PHASIS BIOTECH

Biotechnology & drug discovery company

Funding Objective: **$2.5M**

Founder: **Cliff Brangwynne**

Harvard-trained PhD

Professor of Chemical & Biological Engineering
Princeton University
Howard Hughes Medical Institute Investigator

Discoverer of a paradigm-shifting concept of biological organization via liquid-liquid phase separation

IP: Princeton University

Provisional patents 2017,2018
Available for licensing to Phasis Biotech

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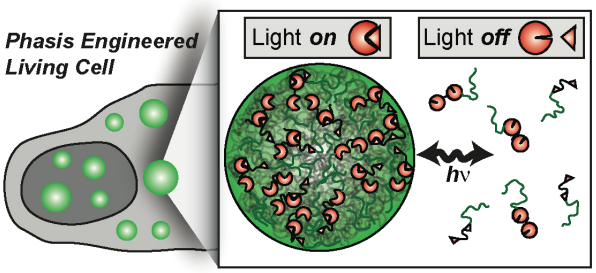

HIGHLIGHTS:

-Disruptive new approach for multi-billion dollar drug discovery market

-Suite of technologies that use light for controllable aggregation of proteins underlying Alzheimer's, ALS, Huntington's and other devastating diseases

-Compatible with high-throughput screening platform for evaluating aggregation-retarding potential of millions of compounds in the context of living cells, on dramatically reduced timescales

-Additional product development applications include new protein purification strategies

Over the last several years, there has been a paradigm shift in our understanding of biological organization, with Brangwynne's discovery that a process known as liquid-liquid phase separation underlies many processes in living cells. ***This discovery has broad implications for basic cell physiology, and also the development of breakthrough therapies to address currently untreatable diseases caused by protein aggregation.*** The Brangwynne lab has followed up on this fundamental work with the invention of several new methods. Our technology, to be commercialized via the creation of a new startup, Phasis Biotech, allow for a range of biotechnology applications, with a focus on drug discovery for neurodegenerative aggregation diseases.

The technology we have developed uses light to reversibly cluster proteins into phase-separated droplets. We take advantage of the ability of light to induce conformation changes to proteins, which allows for controlling the phase separation and pathological conversion of aggregation-prone proteins. Our technologies are adaptable and can be applied to many different cell types and model organisms, enabling new approaches to drug development, but also additional applications including protein purification and control of various intracellular biological processes.

Market

Drug Discovery

Our work has shown that the Phasis technology can be utilized to study and control the aggregation behavior of proteins in living cells, with unprecedented control. The power of these technologies is that they allow for controllable assembly of high concentration condensed phases of both physiological and disease-causing proteins, which appears to dramatically decrease the timescale associated with pathological conversion to solid-like pathological aggregates. The technology thus represents a tremendous opportunity for

examining the effect of compounds that decrease this tendency to pathological conversion, within the native intracellular context. **This will translate into a drastic reduction in the timescale for discovery of novel aggregation-retarding drugs, using high-throughput microscopy-based screening approaches.** We have held multiple conversations with biotechnology companies - the most prominent of our potential partners is a large biotech based in Boston; this company's experts, in numerous teleconferences, have expressed strong interest in working with Phasis to further develop and implement the technology in their drug discovery pipeline. **These companies recognize that Phasis technology has the potential to overturn the existing drug discovery paradigm.**

Protein Purification

Phasis technology could also be used to accelerate protein purification, an industrially important process for which many challenges remain. For example, membrane-bound or highly hydrophobic proteins are difficult to purify, while even relatively well-behaved proteins typically require multiple elution and "clean-up" steps before high purity protein is obtained, with each additional purification step decreasing yield. Phasis technology could enable a resin-free method using a centrifuge-based protocol to spin out these phase-separated droplets before cleaving the desired protein with an intein or protease. **The end result would be a simpler and cheaper protein purification strategy.** We are engaged in discussions with a second established Biotech company that is interested in working with Phasis to determine if our technology can be used to purify various restriction enzymes that they have had trouble purifying. They are also open to the potential for marketing our purification technology as part of a kit.

