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PERSPECTIVE

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Generic nature of the condensed states of proteins

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Proteins undergoing liquid–liquid phase separation are being discovered at an increasing rate. Since at the high concentrations present in the cell most proteins would be expected to form a liquid condensed state, this state should be considered to be a fundamental state of proteins along with the native state and the amyloid state. Here we discuss the generic nature of the liquid-like and solid-like condensed states, and describe a wide variety of biological functions conferred by these condensed states.

Although proteins perform a wide variety of functions in their native states (Box 1 and Table 1), they can also populate other states (Fig. 1). Most proteins are capable of forming a condensed phase with solid-like character (Box 1), known as the amyloid state[1], since they are near their supersaturation limits at their typical cellular concentrations[2]. Rapidly accumulating evidence indicates that a wide range of proteins, perhaps even most of them[3], can be found in a condensed phase with a liquid-like character, referred to as the droplet state (Box 1), which is formed through a liquid–liquid phase-separation process[4–6]. These observations prompt questions about the nature and biological roles of the condensed states of proteins.

In this Perspective, we describe the fundamental nature of the droplet and amyloid states of proteins, discuss how these states are formed, and examine the wide range of cellular functions associated with these condensed states. In essence, it is becoming increasingly clear that proteins can be active in the multiple states that they populate under cellular conditions, thus generalizing the well-established structure–function paradigm towards a wider state–function paradigm, and greatly expanding the repertoire of cellular processes enabled by these molecules (Table 1).

Concentration and order in the native, amyloid and droplet states

Whether proteins populate the native or condensed states depends on their concentrations (Fig. 1). At low concentrations, proteins tend to remain in their native states, while at high concentrations condensed states are thermodynamically more favourable[1,5–7] (Box 1). Proteins in their native states are largely free to diffuse in the cellular environment, less so in the droplet state, and almost not at all in the amyloid state. Thus, in terms of inter-molecular ordering, which determines the collective behaviour of protein molecules, the concentration determines the balance between order and disorder (Fig. 1). Because of their polymeric nature, proteins can also be ordered at the intra-molecular level. Although we are used to thinking about proteins as fully folded in their native states, it is becoming increasingly evident that these molecules can display a continuum of intra-molecular order, ranging from fully structured conformations (high intra-molecular order) to highly heterogeneous conformations (low intra-molecular order)[8] (Figs. 1 and 2). Proteins in native states may also contain disordered regions, which can drive the formation of the droplet state under appropriate conditions[9], although phase separation can also be achieved by multivalent interactions between structured domains[10]. In terms of intra-molecular ordering, which determines the individual behaviour of protein molecules, the native and amyloid states are more ordered[11], and the droplet state tends to be more disordered[12] (Figs. 1 and 2). Thus, the different characteristics of the three states arise because proteins have both an internal organisation as individual molecules and a collective organisation as molecular assemblies (Fig. 1).

State-dependent cellular activities of proteins

Under physiological conditions most proteins can sample the native, droplet and amyloid states[2,3,7]. Emerging evidence indicates that these states contribute to different cellular functions in accordance with their physical and structural properties (Table 1 and Box 1).

Biological functions associated with the native state. Proteins in native states often exhibit high intra-molecular and low inter-molecular order. The folding of proteins creates ordered regions where a wide range of specific recognition events and chemical processes can take place (Table 1 and Fig. 2). The folding of proteins into well-defined structures precisely positions residues to assist chemical reactions[13,14] and provides electrostatically pre-organized environments to enable catalysis[15]. Structured protein regions contribute to specific recognition processes[16–22], where a selected set of residues can form specific patterns[19], signatures[20], pores[21] or scaffolds[22]. In addition, conformational heterogeneity in the native state enables functional adaptability[23–27]. Disordered regions in native states may fold upon binding or remain disordered in the bound form[28,29]. These regions facilitate a remodelling of native conformational ensembles between functionally distinct forms via allostery[30] and increase sensitivity towards post-translational modifications[31]. Extended disordered regions may preferentially adsorb at interfaces and modulate their surface properties[32,33]. Disordered regions may also mediate transient, weak interactions in stoichiometric complexes[28,34,35]. These interactions, which can guide formation of specific assemblies, may also be induced to drive the formation of the condensed states at high protein concentrations.

