric potential difference between the dilute and dense phases as demonstrated by an ion concentration gradient[37–39,42,43]. This ion gradient between the dilute and dense phases correlates directly with the strength of the interfacial electric field and the ability of condensates to drive redox reactions[37] (Box 3). This finding suggests that the the electrochemical properties of condensates can encode non-enzymatic reaction pathways for cellular biochemistry.

From the perspective of the assembly mechanism, condensate formation is similar to amyloid formation, in which the protein-containing solution lowers its free energy by transitioning from a solvated monomer structure into a higher-order state[97], resulting in a density difference within the system. Studies have shown that ionic strength and ion type can affect amyloid aggregation[98], and the hydration property of amyloid aggregates is different from that of the amyloid monomer[32]. This evidence aligns with our previously discussed physiochemical features of macromolecular condensation and suggests that amyloid formation could possibly form a surface electrostatic layer, thereby generating an interfacial electric field. In a work studying the toxicity of amyloid fibers[99], in a simple in vitro setting with amyloid monomer in phosphate buffer, the generation of hydrogen peroxide was indeed observed at the early stage of amyloid assembly, in which the $A\beta_{1-40}$ peptide formed distinct small (~100-nm) protofibrils. However, at a later stage, when the small protofibrils expanded to be a large fibril structure (~100 µm), the ability to generate hydrogen peroxide decreased substantially. This study showed that, even though the biomolecular constituents of amyloid at different stages are the same, the change in fibril size affects the ability of amyloid to drive redox reactions. These observations imply that the interface of amyloid assembly can be the key determinant for the ability to promote redox reactions, because the

BOX 3

Experimental evaluation of the electrochemical redox activities of condensates

Spontaneous redox reactions are a generic feature found at a wide range of interfaces: the liquid–air interface between air and water microdroplets, the liquid–solid interface between water and glass and the liquid–liquid interface of water-in-oil emulsions[88,89,94,134–136]. The common attribute of these interfaces is an established electric double layer, which functions as an electric field[90]. A key consequence of electric field effects at the liquid–liquid interface of condensates is the generation of ROS[37,101], which are highly relevant to cell physiology[137]. Studies have shown the relevance of this phenomenon in in vitro reconstituted stress granules[101,104]. Here, we propose some established and highly sensitive methods to test the redox ability in the case of condensates. For comprehensive techniques on the analysis of ROS, we suggest the following review articles on the topic of ROS detection[138–140]. For in vitro systems, in which there is no existing source of ROS, the use of a commercially available ROS-specific fluorogenic probe can be convenient. This type of probe depends on the hydrogen peroxide-specific boronate oxidation reaction based on the enhanced nucleophilicity caused by the alpha effect, through which the fluorescence property is activated[137,139–141]. This allows qualitative analysis of the ability of condensates to generate ROS. However, to attain relative quantification of ROS, the partitioning of fluorescent probes into the condensates needs to be accounted for. As such, the use of a ratiometric fluorescent probe is ideal because quantification of the activity of the probe can be realized by quantifying the ratio of the fluorescence emission at two distinct wavelengths (one represents the amount of the inactive probe; the other represents the amount of the reacted probe) of the same probe[37,142], thereby eliminating the concentration-dependent fluorescence of the probe. A more advanced technique depends on the use of a particle collision-dependent nano-electrode[101,143–146], which can detect ROS based on the electrochemical redox potential of the target (for example, the standard redox potential for the reduction half-reaction of hydrogen peroxide at 25 °C is 1.763 V (versus standard hydrogen electrode, SHE). The detectable signal only comes from the collision event between the stress granules and the electrode. This feature enables real-time monitoring of the generation of ROS[101]. Compared to the fluorescence-based approach, which is nonspecific to the rich ROS environment in living cells, electrochemical collision-dependent techniques provide a highly selective and useful strategy for the intracellular study of the electrochemical activity of condensates in a real-time manner. From the perspective of biochemistry, ROS serves as a signaling agent for biomolecular interactions through oxidation-dependent mechanisms[147]. For example, the oxidation of protein thiol groups by hydrogen peroxide leads to the formation of disulfide bonds, which can mediate the formation of protein dimers and alter protein structures[104,148]. This feature can also be implemented to develop simple biochemical assays to understand the biochemical consequences of the redox activity of condensates.

ratio of interfacial volume to total volume (ratio = $1-((r-a)/r)^3$, where r is the radius of the particle and a is the width of the interfacial region) increases with decreasing particle size. For example, comparing a condensate with a radius of 1,000 nm and a cluster with a size of 100 nm, both with an interfacial depth of 10 nm, the ratio of interfacial volume to total volume would grow from 3% to 27% (Fig. 4b,c)[100]. This suggests that small clusters in subsaturated solution can possibly define a more pronounced electrochemical function by their interfacial fields.