Business Concept

Our initial efforts will focus on deliverables achieved within the first two years. These include:

1. Establishing proof-of-concept that small molecules can inhibit protein aggregation induced by our technologies, for at least one disease model, with an initial focus on targeting Alzheimer's proteins (e.g. tau), ALS proteins (e.g. TDP43), and Huntingtons disease proteins (Huntington/polyQ).
2. Establishing proof-of-concept that the Phasis technology can be used as protein purification platform.
3. Identification of additional product development applications, iterative refinement of the business plan, and market discovery.

For the initial startup phase (roughly two years), we have identified capital needs of roughly $2.5M. We have estimated these needs based on the cost for leasing physical space within a local biotech incubator, capital equipment and consumables, 3-4 full time scientists, 1 CEO, and several consultants. A CEO will be recruited to drive the early stage growth of Phasis, but Cliff Brangwynne will continue to play a central role as a consultant and Chairman of Phasis' Scientific Advisory Board. Moreover, several Brangwynne lab graduate students with upcoming thesis defense dates have expressed interest in working for Phasis; these students have developed and trained on these technologies during their time in the Brangwynne lab, ensuring a seamless transition to the commercialization of the technologies.

What Makes Phasis Technology So Exciting?

The work behind Phasis Biotech has been pioneered by Cliff Brangwynne, an HHMI Investigator and Associate Professor of Chemical and Biological Engineering at Princeton University, whose work has revealed that membrane-less organelles and other structures inside living cells assemble through a mechanism known as liquid-liquid phase separation or condensation (Figure 1); recent press underscores this paradigm shift in biology (*1*). This revolution in our understanding builds from Brangwynne's initial discoveries of the liquid phase nature of P granules and nucleoli (*2, 3*), a list of phase separated condensates which now includes dozens of different types of structures (*4*). Liquid-liquid phase separation is a type of phase transition similar to those common in nature and familiar from everyday experiences of dewdrops condensing on blades of grass or water freezing into ice. Work from Brangwynne and colleagues has identified key molecular driving forces controlling this intracellular protein condensation, in particular the role of intrinsically disordered proteins regions (IDRs), which are associated with not only healthy intracellular condensates, but also their pathological conversion in protein aggregation disease (*5, 6*).

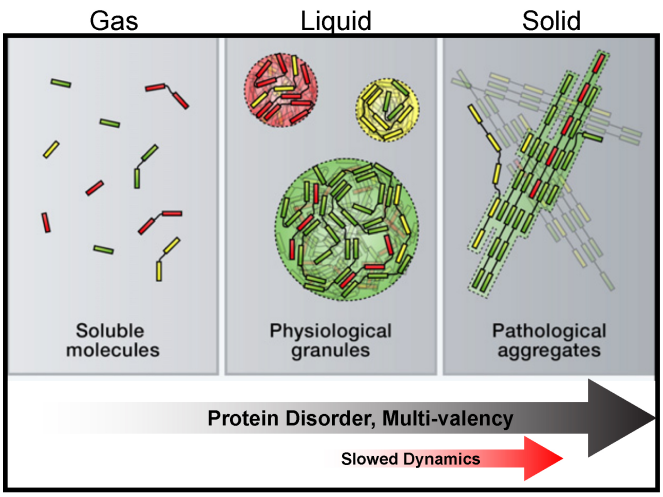

Figure 1. Brangwynne's work has revealed that protein and RNA in living cells can condense into dynamic liquid states (center panel), which represent physiological organelles. However, under some conditions these liquid phases can transition to form amyloid-like solid phases found in aggregation diseases (right panel). Adapted from a Brangwynne lab publication (*7*).

The technologies described below have been developed in Brangwynne's lab to control protein phase separation and associated pathological aggregation. The methods that have been developed bring together the insights obtained on how IDRs can drive phase separation, together with various strategies for optogenetic control. These technologies are adaptable and can be broadly applied to many systems. For example, in published and submitted work, Brangwynne and colleagues have utilized these tools in human and mouse cell lines (e.g. HEK, U2OS, HeLa, 3T3), the worm *C.elegans* (nematode), *X.laevis* (frog), and *S.cerevisiae* (yeast). They have also applied the technologies to study and control purified proteins. The broad applicability allows a multi-pronged approach to product development.

