Leading Edge
Perspective

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Mechanisms and Consequences of Macromolecular Phase Separation

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Over a century ago, colloidal phase separation of matter into non-membranous bodies was recognized as a fundamental organizing principal of cell "protoplasm." Recent insights into the molecular properties of such phase-separated bodies present challenges to our understanding of cellular protein interaction networks, as well as opportunities for interpreting and understanding of native and pathological genetic and molecular interactions. Here, we briefly review examples of and discuss physical principles of phase-separated cellular bodies and then reflect on how knowledge of these principles may direct future research on their functions.

Introduction

Just prior to the beginning of this century, Bruce Alberts eloquently described how the next generation of molecular biologists would need to be trained in order to meet the challenges of studying the organization of matter in living cells (Alberts, 1998). He argued that students would need an appreciation of physicochemical principles and techniques for them to perform the future research necessary to understand the structures and dynamics of well-organized protein complexes, or "protein machines," and how they are spatially and temporally arranged to perform distinct cellular functions. More recently, Alberts has emphasized that important challenges in molecular biology also include understanding other types of cellular molecular organizations, including what he referred to as "subcompartments that are not membrane enclosed" (Alberts, 2010; Alberts et al., 2007).

To begin to understand these machines and their subcompartments, it's important to understand their constituent components. What has emerged from extensive large-scale protein-protein interaction network studies is that few of the proteins in these networks form stable complexes with fixed stoichiometry (of equal or nearly equivalent numbers of each subunits) that one would expect of protein machines (Collins et al., 2007; Gavin et al., 2006; Gavin et al., 2002; Hein et al., 2015; Ho et al., 2002; Krogan et al., 2006; Tarassov et al., 2008). For example, in vivo and in vitro proteome-wide protein-protein interaction mapping resulted in successful detection of stable complexes such as the nuclear pore complex (Figures 1A and 1B), but large regions of protein interactomes are not composed of stable, defined complexes (Figure 1C). In addition, in a recent large-scale study of human protein-protein interactions, the investigators observed few examples of complexes with high subunit stoichiometry (Hein et al., 2015).

What does the apparent lack of order in protein interactomes tell us about how proteins usually behave? We have argued that many, perhaps >50% of protein-protein interactions are "noise." By noise, we mean protein-protein interactions that serve no discernable function (Kanshin et al., 2015; Landry et al., 2013; Landry et al., 2009; Levy et al., 2012; Tarassov et al., 2008). This kind of noise may also arise in protein-RNA and protein-DNA interactions (Landry et al., 2013). Although a large proportion of protein-protein interactions may be noise, non-complex patterns of interactions that we see in protein-protein interaction networks could also represent other kinds of order created by interactions among proteins, RNA, DNA, and membrane surfaces.

Over a century ago, Edmund Wilson summarized a body of work on how the protoplasm of cells appeared to be composed of phase-separated mixtures of distinct globular objects (Hyman and Brangwynne, 2011; Wilson, 1899). What precisely accounted for these observations remained a mystery until just recently. Evidence has now emerged for alternative organizations of matter in cells that could explain some of the transient, complex-less interactions observed in protein-protein interaction networks. Notable among this body of work are the observations that proteins and protein-nucleic-acid mixtures can undergo liquid-liquid phase separation (LLPS) to form liquid droplets or what have been called, "non-membranous organelles" (Table 1 for definitions) (Brangwynne, 2011, 2013; Brangwynne et al., 2009, 2011; Lee et al., 2013b; Li et al., 2012a; Weber and Brangwynne, 2012). Pioneering efforts of Cliff Brangwynne, Tony Hyman, and Michael Rosen established that these bodies behave like liquids and presented examples of their biological functions.

What we now know about liquid droplets has been extensively reviewed and it is not our intention here to cover the state of the field in detail (Brangwynne, 2011; Brangwynne et al., 2015; Hyman and Brangwynne, 2011; Hyman and Simons, 2012; Hyman et al., 2014; Sear et al., 2015; Zhu and Brangwynne, 2015). Our goal with this Perspective is to focus on basic observations about the functions of and molecular mechanisms of formation

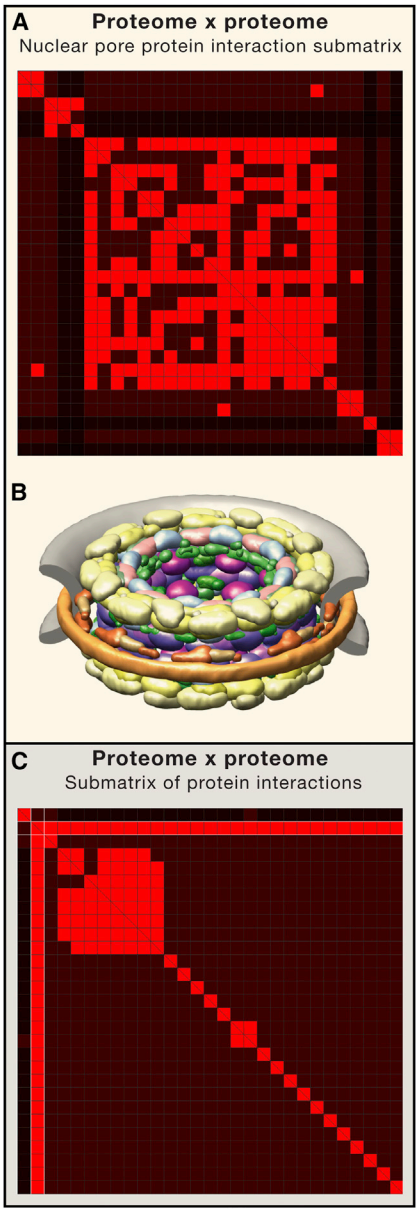

Figure 1. Protein Interactomes Contain More Disordered than Ordered Protein Complexes

(A) Clustered protein-protein interactions among the yeast *Saccharomyces cerevisiae* proteins that are subunits of the nuclear pore complex. The protein-protein interactions were retrieved from Biogrid and organized into a binary association matrix. A hierarchical agglomerative average linkage clustering with the uncentered correlation coefficient as the distance matrix was then applied to this association matrix, and the interactions were visualized with the iVici software (http://michnick.bcm.umontreal.ca/resources/software/iVici.jar) (Tarassov and Michnick, 2005).

(B) Architecture of the ordered assembly of the nuclear pore complex determined from biophysical and proteomic data (Alber et al., 2007).

(C) Another submatrix of the yeast protein-protein interaction network does not show multiple intersubunit protein-protein interactions that would be expected of a multiprotein complex. DSN1, a component of the MIND kineticore complex, makes a number of binary interactions; however, most make no other interactions with each other.

of liquid droplet and to reflect on outstanding questions that arise from recognition of their existence, including: what are the hallmarks and the distinct properties of proteins that undergo LLPS? What is the nature of the interactions among molecules that form a liquid droplet and other molecules that encounter or enter these bodies? What do molecular interactomes mean in the context of liquid droplets? Is phase separation an important and extensive form of organized matter in the cell? As our understanding of this form of organization of matter develops, the challenge then becomes figuring out how many distinct droplets exist, how they could mediate biochemical processes, and (perhaps optimistically) how they may explain molecular mechanisms that, to date, have remained mysteries.

What Are the Hallmarks of Liquid-Liquid Phase Separated Protein Droplets?

A number of mesoscale bodies (hundreds of nanometers to micrometers), particularly in the nucleus, have been described as non-membranous organelles, including nucleoli, nuclear Speckles, Cajal bodies, and Promyelocytic leukemia (PML) bodies (Brangwynne, 2011; Nott et al., 2015). More recently, these and other cellular structures have been demonstrated to behave as liquid droplets, including a number of RNA-protein assemblies such as germline P granules, processing (P) bodies, and stress granules, as well as DNA-protein complexes such as centrosomes, mitotic spindles, and signaling and actin polymerization complex activation bodies (Banjade and Rosen, 2014; Brangwynne et al., 2009; Jiang et al., 2015; Kroschwald et al., 2015; Li et al., 2012a; Lin et al., 2015; Molliex et al., 2015; Sear, 2007; Zwicker et al., 2014). Across this broad swath of biology, three basic principles underlie all of these membrane-less organelles. They arise from a phase separation of proteins or proteins and nucleic acids from the surrounding milieu. They remain in a liquid state but with properties distinct from those of the surrounding matter, and importantly, proteins exchange with these bodies in seconds instead of minutes, hours, or longer, as occurs for subunits of stable complexes (Brangwynne, 2013; Brangwynne et al., 2009, 2011; Hyman and Brangwynne, 2011; Lee et al., 2013a; Li et al., 2012a; Patel et al., 2015; Weber and Brangwynne, 2012). Are there any common characteristics of proteins or nucleic acids that are found to form liquid droplets? As we describe next, the characteristics of amino acid sequences of droplet-forming proteins are remarkably simple.