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Box 1 | Fundamental states of proteins

The different states of proteins have traditionally been defined on the basis of their structures and functions. However, it is becoming increasingly clear that these criteria may not be sufficient to capture the complex properties of these molecules, and that definitions based on thermodynamics and kinetics could be more appropriate (Fig. 1).

The native state of a protein is typically the most frequently observed state under cellular conditions. Thermodynamically, the native state may not have the lowest free energy, but may still be the most populated one because of its favourable kinetic accessibility, while being separated by high free-energy barriers from possibly more stable condensed states (Fig. 1)[1,2,7]. Structurally, the native state is stabilized predominantly by intra-molecular interactions, although inter-molecular interactions are also important for the formation of functional stoichiometric complexes. To enable these interactions and functions, native states exhibit a continuum of ordering between ordered and disordered regions (Fig. 2)[8].

The droplet state is a liquid-like condensed state formed by the non-stoichiometric assembly of protein molecules. This state can have a thermodynamic stability comparable to that of the native state, as it can be reversibly generated from the native state through liquid–liquid phase separation, and it may evolve into a solid-like condensed state through a maturation process (Fig. 1)[3–6]. The droplet state has high conformational entropy, and it is stabilized by a variety of weak intra-molecular and inter-molecular interactions, from both disordered and ordered protein regions (Fig. 2). The biological activities of protein droplets originate from their disordered binding modes and their ability to form dense assemblies that concentrate cellular components.

The amyloid state is a solid-like condensed state that is typically thermodynamically stable at the cellular concentrations of proteins, and therefore its formation tends to be irreversible (Fig. 1)[1,2,7]. The amyloid state is stabilized predominantly by inter-molecular interactions, including in particular backbone hydrogen bonding to form characteristic cross-β structures (Fig. 2). Because the amyloid state is stable, it is difficult for the protein homeostasis system to regulate its presence. For this reason, although this state can be functional, its appearance is often associated with disease[1].

Biological functions associated with the condensed states. While cellular activities associated with native states rely on the individual properties of the polypeptide chains, functions associated with the droplet and amyloid states emerge from collective behaviours of these molecules (Figs. 1 and 2). In the condensed states, the generic inter-molecular interactions enable a vast repertoire of functionalities via a wide range of collective interactions (Table 1 and Fig. 2).

A common feature of the droplet and amyloid states that is relevant for their functions is that they create macroscopic compartments capable of increasing the local concentration of proteins (Table 1). This feature enables protein functions in various cellular processes observed at different scales[36], as illustrated here by the following four examples. First, local increases in concentration of enzymes and substrates can accelerate enzymatic reactions, as observed in the droplets formed by the CO_2-fixing enzyme Rubisco in algae[37] and in the Pmel17 amyloids in mammalian melanosomes[38]. Second, local increases in concentration can cluster binding sites to improve their avidity for low-affinity effectors or ligands, as observed during T cell receptor clustering[39]. Likewise, the dynamic polymerization of proteins in the Dishevelled family generates signalosomes with highly concentrated binding sites for transient Wnt signalling partners[40]. Third, the nucleation of microtubule and actin filaments can be enhanced by droplet formation that concentrates tubulin and actin assembly factors, respectively[41–43]. Fourth, high concentrations can lead to signal amplification, as observed in the case of cGAS droplets[44] and RIP1–RIP3 amyloids[45].

Condensed states can orchestrate components of biochemical pathways to increase their encounter rates and facilitate coordinated events (Table 1). For instance, during DNA repair, 53BP1 forms liquid droplets to recruit repair factors and p53, thus coordinating local repair factory assembly and p53-mediated gene activation[46]. Phase-separated condensates formed by dynamic networks of transactivators, such as OCT4 and GCN4, and coactivators have been shown to enhance gene activation[47]. Orchestrating chromatin components by phase-separated HP1α droplets probably has a role in heterochromatin-mediated gene silencing[48]. p62 can drive liquid–liquid phase separation of ubiquitinated proteins, leading to autophagy by recruitment of further autophagy components, such as Atg family proteins[49,50] (Table 1).