Intellectual Property

We have established a solid base of unique and powerful IP for controlling intracellular phase behavior. Princeton University has agreed to license this technology to Phasis on a worldwide, exclusive basis, with rights to sublicense, on market-appropriate economic terms. This including the following technologies, which are all pending patent protection:

optoDroplets: Combining disordered protein motifs with light-activated oligomerization.

A seminal paper from the Brangwynne lab published in early 2017 introduced the first-generation "optoDroplets" system for controlling intracellular phase behavior in tissue culture cells (*8*). This

system takes advantage of two central features of endogenous phase separated organelles: mutlivalency and protein disorder (Fig. 4). We utilized IDRs from proteins known to drive phase separation in living cells, such as FUS, which we fuse to Cry2, a blue-light activatable oligomerization domain from *Arabidopsis*. Cells expressing these constructs undergo liquid-liquid phase separation in response to blue light activation. Moreover, we showed that cells driven to a highly supersaturated state exhibit more solid-like condensates, suggesting the ability to tuning liquid-to-solid phase transitions within living cells, which a large number of recent studies implicate as key steps in protein aggregation pathologies such as Alzheimers and Amyotrophic lateral schlerosis(ALS) (*7, 9, 10*). This system was the first to show the power of combining optogenetics and the biophysics of IDR-driven phase separation. This and the variant optogenetic approaches described below can also be readily combined with traditional molecular biology techniques, such as transcriptional profiling (e.g., RNA-seq), immunoprecipitation, and mass spectrometry. A US Patent application was filed on June 9, 2017 "Optogenetic tool for rapid and reversible clustering of proteins".

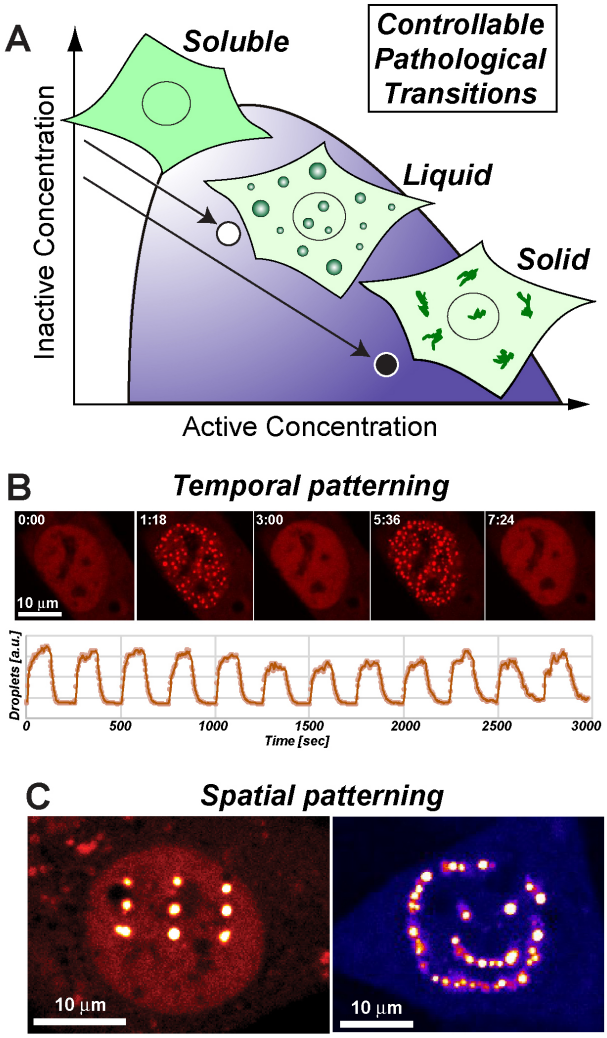

Figure 2. (A) Phasis technologies take advantage of light to control the concentration of phase separation-prone molecules, which can driven to form liquid droplets, or pathological solids. (B) Many cycles of controllable droplet assembly and disassembly can be tested, e.g. to determine the impact of small molecules on the reversibility of disease-associated protein mutations. (C) Precise spatial droplet patterns can be achieved, for example using a 3x3 light activation array (left), or a "smiley face" activation pattern (right). Times are shown as min:sec. All images from our unpublished work.