Amino Acid Sequence Characteristics of Droplet-Forming Proteins

The catalog (to date) of proteins that phase separate into droplets is enriched for those having low complexity amino acid composition domains (LCDs) including tandem repeats (TRs) of individual amino acids or amino acid motifs, such as polyglutamine (polyQ) and polyasparagine (polyN) tracts (Altmeyer et al., 2015). These LCD-containing proteins belong to the general class of intrinsically disordered proteins (IDP)s that make up about a third of eukaryotic proteome peptide sequence (Dunker et al., 2015; Toretsky and Wright, 2014; van der Lee

Table 1. Definition of Terms Related to Protein Phase Separation

Term	Definition
Liquid-liquid phase separation (LLPS)	demixing of two liquid phases with identical chemical potentials, but distinct molecular composition
Low complexity domains (LCDs)	amino acid sequence regions composed of unusually few distinct types of amino acids
Prion-like domains (PrDs)	LCDs predicted to cause a protein to form prionic aggregates
Tandem repeat (TR)	amino acid sequence region of a protein in which each amino acid is identical
Janus particles	particles with two or more different properties on different regions of the particle surface
Blobs	typical length of amino acids (typically 7-10) within proteins over which there is a net balance of all intra- and inter-peptide and peptide-solvent interactions versus thermal energy of the same peptide; blobs will be found in globules or, above a critical concentration, phase-separate from solvent, if the intra- and inter-peptide interactions are greater than blob-solvent interactions
Globules	compact multi-blob arrangements within a single polypeptide chain
Droplet	fluid body formed by condensation of elements (small molecules or macromolecules) in space and time
Colloid	homogeneous suspension of large molecules or particles of 1 to 1000 nanometer dispersed in a second material that do not sediment
Hydrogel	rigid network of hydrophilic polymer chains
Amyloid	insoluble solid protein fibrillar aggregates
Surface tension	property of a fluid surface that resists external forces due to the cohesive force between liquid molecules

et al., 2014). We have calculated that there are over 800 such proteins in the yeast *S. cerevisiae* proteome.

One class of droplet-forming proteins have TRs contained within LCDs called prion domains (PrDs) (Alberti et al., 2009; Alexandrov and Ter-Avanesyan, 2013; Brown and Lindquist, 2009; Caudron and Barral, 2013; Costanzo et al., 2010; Decker et al., 2007; Duennwald et al., 2012; Heinrich and Lindquist, 2011; Holmes et al., 2013; Malinovska et al., 2013; Patel et al., 2009; Raveendra et al., 2013; Sabate et al., 2015; Toombs et al., 2010; Webb et al., 2014). PrDs have been implicated as epigenetic agents, driving the formation of inheritable protein complexes through conformational changes in the protein itself or in other PrD-containing proteins (Brown and Lindquist, 2009; Chung et al., 2013; Heinrich and Lindquist, 2011; Holmes et al., 2013). PrD-containing proteins have been demonstrated to underlie the molecular basis of long-term memory in eukaryotes from budding yeast to fruit flies, snails, and mice (Caudron and Barral, 2013; Drisaldi et al., 2015; Fioriti et al., 2015; Heinrich and Lindquist, 2011; Keleman et al., 2007; Raveendra et al., 2013; Si et al., 2003, 2010; Stephan et al., 2015). TR length variation has also been implicated in neutral or beneficial variations, for example in cell-surface adhesion and transcription factor activities in yeast and canine skull morphology (Fondon and Garner, 2004; Gemayel et al., 2015; Verstrepen et al., 2005). By contrast, mutants of PrD-containing proteins have been implicated in a number of neuro- and neuromuscular-degenerate diseases through formation of insoluble amyloid fibrils (Duim et al., 2011; Jayaraman et al., 2012; Kim et al., 2009; Liebman and Meredith, 2010; Mishra et al., 2012). For example, expansion of the polyQ TRs in huntingtin protein contributes to Huntington's disease. A number of other devastating inherited or acquired neurodegenerative disorders such as myotonic dystrophy and Parkinsonism may be developed through similar mechanisms (La Spada and Taylor, 2010).

We are only beginning to understand what the few known droplets do and which biochemical and physical processes drive their formation. Since proteins with LCDs represent ~30% of the eukaryotic proteome (Sim and Creamer, 2002) and a large number of these proteins are putative droplet components, we speculate that many unique liquid droplets could exist. Microscopists have long observed numerous cellular punctate structures, revealed in fluorescence microscopy of proteins fused to fluorescent proteins. What proportion of these corresponds to liquid droplets of some stripe?

Proteins containing multiple-folded binding domains (to mediate interactions with other peptides or nucleic acids) separated by low complexity linkers are also proposed to phase separate into liquid droplets. Looking at a network of the proteins Nephrin, Nck, and the Wiscott-Aldrich protein (WASP) N-terminal domain, studies describe evidence, in vivo and in vitro, for tyrosine phosphorylation of the membrane-associated Nephrin as creating linear docking sites for the binding of an SH2 domain in Nck, which in turn organizes its three SH3 domains in space (Banjade and Rosen, 2014; Banjade et al., 2015; Li et al., 2012a). These reorganized SH3 domains then bind to complementary peptide motifs in N-WASP. The multivalency of these interactions generates a protein-protein interaction network as a phase-separated droplet. This phase separation was shown to induce F-actin polymerization in the presence of Arp2/3 (Banjade and Rosen, 2014). Recently, the adaptor protein Grb2, which contains two SH3 domains, was shown to be essential to liquid phase separation of an intermediate module of T cell receptor signaling (Su et al., 2016). Both SH3 domains of Grb2 were necessary for phase separation. The implications from the organization of these proteins to the mechanism of phase separation are discussed below.

Does Biological Matter Transform into Distinct Coalesced States?

Having considered some basic definitions for liquid droplets and the patterns of protein sequences that support their formation, it's important to bring in a more general view on phase

separation that is useful for considering proteins and other biological molecules in various states of matter. Phase transitions are not uncommon. Matter can transit between many states, from gas to solid, as a function of temperature, pressure, and concentration, for example, sublimation of snow to vapor on a sunny winter day.

Similarly, simple organic molecules can undergo phase transitions under the right conditions. For example, Brian Shoichet has described asymmetric aromatic compounds that are soluble in aqueous solution but that phase-separate into liquid droplets in a concentration- and temperature-dependent manner (Sassano et al., 2013). Interestingly, the change in state of the small molecule can be used to influence biomolecules. Among these, one series of compounds was shown to spontaneously form extended amyloid-like solid fibrils that could act as scaffolds for caspases, leading to the activation of their self-cleavage, the key step in their full activation. Interestingly, beta-amyloid peptides were shown to bind similarly to these caspases (Zorn et al., 2011). Another remarkable example is a biotinylated isoxazole molecule reported by Steven McKnight's group, which upon exposure to cell lysates, concentrates RNA binding proteins into ordered arrangements. They went on to show that the LCD-containing RNA binding protein FUS also formed solid hydrogels that could concentrate RNAs (Han et al., 2012; Kato et al., 2012).

It's important to keep in mind that, liquid-liquid and liquid-solid phase separations of proteins are not unheard of nor are they specific to the LCD-containing proteins. Any stably folded, single-domain protein could phase separate into a liquid droplet under the right conditions. For instance, the art of protein crystallization involves placing proteins under a number of different conditions, such as various concentrations of certain salts, buffers, detergents, and crowding agents (e.g., polyethylene glycols or polyanionic polymers of different lengths). Screened under these different conditions, proteins can be driven to undergo a number of phase transitions to liquid droplets, to solid precipitates, and either directly or through one of these states (if you are lucky), to ordered crystals (Zhang et al., 2012). What makes LCD-containing proteins special is that they can undergo liquid-liquid phase separation more readily, either self-coalescing at critical pH or salt concentrations or upon binding to nucleic acids or other proteins. As we discuss later, post-translational modifications of LCD-containing proteins have also been shown to induce phase separation into liquid droplets.

Liquid droplets are not the only state that LCD-containing proteins can take on. The most well-known and extensively studied forms are amyloid fibrils. Recent in vitro studies show a slow transition of LCD-containing proteins from weakly associated to fibrillar arrangements, and the evolution of this process may be accelerated, in vivo, in the naturally occurring mutant variants of these proteins, some of which are known to be pathogenic (Lin et al., 2015; Molliex et al., 2015; Patel et al., 2015; Zhang et al., 2015). However, we do not know if fibrillar arrangements of LCD proteins exist in vivo under normal conditions. We do know that homogeneous preparations of liquid droplets in vitro, made of individual LCD-containing proteins, start out as dispersed, weakly associated molecules that exchange with surrounding solutions rapidly, some of which gradually transit to a fibrillar state in which individual monomers no longer exchange rapidly with the surroundings (Molliex et al., 2015; Patel et al., 2015; Zhang et al., 2015). Could this happen in vivo? We do know that protein-RNA assemblies can have a range of liquid to solid states depending on their compositions (Hubstenberger et al., 2015). A key difference between the in vitro and in vivo settings centers on the complexity of the available protein population. It is possible that the transition of cellular liquid droplets, such as P-bodies, into fibrillar states is prevented because of their heterogeneous composition. Cellular droplets are mixtures of interacting proteins or proteins and nucleic acids. In such mixtures, competing heterogeneous interactions among the different types of molecules may somehow frustrate the formation of higher-ordered fibrillar states. The cell protein quality control machinery is also proposed to help preserve the liquid or solid states of protein and protein-RNA assemblies. For example, recent work on budding yeast showed that the action of Hsp104 disaggregase is required to maintain liquid-like P-bodies or that the cellular disaggregation activity is needed to dissolve functional stress granule aggregates (Kroschwald et al., 2015).

What Is the Nature of the Interactions among Molecules in a Liquid Droplet?

What is it about low complexity sequences that make them susceptible to forming a continuum of states? How do the various liquid droplets keep their individual compositions in the cellular milieu? Do proteins within droplets further separate into subphases? Very little is known and there is no all-encompassing theory for how liquid droplets form or how they have evolved. There are, however, some compelling theories, intuitions, and specific cases that can serve as starting points to understand liquid-liquid phase transitions of proteins.