The biological functions of condensed states can also be associated with their material properties (Table 1). In *Streptomyces coelicolor*, amphipathic films supported by chaplin amyloid fibres lower the surface tension of the air–water interface to facilitate the growth of the bacterial hyphae, which then separate into spores[51]. Similarly, in *Neurospora crassa*, hydrophobins enable the formation of aerial structures by assembling into amyloid-like amphipathic fibres[52]. Liquid–liquid phase separation of filaggrin in epidermal cells leads to the formation of keratohyalin granules, which have a role in skin protection[53]. In silk moth larvae, peptide fibres provide mechanical and chemical stability to the eggshell chorion to protect oocytes and embryos[54], and in *Xenopus laevis* amyloid-like Balbiani bodies formed by Xvelo, a disordered protein with an amino-terminal prion-like domain, protect mitochondria and other organelles in oocytes[55]. Peptide hormones are stored in amyloid-like form in secretory granules[56]. Extracellular curli, amyloid fibres formed by CsgA in an environment-regulated manner, enable surface adhesion of *Escherichia coli*[57], and FapC amyloids have a role in biofilm scaffolding in *Pseudomonas* bacteria[58] (Table 1).

The decreased intra-molecular order in the droplet state compared with the native and amyloid states (Fig. 2) enables rapid responses to the cellular contexts via dynamic and heterogeneous contacts[59]. An example is the signal-driven assembly and disassembly of DYRK3[60] that control stress granule dissolution and release of mTORC1 (Table 1). A combination of weak but ultra-fast interactions among multiple disordered motifs of nucleoporins (for example, Nup153) enables a rapid interaction with nuclear transport receptors during selective transport[61]. Upon heat stress, misfolded proteins enter the nucleolus, where they transiently interact with NPM1, which shields them to prevent their irreversible aggregation[62] (Table 1). NPM1 harbours extensive unstructured regions to form liquid droplets, which are enriched in the outer shell of the co-existing liquid phases in the nucleolus[63]. In contrast to the heterogeneous, context-dependent interactions in droplet-like assemblies, the high intra-molecular order in the amyloid state leads to a high stability that is hardly amenable to reversible control.

The amyloid state is usually viewed as non-functional for many proteins[1] and sometimes pathological since it can lead to protein inactivation, as for example in the cases of Sup35[64], Ure2p[65] and p53[66] (Table 1). However, increasing evidence of functional amyloid assemblies has come to light over the past two decades. As mentioned above, the amyloid state can concentrate proteins to offer a platform for chemical reactions (Pmel17[38]), or assemblies of the death-fold domain signalosomes in the innate immune system[67,68], as well as to facilitate inheritance through yeast prions[69]. During antiviral responses, the mitochondrial antiviral protein MAVS forms amyloid assemblies, offering an efficient way to relieve the

Table 1 | Molecular mechanisms enabling proteins in the native, droplet and amyloid states to perform cellular functions