Furthermore, a study using an in vitro electrochemical method uncovered the existence of ROS in isolated stress granules[101], which are multicomponent biomolecular condensates formed in response to diverse cellular stresses[102,103]. A very recent study also showed that in vitro constituted G3BP stress granule assembly factor 1 (G3BP1)–RNA condensates mediate the formation of disulfide bonds between TAR DNA-binding protein 43 (TDP-43) monomers in a redox-dependent manner[104]. By contrast, a study found that the oxidation of TIA1, a component of stress granules, by hydrogen peroxide can prevent stress granule assembly under stress[105]. This opposite signaling effect raises the possibility that condensates might use interfacial chemical activities to modulate cellular signaling as a feedback loop to attain homeostasis of cellular functions through control over the quantity and size of condensates (Fig. 4d). Another study also showed direct evidence on the spontaneous promotion of redox reactions by condensates, in which condensates can mediate the oxidative ligation of amino thioacids[87]. These studies imply that decoding the electrochemical functions of such macromolecular structures can be the basis to expand our understanding of the underlying connections between the assembly of high-order cellular structures and the dynamic functions of the correlated cellular processes. These interface-dependent condensate functions also shed light on the emergent molecular grammars of condensate surface and interfacial interactions[13,106,107] (Supplementary Note 2).

Prospects and conclusions

A physical chemistry perspective of condensates

The organization of molecules is essential to the emergence of biochemical functions from prebiotic systems to living cells[1,9,108]. The best-known type of organization is membrane-bound structures, such as a mitochondria, which contains a distinct set of molecules that are different from those in the surrounding environment. Such divergence in molecular constituents mediates essential electrochemical features emerging between the environment and the mitochondria (Fig. 5a), such as membrane potential and various ion motive forces[109,110]. These features are controlled by diverse modulation systems, such as ion-selective pumps and membrane surface modifications[111]. If we approach our understanding of such structures from what we have discussed in this Perspective, it is not the physical boundary or the phenotypic biochemical features that define the environments, but distinct molecular constituents that define the environments, which in turn establish these electrochemical functions. This implies that chemical environments can be understood through the electrochemical equilibrium between the two separated environments or phases. For example, there is always a resting cross-membrane electric potential that is determined by differences between the electrostatic potential of all the constituents inside and outside the membrane-bound structure. Similarly, condensates or other macromolecular assemblies without a physically defined membrane possess a high concentration of biomolecules, thus creating distinct compositions of water molecules and ions compared to the bulk environment under the same occupied volume. Thus, understanding the organization of molecules in a confined volume essentially amounts to understanding the buffering capacity of a solution containing various charged species. We anticipate the application of laws and methods from electrochemistry and physical chemistry to the field of condensate biology to define and examine these fundamental physicochemical features. Furthermore, the combination of computational methods[24,34,35,112] that can account for the physical and chemical properties of proteins and solvent with theories[38,39,42,113] describing the electrochemical equilibrium in a two-phase aqueous system can be informative to establish the relationship between sequences and the electrochemical features of condensates.

Mechanisms of condensates in regulating ion gradients

An established interfacial electric potential raises the question of whether the condensate interface can act in a manner similar to that of the cellular membrane to regulate the dynamics of ion gradients[37–39,114]. A key difference between the condensate surface and the membrane surface is that the membrane contains a responsive set of ion regulators (such as ion pumps and channels[114]), which can actively regulate translocation and exchange of ions. By comparison, current knowledge has yet to reveal any active mechanism by which condensates can regulate ion flux. We propose that such regulation might be linked with the aging of condensates, which is shown by the change in material properties of the condensates over time, such as the diffusion coefficient and the exchange rate of phase-separation drivers between the dilute and dense phases. The phenotypic change in material properties of condensates upon aging is caused by an enhanced molecular interaction within the percolated network[115]. Therefore, it is possible that the dense-phase solvent environment will be altered by these newly established inter-residue interactions (Fig. 5b). For example, long-range electrostatic interaction can initiate phase transition, while short-range π-based interactions can then contribute to the stability of the condensate network during the aging process of condensates[115]. In this case, the effect on solvent environments, dictated first by the phase transition and second by aging of the condensates, will occur sequentially and operate at different time points and timescales. The same driving force can also lead to a phenotypic change in the material properties of condensates[115,116]. This process might result in the generation of an active ion translocation to dictate physicochemical functions. Establishing the detailed molecular driving forces of this aspect might require correlating the dynamics of proteins and ions at the molecular scale across a wide range of timescales. This effort can possibly yield understanding of the dependence of the kinetics of interconversion among protein ensembles on internal ion dynamics[24,117,118].