Theories of beta-amyloid fibril formation have either explicitly or implicitly implied formation of a liquid-liquid phase separated intermediate (Padrick and Miranker, 2002). The general idea is that a more disordered and dynamic intermediate is needed for formation of a structured and static aggregate. A model for amyloid formation by the protein huntingtin, the pathological agent in the neurodegenerative Huntington's disease, explicitly invokes a transient coiled-coil intermediate, formed from a variable-length glutamine tract. The length of this polyQ tract determines the tendency for huntingtin to form pathological beta-amyloid structures (Crick et al., 2013; Jayaraman et al., 2012; Kim et al., 2009; Liebman and Meredith, 2010; Williamson et al., 2010). This model has also contributed to a general model of fibrillar structural transitions of polyN/polyQ TR proteins (Fiumara et al., 2010). Although a liquid-liquid phase transition is not specifically invoked, it has inspired explanations for their potential occurrence. For instance, in a recent study of the partial reconstitution of the P-body, the authors propose that the LCDs contain sequence motifs that undergo spontaneous coil-to-helix transitions, allowing them to serve as nucleation sites for formation of a network of intermolecular coiled-coils at a critical concentration (Fromm et al., 2014). The authors showed that disruption of helix-forming sequences could prevent formation of liquid droplets. Valency, the number of repeated or distinct binding domains or amino acid motifs in proteins, can also be important to liquid-liquid phase separation. For example, phase-separation of SH3-domain-prolyl peptide interactions between Nck

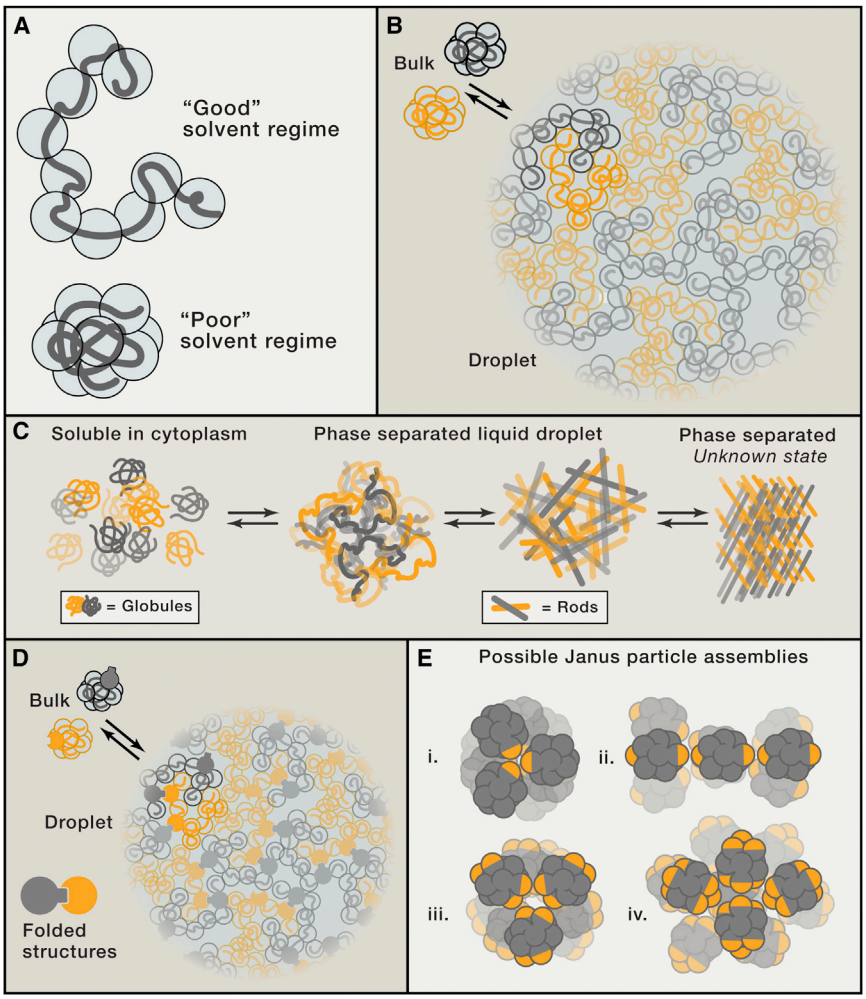

Figure 2. Molecular Structures and Interactions in Distinct Solvent Regimes

(A) Intrinsically disordered proteins (IDPs) in dilute solution take on expanded (top) or compact globular (bottom) conformations depending on whether they are in a good or poor solvent for an individual protein. Compactness of the polypeptide chain depends on solvent conditions and characteristics and patterns of amino acid distribution, notably of charged residues (adapted from Pappu et al., [2008]). Circled peptide segments of about seven to ten residues are called "blobs," segments over which the net balances of all interactions are less than or equal to thermal energy. Interactions between blobs or between blobs and solvent determine how expanded or compact the polypeptide is.

(B) At critical concentrations of a protein or under specific conditions, proteins phase separate when intermolecular blob interactions overcome the intramolecular blob and blob-solvent interactions.

(C) Unified theory of blob length scale in peptide solution predicts that the peptide volume fraction decreases when the phase-separated peptide concentration increases. In this model, the peptide chain is predicted to undergo distinct changes from collapsed globule, to expanded globule, to flexible-rod-like, and to rigid-rod-like structures. This model could account for the distinct coalesced states of proteins that result in transition from liquid droplet to fibrous aggregates.

(D) Blobs can also be folded structural domains and make stereospecific and shape-complementary interactions with themselves or other proteins.

(E) Proteins can have two segments with distinct properties (Janus particles) and these may interact and phase-separate in different ways and under different conditions. Different configurations can result in heterogeneous arrangements of phase-separated proteins in a liquid droplet (Figure 3).

and N-WASP described above depends on the number of repeats of SH3 domains or SH3-domain-binding motifs (Li et al., 2012a). In analogy to this case, Fromm et al., (2014) argue that phase separation depends on the number of possible intermolecular coiled-coils that the P-body proteins they studied could make.

The multivalent domain interaction network model described above is compelling because it explains liquid-liquid phase transitions in terms of structure and chemical complementarity (Falkenberg et al., 2013). It is, however, a model that is based on a limited number of examples and studied under non-physiological conditions and that cannot explain other aspects of droplet formation such as charge distribution dependencies. Furthermore, droplets can form from LCD-containing proteins that show no propensity or evidence of forming structured domains. Droplet formation can, for instance, be driven by simple post-translational modifications. More general theories that could account for and predict liquid droplet formations of LCD-containing proteins are needed.

Rohit Pappu has adapted a general theory to explain amyloid fibril formation from first principles of polymer theory that can also serve as a working theory for liquid-liquid phase separation of proteins (Pappu et al., 2008). In this model, IDPs with specific sequence properties do not take on extended conformations in a dilute solution but, rather, are collapsed into a compact globular form. The sequence property central to this theory is the existence of a specific length scale to intra- and inter-segmental peptide interactions of 7–10 amino acids. These segments, referred to as "blobs," constitute the length scale beyond which the balance of chain-chain, chain-solvent, and solvent-solvent interactions is at least of the order of the thermal energy of the blob (Tran and Pappu, 2006). Under conditions where chain-solvent interactions are favorable (a so-called "good solvent") or above a critical temperature, the interactions between blobs are net repulsive and the chain will swell to maximize interactions of the blobs with solvent, but under the opposite conditions (in a "poor" solvent or below the critical temperature), the polypeptide chain collapses into a globular form (Figure 2A). Vitalis et al. have shown that this model is consistent with scattering and spectroscopic data as well as molecular dynamic simulations (Vitalis and Pappu, 2011; Vitalis et al., 2007, 2008, 2009).

The key to understanding liquid-liquid phase transition of a peptide is the notion that, when the peptide reaches a critical

concentration, the intermolecular blob interactions are more attractive than the intramolecular interactions and peptide-solvent interactions and the peptides demix from the surrounding milieu into a liquid droplet. In the liquid droplet, the peptide expands because now it can make favorable intermolecular blob interactions (Figure 2B). In a sense, the peptide is now acting as its own good solvent. The concentration of the peptides in the droplet could also influence the blob variables, including the length and volume fraction as a function of inter-chain contact distance. As the peptide volume fraction decreases, the peptide chain is predicted to undergo conformational changes from expanded globules to form flexible rods and then rigid-rod structures (Figure 2C) (Uematsu et al., 2005). The rigid-rod structures could coalesce into fibrillar states of the proteins noted to occur over time in droplets in vitro (Lin et al., 2015; Molliex et al., 2015; Patel et al., 2015; Zhang et al., 2015)

It is notable that this theory requires no structural transformations, and blob interactions do not have to be shape complementary (Burke et al., 2015; Hyman and Brangwynne, 2011). Shape-complementary interactions can, however, also occur in liquid droplet. It is notable that the length scale of blobs is the same as that of typical secondary structure elements in folded proteins, and in this theory, folded domains can be thought of as blobs themselves and phase separate with stereospecific or shape complementary interactions (Figure 2D) (Li et al., 2012a).