Cellular processes	State-dependent functions		
	Native state	**Droplet state**	**Amyloid state**
Enzymatic reactions	Positioning of catalytic residues (lysozyme[13,108]). Electrostatic pre-organization to favour transition-state formation (acetylcholinesterase[15]).	Increasing local concentrations to accelerate reactions (Rubisco[37]). Kinetic proofreading (SOS[109]).	Catalysis of melanin synthesis (Pmel17[38]).
Polymerization	Catalysis of polymerization reactions (RNA polymerase[23]). Microtubule formation (tubulin[110]).	Increasing local concentrations for nucleation of polymerization (SPD-5 in centrosomes[41] and N-WASP, Nck and Nephrin in actin polymerization[43]).	Formation of aerial hyphae (chaplins[51]).
Innate immunity	Proteolytic activity (caspase-1[14]).	Production of the secondary messenger cGAMP (cGAS[44]).	Activation of the IRF3 and NF-κB pathways (MAVS[70,71]).
Signalling	Specific binding of hormones (β-adrenergic receptor[25]). Catalysis of post-translational modifications (MKK4[34]). Allosteric regulation (Abl kinase[31]).	Increasing local concentrations of cluster binding sites for low-affinity ligands or effectors (Dishevelled[40] and T cell receptor clusters[39]). Phosphorylation-controlled stress-granule assembly and disassembly (DYRK3[60]).	Increasing local concentrations for signal amplification (RIP1–RIP3[45]).
Transcription	Specific recognition of DNA (phage 434 repressor[16]). Catalysis of post-translational modifications for epigenetic marking (MHhal[26]). Formation of dynamic, weak interactions (p53[35]).	Enhancement of cooperativity via dynamic, low-specificity interactions (OCT4, GCN4 and Mediator[47]). Organisation of chromatin structure (HP1α[48] and BRD4[87]).	Regulation of transcriptional repression (Ure2p[65]).
Translation	Catalysis of peptide bond formation (ribosome[24]). Specific recognition of splicing factors (SF1[29]).	Regulation of RNA assemblies (DEAD-box ATPases[91]).	Regulation of translation termination (Sup35[64]).
DNA repair	Specific recognition of damage sites (uracil–DNA glycosylase[17]). Specific recognition of dsDNA and catalysis of glycosidic or phosphodiester bond cleavage (endonuclease IV[18]).	Orchestration of repair processes (53BP1[46]).	Not reported
Autophagy	Catalysis of post-translational modifications (ubiquitin ligase[111]). Forming signatures for degradation (ubiquitin[20]).	Ubiquitination-regulated assembly and disassembly of autophagic cargo proteins (p62[49]). Organisation of autophagosome (Atg proteins[50]).	Not reported
Synaptic processes	Neurotransmitter binding and ion channel gating (AMPA receptors[73]).	Clustering of synaptic vesicles (synapsin[74]). Post-synaptic density organisation (PSD-95, Homer3 and Shank3[75]).	Formation of distinct activity states for molecular memory (CPEB[76]).
Structural scaffolding	Structural scaffolding of extracellular matrix (laminin[22]).	Centriole biogenesis (Plk4[112])	Protection of oocytes and embryos (silk moth chorion proteins[54] and Xvelo in Balbiani bodies[55]). Biofilm scaffolding (FapC[58]).
Defence or invasion	Pore-forming assembly (α-haemolysin[21]).	Formation of keratohyalin granules (filaggrin[53]).	Protective biofilm stabilization (CsgA in curli[57]).
Modulation of surface properties	Modulation of lipid membrane curvature (α-synuclein[32]). Preferential adsorption at air–water interfaces (late embryogenesis abundant proteins[33]).	Nucleolus organisation (NPM1 and FIB1[63]).	Amphiphatic film formation at water–air interfaces (hydrophobins[52]).
Selective transport	Membrane channels (KcsA ion channel[19]).	Nuclear pore complex (Nup153[61]).	Facilitation of secretion across membranes (CsgA in curli[57]).
Protein homeostasis	ATP-dependent conformational changes (GroEL[30]). Dynamic, multivalent binding with unfolded clients (Hsp40[27]).	Granular component of the nucleolus (NPM1[62]).	Storage and protection of endocrine hormones (endocrine hormones in secretory granules[56]).

Representative cellular processes to compare the functions of the native, droplet and amyloid states are shown with examples in parentheses. The interconversions of proteins between their different states affect their functions based on these mechanisms.