Corelets: controlling disordered protein-driven phase separation using defined high-valency scaffolds

The Corelets system is a second generation optogenetic system we recently developed (*11*), which mimics the radial architecture of many endogenous proteins implicated in phase separation, such as the nucleolar protein Npm1, which pentamerizes to drive phase separation. CORELET use a spherical self-assembling protein core tethered to light-activatable proteins. Upon activation, these proteins are able to bind with their cognate partners, selectively localizing any fusion proteins containing this light-activatable partner. By incorporating intrinsically disordered regions (IDRs) into these fusion proteins, single Corelets are induced to phase separate into large droplet condensates. Along with IDRs, the fusions can be engineered to contain enzymes, fluorescent tags, or other proteins of interest (Fig. 2). A unique feature of Corelets, as compared to the original

Cry2-based optodroplet system, is that while the strong light-driven interactions effectively switch the system into a one component system of IDR coated cores, they do not directly contribute to the cohesive interactions of the emergent liquid phase, which instead rely exclusively on homotypic IDR-IDR interactions. Thus, Corelets can be designed using any number of different IDRs with sequence programmed to achieve the desired material properties. The Corelet system has also allowed us to precisely quantify the concentration- and valency-dependence of phase behavior, generating the first full phase diagrams in living cells. A manuscript describing the Corelet technology is in revision at *Cell* (see also Biorxiv: https://doi.org/10.1101/283655). US Patent application was filed on June 9, 2017 "Disordered protein based seeds for molecular clustering".

CasDrop: Targeting tunable phase separation at addressable genomic loci

The CasDrop system builds upon the technological advances of optoDroplet and Corelet technologies, to enable the targeting of IDR-driven phase separation to defined locations on the genome of living cells. CasDrop utilizes the well-characterized CRISPR/Cas9-based genome targeting technology, and can lead to the targeted assembly of gene regulatory proteins which form a controlled microenvironment for modulating local genome architecture and transcriptional output. As with the optoDroplet and Corelet systems, the CasDrop technology can be delivered into a variety of biological systems including mammalian cell cultures by standard transfection methods or viral delivery. A manuscript describing the CasDrop technology and its utilization for interrogating the principles of nuclear organization is currently under review at *Nature*. Patent protection is pending.

Conclusions

Phasis technologies build on recent paradigm shifting discoveries in cell biology, which have been led by Phasis founder Cliff Brangwynne. Phasis will build upon the strong intellectual property portfolio developed in the Brangwynne lab, to develop a suite of commercial applications. The initial focus will be on establishing a platform for drug discovery, to address the growing and still untreatable global health crisis of neurodegenerative protein aggregation diseases. The tremendous interest in these technologies, both in the academic and commericial sectors, underscores the broad recognition of their market-disrupting potential. We estimate that the initial 2-year phase of commercialization of these technologies through the establishment of Phasis will require an investment of $2.5M. We anticipate that at the end of this initial stage, Phasis will be well-positioned to successfully exit through acquisition by an existing Biotech, or through an additional round of funding will enable continued growth as the pioneer of a new set of platforms that disrupt several different biotech sub-markets.

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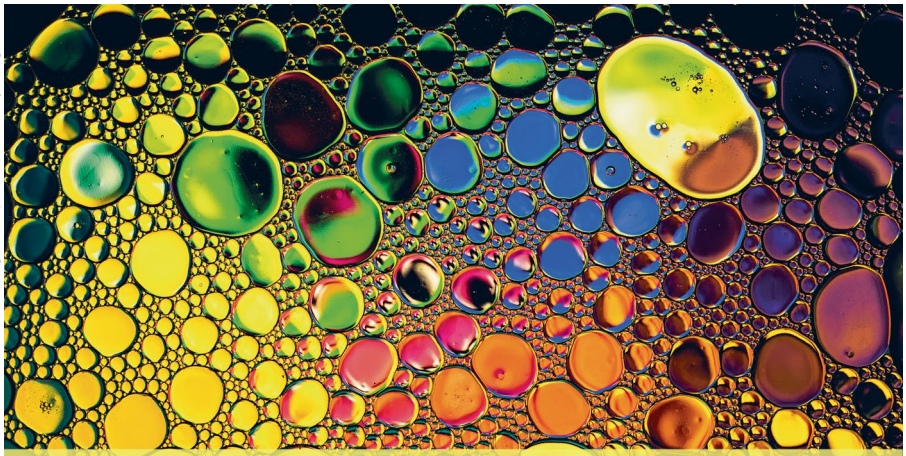

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Biomolecular condensates pique drug discovery curiosity

Transient liquid-like droplets made up of proteins and RNA are scattered throughout the cell — with potentially broad drug discovery implications.