Roles of the interfacial electrical potential gradient

The ability of condensates to define an electrochemical potential gradient of ions points to a role of condensates in energy transduction, akin to the role of the cellular membrane in modulating ion motive forces[119]. The electrochemical potential of each charged species is a form of energy that can move the charged species from one state to another[120]. Thus, when the system is out of equilibrium, with a charged molecule diffusing from one place to the other, which can lead to a decrease in electrochemical potential, there must be a corresponding release of free energy. This free energy can be harnessed for work. This principle is demonstrated as the ion motive force in cellular systems[121]. For example, in bacteria, an ion motive force-directed flux of ions can generate energy to power the flagellar motor, which is critical for bacterial motility[122]. The rotation speed of the flagellar motor is sensitive to the change in ion gradient between the extracellular and intracellular environments[123], which contributes directly to the ion motive force. Thus, aside from the fact that the non-equilibrium state of condensates might drive ion flux as discussed previously, we propose that the formation and dissolution processes of condensates can be an active mechanism to generate intracellular ion flux, which might be used to power intracellular chemical reactions and regulate reaction directionality. Considering that condensates can interface with other condensates and cellular structures[124,125], establishing correlations between the capillary forces generated by condensates and such interfacial electrochemical gradients can possibly uncover new physics of biology in the framework of continuum thermo-electrodynamics (for example,

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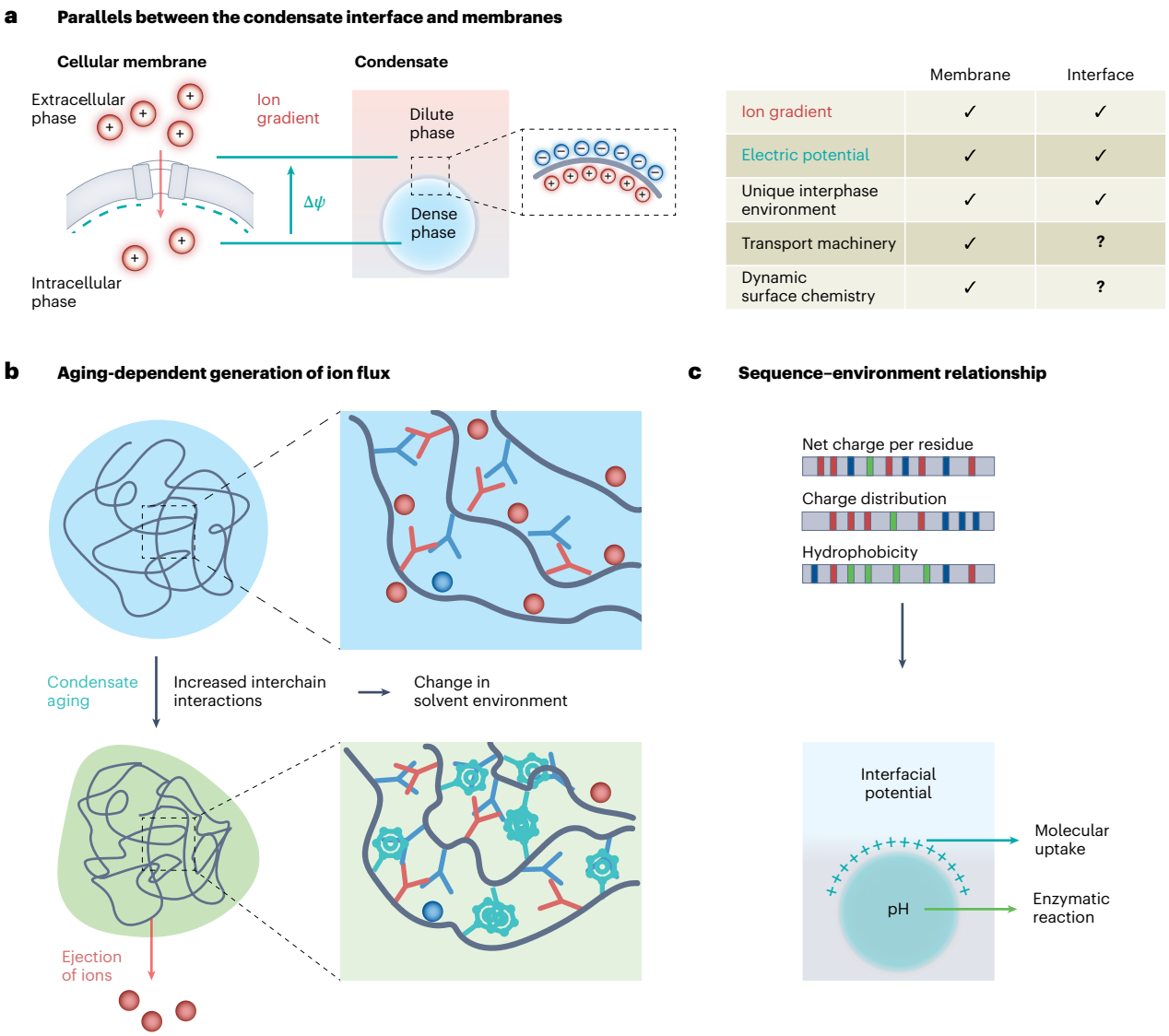

