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The Convergence of Artificial Protein Polymers and Intrinsically Disordered Proteins

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Abstract

A flurry of research in recent years has revealed the molecular origins of many membrane less organelles to be the liquid phase separation of intrinsically disordered proteins (IDPs). Consequently, protein disorder has emerged as an important driver of intracellular compartmentalization, by providing specialized microenvironments chemically distinct from the surrounding medium. Though the importance of protein disorder and its relationship to intracellular phase behavior is clear, a detailed understanding of how such phase behavior can be predicted and controlled remains elusive. While research in IDPs has largely focused on the implications of structural disorder on cellular function and disease, another field, that of artificial protein polymers, has focused on the de novo design of protein polymers with controllable material properties. A subset of these polymers, specifically those derived from structural proteins such as elastin and resilin, are also disordered sequences that undergo liquid-liquid phase separation. This phase separation has been used in a variety of biomedical applications, and researchers studying these polymers have developed methods to precisely characterize and tune their phase behavior. Despite their disparate origins, both fields are complementary as they study the phase behavior of intrinsically disordered polypeptides. This perspective hopes to stimulate collaborative efforts by highlighting the similarities between these two fields and by providing examples of how such collaboration could be mutually beneficial.

Graphical abstract

1.0 Introduction

Membrane less organelles have fascinated biologists since the observation of the nucleolus almost two centuries ago^{1,2}. Only in the last two decades, however, has the origin of these curious intracellular structures been elucidated. We now know that membrane less organelles

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are largely composed of intrinsically disordered proteins (IDPs) that phase separate^{3–7} from the aqueous interior of the cell. With the emergence of IDPs as a new and exciting field in biochemistry and cell biology, another field in materials science, that of protein polymers, has been quietly maturing for the past few decades. Behind this research lies the idea that repeat units composed of collections of amino acids can be used to create new classes of polymers with useful material properties^{8–10}. While research in membrane less organelles has focused on the consequences of disorder on cellular function and disease, research in protein polymers has focused on the bottom-up, *de novo* design of macromolecules with tunable material properties. Similar to IDPs that constitute membrane less organelles, many unstructured protein polymers exhibit aqueous phase behavior, highlighting the fact that structural disorder plays an essential role in their material properties^{11–15}.

Given that both these classes of biomolecules are unstructured —disordered— macromolecules, we propose that there is a clear connection between the liquid-liquid demixing of protein polymers and membrane less organelles, yet there has been little crossover or communication between these two fields of research. This perspective hopes to stimulate collaborative efforts by highlighting the many commonalities shared by these two related fields.

2.0 A Historical Perspective

The membrane less organelle and protein polymer communities have predominantly coexisted as two separate communities with brief periods of intersection¹⁶. Although a full review of both fields is beyond the scope of this perspective, some of the most important discoveries that impacted both polymer science/protein polymer and membrane less organelles/IDPs are documented in Figure 1. As early as 1826, the connection was made between the similarity of synthetic rubber to ‘albumen-like’ material contained within cells¹⁷. Naegli’s observations of granules of various biologic materials is mentioned in the polymer chemistry literature, especially in regard to the separation of large polymers from solution¹⁸. By the time that membrane less organelles had first been robustly characterized by electron microscopy in 1962¹⁹, polymer chemists had developed the experimental techniques to accurately determine the molecular weight of large, unstructured polymers^{20, 21} and had a clear statistical understanding of their solution conformations^{21–24}. This understanding enabled the rapid acceptance of these ‘nuages’ as disordered, high molecular weight, protein and nucleic acid condensates^{20–22, 25}. Historically, the recognition that membrane less organelles contain unstructured proteins and that synthetic polymers are frequently unstructured was a moment in time when these fields could have overlapped productively, but this was not be, as the emergence of molecular and structural biology took biochemistry down a different road.

Changing research motivations in the middle of the 20th century reduced the collaborative efforts between polymer science and biologists. This was driven in part the rise of molecular biology and recombinant protein expression that provided the ability to design, synthesize and purify proteins at the single residue level, and the emergence of x-ray crystallography as the dominant structural characterization technique of proteins. This led to the era of modern structural biology that naturally focused on the major class of known polypeptides that are

folded and have a clearly defined function and are amenable to structural characterization by x-ray diffraction.²⁶ Recombinant protein expression and site-directed mutagenesis reinforced the structure-function paradigm by enabling study of structure-function at the single residue level, providing an unprecedented understanding of how many proteins function. An unfortunate consequence of the structure-function era, however, was the fact that disordered elements within proteins or proteins that were largely unstructured became the “dark matter” of the cell, given their lack of structure and somewhat opaque—difficult to discern—function. As their structure could not be resolved with x-ray crystallography, their role was subsumed within the dominant protein structure-function paradigm as nonfunctional connecting segments. As we now know, these unstructured—intrinsically disordered—proteins are not an evolutionary dead end, but play an important role in biology, and in human health and disease²⁷.