Asher Mullard

According to most biology textbooks, the main organizing principle of the cell is the membrane. Lipid bilayers wrap organelles including the nucleus, mitochondria and the endoplasmic reticulum to keep some proteins in and others out. The rest of a cell's internal machinery has been depicted as floating awash in the cytoplasm, with proteins occasionally running into binding partners, substrates and small-molecule drugs. Now, a growing appreciation for the importance of biomolecular condensates — transient liquid-like droplets made up of proteins and RNA — is forcing cell biologists to rethink this model.

Preliminary evidence already suggests that these so-called membraneless organelles, which form through a process called liquid–liquid phase separation, are important in health and disease. They seem to act as crucibles to speed up reactions between their component proteins in some cases, and as a means of keeping reactants away from one another in others. And mutations that perturb the assembly and disassembly of these structures appear to affect the course of neurodegenerative diseases, cancer and more.

Drug hunters are taking note. Dewpoint Therapeutics launched in January as the first company to publicly stake a claim in this space. A few other start-ups are in stealth mode, set to launch soon. And large pharma is watching closely. "This is a really interesting and exciting area of fundamental biology," says Jason Imbriglio, a researcher at Merck & Co. who recently co-organized a New York Academy of Sciences symposium on the role of these structures. "We are looking to better understand the role of these structures in disease and potential entry points for therapeutics."

The opportunity here might one day be huge, adds Mark Murcko, CSO of Dewpoint. "As we learn how condensates function, we can go back to that list of previously undruggable targets that everyone loves and we can ask, 'OK, now that we have this new insight, does that target appear to be somewhat more tractable because we have a different way of thinking about it?'"

There is a long list of open questions for drug hunters to consider. What happens if a prioritized target clusters in transient biomolecular condensates? Does the diffusion of drugs into these membraneless organelles affect exposure? And, can small molecules sometimes have unintended and harmful effects on condensate formation and disassembly?

"It's not quite cosmology, but there are a lot of unknowns," says Cliff Brangwynne, a biophysical engineer at Princeton University whose work has helped to revitalize interest in liquid–liquid phase separation and membraneless organelles.

> "You don't want to turn your back on this stuff and ignore it because it might sneak up behind you"

"You don't want to turn your back on this stuff and ignore it because it might sneak up behind you," adds Derek Lowe, a medicinal chemist at the Novartis Institutes for BioMedical Research who is following this space closely.

Hiding in plain cytoplasm

Membraneless organelles have been popping up in the literature for over a century. Edmund Beecher Wilson, a cell biology pioneer, described the widespread existence of liquid-like organelles in a review in *Science* in 1899, and these structures bespeckle the drawings of cells throughout the decades. But without a clear understanding of the role of these structures in living cells or the biophysics of their assembly, few researchers have paid them much heed.

That started changing about a decade ago.

When Brangwynne, who was then doing a post doc at the Max Planck Institute, and his supervisor Tony Hyman started to peer at embryos of the worm *Caenorhabditis elegans* through the microscope, their initial aim was to understand the origins of P granules, clusters of RNA and RNA-binding proteins that were poorly biophysically characterized at the time. But what they found was that P granules behaved like oil droplets in vinaigrette, fusing with one another, dripping and rapidly condensing and dissolving in and out of solution. They reported this work in *Science* in 2009, in a landmark paper that applied the concept of phase separation to a specific membraneless organelle.

Two years later Brangwynne and colleagues reported in *Proceedings of the National Academy of Sciences* that nucleoli, structures that form in the nucleus with a key role in ribosome assembly, have similar liquid-like properties and reliance on phase separation. Soon after, researchers started to spot these phenomena everywhere, including in Cajal bodies, nuclear speckles, stress granules, RNA transport granules and more (FIG. 1).