Patterns of amino acids in blobs may also be critical to inter- and intra-blob interactions. Furthermore, this model indirectly distinguishes between IDPs with regard to their ability to form liquid droplets. For example, as discussed below, the pattern and number of charged amino acids, lengths of TRs, and presence of peptide-backbone-constraining amino acids in an IDP can have a profound impact on blob interactions and their tendency to phase separate (Das and Pappu, 2013). Finally, like multi-domain folded proteins, it is quite conceivable that a single polypeptide chain could form multi-globule arrangements (Figure 2E). These globules may have different properties under different conditions. Some of these conditions may lead to certain arrangements, polarizations, or interactions with other macromolecules that result in phase separation of the associated molecules into a droplet with $\geq$2 sub-compartments, or some other extended periodic arrangement (Li et al., 2012b). In the simplest case, an entire polypeptide may collapse into a single globule, but we can also picture different regions of a protein collectively forming different interactions with itself, the rest of the protein sequence, and the solvent. Such, so-called ''Janus'' particles could, for example, be more hydrophobic in one region and more polar in another (Das and Pappu, 2013). These particles are of interest in the soft matter material sciences because many interesting configurations of matter can theoretically be generated from different arrangements of the substructure of Janus particles (Figure 2E) (Li et al., 2012b). They are interesting to us because, as discussed below, sub-compartmentalization within the droplets can occur and the possibility that they are generated from Janus protein surfaces has important functional implications (Brangwynne, 2011).

Understanding the behaviors of IDPs in general requires suspending commonly held notions of classifications of amino acids into subcategories of charged or polar ''hydrophilic'' and apolar aliphatic or aromatic ''hydrophobic'' amino acids. These notions work for most purposes in understanding, for instance, how amino acids are arranged with respect to the solvent in folded proteins or at protein-protein or protein-nucleic acid interfaces, but they fail when trying to understand the behaviors of IDPs, particularly those with LCDs. For instance, the archetypical polyQ TR-containing protein, huntingtin, forms fibrils via collapsing into beta-hairpins, driven by intramolecular backbone and side-chain hydrogen bonds, disrupting interactions with water. Thermodynamically, this process looks like the hydrophobic collapse of a folded protein, and yet, the amino acids involved are thought of as polar, hydrophilic amino acids. Thus, in the context of a tandem repeat, glutamine or asparagine prefer to interact more with themselves than with water and can therefore be viewed to behave hydrophobically. Equally paradoxical, a domain C-terminal to the polyQ tract of huntingtin that contains a preponderance of what is commonly thought to be a hydrophobic amino acid, proline, actually prevents huntingtin from collapsing into fibrils, favoring interactions with the solvent and therefore behaving as hydrophilic agents (Crick et al., 2013). The prolines have not magically changed chemical properties, they are as hydrophilic as other polar amino acids such as Gln and Asn. However, the barrier to cis-trans isomerization of prolyl peptides disfavors the collapse of peptide into a globule, a necessary intermediate state to fibril formation and resulting in the peptide remaining soluble. Future understanding of patterns of amino acids and the properties that favor phase separation will require empirical studies, searches for heuristic rules, and ultimately, theoretical models, such as has been applied to predicting prion-forming domains or to tuning the temperature-dependent phase transition of elastin proteins (Alberti et al., 2009; Quiroz and Chilkoti, 2015).

IDPs tend to be enriched in charged amino acids compared to folded proteins (Das and Pappu, 2013). This observation might seem explainable as an obvious evolutionary adaption to these proteins being unfolded and thus requiring as many polar amino acids be exposed to solvent as possible. Though intuitively reasonable, such an argument contradicts the theory described above and the evidence that IDPs can exist as compact globules, obviating the need for an enrichment of polar or charged amino acids. What could be interesting and important to the way that these proteins behave is the arrangement of charged amino acids within a protein. Again, based on first principles of polymer theory, it has been proposed that the compactness of a polypeptide will vary with the linear polarization of oppositely charged amino acids. This leads to some interesting predictions, notably that a full range of oppositely charged residues creates a phase diagram of states, in which sharply defined regions of compactness, from completely swelled to compact globules and Janus-like objects in between, can exist (Das and Pappu, 2013). Second, given that the phase diagram predicts sharp transitions between states, one can imagine that subtle changes in charge could switch a polypeptide from one state to another. An obvious way this could be achieved is through post-translational modifications. Phosphorylation of serine, threonine, and tyrosine, methylation of arginine, and methylation and acetylation of lysine, among the most common of these, result in the addition of a negative or reduction of a positive charge.

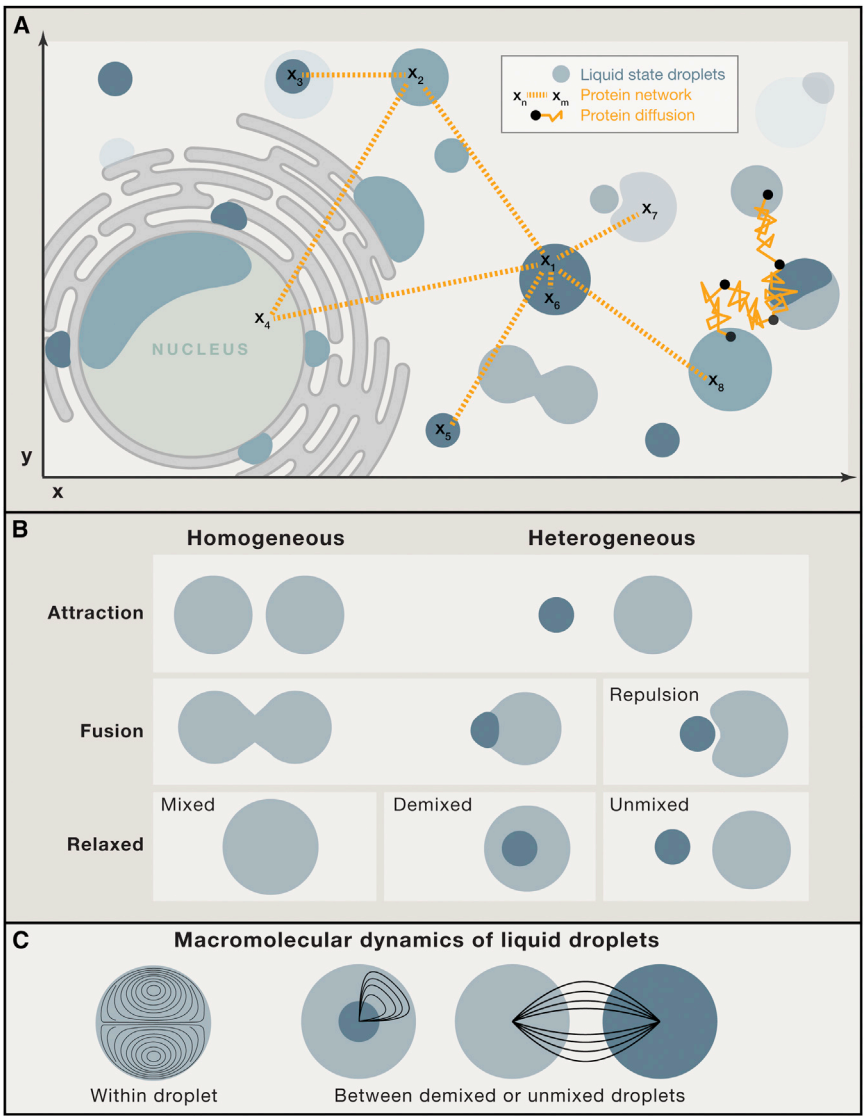

Figure 3. A Liquid Droplet View of Protein and Protein-Nucleic Acid Interactomes

(A) Liquid droplets of different sizes and properties may repel or attract one another, depending on the chemical properties of their interfaces and their distances from each other. Individual molecules that compose droplets can diffuse and exchange readily (black dots and gold lines) and interact with molecules in the surrounding milieu or within other droplets (black letters and dashed gold lines). In addition, droplets wet surfaces such as membranes, creating sites of high concentrations of molecules that could be involved in transport or signaling processes.

(B) Attraction and/or repulsion of droplets with each other can result in mixed, demixed, or unmixed droplets, depending on the properties of each droplet. Large complexes such as ribosomes or F-actin networks may also be repelled from droplet surfaces.

(C) The flow of molecules between or within droplets could partition distinct chemical reactions and generate different types of biochemical dynamics.

What Do Molecular Interactomes Mean in the Context of Liquid Droplets?

We can now put together the principles discussed above to describe how liquid droplet interactomes are distinct from what we usually think of as protein interactomes (Figure 3A). The difference and its importance to understanding liquid phase separation in cellular biology was eloquently captured by Daniel Needleman, who wrote in a recent essay about the material nature of life that, "individual molecules of water are not wet and the individual molecules that compose glass are not brittle, the wetness of water and the brittleness of glass are collective phenomena that arise from the interactions of billions of molecules" (Needleman, 2015).

For example, precedence for phosphorylation- or methylation-dependent liquid droplet formation has been reported (Scaramuzzino et al., 2015; Wang et al., 2014; Wippich et al., 2013).

Formation of Nephrin-Nck-N-WASP liquid droplets described above depends on an intrinsically unstructured linker peptide between the first two SH3 domains of Nck. The linker amino acid sequence has a polarized distribution of oppositely charged amino acids with a highly conserved positively charged sequence motif at the N terminus of the linker and negatively charged residues at the C terminus (Banjade et al., 2015). The authors showed that this motif interacts weakly with the negatively charged second SH3 domain but may also interact with a negative motif at the C-terminal end of the linker. These results illustrate the essentiality of charge and the notion of both regions, an intrinsically disordered motif and the folded SH3 domains as blobs, whose interactions with each other are essential to phase separation.