3.0 Disorder and phase behavior are two features that unite protein polymers and IDPs.

The importance of disordered protein regions and their ability to affect function was eventually realized independently by researchers in the membrane less organelle and protein polymer fields. While there was limited communication between these fields, they are united by polypeptide sequences that: (1) can be described as intrinsically disordered and (2) rely on intrinsic disorder for liquid-liquid phase separation.

3.1. Disorder

Chain disorder is the first common ‘structural’ feature shared by many membrane less organelles and protein polymers. This is a negative description of protein structure—a lack of order—and therefore its precise meaning has been a source of constant debate¹¹. Recently, there has been a concerted effort among researchers in the IDP field to clarify this definition, consolidating a variety of descriptions—from the more analytical ‘natively denatured’ to the more creative ‘dancing proteins’—under the umbrella term of ‘intrinsically disordered’^{3, 75}. IDPs and intrinsically disordered regions (IDRs) within proteins are generally composed of tandem repeats of low complexity sequence domains which can be polymorphic⁷⁶ or adopt multiple conformations in solution³. Mechanisms ranging from electrostatic repulsion to the presence of structurally disruptive amino acids (Gly-Pro), have been proposed, with a flexible chain conformation as the unifying feature⁷⁰. Despite the challenge of predicting intrinsic disorder in a multidomain protein that contains other structured elements, prediction algorithms that account for mean net charge, hydrophathy, and relative Gly/Pro content within a sliding window have enabled the identification hundreds of IDPs and IDRs⁷⁷. This ability to broadly predict disorder has accelerated our understanding of the functional importance of IDPs on gene regulation⁷⁸, intra-cellular communication⁷⁹, and disease⁸⁰ in many biological organisms. Intra-cellular IDPs have been implicated in the formation of membrane less organelles that appear to play a role in cell regulation and communication. While not all membrane less organelles require disorder, we know that many require disorder as a ‘structurally’ necessary feature for their formation^{73, 74, 81, 82}.

In contrast to membrane less organelles, understanding the molecular origins of chain disorder in protein polymers was a more straightforward process. The sequence origins of disorder were readily identified during early work with tropoelastin and resilin, two native proteins that have provided much inspiration for the design of protein polymers^{53, 83}. Tropoelastin, the soluble precursor to elastin, is perhaps the most extensively studied IDP, and characterization of its unstructured regions predates our current understanding of protein disorder by half a century^{83–86}. The conformational flexibility of tropoelastin was experimentally verified in the 1960's when the protein was observed to be highly disordered at low temperatures⁸⁷. Subsequent sequencing of both animal and human tropoelastin led to the discovery that elastin is composed of alternating ordered, hydrophilic domains and disordered, hydrophobic domains^{59, 60, 88}. Noting the conserved low sequence complexity tandem repeats, Urry pioneered the development of an even more reductionist version of the disordered hydrophobic domains of tropoelastin, which he termed elastin-like polypeptides (ELPs), that consist of polymers of VPGXG units, a pentapeptide^{65, 89} that recurs in the primary sequence of the hydrophobic, disordered domains of tropoelastin.

The evolution of resilin-like polypeptides followed a similar trajectory. Resilin was first noted for its remarkable mechanical properties in the 1960s^{53, 55} and although it would take another 40 years to derive a consensus amino acid sequence for *Drosophila* Rec-1 resilin⁹⁰, it turned out to also be a highly disordered protein with a repetitive sequence motif⁹¹. The presence of a high degree of disorder was postulated as an important source of the elastomeric mechanical properties of elastin⁹² and resilin⁹³, which motivated the production of recombinant mimics such as ELPs and resilin-like polypeptides (RLPs), in an effort to design protein polymers with similar properties⁹⁴.