At the same time, researchers were making headway in deciphering the biophysical underpinnings of the rapid formation and dissolution of these structures. In 2012, for example, UT Southwestern Medical Center biophysicist Michael Rosen and colleagues described in *Nature* how multivalent macromolecules that can bind multiple partners enable sharp liquid–liquid phase separations and the rapid condensation of micrometre-sized liquid-like droplets. Subsequent work has shown that the multivalent macromolecules in play include proteins with intrinsically disordered regions (IDRs), RNA molecules and more.

Disease associations started to crystallize shortly afterwards. Paul Taylor, a neurologist at St. Jude Children's Research Hospital, was working to understand the genetics of neurodegenerative diseases, and reported in 2013 in *Nature* that conserved mutations in the IDRs of HNRNPA2B1 and HNRNPA1 were associated with amyotrophic lateral sclerosis (ALS). When one of Taylor's students subsequently realized that purified HNRNPA1 behaves bizarrely in the test tube — flitting reversibly in and out solution — Taylor too found himself captivated by the biology of membraneless organelles.

By 2015, a full renaissance was underway. That year, five papers independently demonstrated that IDRs were crucial to the phase transitions of biomolecular condensates. "All of a sudden there was enormous interest in it. It's just been explosive," says Brangwynne.

Because IDRs are widespread throughout the proteome, these findings have captured the imagination of cell biologists everywhere, adds Taylor. "Many scientists suddenly realized that their favorite proteins undergo biologically relevant phase transitions and they hadn't even realized it," he adds.

Research on the importance of phase transitions in ALS, especially, has taken off. Hyman, a co-founder of Dewpoint, reported with colleagues that FUS forms membraneless organelles at sites of DNA damage and in the cytoplasm upon stress, and that mutations in FUS that are linked with ALS lead to aberrant phase transitions. Taylor reported with colleagues that HNRNPA1 also undergoes liquid–liquid phase separation, and that ALS-related mutations in this protein can also affect liquid–liquid phase separation.

ALS-related mutations that affect the dynamics of membraneless organelle formation, it seems, make some of these structures stickier and more viscous than they would otherwise be. This, in turn, appears to trigger the fibrillization of the inclusion bodies that are a hallmark of disease. Taylor estimates that disturbances in phase transitions account for more than 90% of ALS cases.

Other neurodegenerative diseases might also be linked to liquid–liquid phase separation. In 2017, Ankur Jain and Ron Vale proposed that a broad set of repeat expansion disorders — including Huntington disease and muscular dystrophy as well as ALS — might involve aberrant RNA droplet formation. And in 2018, Taylor and colleagues reported that soluble tau species, a key culprit in Alzheimer disease, can form condensates.

Cancer, too, seems to involve membraneless organelle biology. In 2016, Massachusetts General Hospital pathologist Miguel Rivera and colleagues reported that phase transition mechanisms might establish and maintain oncogenic gene regulatory programmes in Ewing sarcoma. And last year, structural biologist Tanja Mittag, at St. Jude Children's Research Hospital, showed that the tumour suppressor protein SPOP is active inside membraneless organelles. Cancer-related SPOP mutations, moreover, interfere with the protein's ability to phase separate, colocalize with its substrate and suppress tumour formation.

When it comes to drug development programmes, oncology could offer the easiest path to applications. Whereas medicinal chemists may struggle to restore the finely tuned balances of condensate formation in neurodegenerative settings, they can focus on wreaking havoc with the system to kill cancer cells. "I'm not proud of this as a medicinal chemist, but we have a much better chance of messing stuff up than we have of making it work better," says Lowe.

Transcription factors and super-enhancers seem to rely on liquid–liquid phase separation biology to control gene expression, for example, providing one example of how this could play out. "If you just want to go in there and throw a monkey wrench into transcription by messing up condensate behaviour, oncology would be the place to do it," says Lowe.

Beyond neurodegeneration and cancer, infectious diseases and autoimmunity are also worth watching, says Taylor. Infectious disease agents can for example exploit biomolecular condensates to facilitate their replication cycles, he explains, and phase transitions seem to control signalling cascades that modulate innate immunity. "The proof of concept is not there yet to the same extent that it is in neurodegeneration and cancer, but I think it's coming down the pike," he says.