A first key distinction between droplets and other types of protein organization is that the macromolecules that compose a liquid droplet can exchange rapidly with the surrounding cyto- or organelle-plasms. Proteins associated with the droplets are highly dynamic, diffusing into, out of, and within the droplets rapidly. There could be some drag on proteins leaving the non-membranous bodies due to the intermolecular interactions within the droplet, which may be among the steady-state conditions for maintenance of the droplet. Nonetheless, the observed exchange rates between bulk solution (reflecting the cytoplasm or other subcellular compartment milieu) and droplets have been measured to be in the range of seconds to tens of seconds (Brangwynne et al., 2009; Li et al., 2012a). Exchange rates of proteins and nucleic acids determine the droplet size and could be scaled with the cellular concentration of the proteins and size of the cell (Weber and Brangwynne, 2015; Zhu and Brangwynne, 2015). Thus, compared to complexes of folded proteins, the

compositions of liquid droplets are transient and can dictate rapid formation or disintegration upon slight changes in local concentrations and chemical states of components or physical or chemical composition of the environment.

Second, the most profound difference between simple molecular interactions and those of droplets is that collective properties of these objects can result in both attractive and repulsive interactions between droplets or between droplets and other objects (Figure 3B). Depending on the physical characteristics of a droplet, it may fuse and mix with components of other droplets, demix as a result, for example, of a change in a subdroplet's surface properties, or unmix, that is, repel another encountered droplet. The materials within the droplet phase separate from the surroundings because the net interactions they make among each other are more favorable than those with the surrounding milieu. Molecules at the interface are, thus, not as likely to form favorable interactions as those in the interior of the droplet. Consequently, the droplet curvature will increase to minimize contact surface area with its surroundings, as a water droplet does with air. For individual small molecules, proteins and RNAs, interfacial tension of a droplet is not a barrier to their entry into the droplet, provided that they are small enough to slip between intermolecular spaces and either make favorable interactions or no interactions with the molecules composing the droplet. We speculate that contact with large complexes (e.g., ribosomes), however, causes distortion of the droplet surface, increasing its surface area and therefore the interfacial tension between the droplet and its milieu (Brangwynne et al., 2011). One can think of the droplet as behaving like a porous balloon, in which small objects can enter while larger objects can distort the surface but cannot enter the droplet. The exclusion of large particles creates regions in the bulk that are free of such large and unfavorably interacting molecules, so-called exclusion zones. Evidence so far for such exclusion zones created by liquid droplets in cells is limited but compelling. For instance, electron micrographs of sections of P-bodies show that otherwise widespread ribosomes are excluded from the P-bodies (Cougot et al., 2012). Feric and Brangwynne (2013) recently demonstrated that the large nucleoli of *Xenopus* oocytes are suspended in the nucleoplasm within a network of branched actin. Interestingly though, they did not see actin persistently present within the nucleoli themselves, suggesting that these dense networks of protein fibers could form extended rough surfaces that cannot penetrate the nucleoli droplets. Finally, in *C. elegans* embryos, perinuclear germline P granules work as size-exclusion barriers that exclude dextran molecules of $\geq$70 kD but are permeable to dextran molecules of $\leq$40 kD (Updike et al., 2011). The barrier properties of these perinuclear P granules are proposed, through interacting with the Phe/Gly-rich regions, to be coupled to and possibly extend the nuclear pore complex.

Water and its interactions with components of a droplet, at its interior or interface, is an important factor contributing to the droplet's properties, including viscosity and interfacial tension (Chandler, 2002). For example, if molecules interact more favorably with the constituents of the droplet than they do with those of the surrounding milieu, they will be drawn into the droplet, creating a sort of liquid-vapor interface. Depending on the vapor pressure at such interfaces, one droplet could repel or attract another (Cira et al., 2015; Zwicker et al., 2015). This may be one way that droplets with different compositions and functions have evolved to remain separate. One could easily imagine attractive or repulsive interactions of droplets with other structures in the cell, for example membranes and chromatin, which may serve to shape or organize these structures. We could further speculate that liquid droplets might generate force or act as a liquid couple between force-generating machines like actin filaments to distort or organize matter as part of cellular processes. We know, for instance, that liquid droplets associate with sites of damaged DNA or transcriptionally active regions and may serve essential roles in organizing chromatin, either directly or, in the case of transcriptionally active sites, through interactions with RNA (Altmeyer et al., 2015; Gemayel et al., 2015; Patel et al., 2015; Pennisi, 2013; Weber et al., 2015). It remains to be determined precisely how the droplets are organized and whether they are essential to forcing the arrangements of matter in active regions or sequestering certain molecules in a regulatory manner.

Finally, as discussed above, phase separation of matter could further occur inside of the already phase-separated droplets and several examples of sub-compartmentalization of droplets have been reported (Brangwynne, 2011; Cougot et al., 2012). Nucleoli suborganization can be recapitulated in vitro, due to the distinct interfacial tension generated by the distinct phase-separated proteins that make up the different subcompartments of the nucleoli (Feric et al., 2016). Specifically, they observed that a droplet with a relatively high surface tension could be enveloped by a droplet with relatively lower surface tension. It should also be noted that the high surface tension enveloped droplet has other distinct properties including being not liquid but viscoelastic, a property anyone who has played with silly putty will understand: such materials will take on any shape depending on the forces applied to them but, if a force is applied rapidly, they will bounce back to their original shape. Regardless of their nature, such sub-compartmentalization creates a strategy to isolate catalytic processes and could also generate gradients and therefore chemical potentials (Figure 3C) (Scaramuzzino et al., 2015; Wang et al., 2014). For example, frog oocyte nucleolus organization described above is thought to partition the sequential steps of ribosome biogenesis into the distinct nucleolar subcompartments (Feric et al., 2016). P-bodies have also been shown to separate into at least two regions, a core, where proteins responsible for 5′-mRNA-decapping activity (such as Dcp1) are found and a mantle where the proteins involved in translational repression (including Dhh1) accumulate (Cougot et al., 2012). What the functional significance of this layered organization is remains a subject of investigation, but the results point to the possibility that such arrangements may be common. Sub-compartments of stable complexes within a droplet have been described for mammalian and yeast stress granules. In this study, the authors referred to a stable "core" structure that was demonstrated to form in an ATP-dependent manner within stress granules (Jain et al., 2016). In summary, liquid droplets we have described, like other subcellular compartments and the cell itself, may not be just simple concentrators of molecules, they may organize matter and chemical reactions in very specific ways.

Are Droplets, and Other Alternative Organizations of Matter, Important?

The discovery of liquid properties among known non-membranous organelles will remain subjects of research in and of themselves, but whether phase separation is more generally important to our understanding of the organization of matter in the cell will depend on whether it is a very interesting but rare phenomenon or more common and can explain known or reveal novel biological phenomena. There are reasons to hope that the latter may be the case. First of all, there are hundreds, perhaps thousands of proteins that have been localized into puncta or "granules" in cells of different origins. It is conceivable that many of these bodies are liquid droplets and that the cell might be sub-compartmentalized into many such structures. For instance, some objects that to date have been referred to as granules, implying some kind of solid aggregates, have turned out to have liquid properties, including so-called stress and axonal mRNA transport granules and other local RNA translation regulatory bodies (Buxbaum et al., 2014; Cougot et al., 2008; Park et al., 2014). Second, about one-third of eukaryotic proteomes consist of intrinsically unstructured peptide sequences and at least half of these contain low complexity sequences (Toretsky and Wright, 2014; van der Lee et al., 2014). It is conceivable that some or all of these proteins phase separate under some circumstances.

In some organisms the proportion of low complexity sequences is unusually high. For instance the slime mold *Dictyostelium discoideumhas* has a high proportion of LCD-containing proteins and, interestingly, pathological LCD-containing proteins that form aggregates in yeast and mammals and are soluble when expressed in this organism (Malinovska et al., 2015). What purpose it may serve that some organisms have more LCD-containing proteins than others remains to be explored, but it will be interesting to see if one consequence is simply more utilization of non-membranous organelles to partition biochemical processes.

Finally, there are many genetic interactions that cannot be explained through any obvious functional or structural interactions. It is possible that a proportion of such interactions could be explained by the phase separation of the encoded proteins into a droplet to perform cellular functions (Costanzo et al., 2010). In the past, if you knew that proteins A, B, and C were important to a function, likely you'd seek to show that they interact physically with each other or are somehow in functionally linked consecutive or parallel pathways. If they were not, you would just scratch your head and move on. Now, you might be able to explain their genetic interaction by the fact that they phase separate together.

Different non-membranous organelles may have similar functions in organizing cellular material. For example, the first clear example of liquid droplets, the germ cell P granules of *C. elegans* embryos polarize the distribution of mRNA along the embryo polar axis through a protein-concentration-dependent mechanism (Brangwynne et al., 2009). The rate of P granules condensation, and consequently mRNA, was shown to increase with PGL-1 concentration. This could be a more general mechanism for polarization of cellular material. In another example, an essential step in assembly of functional spliceosomal small nuclear ribonucleoproteins (snRNPs) requires a protein coilin that concentrates the snRNP components in Cajal bodies (Strzelecka et al., 2010). The authors propose that coilin may phase separate into a droplet with other components of Cajal bodies, concentrating the snRNP components so that they assemble into an active complex. Detailed evidence that phase separation drives this process remains to be demonstrated.

The all-or-none nature of phase separation may also provide explanations for some abrupt cellular processes. For example, Richard Sear proposed that the Wnt pathway effector protein Dishevelled (Dvl) undergoes a liquid phase transition upon phosphorylation and that this transition is the mechanism underlying switch-like cell-fate decisions that are driven by Dvl in this pathway during embryogenesis (Sear, 2007). It is possible that other switch-like activation/deactivation of cellular processes is driven by phase separation.