3.2 Phase Behavior

Chain disorder is a prerequisite to the second shared capability of membrane less organelles and protein polymers — aqueous liquid-liquid phase separation. Phase separation is a complicated balance of entropic and enthalpic forces which rely on both polymer chemistry and solvent quality in a given state. Thus, if an IDP does not phase separate under a certain set of physiological conditions that does not exclude phase separation under a different set. We note that though there are many examples of IDPs that do not phase separate, there are relatively few examples of proteins which phase separate that do not contain an obvious degree of disorder. As a result, there is a growing understanding in both the protein polymer community and membrane less organelle community that IDPs and IDRs are required to observe phase separation, although the exact amount remains unclear. To move the discussion forward, we accept the mounting evidence of permissive responsibility of intrinsic disorder in both protein polymers and membrane less organelles.

In the protein polymer community, phase behavior is generally studied as a function of temperature and is manifested as lower critical solution temperature (LCST) or upper critical solution temperature (UCST) phase behavior^{95, 96}. Polymers with LCST behavior become immiscible with heat while UCST polymers become miscible with heat. In principle, both UCST and LCST phase behaviors can be observed in the same system⁹⁷. Changes in temperature as a driving force for LCST or UCST phase transition, while eminently useful

in the application of such polymers in an abiotic and technological context, are less relevant to natural biological conditions. In the largely isothermal environment of life, nature frequently uses other variables to drive phase behavior within cells such as changes in protein concentration⁹⁸, post-translation modifications of key proteins⁸², or ligand binding induced coacervation⁹⁹. What unites these myriad and seemingly disparate manifestations of aqueous phase behavior is the concept of the miscibility, specifically the miscibility of a two component solution consisting of polymer chains and solvent molecules¹⁰⁰. All environmental variables that drive aqueous —LCST or UCST— phase behavior in a test tube or within a cell, at their core, simply do so by altering solvent quality between miscible and immiscible states. These observations suggest a testable hypothesis that membrane less organelle formation in cells is driven by the LCST or UCST phase behavior of their constituent IDP(s) in many, if not all, cases (Figure 2).

Tropoelastin provides a concrete example of how the IDP field has influenced the development of protein polymers. As early as the mid-1950's —much before formal recognition of IDPs as a unique class of proteins— it was observed that solubilized elastin reversibly phase separates into liquid-like coacervates, and it was postulated that their liquid nature was due to “loose skeins of random kinks or coils” within the protein⁸³. Seminal work by Keeley and coworkers has shown through recombinant expression of different tropoelastin exons that the unstructured hydrophobic domains promote coacervation with their ordered counterparts responsible for the maturation of elastin fibers^{13, 101–103}. Concurrently, the rise of ELPs inspired by motifs in the unstructured regions of tropoelastin further suggested that the disordered domains were responsible for its coacervation in addition to their contributions to elastin's material properties^{65, 101, 104}. Their facile synthesis by recombinant expression, and the ability to tune their LCST phase behavior precisely by controlling their composition and sequence led to their widespread application in drug delivery and tissue engineering^{105–109}. Similar to ELPs, minimalist resilin-like motifs have been used as repeat units for RLPs, and have been produced recombinantly to understand their dual —LCST and UCST— phase separation behavior, characterized by a low temperature UCST transition and a high temperature LCST phase transition under physiological conditions^{91, 110}.

Until recently, the phase behavior of stimulus-responsive protein polymers was only studied in the context of very specific repetitive amino acid motifs, as LCST phase behavior was thought to be restricted to specific sequences, such as (VPGXG)_n and its close analogs, such as VPAXG and IPGVG^{111, 112}. However, recent research on both protein polymers and IDPs that form membrane less organelles clearly demonstrates that disorder, and not sequence, is the most important structural feature that drives aqueous phase behavior^{103, 113}. We have, for example, recently shown that the periodic inclusion of structurally disruptive Gly and Pro residues within protein polymers inspired by elastin and resilin is a common feature of diverse class of protein polymers that exhibit LCST and UCST phase behavior⁹⁴. On this Pro and Gly rich template, the remaining amino acid composition simply determines whether the polypeptide exhibits LCST or UCST phase separation and the window of temperature that this phase behavior occurs in; these observations then led us to postulate a set of simple design heuristics to encode LCST or UCST phase behavior in protein polymers⁹⁴. Similarly, recent work on the nephrin intracellular domain confirmed that the

overall amino acid composition and its propensity for disorder was a more important determinant of its intracellular phase behavior than its precise amino acid sequence⁷. Teasing out the relative importance of structural disorder on the phase behavior in membrane less organelles and protein polymers remains an open challenge, but the evidence that structural disorder in these systems promotes phase separation is compelling. Contextualizing protein polymers and membrane less organelles under the umbrella of intrinsic disorder and phase behavior has the potential to motivate collaboration between both fields.