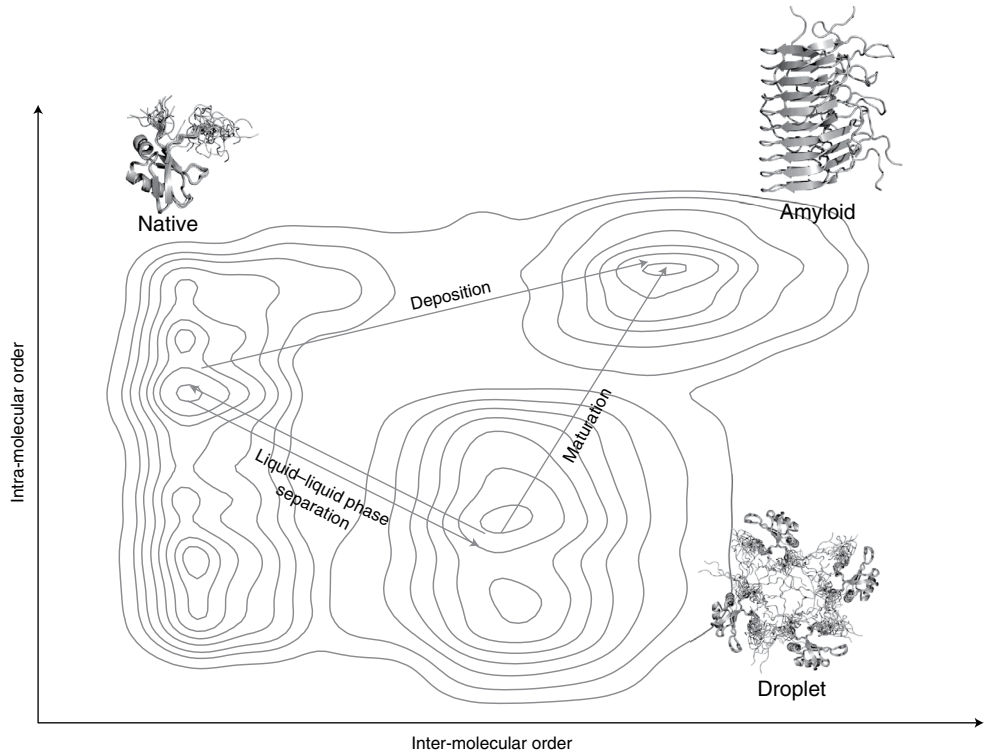

Fig. 1 | Fundamental nature of the native, droplet and amyloid states of proteins. Thermodynamically and kinetically, under cellular conditions most proteins can populate all of these states (Box 1) and interconvert between them[1,5–7], as illustrated here using a free-energy landscape as a function of two order parameters (intra-molecular order and inter-molecular order). At low concentrations, inter-molecular interactions are thermodynamically disfavoured over intra-molecular ones, so that the native state tends to be highly populated and the kinetic barriers towards condensation are high[1,2]. By contrast, at high concentrations, the condensed states become thermodynamically favourable and the kinetic barriers towards condensation are low. Under these conditions, the native state can convert to the droplet state by a reversible liquid–liquid phase-separation process, which can then progress to the amyloid state through a maturation process. The amyloid state can also be formed directly from the native state through a deposition process[98]. There is increasing evidence that the condensed states can participate in a wide range of cellular functions (Table 1). The native state is represented by the RNA-binding domain of TDP43 (Protein Data Bank ID (PDB): 2CQG, residues 96–185), which was also used to generate the structure of the droplet state. The amyloid state was generated from the HET-s structure (PDB: 2RNM [113], residues 218–289).

autoinhibitory conformation present in the monomeric state and exposing interaction motifs for downstream signalling[70,71]. The prion-like activity of MAVS amyloid assemblies also converts endogenous MAVS proteins into functional aggregates to propagate antiviral signalling[71]. The structural polymorphism of the amyloid state may also be exploited in complex decision-making mechanisms, such as cell-fate decision between mitotic proliferation and meiotic differentiation in yeast regulated by SMAUG amyloids[72].

Synaptic processes illustrate how the three fundamental states of proteins are used for related biological activities. Glutamate-gated AMPA receptors in their native state form well-defined architectures for neurotransmitter-driven gating of ion channels[73]. Liquid–liquid phase separation of synapsin at the synapse results in the clustering of synaptic vesicles[74], while phase-separated condensates of post-synaptic density proteins (Shank3, Homer3 and PSD-95) cluster receptors to enhance signalling fidelity and sequester inhibitory post-synaptic proteins[75]. Long-lasting morphological changes at the synapse are related to self-perpetuating conversion of CPEB into an amyloid state. This process generates distinct strains with distinct activities[76], which represent the molecular memory encoded by amyloid conformations.

Evolutionary selection of protein sequences

To enable specific molecular functions, protein sequences that preferentially populate native states over condensed states have emerged through the course of evolution. In order to fold into native conformations, proteins form specific intra-molecular interactions and minimize the collective interactions favoured by high concentrations that lead to the formation of condensates (Fig. 1). Because of their generic nature, however, condensed states of proteins are ultimately unavoidable[1–3]. It is therefore remarkable that the evolution of amino acid sequences balances the need to minimize the population of the condensed states and the opportunistic exploitation of these states for functional processes[36]. Robustness and versatility of a variety of cellular processes, in particular related to regulation of protein activities and development, may indeed depend on the formation of condensed states[77].

Quite generally, one would expect the evolutionary selection rules operating on protein sequences to concern both order-promoting and disorder-promoting interactions. The primary role of order-promoting interactions is to create microenvironments for specific molecular functions[15], whereas the main role of disorder-promoting interactions is to regulate these functions[34,35]. Order-promoting interactions are generally found in native-like, well-defined functional conformations, whereas disorder-promoting interactions result in non-native contacts, leading to variations or heterogeneity of the native conformation. These non-native contacts can promote functional adaptability, which is often exploited for regulation of protein activities and thus encoded by the native sequence[77,78]. Disorder-promoting intra-molecular interactions—for example, generated by low-complexity regions—disfavour the stabilization of the native state and promote the