Fig. 5 | Proposed regulation strategies, electrochemical functional features and engineering opportunities of biomolecular condensates. a, The surface and interface of biomolecular condensates share similar physicochemical features with cellular membranes. Many of the features known for a membrane are unexplored in biomolecular condensates. **b**, Aging of biomolecular condensates is a result of enhanced dense-phase interactions. The change of solvent–chain interactions in a new condensate to chain–chain interactions in an older condensate can possibly change the solvent environments, which might lead to the ejection of ions. This can be a possible mechanism of condensates in generating ion flux. **c**, Establishing sequence–environment relationships to engineer condensates to encode unique electrochemical and chemical features for chemical engineering, such as metabolic engineering.

the Lippmann equation[126]), which will in turn establish new principles for interorganelle communication (for example, wetting-dependent autophagy[125]). Furthermore, understanding how the soft nature of condensate interface can modulate the ion gradient across the liquid–liquid interface can further allow us to expand the structure-property relationship of condensate interface.

A new engineering opportunity

Engineering biomolecular condensates provides a new design opportunity for synthetic biology[14]. Current research focuses primarily on articulating condensate functions by rational engineering of the biomolecules participating in or driving condensate formation[127–129]. Based on the biochemical basis we have discussed, new design parameters and functioning mechanisms can be introduced into condensate-mediated cellular engineering. One exciting area would be programming sequence information to encode the chemical environments of condensates (Fig. 5c), such as pH. One study showed that, based on the charge condition of IDP sequences[37], highly charged sequences can drive a pH gradient between the dilute and dense phases, while noncharged hydrophobic sequences cannot establish a notable pH gradient between phases. This observation suggests that the chemical environments of condensates can be encoded into the IDP sequences. This concept can be applied toward condensate-mediated metabolic engineering[130], in which ideal chemical environments of condensates can be customized for the enzymatic pathways of interest. Furthermore, the same mechanism dictating a unique chemical environment of condensates can also possibly affect the cytoplasmic environment through the established ion gradients. Given that the impact of condensates on cellular environments depends on the formation of condensates[37,59], which can encode certain levels of thermal hysteresis[131], condensate formation can establish a cellular memory that directly modifies cellular states, which provides a new tunable knob for multicellular engineering in synthetic biology.

From the perspective of the electrochemical properties of condensates, the interfacial electric potential might dictate charge-dependent molecular diffusion. Similar to membrane potential-dependent

molecular uptake[56], in which hyperpolarization of membranes (more negatively charged) can lead to enhanced uptake of positively charged small molecules, charged small molecules will experience the electric potential profile of condensates with barriers and traps that affect their diffusion kinetics. This feature can be engineered to program selective molecular uptake by condensates, which can be a powerful fundamental capability for condensate-mediated biochemical reactors.

Summary

Since the nascent stage of studying two-phase equilibrium systems of biologics in the 1950s, phase separation has long been considered a question of molecular immiscibility due to the solvent environment[132]. Inspired by this line of thought, in this Perspective, we have highlighted underexplored aspects in condensate research and the physical chemistry and the potential physicochemical functions of biomolecular condensates. We hope that the presented early evidence and the proposed conceptual ideas can stimulate further thoughts on how condensates might function from the perspective of physical chemistry. We hope that this work will help open new directions for condensate research, with the goal of delivering both improved understanding of cell biology and design principles for engineering biology and for treatments of condensate-related diseases.

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Competing interests

The authors declare no competing interests.

Additional information

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