4.0 Opportunities for Collaboration

We believe that this is an opportune time for renewed collaboration between materials scientists working on protein polymers and biologists working on membrane less organelles/IDPs. This section highlights directions of inquiry explored in the development of protein polymers that could inform the study of IDPs and, conversely, questions explored by research into IDPs that could inform the protein polymer discipline (Figure 4).

4.1 Protein Polymers can Inform Membrane less Organelles

A critical component in the development of protein polymers was the recognition that there is immense value in studying proteins outside of their biological context. Although our understanding of membrane less organelles must be relevant to their biological context, a limitation of solely studying them within cells is the complexity of the intracellular environment, coupled to the difficulty of controlling and modulating that environment. Detailed in vitro characterization of artificial peptide polymers has revealed that variations in the concentration of the polymer, the types of other cosolutes and their concentration can affect phase behavior by moving phase boundaries^{114, 115}. Furthermore, we have shown that ligand binding to a protein fused to an LCST peptide polymer such as ELPs can dramatically impact phase behavior^{116, 117}. This is especially relevant to IDPs because there can be considerable spatio-temporal changes in protein expression levels, pH, and ligand concentration within a cell and from cell to cell¹¹⁸, which can greatly impact their phase behavior¹¹⁹. While the biological complexity of the intracellular environment cannot fully be recapitulated in an in vitro system, in vitro studies can be highly relevant to developing a deeper, physics driven understanding of aqueous demixing phase behavior within cells (Figure 3). For example, a systematic study of how ELP molecular architecture affects protein phase behavior^{65, 89, 105}, informed the mechanistic study of elastin fiber formation—which we now know to be a multi-step process involving the maturation and crosslinking of phase separated coacervates¹⁰².

The ability to analyze protein polymers in a simple, well-defined solution environment is predicated on recombinant expression of these polymers and purifying them to homogeneity; fortunately, both ELPs and RLPs are amenable to over-expression in bacterial expression systems. The availability of large amounts of recombinant protein polymers has allowed understanding of their phase behavior within a thermodynamic framework and provides a rational basis for the design of stimulus responsive protein polymers. The phase separation of a polymer in a solvent can be described by three pairwise interactions:

polymer-solvent, solvent-solvent, polymer-polymer. As with protein polymers, the phase separation of many IDPs can be mathematically described by the Flory interaction parameter, χ , which accounts for competition among these interactions as a function of temperature^{6, 82}. Because this type of phase behavior is driven by the thermodynamic favorability of the polymer interacting with itself, the complex intracellular environment has to be distilled down to a set of intrinsic variables such as ionicity, pH, and viscosity that impact phase behavior of a single IDP. As demonstrated by the low sequence complexity domains from RNA/protein assemblies^{73, 81, 122, 123}, single protein sequences are often sufficient to drive phase separation. Therefore, binary phase diagrams are potentially informative even in the context of the complex intracellular environment of membrane less organelles that may contain other components.

A critical issue facing the membrane less organelle community is explaining the relative importance of the many intrinsic —protein related— and extrinsic —environment related— variables that influence liquid-liquid phase separation. For example, it is clear that phase separation in some membrane less organelles is driven by ligand binding such as RNA⁷³, which triggers coacervation of the IDP into a membrane less organelle with the cell^{7, 72, 124, 125}. On the other hand, recent reports show that nature also uses many other triggers to drive phase separation within the cell; Sup35 makes pH sensitive droplets that is driven by modulation of the cloud point temperature by a shift in the pKa of the protein¹²⁶ while TIA-1, exhibits Zn²⁺ dependent phase separation in the cell¹²⁷. It is interesting that both pH and Zn²⁺ concentration have been independently used to drive LCST phase behavior in ELPs. We have previously shown that ELPs with ionizable residues exhibit a sharp pH dependent phase transition¹²⁸, while protonation of the imidazole side chain of His-containing ELPs as a function of pH in a narrow range for 7.4 to 6.4 causes a drastic shift in the cloud point temperature that enables the LCST phase transition to isothermally triggered by a change of pH in this range. The LCST phase transition of these His-containing ELPs can also be isothermally triggered by changing the Zn²⁺ concentration. It is notable that these results predate recent observations that these same mechanisms are used by IDPs to drive droplet formation within the cell^{127, 129}, suggesting that stimulus responsive protein polymers have useful insights to offer the IDP community. What unites the seemingly disparate observations is the likely fact that all IDPs exist near phase boundaries and that Nature has found clever ways of crossing phase boundaries by an intracellular trigger such as binding of a ligand with RNA or metal ions, or by a change in pH.