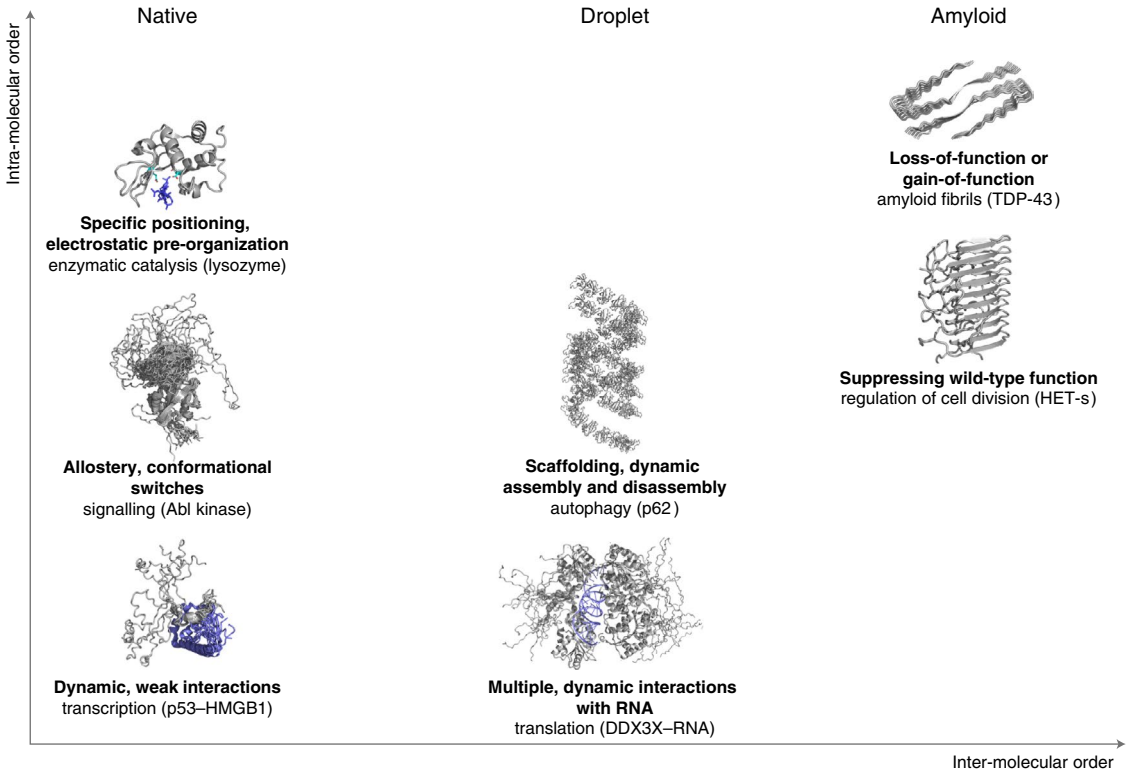

Fig. 2 | Cellular functions of proteins in the native and condensed states. Native states often exhibit high intra-molecular and low inter-molecular order, as illustrated here in the case of lysozyme, where intra-molecular interactions precisely position the catalytic residues (coloured residues) to form the binding pocket for the substrate (blue) and provide electrostatic stabilization for the transition state (PDB: 1LZR[108]). Proteins in their native states can also harbour intrinsically disordered regions, which enable a wide range of functions including signalling regulation, as shown in the case of Abl kinase (PDB: 6AMW[31]) by switching between active and inhibited conformations upon myristoylation, and transcriptional regulation, as shown here for p53 (grey) in complex with HMGB1 (blue, PDB: 2LY4[35]) via weak disorder-promoting interactions that facilitate binding to both transactivator domains and DNA. The formation of the droplet state is driven by sequence regions with a low propensity for intra-molecular order, as shown for p62 (PDB: 4UF9[114]), which undergoes rapid head-to-tail assembly and disassembly upon sensing of signals in the autophagy pathway, and for DDX3X (PDB: 6O5F[115] and PDB: 1RF8[116]), which forms dynamic interactions with other cellular components. The amyloid state is characterized by high levels of intra-molecular and inter-molecular order, which can be functional, as in the case of HET-s (PDB: 2RNM[113]) or dysfunctional, as for TDP43 (PDB: 6N37[117]). Most proteins can populate all these states (Box 1 and Fig. 1), enabling a wide range of cellular functions depending on the level of inter-molecular and intra-molecular orders of corresponding states. Only some of these functions are illustrated here; Table 1 presents a more extensive summary of state-associated functions.

formation of multimolecular assemblies of high inter-molecular order. By contrast, high-complexity regions leverage the physicochemical properties of individual amino acids, including hydrophobicity and electrostatic charge, to enable the formation of specific interactions favoring the native state[77,79].