Another example of all-or-none phase separation of signal-transduction machinery has recently emerged that explains a well-known and curious phenomenon of clustering of activated cell-surface membrane receptors for a wide variety of hormones and other signaling molecules (Su et al., 2016). They showed that intermediate signaling modules phase separated into liquid-like clusters upon T cell receptor phosphorylation. This central module included the adaptor- and effector-binding proteins Grb2 and SOS, respectively, both canonical components of many cell-surface receptors. They also showed that these clusters partitioned distinct enzyme activities, allowing protein kinases that catalyze phase separation to segregate with the clusters but repelling a protein phosphatase. They also result in activation of the canonical MAP kinase signaling cascade and recruit actin regulators, catalyzing actin filament synthesis.

While the number of cellular droplet types appears to be growing and we are beginning to understand some fundamental facts about them, many questions remain. Concerning droplet origins—how many are there? What is it about proteins and nucleic acids that cause them to phase separate with some, but not with other molecules?

Concerning function—what do the droplets actually do and how do their organizations or sub-organizations determine what they do and how cellular processes involving them work?

Concerning evolution—how did droplets evolve and, if they are evolvable, what is it about them that evolves? Is the evolution and selection of some, for example LCD-containing, proteins associated with the evolution of droplets? Finally, assuming that droplets are evolved objects, it is reasonable to ask then how the existence of droplets could contribute to theories of the origins of life since phase transitions are an obvious and simple way to organize matter (Wilson, 1899).

One question that needs close attention is whether droplets have unique compositions or do they share components and what makes one distinct from another, seem within reach. For example, two mRNA-processing bodies, P-bodies and stress granules, share a number of common components, but also have distinct protein species (Parker and Sheth, 2007; Ramaswami et al., 2013; Teixeira and Parker, 2007). How such partitioning occurs will have to be determined empirically through analysis of the sequences and properties of proteins that make

up individual droplets, as has been done as mentioned above, as well as to determine proteins that tend to form prions or undergo temperature-dependent soluble aggregate transformations (Alberti et al., 2009; Malinovska et al., 2013; Quiroz and Chilkoti, 2015).

Since the same proteins that form droplets are also those implicated in protein homeostasis pathologies, could their behavior in droplets provide new insights into diseases (e.g., neuromuscular disorders) and could these behaviors be "druggable"?

Conclusions

Liquid droplets can be individually appreciated as interesting biological phenomena. From the perspective of molecular interaction networks, the discovery of liquid droplets presents fresh challenges and quandaries, which we've endeavored to explore. Furthermore, although the most obvious and cited functional significance of droplets is their ability to store molecules or concentrate chemical processes in space in an efficient way, we believe that droplets may serve more subtle and interesting functions. Here, we've attempted to provide possibilities that we could imagine, but doubtless, nature may have many surprises in store. Only time will tell.

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REFERENCES

Alber, F., Dokudovskaya, S., Veenhoff, L.M., Zhang, W., Kipper, J., Devos, D., Suprapto, A., Karni-Schmidt, O., Williams, R., Chait, B.T., et al. (2007). The molecular architecture of the nuclear pore complex. Nature *450*, 695–701.

Alberti, S., Halfmann, R., King, O., Kapila, A., and Lindquist, S. (2009). A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell *137*, 146–158.

Alberts, B. (1998). The cell as a collection of protein machines: preparing the next generation of molecular biologists. Cell *92*, 291–294.

Alberts, B. (2010). Cell biology: the endless frontier. Mol. Biol. Cell *21*, 3785.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2007). Molecular Biology of the Cell, 5 edn (New York: Garland Science).

Alexandrov, A.I., and Ter-Avanesyan, M.D. (2013). Could yeast prion domains originate from polyQ/N tracts? Prion *7*, 209–214.

Altmeyer, M., Neelsen, K.J., Teloni, F., Pozdnyakova, I., Pellegrino, S., Grøfte, M., Rask, M.-B.D., Streicher, W., Jungmichel, S., Nielsen, M.L., and Lukas, J. (2015). Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). Nat. Commun. *6*, 8088.

Banjade, S., and Rosen, M.K. (2014). Phase transitions of multivalent proteins can promote clustering of membrane receptors. eLife *3*.

Banjade, S., Wu, Q., Mittal, A., Peeples, W.B., Pappu, R.V., and Rosen, M.K. (2015). Conserved interdomain linker promotes phase separation of the multivalent adaptor protein Nck. Proc. Natl. Acad. Sci. USA *112*, E6426–E6435.

Brangwynne, C.P. (2011). Soft active aggregates: mechanics, dynamics and self-assembly of liquid-like intracellular protein bodies. Soft Matter *7*, 3052–3059.

Brangwynne, C.P. (2013). Phase transitions and size scaling of membrane-less organelles. J. Cell Biol. *203*, 875–881.

Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science *324*, 1729–1732.

Brangwynne, C.P., Mitchison, T.J., and Hyman, A.A. (2011). Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. Proc. Natl. Acad. Sci. USA *108*, 4334–4339.

Brangwynne, C.P., Tompa, P., and Pappu, R.V. (2015). Polymer physics of intracellular phase transitions. Nat. Phys. *11*, 899–904.

Brown, J.C.S., and Lindquist, S. (2009). A heritable switch in carbon source utilization driven by an unusual yeast prion. Genes Dev. *23*, 2320–2332.

Burke, K.A., Janke, A.M., Rhine, C.L., and Fawzi, N.L. (2015). Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II. Mol. Cell *60*, 231–241.

Buxbaum, A.R., Wu, B., and Singer, R.H. (2014). Single β-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. Science *343*, 419–422.

Caudron, F., and Barral, Y. (2013). A super-assembly of Whi3 encodes memory of deceptive encounters by single cells during yeast courtship. Cell *155*, 1244–1257.

Chandler, D. (2002). Hydrophobicity: two faces of water. Nature *417*, 491.

Chung, C.Y., Khurana, V., Auluck, P.K., Tardiff, D.F., Mazzulli, J.R., Soldner, F., Baru, V., Lou, Y., Freyzon, Y., Cho, S., et al. (2013). Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. Science *342*, 983–987.

Cira, N.J., Benusiglio, A., and Prakash, M. (2015). Vapour-mediated sensing and motility in two-component droplets. Nature *519*, 446–450.

Collins, S.R., Kemmeren, P., Zhao, X.C., Greenblatt, J.F., Spencer, F., Holstege, F.C., Weissman, J.S., and Krogan, N.J. (2007). Toward a comprehensive atlas of the physical interactome of Saccharomyces cerevisiae. Mol. Cell. Proteomics *6*, 439–450.

Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E.D., Sevier, C.S., Ding, H., Koh, J.L., Toufighi, K., Mostafavi, S., et al. (2010). The genetic landscape of a cell. Science *327*, 425–431.

Cougot, N., Bhattacharyya, S.N., Tapia-Arancibia, L., Bordonné, R., Filipowicz, W., Bertrand, E., and Rage, F. (2008). Dendrites of mammalian neurons contain specialized P-body-like structures that respond to neuronal activation. J. Neurosci. *28*, 13793–13804.

Cougot, N., Cavalier, A., Thomas, D., and Gillet, R. (2012). The dual organization of P-bodies revealed by immunoelectron microscopy and electron tomography. J. Mol. Biol. *420*, 17–28.

Crick, S.L., Ruff, K.M., Garai, K., Frieden, C., and Pappu, R.V. (2013). Unmasking the roles of N- and C-terminal flanking sequences from exon 1 of huntingtin as modulators of polyglutamine aggregation. Proc. Natl. Acad. Sci. USA *110*, 20075–20080.

Das, R.K., and Pappu, R.V. (2013). Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. Proc. Natl. Acad. Sci. USA *110*, 13392–13397.

Decker, C.J., Teixeira, D., and Parker, R. (2007). Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in Saccharomyces cerevisiae. J. Cell Biol. *179*, 437–449.

Drisaldi, B., Colnaghi, L., Fioriti, L., Rao, N., Myers, C., Snyder, A.M., Metzger, D.J., Tarasoff, J., Konstantinov, E., Fraser, P.E., et al. (2015). SUMOylation Is an Inhibitory Constraint that Regulates the Prion-like Aggregation and Activity of CPEB3. Cell Rep. *11*, 1694–1702.

Duennwald, M.L., Echeverria, A., and Shorter, J. (2012). Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. PLoS Biol. *10*, e1001346.

Duim, W.C., Chen, B., Frydman, J., and Moerner, W.E. (2011). Sub-diffraction imaging of huntingtin protein aggregates by fluorescence blink-microscopy and atomic force microscopy. ChemPhysChem *12*, 2387–2390.

Dunker, A.K., Bondos, S.E., Huang, F., and Oldfield, C.J. (2015). Intrinsically disordered proteins and multicellular organisms. Semin. Cell Dev. Biol. *37*, 44–55.

Falkenberg, C.V., Blinov, M.L., and Loew, L.M. (2013). Pleomorphic ensembles: formation of large clusters composed of weakly interacting multivalent molecules. Biophys. J. *105*, 2451–2460.

Feric, M., and Brangwynne, C.P. (2013). A nuclear F-actin scaffold stabilizes ribonucleoprotein droplets against gravity in large cells. Nat. Cell Biol. *15*, 1253–1259.

Feric, M., Vaidya, N., Harmon, T.S., Mitrea, D.M., Zhu, L., Richardson, T.M., Kriwacki, R.W., Pappu, R.V., and Brangwynne, C.P. (2016). Coexisting liquid phases underlie nucleolar sub-compartments. Cell. Published online May 19, 2016. http://dx.doi.org/10.1016/j.cell.2016.04.047.