Another benefit of recombinant expression of IDPs is that it provides enough pure material to allow detailed in vitro analysis by a variety of experimental techniques that can provide a mechanistic understanding of IDP function. In contrast, microscopy has been the dominant technique used to study membrane less organelles within the cell for over 100 years despite the fact that much of their behavior —such as their phase diagrams— cannot be easily elucidated by visible light microscopy in the complex environment of the cell. Biophysical and structural analysis techniques that provide detailed structural information have recently helped to inform work on membrane less organelles include circular dichroism⁸¹, NMR¹²⁴, light scattering⁷², electron microscopy^{72, 123, 130}, and rheology⁸¹.

Detailed biophysical studies of the aqueous phase separation of a large set of LCST and UCST protein polymers can provide useful insights of how amino acid sequence controls phase behavior that may translate to the sequence determinants of the coacervation behavior of IDPs. Conventional thinking suggests that phase behavior of stimulus responsive protein polymers is controlled to a first approximation by the average amino acid composition rather than the specific amino acid sequence⁷. For example, for a ~30kDa ELP, changing just 5% of the amino acids from valine to alanine—differentiated by a single methyl group—increases the concentration necessary for phase transition at 37°C by more than an order of magnitude, suggesting that a simple post-translational modification—methylation—could easily trigger the phase behavior of an IDP in vivo¹⁰⁶. Furthermore, the distribution of amino acids can also affect both the critical concentration and the architecture of the resultant coacervate in ELPs. This concept has been theoretically explored in the context of IDPs through atomistic simulations on the impact of the distributions of charged amino acids within a polymer chain on the radius of gyration¹³¹. In a similar vein, we recently produced a library of ELPs where two guest residues of differing hydrophilicities were precisely distributed throughout the polymer, ranging from alternating copolymer on one end, to blocky copolymers in the middle, and di-block copolymers on the other end of the mixing scale¹⁰⁷. Alternating polymers, where the two “monomers” are perfectly mixed along the polymer chain, display classic coacervation phase behavior in response to temperature, whereas polymers with poor mixing of monomers in the polymer chain form of nanoparticles upon undergoing phase separation. These studies suggest that much work remains to be done to understand the role of amino acid composition and distribution on the phase separation behavior of IDPs that form membrane less organelles.

The primary utility of artificial LCST or UCST protein polymers for the IDP field is to provide simple but relevant models to understand how amino acid sequence controls the formation of membrane less organelles in the cell. For example, can the shape of a LCST or UCST phase diagram be predicted from the primary amino acid sequence, enabling de novo prediction of phase behavior of IDPs? Conversely, what lessons can we learn from IDPs to repurpose their intracellular phase separation to create novel compartments within cells? Could wild type IDPs be replaced with engineered versions, without affecting wild type functionality, but that are more resistant to pathogenic structural changes (i.e. liquid to amyloid transition) such as those seen in Alzheimer’s disease? These are just some examples of the new directions that could be explored through collaborative efforts between these two communities.

4.2 Membrane less Organelles can Inform Protein Polymers

Biological structures have long been a source of inspiration for polymer chemists, and IDPs that form membrane less organelles are no exception. In fact, developments in protein-based polymers are only a recent subset of the centuries old field of bio-inspired materials. Since the initial discovery of disordered protein polymers capable of phase separation, until recently, only a handful of sequences from IDPs have been used to create phase separating protein polymers¹¹. This limited set of available sequences is due to researchers working on protein polymers placing a higher priority on manipulating the phase behavior of existing sequences for specific applications over the discovery of new sequences that exhibit phase

behavior. Even elastin biopolymers, with their rich history of sequence modifications, have remained largely restricted to polymers of the pentapeptide motifs (VPGXG)_n and (VPAXG)_n. While working towards the development of design rules for IDPs with phase behavior, we have recently shown that the amino acid sequence space available to biopolymers with LCST or UCST is much larger than previously thought⁹⁴. This finding is consistent with a growing body of literature on IDPs, which has further uncovered a diverse sequence space that exhibit aqueous phase behavior^{3, 7}. These new sequences provide building blocks for the design of the next generation of stimulus-responsive protein polymers.