A sequence code for droplet formation

The evidence based on the 500 or so proteins reported to date to spontaneously undergo liquid–liquid phase separation indicates that a wide variety of sequence motifs can drive the formation of liquid-like condensates[3]. These motifs contribute to non-specific interactions, such as those arising from different combinations of charged, dipole and quadrupole interactions, some of which can be distinguished as cation–π or hydrophobic contacts[80,81]. These interactions are frequently modulated by post-translational modifications that in turn influence liquid–liquid phase separation[82]. Thus, whether or not a given sequence motif is capable of promoting droplet formation is primarily determined by its sequence complexity[3].

In this view, the sequence composition of low-complexity domains of a protein that favour a low degree of intra-molecular ordering and a high degree of inter-molecular ordering gives rise to droplet formation[79]. For example, within a pool of polar or charged amino acids that promote low intra-molecular order and form disordered domains, a relatively small number of hydrophobic or aromatic residues can drive inter-molecular ordering and facilitate liquid–liquid phase separation, as observed in the case of α-synuclein[83]. In addition, the solubility of the low-complexity domain of FUS is weakly dependent on salt concentration, suggesting that hydrophobic contacts, in particular those formed by tyrosine residues, can drive the condensation of this protein[81]. Similarly, the presence of charged residues within a pool of hydrophobic amino acids can drive disorder-promoting, collective interactions, leading to the formation of liquid droplets. Phosphorylation, for example, facilitates the liquid–liquid phase separation of tau by generating a local increase of charged interactions[84]. Overall, high conformational entropy and multiple binding configurations are characteristic of the droplet state, which is promoted by increasing the proportion of disorder-promoting residues such as glycine[12,85], or by the redundancy of the interaction motifs[10,28].

Cellular context-dependent formation of condensed states

Most human proteins include sequence regions capable of forming disorder-promoting interactions[3]. The generic nature of the collective interactions between proteins makes the properties of the droplet state particularly responsive to the cellular environment[59], which influences the balance between intra-molecular

and inter-molecular disorder-promoting interactions through a wide range of factors[3]. First, post-translational modifications can exert both positive and negative effects on the formation of condensed states. Phosphorylation has been reported to shift the conformational ensemble towards amyloid aggregation[45] or droplet formation[48,86], as the incorporation of negatively charged phosphate groups promotes the exposure of sequence motifs for inter-molecular interactions. For example, specific phosphorylation patterns of FMRP and Caprin1 control their phase separation and regulate translation rates[86], and binding of HP1α ligands at phosphorylation sites can reverse phosphorylation-driven phase separation of HP1α[48]. Nevertheless, phosphorylation may also induce condensate dissolution, as in the case of synapsin[74]. Similarly, histone acetylation by p300 inhibits chromatin phase separation, which can be reversed by interactions with BRD4[87]. Second, changes in cellular localization can alter the interaction networks associated with the formation of condensed states[62,88] or modulate their biophysical properties[63]. Third, owing to the context dependence of the intra-molecular and inter-molecular interactions, the biophysical properties of the condensates are influenced by the concentration of ATP[89], ions, osmolytes and metabolites[90]. For example, it has been shown that ATP binding and hydrolysis can facilitate condensate formation of RNA-dependent DEAD-box ATPases[91].

Proteins expressed at low concentrations can be incorporated into condensates as clients[3,4]. Such client proteins typically partition into droplets through context-sensitive sequence motifs[3], but may retain their native states within droplets. The activity of client proteins is influenced by changes in the concentrations of substrates and effectors within the condensates[36]. These proteins, however, may also form droplets themselves when the conditions are appropriate. β-synuclein, for example, includes a droplet-promoting carboxy-terminal region, but lacks a hydrophobic patch present in the non-amyloid-β component region of the α-synuclein sequence, and thus does not readily form droplets[83]. However, raising the temperature above physiological values increases the strength of hydrophobic interactions and can drive liquid–liquid phase separation of this client protein[3]. In another example, under energy-stress conditions, glycolytic enzymes with high intra-molecular order such as phosphofructokinase-1 can form liquid-like metabolic subcompartments using short disordered regions[92,93].