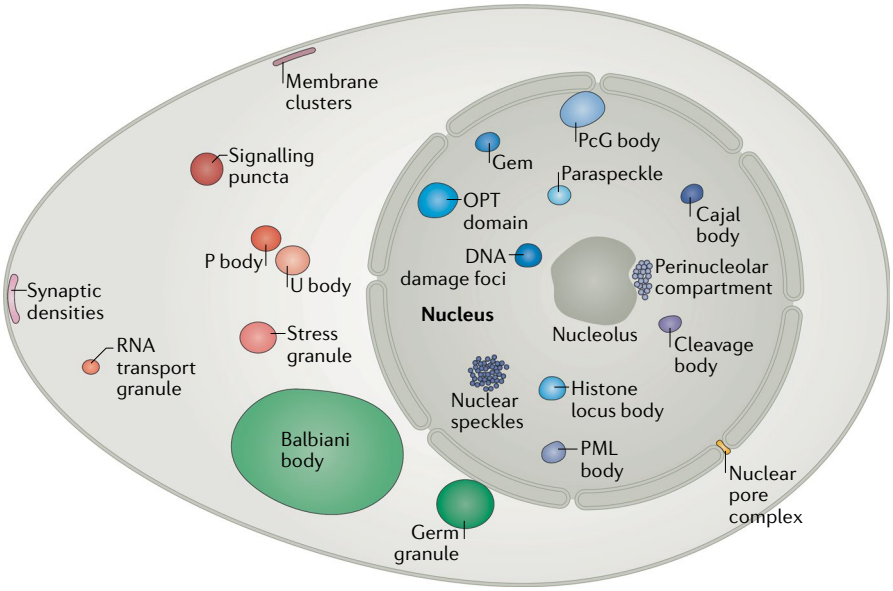

Fig. 1 | **Membraneless organelles in the cell.** A growing number of biomolecular condensates have been identified in the cytoplasm and nucleus. Not all compartments are present in all cell types. Gem, Gemini of Cajal body; OPT, OCT1/PTF/transcription; PcG, Polycomb group; PML, promyelocytic leukaemia. Reproduced from *Nat. Rev. Mol. Cell Biol.* **18**, 285–298; 2017, Springer Nature Limited.

Controlling condensates

Some researchers still have some doubts. Will work done with purified condensates in in vitro systems translate into in vivo models? Are the disease associations causal? And, could condensates just be biophysical artifacts with no real role, rather than critical cellular structures?

Hyman and Rosen trod carefully in a 2017 review of membraneless organelles. "We do not understand in most cases what biochemical or cellular functions uniquely emerge from organizing molecules into such structures," they wrote with colleagues. "Phenotypes resulting from the disruption of condensates are relatively subtle and the structures do not appear to be essential for the viability of cells or organisms." The field, in other words, is still young.

But Taylor is convinced about the pathophysiological importance of condensates, even if he concedes that the path from this new organizational paradigm

to new drugs remains fraught. "We are at this weird point in the field where the concepts and principles have emerged, but precise targets are as yet not clear," says Taylor.

He nevertheless points to two main ways forward. On the one hand, researchers can hunt for small molecules that will bind directly to the proteins that make up the biomolecular condensates themselves — potentially stabilizing or destabilizing these proteins to tweak their ability to form clusters, for example. However, many of these components are proteins with IDRs, which have long eluded small-molecule discovery work.

Alternatively, researchers can target the upstream regulatory machinery that controls condensate biology. ATPases, helicases and ubiquitinases seem critical to liquid–liquid phase dynamics, as do proteins that control the post-translational modification of condensate members. It remains to be seen whether any of these targets are selective enough to control only the dynamics of disease-associated condensates, and whether redundant regulatory mechanisms might complicate matters.

Taylor's hopes are nevertheless high. "Both of those are viable strategies, but it's the second strategy that I would place my money on," he says.

Dewpoint is keeping its options open. "Depending on the specific therapeutic situation and the specific target and the specific condensate, it'll be valuable to have a whole range of approaches at our disposal," says Murcko.