Fioriti, L., Myers, C., Huang, Y.-Y., Li, X., Stephan, J.S., Trifilieff, P., Colnaghi, L., Kosmidis, S., Drisaldi, B., Pavlopoulos, E., and Kandel, E.R. (2015). The Persistence of Hippocampal-Based Memory Requires Protein Synthesis Mediated by the Prion-like Protein CPEB3. Neuron *86*, 1433–1448.

Fiumara, F., Fioriti, L., Kandel, E.R., and Hendrickson, W.A. (2010). Essential role of coiled coils for aggregation and activity of Q/N-rich prions and PolyQ proteins. Cell *143*, 1121–1135.

Fondon, J.W., 3rd, and Garner, H.R. (2004). Molecular origins of rapid and continuous morphological evolution. Proc. Natl. Acad. Sci. USA *101*, 18058–18063.

Fromm, S.A., Kamenz, J., Nöldeke, E.R., Neu, A., Zocher, G., and Sprangers, R. (2014). In vitro reconstitution of a cellular phase-transition process that involves the mRNA decapping machinery. Angew. Chem. Int. Ed. Engl. *53*, 7354–7359.

Gavin, A.C., Bösche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M., et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature *415*, 141–147.

Gavin, A.C., Aloy, P., Grandi, P., Krause, R., Boesche, M., Marzioch, M., Rau, C., Jensen, L.J., Bastuck, S., Dümpelfeld, B., et al. (2006). Proteome survey reveals modularity of the yeast cell machinery. Nature *440*, 631–636.

Gemayel, R., Chavali, S., Pougach, K., Legendre, M., Zhu, B., Boeynaems, S., van der Zande, E., Gevaert, K., Rousseau, F., Schymkowitz, J., et al. (2015). Variable Glutamine-Rich Repeats Modulate Transcription Factor Activity. Mol. Cell *59*, 615–627.

Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G., and McKnight, S.L. (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell *149*, 768–779.

Hein, M.Y., Hubner, N.C., Poser, I., Cox, J., Nagaraj, N., Toyoda, Y., Gak, I.A., Weisswange, I., Mansfeld, J., Buchholz, F., et al. (2015). A human interactome in three quantitative dimensions organized by stoichiometries and abundances. Cell *163*, 712–723.

Heinrich, S.U., and Lindquist, S. (2011). Protein-only mechanism induces self-perpetuating changes in the activity of neuronal Aplysia cytoplasmic polyadenylation element binding protein (CPEB). Proc. Natl. Acad. Sci. USA *108*, 2999–3004.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., et al. (2002). Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. Nature *415*, 180–183.

Holmes, D.L., Lancaster, A.K., Lindquist, S., and Halfmann, R. (2013). Heritable remodeling of yeast multicellularity by an environmentally responsive prion. Cell *153*, 153–165.

Hubstenberger, A., Cameron, C., Noble, S.L., Keenan, S., and Evans, T.C. (2015). Modifiers of solid RNP granules control normal RNP dynamics and mRNA activity in early development. J. Cell Biol. *211*, 703–716.

Hyman, A.A., and Brangwynne, C.P. (2011). Beyond stereospecificity: liquids and mesoscale organization of cytoplasm. Dev. Cell *21*, 14–16.

Hyman, A.A., and Simons, K. (2012). Cell biology. Beyond oil and water–phase transitions in cells. Science *337*, 1047–1049.

Hyman, A.A., Weber, C.A., and Jülicher, F. (2014). Liquid-liquid phase separation in biology. Annual Review of Cell and Dev. Biol. *30*, 39–58.

Jain, S., Wheeler, J.R., Walters, R.W., Agrawal, A., Barsic, A., and Parker, R. (2016). ATPase-Modulated Stress Granules Contain a Diverse Proteome and Substructure. Cell *164*, 487–498.

Jayaraman, M., Kodali, R., Sahoo, B., Thakur, A.K., Mayasundari, A., Mishra, R., Peterson, C.B., and Wetzel, R. (2012). Slow amyloid nucleation via α-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. J. Mol. Biol. *415*, 881–899.

Jiang, H., Wang, S., Huang, Y., He, X., Cui, H., Zhu, X., and Zheng, Y. (2015). Phase transition of spindle-associated protein regulate spindle apparatus assembly. Cell *163*, 108–122.

Kanshin, E., Bergeron-Sandoval, L.P., Isik, S.S., Thibault, P., and Michnick, S.W. (2015). A cell-signaling network temporally resolves specific versus promiscuous phosphorylation. Cell Rep. *10*, 1202–1214.

Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., et al. (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell *149*, 753–767.

Keleman, K., Krüttner, S., Alenius, M., and Dickson, B.J. (2007). Function of the Drosophila CPEB protein Orb2 in long-term courtship memory. Nat. Neurosci. *10*, 1587–1593.

Kim, M.W., Chelliah, Y., Kim, S.W., Otwinowski, Z., and Bezprozvanny, I. (2009). Secondary structure of Huntingtin amino-terminal region. Structure *17*, 1205–1212.

Krogan, N.J., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., Li, J., Pu, S., Datta, N., Tikuisis, A.P., et al. (2006). Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature *440*, 637–643.

Kroschwald, S., Maharana, S., Mateju, D., Malinovska, L., Nüske, E., Poser, I., Richter, D., and Alberti, S. (2015). Promiscuous interactions and protein disaggregases determine the material state of stress-inducible RNP granules. eLife *4*, e06807.

La Spada, A.R., and Taylor, J.P. (2010). Repeat expansion disease: progress and puzzles in disease pathogenesis. Nat. Rev. Genet. *11*, 247–258.

Landry, C.R., Levy, E.D., and Michnick, S.W. (2009). Weak functional constraints on phosphoproteomes. Trends Genet. *25*, 193–197.

Landry, C.R., Levy, E.D., Abd Rabbo, D., Tarassov, K., and Michnick, S.W. (2013). Extracting insight from noisy cellular networks. Cell *155*, 983–989.

Lee, C., Zhang, H., Baker, A.E., Occhipinti, P., Borsuk, M.E., and Gladfelter, A.S. (2013a). Protein aggregation behavior regulates cyclin transcript localization and cell-cycle control. Dev. Cell *25*, 572–584.

Lee, C.F., Brangwynne, C.P., Gharakhani, J., Hyman, A.A., and Jülicher, F. (2013b). Spatial organization of the cell cytoplasm by position-dependent phase separation. Phys. Rev. Lett. *111*, 088101.

Levy, E.D., Michnick, S.W., and Landry, C.R. (2012). Protein abundance is key to distinguish promiscuous from functional phosphorylation based on evolutionary information. Philos. Trans. R. Soc. Lond. B Biol. Sci. *367*, 2594–2606.

Li, P., Banjade, S., Cheng, H.-C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012a). Phase transitions in the assembly of multivalent signalling proteins. Nature *483*, 336–340.

Li, Z.-W., Lu, Z.-Y., Sun, Z.-Y., and An, L.-J. (2012b). Model, self-assembly structures, and phase diagram of soft Janus particles. Soft Matter *8*, 6693–6697.

Liebman, S.W., and Meredith, S.C. (2010). Protein folding: sticky N17 speeds huntingtin pile-up. Nat. Chem. Biol. *6*, 7–8.

Lin, Y., Protter, D.S.W., Rosen, M.K., and Parker, R. (2015). Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins. Mol. Cell *60*, 208–219.

Malinovska, L., Kroschwald, S., and Alberti, S. (2013). Protein disorder, prion propensities, and self-organizing macromolecular collectives. Biochim. Biophys. Acta *1834*, 918–931.

Malinovska, L., Palm, S., Gibson, K., Verbavatz, J.-M., and Alberti, S. (2015). Dictyostelium discoideumhas a highly Q/N-rich proteome and shows an unusual resilience to protein aggregation. Proceedings of the National Academy of Sciences of the United States of America, 201504459.

Mishra, R., Jayaraman, M., Roland, B.P., Landrum, E., Fullam, T., Kodali, R., Thakur, A.K., Arduini, I., and Wetzel, R. (2012). Inhibiting the nucleation of amyloid structure in a huntingtin fragment by targeting α-helix-rich oligomeric intermediates. J. Mol. Biol. *415*, 900–917.

Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A.P., Kim, H.J., Mittag, T., and Taylor, J.P. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Cell *163*, 123–133.

Needleman, D. (2015). The Material Basis of Life. Trends Cell Biol. *25*, 713–716.

Nott, T.J., Petsalaki, E., Farber, P., Jervis, D., Fussner, E., Plochowietz, A., Craggs, T.D., Bazett-Jones, D.P., Pawson, T., Forman-Kay, J.D., and Baldwin, A.J. (2015). Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. Mol. Cell *57*, 936–947.

Padrick, S.B., and Miranker, A.D. (2002). Islet amyloid: phase partitioning and secondary nucleation are central to the mechanism of fibrillogenesis. Biochemistry *41*, 4694–4703.

Pappu, R.V., Wang, X., Vitalis, A., and Crick, S.L. (2008). A polymer physics perspective on driving forces and mechanisms for protein aggregation. Arch. of Biochem. Biophys. *469*, 132–141.

Park, H.Y., Lim, H., Yoon, Y.J., Follenzi, A., Nwokafor, C., Lopez-Jones, M., Meng, X., and Singer, R.H. (2014). Visualization of dynamics of single endogenous mRNA labeled in live mouse. Science *343*, 422–424.