Similarly to the formation of droplets, the formation of amyloid states are also subject to context-sensitive regulation[94]. Amyloid formation associated with functional necrosomes is facilitated by the RIP1 and RIP3 kinases, and only the hyperphosphorylated forms of RIP1 and RIP3 are present in the insoluble fraction[45]. Phosphorylation leads to charge repulsion and extended conformations of the disordered linker, thus exposing the RIP interaction motifs for oligomerisation[45]. Similarly, context-sensitive conformational changes of disordered linkers facilitate polymerization of ASC-dependent inflammasomes by relieving the autoinhibition between the PYD and HIN domains of AIM2 and by nucleating ASC filaments[94].

Condensed states and human disease

The formation of condensed states of proteins is associated with a variety of pathological processes[95]. Many protein-misfolding diseases are characterized by the formation of aberrant amyloid deposits[1] or the maturation of liquid-like condensates into solid-like condensates[96–98].

Since the formation of the droplet state is sensitive to the cellular context, this state could be particularly vulnerable to dysfunction. For instance, RNA interactions drive condensate assembly via context-dependent mechanisms[99]. RNA-binding proteins such as TDP43 and FUS phase separate into liquid-like droplets in the nucleus where RNA concentration is high, but form solid-like pathological condensates in the cytoplasm where RNA concentration is low[88]. The P525L mutation in the nuclear-localization signal of FUS induces its accumulation in the cytoplasm and reduces its solubility, resulting in accelerated ageing[100]. Furthermore, the neurodegeneration-related maturation of stress granules[101] is modulated by the phosphorylation of intrinsically disordered regions of G3BP1[102] and Caprin1[86], which affects RNA interactions to influence the liquid–liquid phase separation and material properties of the condensates. Altered activities of condensation regulators, such as the dual-specificity kinase DYRK3[60], may also affect the assembly and disassembly of condensates and disrupt signalling activity.

Mutations that perturb the properties of the condensed states may lead to protein dysfunction and disease. For example, oncogenic mutations of AKAP95, a nuclear protein that regulates transcription and splicing, were found to impair the liquidity of AKAP95 condensates[103]. As part of the protein homeostasis system, the amino acid code that controls the balance between the native, droplet and amyloid states can be viewed as an evolutionarily acquired first line of defence. For example, amyloid-promoting and droplet-promoting regions are predicted to be in proximity to the sequences of most human proteins[104], which may inhibit interactions between aggregation-prone regions. The conversion from the droplet to amyloid states takes place through the formation intermediate species that are stabilized by both order-promoting and disorder-promoting interactions. These intermediate species probably exhibit promiscuous binding to a variety of cellular components, thus causing toxicity[104].

The context-sensitive behaviour of the droplet state, which manifests in the ability to switch between order-promoting and disorder-promoting interaction modes, may offer opportunities for drug discovery. Sequence motifs that drive the formation of condensates with different properties could serve as drug targets, as for instance in the case of nuclear-localization sequences and their regulation by nuclear import proteins[105]. Pharmacological targeting of these sequence motifs could inhibit the conversion between the liquid-like and solid-like states to avoid the appearance of the toxic intermediates. For example, disorder-promoting interactions have been targeted by small molecules in drug discovery for Alzheimer's disease[106]. As an alternative approach, protein condensates may selectively partition small-molecule drugs, thus influencing their pharmacodynamic properties[107].

Concluding remarks and outlook to the future

The native states of proteins have simultaneously high intra-molecular order and high inter-molecular disorder. From the point of view of thermodynamics, this type of ordering is marginally stable at the high concentrations present in the cellular environment. Therefore, the formation of condensed states, both liquid-like and solid-like, could be expected for most proteins.

We anticipate that a greater recognition of the ubiquitous nature of the condensed states of proteins will have a great impact in research in cell biology. We have only relatively recently begun to realize that cellular functions are possible in these states, thereby expanding the repertoire of the possible roles of proteins in biological processes.

To advance this process of discovery, it will be important to develop quantitative conceptual and experimental tools to characterize the biophysical properties of the condensed states of proteins. Since these properties are determined by the thermodynamics and kinetics of the protein molecules, analytical methods for accurately measuring protein concentrations and interactions in the cellular environment will be useful for providing mechanistic understanding. These investigations will reveal the links between the phase behaviour and the function of proteins, as well as the factors and cellular processes that regulate such links.

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Competing interests

The authors declare no competing interests.

Additional information

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