Studies on IDPs and membrane less organelles have also revealed the breadth of bioactive domains that can be encoded into IDPs without loss of phase behavior^{3-5, 132}. In the field of LCST protein polymers, the dogma that perturbation of the repeat sequence is likely to abolish phase behavior has largely restricted the incorporation of proteins or peptides to the chain ends of the polymer or to periodic incorporation of very short peptide sequence such as RGD within the polymer sequence that were thought to be minimally disruptive¹³³⁻¹³⁷. There are two useful insights from IDPs that are relevant for the design of protein polymers that exhibit aqueous phase behavior that suggest that these restrictions are irrelevant. First, intrinsically disordered proteins have evolved to not only create unique biochemical environments within the cell, but also to actively interact with other macromolecules. These interactions are often controlled by short linear motifs (SLiMs), which, like the RGD peptide, do not rely on a unique three-dimensional structure for their activity and therefore can be easily encoded into disordered proteins¹³². Second, the entire length of an IDP is frequently not disordered and yet they still exhibit reversible phase behavior that is driven primarily by their intrinsically disordered regions^{73, 81, 82}. These observations suggest that there is considerable flexibility in incorporating extrinsic —folded and functional— domains into artificial protein polymers that will retain their aqueous phase behavior, as long as a critical —but as yet undefined— fraction of the polypeptide chain is unstructured.

5.0 Conclusion

Despite their divergent origins, the underlying physics of membrane less organelles and protein polymers are likely to be similar. Researchers in both fields are working to understand the functional implications of phase separation driven by protein disorder. Until recently, IDPs responsible for membrane less organelles have only been studied within the confines of the intracellular environment through live cell microscopy and mutagenesis. While this approach has been highly informative, a detailed understanding of how intracellular phase behavior can be predicted and controlled remains elusive. On the other hand, the imperatives of industrial applications have driven biomaterial researchers to design disordered protein polymers with sharp, tunable phase boundaries. Reflective of the sentiment of Richard Feynman, “what I cannot create, I do not understand,” we propose that the insights discovered through the bottom-up design of protein polymers may provide the missing link to understanding intracellular phase behavior. Conversely, the diversity of IDPs that exhibit aqueous phase behavior provides inspiration for the design of new protein polymers. It is our hope that increased collaboration across these two fields will eventually lead to their coalescence.

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Figure 1. Significant events in the history of protein polymers and membrane less organelles.

Protein polymers are an offshoot of polymer science, both of which are denoted by the blue line. Membrane less organelles emerged from biochemistry and cell biology and are denoted by the red line. At various points in the 20th century, advancements made by polymer science and biochemistry and cell biology influenced one another, which is symbolically represented in the two lines crossing over one another. With time, however, both fields drifted away from each other, as graphically represented by the divergence of the parallel red and blue lines away from one another. With the recent discovery of myriad IDPs and protein polymers derived from IDP building blocks and their common features, the time is now right for renewed communication and collaboration between these two fields 1, 2, 16–26, 28–74.

Figure 2. Phase Behavior

(a) Polymers with upper critical solution temperature (UCST) phase behavior transition at a critical point that shifts along the phase boundary. At a constant concentration, a specific temperature is required for phase separation, and at a constant temperature, a specific concentration is required. (b) The shape of the phase diagram changes between systems—either narrowing, broadening, or shifting up and down—with protein sequence and solvent conditions.

Figure 3. Intracellular and extracellular phase transitions

(a) Phase behavior is a robust process that largely depends on a small set of variables which readily translate between intracellular and extracellular environments. (b) Recombinantly expressing IDPs responsible for the formation of membrane less organelles, such as (bi/iii) the nephrin intracellular domain (NICD)⁷, has allowed meaningful investigation into the conditions necessary for intracellular phase separation. Likewise, (bii/iv) elastin-like polypeptides (ELPs), disordered protein peptides historically studied in extracellular environments, retain their phase behavior when constitutively expressed in mammalian cells^{120, 121}. This consistency in behavior opens the door for the *de novo* design of IDPs with specific intracellular function.

Figure 4. Specific research opportunities for collaboration between researchers working on protein polymers and membrane less organelles

Scientists studying protein polymers and membrane less organelles have many opportunities to contribute to each other's disciplines. Protein polymer chemists have adapted the approaches and experimental methodologies of polymer chemistry to study the effect of chain architecture and solution conditions on phase separation. These same techniques could be easily adopted by the membrane less organelle/IDP community to provide new molecular insights into the origins and mechanisms of phase separation of these macromolecules. Likewise, the prevalence of phase separation across biology provides new inspiration for

polymer chemists to expand the diversity of motifs that when polymerized will exhibit phase behavior. The recognition of bioactive sequences that are short and disordered (short linear motifs) provides new building blocks for protein polymers.

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