Parker, R., and Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. Mol. Cell *25*, 635–646.

Patel, B.K., Gavin-Smyth, J., and Liebman, S.W. (2009). The yeast global transcriptional co-repressor protein Cyc8 can propagate as a prion. Nat. Cell Biol. *11*, 344–349.

Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M., et al. (2015). A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell *162*, 1066–1077.

Pennisi, E. (2013). Long Noncoding RNAs May Alter Chromosome's 3D Structure. Science *340*, 910–910.

Quiroz, F.G., and Chilkoti, A. (2015). Sequence heuristics to encode phase behaviour in intrinsically disordered protein polymers. Nat. Mater. *14*, 1164–1171.

Ramaswami, M., Taylor, J.P., and Parker, R. (2013). Altered ribostasis: RNA-protein granules in degenerative disorders. Cell *154*, 727–736.

Raveendra, B.L., Siemer, A.B., Puthanveettil, S.V., Hendrickson, W.A., Kandel, E.R., and McDermott, A.E. (2013). Characterization of prion-like conformational changes of the neuronal isoform of Aplysia CPEB. Nat. Struct. Mol. Biol. *20*, 495–501.

Sabate, R., Rousseau, F., Schymkowitz, J., and Ventura, S. (2015). What makes a protein sequence a prion? PLoS Comput. Biol. *11*, e1004013.

Sassano, M.F., Doak, A.K., Roth, B.L., and Shoichet, B.K. (2013). Colloidal aggregation causes inhibition of G protein-coupled receptors. J. Med. Chem. *56*, 2406–2414.

Scaramuzzino, C., Casci, I., Parodi, S., Lievens, P.M.J., Polanco, M.J., Milioto, C., Chivet, M., Monaghan, J., Mishra, A., Badders, N., et al. (2015). Protein arginine methyltransferase 6 enhances polyglutamine-expanded androgen receptor function and toxicity in spinal and bulbar muscular atrophy. Neuron *85*, 88–100.

Sear, R.P. (2007). Dishevelled: a protein that functions in living cells by phase separating. Soft Matter *3*, 680.

Sear, R.P., Pagonabarraga, I., and Flaus, A. (2015). Life at the mesoscale: the self-organised cytoplasm and nucleoplasm. BMC Biophys. *8*, 4.

Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A.M., Miniaci, M.C., Kim, J.H., Zhu, H., and Kandel, E.R. (2003). A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in aplysia. Cell *115*, 893–904.

Si, K., Choi, Y.-B., White-Grindley, E., Majumdar, A., and Kandel, E.R. (2010). Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. Cell *140*, 421–435.

Sim, K.L., and Creamer, T.P. (2002). Abundance and distributions of eukaryote protein simple sequences. Mol. Cell. Proteomics *1*, 983–995.

Stephan, J.S., Fioriti, L., Lamba, N., Colnaghi, L., Karl, K., Derkatch, I.L., and Kandel, E.R. (2015). The CPEB3 Protein Is a Functional Prion that Interacts with the Actin Cytoskeleton. Cell Rep. *11*, 1772–1785.

Strzelecka, M., Trowitzsch, S., Weber, G., Lührmann, R., Oates, A.C., and Neugebauer, K.M. (2010). Coilin-dependent snRNP assembly is essential for zebrafish embryogenesis. Nat. Struct. Mol. Biol. *17*, 403–409.

Su, X., Ditlev, J.A., Hui, E., Xing, W., Banjade, S., Okrut, J., King, D.S., Taunton, J., Rosen, M.K., and Vale, R.D. (2016). Phase separation of signaling molecules promotes T cell receptor signal transduction. Science *352*, 595–599.

Tarassov, K., and Michnick, S.W. (2005). iVici: Interrelational Visualization and Correlation Interface. Genome Biol. *6*, R115.

Tarassov, K., Messier, V., Landry, C.R., Radinovic, S., Serna Molina, M.M., Shames, I., Malitskaya, Y., Vogel, J., Bussey, H., and Michnick, S.W. (2008). An in vivo map of the yeast protein interactome. Science *320*, 1465–1470.

Teixeira, D., and Parker, R. (2007). Analysis of P-body assembly in Saccharomyces cerevisiae. Mol. Biol. Cell *18*, 2274–2287.

Toombs, J.A., McCarty, B.R., and Ross, E.D. (2010). Compositional determinants of prion formation in yeast. Mol. Cell. Biol. *30*, 319–332.

Toretsky, J.A., and Wright, P.E. (2014). Assemblages: functional units formed by cellular phase separation. J. Cell Biol. *206*, 579–588.

Tran, H.T., and Pappu, R.V. (2006). Toward an accurate theoretical framework for describing ensembles for proteins under strongly denaturing conditions. Biophys. J. *91*, 1868–1886.

Uematsu, T., Svanberg, C., and Jacobsson, P. (2005). A Unified Picture of Static and Dynamic Length Scales in Polymer Solutions. Macromolecules *38*, 6227–6230.

Updike, D.L., Hachey, S.J., Kreher, J., and Strome, S. (2011). P granules extend the nuclear pore complex environment in the C. elegans germ line. J. Cell Biol. *192*, 939–948.

van der Lee, R., Lang, B., Kruse, K., Gsponer, J., Sánchez de Groot, N., Huynen, M.A., Matouschek, A., Fuxreiter, M., and Babu, M.M. (2014). Intrinsically disordered segments affect protein half-life in the cell and during evolution. Cell Rep. *8*, 1832–1844.

Verstrepen, K.J., Jansen, A., Lewitter, F., and Fink, G.R. (2005). Intragenic tandem repeats generate functional variability. Nat. Genet. *37*, 986–990.

Vitalis, A., and Pappu, R.V. (2011). Assessing the contribution of heterogeneous distributions of oligomers to aggregation mechanisms of polyglutamine peptides. Biophys. Chem. *159*, 14–23.

Vitalis, A., Wang, X., and Pappu, R.V. (2007). Quantitative characterization of intrinsic disorder in polyglutamine: insights from analysis based on polymer theories. Biophys. J. *93*, 1923–1937.

Vitalis, A., Wang, X., and Pappu, R.V. (2008). Atomistic simulations of the effects of polyglutamine chain length and solvent quality on conformational equilibria and spontaneous homodimerization. J. Mol. Biol. *384*, 279–297.

Vitalis, A., Lyle, N., and Pappu, R.V. (2009). Thermodynamics of beta-sheet formation in polyglutamine. Biophys. J. *97*, 303–311.

Wang, J.T., Smith, J., Chen, B.-C., Schmidt, H., Rasoloson, D., Paix, A., Lambrus, B.G., Calidas, D., Betzig, E., and Seydoux, G. (2014). Regulation of RNA granule dynamics by phosphorylation of serine-rich, intrinsically disordered proteins in C. elegans. eLife *3*, e04591.

Webb, S., Hector, R.D., Kudla, G., and Granneman, S. (2014). PAR-CLIP data indicate that Nrd1-Nab3-dependent transcription termination regulates expression of hundreds of protein coding genes in yeast. Genome Biol. *15*, R8.

Weber, S.C., and Brangwynne, C.P. (2012). Getting RNA and protein in phase. Cell *149*, 1188–1191.

Weber, S.C., and Brangwynne, C.P. (2015). Inverse size scaling of the nucleolus by a concentration-dependent phase transition. Curr. Biol. *25*, 641–646.

Weber, S.C., Vaidya, N., and Haataja, M. (2015). RNA transcription modulates phase transition-driven nuclear body assembly. In Proceedings of the….

Williamson, T.E., Vitalis, A., Crick, S.L., and Pappu, R.V. (2010). Modulation of polyglutamine conformations and dimer formation by the N-terminus of huntingtin. J. Mol. Biol. *396*, 1295–1309.

Wilson, E.B. (1899). THE STRUCTURE OF PROTOPLASM. Science *10*, 33–45.

Wippich, F., Bodenmiller, B., Trajkovska, M.G., Wanka, S., Aebersold, R., and Pelkmans, L. (2013). Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. Cell *152*, 791–805.

Zhang, F., Roth, R., Wolf, M., Roosen-Runge, F., Skoda, M.W.A., Jacobs, R.M.J., Stzucki, M., and Schreiber, F. (2012). Charge-controlled metastable liquid–liquid phase separation in protein solutions as a universal pathway towards crystallization. Soft Matter *8*, 1313–1316.

Zhang, H., Elbaum-Garfinkle, S., Langdon, E.M., Taylor, N., Occhipinti, P., Bridges, A.A., Brangwynne, C.P., and Gladfelter, A.S. (2015). RNA Controls PolyQ Protein Phase Transitions. Mol. Cell *60*, 220–230.

Zhu, L., and Brangwynne, C.P. (2015). Nuclear bodies: the emerging biophysics of nucleoplasmic phases. Curr. Opin. Cell Biol. *34*, 23–30.

Zorn, J.A., Wille, H., Wolan, D.W., and Wells, J.A. (2011). Self-assembling small molecules form nanofibrils that bind procaspase-3 to promote activation. J. Am. Chem. Soc. *133*, 19630–19633.

Zwicker, D., Decker, M., Jaensch, S., Hyman, A.A., and Julicher, F. (2014). Centrosomes are autocatalytic droplets of pericentriolar material organized by centrioles. Proceedings of the National Academy of Sciences of the United States of America.

Zwicker, D., Hyman, A.A., and Jülicher, F. (2015). Suppression of Ostwald ripening in active emulsions. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *92*, 012317.