Even IDRs — in everything from kinases to transcription factors — are on the table, he adds. For more than 15 years, Murcko has had a folder on his computer for papers about shape-shifting proteins with IDRs. "Every single time I would see a paper on this topic I would read it and I would get mad because I didn't really understand what these proteins were doing," says Murcko, who has harnessed protein structure to design better drugs throughout his career. "Now, for the first time, we are getting a clear sense of a big part of what these IDRs are doing," he adds.

IDRs, with all the structure of limp spaghetti, it turns out are multivalent actors that can bind to other proteins and RNAs around them to nucleate liquid-like condensates when the conditions are just right.

The hunt for small molecules that can interact with amorphous IDR regions is likely to remain frustrating. "It is beastly hard," says Lowe, "enough to give the man the willies." But Murcko points to encouraging proof-of-concept data as cause for optimism. In 2016, for example, researchers identified a small-molecule drug candidate that binds to the disordered region of the androgen receptor.

Regardless of what targets drug hunters choose to go after, they are going to need a wide array of tools at their disposal. Not only will they have to be able to individually study the protein components and the biophysics of condensates, but they'll also have to use advanced imaging techniques to screen for small molecules that can modulate these processes in relevant cells.

"Unless you really have thought pretty carefully about a wide range of technologies to tackle these problems, you are in for a world of hurt, full of false readings, artifacts and outright failures," says Murcko.

Established technologies such as cross-linking mass spectrometry are being used to see how single amino acid changes to condensate components affect protein structure, and high-content imaging can be used to watch membraneless organelles form and to screen for small molecules that perturb these dynamics. But new techniques are also needed.

Hyman points to the need for more work with Bouillon microscopy as a means of assessing the material properties of condensates inside a cell, to assess for example whether a condensate is liquid-like or gel-like. "Those are the new kinds of tools that are the cutting edge."

Brangwynne meanwhile has focused on developing optogenetic tools that can control condensate formation inside the cell. OptoDroplets, Corelets and CasDrop combine the IDR regions of condensate components with light-dependent oligomerization domains from other proteins. By shining light onto cells that express these constructs, researchers can essentially force oligomerization and the resulting condensate formation.

"No one's really thinking quantitatively about the rules that govern the organization inside our cells. They just see some puncta and call it phase separation, and I think there's a real danger with that," says Brangwynne. "What this technology does is it allows you to quantitatively map phase diagrams in living cells," he says.

Taylor has built on the OptoDroplet format to create OptoGranules, another way to initiate membraneless organelle assembly.

Such tools should help researchers to figure out the functions of condensates. And for drug hunters who want to find small molecules that can control condensate biology, they could provide a way to assess whether small molecules are making a difference to phase separation dynamics in cells.

Brangwynne is moving towards commercializing his tools for therapeutic and drug discovery applications, and says he has had interest from both investors and existing biotech companies. But he also likes to borrow a quote from late Nobel prize winner Sydney Brenner to emphasize the need for more tool development. "Progress in science depends on new techniques, new discoveries and new ideas, probably in that order."

Early phase

Drug hunters have never had a great grasp on where exactly a small molecule goes once it penetrates into a cell, and how it finds a free-floating target within the chaos of the cytoplasm. Now, these new insights into the organizational principles of cells suggest that things might be even more complicated than previously anticipated.

"Not only would you have to track your compounds in the cell, you'd also have to track them into locations that are so small you need advanced imaging techniques just to convince yourself they exist," says Lowe.

Researchers have their work cut out for them to get a better grasp on what this all might mean for drug discovery. What targets can be found in membraneless organelles? How, if at all, do biocondensates directly or indirectly impact the activity of various pathways? Do small molecules traffic in and out these structures, and does this impact exposure? Can small molecules meaningfully modulate condensate formation and function? None of this work will be straightforward, given the ephemeral and microscopic nature of these organelles.

Depending on how things play out, this new field could also open up new avenues in toxicity testing. "My personal opinion is that as the field matures, no drug company should feel comfortable taking a drug candidate into clinical trials unless they fully understood what effect that drug candidate has on cellular condensates," says Murcko.

As with many emerging fields, these opportunities and open questions leave industry drug hunters in a tricky place. "It's like trying to catch a falling knife," says Lowe. "You might want to let it drop and vibrate on the floor for a second before you pick it up. But you don't want to sit around so long that other people have already got all the good stuff staked out."